ISSCR INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH

KSSCR 한국줄기세포학회 KOREAN SOCIETY FOR STEM CELL RESEARCH

ISSCR / KSSCR 2019 INTERNATIONAL SYMPOSIUM

Stem Cell Research: SEOUL Present and Future

KOREA

Abstract Book

Mugunghwa (Rose of Sharon) is Korea's national flower

GRAND HILTON SEOUL HOTEL, KOREA

26-27 SEPTEMBER, 2019

Co-hosted by ISSCR INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH, KSSCR 한국줄기세포학회 KOREAN SOCIETY FOR STEM CELL RESEARCH

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WELCOME MESSAGE

한국줄기세포학회 KOREAN SOCIETY FOR STEM CELL RESEARCH

Dear Colleagues,

On behalf of the Korean Society for Stem Cell Research (KSSCR), we are delighted to welcome you to the 2019 ISSCR/KSSCR International Symposium held on 26-27 September, 2019 in Seoul, Korea. This is a joint meeting of the KSSCR and the International Society for Stem Cell Research (ISSCR), the largest and most authoritative stem cell society in the world.

With no doubts, the 2019 ISSCR/KSSCR International Symposium will be the biggest and the most successful stem cell symposium in the history of Korean stem cell research which will greatly contribute to the development of KSSCR and stem cell research in Korea in the future.

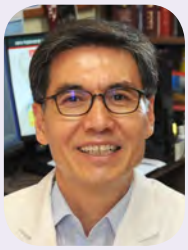
The 2019 ISSCR/KSSCR International Symposium will consist of excellent scientific programs and presentations, including a keynote lecture by Professor Shinya Yamanaka from Kyoto University, Japan, who was awarded a Nobel Prize in 2012 for his groundbreaking discovery of induced pluripotent stem cells (iPSC). Another keynote lecture will be delivered by Professor Deepak Srivastava from Gladstone Institutes, USA, who is the president of ISSCR. In addition, there will be seven special sessions and six concurrent sessions, covering the hottest and most important topics of the stem cell field, from basic research to clinical applications. We believe that these excellent scientific programs, presented by dozens of world-renowned stem cell experts, will provide participants with a great forum to exchange ideas and information in the fields of stem cells and regenerative medicine.

We hope the 2019 ISSCR/KSSCR International Symposium offers you wonderful opportunities to broaden your view of stem cell research and to create interactive networks with other stem cell scientists. In addition to attending the Symposium, we hope you have the chance to visit some picturesque places in Seoul and other areas of Korea.

Thank you again for your great interest in the 2019 ISSCR/KSSCR International Symposium.

Sincerely,

Dong-Wook Kim
KSSCR president



About the ISSCR

Mission Statement

The mission of the International Society for Stem Cell Research (ISSCR) is to promote excellence in stem cell science and applications to human health.

History & Philosophy

With roughly 4,000 members from more than 60 countries, the ISSCR is the preeminent global, cross-disciplinary, science-based organization dedicated to stem cell research and its translation to the clinic.

Formed in 2002, the Society promotes global collaboration among the world's most talented and committed stem cell scientists and physicians, and plays a catalyzing role in the development of effective new medical treatments.

The Society brings together researchers, clinicians, academics, and industry representatives engaged in both fundamental and applied research, and provides international opportunities to share science and advance the field. The ISSCR supports the strong scientific and medical consensus that continued research on all types of stem cells is critical to developing research strategies that will ultimately provide new therapies for patients with debilitating diseases and injuries.

The ISSCR represents academia and industry on a broad range of issues that affect the well-being of patients and their families, and strives to educate the public and government regulators on the basic principles of stem cell science and the realistic potential for new medical treatments and cures.

The leadership of the ISSCR is acutely aware of the responsibility the Society bears to promote the highest scientific and ethical standards, and is dedicated to integrity in the rigor and quality of the research community's scientific work, the public policy stands it takes on stem cell related issues, and the organization's relations with its key constituents and the public. Only such an abiding commitment to integrity can ensure that as the ISSCR grows, it will continue to be seen as a fair and trusted advocate by both its internal and external stakeholders.

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Mohammad Jaber, Hebrew University, Israel
Sang Yoon Moon, University of Western Australia, Australia

PROGRAM

Day 1 / Thursday, 26 September, 2019

08:45-09:00 Opening ceremony and welcome remarks: Dong-Wook Kim
Introduction to ISSCR Symposium: Nancy Witty

Keynote Lecture 1 — Room A+B+C

09:00-09:45 — **Introduction: Deepak Srivastava**

Recent Progress in iPS Cell Research and Application

Shinya Yamanaka
(Gladstone Institutes, USA and Center for iPS Cell Research & Application, Kyoto University, Japan)

Special Session 1 : Cell Fate Decisions — Room A+B+C

09:45-10:45 — **Chair: Christine Mummery**

The Blastocyst and its Stem Cells: Understanding Early Cell Fate Decisions and Modeling Early Development

Janet Rossant (Hospital for Sick Children, Canada)

SHP2 Mutations Induce Early Cell Fate Determination of Noonan Syndrome-iPSCs to the Glial Lineage During Neural Development In Vitro

Yong-Mahn Han (Korea Advanced Institute of Science and Technology, Korea)

Special Session 2 : Cellular Reprogramming — Room A+B+C

11:05-12:35 — **Chair: Kevin Eggan**

Unleashing the Developmental Potential of iPSCs

Hans Schöler (Max Planck Institute for Molecular Biomedicine, Germany)

Recent Advances in Direct Reprogramming and Gene Targeting as New Therapies for Neurodegenerative Diseases

Jongpil Kim (Dongguk University, Korea)

Using Chemical Approaches to Generate Desired Functional Cells

Hongkui Deng (Peking University, China)

Luncheon Symposium 1: Molecular Devices — Room A

12:35-13:35

Overcoming Challenges of High Content Imaging and Analysis for 3D Cell Culture

Minho Tak (Molecular Devices Korea, Korea)

Luncheon Symposium 2: JSK Biomed Inc. **Room B**

12:35-13:35

What's in Your Media Matters: Applications of Cellular Metabolic Profiling in Stem Cell Research

Yoonseok Kam (Cell Analysis Group, Agilent Technologies, Inc., Korea)

Luncheon Symposium 3: Thermo Fisher Scientific **Room C**

12:35-13:35

Integrated Work Flow Solutions from Discovery to Translational Research

Uma Lakshmipathy (Thermo Fisher Scientific, USA)

Concurrent Session 1 : Organoids and Development **Room A**

13:35-15:25 **Chair: Il-Hoan Oh**

The Inflammatory Niche Shapes Lung Regeneration

Joo-Hyeon Lee (University of Cambridge, UK)

In Vitro Vascularization of Human Organoids and Tissue Fragments: Cell Cultures' Missing Link?

Paul Vulto (Mimetas B.v., The Netherlands)

Single Cell RNA-sequencing Analysis Reveals the Absence of Transient iPSCs During Pluripotency Factor-Mediated Direct Reprogramming

Janghwan Kim (Korea Research Institute of Bioscience and Biotechnology, Korea)

Comparative Analysis of Diverse Cell States Establishes an Epigenetic Basis for Inferring Regulatory Genes Governing Cell Identity

Enakshi Sinniah (University of Queensland, Australia)

Direct Induction of the Three Pre-implantation Blastocyst Cell Types from Fibroblast

Mohammad Jaber (The Hebrew University, Israel)

Chemical Derived Hepatic Progenitors, a Silver Lining of Regenerative Medicine of the Liver

Dongho Choi (Hanyang University, Korea)

Concurrent Session 2 : Stem Cells and Tissue Engineering **Room B**

13:35-15:25 **Chair: Peter Zandstra**

Biomimetic Hydrogels for Stem Cell and Reprogrammed Cell Therapy

Seung-Woo Cho (Yonsei University, Korea)

Bone Marrow-Derived Epithelial Cells and Hair Follicle Bulge Stem Cells Initiate and Promote Chronic Inflammation-Associated Cutaneous Neoplasms in Mice

Heuijoon Park (Columbia University, USA)

Printing Human Pancreatic Tissues for the Treatment of Diabetes

Jinah Jang (Postech, Korea)

Modelling of Retinitis Pigmentosa Caused by a Nonsense Mutation in the RP1 Gene Using Induced Pluripotent Stem Cells

Sang Yoon Moon (The University of Western Australia, Australia)

Generation of Epithelial Organoids from Human Tonsils of Waldeyer's Ring in a Chemically Defined Medium

Jongman Yoo (CHA University, Korea)

Vascularized Spheroids/Organoids Using Microfluidics

Noo Li Jeon (Seoul National University, Korea)

Concurrent Session 3 : Stem Cells and Regenerative Medicine in Asia

Room C

13:35-15:25 **Co-Chairs: Youngsook Son, Ssang-Goo Cho**

Present and Future Perspective of Myocardial Regeneration Therapy

Yoshiki Sawa (President, Japanese Society for Regenerative Medicine, Japan)

Neural Differentiation for Discovery and Therapy

Baoyang Hu (Innovation Academy for Stem Cell and Regeneration, Chinese Academy of Sciences, China)

Modeling Human Hepato-Biliary-Pancreatic Organogenesis from the Foregut-Midgut Boundary

Takanori Takebe
(Tokyo Medical and Dental University, Japan and Cincinnati Children's Hospital, USA)

National Stem Cell Resource Center (NSCRC) of China, and its Role in Delivering Stem Cell-based Therapies

Jie Hao (Innovation Academy for Stem Cell and Regeneration, Chinese Academy of Sciences, China)

Differential Regulation of Neural Stem Cell Differentiation by MEK Inhibitors

Hyunjung Kim (Chung-Ang University, Korea)

Deciphering Brain Somatic Mutations in Human Neurological Disorders

Jeong Ho Lee (Korea Advanced Institute of Science and Technology, Korea)

Special Session 3 : Tissue and Cancer Stem Cells

Room A+B+C

15:45-17:15 **Chair: Paul Frenette**

Modelling of Human Neurodegenerative Diseases Using iPSCs and Genetically Modified Non-Human Primates

Hideyuki Okano (Keio University School of Medicine, Japan)

Pharmacogenomic Landscape of Patient-Derived Cancer Stem Cells Informs Precision Oncology Therapy

Do Hyun Nam (Sungkyunkwan University School of Medicine, Korea)

Stress-Mediated Hyperactivation of Sympathetic Nerves Drives Melanocyte Stem Cell Depletion

Ya-Chieh Hsu (Harvard University, USA)

Special Session 4 : Microenvironment and the Stem Cell Niche

Room A+B+C

17:25-18:25 **Chair: Amy Wagers**

Engineering Stem Cell Niche for Regeneration and Therapy

Il-Hoan Oh (The Catholic University of Korea, Korea)

A Novel Mechanism for Innate Immune Tolerance of Hematopoietic and Leukemic Stem Cells

Paul Frenette (Albert Einstein College of Medicine, USA)

18:30-19:30 Posters I (odd numbers) and Mixer

Exhibition Hall (2F, Convention Center)

19:30-20:30 Posters II (even numbers) and Mixer

Exhibition Hall (2F, Convention Center)

Day 2 / Friday, 27 September, 2019

08:45-09:00 Opening remarks: Dong-Youn Hwang

Special Session 5 : Epigenetics and Aging of Stem Cells **Room A+B+C**

09:00-10:00 **Chair: Ya-Chieh Hsu**

Activating Muscle Stem Cell Function through Immediate Early Transcription Factors and Lipid Mediators

Amy Wagers (Harvard University, USA)

Restoring the Human Germline

Amander Clark (University of California, Los Angeles, USA)

Special Session 6 : New Technologies in Stem Cells **Room A+B+C**

10:00-11:00 **Chair: Hongkui Deng**

Haploid Human Embryonic Stem Cells: Derivation and Applications

Nissim Benvenisty (The Hebrew University, Israel)

Patterning Mesoderm and Blood Development from Human Pluripotent Stem Cells

Peter Zandstra (University of British Columbia, Canada)

Special Session 7 : Disease Modeling and Stem Cells — **Room A+B+C**

11:20-12:50 — **Chair: Malin Parmar**

Villages in a Dish: Scaling the Use of Human Cell Models to Detect Drug Genotype Interactions
Kevin Eggan (Harvard University, USA)

Harnessing the Potential of ES Cells & iPS Cells: New Opportunities for Therapeutics, Disease Modeling and Genome Editing
Dong-Wook Kim (Yonsei University, Korea)

Cardiovascular Diseases and Drugs: Where Are We with hiPSC Models?
Christine Mummery (Leiden University, The Netherlands)

Luncheon Symposium 4: Kangstem Biotech — **Room A**

12:50-13:50

Development of First-in-Class and Best-in-Class Stem Cell Therapeutics for Immune Related Diseases
Speaker: Seunghee Lee (Kangstem Biotech, Korea)

Luncheon Symposium 5: Bio-Techne — **Room B**

12:50-13:50

Organoid Technology and Medicine
KyungJin Lee (ORGANOIDSCIENCES, Ltd., Korea)

Luncheon Symposium 6: Ajinomoto Co., Inc. + CHAYON Laboratories, Inc. — **Room C**

12:50-13:50

Use of Biomaterials to Regulate Neural Differentiation from Human iPSCs
Yoichi Kosodo (Korea Brain Research Institute, Korea)

Concurrent Session 4: Genome Editing in Stem Cells — **Room A**

13:50-15:15 — **Chair: Hans Schöler**

Highly Efficient Genome Editing by CRISPR-Cpf1 Using CRISPR RNA with a U-rich 3'-Overhang
Yong-Sam Kim (Korea Research Institute of Bioscience and Biotechnology, Korea)

A Lin28a Loss-of-Function Associated with Early-Onset Parkinson's Disease
Mi-Yoon Chang (Hanyang University, Korea)

Enhanced Immunocompatibility of iPS Cells by CRISPR-Cas9 Targeted Disruption of HLA Genes
Huaigeng Xu (Center for iPS Cell Research and Application, Japan)

Genome-Wide Screening of Functional Deubiquitinating Enzymes Regulating Stemness-Related Proteins Using CRISPR/Cas9-Mediated Dubs Knockout Library
Suresh Ramakrishna (Hanyang University, Korea)

Efficient Detection and Purification of Human PSC-Derived Cell Populations Using RNA Switches
Shin-Il Kim (AceRNA Technologies Co., Japan)

Concurrent Session 5 : Application of Stem Cell Technologies **Room B**

13:50-15:15 **Chair: Janet Rossant**

Real-Time Monitoring of Dynamic Cellular Properties of Ex Vivo Expanded or In Vivo Engrafted Mesenchymal Stem Cells
Dong-Myung Shin (University of Ulsan College of Medicine, Korea)

Human iPSC-Derived Astroglia Delay Disease Progression in YAC128 Huntington's Disease Mice
Hyun Jung Park (CHA University, Korea)

Promotion of Pancreatic Beta Cell Differentiation by Modulating Organ-Specific Stromal Niche Signals
Tae-Hee Kim (The Hospital for Sick Children/University of Toronto, Canada)

Therapeutic Potential of Prodrug Solid Tumour Therapy by Non-Viral Modified Mesenchymal Stem Cells in Mice Model and Companion Animal
Yoon Khei Ho (National University of Singapore, Singapore)

Therapy of Ischemic Diseases Using Human Induced Pluripotent Stem Cells
Jae Ho Kim (Pusan National University College of Medicine, Korea)

Concurrent Session 6 : Stem Cell Quality Control for Cell Therapy
Co-organized by GAiT/K-NIH/ISCBI **Room C**

13:50-15:15 **Co-chairs: Jihwan Song and Glyn Stacey**

Usefulness of the Korean HLA-homozygous iPSC lines to Multiple Populations
Jihwan Song (CHA University and GAiT, Korea)

The Global Alliance for iPSC Therapies (GAiT): Quality Testing of Clinical Grade Induced Pluripotent Stem Cells
Stephen Sullivan (The Global Alliances for iPSC Therapies (GAiT), UK)

Latest Accomplishments of National Center for Stem Cell and Regenerative Medicine
Soo Kyung Koo (Korea NIH, Korea)

Suitability of Pluripotent Stem Cell Lines for Clinical Applications
Glyn Stacey (International Stem Cell Banking Initiative, UK)

Integrated iPSC Characterization Optimal for Cell Therapy Manufacturing
Uma Lakshmipathy (Thermo Fisher Scientific, USA)

Cancer-related Mutations in Primed and Naive Human Pluripotent Stem Cells
Nissim Benvenisty (The Hebrew University, Israel)

Autologous iPS Cell Therapy for Macular Degeneration: From Bench-To-Bedside
Kapil Bharti (NIH/National Eye Institute, USA)

Special Session 8 : Stem Cells in Translation	**Room A+B+C**
15:35-17:05	**Chair: Hideyuki Okano**

Towards a Patient-Specific Treatment for Parkinson's Disease

Malin Parmar (Lund University, Sweden)

Trafficking of Endogenous Stem Cells and M1/M2 Polarization of Macrophage for Tissue Repair; A Story of Substance-P

Youngsook Son (Kyung Hee University, Korea)

Stem Cell Ethics and Policy: Converging Paths and New Synergies

Insoo Hyun (Case Western Reserve University, USA)

Keynote Lecture 2	**Room A+B+C**
17:15-18:00	**Introduction: Christine Mummery**

Cellular Reprogramming Approaches in Human Genetics and Regenerative Medicine

Deepak Srivastava (Gladstone Institutes, USA)

18:00-18:25 Poster award and closing ceremony: Eekhoon Jho
Closing remarks: Youngsook Son

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Thursday, 26 September, 09:00-09:45, Room A+B+C

Keynote Lecture 1 **Introduction: Srivastava, Deepak**

Yamanaka, Shinya

Gladstone Institutes, San Francisco, CA, USA and Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan

Professor Shinya Yamanaka is most recognized for his discovery of induced pluripotent stem (iPS) cells, which are reprogrammed cells from the differentiated state to the pluripotent state. He is Director of the Center for iPS Cell Research and Application (CiRA), Kyoto University (2008~), and Senior Investigator at the Gladstone Institutes (2007~). Since his breakthrough finding, he has been the recipient of many prestigious awards, including the Nobel Prize in Physiology or Medicine (2012).

09:00-09:45

RECENT PROGRESS IN IPS CELL RESEARCH AND APPLICATION

Yamanaka, Shinya

Gladstone Institutes, San Francisco, CA, USA and Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan

Induced pluripotent stem cells (iPSCs) can proliferate almost indefinitely and differentiate into multiple lineages, giving them wide medical application. As a result, they are being used for new cell-based therapies, disease models and drug development around the world. In 2014, the world's first clinical study using iPSCs began for the treatment of age-related macular degeneration (AMD). iPSCs can be used for regenerative medicine to restore organ function. To push these efforts, we are proceeding with an iPSC stock project in which clinical-grade iPSC clones are being established from "super" donors with homologous HLA haplotypes, which are associated with decreased immune response and therefore less risk of transplant rejection. In 2015, we started distributing an iPSC stock clone to organizations in Japan, and clinical study using the iPSC stock began for the people with AMD in 2017, and 1-year follow-up study of 5 cases turned out the safety of allogenic transplantation using iPSC stock. Additionally, clinical trial for Parkinson's disease started using the iPSC stock-originated neurons in 2018 and the first surgery to transplant dopaminergic progenitors into the brain was conducted at Kyoto University Hospital. Other applications of iPSCs include drug screening, toxicity studies and the elucidation of disease mechanisms using disease-specific iPSCs from the people with intractable diseases. We reported a new drug screening system using iPSCs derived from the people with fibrodysplasia ossificans progressiva (FOP), revealing one drug candidate, Rapamycin. Based on these findings, we have achieved to initiate a clinical trial to treat FOP in 2017. Additionally, Bosutinib, a drug for leukemia was revealed to be efficacious for the treatment of amyotrophic lateral sclerosis (ALS) using a disease model established from disease-specific iPSC. We have just recently initiated a new clinical trial for the Bosutinib to treat ALS at the Kyoto University Hospital and the other centers in 2019. Over the past decade iPSCs research made a great progress. However, there are still various hurdles to be overcome, iPSC-based science is certainly moving forward for delivering innovative therapeutic options to the people with intractable diseases.

Keywords: Induced pluripotent stem cells (iPSCs), cell-based therapies, drug development

Friday, 27 September, 17:15-18:00, Room A+B+C

Keynote Lecture 2 — **Introduction: Mummery, Christine**

Srivastava, Deepak

Gladstone Institutes, San Francisco, CA, USA and School of Medicine, University of California, San Francisco, CA, USA

Dr. Srivastava received his B.S. from Rice University, M.D. from University of Texas, trained in pediatrics at UCSF, and in pediatric cardiology at Harvard Medical School. Dr. Srivastava's laboratory discovered genetic bases for cardiac defects and revealed complex signaling, transcriptional, and translational networks that regulate progenitor cells to adopt a cardiac cell fate and subsequently fashion a functioning heart. He has leveraged this knowledge to understand disease mechanisms using iPS cells and to reprogram fibroblasts directly into cardiomyocyte-like cells for regenerative purposes.
Dr. Srivastava is a member of the American Academy of Arts and Sciences and the National Academy of Medicine.

17:15-18:00

CELLULAR REPROGRAMMING APPROACHES IN HUMAN GENETICS AND REGENERATIVE MEDICINE

Srivastava, Deepak

Gladstone Institutes, San Francisco, CA, USA and School of Medicine, University of California, San Francisco, CA, USA

Heart disease is a leading cause of death in adults and children. We have revealed complex signaling, transcriptional and translational networks that guide early differentiation of cardiac progenitors and later morphogenetic events during cardiogenesis. By leveraging these networks, we have reprogrammed disease-specific human cells in order to model genetically defined human heart disease in patients carrying mutations in cardiac developmental genes. These studies revealed mechanisms of haploinsufficiency and more recently the contribution of genetic variants inherited in an oligogenic fashion in heart disease. Integration of large-scale DNA-sequencing in patients with CRISPR and single cell technologies are revealing complex inheritance patterns for human disease. We also utilized a combination of major cardiac developmental regulatory factors to induce direct reprogramming of resident cardiac fibroblasts into cardiomyocyte-like cells with global gene expression and electrical activity similar to cardiomyocytes, and now have revealed the epigenetic mechanisms underlying the cell fate switch. Most recently, we identified an approach to unlock the cell cycle in adult cardiomyocytes by introducing fetal cyclins and cyclin dependent kinases, and have been able to induce resident, post-mitotic cardiomyocytes to undergo cell division efficiently enough to regenerate damaged myocardium. Knowledge regarding the early steps of cardiac differentiation in vivo has led to effective strategies to generate necessary cardiac cell types for disease-modeling and regenerative approaches, and may lead to new strategies for human heart disease.

Keywords: Cellular reprogramming, heart disease, developmental biology

Thursday, 26 September, 09:45-10:45

Special Session 1 : Cell Fate Decisions, Room A+B+C

Chair: Mummery, Christine

THE BLASTOCYST AND ITS STEM CELLS: UNDERSTANDING EARLY CELL FATE DECISIONS AND MODELING EARLY DEVELOPMENT

Rossant, Janet
Program in Developmental and Stem Cell Biology, Hospital for Sick Children, and Department of Molecular Genetics, University of Toronto, ON, Canada

SHP2 MUTATIONS INDUCE EARLY CELL FATE DETERMINATION OF NOONAN SYNDROME-IPSCS TO THE GLIAL LINEAGE DURING NEURAL DEVELOPMENT IN VITRO

Han, Yong-Mahn
Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon, Korea

Thursday, 26 September, 11:05-12:35

Special Session 2 : Cellular Reprogramming, Room A+B+C

Chair: Eggan, Kevin

UNLEASHING THE DEVELOPMENTAL POTENTIAL OF IPSCS

Schöler, Hans R.
Max Planck Institute for Molecular Biomedicine, Münster, Germany

RECENT ADVANCES IN CELL REPROGRAMMING AND GENE EDITING AS NEW THERAPEUTICS FOR NEURODEGENERATIVE DISEASES

Kim, Jongpil
Department of Chemistry and Biomedical Engineering, Dongguk University, Seoul, Korea

USING CHEMICAL APPROACHES TO GENERATE DESIRED FUNCTIONAL CELLS

Deng, Hongkui
Stem Cell Research Center, Peking-Tsinghua Center for Life Sciences, Peking University, Beijing, China

Thursday, 26 September, 09:45-10:45, Room A+B+C

Special Session 1 : Cell Fate Decisions **Chair: Mummery, Christine**

Rossant, Janet

Program in Developmental and Stem Cell Biology, Hospital for Sick Children, and Department of Molecular Genetics, University of Toronto, ON, Canada

Janet Rossant, CC, PhD, FRS, FRSC is President and Scientific Director of the Gairdner Foundation, and Senior Scientist and Chief of Research Emeritus at the Hospital for Sick Children in Toronto. She is an internationally recognized developmental and stem cell biologist, exploring the origins of stem cells in the early embryo and their applications to understanding and treating human disease.

09:45-10:15

THE BLASTOCYST AND ITS STEM CELLS: UNDERSTANDING EARLY CELL FATE DECISIONS AND MODELING EARLY DEVELOPMENT

Rossant, Janet

Program in Developmental and Stem Cell Biology, Hospital for Sick Children, and Department of Molecular Genetics, University of Toronto, ON, Canada

The blastocyst stage of development marks the final transition from totipotency to lineage restriction in the early mammalian embryo, with the formation of the pluripotent epiblast and the extraembryonic lineages of trophectoderm and primitive endoderm. In the mouse, self-renewing stem cell lines reflecting the lineage specificity of these three cell types have been generated, namely embryonic stem cells, trophoblast stem cells and XEN cells, respectively. The key transcription factors and signaling pathways specifying cell fate in the blastocyst and its derived stem cells have been identified. It is often stated that the 2-cell stage is the last truly totipotent stage of development, but by undertaking a careful experimental assessment of the dynamic processes of lineage commitment, combined with in vivo imaging and single cell gene expression analysis, we have shown that loss of totipotency is a gradual process and not complete until close to the blastocyst stage. The exact lineage equivalence between the in vitro blastocyst-derived stem cells and the lineage restriction of their in vivo counterparts is still a matter of discussion and is relevant to their proposed use in generating stem-cell-derived embryo models. Development of human and mouse blastocysts show similarities and differences that impacts on the potential to develop human blastocyst stem cell lines for exploring human early developmental events.

Keywords: Blastocyst, lineage development, stem cell lines

Han, Yong-Mahn

Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon, Korea

Professor Han a professor of Department of Biological Sciences at KAIST. He received B.S. and M.S. degrees from Konkuk University, Korea and Ph.D. degree in molecular biology from KAIST. During his researcher position at KRIBB for 20 years (1986 to 2006), Professor Han worked on diverse research topics in mammalian development, including transgenic mice, somatic cell nuclear transfer, and epigenetic reprogramming of preimplantation embryos. Since Professor Han moved to KAIST, his group has focused on studying differentiation of human pluripotent stem cells and modeling human diseases via cellular reprogramming.

10:15-10:45

SHP2 MUTATIONS INDUCE EARLY CELL FATE DETERMINATION OF NOONAN SYNDROME-IPSCS TO THE GLIAL LINEAGE DURING NEURAL DEVELOPMENT IN VITRO

Han, Yong-Mahn

Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon, Korea

Noonan syndrome (NS) is a genetic disorder caused by gain-of-function mutations in Src homology 2 domain-containing protein tyrosine phosphatase 2 (SHP2). Although approximately 30-50% of NS patients have the cognitive deficits such as lower intelligence and neuropsychological complications, mechanisms how SHP2 mutations are associated with the neurological dysfunction in NS patients are poorly understood. For modeling the neurological dysfunction of NS, induced pluripotent stem cells (NS-iPSCs) were generated from dermal fibroblasts of three NS-patients, and differentiated into neural cells. NS-embryoid bodies (EBs) derived from NS-iPSCs showed abnormal morphologies and defective development to neural rosettes (NRs). Inhibition of both BMP and TGF-β signaling pathways rescued impaired early neuroectodermal development of NS-iPSCs. Recued NS-EBs normally differentiated into NRs and neural precursor cells (NPCs). NS-neural cells further differentiated from NS-NPCs exhibited phenotypic abnormalities such as increment of glial cells and shortened neurites of neuronal cells. Multielectrode assay represented reduced extracellular spontaneous firing in NS-neural cells. SHP2 inhibition helps partially restore defective phenotypes and dysfunctional electrophysiology of NS-neural cells. Recently, cerebral organoids developed from NS-iPSCs also represented diverse neurodevelopmental anomalies, including enhanced gliogenesis, delayed cortical layers development, and decreased electrophysiological activity. Transient SHP2 inhibition ameliorated their abnormalities in the neurodevelopmental process of NS-cerebral organoids. The results indicate that SHP2 mutations lead to aberrant gliogenesis in NS-iPSCs during neural development, and the precocious development of glial cells may contribute to cognitive deficits in NS patients.

Keywords: Noonan syndrome, iPSCs, neural differentiation

Thursday, 26 September, 11:05-12:35, Room A+B+C

Special Session 2 : Cellular Reprogramming

Chair: Eggan, Kevin

Schöler, Hans R.

Max Planck Institute for Molecular Biomedicine, Münster, Germany

Hans Schöler is a Director of the Max Planck Society, as well as a professor or adjunct professor at several universities in Germany, the United States, and Korea. Schöler is a member of the BOD of the International Society for Stem Cell Research and will serve as the president of the German Stem Cell Network for the 2021–2022 term. His major research interests are outlined here https://www.mpi-muenster.mpg.de/97787/schoeler .

11:05-11:35

UNLEASHING THE DEVELOPMENTAL POTENTIAL OF IPSCS

Schöler, Hans R., Velychko Sergiy, Adachi Kenjiro, Hou Yanlin, Kim, Kee-Pyo, MacCarthy, Caitlin M., Wu Guangming

Max Planck Institute for Molecular Biomedicine, Münster, Germany

A current aim in cell and developmental biology is to program cells at will. The first step towards converting a given cell type into another one is achieved via a pluripotent stem cell state that resembles that of ES cells. In most cases, somatic cells are pushed into a pluripotent state by the introduction of exogenous factors, mostly transcription factors. Reprogramming of mouse and human somatic cells into pluripotent stem cells designated as induced pluripotent stem cells (iPSCs) was first described in 2006. Fibroblasts were used as the somatic cell source and initially required introduction of the virally expressed transcription factor quartet Oct4, Sox2, Klf4, and c-Myc (OSKM). We previously reported that Oct4 alone is sufficient for directly reprogramming adult mouse and human fetal neural stem cells into iPSCs, thus highlighting the crucial role played by Oct4 in the process. Surprisingly, we have now shown that the combination of SKM is sufficient for reprogramming mouse somatic cells into iPSCs. Actually, SKM even activates the pluripotency network in Oct4-knockout fibroblasts. Retroviral silencing requires the simultaneous expression of Sox2 and c-Myc, perhaps accounting for the discrepancy with respect to previous studies that used retroviral vectors to generate iPSCs without Oct4. Reprogramming in the absence of exogenous Oct4 results in iPSCs that are characterized by more faithful gene expression and greatly improved developmental potential. Our data suggests that expression of exogenous Oct4 during reprogramming leads to off-target gene activation, thereby worsening the quality of the generated iPSCs with major implications for further development and application of iPSC technology.

Kim, Jongpil

Department of Chemistry and Biomedical Engineering, Dongguk University, Seoul, Korea

Jongpil Kim is an Associate Professor of Chemistry & Biomedical Engineering and Director of Center for Regenerative medicine at Dongguk University, Seoul, Korea. Before coming to Dongguk University, he completed the Postdoctoral training at Rudolf Jaenisch's lab at MIT/Whitehead institute. In 2008, Jongpil Kim received a PhD in Neurobiology from Columbia University.

11:35-12:05

RECENT ADVANCES IN CELL REPROGRAMMING AND GENE EDITING AS NEW THERAPEUTICS FOR NEURODEGENERATIVE DISEASES

Kim, Jongpil

Department of Chemistry and Biomedical Engineering, Dongguk University, Seoul, Korea

Recent advances in direct reprogramming have garnered considerable interest for human disease modelling and cell replacement strategies. We are interested in developing new technologies of cell reprogramming and gene editing for the treatment of neurodegenerative diseases such as Parkinson & Alzheimer's diseases. Recently, our group have developed approaches to control cell fate through nanotechnology for regenerative medicine. This study provide a proof of principle for in vivo reprogramming as a potentially viable and safe therapeutic strategy for the treatment of Parkinson disease. Moreover, we have reported the generation of 3D midbrain organoids for modeling sporadic Parkinson's disease, and used this system to explore the pathogenic mechanisms resulted from the LRRK2 mutant in the Parkinson's disease. Finally, our group is also developing CRISPR/Cas9 nanocomplex for in vivo gene editing technology for treating neurodegenerative diseases. I will discuss the recent updates on the in vivo gene targeting using Cas9 nanocomplexes as a novel therapeutic agent for Alzheimer's disease.

Keywords: cell reprogramming, gene editing, neurodegenerative diseases

Deng, Hongkui

Stem Cell Research Center, Peking-Tsinghua Center for Life Sciences, Peking University, Beijing, China

Hongkui Deng earned his B.Sc. in Cell Biology from Wuhan University and his Ph.D. in immunology from the University of California, Los Angeles. From 1995 to 1997 he was an Aaron Diamond Postdoctoral Fellow with Dan R. Littman at the NYU School of Medicine's Skirball Institute, where he identified major co-receptors responsible for HIV entry into cells. From 1998 to 2000, Hongkui was the director of molecular biology at Viacell Inc. working on ex vivo expansion of human hematopoietic stem cells. Hongkui Deng was awarded the prestigious Cheung Kong Scholarship in 2000 and became a professor at Peking University in 2001. Since 2013, he has been the director of the Peking University Stem Cell Research Center. Professor Deng's research focuses on somatic cell reprogramming and lineage specific differentiation of human pluripotent stem cells. His lab also explores chemical biological approaches for manipulating cell fate and function. His group was the first to report a chemical approach to induce pluripotent stem cells. He has been awarded several awards and honors including the Tan Jiazhen Life Science Award in 2014 and Wu-Jieping-Paul Janssen Medical & Pharmaceutical Award in 2017. He also serves on a number of editorial boards including Cell, Cell Stem Cell, Stem Cell Reports, and Cell Research. Professor Deng was elected to the ISSCR Board of Directors in 2010 and re-appointed for a second term in 2013.

12:05-12:35

USING CHEMICAL APPROACHES TO GENERATE DESIRED FUNCTIONAL CELLS

Deng, Hongkui

Stem Cell Research Center, Peking-Tsinghua Center for Life Sciences, Peking University, Beijing, China

Cell fate manipulation is a fundamental question in biology and shows great potential for application in the development of new disease models, drug screening and cell-based therapies. In recent years, we have been developing chemical approaches completely using small molecules to direct cell-fate conversion; hence permitting the generation of diverse cell types including extended pluripotent stem cells and functional lineages such as neurons and hepatocytes. The chemical approach is a non-integrative method to modulate the genome and is versatile as it provides spatiotemporal orchestrations of molecular targets in a synergistic manner, which is a simple way to manipulate cell fates and favorable for clinical translations. Very recently, we have employed this approach to foster functional maturations of differentiated cells, and we have been able to produce a large amount of competent human hepatocytes in vitro. These cells highly resemble and functionally rival freshly isolated primary human hepatocytes for a series of in vitro applications including drug-metabolizing activities, toxicity prediction and modelling hepatitis B virus infection. Furthermore, to apply these cells in the treatment of acute liver failure, we have developed a novel bio artificial liver system, which exhibited high efficacy in preclinical studies using large animal models.

Thursday, 26 September, 15:45-17:15

Special Session 3 : Tissue and Cancer Stem Cells, Room A+B+C

Chair: Frenette, Paul

MODELLING OF HUMAN NEURODEGENERATIVE DISEASES USING IPSCS AND GENETICALLY MODIFIED NON-HUMAN PRIMATES

Okano, Hideyuki
Department of Physiology, Keio University School of Medicine, Keio, Japan

PHARMACOGENOMIC LANDSCAPE OF PATIENT-DERIVED CANCER STEM CELLS INFORMS PRECISION ONCOLOGY THERAPY

Nam, Do Hyun
Institute for Refractory Cancer Research, Samsung Medical Center, Seoul, Korea; Department of Neurosurgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea: Department of Health Sciences and Technology, Samsung Advanced Institute for Health Science and Technology, Sungkyunkwan University, Seoul, Korea

STRESS-MEDIATED HYPERACTIVATION OF SYMPATHETIC NERVES DRIVES MELANOCYTE STEM CELL DEPLETION

Hsu, Ya-Chieh
Department of Stem Cell and Regenerative Biology, Harvard University and Harvard Stem Cell Institute, Cambridge, MA, USA

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Thursday, 26 September, 17:25-18:25

Special Session 4 : Microenvironment and the Stem Cell Niche, Room A+B+C

Chair: Wagers, Amy

ENGINEERING STEM CELL NICHE FOR REGENERATION AND THERAPY

Oh, Il-Hoan
Catholic High-Performance Cell Therapy Center, The Catholic University of Korea, Seoul, Korea

A NOVEL MECHANISM FOR INNATE IMMUNE TOLERANCE OF HEMATOPOIETIC AND LEUKEMIC STEM CELLS

Frenette, Paul S.
Ruth L. and David S. Gottesman Institute for Stem Cell and Regenerative Medicine Research, Albert Einstein College of Medicine, New York, NY, USA

Thursday, 26 September, 15:45-17:15, Room A+B+C

Special Session 3 : Tissue and Cancer Stem Cells **Chair: Frenette, Paul**

Okano, Hideyuki

Department of Physiology, Keio University School of Medicine, Keio, Japan

Hideyuki Okano graduated from Keio University School of Medicine in 1983. After post-doctoral training in Johns Hopkins University School of Medicine, he worked at Tsukuba University and Osaka University, and was appointed as a full professor of Physiology in Keio University Medical School in 2001. Since 2007 to date, he has been a Dean of Keio University Graduate School of Medicine or School of Medicine. He is well known in the field of regenerative medicine of CNS diseases.

15:45-16:15

MODELLING OF HUMAN NEURODEGENERATIVE DISEASES USING IPSCS AND GENETICALLY MODIFIED NON-HUMAN PRIMATES

Okano, Hideyuki

Department of Physiology, Keio University School of Medicine, Keio, Japan

There is an increasing aged population across the world. Also, we have to notice that ageing is the biggest risk factor for neurodegenerative diseases including dementia, Parkinson Disease (PD) and ALS. In the major neurodegenerative diseases, such as Alzheimer Disease (AD), PD and ALS, abnormal protein assembly is a common mechanism for triggering neurodegeneration. To investigate the pathogenic mechanisms and develop new interventions for these diseases, we took advantage of induced pluripotent stem cells (iPSCs) technologies. So far, we have established iPSCs from the patients of about 40 human neurological/psychiatric disorders and characterized their pathophysiology. Using iPSC-technology to generate stem and differentiated cells retaining the patients' full genetic information, we have established a large number of in vitro cellular models of familial ALS (FALS) and sporadic ALS (SALS). These SALS models showed phenotypic differences in their pattern of neuronal degeneration, types of abnormal protein aggregates, cell death mechanisms, and onset and progression of these phenotypes in vitro among cases. We therefore developed a system for case clustering capable of subdividing these heterogeneous SALS models by their in vitro characteristics. We further evaluated multiple-phenotype rescue of these sub-classified SALS models using agents selected from non-SOD1 FALS models, and identified ropinirole (ROPI: known as D2 receptor agonist) as a potential therapeutic candidate. Furthermore, we found that i) ROPI's anti-ALS action is mostly D2R-independent, by improving mitochondrial function, and suppressing ROS production, ii) ROPI's anti-ALS action exceeds the effects of pre-existing anti-ALS drugs (Riluzole, Edaravone) in a dish, iii) ROPI is effective about 70% of SALS patients (16/22) as assayed in our in vitro system and iv) localization of ALS related RNA-binding proteins (TDP-43 and FUS) potentially acts as biomarker to predict Responders vs Non-Responders. Based on these findings on the potential anti-ALS action of ROPI, we started A Phase I/IIa, randomized, double-blind, placebo-controlled study followed by a continuing open label study, to verify the safety and tolerability of "ROPI" in subjects with ALS (ROPALS trial) from December, 2018. Notably, we will also generate iPSCs from patients and compare in vitro and in vivo effects of ROPI on ALS phenotypes.

Keywords: ALS, iPSCs, Clinical trial

Nam, Do Hyun

Institute for Refractory Cancer Research, Samsung Medical Center, Seoul, Korea; Department of Neurosurgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea: Department of Health Sciences and Technology, Samsung Advanced Institute for Health Science and Technology, Sungkyunkwan University, Seoul, Korea

Professor Nam started his career at Department of Neurosurgery, Seoul National University Hospital and Samsung Biomedical Research Institute. Presently he serves as the Director of the Institute of Refractory Cancer Research, Samsung Medical Center and the Chair of Graduate School for Health Sciences & Technology, Sungkyunkwan University. Prof Nam has extensive experience and expertise in the field of brain tumor research. His major scientific achievements include the creation of AVATAR System, which recommends effective therapeutic options for individual patients based on the tumors' genomic alterations and drug response profiles.

16:15-16:45

PHARMACOGENOMIC LANDSCAPE OF PATIENT-DERIVED CANCER STEM CELLS INFORMS PRECISION ONCOLOGY THERAPY

Nam, Do Hyun[1,2,3], Sa, Jason K.[1]

[1]Institute for Refractory Cancer Research, Samsung Medical Center, Seoul, Korea, [2]Department of Neurosurgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea, [3]Department of Health Sciences and Technology, Samsung Advanced Institute for Health Science and Technology, Sungkyunkwan University, Seoul, Korea

Cancer is a complex disease with profound molecular and transcriptional heterogeneity, exhibiting a wide spectrum of genomic and epigenomic dysregulations. Intra-tumoral heterogeneity confounds accurate diagnosis and complicates design of effective treatments within clinical framework. Substantial evidences have suggested an existence of tumor cellular hierarchy, with cancer stem cells (CSCs) occupying at its apex. CSCs are functionally defined by their enhanced ability for perpetual self-renewal and govern essential programs that modulate tumor survivability. Given the recognition of CSC significance, use of primary human cancer models that harbor cellular hierarchy (cancer stem cells) and recapitulate the genomic and molecular phenotypes of the parental tumors in situ will greatly enhance clinically relevant studies. A fundamental tenet of precision oncology in cancer proposes that genomic and molecular characterization of the tumor could inform patient tailored therapy. However, as most solid tumors harbor multiple genetic aberrations, predicting successful treatments based on computational approach alone remains challenging. An integrated approach consisting of genomic analysis of the patient tumor, in parallel with direct assessments of drug response on patient tumor derivatives is the next step towards precision oncology. Toward this goal, we have established a library of 462 patient-derived cancer stem cells across 14 tumor types, coupled with molecular and transcriptional profiling. Here, we provide comprehensive insights into dynamic pharmacogenomic associations, including molecular determinants that elicit therapeutic resistance to EGFR inhibitors and potential repurposing of ibrutinib (currently used in hematological malignancies) for EGFR-specific therapy in gliomas. Lastly, we present a potential implementation of PDC-derived drug sensitivities for the prediction of clinical response to targeted therapeutic using retrospective clinical studies. Collectively, our work presents that evolutionary inference from integrated genomic analysis with chemical screening of the CSCs can inform optimal targeted therapeutic intervention for cancer patients.

Keywords: Cancer Stem Cell, Precision Oncology, Gene-Drug Response Association

Hsu, Ya-Chieh

Department of Stem Cell and Regenerative Biology, Harvard University and Harvard Stem Cell Institute, Cambridge, MA, USA

Ya-Chieh Hsu is the Alvin and Esta Star Associate Professor at the Department of Stem Cell and Regenerative Biology at Harvard University. She is also a principal faculty member at the Harvard Stem Cell Institute and an associate member of the Broad Institute. Her lab focuses understanding how stem cell behaviors are shaped by niche factors and systemic changes using skin as a model.

16:45-17:15

STRESS-MEDIATED HYPERACTIVATION OF SYMPATHETIC NERVES DRIVES MELANOCYTE STEM CELL DEPLETION

Hsu, Ya-Chieh[1], Zhang, Bing[1], Ma, Sai[1,2,10], Rachmin, Inbal[3], He, Megan[1,4], Baral, Pankaj[5], Choi, Sekyu[1], Gonçalves, William A.[6], Shwartz, Yulia[1], Fast, Eva M.[1,7], Su, Yiqun[3], Zon, Leonard I.[1,7,8], Regev, Aviv[2,9,10], Buenrostro, Jason D.[1], Cunha, Thiago M.[5,11], Chiu, Isaac M.[5], Fisher, David E.[3]

[1]Department of Stem Cell and Regenerative Biology, Harvard University and Harvard Stem Cell Institute, Cambridge, MA, USA, [2]Broad Institute of MIT and Harvard, Cambridge, MA, USA, [3]Cutaneous Biology Research Center, Department of Dermatology, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA, USA, [4]Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA, USA, [5]Department of Immunology, Harvard Medical School, Boston, MA, USA, [6]Institute of Biological Science, Federal University of Minas Gerais, Belo Horizonte, Brazil, [7]Stem Cell Program and Division of Hematology/Oncology, Boston Children's Hospital and Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA, [8]Howard Hughes Medical Institute, Boston Children's Hospital and Harvard University, Boston, MA, USA, [9]Howard Hughes Medical Institute, Chevy Chase, MD, USA, [10]Department of Biology and Koch Institute, Massachusetts Institute of Technology, Cambridge, MA, USA, [11]Center for Research in Inflammatory Diseases (CRID), Department of Pharmacology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil

Stress is an out of the norm state caused by emotional or physical insults. While chronic stress is considered harmful, acute stress is thought to cause transient and reversible changes essential for fight or flight. Stress has been anecdotally associated with hair greying, but scientific evidence linking the two is scant. Here, using mouse stress models, we found that acute stress leads to hair greying through rapid depletion of melanocyte stem cells (MeSCs). Combining adrenalectomy, denervation, chemogenetics, cell ablation, and MeSC-specific adrenergic receptor knockout, we found that stress-induced MeSC loss is independent of the immune attack or adrenal hormones. Instead, hair greying results from activation of the sympathetic nervous system that innervates the MeSC niche. Upon stress, sympathetic nerve activation leads to burst release of the neurotransmitter norepinephrine, which acts directly on MeSCs. Norepinephrine drives quiescent MeSCs to proliferate rapidly, followed by migration and differentiation, leading to their permanent depletion from the niche. Transient suppression of MeSC proliferation with topical application of cell cycle inhibitors rescues stress-induced hair greying. Our studies demonstrate that stress-induced neuronal activity can be an upstream trigger that forces stem cells out of quiescence, and suggest that acute stress stimuli can be more detrimental than anticipated by causing rapid and irreversible loss of somatic stem cells.

Keywords: stem cell niche, sympathetic nerve, melanocyte stem cells

Thursday, 26 September, 17:25-18:25, Room A+B+C

Special Session 4 : Microenvironment and the Stem Cell Niche **Chair: Wagers, Amy**

Oh, Il-Hoan

Catholic High-Performance Cell Therapy Center, The Catholic University of Korea, Seoul, Korea

Dr. Il-Hoan Oh obtained MD degree from the Catholic University, College of Medicine, and Ph.D from Temple University, USA. His initial study was focusing on the molecular mechanisms controlling self-renewal of hematopoietic stem cells (HSCs). Recently, he focuses on the microenvironmental regulation of HSCs and leukemia stem cells. He served as president of KSSCR and currently, as director of Catholic High-Performance Cell Therapy Center, Catholic University of Korea.

17:25-17:55

ENGINEERING STEM CELL NICHE FOR REGENERATION AND THERAPY

Oh, Il-Hoan[1], Jeong, Seon-Young[2], Jeon, So-Hee[2], Kim, Jin-A[2]

[1]*Catholic High-Performance Cell Therapy Center, The Catholic University of Korea, Seoul, Korea,* [2]*The Catholic University of Korea, Seoul, Korea*

Microenvironment in bone marrow plays a pivotal role to regulate normal and leukemic stem cell fates through cellular interaction with stem cell niche. Therefore, the mechanisms controlling the functional status of microenvironmental niche could open alternative approaches to control the in-vivo behavior. In this presentation, we would show the changes in the cellular characteristics of BM niche cells during their functional activation for stimulating hematopoietic stem cells (HSCs). First, using 3D-spheroid culture model of mesenchymal stromal cells, we observed an increase of niche activity associated with molecular remodeling in MSCs. We show that these molecular remodeling could promote self-renewing regeneration of HSCs. Secondly, we show that the BM niche is stimulated by myeloablative agents and induce an adaptive remodeling of niche cells acquiring new cellular characteristics. Interestingly, these mesenchymal niche cells generated by remodeling exerted counteractive effects on normal and leukemic stem cells in a manner that stimulates regeneration of normal HSCs, but suppress leukemia stem cells. These counteractive anti-leukemic/pro-normal effects led to therapeutic benefit prolonging survival of leukemic mice. Lastly, we show that BM stromal cells play a role to generate new subsets of leukemic cells with stem cell-like, drug resistant clones in a reversible, stochastic manner. Together, we propose that BM microenvironment could be an attractive target of therapy for regeneration of normal HSCs and therapy against hematological malignancy.

Frenette, Paul S.

Ruth L. and David S. Gottesman Institute for Stem Cell and Regenerative Medicine Research, Albert Einstein College of Medicine, New York, NY, USA

Paul Frenette is the founding Director and Chair of the Ruth L. and David S. Gottesman Institute for Stem Cell and Regenerative Medicine at Albert Einstein College of Medicine. His laboratory has uncovered the critical role of the sympathetic nervous system in the regulation of hematopoietic stem cell (HSC) egress from their niches. He has elucidated circadian rhythmicity in HSC release from the bone marrow, and contributed to identify major cellular constituents forming niches in the bone marrow.

17:55-18:25

A NOVEL MECHANISM FOR INNATE IMMUNE TOLERANCE OF HEMATOPOIETIC AND LEUKEMIC STEM CELLS

Frenette, Paul S.

Ruth L. and David S. Gottesman Institute for Stem Cell and Regenerative Medicine Research, Albert Einstein College of Medicine, New York, NY, USA

Hematopoietic stem cells (HSCs) home to the bone marrow niche via the regulated expression of chemokines and adhesion receptors. Upon migrating into the marrow space, HSCs are vetted by perivascular phagocytes that ensure their self-integrity. Here, I will describe a mechanism by which mononuclear phagocytes serve as quality-control checkpoint for entry in the bone marrow, and discuss the implications for novel strategies to eliminate leukemic cells via modulation of innate immune tolerance.

Keywords: Hematopoietic stem cells, innate immunity, leukemic stem cells

Friday, 27 September, 09:00-10:00

Special Session 5 : Epigenetics and Aging of Stem Cells, Room A+B+C

Chair: Hsu, Ya-Chieh

ACTIVATING MUSCLE STEM CELL FUNCTION THROUGH IMMEDIATE EARLY TRANSCRIPTION FACTORS AND LIPID MEDIATORS

Wagers, Amy J.
Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA

RESTORING THE HUMAN GERMLINE

Clark, Amander
Department of Molecular Cell and Developmental Biology, Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research University of California, Los Angeles, CA, USA

Friday, 27 September, 10:00-11:00

Special Session 6 : New Technologies in Stem Cells, Room A+B+C

Chair: Deng, Hongkui

HAPLOID HUMAN EMBRYONIC STEM CELLS: DERIVATION AND APPLICATIONS

Benvenisty, Nissim
The Azrieli Center for Stem Cells and Genetic Research, The Hebrew University of Jerusalem, Israel

PATTERNING MESODERM AND BLOOD DEVELOPMENT FROM HUMAN PLURIPOTENT STEM CELLS

Zandstra, Peter
School of Biomedical Engineering, Michael Smith Laboratories, University of British Columbia, Vancouver, BC, Canada

This program was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (MSIT) (No. 2017M3A9B4042583).

Friday, 27 September, 09:00-10:00, Room A+B+C

Special Session 5 : Epigenetics and Aging of Stem Cells

Chair: Hsu, Ya-Chieh

Wagers, Amy J.

Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA

Dr. Amy Wagers is the Forst Family Professor and Co-Chair of the Department of Stem Cell and Regenerative Biology at Harvard University, a Senior Investigator at the Joslin Diabetes Center and a member of the Paul F. Glenn Center for the Biology of Aging at Harvard Medical School. Dr. Wagers' laboratory investigates how changes in stem cell activity impact tissue homeostasis and repair throughout life, and how stem cells may be harnessed for regenerative medicine using cell transplantation and gene editing approaches.

09:00-09:30

ACTIVATING MUSCLE STEM CELL FUNCTION THROUGH IMMEDIATE EARLY TRANSCRIPTION FACTORS AND LIPID MEDIATORS

Wagers, Amy J.

Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA

Skeletal muscle stem cells, also known as satellite cells, are largely non-proliferative in uninjured adult muscle but transition at sites of injury into rapidly dividing progenitors that mediate muscle repair. As early events in satellite cell activation are rate-limiting for recovery from muscle damage and can impact the efficiency of engraftment in transplantation scenarios, there is substantial interest in discovering their molecular driver(s). Using comparative transcriptomics and chemical screening approaches, we have uncovered a unique molecular signature associated with recently activated muscle satellite cells, including new molecular effectors of satellite cell function, and identified two bioactive lipids that promote myogenic progenitor cell engraftment in transplantation models. Results from these studies strongly implicate immediate early response genes of the AP-1 family in licensing satellite cells for optimal regenerative activity and uncover previously unsuspected roles for targets of AP-1 in the proliferation and expansion of regenerative muscle stem cells. They further suggest new potential therapeutic targets and opportunities for treating muscle disease and reversing age-related loss of muscle repair by mobilizing endogenous muscle stem cells and/or boosting the myogenic contributions of transplanted progenitors.

Keywords: Satellite cells, activation, regeneration

Clark, Amander

Department of Molecular Cell and Developmental Biology, Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research University of California, Los Angeles, CA, USA

Amander Clark PhD is Professor and Chair of the Department of Molecular Cell and Developmental Biology, University of California, Los Angeles (UCLA). She is a key member of the UCLA Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research and Co-Director of the UCLA Human Embryonic Stem Cell Derivation laboratory. As an independent investigator, Dr. Clark's research is focused on germline development and epigenetic reprogramming using pluripotent stem cells as a model.

09:30-10:00

RESTORING THE HUMAN GERMLINE

Clark, Amander

Department of Molecular Cell and Developmental Biology, Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research University of California, Los Angeles, CA, USA

At birth the human ovary contains an estimated 1-2 million primordial follicles. This is referred to as the ovarian reserve. With healthy aging in women, the ovarian reserve decreases due to loss of follicles and in the fifth decade of life the ovarian reserve depletes resulting in menopause. In the decade prior to menopause, women experience age related fertility decline, and this is associated with the generation of poor-quality oocytes that if fertilized can result in miscarriage or adverse health outcomes. Accelerated ovarian aging can also occur when the ovary is injured, for example as a side effect of cancer therapy, notably cisplatin-based chemotherapy. As a result, child hood cancer survivors are at risk for entering menopause as adolescents or young adults. Therefore, new approaches to restore the human germline are required to help women with premature fertility decline. In this project, we are using human pluripotent stem cells to differentiate human germline cells called primordial germ cell like cells (PGCLCs) in vitro. PGCLCs are the precursors to oogonia and oocytes that are central to the control of ovarian aging. Using assay for transposase accessible chromatin followed by Sequencing (ATAC-Seq), single cell RNA-Seq, Whole genome bisulfite sequencing (WGBS) and Chromatin immunoprecipitation followed by Sequencing (ChIP-Seq), my group are focused on establishing the cell and molecular principles required for human germline cell development. In particular, we are focused on the role of the transcription factor AP2 (TFAP2) family, and the human-specific roles these transcription factors play in establishing the human germline. Collectively, results from this work will be essential in the future to differentiating high quality gametes from stem cells that could one day be used to restore fertility and reverse ovarian aging.

Keywords: pluripotent stem cells, germ cells, and epigenetics

Friday, 27 September, 10:00-11:00, Room A+B+C

Special Session 6 : New Technologies in Stem Cells

Chair: Deng, Hongkui

Benvenisty, Nissim

The Azrieli Center for Stem Cells and Genetic Research, The Hebrew University of Jerusalem, Israel

Prof. Nissim Benvenisty is the Herbert Cohn Chair in Cancer Research and the Director of The Azrieli Center for Stem Cells and Genetic Research at the Hebrew University. He earned his M.D. and Ph.D. degrees from the Hebrew University, and conducted postdoctoral studies at Harvard University. Prof. Benvenisty's research projects focus on stem cell biology, tissue engineering, human genetics, and cancer research. He published numerous original and review papers on human pluripotent stem cells, and serves on the editorial board of various stem cell related journals. He is a member of the steering committee of the International Stem Cell Initiative (ISCI), the Board of Directors of the International Society of Differentiation (ISD), and serves as the academic advisor for the International Symposia of the International Society for Stem Cell Research (ISSCR). Prof. Benvenisty presents the issue of human embryonic stem cells in many international conferences, and gave testimonies before the US Senate and the European Union. He was awarded several prizes among them the Foulkes Prize (London), the Hestrin Prize, the Teva Prize, the Kaye Prize, the Milken Prize and the ACTO Award.

10:00-10:30

HAPLOID HUMAN EMBRYONIC STEM CELLS: DERIVATION AND APPLICATIONS

Benvenisty, Nissim

The Azrieli Center for Stem Cells and Genetic Research, The Hebrew University of Jerusalem, Israel

We have recently generated haploid human embryonic stem (ES) cells from unfertilized human oocytes. The haploid human ES cells exhibited typical pluripotent stem cell characteristics, such as self-renewal capacity and a pluripotency-specific molecular signature. Although haploid human ES cells resembled their diploid counterparts by several aspects, they also displayed distinct properties including differential regulation of X-chromosome inactivation and genes involved in oxidative phosphorylation, alongside reduction in absolute gene expression levels and cell size. Interestingly, we found that a haploid human genome is compatible not only with the undifferentiated pluripotent state, but also with differentiated somatic fates representing all three embryonic germ layers both in vitro and in vivo. Furthermore, we demonstrated the utility of haploid human ES cells for loss-of-function genetic screening by analyzing a haploid gene-trap mutant library. To define the essentialome of human pluripotent stem cells we generated a genome-wide loss-of-function library in haploid human ES cells utilizing the CRISPR/Cas9 technology using about 180,000 guide RNAs, targeting virtually all coding genes. Using this library, we characterized the essential genes in human pluripotent stem cells, showed the relative role of each cellular compartment in promoting or restricting cell growth, and categorized human genetic disorders according to their role in early embryogenesis. Thus, haploid human ES cells hold a great potential for biomedically-relevant functional genomics to unravel genotype-phenotype interactions in the context of human development and disease.

Zandstra, Peter

School of Biomedical Engineering, Michael Smith Laboratories, University of British Columbia, Vancouver, BC, Canada

Peter Zandstra graduated with a Bachelor of Engineering degree from McGill University in the Department of Chemical Engineering, obtained his Ph.D. degree from the University of British Columbia in the Department of Chemical Engineering and Biotechnology and continued his research training as a Post-Doctoral Fellow in the field of Bioengineering at MIT. In 1999, Dr. Zandstra began his faculty appointment at the University of Toronto's Institute of Biomaterial and in 2016 was appointed University Professor, the university's highest academic rank. In July 2017, Zandstra joined the University of British Columbia as the Founding Director the School of Biomedical Engineering and as the Director of the Michael Smith Laboratories. In these roles, he aims to build programs with deeper interactions between the Faculties of Applied Science, Science and Medicine, especially as related to innovative research and training programs. Peter is the Canada Research Chair in Stem Cell Bioengineering and is a recipient of a number of awards and fellowships including the Premiers Research Excellence Award (2002), the E.W.R. Steacie Memorial Fellowship (2006), the John Simon Guggenheim Memorial Foundation Fellowship (2007), and the University of Toronto's McLean Award (2009). Dr. Zandstra is a fellow of the American Institute for Medical and Biological Engineering and the American Association for the Advancement of Science. Peter's research focuses on understanding how complex communication networks between stem cells and their progeny influence self-renewal and differentiation, and how this information can be applied to the design of novel culture technologies capable of controlling cell fate.

10:30-11:00

PATTERNING MESODERM AND BLOOD DEVELOPMENT FROM HUMAN PLURIPOTENT STEM CELLS

Zandstra, Peter

School of Biomedical Engineering, Michael Smith Laboratories, University of British Columbia, Vancouver, BC, Canada

The blood forming system arises de novo during embryogenesis. After dynamic changes during organismal development, blood stem cells settle down in the adult in specialized niches where they support the continuous generation of a diverse population of mature and functional blood cells throughout our lifetime. In this presentation I will review our efforts to establish an integrated understanding of the effects of cell type heterogeneity, spatial organization and multi-scale regulatory network engagement on defined and measurable stem cell fate transitions. Specific examples using new technologies for pluripotent stem cell fate control will be highlighted and new data on modeling human pluripotent stem cell development into definitive blood cells, including functional T-cells, will be presented.

Keywords: Blood development, immune-engineering, niche engineering

Friday, 27 September, 11:20-12:50

Special Session 7 : Disease Modeling and Stem Cells, Room A+B+C

Chair: Parmar, Malin

VILLAGES IN A DISH: SCALING THE USE OF HUMAN CELL MODELS TO DETECT DRUG GENOTYPE INTERACTIONS

Eggan, Kevin
Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA

HARNESSING THE POTENTIAL OF ES CELLS & IPS CELLS: NEW OPPORTUNITIES FOR THERAPEUTICS, DISEASE MODELING AND GENOME EDITING

Kim, Dong-Wook
Yonsei University College of Medicine, Seoul, Korea

CARDIOVASCULAR DISEASES AND DRUGS: WHERE ARE WE WITH HIPSC MODELS?

Mummery, Christine
Department of Anatomy and Embryology, Leiden University Medical Centre, Leiden, The Netherlands

This session was supported by a grant (HI18C0096) from the Ministry of Health and Welfare.

Friday, 27 September, 15:35-17:05

Special Session 8 : Stem Cells in Translation, Room A+B+C

Chair: Okano, Hideyuki

TOWARDS A PATIENT-SPECIFIC TREATMENT FOR PARKINSON'S DISEASE

Parmar, Malin
Developmental and Regenerative Neurobiology, Lund University, Lund, Sweden

TRAFFICKING OF ENDOGENOUS STEM CELLS AND M1/M2 POLARIZATION OF MACROPHAGE FOR TISSUE REPAIR; A STORY OF SUBSTANCE-P

Son, Youngsook
Department of Genetic Engineering and Graduate School of Biotechnology, Kyung Hee University, Seoul, Korea and Center for Kyung Hee Institute of Regenerative Medicine (KIRM), Kyung Hee University Hospital, Seoul, Korea

STEM CELL ETHICS AND POLICY: CONVERGING PATHS AND NEW SYNERGIES

Hyun, Insoo
Center for Bioethics and the Department of Global Health and Social Medicine Harvard Medical School, Boston, MA, USA and Department of Bioethics, Case Western Reserve University School of Medicine, Cleveland, OH, USA

This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number : HI13C1479).

Friday, 27 September, 11:20-12:50, Room A+B+C

Special Session 7 : Disease Modeling and Stem Cells

Chair: Parmar, Malin

Eggan, Kevin

Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA

Kevin Eggan was a graduate student in Rudolf Jaenisch's lab in the Department of Biology at MIT, where he studied fundamental aspects of stem cell biology and reprogramming. In 2003, he became a Harvard Junior Fellow in the Harvard Society of Fellows. He established his lab in the Department of Molecular and Cellular Biology at Harvard University where he became an Assistant Professor in 2005. In 2007, he joined the Department of Stem Cell and Regenerative Biology.

11:20-11:50

VILLAGES IN A DISH: SCALING THE USE OF HUMAN CELL MODELS TO DETECT DRUG GENOTYPE INTERACTIONS

Eggan, Kevin

Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA

A maturing application of reprogramming and stem cell technologies is their application to understanding how genetic variation that underlies disease risk impinge the function of affected cell types. However, a major limitation of this approach has been the number of patients and genetic variants that can be reasonably analyzed. I will describe a new strategy we have developed that allows us to simultaneously measure phenotypes in cell types derived from as many as 100 individuals in a single tissue culture well. These approaches, we call "Dropulation Genetics" and "Census sequencing" not only have allowed us to probe how genotype underlies phenotype at previously impractical scales, they have also provided a remarkable improvement in sensitivity and assay reproducibility. I will describe practical application of these approaches in psychiatry, neuromuscular disease and susceptibility to infectious agents.

Kim, Dong-Wook

Yonsei University College of Medicine, Seoul, Korea

Dr. Dong-Wook Kim is a professor of Department of Physiology, Yonsei University College of Medicine, Korea and President of Korean Society for Stem Cell Research (KSSCR). He earned his B.Sc. in 1986 from Korea University, and Ph.D. in biotechnology at the University of Tokyo in 1996. Dr. Kim worked in the field of protein trafficking at Yale University from 1997 to 2001. While working at Harvard Medical School and Mclean Hospital as a junior faculty member since 2001, he focused on neural differentiation using ES cells. Dr. Kim joined the Dept. of Physiology at Yonsei University as a faculty member from 2003. Dr. Kim served as a Director of Korean Stem Cell Research Center from 2006 to 2012 and made the 10-year Korean Plan for Stem Cell Research as a Task Force team leader in 2006. Dr. Kim also played a role in ISSCR's International Committee (2005-2009), Government Affairs Committee (2008-2009) and Program Committee (2006-2007, 2018-2019) as a Korean representative. Dr. Kim served as a special advisory board member for the Korean FDA in the field of cell-based therapeutics. Dr. Kim's research areas include disease modeling and drug discovery using iPSCs, gene correction in patient-derived iPSCs, neural differentiation of ESCs/iPSCs, and development of cell-based therapeutics on spinal cord injury and Parkinson's disease, etc.

11:50-12:20

HARNESSING THE POTENTIAL OF ES CELLS & IPS CELLS: NEW OPPORTUNITIES FOR THERAPEUTICS, DISEASE MODELING AND GENOME EDITING

Kim, Dong-Wook

Yonsei University College of Medicine, Seoul, Korea

Human pluripotent stem cells (hPSCs) such as embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) emerged as promising cell sources for transplantation due to their properties of unlimited self-renewal and ability to differentiate into all cell types in the body. In addition, hPSCs have potential applications for the development of cellular models to study diseases, drug discovery and genome editing. In the first part of my talk, I will introduce our efforts of using hESCs to develop cell replacement therapeutics to treat two incurable neurological diseases, spinal cord injury (SCI) and Parkinson's disease (PD). PSA-NCAM-positive neural cells differentiated from hESCs are the cell source for SCI. We previously reported that purified PSA-NCAM-positive neural precursors, unlike neural rosette-derived neural precursors, did not generate any tumors or unwanted tissues after transplantation, and hence were a safe cell source for cell therapy. For cell therapy for PD, we developed an efficient differentiation protocol that generated a large number of ventral mesencephalic dopamine (vmDA) precursors for transplantation. We also discovered a novel surface marker to purify and enrich vmDA precursors. Based on these studies, we are currently preparing clinical trials for SCI and PD. In the second part of my talk, I will focus on how patient-derived iPSCs or genome editing with WT-iPSCs can be used to generate cellular disease models. First, we generated hemophilia patient-derived iPSCs and tried to correct hemophilia structural variations such as chromosomal inversions which were generally more complicated and difficult to correct than small nucleotide changes. In this study, we established an efficient method to edit the structural variations and applied it to model or correct large chromosomal rearrangements existing in many hemophilia A patients. Finally, I will briefly explain our research on X-linked adrenoleukodystrophy (X-ALD). In this study we generated X-ALD patient-iPS cells as a disease model and used it to develop new drugs and to study mechanism underlying the disease.

Mummery, Christine

Department of Anatomy and Embryology, Leiden University Medical Centre, Leiden, The Netherlands

Christine Mummery is Professor of Developmental Biology at Leiden University Medical Centre. Her research concerns multidisciplinary approaches to understanding heart and vascular development, the differentiation of pluripotent stem cells into the cardiac and vascular lineages and using these cells as disease models. She is a member of the Royal Netherlands Academy of Science, was editor in chief of the ISSCR journal Stem Cell Reports until 2018 and is is presently vice president of ISSCR.

12:20-12:50

CARDIOVASCULAR DISEASES AND DRUGS: WHERE ARE WE WITH HIPSC MODELS?

Mummery, Christine, Giacomelli, Elisa, Tertoolen, Leon, van Meer, Berend, Sala, Luca, Orlova, Valeria, Bellin, Milena

Department of Anatomy and Embryology, Leiden University Medical Centre, Leiden, The Netherlands

Derivation of cardiovascular cell types from human pluripotent stem cells derived from patients or introducing targeted mutations is an area of growing interest as a platform for disease modelling, drug discovery and toxicity. Our lab has been investigating microtissue solutions in which cardiomyocytes and cardiac vascular and stromal cells are present. This promotes cardiomyocyte maturation and in combination with new methods for functional phenotyping, we have been able to quantify the outcomes of drug and disease mutation responses in situ. The use of isogenic pairs of hiPSC lines with and without mutations has proven very important since variability between "healthy control" hiPSC lines is often greater than the difference between a diseased cells and its isogenic control. hiPSC derived cardiomyocytes with mutations in ion channels and other genes can accurately predict changes in cardiac electrical properties and reveal drug sensitivities also observed in patients.

Keywords: cardiovascular disease, cardiomyocyte maturation, biophysical analysis

Friday, 27 September, 15:35-17:05, Room A+B+C

Special Session 8 : Stem Cells in Translation

Chair: Okano, Hideyuki

Parmar, Malin

Developmental and Regenerative Neurobiology, Lund University, Lund, Sweden

Malin Parmar is a professor in cellular neuroscience at Lund University in Sweden and a New York Stem Cell Foundation – Robertson investigator. Her research has a strong translational focus. Together with her lab she has shown in a series of high-profile publications how human fibroblasts can be converted into neurons, how glial cells can be reprogrammed into neurons in vivo, and how therapeutic dopamine neurons can be generated from human embryonic stem cells. She is the recipient of an ERC starting grant and an ERC Consolidator grant. She leads the European effort STEM-PD, designed to bring stem cell-derived dopamine neurons to clinical trials, and she is a key partner within European and International networks as well as Industry partners to develop new, cell based therapies for Brain Repair with focus on Parkinson's Disease.

15:35-16:05

TOWARDS A PATIENT-SPECIFIC TREATMENT FOR PARKINSON'S DISEASE

Parmar, Malin

Developmental and Regenerative Neurobiology, Lund University, Lund, Sweden

The adult brain has a very limited capacity for generation of new neurons. For decades, researchers have therefore developed strategies for brain repair using exogenous cell sources for cell replacement by transplanting them into the adult brain. In Parkinson's disease, clinical trials using fetal cells have demonstrated that effective repair can indeed be achieved by cell transplantation of developing human fetal dopamine (DA) neurons. Current approaches using pluripotent stem cells to replace the scarcely available fetal tissue as a source for DA neurons is underway. It is now also possible to by-pass the pluripotent stage and directly convert somatic cells into induced neurons (iNs) represent an interesting alternative to induced pluripotent stem cells (iPSCs) for obtaining patient specific neurons for personalized cell therapy. However, when developing autologous therapies where the transplanted cells are derived from the patients themselves, the issue of increased sensitivity of the transplanted cells to developing disease-associated pathology in the donor-derived transplanted cells arise. To investigate this, we have developed new models that allows us to study sensitivity to acquire disease pathology in patient derived cells in vitro, and after transplantation in vivo.

Keywords: Cell replacement therapy, Parkinson's Disease, Cellular reprogramming

Son, Youngsook

Department of Genetic Engineering and Graduate School of Biotechnology, Kyung Hee University, Seoul, Korea and Center for Kyung Hee Institute of Regenerative Medicine (KIRM), Kyung Hee University Hospital, Seoul, Korea

She is a Professor of Department of Genetic Engineering and Director of Kyung Hee Institute of Regenerative Medicine, Kyung Hee University. She received Ph.D., Department of Pharmacology & Cell Biology, UCSF (1989), M.A. (1982), and B.A. (1980) at Seoul National University. She has served as a president of KSSCR2018 and is a chairman of KSSCR board of directors. Her major research areas are Trafficking of endogenous stem cells, cartilage and skin tissue engineering, and inflammation control for tissue repair

16:05-16:35

TRAFFICKING OF ENDOGENOUS STEM CELLS AND M1/M2 POLARIZATION OF MACROPHAGE FOR TISSUE REPAIR; A STORY OF SUBSTANCE-P

Son, Youngsook[1,2], Hong, Hyun Sook[1,2,3], Jiang, Mei Hwa[1], Ahn, Woosung[1], Zhang, Mingzi[1], Lim, Ji Eun[1], Kim, Sumin[1], Chung, Eun Kyung[1], Lee, Jung Sun[4], Kim, Jae Chan[5]

[1]Department of Genetic Engineering and Graduate School of Biotechnology, Kyung Hee University, Seoul, Korea, [2]Center for Kyung Hee Institute of Regenerative Medicine (KIRM), Kyung Hee University Hospital, Seoul, Korea, [3]College of Medicine, Kyung Hee University, Seoul, Korea, [4]Biosolutions Corporation, Seoul, Korea, [5]Department of Ophthalmology, Chung Ang University, Seoul, Korea

Tissue injury may create a specific microenvironment, which brings up the systemic participation of reparative stem cells in the repair process. Previously, we identified a new role of substance-P (SP) as an injury-inducible messenger to mobilize bone marrow stromal cells, namely mesenchymal stem cells (BMSC or MSC) from the marrow to the blood, home to the injured tissue, and be engaged in the tissue repair in the alkali-burn corneal injury model. This elucidates endogenous healing mechanism recalling BMSC to the wound site. In addition to SP's BMSC mobilizer function, SP also mobilizes endothelial precursor cells (EPC) from the bone marrow to the peripheral blood and recruits them to the injured tissue for neovascularization as pericytes and endothelial cells respectively. We explored SP's efficacy in a variety of ischemic vascular disease and chronic disease animal models such as spinal cord injury, acute myocardiac infarction, stroke, diabetes, radiation-induced BM injury and gastrointestinal injury. At the early stage, SP exerts its anti-inflammatory role through specific trafficking of CD163+/CD206+ subset of monocytes from the bone marrow to the blood and direct induction of M2 type polarization of monocytes and macrophages, which in turn suppresses the injury-evoked tissue inflammation and clean up dead cells for tissue repair. This unique role of SP seems to reduce the inflammation-induced secondary cell death of neighboring cells and creates favorable microenvironment for the engraftment of incoming stem cells. All of these events comes much earlier, approximately 4-6 hours after the SP injection, than its action on BMSC and EPC mobilization from the bone marrow initiating 2-3 days after SP injection. Finally, SP-mobilized BMSC and EPC homes to the injured tissue and participates as reparative stem cells in the tissue repair. Collectively, SP may orchestrate tissue repair by reducing inflammation-provoked tissue damages at early stage and then recruiting endogenous stem cells from bone marrow to the injured tissue for the tissue repair, which stimulate its development as a potential stem cell stimulating agent to cure a variety of acute and chronic diseases requiring the reduction of inflammation load and engagement of endogenous reparative stem cells.

Funding Source: This work was supported by Projects from Ministry of Science, ICT and Future Planning (NRF-2016M3A9B4917320) and Translational medicine project from Ministry of Health and Welfare (HI13C1479, HI18C1492).

Hyun, Insoo

Center for Bioethics and the Department of Global Health and Social Medicine Harvard Medical School, Boston, MA, USA and Department of Bioethics, Case Western Reserve University School of Medicine, Cleveland, OH, USA

Insoo Hyun is Professor of Bioethics at Case Western Reserve University School of Medicine and Senior Lecturer at Harvard Medical School. Dr. Hyun has been involved for many years with the ISSCR, for which he has helped draft all of the ISSCR's guidelines and has served as the Chair of the Ethics Committee. He is a regular contributor to Nature, Science, Cell Stem Cell, among many other journals.

16:35-17:05

STEM CELL ETHICS AND POLICY: CONVERGING PATHS AND NEW SYNERGIES

Hyun, Insoo

Center for Bioethics and the Department of Global Health and Social Medicine Harvard Medical School, Boston, MA, USA and Department of Bioethics, Case Western Reserve University School of Medicine, Cleveland, OH, USA

Like the field of human stem cell research itself, stem cell ethics and policy issues have been rapidly evolving alongside astonishing developments in cell reprogramming, organoid research, and embryo culture and modeling. We have now entered an era where bioengineering and stem cell science are starting to converge in evermore exciting combinations. This talk explores the ways in which a renewed focus on ethics and policy considerations can further reinforce these growing scientific synergies. To this end, the development of a collaborative approach to "bioengineering ethics" can help pave the way for the next chapter of stem cell research.

Keywords: Ethics, science policy, bioengineering

Thursday, 26 September, 13:35-15:25

Concurrent Session 1 : Organoids and Development, Room A

Chair: Oh, Il-Hoan

THE INFLAMMATORY NICHE SHAPES LUNG REGENERATION

Lee, Joo-hyeon
Wellcome Trust - MRC Cambridge Stem Cell Institute, University of Cambridge, UK, Department of Physiology, Development and Neurobiology, University of Cambridge, UK

IN VITRO VASCULARIZATION OF HUMAN ORGANOIDS AND TISSUE FRAGMENTS: CELL CULTURES' MISSING LINK?

Vulto, Paul
Research and Development, Mimetas B.v., Leiden, the Netherlands

SINGLE CELL RNA-SEQUENCING ANALYSIS REVEALS THE ABSENCE OF TRANSIENT IPSCS DURING PLURIPOTENCY FACTOR-MEDIATED DIRECT REPROGRAMMING

Kim, Janghwan
Stem Cell Convergence Research Center, Korea Research Institute of Bioscience and Biotechnology, Korea

COMPARATIVE ANALYSIS OF DIVERSE CELL STATES ESTABLISHES AN EPIGENETIC BASIS FOR INFERRING REGULATORY GENES GOVERNING CELL IDENTITY

Sinniah, Enakshi
Institute for Molecular Bioscience, The University of Queensland, Brisbane, Australia

DIRECT INDUCTION OF THE THREE PRE-IMPLANTATION BLASTOCYST CELL TYPES FROM FIBROBLAST

Jaber, Mohammad
Department of Developmental Biology, Hebrew University, Jerusalem, Israel

CHEMICAL DERIVED HEPATIC PROGENITORS, A SILVER LINING OF REGENERATIVE MEDICINE OF THE LIVER

Choi, Dongho
Department of Surgery, College of Medicine, Hanyang University, Seoul, Korea

Lee, Joo-Hyeon

Wellcome Trust - MRC Cambridge Stem Cell Institute, University of Cambridge, UK, Department of Physiology, Development and Neurobiology, University of Cambridge, UK

Dr Joo-Hyeon Lee undertook her postdoctoral training at Carla Kim's laboratory in Children's Hospital Boston, Harvard Medical School. She established her own laboratory at Cambridge Stem Cell Institute in 2016 and focuses on understanding cellular behavior and regulatory networks of stem and niche cells. Joo-Hyeon is currently Faculty member at the Department of Physiology, Development, and Neuroscience, University of Cambridge and was awarded with the Sir Henry Dale Fellowship and ERC starting grant.

13:35-14:00

THE INFLAMMATORY NICHE SHAPES LUNG REGENERATION

Lee, Joo-hyeon[1,3], Choi, Jinwook[1], Park, Jong-eun[2]

[1]*Wellcome Trust - MRC Cambridge Stem Cell Institute, University of Cambridge, UK,* [2]*Wellcome Sanger Institute, Cambridge, UK,* [3]*Department of Physiology, Development and Neurobiology, University of Cambridge, UK*

Numerous epithelial stem/progenitor cells have been identified and shown to play a role in lung homeostasis and regeneration. In the alveoli, the site of gas exchange, alveolar type II cells (AT2) serve as stem cells that slowly produce alveolar cells throughout the life time but dramatically expand to regenerate a large proportion of the alveolar cells after lung injury. However, it is not yet known how these cells are rapidly expand and properly generate differentiated AT1 cells. Despite the vulnerability of epithelial cells to inflammatory environment upon damage, the primary response of stem cells to an inflammation is poorly understood. We have used a combination of genetic lineage tracing, single cell RNA-seq and 3D organoid co-culture approaches to define the dynamic inflammatory niche that enhances AT2 proliferation and differentiation into AT1 cells during alveolar injury repair. We discovered a detailed trajectory of AT2 differentiation during lung regeneration and the underlying mechanisms to direct these paths. Our study demonstrates that the inflammatory niche regulates both the activity and fate behaviour of AT2 cells enabling effective repair after lung injury.

Keywords: Lung stem cells, Regeneration, Inflammation

14:00-14:15

IN VITRO VASCULARIZATION OF HUMAN ORGANOIDS AND TISSUE FRAGMENTS: CELL CULTURES' MISSING LINK?

Vulto, Paul[1], Kurek, Dorota[1], Previdi, Sara[1], Trietsch, Sebastiaan[1], Nicolas, Arnaud[1], Hendriks, Delilah[2], Hu, Delilah[2], Clevers, Hans[2], Zhang, Luc[1]

[1]Research and Development, Mimetas B.v., Leiden, Netherlands, [2]Research and Development, Hubrecht Institute, Utrecht, Netherlands

Vasculature is a crucial ingredient of human organs and tissues. In addition to being a vehicle for blood circulation, exchange of angiocrine factors contribute to tissue function and homeostasis. Although the importance of perfusion systems in cell culture is now widely recognized in the cell culture community, true vascularization of tissues has been lacking thus far. Here, we introduce a microfluidic platform that is capable of vascularization of tissues such as organoids, spheroids, and tissue fragments. The platform comprises 64 independent microfluidic chips arranged in a microtiter plate format. Each chip comprises an extracellular matrix gel that is patterned by means of a surface tension technique called PhaseGuiding. Two blood vessels are grown on each lateral side of the free-standing ECM gel, by seeding cells against the matrix and subsequently applying perfusion flow of growth medium. The blood vessels are optionally induced to form microvessels by application of a gradient of angiogenic factors. The resulting microvascular bed is used as a scaffold for subsequent vascularization. Tissues are grafted onto the vascular bed by placing it on top of the ECM gel, containing the vascular bed. We show vascularisation of liver 3d structures as liver spheroids and liver organoids. The microvessels are connecting to the tissue as witnessed by an increase in CD31 positive cells in relation to culture time. Upon anastomosis, microvessels widen and become leaktight as assessed by perfusion with FITC labeled dextran. Scanning electron micrographs revealed basement membrane formation between vessels and the hepatocyte organoid, and microvesicle transport is observed through the endothelial cell membrane. The take rate of tissues on top of an extracellular matrix dramatically increased in the presence of a microvascular bed. In addition, immunohistochemical staining of the core of dense spheroids in presence is improved. The method provides an in vitro alternative to current xenograft techniques and may fill up a crucial gap in current day cell culture.

14:15-14:30

SINGLE CELL RNA-SEQUENCING ANALYSIS REVEALS THE ABSENCE OF TRANSIENT IPSCS DURING PLURIPOTENCY FACTOR-MEDIATED DIRECT REPROGRAMMING

Kim, Janghwan[1], Im, Ilkyun[1], Kim, Beomseok[2], Choi, Younha[2], Ha, Jeongmin[1], Son, Mi-young[1], Ding, Sheng[3], Kim, Jong Kyoung[2]

[1]Stem Cell Convergence Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea, [2]Department of New Biology, Daegu Gyeongbuk Institute of Science and Technology (DGIST), Daegu, Korea, [3]The J. David Gladstone Institute, San Francisco, CA, USA

Forced expression of four transcription factor Oct4, Sox2, Myc, and Klf4 (OSKM) is capable of inducing direct reprogramming into three germ layer cell types under the cell type-specific conditions as well as induced pluripotent stem cells (iPSCs) from somatic cells. In 2015, two groups reported that direct reprogramming using OSKM involves transient iPSCs by using lineage-tracing technique. However, recent studies raised the possibility that transient iPSCs was emerged in specific polycistronic expression system. In this study, we analyzed OSKM-mediated direct reprogramming using single cell RNA sequencing to investigate whether the transient iPSCs are formed during this process when we utilize monocistronic expression system. Trajectory analysis toward iPSCs and induced neural stem cells (iNSCs) revealed that two reprogramming processes completely bifurcated after day 5. Although we observed the activation of endogenous Oct4 in the major population toward iNSCs, the Oct4-expressing cells did not co-express other mandatory pluripotency markers including Nanog. In further analysis on the trajectory of iNSC reprogramming, we found three distinct reprogramming paths: two Oct4-positive paths and one

Oct4-negative path. These three reprogramming paths diverged by the different expression status of the exogenous OSKM. The higher and longer maintenance of OSKM expression induced the stronger activation of endogenous Oct4. In conclusion, our single-cell resolution analysis of the pluripotency factor-mediated direct reprogramming shows the absence of iPSC-like cells and the presence of novel reprogramming trajectories to iNSCs.

Funding Source: This work was supported by Samsung Research Funding Center of Samsung Electronic under Project Number SRFC-MA1601-06.

14:30-14:45

COMPARATIVE ANALYSIS OF DIVERSE CELL STATES ESTABLISHES AN EPIGENETIC BASIS FOR INFERRING REGULATORY GENES GOVERNING CELL IDENTITY

Sinniah, Enakshi[1], Shim, Woo Jun[2], XU, Jun[1], Vitrinel Burcu[3], Alexanian, Michael[4], Andreoletti, Gaia[5], Shen, Sophie[1], Balderson, Brad[2], Peng, Guangdun[6], Jing, Naihe[7], Sun, Yuliangzi[1], Chhabra, Yash[8], Wang, Yuliang[9], Tam, Patrick P L[10], Smith, Aaron[8], Piper, Michael[11], Christiaen, Lionel[3], Nguyen, Quan[1], Boden, Mikael[2], Palpant, Nathan J[1]

[1]Institute for Molecular Bioscience, The University of Queensland, Brisbane, Australia, [2]School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Australia, [3]Center for Developmental Genetics, New York University, NY, USA, [4]The Gladstone Institute, University of California, San Francisco, CA, USA, [5]Institute for Computational Health Sciences, University of California, San Francisco, CA, USA, [6]Cas Key Laboratory of Regenerative Biology, University of Chinese Academy of Sciences, Guangzhou, China, [7]State Key Laboratory of Cell Biology, University of Chinese Academy of Sciences, Shanghai, China, [8]Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia, [9]Department of Computer Science, University of Washington, Seattle, WA, USA, [10]Children's Medical Research Institute, The University of Sydney, Westmead, Australia, [11]School of Biomedical Sciences, The University of Queensland, Brisbane, Australia

Understanding genetic control of cell diversification is essential for establishing mechanisms controlling biological complexity. We analyzed 111 NIH epigenome roadmap data sets to identify distinguishing features of genome regulation associated with cell-type specification. We show that the a priori deposition of H3K27me3, which we call a gene's repressive tendency (RT), provides a genome-wide enrichment for genes governing fundamental mechanisms underlying biological complexity in cell differentiation, organ morphogenesis and drivers of disease. We tested the ability to infer regulatory genes controlling theoretically any somatic cell by interfacing genome-wide RT values with cell-specific genome-wide sequencing data. Using more than 1 million genome-wide data sets from diverse omics platforms including bulk and single cell RNA-seq, CAGE-seq, ChIP-seq and quantitative proteomics, we identify cell-type specific regulatory mechanisms underlying diverse cell-states, organ systems and disease pathologies. Since regulatory control of cell identity is highly evolutionarily conserved across species, we demonstrate that this computational logic enriches for cell- type specific regulatory genes from species across the animal kingdom including chordates and arthropods. Lastly, we use this computational inference approach for novel gene discovery. Analysis of single cell RNA-seq data from in vitro human iPSC cardiac differentiation predicted SIX3 as a novel transcription factor controlling derivation of definitive endoderm, which we confirmed by SIX3 genetic loss of function using CRISPRi hPSCs. Moreover, analysis of transcriptional data from heart development of the invertebrate chordate Ciona robusta, predicted RNF220 to underlie tunicate heart field formation. This was confirmed with CRISPR knockout in vivo showing that RNF220 loss of function results in pharyngeal muscle morphogenesis defects. This study demonstrates that the conservation of epigenetic regulatory logic provides an effective strategy for utilizing large, diverse genome-wide data to establish quantitative basic principles of cell-states to infer cell-type specific mechanisms that underpin the complexity of biological systems.

Funding Source: E.S acknowledges funding by Children's Hospital Foundation Queensland (Award Reference Number: 50268). B.V. acknowledges funding by American Heart Association grant #18PRE33990254. The Ciona work was supported by NIH/NHLBI award R01 HL108643 to L.C. M.A. was supported by the Swiss National

Science Foundation (project P2LAP3_178056). N.P is supported by the National Health and Medical Research Council of Australia (Grant APP1143163) and the Australian Research Council (Grant SR1101002).

14:45-15:00

DIRECT INDUCTION OF THE THREE PRE-IMPLANTATION BLASTOCYST CELL TYPES FROM FIBROBLAST

Jaber, Mohammad, Benchetrit, Hana, Zayat, Valery, Buganim, Yosef

Department of Developmental Biology, Hebrew University, Jerusalem, Israel

Following fertilization, totipotent cells undergo asymmetric cell divisions, resulting in three distinct cell types in the late pre-implantation blastocyst: epiblast (Epi), primitive endoderm (PrE), and trophectoderm (TE). Here, we aim to understand whether these three cell types can be induced from fibroblasts by one combination of transcription factors. By utilizing a sophisticated fluorescent knockin reporter system, we identified a combination of five transcription factors, Gata3, Eomes, Tfap2c, Myc, and Esrrb, that can reprogram fibroblasts into induced pluripotent stem cells (iPSCs), induced trophoblast stem cells (iTSCs), and induced extraembryonic endoderm stem cells (iXENs) , concomitantly. In-depth transcriptomic, chromatin, and epigenetic analyses provide insights into the molecular mechanisms that underlie the reprogramming process toward the three cell types. Mechanistically, we show that the interplay between Esrrb and Eomes during the reprogramming process determines cell fate, where high levels of Esrrb induce a XEN-like state that drives pluripotency and high levels of Eomes drive trophectodermal fate.

Choi, Dongho

Department of Surgery, College of Medicine, Hanyang University, Seoul, Korea

Dr. Dongho Choi was born in 1969 in South Korea. In terms of educational career, he graduated from Hanyang University in 1993 and became M.D. he also obtained his Ph. D. degrees at the Hanyang University in 2003. His research interest is hepatobiliary surgery, tissue engineering, liver transplantation and stem cell research. Dr. Choi is a member of Korean Surgical Society and many other scientific societies. He has published over 100 original research papers.

15:00-15:25

CHEMICAL DERIVED HEPATIC PROGENITORS, A SILVER LINING OF REGENERATIVE MEDICINE OF THE LIVER

Choi, Dongho

Department of Surgery, College of Medicine, Hanyang University, Seoul, Korea

The liver is the biggest organ in the body with complex architecture, wide range of functions and unique regenerative capacity. Growing incidence of liver diseases worldwide demands increased number of liver transplantation and leads to ongoing shortage of donor livers, resulting in morbidity and mortality. As an alternative to meet the huge demand, various approaches are being pursued including, in vitro and in vivo devices, hepatic cell transplantation and bioprinting of the organ itself. Adult hepatocytes are the most preferred cell sources for it, but they have limitations regarding their scanty availability, difficult isolation, poor in-vitro propagation and rapid functional deterioration. Thus, there have been efforts to overcome the drawback, regarding adequate extracellular matrix and co-culture of extra-parenchymal cells of liver and finding alternative cell sources. Hepatocytes have been successfully generated from iPSC, ESC and even direct differentiation. They are considered to be an attractive tool for treating end-stage liver diseases hampered by a shortage of donor organs for transplantation and the difficulties in cryopreservation and long term culture of mature hepatocytes in the near future. Recently, We developed chemical derived hepatic progenitors and it is regarded as new emerging cutting edge technology to overcome various hurdles for regenerative medicine of the liver. This presentation will provide summary of issues and challenges of 3 D hepatic structure and current status and personal experience of author for this field.

Keywords: regenerative medicine, chemical derived hepatic progenitors (CdHs), liver

Thursday, 26 September, 13:35-15:25

Concurrent Session 2 : Stem Cells and Tissue Engineering, Room B

Chair: Zandstra, Peter

BIOMIMETIC HYDROGELS FOR STEM CELL AND REPROGRAMMED CELL THERAPY

Cho, Seung-woo
Department of Biotechnology, Yonsei University, Seoul, Korea

BONE MARROW-DERIVED EPITHELIAL CELLS AND HAIR FOLLICLE BULGE STEM CELLS INITIATE AND PROMOTE CHRONIC INFLAMMATION-ASSOCIATED CUTANEOUS NEOPLASMS IN MICE

Park, Heuijoon
Department of Pathology and Cell Biology, Columbia University, New York, NY, USA

PRINTING HUMAN PANCREATIC TISSUES FOR THE TREATMENT OF DIABETES

Jang, Jinah
Creative IT Engineering, Postech, Pohang, Korea

MODELLING OF RETINITIS PIGMENTOSA CAUSED BY A NONSENSE MUTATION IN THE RP1 GENE USING INDUCED PLURIPOTENT STEM CELLS

Moon, Sangyoon
Centre for Ophthalmology and Visual Sciences, The University of Western Australia, Perth, Australia

GENERATION OF EPITHELIAL ORGANOIDS FROM HUMAN TONSILS OF WALDEYER'S RING IN A CHEMICALLY DEFINED MEDIUM

Yoo, Jongman
School of Medicine, CHA University, Seongnam, Korea

VASCULARIZED SPHEROIDS/ORGANOIDS USING MICROFLUIDICS

Jeon, Noo Li
School of Mechanical and Aerospace Engineering, Seoul National University, Seoul, Korea

Cho, Seung-woo

Department of Biotechnology, Yonsei University, Seoul, Korea

Seung-Woo Cho is a Professor at the Department of Biotechnology at Yonsei University, Korea. He obtained his B.S, M.S, and Ph.D. degrees in Chemical Engineering from Seoul National University in 1999, 2001, and 2006, respectively. He received his postdoctoral training at the Department of Chemical Engineering at the Massachusetts Institute of Technology. He later joined Yonsei University as a faculty member in 2010 and was awarded the 9th Asan Award for Young Medical Scientists in 2016. His research interests include stem cell engineering, reprogramming, and tissue engineering with functional biomaterials and biomedical devices. He has published 140 peer-reviewed papers and 32 pending/filed patents.

13:35-14:00

BIOMIMETIC HYDROGELS FOR STEM CELL AND REPROGRAMMED CELL THERAPY

Cho, Seung-woo

Department of Biotechnology, Yonsei University, Seoul, Korea

Stem cell engineering and reprogramming have been applied for the generation of tissue-specific cell types for cell therapy and tissue engineering. However, low efficiency in stem cell differentiation and direct reprogramming process still remains a major challenge. Thus, three-dimensional (3D) microenvironments to provide tissue-specific biochemical cues and native tissue-mimicking structures have received a great attention to facilitate differentiation and reprogramming for lineage specification. In my presentation, biomimetic hydrogel platforms are demonstrated to improve regenerative efficacy of stem cells and reprogrammed cells. The engineered hydrogels reconstituting tissue-specific 3D microenvironments were applied for increasing the efficiency of non-viral direct reprogramming of fibroblasts to induced neuronal, hepatic, and muscle cells. The induced cells generated by our biomimetic hydrogels displayed mature phenotypes and functions, and significantly improved in vivo therapeutic efficacy for treating degenerative diseases and repairing tissue injuries. Cell patterning in 3D hydrogels was employed to reconstitute native tissue-like structures, thereby enhancing stem cell-mediated regeneration for ischemic diseases. The biomimetic hydrogels reported herein would be able to provide highly effective biomedical platforms for improving stem cell engineering and reprogramming technology.

Funding Source: This work was supported by a grant (2017R1A2B3005994) from the National Research Foundation of Korea (NRF) funded by the Ministry of Science and ICT (MSIT), Korea.

14:00-14:15

BONE MARROW-DERIVED EPITHELIAL CELLS AND HAIR FOLLICLE BULGE STEM CELLS INITIATE AND PROMOTE CHRONIC INFLAMMATION-ASSOCIATED CUTANEOUS NEOPLASMS IN MICE

Park, Heuijoon[1], Lad, Sonali[3], Boland, Kelsey[3], Johnson, Kelly[3], Nyssa, Readio[3], Jin, Guangchun[2], Asfaha, Samuel[2], Patterson Kelly[2], Singh, Ashok[3], Yang, Xiangdong[3], Londono, Douglas[4], Singh, Anupama[3], Trempus, Carol[5], Gordon, Derek[4], Wang, Timothy[2], Morris, Rebecca[3]

[1]Department of Pathology and Cell Biology, Columbia University, New York, NY, USA, [2]Division of Digestive and Liver Diseases, Department of Medicine and Irving Cancer Center, Columbia University, New York, NY, USA, [3]Stem Cells and Cancer, The Hormel Institute, University of Minnesota, Austin, MN, USA, [4]Department of Genetics, Rutgers University, NJ, USA, [5]Matrix Biology Group, Immunity, Inflammation, and Disease Laboratory, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA

Bone marrow-derived epithelial cells (BMDECs) are recruited to sites of injury and contribute to wound healing; however, their clinical significance has not been determined in chronic inflammation-associated cutaneous malignancies such as tumors and ulcers. Here, we used gender-mismatched allogeneic bone marrow transplantation (BMT) in the context of the classical multistage murine cutaneous carcinogenesis model to probe the recruitment of BMDECs in skin tumors initiated with the carcinogen, dimethylbenz[a]anthracene (DMBA), and promoted with the phorbol ester, 12-O-tetradecanolyphorbol-13-acetate (TPA). We observed that the number of cytokeratin-immunoreactive bone marrow-derived cells (BMDCs) is increased in the hyperplastic epidermis after long term treatment with TPA. Furthermore, we detected clusters of BMDCs in 38% (17/45) of papilloma samples, where they occupied 25% or more of the epithelial areas. In addition, clusters of BMDCs were identified in 35% of the epithelial lesional areas in 53% (26/49) of the dysplastic ulcer samples. The BMDCs clustered in the cutaneous epithelium, where they became immunoreactive to keratin 14 (K14), proliferated (BRDU+ and Ki67+) and stratified, thereby contributing to the lesions comparably with the progeny of hair follicle stem cells in engrafted Krt1-15Cre;R26R mice. Moreover, a subset of K14+ plastic-adherent bone marrow cells (BMCs) was detected by immunostaining and Q-PCR after coculture with filter-separated epidermal keratinocytes (KCs) and treatment of bone morphogenetic protein 5. Further ex vivo invasion assays demonstrated that BMCs migrated towards the alarmin protein, High Mobility Group Box 1, and KCs. Finally, naïve female mice receiving BMTs from carcinogen-exposed donors developed benign and malignant lesions after TPA promotion alone. We conclude that a significant number of BMDECs contribute to a subset of cutaneous papillomas and dysplastic ulcers, demonstrating a systemic contribution to these lesions. Furthermore, carcinogen-exposed BMCs can initiate benign and malignant lesions upon tumor promotion. Ultimately, these findings demonstrated systemic support of cellular sources for local skin cancer development and suggest new novel targets for treatment of non-melanoma skin cancers as well as other solid cancers.

Funding Source: NIH R01 CA097957, NIH R01 CA097957-APRC, NIH R01 AR052713, and NIH R21 CA124942.

14:15-14:30

PRINTING HUMAN PANCREATIC TISSUES FOR THE TREATMENT OF DIABETES

Jang, Jinah[1], Kim, Jaewook[4], Hwang, Donggyu[2], Kim, Myungji[2], Shim, Inkyoung[3], Kim, Songcheol[3]

[1]Creative IT Engineering, Postech, Pohang, Korea, [2]School of Interdisciplinary Bioscience and Bioengineering, Postech, Pohang, Korea, [3]Asan Institute for Life Science, Asan Medical Center, Seoul, Korea, [4]Mechanical Engineering, Postech, Pohang, Korea

Type 1 diabetes mellitus (T1DM) is a form of diabetes that inhibits or halts insulin production in the pancreas. Although various therapeutic options are widely applied in clinical settings, not all patients are treatable with such

methods due to the unstable T1DM or hypoglycemia unawareness. Islet transplantation using a tissue engineering-based approach may mark a significant advance in this field, but finding ways to increase the function of islets in 3D constructs is a major challenge. In this study, we suggest pancreatic tissue-derived extracellular matrix as a potential candidate to recapitulate the native microenvironment in transplantable 3D pancreatic tissues. Notably, insulin secretion and the maturation of insulin-producing cells derived from human pluripotent stem cells were highly up-regulated when cultured in pdECM bioink. In addition, co-culture with human umbilical vein-derived endothelial cells decreased the central necrosis of islets in 3D culture conditions. Through the convergence of 3D cell printing technology, we validated the possibility of fabricating 3D constructs of a therapeutically applicable size that can be used for transplants with a potentially allogeneic source of islets, such as patient-induced pluripotent stem cell-derived insulin-producing cells.

Funding Source: This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (2017M3A9C6032067) and, under the "ICT Consilience Creative Program" (IITP-R0346-16-1007).

14:30-14:45

MODELLING OF RETINITIS PIGMENTOSA CAUSED BY A NONSENSE MUTATION IN THE RP1 GENE USING INDUCED PLURIPOTENT STEM CELLS

Moon, Sangyoon[1], Zhang, Xiao[1], Zhang, Dan[2], Chen, Shang-chih[2], Mellough, Carla[2], Thompson, Jennifer[3], Mclaren, Terri[3], Tina, Lamey[3], De roach, John[3], Chen, Fred[4], Mclenachan, Samuel[2]

[1]Centre for Ophthalmology and Visual Sciences, The University of Western Australia, Perth, Australia, [2]Lions Eye Institute, Western Australia, Australia, [3]Australian Inherited Retinal Disease Registry and DNA Bank, Sir Charles Gairdner Hospital, Western Australia, Australia, [4]Department of Ophthalmology, Royal Perth Hospital, Western Australia, Australia

Nonsense mutations in the last exon of the RP1 gene cause autosomal dominant retinitis pigmentosa through a presumed dominant- negative mechanism. It has not been possible to confirm this mechanism due to the lack of human retinal tissue early in the disease process. Cellular reprogramming and gene editing techniques that enable human fibroblast cells to be converted into many different kinds of tissue has provided access to patient-specific retinal cells for analysis. In this study we first examined post-mortem retinal tissue from an 85year old female donor with a nonsense mutation in the RP1 gene (NM_006269. 1: c.2098G>T, p.E700X). To provide a model for investigating the molecular consequences of this mutation, we reprogrammed dermal fibroblasts from this donor into induced pluripotent stem cells (iPSC). RP1-iPSCs were characterised by immunostaining and qRT-PCR. RP1-iPSC were differentiated into retinal organoids for 60 days and RP1 expression analysed by qRT-PCR and western blot. CRISPR/Cas9 gene editing was used to insert a HiBiT sequence tag into the N-terminus of the RP1 gene. Histological examination of post-mortem retinal tissue revealed severe degeneration in all regions of the retina, with a loss of retinal nuclear layers, photoreceptor outer segments and retinal pigmented epithelial cells. Patient-derived iPSCs were positive for pluripotency markers and could be differentiated into embryoid bodies expressing markers of ectoderm, mesoderm and endoderm, confirming their pluripotent potential. RP1-iPSC-derived retinal organoids contained photoreceptor progenitors which expressed RP1, indicating that the HiBiT sequence was successfully inserted into the RP1 gene at high efficiency. In this study, patient fibroblasts were successfully reprogrammed into iPSC lines containing the RP1 nonsense mutation. RP1-iPSCs could be differentiated into retinal tissues to investigate RP1 pathophysiology. Such HiBiT-tagged RP1-iPSCs can be used to screen novel drugs aimed at reducing mutant RP1 protein expression.

Funding Source: This project was funded by the National Health and Medical Research Council of Australia (grants 1142962 and 1116360), the Ophthalmic Research Institute of Australia and generous donations from the Hogg family.

14:45-15:00

GENERATION OF EPITHELIAL ORGANOIDS FROM HUMAN TONSILS OF WALDEYER'S RING IN A CHEMICALLY DEFINED MEDIUM

Yoo, Jongman, Kim, Han Kyung, Kim, Hyeryeon, Lim, Young Chang

School of Medicine, CHA University, Seongnam, Korea

Tonsils, a collection of the mucosa associated lymphoid tissues, are the gateway of respiratory and digestive tract as the first line of defense against ingested or inhaled pathogens such as bacteria and viruses. Tonsils are vulnerable to bacterial and viral infections, which can lead to tonsillitis, tonsil hypertrophy and oropharyngeal cancer. There is no suitable in vitro model to recapitulate human tonsils, and experimental animal model for tonsils is not available, making it difficult to study the tonsil and its pathophysiology. Tractable methods to identify and interrogate pathways involved in tonsil related disorders, especially in vitro setting, are urgently needed. We developed defined culture protocols of palatine and nasopharyngeal tonsil (adenoid)-derived epithelial organoids that preserve essential features of the tonsil epithelium, such as its cellular composition and microscopic structures. Tonsil organoids can be rapidly generated from resected biopsies, expanded over several months, and exhibit histologic characteristics of stratified squamous epithelium. Moreover, a substantial proportion of EpCAM-positive cells and subsequent culturing efficiently generate tonsil organoids containing tonsil epithelium-like structures expressing markers of all-lineage cells in an organized, continuous arrangement and pathogenic responses for that resembles the tonsil in vivo. Furthermore, lentiviral transduction of human papillomavirus 16-encoded E6/E7 induced precancerous changes such as aberrant differentiation and hyperplastic proliferation. Also, supernatant post-LPS challenge increases neutrophil chemotactic activity and to elucidate the role of CXCL8-CXCR1/CXCR2 pathways in this process. In conclusion, we developed an organoid technology established from human tonsils, and it can offer the valuable complements to analyze human specific tonsil disorders using tissues from HPV infected or cancer patients and to test potential therapeutic compounds.

Funding Source: This work was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute, funded by the Ministry of Health & Welfare, Korea (HI16C1559, HI16C1634, HI17C2094, HI18C2458) and by the Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Science, ICT & future Planning, Korea (NRF-2018R1D1A102050030).

Jeon, Noo Li

School of Mechanical and Aerospace Engineering, Seoul National University, Seoul, Korea

Noo Li Jeon is a Professor of School of Mechanical and Aerospace Engineering at Seoul National University (SNU). He studied Materials Science and Engineering at Northwestern University (B.S.) and University of Illinois Urbana-Champagne (Ph.D.). He worked on soft lithography applications in Prof G.M. Whitesides' laboratory at Harvard University and at Prof M. Toner's group at Harvard Medical School. He was an Assistant and Associate Professor at UC Irvine from 2001-2009 in the Department of Biomedical Engineering. In 2009, he moved to Seoul National University to join School of Mechanical Engineering at Seoul National University. He is developing high-throughput organ-on-a-chip systems with emphasis on blood and lymphatic vessel networks and vascularized organoids and spheroids.

15:00-15:25

VASCULARIZED SPHEROIDS/ORGANOIDS USING MICROFLUIDICS

Jeon, Noo Li

School of Mechanical and Aerospace Engineering, Seoul National University, Seoul, Korea

The field of microfluidics-based three-dimensional (3D) cell culture systems is rapidly progressing from academic proof-of-concept studies to valid solutions to real-world problems. Polydimethylsiloxane (PDMS)-based microfluidics platforms have been widely adopted for organ-on-a-chip systems. However, due to the inherent material limitations that make it difficult to scale-up production, PDMS has not been widely used in standardized commercial applications for preclinical screening testing. In this presentation, injection-molded tumor spheroid chip made of polystyrene (PS) in a standardized 96-well plate format with a user-friendly design. Spontaneous liquid patterning is achievable with high repeatability. To demonstrate the feasibility of the device, we fabricated array of vascularized organdies and spheroids and developed a 3D tumor angiogenesis model for drug screening. We describe a reproducible, in vitro approach to grow perfusable 3D microvascular networks and vascularized organoids and spheroids on microfluidic chip. This model is easy- and ready-to-use and suitable for mass-production, with the ability to deliver robust and reproducible results.

Keywords: vascularized organoids, microfluidics, blood vessel networks

Thursday, 26 September, 13:35-15:25

Concurrent Session 3 : Stem Cells and Regenerative Medicine in Asia, Room C

Co-Chairs: Son, Youngsook, Cho, Ssang-Goo

PRESENT AND FUTURE PERSPECTIVE OF MYOCARDIAL REGENERATION THERAPY

Sawa, Yoshiki
Department of Cardiovascular Surgery, Osaka University Graduate School of Medicine, Osaka, Japan

NEURAL DIFFERENTIATION FOR DISCOVERY AND THERAPY

Hu, Baoyang
Innovation Academy for Stem Cell and Regeneration, Chinese Academy of Sciences, Beijing, China

MODELING HUMAN HEPATO-BILIARY-PANCREATIC ORGANOGENESIS FROM THE FOREGUT-MIDGUT BOUNDARY

Takebe, Takanori
Tokyo Medical and Dental University, Tokyo, Japan and Cincinnati Children's Hospital, Cincinnati, OH, USA

NATIONAL STEM CELL RESOURCE CENTER (NSCRC) OF CHINA, AND ITS ROLE IN DELIVERING STEM CELL-BASED THERAPIES

Hao, Jie
National Stem Cell Resource Center (NSCRC) of China, and Institute of Zoology, Chinese Academy of Sciences, Beijing, China

DIFFERENTIAL REGULATION OF NEURAL STEM CELL DIFFERENTIATION BY MEK INHIBITORS

Kim, Hyun-jung
College of Pharmacy, Chung-Ang University, Seoul, Korea

DECIPHERING BRAIN SOMATIC MUTATIONS IN HUMAN NEUROLOGICAL DISORDERS

Lee, Jeongho
Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, Korea

This program was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (MSIT) (No. 2017M3A9B4042580).

Sawa, Yoshiki

Department of Cardiovascular Surgery, Osaka University Graduate School of Medicine, Osaka, Japan

Professor at Department of Cardiovascular Surgery, Osaka University Graduate School of Medicine. Research activities include heart transplantation, artificial organs, gene and regenerative therapies. Dedication to the research led to receive numerous awards and honors, such as Japan Biomaterial Association Award, Scientific Technology Award sponsored by Minister of Education, Culture, Sports, Science and Technology, Minister of Health, Labor and Welfare award. He is also the President of Japanese Society of Regenerative Medicine and the Director of Japanese Surgical Society. Earned a medical degree from Osaka University Medical School in 1980 and joined the First Department of Surgery, Osaka University School of Medicine. In 1989, earned Humboldt scholarship to pursue further education in both the departments of cardiovascular physiology and cardiac surgery at the Max-Planck Institute in Germany. After returning to Japan, became Chief surgeon at the Department of Cardiovascular Surgery in 2004, Professor and Chief at the Department of Cardiovascular Surgery, the Director at Medical Center for Translational Research at Osaka University Hospital in 2006. He was appointed to the Dean at Osaka University Graduate School of Medicine from 2015 – March 2017.

13:35-14:00

PRESENT AND FUTURE PERSPECTIVE OF MYOCARDIAL REGENERATION THERAPY

Sawa, Yoshiki

Department of Cardiovascular Surgery, Osaka University Graduate School of Medicine, Osaka, Japan

Heart failure is a life-threatening disorder worldwide, and the current end-stage therapies for severe heart failure are replacement therapies such as ventricular-assist devices and heart transplantation. Although these therapies have been reported to be useful, there are many issues in terms of the durability, complications, limited donors, adverse effect of continuous administration of immunosuppressive agents, and high costs involved. Recently, regenerative therapy based on genetic, cellular, or tissue engineering techniques has gained attention as a new therapy to overcome the challenges encountered in transplantation medicine. We focused on skeletal myoblasts as the source of progenitor cells for autologous cell transplantation and the cell-sheet technique for site-specific implantation. In vitro studies have reported that myoblast sheets secrete cytoprotective and angiogenic cytokines such as hepatocyte growth factor (HGF). Additionally, in vivo studies using large and small animal models of heart failure, we have shown that myoblast sheets could improve diastolic and systolic performance and enhance angiogenesis and antifibrosis as well as the expression of several cytokines including HGF and vascular endothelial growth factor (VEGF) in the tissues at the transplanted site. Based on the results of these studies, we performed clinical trials using autologous myoblast sheets in ischemic cardiomyopathy (ICM) and dilated cardiomyopathy patients. Some patients showed left ventricular reverse remodeling and improved symptoms and exercise tolerance. Recently, multiple medical institutions including our institution successfully conducted an exploratory, uncontrolled, open-label phase II study in subjects with ICM to validate the efficacy and safety of autologous myoblast sheets. Thus, we could get the evidence that autologous skeletal muscle sheet might occur reverse remodeling in the responder of severe heart failure patients.

Hu, Baoyang

Innovation Academy for Stem Cell and Regeneration, Chinese Academy of Sciences, Beijing, China

Baoyang Hu is a Principal Investigator at the Institute of Zoology, Chinese academy of Sciences (IOZ/CAS), known for his work in stem cell based neural regeneration and transplantation. He is the executive director of the Innovation Academy for Stem Cell and Regeneration of CAS, the deputy director of State Key Laboratory of Stem Cell and Reproductive Biology, and the executive dean of Medical School at the University of the CAS (UCAS). He has a Ph.D. from Fudan University (2004) and got his postdoctoral training in UW-Madison (2005 - 2010). He joined the IOZ/CAS in 2011. Dr. Hu's research interests focus on human brain development, neural differentiation and neural degenerative diseases. He pioneered the neural differentiation of subtype specific neurons from hESCs and discovered the variable neural differentiation potency of human iPSCs (PNAS, 2010). Using hESC and neural differentiation as tool, his team discovered that SIRT6 represses H19 for proper development in primates, and knockout of SIRT6 cause development retardation of monkeys (Nature, 2018). He and his colleagues initiated the first clinical trial of treating Parkinson's disease using hESC-differentiated DA neuronal progenitors.
He has published more than 40 papers in prestigious journals such as Nature, Science, and Cell Stem Cells, and was granted 2 US patents. He is actively involved in national scientific planning and co-leads a steering committee of the National Major R&D Program on stem cell and translational research. He also serves as director of CSSCR and the Chinese Society of Cell Biology.

14:00-14:25

NEURAL DIFFERENTIATION FOR DISCOVERY AND THERAPY

Hu, Baoyang

Innovation Academy for Stem Cell and Regeneration, Chinese Academy of Sciences, Beijing, China

Neural differentiation of human pluripotent stem cells (hPSCs) together with novel technologies such as genome editing and brain organoids promise novel opportunities for mechanistic discovery and novel therapy for yet incurable neurological diseases. Here, we demonstrate three breakthroughs we have made based on such concepts, which represent disease modeling, drug discovery and replacement therapy, respectively. Using CRISPR/Cas9 and SMASh technology, we engineered hPSCs so that we can precisely control the dosage of endogenous proteins. This allows us temporally manipulating the FOXG1 dosage during neural differentiation, and successfully modeled FOXG1 syndrome and dissected its pathological alterations. Using hESC derived cerebral organoids, we also identify potent antiviral RNAi in neural stem cells. We demonstrated that enoxacin, a broad-spectrum antibiotic that is known as an RNAi enhancer, exerts potent antiviral activity against diverse ZIKV strains in hNPCs. Strikingly, enoxacin treatment completely prevents ZIKV infection and circumvents the ZIKV-induced microcephalic phenotypes in brain organoids that recapitulate human fetal brain development. To support clinical and industrial translation of stem cell research, we established a stem cell resource bank that deposits clinical-grade hESC lines following good manufacturing practice (GMP). This stem cell bank is certified by ISO9001:2015 and accredited by ISO20387. We have initiated several stem cell therapies based on this bank and the deposited resources. As a proof of concept demonstration, we systematically evaluate the safety and efficacy of hESC-derived DA neurons,

including transplanted clinical-grade hESC-derived DA neurons into brains of MPTP induced monkey models of Parkinson's disease. The transplanted cells survive, migrate and differentiate into mature DA neurons that express TH. Monkeys received transplantation exhibit significant locomotive improvement for up to 2 years post transplantation. These results support the ongoing China's first embryonic stem cell-based Phase I/IIa clinical study on PD.

Keywords: Neural differentiation, brain organoids, Parkinson's disease

14:25-14:40

MODELING HUMAN HEPATO-BILIARY-PANCREATIC ORGANOGENESIS FROM THE FOREGUT-MIDGUT BOUNDARY

Takebe, Takanori

Center for Stem Cell and Organoid Medicine (CuSTOM), Division of Gastroenterology, Hepatology and Nutrition, Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, OH, USA; Department of Pediatrics, University of Cincinnati College of Medicine, OH, USA; and Institute of Research, Tokyo Medical and Dental University (TMDU), Tokyo, Japan

Organogenesis is a complex and inter-connected process, orchestrated by multiple boundary tissue interactions. However, it is currently unclear how individual, neighboring components coordinate to establish an integral multi-organ structure. The hepato-biliary-pancreatic (HBP) anlage, which is demarcated by HHEX (Hematopoietically-expressed homeobox protein) and PDX1 (Pancreatic and duodenal homeobox 1) expression is first specified at the boundary between the foregut-midgut. Here, we leverage a three-dimensional differentiation approach using human pluripotent stem cells (PSC) to specify gut spheroids with distinct regional identities comprised of both endoderm and mesoderm. We show that antero-posterior interactions recapitulate the foregut (marked by SOX2, SRY-Box 2) and the midgut (marked by CDX2, Caudal type homeobox 2) boundary in vitro, modeling the inter-coordinated specification and invagination of the human hepato-biliary-pancreatic system. The boundary interactions between anterior and posterior gut spheroids enables autonomous emergence of HBP organ domains specified at the foregut-midgut boundary organoids in the absence of extrinsic factor supply. Whereas transplant-derived tissues were dominated by midgut derivatives, long-term culture develop into a segregated HBP anlage, followed by the recapitulation of early morphogenetic events including the invagination and branching of three different and inter-connected organ structures. Together, we demonstrate that the experimental multi-organ integrated model can be established by the juxta-positioning of foregut, midgut tissues, and potentially serves as a tractable, manipulatable and easily-accessible model for the study of complicated endoderm organogenesis and disease in human.

Keywords: Stem cells, iPSC, ESC, Organoid, Liver Bud, Human, Drug development, Transplantation

14:40-14:55

NATIONAL STEM CELL RESOURCE CENTER (NSCRC) OF CHINA, AND ITS ROLE IN DELIVERING STEM CELL-BASED THERAPIES

Hao, Jie

National Stem Cell Resource Center (NSCRC) of China, and Institute of Zoology, Chinese Academy of Sciences, Beijing, China

National Stem Cell Resource Center (NSCRC), formerly known as Beijing Stem Sell Bank, is the first Chinese clinical-grade human embryonic stem cell (hESC) bank as well as the first national stem cell bank , NSCRC successfully derived the first clinical-grade hESC line under current Good Manufacturing Practices (cGMP) conditions and the cell quality was fully reviewed by the National Institute for Food and Drug Control (NIFDC) according to the Chinese regulations. After 12 years of development, there are nearly 2000 cell lines with different differentiation potential and over 500 clinical-grade stem cell lines that have been generated and banked. NSCRC also received onsite assessment and passed the verification during the biobanking accreditation using ISO 2038 standard by China National Accreditation Service (CNAS) firstly in the world. NSCRC has developed a unique culture system which can maintain hESCs' pluripotent state under a completely xeno-free condition. Several clinical-grade hESCs derived functional cells, such as neural progenitors, cardiomyocytes, hepatocytes, retinal pigment epithelium (RPE) cells and multipotent mesenchymal stromal cells have been achieved with following quality reviews by NIFDC, and their pre-clinical biosafety and efficacy were further investigated. NSCRC launched first-in-

human (FIH) clinical studies using clinical-grade derived dopamine neuron progenitors and RPE cells for the treatment of Parkinson's disease (PD) (NCT03119636) and age-related macular degeneration (AMD) (NCT03046407) respectively, which firstly adopted cellular HLA matching strategies into the cell transplantation. Up to 2019, NSCRC has launched 8 clinical studies using hESCs derived functional cells, which account for around 15% of the country's stem cells based clinical studies in China.

14:55-15:10

DIFFERENTIAL REGULATION OF NEURAL STEM CELL DIFFERENTIATION BY MEK INHIBITORS

Kim, Hyun-jung[1], Lee, Ha-rim[1], Kim, Youngmin[1], San, Thinthin[1], Lee, Jeewoo[2]

[1]College of Pharmacy, Chung-Ang University, Seoul, Korea, [2]College of Pharmacy, Seoul National University, Seoul, Korea

Neural stem cells (NSCs) proliferate and differentiate into neurons and glia depending on the culture environment. However, the underlying mechanisms determining the fate of NSCs are not fully understood. Growth factors facilitate NSC proliferation through MAPK/ERK kinase (MEK) and MAPK activation, and NSCs differentiate into neurons, astrocytes, or oligodendrocytes when mitogens are withdrawn from the culture media. Here, we aimed to identify the effects and roles of MEK signaling on the determination of NSC fate. In our study, we suggest that MEK inhibitors have distinct functions in determining NSC fate. Inhibition of MEK2 is important for induction of neurogenesis in NSCs. U0126 and SL327 increase neurogenesis through MEK2 inhibition, whereas PD98059 induced astrocytogenesis in NSCs, which is mediated by the chemical structure, particularly the 3′-methoxy group rather than its renowned MEK1 inhibition.

Keywords: MEK inhibitors; MEK1; MEK2; neural stem cells; neurogenesis; astrocytogenesis

15:10-15:25

DECIPHERING BRAIN SOMATIC MUTATIONS IN HUMAN NEUROLOGICAL DISORDERS

Lee, Jeongho

Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, Korea

Mutations occur during cell division in all somatic lineages due to the unavoidable DNA replication errors. Because neural stem cells continue to undergo cell division throughout human life, somatic mutations in human brain can arise during development and accumulate with the aging process. Although somatic diversity is an evident feature of the brain, the extent of somatic mutations affecting the neuronal structure and function and their contribution to neurological disorders remain largely unexplored. Recently, we have provided the molecular genetic evidence that brain somatic mutations indeed lead to the structural and functional abnormalities of the brain observed in neurodevelopmental disorders, brain tumors, and neurodegenerative disorders. In this symposium, I will present our recent findings regarding brain somatic mutations as potential molecular lesions underlying various human brain disorders, thereby providing a new insight into the molecular pathogenesis and therapeutics for the related disorders.

Keywords: Brain, Somatic Mutations, Neural Stem Cells

Friday, 27 September, 13:50-15:15

Concurrent Session 4: Genome Editing in Stem Cells, Room A

Chair: Schöler, Hans

HIGHLY EFFICIENT GENOME EDITING BY CRISPR-CPF1 USING CRISPR RNA WITH A U-RICH 3'-OVERHANG

Kim, Yong-sam
Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea

A LIN28A LOSS-OF-FUNCTION ASSOCIATED WITH EARLY-ONSET PARKINSON'S DISEASE

Chang, Mi-yoon
Biomedical Science, Hanyang University, Seoul, Korea

ENHANCED IMMUNOCOMPATIBILITY OF IPS CELLS BY CRISPR-CAS9 TARGETED DISRUPTION OF HLA GENES

Xu, Huaigeng
Department of Clinical Application, Centre for IPS Cell Research and Application (CiRA), Kyoto University, Kyoto, Japan

GENOME-WIDE SCREENING OF FUNCTIONAL DEUBIQUITINATING ENZYMES REGULATING STEMNESS-RELATED PROTEINS USING CRISPR/CAS9-MEDIATED DUBS KNOCKOUT LIBRARY

Ramakrishna, Suresh
Department of Biomedical Science, Hanyang University, Seoul, Korea

EFFICIENT DETECTION AND PURIFICATION OF HUMAN PSC-DERIVED CELL POPULATIONS USING RNA SWITCHES

Kim, Shin-Il
Research and Development, AceRNA Technologies Co., Ltd, Kyoto, Japan

This program was supported by a faculty research grant of Yonsei University College of Medicine (6-2018-0200).

Kim, Yong-sam

Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea

Dr. Yong-Sam Kim is a principal investigator working for the Korea Research Institute of Bioscience and Biotechnology (KRIBB). He is also the director of Genome Editing Research Center in KRIBB. He holds BA, MS, PhD degrees from the Seoul National University majoring in Agricultural Chemistry. He earned a PhD degree with a thesis on an auxin binding protein. He was a research associate for Fred Hutchinson Cancer Research Center in 2011. Currently, his main research interests include the development of a novel genome editing technology and improvement of CRISPR technologies. His major achievement is the development of a highly efficient CRISPR-Cpf1 system through the engineering of CRISPR RNA. The engineered CRISPR-Cpf1 shows genome editing efficiency that surpasses that of CRISPR-Cas9 system. The highly efficient Cpf1 system is expected to harness genome editing-based gene therapy. Before working in the genome editing field, he was previously dedicated to the development of cancer biomarkers, focused on aberrant glycosylation occurring in tumor cells. Accordingly, in addition to refining genome editing tools, he is also seeking to apply genome editing tools to facilitate the development of cancer-specific biomarkers by genome-engineering model animals. He is a professor of the University of Science and Technology in Korea.

13:50-14:15

HIGHLY EFFICIENT GENOME EDITING BY CRISPR-CPF1 USING CRISPR RNA WITH A U-RICH 3'-OVERHANG

Kim, Yong-sam[1,2], Moon, Su Bin[1,2], Mi Lee, Jeong[1,2]

[1]Genome Editing Research Center, KRIBB, Daejeon, Korea, [2]KRIBB School of Bioscience, UST, Daejeon, Korea

Genome editing has been harnessed through the development of the clustered regularly interspaced short palindromic repeats (CRISPR) system, and the CRISPR/ CRISPR from Prevotella and Francisella 1 (Cpf1) system has emerged as a promising alternative to CRISPR/ CRISPR-associated protein 9 (Cas9) for use under in various circumstances. Despite the inherent multiple advantages of Cpf1 over Cas9, the adoption of Cpf1 has been unsatisfactory because of target-dependent insufficient indel efficiencies, compared with that of CRISPR/Cas9. Here, we report the use of an engineered CRISPR RNA (crRNA) for highly efficient genome editing by Cpf1, which includes a 20-base target-complementary sequence and a uridinylates-rich 3'-overhang. For the chemically synthesized crRNA, the 3'-proximal addition of 8-mers of uridinylates (U8) showed the highest indel efficiency. However, where the crRNA is transcriptionally produced, crRNA with a 20-base target-complementary sequence plus a U4AU4 3'-overhang was the optimal configuration for maximum Cpf1 activity. U-rich crRNA also maximized the utility of the AsCpf1 PAM variants and multiplexing genome editing using mRNA as the source of multiple crRNAs. Furthermore, U-rich crRNA enabled a highly safe and specific genome editing using Cpf1 in human cells. This engineered U-rich crRNA contributes to the development of a more powerful genome-editing toolbox.

Keywords: Genome editing, CRISPR-Cpf1, CRISPR RNA

14:15-14:30

A LIN28A LOSS-OF-FUNCTION ASSOCIATED WITH EARLY-ONSET PARKINSON'S DISEASE

Chang, Mi-yoon, Lee, Sang-hun

Biomedical Science, Hanyang University, Seoul, Korea

The etiology of Parkinson's disease (PD), a common movement disorder characterized by degeneration of midbrain-type dopamine (mDA) neurons in the substantia nigra (SN), is partially understood. Developmental aspects are recently emphasized as an etiology of PD. Lin28 is a heterochronic RNA-binding protein, identified to have roles in the developing brain. In this study, we found an early PD pathologic finding, associated with mDA neuron vulnerability in the SN, and PD-related behavioral deficits in Lin28 conditional knockout (cKO) mice. At the same time, we detected a loss-of-function variant of LIN28A (arginine to glycine at aa 192, R192G) from two young age-onset PD patients, and assessed the contribution of this variant to the patients' disease using an isogenic human embryonic stem cell (hESC)/ human induced pluripotent stem cell (hiPSC)-based disease model. Severe developmental defects and PD-related pathologic phenotypes were present in mDA neurons differentiated from the hESCs and the patient-derived hiPSCs carrying this variant, but these phenotypes were substantially rescued by correction of the variant. We further show that behavioral recovery of rats with PD after cell transplantation requires correction of the LIN28A variant in the donor patient (pt)-hiPSCs. Our data implicate LIN28A in PD pathogenesis and suggest future personalized medicine targeting this variant in patients.

Funding Source: Medical Research Center (2017R1A5A2015395), 2017M3A9B4062401, NRF-2017R1A2B2002220 and NRF-2016R1A2B4016342, funded by the National Research Foundation of Korea (NRF) of the Ministry of Science and ICT, Korea.

14:30-14:45

ENHANCED IMMUNOCOMPATIBILITY OF IPS CELLS BY CRISPR-CAS9 TARGETED DISRUPTION OF HLA GENES

Xu, Huaigeng, Wang, Bo, Ono, Miyuki, Kagita, Akihiro, Fujii, Kaho, Sasakawa, Noriko, Ueda, Tatsuki, Gee, Peter, Okita, Keisuke, Yoshida, Yoshinori, Kaneko, Shin, Hotta, Akitsu

Department of Clinical Application, Centre for iPS Cell Research and Application (CiRA), Kyoto University, Kyoto, Japan

Induced pluripotent stem cells (iPSCs) have strong potential in regenerative medicine applications, however, immune rejection especially caused by HLA-mismatching is one of the major concerns. B2M gene knockout and HLA-homozygous iPSC stocks can potentially address this issue, but the former approach may induce NK cell activity and fail to present antigens in the event of pathogen infection or oncogenesis, and latter approach is challenging to recruit rare donors. To overcome these issues, here, we show two genome-editing strategies for making immune compatible donor iPSCs. First, we generated HLA pseudo-homozygous iPSCs by allele- specific knockout of HLA heterozygous iPSCs. Second, we generated HLA-C-retained iPSCs by disrupting both HLA-A and -B alleles and one of HLA-C allele in order to suppress the NK cell response while maintaining antigen presentation. Importantly, HLA-C-retained iPSCs, which retain endogenous single allele of HLA-C and non-canonical HLA-E, -F, -G, could evade from T cells response and suppress NK cells more than HLA-E only expressing cells. We also confirmed HLA-Class II null HLA-C-retained cells could evade total allo-response from bulk PBMCs including CD8+ T cells CD4+ T cells and NK cells. Finally we estimated that 12 lines of Class II null HLA-C-retained iPSCs are immunologically compatible with over 90% of the world-wide population, greatly facilitating iPSC-based regenerative medicine applications.

Funding Source: Japan Agency for Medical Research and Development (AMED) grants for the Core Center for iPS cell Research. Research Center Network for Realization of Regenerative Medicine. Japan Society for the Pro-

motion of Science (JSPS) KAKENHI.

14:45-15:00

GENOME-WIDE SCREENING OF FUNCTIONAL DEUBIQUITINATING ENZYMES REGULATING STEMNESS-RELATED PROTEINS USING CRISPR/CAS9-MEDIATED DUBS KNOCKOUT LIBRARY

Ramakrishna, Suresh, Das, Soumyadip, Chandrasekaran, Arun Pandian, Karapurkar, Janardhan, Kim, Kye-seong

Department of Biomedical Science, Hanyang University, Seoul, Korea

Post-translational modification of proteins by ubiquitin has emerged as a key regulator of protein stability including various signal transduction cascades. Recently, extensive progress has been made in the characterization of ubiquitin proteases that remove ubiquitin from the substrates called deubiquitinating enzymes (DUBs). Although the activity of DUBs has been implicated in several important pathways including cell growth and differentiation, development, oncogenesis, neuronal disease and transcriptional regulation, their function and regulation remains insufficiently understood. The “loss-of-function” studies based on knockout in diploid mammalian cells can provide an excellent model to elucidate the DUB function. Here, we applied targeted genome editing technology based on CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated) to identify DUBs regulating diverse cellular functions. We constructed two sets of sgRNAs and CRISPR/Cas9 targeting each DUBs in human genome and performed genome-wide screening of functional DUBs regulating stemness-related proteins. Our DUBs knock out kit was used to identifying DUBs for protein of our interest based on protein stability by Western blotting. Using our method, we have identified several potential DUB genes regulating self-renewal of human embryonic stem cell and differentiation. We envision that our DUBs knockout library system will facilitate to identify several functional DUBs for any target protein of our interest from wide range of biomedical research in a high-throughput setting.

Funding Source: National Research Foundation of Korea

15:00-15:15

EFFICIENT DETECTION AND PURIFICATION OF HUMAN PSC-DERIVED CELL POPULATIONS USING RNA SWITCHES

Kim, Shin-Il

Research and Development, AceRNA Technologies Co., Ltd, Kyoto, Japan

Despite significant progress in improving an efficiency on a differentiation of various cell lineages derived from human pluripotent stem cells (PSCs), a purification of target cells or an elimination of unwanted cell population are highly required. Based on an endogenous microRNA (miRNA) activity in living cells, we developed an efficient method for purifying cells. We designed synthetic messenger RNAs (mRNAs) encoding a reporter tagged with sequences targeted by cell-type specific miRNAs. These RNA switches control their translation levels by sensing miRNA activities. For example, miR-208a-switch efficiently purified cardiomyocytes differentiated from human PSCs, and a switch encoding a proapoptotic protein Bim enriched for cardiomyocytes without cell sorting. This approach is widely applicable, as several miRNA-responsive switches precisely and efficiently isolated specific cell types including endothelial cells, hepatocytes and insulin-producing cells. Thus, RNA switches could be useful to purify desired cell types for future therapeutic applications.

Friday, 27 September, 13:50-15:15

Concurrent Session 5: Application of Stem Cell Technologies, Room B

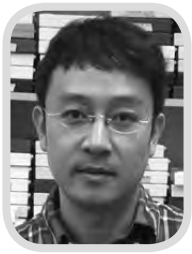
Chair: Rossant, Janet

REAL-TIME MONITORING OF DYNAMIC CELLULAR PROPERTIES OF EX VIVO EXPANDED OR IN VIVO ENGRAFTED MESENCHYMAL STEM CELLS

Shin, Dong-Myung
Department of Biomedical Sciences, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

HUMAN IPSC-DERIVED ASTROGLIA DELAY DISEASE PROGRESSION IN YAC128 HUNTINGTON'S DISEASE MICE

Park, Hyun Jung
Department of Biomedical Science, CHA University, Gyeonggi-do, Korea



PROMOTION OF PANCREATIC BETA CELL DIFFERENTIATION BY MODULATING ORGAN-SPECIFIC STROMAL NICHE SIGNALS

Kim, Tae-hee
Developmental and Stem Cell Biology, The Hospital for Sick Children/University of Toronto, ON, Canada

THERAPEUTIC POTENTIAL OF PRODRUG SOLID TUMOUR THERAPY BY NON VIRAL MODIFIED MESENCHYMAL STEM CELLS IN MICE MODEL AND COMPANION ANIMAL

Ho, Yoon Khei
Biochemistry, National University of Singapore, Singapore

THERAPY OF ISCHEMIC DISEASES USING HUMAN INDUCED PLURIPOTENT STEM CELLS

Kim, Jae Ho
Department of Physiology, Pusan National University College of Medicine, Yangsan, Korea

This program was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (MSIT) (No. 2017M3A9B4042581).

Shin, Dong-Myung

Department of Biomedical Sciences, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

Dong-Myung Shin, male, molecular biologist, got PhD degree in Seoul National University College of Medicine, Korea and got postdoctoral training at University of Louisville, USA (20082011) and then came back Korea as assistant-professor in current institute. He has published more than 80 papers including Leukemia, Theranostics, Cell Reports, and Stem Cell Reports. As his main research focus, he has investigated the molecular signature of pluripotency and redox homeostasis in pluripotent and adult stem cells.

13:50-14:15

REAL-TIME MONITORING OF DYNAMIC CELLULAR PROPERTIES OF EX VIVO EXPANDED OR IN VIVO ENGRAFTED MESENCHYMAL STEM CELLS

Shin, Dong-Myung[1], Lim, Jisun[1], Yu, Hwan Yeul[1,2], Heo, Jinbeom[1], Jeong, Eui Man[3], Choi, Kihang[4], Kim, In-Gyu[3], Choo, Myung-Soo[2]

[1]Department of Biomedical Sciences, [2]Department of Urology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea, [3]Department of Biochemistry and Molecular Biology, Seoul National University College of Medicine, Seoul, Korea, [4]Department of Chemistry, Korea University, Seoul, Korea

Transplantation of stem cells, such as mesenchymal stem cells (MSCs), is a promising strategy for treating several types of intractable disorders. Mechanistically, it could not only replace damaged cells by direct contribution, but also establish an anti-inflammatory or immunomodulatory microenvironment. However, the cellular mechanisms underlying molecular and biological properties of stem cells during ex vivo expansion and also after transplantation in pathological environments remain largely elusive. We recently developed the cyanoacrylamide-based coumarin derivatives (Fluorescent real-time thiol tracer; FreSHtracer)** reversibly react with glutathione for monitoring of glutathione levels in living stem cells. These probes revealed that glutathione levels are heterogeneous among subcellular organelles and among individual cells and show dynamic changes and heterogeneity in repopulating stem cells depending on oxidative-stress or culture conditions. Importantly, a subpopulation of stem cells with high-glutathione levels exhibited increased self-renewal and migration activities in vitro and showed improved therapeutic efficiency in treating asthma. Furthermore, employing a novel combination of longitudinal intravital confocal fluorescence imaging and microcystoscopy in living animals, we investigated the distributions and properties of transplanted multipotent MSCs derived from human embryonic stem cells at single-cell resolution in real-time by performing confocal imaging of bladder tissues in a rat model of IC/BPS for up to 6 months post-transplantation. These novel real-time monitoring strategies demonstrate the novel molecular insight for maintaining stem cell functions and also enhance understanding of the in vivo behaviors of the engrafted stem cells, which is crucial to determine the efficacy and safety of stem cell-based therapies. This strategy may facilitate the translation of various stem cell-based approaches into clinical practice.

Keywords : Real-time monitoring, Glutathione, Intravital imaging

** We have been collaborating with Cell2in Inc, a bio-venture company (Seoul, Korea) for research projects related to FreSHtracer based technologies.

14:15-14:30

HUMAN IPSC-DERIVED ASTROGLIA DELAY DISEASE PROGRESSION IN YAC128 HUNTINGTON'S DISEASE MICE

Park, Hyun Jung[1], Choi, Jiwoo[1], Kim, Jiyeon[1], Jeon, Juhyun[1], Windrem, Martha S[2], Kim, Hyunsook[3], Goldman, Steven A[2], Song, Jihwan[1]

[1]Department of Biomedical Science, CHA University, Gyeonggi-do, Korea, [2]Center for Translational Neuromedicine, University of Rochester Medical Center, Rochester, NY, USA, [3]Department of Neurology, CHA Bundang Medical Center, Gyeonggi-do, Korea

Huntington's disease (HD) is a devastating autosomal-dominant neurodegenerative disease, in which medium spiny neurons present in the striatum is selectively degenerated by the neurotoxicity from the extended CAG repeat sequences in the N-terminus of huntingtin gene. Dysfunctional astrocytes have been implicated in the development of various pathological symptoms of HD. The purpose of this study is to investigate the potential of astrocyte differentiation and the neuroprotective effects of human HLA-homozygous iPSC-derived neural precursor cells (iPSC-NPCs) following transplantation into the YAC128 transgenic mouse model of HD. We detected human nuclei (hNu)-positive transplanted cells at 5 months post transplantation, in which the striatal density, and the expression of myelin basic protein (MBP) of the corpus callosum (CC) was recovered significantly. Transplanted animals exhibited a significant improvement in motor functions (i.e., rotarod and grip strength tests) and cognitive functions (i.e., simple swim and novel object recognition tests). We observed that most transplanted cells gave rise to astrocytes, providing various neuroprotective roles for the recovery of YAC128 transgenic mice. First of all, transplanted cells were differentiated either into hGFAP (a human-specific astrocyte marker)-positive astrocytes or they contributed to the change of astrocyte morphology. Using immunohistochemistry and western blot analyses, we observed the increase of EAAT2, a marker for glutamate transporter, as well as Kir4.1, a marker for potassium channel, in the hGFAP-positive cells. These results strongly suggest that the transplanted cells can increase the glutamate reuptake and the expression of potassium channel in the hGFAP-positive cells, thereby reducing the glutamate toxicity in the host brain. We also found that the transplanted hGFAP-positive cells can give rise to the anti-inflammatory effects by astrocytes of physiological condition. Taken together, these results strongly suggest that the transplanted iPSC-NPCs can lead to astrocyte-mediated neuroprotection, followed by functional recovery in the preclinical mouse model of HD.

Funding Source: This work was supported by grants awarded to Jihwan Song (NRF-2017M3A9B4061407), and to Hyun Jung Park (NRF-2018R1C1B6008671) from the National Research Foundation of Korea.

14:30-14:45

PROMOTION OF PANCREATIC BETA CELL DIFFERENTIATION BY MODULATING ORGAN-SPECIFIC STROMAL NICHE SIGNALS

Kim, Tae-hee[1], Yung, Theodora[2], Poon, Frankie[4], Liang, Minggao[2], Coquenlorge, Sabrina[1], Yin, Wen-chi[2], Wilson, Michael[2], Hui, Chi-chung[1], Nostro, Cristina[3]

[1]Developmental and Stem Cell Biology, The Hospital for Sick Children/University of Toronto, ON, Canada, [2]Molecular Genetics, University of Toronto, ON, Canada, [3]McEwen Stem Cell Institute, University Health Network, Toronto, ON, Canada, [4]Physiology, University of Toronto, ON, Canada

The protocol established for the differentiation of human embryonic stem cells (hESCs) into insulin producing beta cells offers a promising new cell-based therapy for diabetes. However, the efficient differentiation into functionally mature beta cells has proven difficult. To define organ-specific niche signals required for beta cell differentiation, we isolated mouse pancreatic and gut stromal cells at E13.5, and analyzed their gene expression, revealing the pancreas-specific downregulation of mesenchymal Hh signaling. To investigate the mechanisms of this regulation, we analyzed mice deleted for Hh negative regulators, Sufu and/or Spop. These mutant mice exhibited severe

defects in pancreatic growth and beta cell neogenesis. To confirm that these phenotypes are caused by abnormal Hh signaling, we analyzed Hh downstream transcription factors and targets, and found that GLI2 becomes highly expressed and stabilized in the mutant mice. To determine the significance of this GLI2 overexpression and stabilization, we conditionally deleted Gli2 in Sufu knockout mice and found significant rescue of pancreatic defects, demonstrating its regulation of Hh signaling in pancreatic development and beta cell differentiation. Interestingly, the mesenchyme of Sufu and Spop knockout mice expressed gut stroma-enriched markers such as alpha-SMA and SM22, indicating a shift of pancreas mesenchymal identity into that of the gut. Recent studies have shown that gut stromal cells express Wnt ligands to promote gut stem cell renewal, and our analysis of GLI2 binding sites genome-wide has revealed its direct transcriptional activation of Wnt ligands in the gut stroma. Indeed, we found that the expression of Wnt ligands is abnormally increased in the Sufu and Spop knockout pancreatic mesenchyme. Therefore, we hypothesized that GLI2-mediated abnormal activation of Wnt niche signals prevents pancreatic beta cell differentiation. Notably, Wnt inhibitors such as WIKI4 significantly increased the number of C-PEP+/NKX6.1+ beta-like cells in hESC differentiation, whereas its agonist, CHIR99021, impaired the expression of pancreatic progenitors and endocrine lineage markers. Our work reveals the requirement for pancreas-specific regulation of stromal niche signals in beta cell differentiation.

Funding Source: SickKids Foundation, Canadian Institutes of Health Research (CIHR), Natural Science Research Council of Canada (NSERC) Discovery.

14:45-15:00

THERAPEUTIC POTENTIAL OF PRODRUG SOLID TUMOUR THERAPY BY NON VIRAL MODIFIED MESENCHYMAL STEM CELLS IN MICE MODEL AND COMPANION ANIMAL

Ho, Yoon Khei, Tu, Xue En, Woo, Jun Yung, Too, Heng-phon

Biochemistry, National University of Singapore, Singapore

Mesenchymal stem cells (MSCs) have emerged as promising vehicles for Gene-directed enzyme prodrug therapy (GDEPT). The inherent tumour-trophic migratory properties of MSCs enable these vehicles to deliver effective, targeted therapies to tumours and metastatic diseases. A critical step in modifying MSCs is the delivery of genes with high efficiency and low cytotoxicity. Due to the poor efficiency of transfection approaches, viral methods are used extensively to transduce MSCs in preclinical and clinical studies.We demonstrate, for the first time, the efficient transfection (>80%) of human adipose tissue derived MSCs (AT-MSCs) using an off-the shelf and cost-effective cationic polymer, polyethylenimine, in the presence of fusogenic lipids and histone deacetylase 6 inhibitor (HDAC6i). Notably, the cellular phenotypes of MSCs remained unchanged after modification. AT-MSCs modified with a fused transgene, yeast cytosine deaminase::uracil phosphoribosyltransferase (CDy::UPRT), exhibited strong cytotoxic effects towards glioma, breast and gastric cancer cells in vitro. The efficiency of eliminating gastric MKN1 and MKN28 cell lines were effective even when using 7 days post-transfected AT-MSCs, indicative of the sustained expression and function of the therapeutic gene. We demonstrate anti cancer efficacy of CD::UPRT_MSCs by direct injection into glioma led to regression of the subcutaneous tumours upon intraperitoneal treatment with the prodrug in the mice model. Similar observation was found in canine patients with sarcoma and indolent lymphoma. The therapeutic effects of canine CD::UPRT_MSCs appeared approximately one weeks post 5-FC administration. In conclusion, this method offers an efficient modification process for MSC-based prodrug therapy as an alternative to the use of viral vectors. The therapeutic cells can potentially secure patient quality of life with the same or more therapeutic effects and fewer side effects than other recommended chemotherapies.

Funding Source: SMART innovation grant (ING-000665 BIO), 2019.

15:00-15:15

THERAPY OF ISCHEMIC DISEASES USING HUMAN INDUCED PLURIPOTENT STEM CELLS

Kim, Jae Ho

Department of Physiology, Pusan National University College of Medicine, Yangsan, Korea

Peripheral artery disease is a condition in which tissue necrosis occurs as blood flow decreases due to arterial occlusion, resulting in limb amputation in severe cases. Both endothelial cells (ECs) and vascular smooth muscle cells (SMCs) are needed for regeneration of peripheral artery in ischemic tissues. However, it is difficult to isolate and cultivate primary endothelial cells and smooth muscle cells from patients for therapeutic angiogenesis. Induced pluripotent stem cells (iPSC) are regarded as useful stem cells due to their pluripotent differentiation potential. In this study, we explored the therapeutic efficacy of human iPSC-derived ECs (iPSC-ECs) and iPSC-derived SMCs (iPSC-SMCs) on peripheral artery disease in a murine ischemic hindlimb model. After induction of mesodermal differentiation of iPSC, CD34-positive vascular progenitor cells were isolated by magnetic-activated cell sorting. Cultivation of the CD34-positive cells in endothelial culture medium induced expression of endothelial markers, including CD31, VE-cadherin, and vWF, and endothelial characteristics, such as endothelial tube forming ability, eNOS expression, and Ac-LDL uptake. Moreover, the CD34-positive cells could be differentiated to not only ECs but also SMCs. Cultivation of the CD34-positive cells in SMC medium induced expression of SMC marker. Conditioned media of iPSC-SMCs were stimulated the migration, proliferation and tube formation of iPSC-ECs in vitro. In a murine hindlimb ischemia model, co-transplantation of iPSC-ECs with iPSC-SMCs accelerated blood perfusion and increased limb salvage rate in the ischemic limbs, compared to the ischemic limbs injected with either iPSC-ECs or iPSC-SMCs alone. Moreover, co-transplantation of iPSC-ECs with iPSC-SMCs further stimulated angiogenesis and that transplanted iPSC-ECs and iPSC-SMCs contributed formation of ILB4-positive capillaries and α-SMA-positive arteries/arterioles. These results suggest that combined treatment of iPSC-ECs and iPSC-SMCs differentiated from iPSC is useful for therapy of peripheral artery diseases.

Funding Source: This research was supported by the MRC program (NRF-2015R1A5A2009656) and the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (NRF-2015M3A9C6030280; NRF-2017M3A9B4051542).

Friday, 27 September, 13:50-15:15

Concurrent Session 6: Stem Cell Quality Control for Cell Therapy, Room C

Co-organized by GAiT/K-NIH/ISCBI

Co-chairs: Song, Jihwan and Stacey, Glyn

USEFULNESS OF THE KOREAN HLA-HOMOZYGOUS IPSC LINES TO MULTIPLE POPULATIONS

Song, Jihwan
Department of Biomedical Science, CHA University, Gyeonggi-do, Korea and The Korea Chapter, Global Alliance for iPSC Therapies (GAiT), Gyeonggi-do, Korea

THE GLOBAL ALLIANCE FOR IPSC THERAPIES (GAIT): QUALITY TESTING OF CLINICAL-GRADE INDUCED PLURIPOTENT STEM CELLS

Sullivan, Stephen
Global Alliance for iPSC Therapies (GAiT), Edinburgh, UK and Hanyang University, Seoul, Korea

LATEST ACCOMPLISHMENTS OF NATIONAL CENTER FOR STEM CELL AND REGENERATIVE MEDICINE

Koo, Sookyung
Korea National Institute of Health, Osong, Korea

SUITABILITY OF PLURIPOTENT STEM CELL LINES FOR CLINICAL APPLICATIONS

Stacey, Glyn
International Stem Cell Banking Initiative, Cambridge, UK and SSCBio Ltd, Royston, UK

INTEGRATED IPSC CHARACTERIZATION OPTIMAL FOR CELL THERAPY MANUFACTURING

Lakshmipathy, Uma
Thermo Fisher Scientific, Carlsbad, CA, USA

CANCER-RELATED MUTATIONS IN PRIMED AND NAIVE HUMAN PLURIPOTENT STEM CELLS

Benvenisty, Nissim
The Azrieli Center for Stem Cells and Genetic Research, The Hebrew University, Jerusalem, Israel

AUTOLOGOUS IPS CELL THERAPY FOR MACULAR DEGENERATION: FROM BENCH-TO-BEDSIDE

Bharti, Kapil
National Eye Institute, National Institutes of Health, Bethesda, MD, USA

This session was supported by the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health and Welfare (HI15C3042), as well as the Korea National Institute of Health (K-NIH), Republic of Korea.

13:50-13:55

USEFULNESS OF THE KOREAN HLA-HOMOZYGOUS IPSC LINES TO MULTIPLE POPULATIONS

Song, Jihwan

Department of Biomedical Science, CHA University, Gyeonggi-do, Korea and The Korea Chapter, Global Alliance for iPSC Therapies (GAiT), Gyeonggi-do, Korea

In order to establish a haplobank of iPSCs, we have recently generated 10 most frequent HLA-homozygous iPSC lines from the repurposed cord blood samples in Korea, which can potentially match 41.07% of the Korean population. Comparative analysis of HLA population data shows that they are also of use in other Asian populations, such as Japan, with some limited utility in ethnically diverse populations, such as the UK. Therefore, the generation of the 10 most frequent Korean HLA-homozygous iPSC lines serves as a useful pointer for the development of optimal methods for iPSC generation and quality control and indicates the benefits and limitations of collaborative HLA driven selection of donors for future stocking of worldwide iPSC haplobanks.

Funding Source: This work was supported by a grant from the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health and Welfare (HI15C3042), Korea.

13:55-14:05

THE GLOBAL ALLIANCE FOR IPSC THERAPIES (GAIT): QUALITY TESTING OF CLINICAL-GRADE INDUCED PLURIPOTENT STEM CELLS

Sullivan, Stephen

Global Alliance for iPSC Therapies (GAiT), Edinburgh, UK and Hanyang University, Seoul, Korea

Development of many stem cell derived therapeutic products has been unnecessarily delayed due to inconsistent terminology use between professional siloes, and also from a lack of consistency in the understanding of, and testing for, Critical Quality Attributes of starting materials. This lack of alignment makes stem cell products less likely to be financially viable, slows the adoption of key technological improvements, and may well lead to a divergence in regulatory requirements across different jurisdictions. The Global Alliance for induced Pluripotent Stem Cell Therapies (GAiT) is an international non-profit organization focused on building of a global haplobank network for clinical-grade induced Pluripotent Stem Cell (iPSC) lines so that these are an appropriate starting material for subsequent development of new allogeneic or 'off-the-shelf' therapeutics. GAiT activity is currently funded by four Founder Organizations: the Centre for Commercialization of Regenerative Medicine, the New York Stem Cell Foundation, the Cell and Gene Therapy Catapult, and the Korean HLA-matched iPSC Banking Initiative. In this talk, we shall review how GAiT is building consistency and reliability of Quality Testing of clinical-grade iPSCs across its network through execution of a Quality Round.

14:05-14:15

LATEST ACCOMPLISHMENTS OF NATIONAL CENTER FOR STEM CELL AND REGENERATIVE MEDICINE

Koo, Sookyung

Korea National Institute of Health, Osong, Korea

National Center for Stem Cell and Regenerative Medicine (NCSR) is a national infrastructure for stem cell and regenerative medicine research. Its purpose is to facilitate stem cell and regenerative medicine research in Korea by providing high-quality stem cells to the researchers and GMP manufacturing services for those preparing early

phase clinical trial of cell therapy. We have been running National Stem Cell Bank since October 2012, and now we are distributing four human embryonic stem cell (ESC) lines and 15 human induced pluripotent stem cell (iPSC) lines for research purposes, which includes 4 HLA haplotype-homozygous hiPSC lines, 4 fluorescent iPSC lines, and 2 iPSC lines originated from genetic disease patients. We are planning to start distributing one GMP-grade HLA haplotype-homozygous hiPSC line this year. We have launched our GMP manufacturing services in 2018. Once we receive requests for the service application, we will review them thoroughly and do our best to support the GMP manufacturing. So far, we have manufactured three items and they are currently undergoing KFDA evaluation process.

14:15-14:30

SUITABILITY OF PLURIPOTENT STEM CELL LINES FOR CLINICAL APPLICATIONS

Stacey, Glyn

International Stem Cell Banking Initiative, Cambridge, UK and SSCBio Ltd, Royston, UK

Human pluripotent stem cells offer unique possibilities for human therapies and hold great promise for the treatment diseases where there is unmet clinical need. Such therapies require complex manufacturing processes involving careful characterisation and control to deliver safe, effective and reliable therapies. Therefore, early development of stable cell lines with characteristic markers, pluripotent potential and absence of microbial contamination, are fundamental features which must be assured if the cells are to be suitable to generate a range of cell types for therapy. It is also important to address intellectual property issues relating to the use of the cell line to avoid risk to the economic delivery of final products. Regarding safety; traceability of candidate cell banks and the materials used to make them is key. This will permit robust, risk assessment for adventitious agents and assure compliance with requirements of GMP manufacture. Furthermore, it will be important to carry out genetic characterisation and assessment of the tumorigenic potential of cell lines. Suitable donor selection procedures will also be necessary to reduce risk of disease and to demonstrate that suitable informed consent has been obtained. Panels of cell lines covering a range of HLA haplotypes could provide for reduced severity of immune response against transplants in patients. However, the evaluation of other cell features relating to chronic as well as acute rejection mechanisms ay need to be addressed. In addition, any change in the source cell lines or any other aspect of the manufacturing which could impact on the final product, will require investigation to assure comparability of the new process in terms of the quality and safety of the final product. This presentation will also review the international consensus on these issues established by the International Stem Cell Banking Initiative (ISCBI, www.iscbi.org) which brings together stem cell banks from more than 20 countries. It will also consider the various avenues adopted for standardisation in this area and in the development of new international guidance on the manufacture of pluripotent stem cell derived therapies.

14:30-14:45

INTEGRATED IPSC CHARACTERIZATION OPTIMAL FOR CELL THERAPY MANUFACTURING

Lakshmipathy, Uma

Thermo Fisher Scientific, Carlsbad, CA, USA

Rapid progress in the translational and clinical applications of induced pluripotent stem cells (iPSC) necessitates the need for robust and consistent workflows that utilize high quality reagents that are xeno-free. When commonly used animal-origin components are replaced with xeno-free alternatives, performance often suffers, thereby necessitating thorough qualification of reagents and manufacturing processes. Methods that enhance consistency will minimize extra effort and costs associated with generation of clones that fail to expand, or do not meet quality standards for downstream use. Previously, we reported CTS CytoTune 2.1, the first off-the-shelf reprogramming kit specifically designed for clinical and translational research, with xeno-free workflows optimized to minimize

the effect of donor to donor variability. Here, we report a complete and comprehensive panel of assays for characterization of iPSCs. iPSC clones derived under xeno-free conditions with cell therapy qualified reagents were confirmed to be foot-print free, authenticated to be derived from the corresponding donor cells, and assessed for quality and safety profile. Clones qualified to be pluripotent via PluriTest and possess trilineage differentiation potential with TaqMan hPSC ScoreCard were thoroughly investigated for genomic stability. All of the clones tested showed a normal karyotype with traditional G-banding and KaryoStat arrays. Further assessment of oncogenic hotspot mutations that are known to occur at a higher frequency in ESC and iPSC, including TP53, indicated the absence of oncogenic structural variants in all of the clones tested. The combination of qualified reagents and defined xenofree workflows, in combination with comprehensive and predictive characterization assays aids in easy transition of early investigation work towards translational and clinical research.

14:45-15:00

CANCER-RELATED MUTATIONS IN PRIMED AND NAIVE HUMAN PLURIPOTENT STEM CELLS

Benvenisty, Nissim

The Azrieli Center for Stem Cells and Genetic Research, The Hebrew University, Jerusalem, Israel

Human pluripotent stem cells (hPSCs) are known to harbor chromosomal aberrations that might affect their tumorigenic potential. More recently, point mutations in the gene coding for the p53 tumor suppressor (TP53) have been found in hPSCs. These mutations gradually take over the culture, suggesting they provide a growth advantage in vitro. However, it remains unclear whether other cancer-related genes acquire recurrent mutations during hPSC propagation. Here we established a strategy to identify such mutations by comparing of genomic data from early and late passage hPSCs. Analysis of over 170 samples (from 46 studies) of the two most commonly used hPSC lines revealed mutations in over 20 verified cancer-related genes other than TP53. Similar mutations were found in an analysis of over 400 induced pluripotent stem cell samples (from 24 studies). Importantly, naive hPSCs were found to harbor four-times more cancer-related mutations on average than their primed counterparts. These mutated genes corresponded to the mechanisms of action of the chemical inhibitors inducing a naive state, suggesting that selective pressures imposed by them resulted in an increased cancer-associated mutational burden. Together, our results suggest that prolonged culturing and pluripotent cell state transition enhance hPSC cancer-related mutagenesis. These mutations should be taken into consideration in future applications, especially in clinical contexts.

15:00-15:15

AUTOLOGOUS IPS CELL THERAPY FOR MACULAR DEGENERATION: FROM BENCH-TO-BEDSIDE

Bharti, Kapil

National Eye Institute, National Institutes of Health, Bethesda, MD, USA

Induced pluripotent stem (iPS) cells are a promising source of personalized therapy. These cells can provide immune-compatible autologous replacement tissue for the treatment of potentially all degenerative diseases. We are preparing a phase I clinical trial using iPS cell derived ocular tissue to treat age-related macular degeneration (AMD), one of the leading blinding diseases in the US. AMD is caused by the progressive degeneration of retinal pigment epithelium (RPE), a monolayer tissue that maintains vision by maintaining photoreceptor function and survival. Combining developmental biology with tissue engineering we have developed clinical-grade iPS cell derived RPE-patch on a biodegradable scaffold. This patch performs key RPE functions like phagocytosis of photoreceptor outer segments, ability to transport water from apical to basal side, and the ability to secrete cytokines in a polarized fashion. We confirmed the safety and efficacy of this replacement patch in animal models as part of a

Phase I Investigational New Drug (IND)-application. Approval of this IND application will lead to transplantation of autologous iPS cell derived RPE-patch in patients with the advanced stage of AMD. Success of NEI autologous cell therapy project will help leverage other iPS cell-based trials making personalized cell therapy a common medical practice.

Thursday, 26 September, 12:35-13:35

Luncheon Symposium 1, Room A

Molecular Devices, Seoul, Korea
OVERCOME CHALLENGES OF HIGH-CONTENT IMAGING AND ANALYSIS FOR 3D CELL CULTURE

Tak, Minho

Luncheon Symposium 2, Room B

JSK Biomed Inc.
WHAT'S IN YOUR MEDIA MATTERS: APPLICATIONS OF CELLULAR METABOLIC PROFILING IN STEM CELL RESEARCH

Kam, Yoonseok

Luncheon Symposium 3, Room C

Thermo Fisher Scientific
INTEGRATED WORK FLOW SOLUTIONS FROM DISCOVERY TO TRANSLATIONAL RESEARCH

Lakshmipathy, Uma

Friday, 27 September, 12:50-13:50

Luncheon Symposium 4, Room A

Kangstem Biotech, Korea
DEVELOPMENT OF FIRST-IN-CLASS AND BEST-IN-CLASS STEM CELL THERAPEUTICS FOR IMMUNE RELATED DISEASES

Lee, Seunghee

Luncheon Symposium 5, Room B

Bio-Techne
ORGANOID TECHNOLOGY AND MEDICINE

Lee, Kyungjin

Luncheon Symposium 6, Room C

Ajinomoto Co., Inc., Daegu, Korea
USE OF BIOMATERIALS TO REGULATE NEURAL DIFFERENTIATION FROM HUMAN IPSCS

Kosodo, Yoichi

Thursday, 26 September, 12:35-13:35

Room A, Molecular Devices

12:35-13:35

OVERCOME CHALLENGES OF HIGH-CONTENT IMAGING AND ANALYSIS FOR 3D CELL CULTURE

Tak, Minho

Application Scientist Manager, Molecular Devices Korea, Seoul, Korea

Development of more complex, biologically relevant, and predictive cell-based assays for compound screening is a primary challenge in drug discovery. The integration of three-dimensional (3D) assay models is becoming more widespread to drive translational biology. Specifically, 3D cultures offer the advantage of closely recapitulating aspects of human tissues including the architecture, cell organization, cell-cell and cell-matrix interactions, and more physiologically-relevant diffusion characteristics. The ImageXpress® Micro Confocal system and the 3D Analysis Tools in MetaXpress® software meet these challenges and enable high-throughput screening of 3D cell models within a single interface, reducing the time to discovery. This session will introduce the 3D cell culture imaging and analysis advantage and includes a few 3D cell model applications as well as tips and techniques for optimizing your 3D assay workflow.

Room B, JSK Biomed Inc.

12:35-13:35

WHAT'S IN YOUR MEDIA MATTERS: APPLICATIONS OF CELLULAR METABOLIC PROFILING IN STEM CELL RESEARCH

Kam, Yoonseok

Cell Analysis Group, Agilent Technologies, Inc., Korea

Cellular metabolism is emerging as a critical factor in stem cell research with recent reports suggesting that both metabolic status and cell culture conditions can significantly alter the differentiation trajectory of stem cells. In vitro metabolic profiling provides information to identify key metabolic changes associated with stem cell differentiation. Agilent Seahorse XF technology enables real-time measurement of glycolytic and mitochondrial function along the cell differentiation axis. By changing the metabolic environment in the design of differentiation experiments, metabolic profiling can be incorporated to probe cell function, and more importantly, to guide improved differentiation outcomes. Here, introduced are the application models of Seahorse XF technology in metabolic phenotyping through stem cell differentiation process. The metabolic poise of a differentiated somatic cell is typically highly aerobic – high mitochondrial function and low glycolytic function. In contrast, undifferentiated or de-differentiated cells are highly glycolytic while maintaining relatively low mitochondrial function. The dynamic metabolic changes between those two different statuses can be assessed by using XF technology. This workshop covers different application examples including an assay design for sequential metabolic profiling during iPSC differentiation toward functional neurons. This design can be applied to better understand the interrelationship between metabolic poise and differentiation, thus enabling the development of improved differentiation protocols.

Room C, Thermo Fisher Scientific

12:35-13:35

INTEGRATED WORK FLOW SOLUTIONS FROM DISCOVERY TO TRANSLATIONAL RESEARCH

Lakshmipathy, Uma

Thermo Fisher Scientific, Carlsbad, CA, USA

Induced pluripotent stem cell (iPSC) research is rapidly moving towards translational and clinical applications. These applications require robust and consistent workflows that utilize high quality reagents that are xeno-free. When commonly used animal-origin components are replaced with xeno-free alternatives, performance often suffers, thereby necessitating thorough qualification of reagents and the process. Robust and reproducible methods minimize or eliminate the extra time and costs associated with clones that fail to expand, or do not meet quality standards for downstream use. Key considerations here are, first, the use of higher grade reagents specifically designed and qualified for clinical and translation research. Second, these systems will then need to be successfully integrated into existing workflows. In some cases, further optimization may be required to ensure consistent generation of high quality iPSCs despite donor to donor variability associated with the starting material. Finally, the resulting iPSC master cell banks need to be thoroughly characterized to establish their quality and safety. For CAR-T research, building integrated CAR-T workflow is important. End-to-end solution for the generation and characterization of CAR-T as so key factor of research. And using prequalified product, comprehensive and scalable characterization and traceability documentation also substantial.

Friday, 27 September, 12:50-13:50

Room A, Kangstem Biotech

12:50-13:50

DEVELOPMENT OF FIRST-IN-CLASS AND BEST-IN-CLASS STEM CELL THERAPEUTICS FOR IMMUNE RELATED DISEASES

Lee, Seunghee

Stem Cell and Regenerative Bioengineering Institute, Kangstem Biotech Co., Ltd., Korea

Mesenchymal stem cells have been developed as stem cell therapeutics for various target diseases because of their several advantages in terms of immunomodulatory ability, safety and low immunogenicity. Among the various tissue derived mesenchymal stem cells, umbilical cord blood-derived mesenchymal stem cell which is developed as stem cell therapeutics, Furestem®, in Kangstem Biotech Co., Ltd. have been found to have excellent proliferative and differentiating ability, and especially immunomodulatory ability. Furestem® exhibits therapeutic efficacy by controlling the underlying cause of disease incidence through multi-pathways. Additionally, since the cells communicate with disease environment, the immunomodulatory factors which have a key role in efficacy are different according to the target diseases. Based on these characteristics, we established mass production technology and are developing stem cell therapeutics which target various immune related disease. In this presentation, we are going to introduce the platform technology and mechanisms of action of Furestem® in atopic dermatitis, rheumatoid arthritis, Crohn's disease and osteoarthritis. Additionally, phase I&IIa clinical trial results of atopic dermatitis and rheumatoid arthritis and long-term observational investigation results of atopic dermatitis would be also presented. Finally, we are going to briefly introduce the current state of basic research on direct conversion technology and gene editing technology to develop incurable disease treatments or cell therapeutics/artificial organs for universal

applications, and their potential and future development plan would be discussed.

Room B, Bio-Techne

12:50-13:50

ORGANOID TECHNOLOGY AND MEDICINE

Lee, Kyungjin

ORGANOIDSCIENCES, Ltd., Seoul, Korea

Organoids are three-dimensional in-vitro-grown cell clusters with near-native microanatomy that arise from self-organizing stem cells. Organoid based models has been highlighted for last few years as breakthrough platforms for studying pathophysiology, screening drug efficacy, and predicting drug toxicity. Furthermore, the organoids are capable of regenerative therapeutics that can restore the damaged organ functions when injected into animal models such as inflammatory bowel diseases. However, there are many limitations to the application of organoids. A high cost for organoid expansion, low viability after cryopreservation, inefficient expansion by spontaneous differentiation and using animal origin Matrigel as an extracellular matrix are major obstacles in the clinical and industrial applications. This presentation will discuss the current limitations for clinical and industrial application of organoids, and introduce our challenges.

Keywords: Organoid, Regeneration, Disease model, Cell therapy, Screening

Room C, Ajinomoto Co., Inc.

12:50-13:50

USE OF BIOMATERIALS TO REGULATE NEURAL DIFFERENTIATION FROM HUMAN IPSCS

Kosodo, Yoichi

Korea Brain Research Institute, Daegu, Korea

Use of biomaterials, defined as materials selected or designed to interact with biological systems, for reprogramming, maintenance, and differentiation of stem cells is often superior to the traditional protocols in terms of safety, efficiency, and scalability. Particularly, generation of specific cell-types not by genetic modifications, but by extrinsic physical factors of biomaterials would become efficacious tools for regenerative therapies in the next generation. In this seminar, I will introduce use of functional culture substrates as novel biomaterial for human iPS cells (hiPSCs) research. The seminar includes the introduction of our recent achievement, that is, a culture substrate that reproduces the stiffness of brain tissue using tilapia collagen for in vitro reconstitution assays. By adding crosslinkers, we obtained gels that are similar in stiffness to living brain tissue. Compared to existing protocols, our material is superior to avoid the risk of neural toxicity and zoonotic diseases. We further examined the capability of the gels serving as a substrate for stem cell culture and the effect of stiffness on neural lineage differentiation using hiPSCs. Taken together, chemically crosslinked tilapia collagen gel is expected to be useful in reconstitution assays to explore the role of stiffness in neural functions, as well as for future therapeutic strategies such as transplantation.

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Poster Abstracts

Mechanisms of Pluripotency and Differentiation

P-101

CORRELATION BETWEEN DNA METHYLATION AND CYP450 GENE EXPRESSION IN HUMAN PLURIPOTENT STEM CELL-DERIVED HEPATOCYTES

Kang, Eun-hye[1], Jo, Seongyea[1], Kim, Ji-woo[1], Kim, Hyemin[1], Park, Han-jin[1], Kang, Eun-hye[2], Jo, Seongyea[3]

[1]Department of Predictive Toxicology, Korea Institute of Toxicology, Daejeon, Korea, [2]Department of Human and Environmental Toxicology, University of Science and Technology, Daejeon, Korea, [3]College of Life Science and Biotechnology, Korea University, Daejeon, Korea

Human pluripotent stem cell-derived hepatocytes (hPSC-Heps) offer a possible solution to the shortage of cell sources in drug discovery and hepatotoxicity testing. Drug metabolism is a biotransformation process, where xenobiotics such as foreign drugs or chemicals are converted into more water-soluble compounds to facilitate their excretion from the body. Cytochrome P450 (CYP) is the key enzyme involved in phase I process of drug metabolism. Although hPSC-Heps as a hepatotoxicity evaluation model have been widely applied, little is known about the regulatory mechanism of CYP gene expression. Here, we showed the expression of nine major CYP genes in each of two types of human embryonic stem cell-derived hepatocytes (hESC-Heps), human induced pluripotent stem cell-derived hepatocyte (hiPSC-Heps), and hPHs. Transcript levels of nine CYP genes in hESC-Heps and hiPSC-Heps were significantly lower than those of hPHs. We hypothesized that low expression of CYP genes in hPSC-Heps would be closely related with epigenetic modification. DNA methylation is a major epigenetic mechanism to regulate gene expression. In order to elucidate the mechanism of limited expression of CYP genes in hPSC-Heps, we analyzed the methylation status of CpG sites at the regulatory region to which transcription factors bind. The methylation patterns at regulatory region of hPSC-Heps were hypermethylated compared to hPHs and consistent with the gene expression patterns. Therefore, our finding suggests that lower expression of CYP genes in hPSC-Heps would be correlated with DNA hypermethylation in transcriptional regulatory regions.

P-102

STUDYING THE MOLECULAR REGULATION OF HEMATOPOIETIC STEM AND PROGENITOR CELLS EMERGENCE DURING EMBRYOGENESIS

Serina Secanechia, Yasmin Natalia[1], Bergiers, Isabelle[1], Le, Stephanie[1], Descostes, Nicolas[1], Arnold, Christian[2], Ganter, Kerstin[1], Lopez Anguita, Natalia[3], Andrews, Tallulah[5], Rogon, Matt[4], Zaugg, Judith[2], Lancrin, Christophe[1]

[1]Epigenetics and Neurobiology Unit, European Molecular Biology Laboratory (EMBL)-Rome, Monterotondo, Italy, [2]Structural and Computational Biology, European Molecular Biology Laboratory (EMBL)-Heidelberg, Heidelberg, Germany, [3]Genome Regulation, Max Planck Institute for Molecular Genetics, Berlin, Germany, [4]EMBL Centre for Biomolecular Network Analysis, European Molecular Biology Laboratory (EMBL)-Heidelberg, Germany, [5]Cellular Genetics, Wellcome Sanger Institute, Hinxton, UK

Hematopoietic Stem-Progenitor Cells (HSPCs) first appear in the vertebrate embryo at mid-gestation, in a region named aorta-gonad- mesonephros. They originate from an endothelial precursor, the Hemogenic Endothelium (HE), through the process of Endothelial to Hematopoietic Transition (EHT). During this process, HE cells progressively transition into HSPCs via an intermediate stage called preHSPCs, characterized by the co-expression of endothelial and hematopoietic genes. Seven transcription factors are particularly important for the regulation of the EHT, namely Gata2, Runx1, Tal1, Lmo2, Fli1, Erg, and Lyl1, and they have been proposed to form a complex termed “Heptad complex” that acts as a master regulator of the transcriptional program that drives preHSPC formation. Recently, the existence of a dual axis between these factors has been revealed, whereby Fli1 and Erg are involved in maintaining the endothelial phenotype and Gata2 and Runx1 promote the acquisition of a hematopoietic identity, while a clear role could not be identified for Lyl1, Tal1 and Lmo2. To investigate whether these genes are also important for the generation of preHSPCs, we exploited the ability of the Heptad factors to induce the transdifferentiation of Vascular Smooth Muscle (VSM) cells into preHSPCs upon overexpression. We removed Lyl1, Tal1 and Lmo2

from the overexpression construct, and observed a reduction in the reprogramming efficiency of VSM cells. The cells that reprogrammed had turned on the expression of the endogenous Tal1 and Lmo2 genes, while the cells that did not reprogram had failed to do so. When we overexpressed Lyl1, Tal1 and Lmo2 alone in VSM cells, we observed no changes at the phenotypic level, but we identified over 8000 differentially open regions of chromatin compared to the untreated cells, and 452 differentially expressed genes, indicating that Lyl1, Tal1 and Lmo2 were able to induce molecular rearrangements even in the absence of the other Heptad proteins. Many of the downregulated genes were related the homeostasis of muscle cells, while many of the overexpressed ones have documented roles in hematopoiesis. Taken together, our results indicate that at least Tal1 and Lmo2 are important for the generation of preHSPCs, and they act at the transcriptional level to prime cells towards the hematopoietic fate.

P-103

NATIONAL STEM CELL BANK OF KOREA : PROGRESS IN HUMAN PLURIPOTENT STEM CELL BANKING ACTIVITY

Ha, Hye-yeong, Yoo, Daehoon, Im, Young Sam, Park, Hyeyeon, Han, Hyo-won, Lee, Youngsun, Kim, Boyoung, Jo, Hye-yeong, Koo, Soo Kyung

Division of Intractable Diseases, National Institute of Health, Cheongju, Korea

Human pluripotent stem cell (hPSC) including human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) provide an unlimited cell source for basic and clinical research. Despite the rapid technical development this fields, there are increasing demand for quality-controlled and well-characterized hPSCs lines for assuring scientific reproducibility. Established in 2012, National Stem Cell Bank of Korea (NSCB) facilitates the use and sharing of quality controlled stem cell lines to support scientific research. NSCB has been making an effort to expand and qualify hPSCs stocks for consistently providing quality assured hPSCs. The expansion of hPSCs is performed in accordance with strict quality control in NSCB. The bank is working on practicing methods for optimizing hPSC cultivation and improving quality assurance measures based on the efficiency and stability of hPSCs. Today, NSCB collected diversified hPSC lines from different type of source cells, diseases, derivation methods, culture conditions, and genetic modifications. The bank creates distribution stocks of hPSC lines on which it assess pluripotency and differentiation potential using cellular and molecular analyses. Currently, there are 19 hPSCs (4 hESCs and 15 hiPSCs) available.

P-104

PROFILING TRANSLATIONAL LANDSCAPES DURING NEURAL DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS

Kim, Yeji[1], Schwarz, Juliane[2], Leidel, Sebastian[1]

[1]*Department of Chemistry and Biochemistry, University of Bern, Switzerland,* [2]*Max Planck Research Group for RNA Biology, Max Planck Institute for Molecular Biomedicine, Muenster, Germany*

Differentiation of human pluripotent stem cells (hPSCs) into the different lineages is regulated by a complex coordination of transcription and translation. While key aspects of the developmental processes are regulated by transcription factors, translation is increasingly recognized as an important regulatory process during differentiation, particularly in fine-tuning gene expression. To decipher the mechanisms underlying global transcriptional and translational changes during neuronal differentiation, we differentiated human embryonic stem cells (hESC) and human induced pluripotent stem cells (hiPSC) into neuron-like cells (NLC). We measured genome-wide transcription and translation in stem cells, neural precursor cells (NPC), and NLC using RNAseq and ribosome profiling. We found that approximately 8000 genes were differentially expressed during differentiation. Interestingly, 1000 genes were mainly controlled at the translational level without significant transcriptional changes. As expected, neural genes were increased both at transcriptional and translational levels, while proliferation and development-related genes were reduced. While most translationally controlled genes only showed mild effects, we found a small number of genes implicated in metabolic and epigenetic processes and mitochondrial genes to be differentially regulated by translation. Particularly, we found that mitochondrial genes were translationally downregulated during early stages of differentiation but rebounded at later stages of differentiation. This implies that translational regulation participates in controlling metabolic reprogramming during differentiation. Taken together, our findings

contribute to our understanding extensive translational control during human neural differentiation.

P-105

THE SPECTROSCOPIC SIGNATURE OF HUMAN PLURIPOTENT STEM CELL-DERIVED HEPATOCYTES STUDIED USING SYNCHROTON FTIR

U-pratya, Yaowalak[1], Thumanu, Kanjana[2], Janan, Montira[3], Kheolamai, Pakpoom[4], Laowtammathron, Chuti[3], Issaragrisil, Surapol[1]

[1]Division of Hematology, Department of Medicine, Faculty of Medicine Siriraj Hosipital, Mahidol University, Nakhon Pathom, Thailand, [2]Synchrotron Light Research Institute, Nakhon Ratchasima, Thailand, [3]Siriraj Center of Excellence for Stem Cell Research, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, [4]Division of Cell Biology, Faculty of Medicine, Thammasat University, Pathumthani, Thailand

Human pluripotent stem cells (hPSCs), such as human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), hold great promise for regenerative medicine due to their unique properties of self-renewal and multilineage differentiation. Although various methods have been used to induce the hepatic differentiation of hPSCs, those hPSC-derived hepatocyte-like cells generally lack important metabolic functions of mature hepatocytes and therefore unsuitable for most research and clinical applications. To improve the efficiency of hepatic differentiation, fast and extensive characterization of hPSC-derived hepatocytes obtained from various culture systems. The present study demonstrated that the FTIR microspectroscopic analysis was superior than other conventional assays, such as morphological examination and detection of hepatic gene and protein expression, for characterizing hPSC-derived hepatocyte-like cells. This technique can also be used to identify and distinguish various stages of hepatic differentiation of hPSCs which other conventional assays failed to accomplish. We therefore believe that the FTIR microspectroscopy can effectively be used to compare the efficiency of various hepatic induction protocols and lead to the establishment of a better procedure for generating hPSC-derived hepatocytes in the future.

P-106

2-(TRIMETHYLAMMONIUM)ETHYL (R)-3-METHOXY-3-OXO-2-STEARAMIDOPROPYL PHOSPHATE ENHANCES THROMBOPOIETIN-INDUCED MEGAKARYOCYTIC DIFFERENTIATION AND PLATELETOGENESIS

Kim, Jusong[1], Jim, Gaunghai[2], Lee, Jisu[2], Lee, Kyeong[2], Bae, Yun Soo[1], Kim, Jaesang[1]

[1]Life Science, Ewha Womans University, Seoul, Korea, [2]College of Pharmacy, Dongguk University, Goyang, Korea

We have previously reported the effects of 2-(trimethylammonium)ethyl (R)-3-methoxy-3-oxo-2-stearamidopropyl phosphate [(R)-TEMOSPho], a synthetic phospholipid, on megakaryocytic differentiation of myeloid leukemia cells. Here, we demonstrate that (R)-TEMOSPho enhances megakaryopoiesis and plateletogenesis from primary hematopoietic stem cells (HSCs) induced by thrombopoietin (TPO). Specifically, we demonstrate at sub-saturation levels of TPO, the addition of (R)-TEMOSPho enhances differentiation and maturation of megakaryocytes (MKs) from murine HSCs derived from fetal liver. Furthermore, we show that production of platelets with (R)-TEMOSPho in combination with TPO is also more efficient than TPO alone and that platelets generated in vitro with these two agents are as functional as those from TPO alone. TPO can thus be partly replaced by or supplemented with (R)-TEMOSPho, and this in turn implies that (R)-TEMOSPho can be useful in efficient platelet production in vitro and potentially be a valuable option in designing cell-based therapy.

Funding Source: This work was supported by Stem Cell Grant (NRF- 2017M3A9B3061850), Aging Project Grant (NRF-2017M3A9D8062955) and Innovative Medicine Research Center for Tumor Remission (NRF 2018R1A5A2023127) from the National Research Foundation of Korea funded by the Ministry of Science and ICT, Korea. This study was also supported by the Ewha Womans University scholarship of 2018.

P-107

NRF2 REGULATES HUMAN EMBRYONIC STEM CELL SELF-RENEWAL THROUGH DIRECT REGULATION OF GLYCOLYSIS

Seo, Hyang Hee, Han, Hyo-won, Kim, Jung-hyun

Division of Intractable Diseases, Korea National Institute of Health, Cheongju, Korea

Glycolysis is a key regulator of human embryonic stem cells (hESC) self-renewal, but how glycolysis is regulated is poorly understood. Nuclear factor E2-related factor 2 (Nrf2) is a basic leucine zipper transcription factor that regulates primarily cellular defense mechanisms. Recently, the role of Nrf2 in hESC has been reported, but its role in hESC metabolism has not been previously investigated. In the present study, we generated keap1, which lead to the proteolysis of Nrf2, knock-out cell by using a CRISPR-Cas9 system and demonstrated that NRF2 maintain the pluripotency of hESC. Besides, keap1 knock-out cells showed a high rate of ATP flux through glycolysis and increased expression of OCT4, NANOG and SOX2 under bFGF withdrawn culture condition, and it was disrupted when glycolysis inhibitor, 2-DG, was added. In addition, we screened genes which directly regulated by NRF2, and the result revealed that NRF2 directly regulates glycolysis-related genes, ENO1, ALDH3A2 and DLD. In conclusion, these data suggest that hESCs maintain pluripotency through glycolysis which regulated by NRF2 in hESC.

Funding Source: 2017-NC61001-00, 2017-NG61004-00.

P-108

TREATMENT WITH SIRT1 INHIBITORS ENHANCES HEMATOPOIETIC DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS

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Embryonic stem cells have pluripotent ability to differentiate into multiple tissue lineages. SIRT1 is a class III histone deacetylase which modulates chromatin remodeling, gene silencing, cell survival, metabolism, and development. SIRT1 is expressed at high levels in mouse embryonic day 4.5 embryos. Although its expression is down-regulated during the subsequent embryogenesis, a high level of expression remains detectable at embryonic day 18.5. SIRT1 is important in embryonic development and contributes to embryonic and adult stages of hematopoietic and endothelial differentiation. Recently, small molecules have emerged as essential tools for understanding and regulating stem cells and manipulating stem cell fate. Nicotinamide, a form of vitamin B3 serving as a precursor of nicotinamide adenine dinucleotide (NAD), is a well-established potent inhibitor of SIRT1. Splitomicin is derived from β-naphthol and is an inhibitor of SIRT1 and SIRT2. In this study, we examined the effects of SIRT1 inhibitors on the hematopoietic differentiation of mouse embryonic stem cells. Treatment with the SIRT1 inhibitors, nicotinamide and splitomicin, during the hematopoietic differentiation of mouse embryonic stem cells enhanced the production of hematopoietic progenitors and slightly up-regulated erythroid and myeloid specific gene expression such as GATA1, βH1, Pu.1, and β-major. Furthermore, treatment with splitomicin increased the percentage of erythroid and myeloid lineage cells. Application of the SIRT1 inhibitor splitomicin during mouse embryonic stem cells differentiation to hematopoietic cells enhanced the yield of specific hematopoietic lineage cells. This result suggests that SIRT1 is involved in the regulation of hematopoietic differentiation of specific lineages and that the modulation of the SIRT1 activity can be a strategy to enhance the efficiency of hematopoietic differentiation.

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P-109

FUNCTIONAL IN VIVO AND IN VITRO EFFECT OF CHROMOSOME 20 GENETIC ABNORMALITIES ON HPSC DIFFERENTIATION

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Human pluripotent stem cells (hPSCs) are promising in therapeutic applications based on their infinite capacity to self-renew and pluripotency. To realize regenerative medicine or cell therapy using hPSCs, understanding the functional consequences of carrying common and subtle variances is necessary to support benefit or risk assessment into hPSCs characteristics. Here, we aimed to assess functional in vivo and in vitro effect of frequently detected chromosome 20 genetic aberrations during early stage of differentiation of hPSCs. Experiments were conducted on genetically identical iPSC sublines with and without the CNV gain, hFSiPS1 and hFSiPS3 lines, respectively, as follows : Single-cell RNA sequencing (scRNA-seq) for embryonic bodies (EBs) from the two lines ; bulk RNA sequencing (RNA-seq) and histological examination for teratomas. The scRNA-seq showed alteration of differentiated cell distribution due to the CNV abnormalities, suggesting that hPSCs harboring the 20q11.21 CNV duplication undergo distinct lineage specification or cell fate decision, compared with normal hPSCs. Moreover, although Teratoscore evaluated similar differentiation potential between two of lines, comprehensive RNA-seq examination of teratomas explained that several lineage-specific marker genes of hPSCs with the CNV are differentially expressed, consistent with the histological results that the ecto/meso/endodermal ratio showed was changed due to the CNV. Taken together, our results suggest that the CNV amplification contributes to cell fate specification, affecting the potential to differentiate into the three germ layers. We expect that our results provide not only genomic evidence of the differentiation mechanism, but also new insights for optimization of the differentiation system to support development of cell therapies and other future applications.

P-110

A SIMPLE DIFFERENTIATION METHOD TO ANALYZE DIFFERENTIATION PROPENSITY

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Assessment of pluripotency is a basic requirement for qualification of human pluripotent stem cells (hPSCs). Here we report a simple differentiation method using fetal bovine serum (FBS) and RNA-seq to estimate differentiation propensity of hPSCs. PluriTest using RNA-seq shows that differentiation progresses after 5% FBS treatment. Expression patterns of three germ layer markers reveal that cells cultured in a chemically defined medium (E8) with recombinant matrix proteins have a propensity to differentiate into ectoderm lineage, while cells cultured in Knockout Serum Replacement-containing medium (KSR) with mouse feeder cells into mesoderm and endoderm lineages.

P-111

YAP IS DISPENSABLE FOR STEMNESS MAINTENANCE BUT REQUIRED FOR PROPER EMBRYOID BODY FORMATION OF HUMAN IPSCS

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The Yes-associated protein (YAP) is a transcription coactivator in the Hippo signaling pathway. It has been shown to regulate the expression of the pluripotent genes, OCT4 and SOX2, which in turns facilitates the self-renewal property of stem cells. It has been suggested that YAP is localized in the nucleus of mouse ES cells (mESCs), and that it plays an important role in the maintenance of mESC pluripotency and self-renewal capacity. However, recent studies claimed that YAP, although localized in the nucleus, is dispensable for self-renewal of mESCs since the knocked-out YAP mESCs still maintained undifferentiated state even

under differentiation promoting culture system. Similar to the lack of clarity and understanding of YAP in mouse model, the role of YAP in human iPSCs is also inconclusive. In this study, we aimed to investigate the effect of YAP on the biological properties of human iPSCs. Gain and loss-of-function experiments were performed on the isogenic iPSCs to reduce the variation in genetic background that might adversely affect the experimental outcomes. We found that expression level of YAP did not alter the expression of pluripotency markers and the genetic stability of the cells. However, ectopic expression of YAP affects EB formation and the survival of the aggregated cells, while depletion of YAP did not show significant different of EB morphology when compared to control. Role of YAP in in vivo teratoma formation was also studied. Smaller teratomas were observed in the YAP overexpressing cells, however, lineage differentiation was not affected since the representative cell types from three embryonic germ layers were found as similar to YAP-depleted and control cell lines. Taken together, our results showed that overexpression of YAP obstructs EB formation in vitro, but it is dispensable for in vivo teratoma formation and lineage differentiation of human iPSCs.

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P-112

GENERATION OF HUMAN HAEMATOPOIETIC STEM CELLS FROM INDUCED PLURIPOTENT STEM CELLS

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Induced pluripotent stem cells (iPSCs) represent a promising new approach in the field of regenerative medicine. iPSCs have the ability to generate hematopoietic stem cells (HSCs) in the laboratory, which enables their use as an autologous treatment for hematological disorders without limitations in terms of cell numbers. In this study, we examined the size of embryoid body (EB) and the role of small molecules in mesodermal formation and definitive HSCs differentiation from iPSCs. We found that size of EB effect on differentiation potential of iPSCs to HSCs. Small EB (2,000 cells/EB) can give rise to HSC that positive for CD34 CD43/CD45 more than those of the big EB (5,000 cell/EB). It has been reported that stage-specific activation of the Wnt signaling pathway affect the generation of definitive hematopoietic progenitors. To determine whether activation of Wnt could also affect mesodermal formation and definitive HSCs differentiation from iPSCs, the iPSCs were induced for differentiation via EB formation method with and without supplementation of Wnt agonist (CHIR) at specific time point. We found that differentiation of iPSCs without supplementation with CHIR resulted in break out EBs on day 2 of differentiation. In contrast, addition of CHIR at the same time point during differentiation resulted in an increase in size of EBs when compared with the control. Moreover, the prominently upregulated T brachyury was elevated 10-fold in the treated group compared to control. These results suggest that supplementation of CHIR at specific time point could promote the differentiation towards mesodermal lineage. We also found that the expression of CD34+CD43+ cells in floating and adherent cells were enriched on day 13 of differentiation. CFU assay revealed the multilineage differentiation capacity of CD34+CD43+ derived from both floating and adherent cells as the can differentiate to all blood cell lineages. The result of RT-qPCR of erythroid colonies (BFU-E and CFU-E) from both floating and adherent cells showed expression of embryonic, fetal, and adult globin (beta-globin). Altogether, our results suggest that the CD34+CD43+ cells derived from both floating and adherent cell populations are definitive hematopoietic cells.

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P-113

OGT REGULATES MEGAKARYOCYTE MATURATION AND PLATELET PRODUCTION

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Metabolic state of hematopoietic stem cells (HSCs) is an important regulator of self-renewal and differentiation. Hexosamine biosynthetic pathway (HBP) is a nutrient-sensing metabolic pathway that produces UDP-GlcNAc, a critical substrate for protein O- GlcNAcylation. In the present study, we found that cellular O-GlcNAcylation was gradually reduced during the course of megakaryocyte differentiation from human cord blood CD34+ stem/progenitor cells. O-GlcNAc cycling is controlled by two enzymes; the O-GlcNAc transferase (OGT) and O-GlcNAcas (OGA) that catalyzes and removes O-GlcNAc, respectively. Inhibition of OGT and subsequent global O-GlcNAcylation increased the number of CD41+ and CD42+ cells as well as platelet-like particles (PLPs) after 10 days of differentiation in culture without affecting its viability. Consistently, we observed a substantial higher portion of megakaryocytes with multinucleated morphology upon OGT inhibition. The regulatory roles of OGT in platelet formation was further validated in megakaryoblastic cell (MEG-01 and MEG-01s) model, where a remarkable increase of CD41+ and CD42+ PLPs were detected. Together, our findings provide novel evidence on the role of OGT and HBP in megakaryocyte differentiation and platelet production, which could be important in understanding of normal megakaryopoiesis and related disorders.

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P-114

ROLE OF LYSOPHOSPHATIDIC ACID RECEPTOR 1 ON THE INFLAMMATORY RESPONSE INDUCED BY LIPOPOLYSACCHARIDE FROM PORPHYROMONAS GINGIVALIS IN HUMAN PERIODONTAL LIGAMENT STEM CELLS

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Lysophosphatidic acid (LPA) is a lipid messenger mediated by G protein-coupled receptors (LPAR1-6). LPA is involved in the pathogenesis of chronic inflammatory and autoimmune diseases and controls the stemness in embryonic stem cells, adult stem cells and cancer stem cells. Recent progress has shown the intimate relationship between periodontitis and various diseases in the human body. However, the precise role of LPA in developing periodontitis has not been studied. We investigate the role of LPARs during the development of periodontitis in the inflammatory condition. When human periodontal ligament stem cells (PDLSC) were treated with lipopolysaccharide from Porphyromonas Gingivalis (LPS-PG), the viability of PDLSC was not affected up to 10 ug/ml LPS-PG treatment. In comparison, treatment of PLDSC with E. coli LPS did not decrease the viability up to 10 ug/ml. When LPAR expression was evaluated in comparison with human dental pulp stem cells (DPSC), LPAR1 expression was significantly higher in PDLSC whereas the expression of LPAR1, 2, 3, 6 was higher in DPSC. In serum-free culture, the increasing dose of LPS-PG gradually decreased PDLSC viability in 24-120 hr. Incubation of LPS-PG with PDLSC meaningfully increase the expression of LPAR1, and TLR4. When PDLSC was treated with LPAR1 antagonist, in serum-free culture, the individual treatment of LPAR1 antagonist did not affect the cellular viability. Levels of pro-inflammatory cytokines including TNF-α and IL-1β decreased meaningfully after LPAR1 antagonist treatment. These results suggest that LPAR1 can be a noble target to resolve inflammatory condition in periodontal disease.

P-115

TROPHOBLASTIC SPHEROID DERIVED FROM HUMAN EMBRYONIC STEM CELLS RESEMBLES HUMAN TROPHECTODERM DURING EARLY IMPLANTATION PERIOD

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Study of the mechanisms of human implantation in vivo is unethical and provision of high-quality human embryos for in vitro research is limited. Our team has established a human embryonic stem cell (hESC)-derived trophoblastic spheroid (BAP-EB) for the study of early embryo implantation and trophoblast development. Here, we used RNA sequencing method to delineate the changes in transcriptome of BAP-EB during differentiation. Pathways like Hippo signaling involved in trophectoderm/trophoblast specification were induced during BAP-EB differentiation. Hippo signaling pathway inhibitor significantly reduced the attachment rate and outgrowth of BAP-EB. We obtained the differential gene lists between epiblast and trophectoderm (TE) of human preimplantation embryos from two published datasets, and found that the undifferentiated hESC, BAP-EB at 0h and 24h clustered with the epiblast samples while BAP- EB at 48h, 72h and 96h were clustered with the TE samples from the two studies. Further gene set enrichment analysis (GSEA) showed that attachment-competent BAP-EB highly resembled human polar trophectoderm prior to implantation. By using a coculture model with BAP-EB and primary endometrial epithelial cell (EEC) isolated from patients, it was found that significantly higher numbers of BAP-EB at72h derived from two different hESC lines attached onto receptive EEC isolated from patients in their natural (LH+7) or stimulated (hCG+7) cycles when compared to those obtained from pre-receptive EEC at day 2 (LH/hCG+2). Together, this study reports the TE-like signature of hESC derived BAP-EB and supports its use as human blastocyst surrogate for studying early embryo implantation process and trophoblast development.

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P-116

A SIMPLE AND STABLE MAINTENANCE CULTURE METHOD FOR HUMAN PLURIPOTENT STEM CELLS USING STEMFIT BASIC04 MEDIUM

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Human pluripotent stem cells (hPSCs), including embryonic and induced pluripotent stem cells, are expected to be one of the cell sources for regenerative medicine. Aside from expansion efficiency and quality of the cells, simplification of operation and robustness should also be considered for the production of cells for clinical applications. StemFit Basic04 is the culture medium for maintenance and expansion of hPSCs with single-cell passaging and weekend-free feeding, which is made of chemically-defined and animal origin-free (CD-AOF) materials. Here, we present a simplified culture method using Basic04, especially focusing on the process of extracellular matrix coating and cell detachment during cell passage. Recently, it was reported that direct supplementation of matrices into media enabled hPSC culture without vessel pre-coating. In this study, we applied this method to hPSC culture using StemFit Basic04 and optimized the whole process including procedure and reagents for cell detachment. We found that this simplified method allows efficient expansion of hPSCs with sufficient quality without vessel pre-coating and utilization of cell scrapers during passage. Application of this culture system would contribute to a time-efficient operation for stable and robust production of hPSCs for clinical application.

P-117

IDENTIFICATION OF NEW LONG NON-CODING RNA THAT REGULATES GROUND-STATE PLURIPOTENCY

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Long noncoding RNAs (lncRNAs) have been identified as key regulators of pluripotency. The analysis of the LIF/STAT3 target genes expressed in mouse embryonic stem cells (mESCs) led us to identify a new lncRNA with the cardinal characteristics of a pluripotency regulator, which is expressed specifically in the epiblast of the E4.5 mouse embryo. In mESCs cultured in 2i (GSK3βi + ERKi)/LIF, this lncRNA is expressed at a high level in all cells; whereas, in the LIF/Serum regimen, its expression is mosaic. Its promoter contains binding sites for both STAT3 and the naïve state-specific transcription factor TFCP2L1. Bioinformatics analysis of the lncRNA sequence has revealed the following two functional domains: one codes for an unknown pre-micro (mi)RNA and the other contains four potential binding sites for this miRNA. The expression of miRNA was detected in the mESCs. Transfection of a miRNA mimic induced mESC differentiation. One thousand copies of this new miRNA were found in the mouse genome, most of which were localized in Alu sequences. We hypothesized that the lncRNA serves as a sponge to titrate the miRNA through the four binding sites. Using a reporter system containing this sponge domain inserted into the 3′UTR of the luciferase gene, we showed that the miRNA bound this domain. Preliminary results indicate that both knockdown of lncRNA expression and deletion of the sponge domain are detrimental to mESC self-renewal. These findings strongly suggest that the function of the lncRNA is to titrate the miRNA, which, in turn, inhibits differentiation. We named this new lncRNA "Asgard."

P-118

STEMNESS/DIFFERENTIATION CONVERSION REQUIRES COOPERATIVE CROSS-TALK BETWEEN VPS26A AND THE NOX/ROS/ERK CASCADE IN EMBRYONIC STEM CELLS

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Despite numerous studies on the molecular switches governing the conversion of stemness to differentiation in embryonic stem cells (ESCs), little is known about the involvement of the retromer complex. Under neural differentiation conditions, Vps26a deficiency (Vps26a-/-) or knockdown suppressed the loss of stemness and subsequent neurogenesis from ESCs or embryonic carcinoma cells, respectively, as evidenced by the long-lasting expression of stemness markers and the slow appearance of neuronal differentiation markers. Interestingly, relatively low reactive oxygen species (ROS) levels were generated during differentiation of Vps26a-/- ESCs, and treatment with an antioxidant or inhibitor of NADPH oxidase (Nox), a family of ROS-generating enzymes, led to restoration of stemness in wild-type cells to the level of Vps26a-/- cells during neurogenesis. Importantly, a novel interaction between Vps26a and Nox4 linked to the activation of ERK1/2 depended highly on ROS levels during neurogenesis, which were strongly suppressed in differentiating Vps26a-/- ESCs. Moreover, inhibition of phosphorylated ERK1/2 (pERK1/2) resulted in decreased ROS and Nox4 levels, indicating the mutual dependency between pERK1/2 and Nox4-derived ROS during neurogenesis. These results suggest that Vps26a regulates stemness by actively cooperating with the Nox4/ROS/ERK cascade during neurogenesis. Our findings have important implications for understanding the regulation of stemness via crosstalk between the retromer molecule and redox signaling, and may contribute to the development of ESC-based therapeutic strategies for the mass production of target cells.

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P-119

DISEASE MODELING AND RECAPITULATION OF DIABETIC ARTHROPATHY WITH IN VITRO CHONDROGENESIS PLATFORM USING INDUCED PLURIPOTENT STEM CELL

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Diabetes usually complicates with various organs and bodily systems. Musculoskeletal system is one of frequently affected organs. Diabetes patients often suffer from arthralgia and joint stiffness. However, pathophysiology of diabetic arthropathy is not well known. In this study, we investigated diabetic arthropathy using disease modeling platform via induced pluripotent stem cell (iPSC). We postulated that high glucose media during chondrogenesis from iPSC may recapitulate the hyperglycemic condition to cartilage of diabetic patients. Human iPSCs were generated from cord blood mononuclear cells (CBMCs) using the Sendai virus. The characterization of human iPSCs was performed by various assays. Embryonic bodies (EBs) were obtained using human iPSCs, and outgrowth cells were induced by plating the EBs onto a gelatin-coated plate. Expanded outgrowth cells were detached and dissociated for chondrogenic differentiation. Outgrowth cells were differentiated into chondrogenic lineage with pellet culture. Chondrogenic pellets derived from outgrowth cells were incubated under normal glucose (5 mM) or high glucose (12.5 mM) concentration. Chondrogenic pellets were maintained for 21 days. The quality of chondrogenic pellets was evaluated using various staining and genetic analysis of cartilage-specific markers. High glucose condition increased cell metabolism. Hyperglycemia facilitated chondrogenesis in early phase of cartilage generation. High glucose condition expressed higher levels of cartilage markers, aggrecan, collagen type II and sex-determining region Y-box 9, compared with normal glucose condition. However, chronic hyperglycemia reduced the expression of chondrogenic markers and increased the expression of matrix metalloproteinase 2, cartilage degrading enzyme, at later stage. The expression of glucose transporter 1 and pyruvate kinase muscle isoenzyme 2 was higher with high glucose than normal glucose-treated chondrogenic pellets in both early and late phase of cartilage differentiation. In the late phase of differentiation, high glucose condition significantly decreased the expression of hexokinase 2. Diabetic arthropathy is difficult to investigate due to chronic evolution and shortage of adequate disease samples. Disease modelling using iPSC can be potential study substitute for classic methods. With this strategy, we became to know acute hyperglycemia facilitate chondrogenesis but chronic hyperglycemia reduces chondrogenesis. Recapitulating hyperglycemia-induced defective chondrogenesis in vitro may give scientists opportunity to develop point-of-care medication, based on mechanism.

P-120

GP130-DEPENDENT SELF-RENEWAL OF HUMAN PLURIPOTENT STEM CELLS

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Pluripotency exists in two main states—naïve and primed. Conventional human pluripotent stem cells (PSCs) self-renew in the primed state. We previously reported a strategy to reprogram human PSCs into the naïve state of pluripotency (Chen et al., Nat Commun, 6:7095, 2015). Naïve-to-primed conversion is based on the synergistic action of tamoxifen-activated STAT3-ER, LIF, and 2i (MEKi and GSK3i) inhibitors. The resulting cells, called TL2i, exhibit JAK dependency, clonogenic self-renewal in the absence of FGF2, and reconfiguration of transcriptome and epigenome. In the present study, we sought to identify the key factor(s) downstream of the LIF receptor that act synergistically with STAT3-ER to reprogram human PSCs into the naïve state. To this end, we generated human PSCs that express both the STAT3-ER and G-CSFR:GP130 chimeric receptors, composed of the extracellular domain of the G-CSF receptor and the cytoplasmic domain of the GP130 signal transducer. The human PSCs that expressed both wild-type

G- CSFR:GP130 chimeric receptor and STAT3-ER acquired clonogenic self-renewal under the combined action of LIF + tamoxifen or of G-CSF + tamoxifen. In contrast, treatment with either factor resulted in differentiation. To identify the key factor(s) downstream of the G-CSFR:GP130 chimeric receptor that are essential to clonogenic self-renewal, we generated human PSCs that expressed five mutant receptors. Three of these harbor point mutations that prevent the recruitment of transcription factor STAT3, phosphatase SHP2, or both. The other two harbor small deletions that prevent the recruitment of the Src-related kinases HCK and YES. Human PSCs that express both the STAT3-ER and each of the five G-CSF:GP130 mutant receptors were generated, and their ability to self-renew in FGF2-free culture media supplemented with both G-CSF and tamoxifen was then assessed. Human PSCs expressing the STAT3-binding-deficient, SHP2-binding-deficient, and HCK-binding-deficient receptors responded to G-CSF stimulation by exhibiting clonogenic self-renewal in the absence of FGF2. In contrast, human PSCs that express the YES-binding-deficient receptor lost their self-renewal ability. These results suggest that YES is a factor that acts synergistically with STAT3 to sustain the pluripotency of FGF2- deprived human PSCs.

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ROLE OF EMBRYONIC STEM CELL SPECIFIC HECAT5 IN CELL PROLIFERATION

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Human embryonic stem cells (hESC) have been regarded as promising resources with the potential for therapeutic use. Resent studies have discovered various transcription factors underlying stemness of hESCs. However, no membrane receptors related with stemness are known so far. In our previous study, we identified a novel GPCR protein HECAT5 in hNSC and HEK293 cells, it dramatically increased cell proliferation as well as S phase population. Nonanoic acid, a ligand for HECAT5, increased cell proliferation in HECAT5 dependent manner. In addition, HECAT5 enhanced cell expansion in soft agar assay. Next, to investigate the interaction with M3R, a modulator of HECAT5 family proteins, we performed cell proliferation assay in the presence of M3R antagonist and agonist. Cell proliferation are increased or decreased with increasing concentration of agonist and antagonist, respectively. Collectively, our data demonstrated that hESC-specific factor HECAT5 modulate stemness properties in human cells through crosstalk with M3R.

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NEURAL CREST STEM CELLS DERIVATION FROM HUMAN PLURIPOTENT STEM CELLS

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Neural crest stem cells (NCSCs) are multipotent stem cells which can become neurons and glial cells of peripheral nervous system (PNS). Furthermore, NCSCs can be differentiated into melanocytes and mesodermal cells. During early embryonic development, specification of NCSCs are tightly regulated in a temporal and spatial manner by various cues, such as WNT, BMP, and FGF. In this study, we established a novel method that could efficiently differentiate hPSCs into NCSCs using the signaling regulators that were known to function in embryonic development. The resulting NCSCs retained multipotency and were able to become peripheral neural cells as well as mesodermal cells. In summary, our differentiation method provides an efficient way to produce NCSCs from hPSCs and hence will expedite clinical applications of NCSCs to treat many incurable PNS diseases

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COMPARATIVE GENE EXPRESSION PROFILING OF HUMAN MESENCHYMAL STEM CELLS FROM UMBILICAL CORD BLOOD AND INDUCED PLURIPOTENT STEM CELLS

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Human mesenchymal stem cells (MSCs) derived from induced pluripotent stem cells (iPSCs) (iMSCs) provide an alternative means of large scale allogenic MSCs for clinical cellular therapy. Studies have shown that iMSCs have typical MSC properties, including cell surface marker expression, differentiation capacity, and transcriptome profile. However, the gold standard for identifying and distinguishing the molecular identity of iMSCs has not been established. Here, we analyzed RNA-Seq data for whole transcriptome profiling and differential splicing of UCB-derived MSC (UCB-MSC), and fibroblast-derived iMSCs (FB-iMSCs). We identified 4,586 significant genes with fold change $|FC| \geq 2$ by DEG analysis. Based on gene ontology (GO) classification and KEGG pathway analysis, we found that the expression levels of genes associated aging, angiogenesis, and extracellular matrix were higher in UCB-MSCs than FB-iMSCs, while the levels of RNA splicing gene expression were higher in FB-iMSCs than UCB-MSCs. Functional enrichment analysis also showed that genes related with extracellular matrix, development process, and genes encoding cell communication, developmental biology, axon guidance, cell junction were more highly expressed in FB-iMSCs than in UCB-MSCs. In addition, we identified that a subset of genes, mainly collagens, extracellular matrix, cytokines, and cytoskeleton-associated proteins, predominantly expressed the skipped exon types of alternative splicing ($BF > 20$) in comparative analysis of two MSCs. Our results would be a valuable in clarifying the molecular level of characteristic information about iMSCs for their use in therapeutic applications.

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GENERATION OF ISTHMIC ORGANIZER-LIKE CELLS FROM HUMAN EMBRYONIC STEM CELLS

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The isthmic organizer (IsO), the secondary organizer which was located at the midbrain-hindbrain border (MHB) of the developing neural tube, influences the induction, proliferation, and differentiation of neural cells between the midbrain and hindbrain by secreting Wnt1 and fibroblast growth factor (FGF) 8. In the present study, we attempted to induce the production of isthmic organizer (IsO)-like cells capable of secreting FGF8 and WNT1 from human embryonic stem cells (ESCs). The precise modulation of canonical Wnt signaling was achieved in the presence of the small molecule CHIR99021 (0.6 μM) during the neural induction of human ESCs, resulting in the differentiation of these cells into IsO-like cells having a midbrain-hindbrain border (MHB) fate in a manner that recapitulated their developmental course in vivo. Resultant cells showed upregulated expression levels of FGF8 and WNT1. The addition of exogenous FGF8 further increased WNT1 expression by 2.6 fold. Gene ontology following microarray analysis confirmed that IsO-like cells enriched the expression of MHB-related genes by 40 fold compared to control cells. Lysates and conditioned media of IsO-like cells contained functional FGF8 and WNT1 proteins that could induce MHB-related genes in differentiating ESCs. The method for generating functional IsO-like cells described in this study could be used to study human central nervous system development and congenital malformations of the midbrain and hindbrain.

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P-125

DEFINED CONDITIONS FOR DIFFERENTIATION OF FUNCTIONAL RETINAL GANGLION CELLS FROM HUMAN PLURIPOTENT STEM CELLS

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Retinal ganglion cell (RGC) is a type of cell in the eye linking the retina and brain, and affected by several pathological conditions including glaucoma and mitochondrial optic neuropathies. In the present study, we attempted to optimize a method for generating RGCs from hPSCs by dissecting the process of RGC differentiation and the role of each signal pathway involved in retinal development. Fine modulation of signaling pathways involved in the eye field specification including BMP, TGFβ, Wnt, insulin growth factor-1, and fibroblast growth factor signaling, along with mechanical isolation of neural rosette cell cluster, significantly enriched cryopreservable RX and PAX6 double-positive eye field progenitors from hPSCs in 12 days. Following Notch signal inhibition facilitated differentiation into MATH5-positive RGC progenitors at 90% of efficiency in 19 days, which further differentiated to BRN3B and ISLET1 double-positive RGCs at 43% of efficiency in 40 days. RGCs differentiated by our method were functional as exemplified by the ability to generate action potentials, expression of microfilament component on neuronal process, and axonal transportation of mitochondria. Therefore, our results offer a solid basis for RGC differentiation from hPSCs, and may provide an in vitro disease model and a cell source of transplantation for diseases related to RGCs.

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WNT SIGNAL ACTIVATION INDUCES MIDBRAIN SPECIFICATION THROUGH DIRECT BINDING OF THE BETA-CATENIN/TCF4 COMPLEX TO THE EN1 PROMOTER IN HUMAN PLURIPOTENT STEM CELLS

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The canonical Wnt signal pathway plays a pivotal role in anteroposterior patterning and midbrain specification during early neurogenesis. Activating Wnt signal has been a strategy for differentiating human pluripotent stem cells (PSCs) into midbrain dopaminergic (DA) neurons; however, the underlying molecular mechanism(s) of how the Wnt signal drives posterior fate remained unclear. In this study, we found that activating the canonical Wnt signal significantly upregulated the expression of EN1, a midbrain- specific marker, in a fibroblast growth factor signal-dependent manner in human PSC-derived neural precursor cells (NPCs). The EN1 promoter region contains a putative TCF4-binding site that directly interacts with the β-catenin/TCF complex upon Wnt signal activation. Once differentiated, NPCs treated with a Wnt signal agonist gave rise to functional midbrain neurons including glutamatergic, GABAergic, and DA neurons. Our results provide a potential molecular mechanism that underlies midbrain specification of human PSC derived NPCs by Wnt activation, as well as a differentiation paradigm for generating human midbrain neurons that may serve as a cellular platform for studying the ontogenesis of midbrain neurons and neurological diseases relevant to the midbrain.

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CHARACTERIZATION AND GENE EXPRESSION PATTERNS OF HUMAN MARROW MESENCHYMAL STEM CELLS RELATED TO CHONDROGENIC DIFFERENTIATION

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Bone marrow mesenchymal stem/stromal cells (BMSCs) have high self-renewal and tri-lineage differentiation capacity. Particularly, the ability to form chondrocytes attracts these cells as a promising therapeutic option for cartilage regeneration. To realize the potential chondrogenesis of BMSCs, in vitro characterization and the data on the time-dependent variations of marker genes implicated in the initiation and regulation of specific lineage is essential. Hence, the present study was aimed to evaluate the biological properties, tri- lineage potential and gene expression of human BMSCs during chondrogenic differentiation. BMSCs were isolated from bone marrow (n=3) from patients undergoing knee arthroscopy procedure for ligament injury with cartilage damage after due consent. BMSCs were isolated by density gradient separation and expanded till passage 5. Cellular properties, including morphology, viability, proliferation and population doubling time (PDT), colony forming unit assay, alkaline phosphatase activity (ALP), surface marker expression, and multilineage potential towards osteocytes, adipocytes and chondrocytes were analyzed. Genes primarily involved in the early, intermediate and late phases of chondrogenesis, such as Aggrecan, Collagen II α1, BMP-6, TGF-β1, Collagen I α1, FGFR-3, MMP-1, AnxA6, CNTN1 and MATN1 were analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR) and quantitative PCR (qPCR). BMSCs exhibited spindle-shape morphology and the viability was above 95% at all passages. They were highly proliferative with colony forming ability and stained positive for alkaline phosphatase (ALP) activity. The cells were positive for CD29, CD90 and CD73 and negative for CD34 and CD45 markers. All BMSCs were successfully differentiated towards osteocytes, adipocytes and chondrocytes upon specific induction as confirmed by respective staining methods. Time-dependent and differential expression of selected genes by qPCR showed the key molecular events that regulate the chondrogenesis. In conclusion, BMSCs were proliferative with expression of phenotypic markers and multilineage potential. Importantly, based on the potency features and expression of chondrogenic lineage committed genes, BMSCs could be an ideal source for cartilage regeneration.

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P-128

TFEB IS ESSENTIAL FOR THE MAINTENANCE OF PLURIPOTENCY OF MOUSE EMBRYONIC STEM CELLS

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Transcription factor EB (TFEB), a well-known master regulator of autophagy and lysosomal biogenesis, is a member of the micropthalmia family of transcription factors (MiT family). Over years, TFEB has gained a lot of attention owing to its diverse role in physiological processes, intracellular pathogenic factors clearance pathway and developmental processes such as dendritic maturation, osteoclast and endoderm differentiation. However, the role of TFEB in pluripotent embryonic stem cells (ESC) is yet to be elucidated. Here, we propose a novel mechanisms on how TFEB governs pluripotency of mouse ESC by regulating pluripotency transcriptional network (PTN). We observed high levels of endogenous TFEB mRNA and protein in undifferentiated mESC. Knockdown of TFEB by using CRISPR/Cas9 system reduced the levels of core stem cell markers (Sox2, Oct4 and Nanog) and impaired the compact colony morphology of mESC. Lentivirus-mediated overexpression of TFEB at D6 –LIF and D7 EB increased the levels of Nanog and Sox2 but not Oct4. In addition, Nanog-luciferase reporter assay suggested that Nanog may be TFEB target gene as luciferase activity increased upon TFEB overexpression and reduced upon TFEB knockdown. Interestingly, co-immunoprecipitation experiments showed that endogenous TFEB binds to Nanog and

Sox2 in mESC. Surprisingly, TFEB promoter contains Sox2, Oct4 and Nanog putative binding sites. Knockdown of these stem cell markers greatly reduced endogenous TFEB mRNA and protein levels in mESC. ChIP-qPCR data showed high enrichment of Sox2, Oct4 and Nanog in TFEB promoter-putative binding sites. Undifferentiated mESC also displayed low basal lysosomal activity as shown by Lysotracker-Red staining suggesting that TFEB may regulates pluripotency independent of autophagy and lysosomal biogenesis. Taken together, our results define a newly recognized role of TFEB in the maintenance of pluripotency of mESC by incorporating and regulating PTN.

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P-129

REGULATION OF FOCAL ADHESION ASSEMBLY IN MESODERMAL PRECURSOR CELLS THROUGH NANOSCALE PILLAR SURFACE

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Nanoscale pillar surface plays a pivotal role in the response to various cells. Nonetheless, little is known about how nanoscale pillar surface stimuli alter the response mesoderm precursor cells. Herein, we adopted nanoscale pillar surface with decreasing diameter ranges [280-360 nm (GP 280/360); 200-280 nm (GP 200/280); and 120-200 nm (GP 120/200)], and explored their cellular effect on multipotent mouse fetal liver kinase 1-positive mesoderm precursor cells (Flk1+MPCs) and human endothelial colony forming cells (hECFCs). We observed the 200-280 nm-sized (GP 200/280) nanoscale pillar surface dramatically increased cardiomyocyte differentiation from Flk1+MPCs. The nanoscale pillar surface-induced cardiomyocytes had sarcomeres with mature cardiac specific gene expression. Moreover, the 120-200 nm-sized (GP 120/200) nanoscale pillar surface caused the cell perimeter and area of hECFCs to decrease and their filopodial protrusion to increase. Both cells were observed on the nanoscale pillar surface with the increasing of Vinculin expression and assembling of focal adhesion. We determined Vinculin-mediated cytoskeleton reorganization during this process. Therefore, the nanoscale pillar surface to regulate cytoskeleton-relative downstream signaling to increase cellular response from Flk1+ MPCs and hECFCs.

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Reprogramming and Transdifferentiation

P-201

STEP-BY-STEP PROTOCOL FOR GENERATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS (IPSCS) USING HEPATIC NON-PARENCHYMAL CELLS (NPCS)

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Human iPSCs are a potentially unlimited source to generate human hepatocyte-like cells (h-iHLCs) for treatment of patients with end- stage liver disease. To understand the impact of donor-specific factors

on generating h-iHLCs, a model for the direct comparison of h- iHLCs and primary human hepatocytes (PHHs) from the same donor is needed. This study proposes a protocol for the generation of human iPSCs using primary hepatic NPCs with subsequent differentiation of these iPSCs into h-iHLCs and ultimately comparison of the metabolic profiles of iHLCs and PHHs from the same human donor. The current protocol describes step-by-step culture, characterization, and the reprogramming method of primary human NPCs by transient overexpression of four Yamanaka factors utilizing Sendai virus. NPCs were expanded in culture following isolation from a human liver. The optimal passage for the initiation of reprogramming was identified by co-infecting NPCs with green fluorescent protein-labeled Sendai virus. Human NPCs from passage 3 were successfully reprogrammed by transient overexpression of OCT4, SOX2, C-MYC, and KLF4. RNAseq analysis and immunofluorescence staining were used to characterize Sendai-infected NPCs, which were found to overexpress α-SMA. NPC- derived iPSCs were characterized by morphology, silencing of exogenous reprogramming factors (C-Myc, KLF4, SeV, KOS) after passage 18, stability of karyotype, confirmation of pluripotency by directed differentiation into the three germ layers, and RNAseq analysis. The generated iPSCs will be differentiated into h-iHLCs by using an established differentiation protocol, and the maturation will be confirmed by HNF-4α and albumin expression on immunofluorescence staining. The current protocol proposes to use a human liver specimen as a single source for the isolation of PHHs and NPCs to generate h-iHLCs, which provides a unique opportunity for a direct comparative study of PHHs and h-iHCLs from the same human donor.

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P-202

GENERATION OF INTEGRATION-FREE INDUCED NEURONS USING GRAPHENE OXIDE-POLYETHYLENIMINE

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The direct conversion of somatic cells into induced neurons (iNs), without inducing pluripotency, has great therapeutic potential for central nervous system diseases. Reprogramming of somatic cells to iNs requires the introduction of several factors to drive cell fate conversion. Common direct reprogramming strategies to deliver these factors into somatic cells use viruses. However, novel gene delivery systems that do not integrate transgenes into the genome should be developed to generate iNs for safe human clinical applications. In this study, we investigated whether graphene oxide-polyethylenimine (GO-PEI) complexes are an efficient and safe system for mRNA delivery for direct reprogramming into iNs. The GO-PEI complexes created in this study exhibited a low cytotoxicity, high delivery-efficiency, and non-integration features for direct conversion into iNs. Moreover, in vivo transduction of reprogramming factors with GO-PEI complexes into the brain was sufficient to facilitate conversion into iNs which alleviated symptoms in mouse models of Parkinson's disease. Thus, these studies demonstrate that the GO-PEI delivery system could be used to obtain safe iNs, and provide a promising for direct reprogramming-based therapies of neurodegenerative diseases.

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P-203

GENERATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS FROM PERIPHERAL BLOOD MONONUCLEAR CELLS OF EACH SENIOR-LOKEN SYNDROM PATIENT AND LEBER CONGENITAL AMAUROSIS PATIENT

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Senior-Loken syndrome (SLS) and Leber congenital amaurosis (LCA) are hereditary eye diseases leading to blindness. Both diseases primarily affects the retina, including an increased sensitivity to light (photophobia), involuntary movements of the eyes (nystagmus), and extreme farsightedness (hyperopia). We generated the human induced pluripotent stem cell (hiPSC) lines, designated DKH005i-A and DKH090i-A, from peripheral blood mononuclear cells of each patient with SLS or LCA respectively using a Sendai virus reprogramming method. We confirmed that both of hiPSC lines harbor the same mutation as the patient and show a normal karyotype. All factors to induce reprogramming were silenced. Both hiPSC lines also have pluripotency and the capacity for differentiation into the three germ layers. These generated hiPSC lines with a WDR19 R1178E mutation (DKH005i-A) and NMNAT1 R237C mutation (DKH090i-A) could facilitate investigations of the pathogenic mechanism underlying SLS and LCA, and should serve as the valuable tools to screen new drugs for improving the symptoms of SLS and LCA patients'. These cell lines are registered and available at the National Stem Cell Bank, Korea National Institute of Health.

P-204

A NEED TO ENHANCE SILENCING EFFICIENCY OF EPISOMAL VECTORS FOR REPROGAMMING IN XENO-FREE CULTIRE CONDITION

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Various methods have been developed to reprogram somatic cells to pluripotent stem cells. Among them episomal vector system has been suggested as a reprogramming tool for clinical application of human induced pluripotent stem cells (hPSCs). After reprogramming the somatic cells the episomal vectors are supposed to disappear by methylation of the component gene for their replication. Our study shows that the silencing efficiency is very low in xeno-free condition compared to non-xeno-free condition using mouse feeder cells. There is a need to enhance silencing efficiency of episomal vectors to reprogram somatic cells in xeno-free condition for clinical application.

P-205

INDUCTION OF PRIMORDIAL GERM CELL-LIKE CELLS BY ECTOPIC EXPRESSION OF MVH IN MOUSE EMBRYONIC STEM CELLS

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Embryonic stem cells (mESCs) has two important character, one is self-renew and another is mESCs can differentiate to different kind of cell, including germ cell. Germ cell can differentiate to sperm or oocyte and carry the genetic material from generation to generation. Primordial germ cells (PGCs) is the precursor cell of germ cell and has a specific marker, Mvh. Because of the knowledge progress about cell signaling, can use the culture condition to induce mESCs to form PGCs-like cell (PGCLCs) now. For these experiment, we want to ectopic express Mvh in mESCs to investigate whether Mvh can induce to form PGCLCs rapidly First, we use the green fluorescent protein (GFP) transgene mouse from our lab to establish a mESCs-GFP cell line. And use immunocytochemistry (ICC), qPCR and Karyotyping to identify its pluripotency and correct rate about number of chromosome. Then, we use embryoid body (EB) and (teratoma) to collect in vitro and in vivo results to assay mESCs-GFP differentiation ability. Mvh coding DNA sequence (CDS) is collect from male mouse testis and build the Mvh overexpression vector, pDsRed-C1-Mvh. In the 3T3 results, we find expression of Mvh let 3T3 to form the colony like situation. After optimize the culture condition, use U bottom suspend culture system can keep Mvh and germ cell related gene expression. Then, we

will ectopic express Mvh in mESC-GFP. In vitro to assay rapidly PGCLCs formation. And in vivo will use blastocyst injection to produce chimera mouse. To evaluate Mvh-mESCs-GFP ability of differentiate to germ cell and Mvh-mESCs-GFP distribution in different germ layer.

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P-206

DNA METHYLATION OF THE IMPRINTED GENES IN HUMAN EMBRYONIC AND INDUCED PLURIPOTENT STEM CELLS USING NEXT GENERATION SEQUENCING PLATFORM

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Genomic imprinting is an epigenetic process resulting in parent-of-origin specific expression. Imprinted genes play key roles in regulating growth and development. Thus the aberrant expression of imprinted genes disrupts development, and is associated with genetic diseases, some cancers and several neurological disorders. Alteration of imprinted gene may contribute to tumorigenesis or alter the differentiation potential of stem cells, would hinder the clinical application. To study the epigenetic status of imprinting in human pluripotent stem cells, we performed DNA methylation sequencing and RNA sequencing of known imprinted genes in human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) from Korea National Stem Cell Bank. We compared DNA methylation level and gene expression of hESC and hiPSC lines derived from the human somatic cell. Our results show that nearly all hESC and hiPSC lines possessed a similar methylation patterns of the imprinted gene and no difference in gene expression, despite differences in genetic background. SNRPN, MEST, PEG10, NDN, and KCNQ1 contained CpG methylation regions that were highly stable and maintained a level of 0.4 to 0.6 methylation. Analysis of genomic imprinting in pluripotent stem cell is a necessary safety step in regenerative medicine. The study of imprinted genes provide a useful indication of epigenetic stability.

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DERIVATION OF MESENCHYMAL STEM CELLS FROM GENERATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS USING EPISOMAL VECTOR

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Human induced pluripotent stem cells (hiPSCs), replaced with human embryonic stem cells(hESCs) due to ethical issue, could be an appropriate source for application of regenerative medicine. However, general culture conditions that depend on the use of feeder and/or xenogeneic medium components show difficulties of use in clinical application. Here, we tried to generate hiPSCs from human fibroblasts using transfection of non-integration/non-virus episomal vectors which carry pCE-hOCT3/4, pCE-hSK, pCE-hUL, pCE- mp53DD and pCXB-EBNA1 on the recombinant laminin-511 E8 fragments-coated dish with completely xeno-free medium, StemFit® Basic02. Even though these hiPSCs could be the ideal cells for clinical medicine, they still have the risk of tumor formation since they are pluripotent. Mesenchymal stem cells (MSCs), which can be differentiated into mesodermal derivatives, have a significant advantage of transplantation-favorable characteristic due to their immunomodulatory effect. MSCs can be separated from various tissues and organs such as bone marrow, liver, muscle and/or fat, however, isolating MSCs is invasive and expensive. MSCs differentiated from hiPSCs represent the valuable substitute for regenerative medicine, therefore, differentiation of hiPSCs into MSC- like cells were attempted by serial passaging strategy using the specified medium. The expressions of mesenchymal markers such as CD166+, CD105+, CD90+, and CD73+ confirmed that hiPSCs have differentiated into MSC-like cells. Hence, hiPSC-derived MSCs showed the characteristic of native MSCs, and, we assume that MSCs differentiated from hiPSCs using non-integration/non-virus vector on feeder-free/xeno-free condition may provide safer application of

stem cell therapy.

P-208

A NOVEL NANO-TRANSFECTION REAGENT FOR THE HIGHLY EFFICIENT, NON-VIRAL CELL CONVERSION

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Concept of the cell conversion arose from the overall effort to generate mass products of cells for therapeutics from the conventional somatic cells which can be obtained relatively easily. Various combinations of factors including genes, macromolecules, and small molecules are involved to control the intracellular environment in the desired manner, which makes such technology difficult to achieve. It has been one of the most challenging issues to develop an intracellular delivery platform to ensure safe and stable delivery of the functional factors involved in cell conversion. Tremendous techniques on transfection have been gone on the trial to artificially take control of the cell fate, including methods incorporating viral vectors and non-viral vectors. Viral vectors have been widely applied to various cell conversion strategies with relatively high efficiency. One of the biggest drawbacks of the viral vector-based method, however, comes from the original traits of the viral genome, which possesses the potential danger of random genetic integration resulting in a fatal mutation. Meanwhile, non-viral cell conversion strategies have been developed with the involvement of various micro/nano-sized particles consisting of biocompatible natural or polymers or even inorganic materials. Yet, several drawbacks including cytotoxicity and low efficiency of intracellular delivery to induce cell conversion have long been under debate. Here, we introduce a modified nanodot-based intracellular delivery reagent for the highly-efficient intracellular delivery of plasmid DNA. The nanodot system successfully supported the incorporation of the reporter gene-encoding plasmid DNA (pGFP), resulting in the enhancement of the gene expression efficiency in the target somatic cell. In recent studies, we are investigating whether the nano- transfection system would mediate the full process of the direct cell reprogramming of human somatic cells into neuronal cells.

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DIFFERENTIATION OF EQUINE INDUCED PLURIPOTENT STEM CELLS INTO MESENCHYMAL LINEAGE FOR THERAPEUTIC USE

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In the previous study, we established an equine induced pluripotent stem cell line (E-iPSCs) using a lentiviral encoding Oct4, Sox2, Klf4, and c-Myc from equine adipose-derived stem cells (E-ASCs). In the current study, differentiation of the established E-iPSCs into mesnchymal stem cells (MSCs) was attempted by serial passaging using MSC-induction media. The expression of CD44 and CD29, which are MSC surface markers, and the absence of Nanog and Oct4, which are pluripotency markers, confirmed that E-iPSCs have differentiated into MSC like cells. The results indicated that the E-iPSC derived MSCs possessed the characteristics of MSCs, including the ability to differentiate into chondrogenic, osteogenic, or myogenic lineages. Subsequently, for therapeutic use, the reduction of immune rejection was required following allogeneic MSC transplantation. The exposure of E-iPSC-MSCs to transforming growth factor beta 2 (TGF-β2) in the culture down-regulated the expression level of major histocompatibility complex class I (MHC class I) proteins that can cause immune rejection in several animals when incompatible with the MHC antigen of the recipient. We reported 16 cases of E-iPSC-MSC transplantations which mostly resulted in positive effects, such as reduced lameness and fracture line into injured horses. The results presented in this study indicate that E-iPSC-MSCs display the characteristics of MSCs and can be safely and practically used as a stem cell source for the treatment of musculoskeletal injuries in horses.

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P-210

PINK1 OVEREXPRESSION ENHANCES CELLULAR REPROGRAMING

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Induced pluripotent stem cells (iPSCS) are very similar to embryonic stem cells (ESCs) in term of self-renewal feature and differentiation potential. Pluripotent stem cells including ESCs and iPSCs have immature structure of mitochondria and lower number of mitochondria compared to somatic cells. In addition, ESCs and iPSCs depend on cytoplasmic glycolysis more than mitochondrial oxidative phosphorylation for generation of ATP. However, cell reprogramming efficiency is low to use iPSCs to clinical purpose (0.01~0.02%) and has a problem about overexpression of oncogenes. To solve these problems, the detailed studies of molecular mechanism for cellular reprogramming is required. Here, we show that mitophagy modulation regulates cellular reprogramming. Park2 knockdown inhibits cellular reprogramming and PINK1 overexpression enhances cellular reprogramming. These data indicates that mitophagy is an essential step for cellular reprogramming.

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CHARACTERIZATION AND QUALITY ASSESMENT OF NEURAL STEM CELL GENERATED BY DIRECT REPROGRAMMING

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Direct reprogramming(direct conversion) is the use of differentiated cells to differentiate into other specific cells bypassing unstable intermediate pluripotent state. Research is actively underway to develop cell therapeutics using direct reprogramming technologies because it reduces the risk of tumorigenecity. In this study, a direct reprogramming technology was introduced to induce neural stem cells using human fibroblast. Oct4, Sox2, Klf4, c-Myc was introduced using sendai viral vectors and induced neural stem cells by adjusting environmental factors such as media and growth factors. To identify the characteristics of induced neural stem cells, we analyzed cell morphology, gene expression analysis through RT-PCR, and related marker expression through immunocytochemistry. And we confirmed genetic stability through karyotyping analysis. We will analyze differentiation ability of NSC to neuron, astrocyte, oligodendrocyte. Based on this study, we will provide basic data and considerations on the quality assessment of cell therapy products based on the direct reprogramming technology.

Funding Source: MFDS(Ministry Of Food And Drug Safety)

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SIMPLE AND DIRECT DIFFERENTIATION PROTOCOL OF HUMAN EMBRYONIC STEM CELLS INTO MESENCHYMAL STEM CELLS

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Mesenchymal stem cells (MSCs) have great potential to generate a wide range of cell types including bone, cartilage, lipid cells, and smooth muscle cells. Moreover, due to their potential for therapeutic applications, MSCs have been explored as a promising option for the treatment of chronic diseases and many injuries. However, MSCs harvested from donors of different ages show different biological characteristics. For those reasons, there are many reports to produce the embryonic stem cell-derived MSCs (ES-MSCs) with multi-differentiation potential and immunomodulation functions in vitro and in vivo as shown by the adult MSCs. However, the most of producing methods previously reported are laborious, time-consuming, costly, and heterogeneous populations. To overcome these hurdles, standard protocols and validation criteria for high quality ES-MSCs need to be created. In this study, we developed improved direct differentiation protocol to generate ES-MSCs (ES-Direct-MSCs) having high-purity and genetic stability within a short period of time. Human ESCs (CHA-hES 15, CHA-hES 52 and CHA-hES 54) were cultured in feeder free condition with mTeSRTM1 and CELLstartTM. For differentiation, hESCs were treated with 1 μM SB431542, TGF-β inhibitors, in DMEM/F12 contained with 20% KSR for 3 d. Then, the cells were additionally treated with 10 μM RS-1, Rad51 activator, and 10 μM Y27632, rho-associated protein kinase (ROCK) inhibitor, simultaneously. After 24 hour, the cells were transferred into new culture dishes with collagenase and cultured in MSC maintenance media. We have got homogenous cell population by serial sub-passaging using trypsin-EDTA, and checked MSC characteristics of our ES-Direct-MSCs around 5 passages. CHA15-EB-MSCs, differentiated by embryoid body (EB) mediated-methods, and hMSCs (Lonza, PT2501; adult MSCs) were used as controls. The ES-Direct-MSCs express typical MSCs surface markers and undergo adipogenesis, osteogenesis, and chondrogenesis similar to CHA15-EB-MSCs and hMSCs. In additions, our ES-Direct MSCs have shown significantly higher proliferation capacity, and differentiation abilities than hMSCs. Therefore, our direct differentiation protocol can save time and cost to produce ES-MSCs which are proposed as an alternative source of MSC for regenerative medicine.

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EFFICIENT DIRECT LINEAGE REPROGRAMMING OF FIBROBLASTS INTO INDUCED CARDIOMYOCYTES USING NANOTOPOGRAPHICAL CUES

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Induced cardiomyocytes (iCMs) generated via direct lineage reprogramming offer a novel therapeutic target for the study and treatment of cardiac diseases. However, the efficiency of iCM generation is significantly low for therapeutic applications. Here, we show the efficient direct conversion of somatic fibroblasts into iCMs using nanotopographic cues. Direct conversion into iCMs on nanopatterned substrates resulted in a dramatic increase in the reprogramming efficiency and maturation of iCM phenotypes compared with that on flat substrates. Additionally, the enhanced reprogramming by the substrate nanotopography was derived from changes in the activation of focal adhesion kinase and specific histone modifications. Taken together, these results suggest that nanotopographic cues can serve as an efficient stimulant for direct lineage reprogramming into iCMs.

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IN VIVO REPROGRAMMING FACTOR OCT4 EXRESSION AMELIORATES DYSMYELINATION IN AN ANIMAL MODEL OF HUNTINGTON'S DISEASE

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Huntington's disease (HD) is caused by genetic mutation which specific sequence repeat produce mutant Huntingtin proteins. As incurable neurodegenerative disease, Previous studies were primarily focusing on regenerating GABAergic neurons in the striatum, but recent studies showed that white matter atrophy is caused by early symptom of HD. In order to overcome this symptom, we used OCT4 that one of reprogramming factors to induce regeneration of oligodendrocyte precursor cells (OPCs). Each group of R6/2 mice was injected with PBS, AAV9-Null, or AAV9-OCT4 on both sides of the lateral ventricles. According to the results of the behavioral tests such as rotarod, grip strength and open-field tests, AAV9-OCT4 group display significant improvement compared to control other groups—PBS and AAV9-Null. Levels of RNA expression in AAV9-OCT4 group were significantly increased in Platelet Derived Growth Factor Receptor Alpha (PDGFRa), Wnt Family Member 3 (WNT3), Myelin Regulatory Factor (MYRF) and Glia cell-derived neurotrophic factor (GDNF). In addition, Nestin, NG2, and Olig2 expression were higher in AAV9-OCT4 than control groups. Contrastively, NeuN did not show any significant difference between groups. Moreover, in Immunohistochemistry, both Nestin+BrdU+ and NG2+BrdU+ cells were increased in the AAV9-OCT4 group compared to the control groups in the SVZ. Furthermore, protein expression of MBP is significantly increased in AAV9-OCT4 in the cortex. In addition, Electron Microscope (EM) and Magnetic Resonance Imaging (MRI) results show that recovery of demyelination in corpus callosm in AAV9-OCT4 group compared to other groups. We also confirmed survival rates of GABAergic neurons in striatum. GABAergic marker (DARPP32) increased in AAV-Oct4 group. In conclusion, our data suggest that in vivo overexpression of OCT4 treatment in SVZ had neuroprotective effects from the OPCs with its secreted factors such as PDGFRa, WNT3, MYRF, GDNF. The findings of this study suggest that improving the function of immature oligodendrocytes by OCT4 should at least be beneficial in HD. Thus, OCT4 could be the important therapeutic factor for HD.

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ENHANCED DELIVERY OF CELL PENETRATING PEPTIDE FUSED PROTEINS TO MAMMALIAN CELLS

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Recent progress in cellular reprogramming technology and lineage-specific cell differentiation has provided great opportunities for translational research. Because virus-based gene delivery is not a practical reprogramming protocol, protein-based reprogramming has been receiving attention as a safe way to generate reprogrammed cells. However, the poor efficiency of the cellular uptake of reprogramming proteins is still a major obstacle. Here, we reported key factors which improve the cellular uptake of these proteins. Purified red fluorescent proteins fused with 9xLysine (dsRED-9K) as a cell penetrating peptide were efficiently delivered into the diverse primary cells. Protein delivery was improved by the addition of amodiaquine. Furthermore, purified dsRED-9K was able to penetrate all cell lineages derived from mouse embryonic stem cells efficiently. Our data may provide important insights into the design of protein-based reprogramming or differentiation protocols. This research was supported by a grant from NRF: 2016R1D1A1B03931915.

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NANOGROOVED SUBSTRATE PROMOTES DIRECT LINEAGE REPROGRAMMING OF FIBROBLASTS TO FUNCTIONAL INDUCED DOPAMINERGIC NEURONS

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The generation of dopaminergic (DA) neurons via direct lineage reprogramming can potentially provide a novel therapeutic platform for the study and treatment of Parkinson's disease. Here, we showed that nanoscale biophysical stimulation can promote the direct lineage reprogramming of somatic fibroblasts to induced DA (iDA) neurons. Fibroblasts that were cultured on flat, microgrooved, and nanogrooved substrates responded differently to the patterned substrates in terms of cell alignment. Subsequently, the DA marker expressions, acquisition of mature DA neuronal phenotypes, and the conversion efficiency were enhanced mostly on the nanogrooved substrate. These results may be attributed to specific histone modifications and transcriptional changes associated with mesenchymal-to epithelial transition. Taken together, these results suggest that the nanopatterned substrate can serve as an efficient stimulant for direct lineage reprogramming to iDA neurons, and its effectiveness confirms that substrate nanotopography plays a critical role in the cell fate changes during direct lineage reprogramming.

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STEMNESS RESTORATION CAPACITY OF INDUCED HEPATOCYTE LIKE CELLS DERIVED FROM EAR FIBROBLAST OF GENETIC MODIFIED PIG

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Stem cell has been known to possess self-renewal and differentiation, and long term self-renewal correlates with bypassing senescence or prolonging lifespan involved with the regulation of telomere length and high levels of DNA repair enzymes. We analyzed cell life span factors (absolute telomere length / TERT) and proliferation factors (TP53 / c-Myc) using real time PCR to confirm whether the stem cell capability was restored or not in induced hepatocyte like cell (iHeps) generated from fibroblast (passage 5) of alpha-1, 3-Galactosytransferase knock out pig. iHeps were generated using two types of episomal vectors; vector with codon optimization (hHnf1α/ hHnf4α/ hFoxa3) and without optimization. iHeps and fibroblast (as control) were cultured until passage 21 for 2 years and 6 months, respectively. We observed positive correlation between telomere length and TERT in iHeps, while they decreased with increasing passage in fibroblast but increased in iHeps regardless of cell type. TP53 was shown to temporary increase in iHeps with or without codon optimization at passage 10 and 15, respectively; moreover, iHep with codon optimization was restored same TP53 expression at passage 21 as fibroblast but iHep without codon optimization did not. There was no difference in c–MYC expression in fibroblasts with increasing passage number, but iHeps maintained higher expression level, regardless of iHep types. From the above results, we revealed that iHeps generated from fibroblast with lost stem cell ability could restore stemness.

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DERIVATION OF INTEGRATION-FREE PORCINE INDUCED PLURIPOTENT STEM CELLS

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Pig is considered as animal models because of its organ anatomy, physiology and metabolism are similar to humans. The main obstacle in fully reprogrammed porcine iPSCs is insufficient silencing of exogenous genes from integrating into the host genome. The non-integration methods are a better method to produce iPSCs for therapeutic application, but its efficiency of reprogramming is low (0.01%) compared to integration vectors. According to episomal plasmid vectors, this method is easy to manipulate and allow a high efficiency compared to the other non-integrating methods. In this study, Sertoli cells were used as a somatic-cell source. We aimed to generate and characterize the porcine iPSCs by non-integration episomal plasmid method. The neonatal pig testes were digested by the two-step enzymatic method in order to isolate the Sertoli cells. We used program EH138 of Nucleofector 4DX Device (Lonza, Germany). Sertoli cells were transfected with plasmid DNA (OCT3/4-shp53, SOX2, KLF4, L-MYC and LIN28). Primary Sertoli cells demonstrated polygonal-shaped morphology. The primary colonies were appeared 5 days after nucleofection. Seven days after transfection, we observed 0.229% of primary colonies formation. Sixteen primary colonies were cultured. Porcine iPS-like colonies represented positive to alkaline phosphatase staining and OCT4 expression. At passage 8, four selected cell lines could form embryoid bodies via the hanging drop method. From RT-PCR result, iPS-like cell lines were expressed in pluripotent genes (OCT4, SOX2, KLF4, c-MYC and NANOG) and three germ layers genes. Moreover, the tumor was formed after injection of porcine iPSCs into immunodeficiency mice for two months. Generation of iPSCs via non-integration reprogramming methods is safer than integration methods in part of clinical applications. The major advantages of non-integrating methods are decreasing host genome insertion-mutagenesis and residual exogenous genes expression. In this study, we firstly reported the generation of porcine iPSCs from Sertoli cells via non-integrating reprogramming method.

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DEVELOPMENT OF INSULIN PRODUCING CELLS EXPRESSING PDX1-GFP FOR CELL TRACKING AND DIFFERENTIATION STUDIES USING HUMAN IPSC

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Differentiation has been tried into various cells for treatment of disease since induced pluripotent stem cells have developed. We purposed to generate human insulin producing cells (IPC) that is enable to track as well as to effective for clinical trial. We using human iPSC. Human iPSC were generated by sendai virus system and were derived by bone marrow. Differentiation protocol of IPC was composed of 5 stage, definitive endoderm, primitive gut tube, posterior foregut, pancreatic progenitor (PP) and insulin producing cells. We used modified pancreatic progenitor kit for differentiation into insulin producing cells. At final stage, pancreatic progenitor cells were cultured in CMRL medium with hGF, nicotinamide, exendin-4, SANT1, T3 and Alk5i. Differentiated cells were aggregated and were cultured in microwell plate during all differentiation steps. Human iPSC were generated EGFP tagging on c-terminal of PDX-1 by CRISPR/Cas9 system (PDX1-GFP). Suspended single cells were uniformly aggregated in microwells. And these cells were verified cell viability, pancreatic β-cell specific markers and function of IPC. As a result, cell viability was no difference in differentiation stage, and differentiated cells expressed pancreatic β-cell specific markers, Ngn3, Nkx6.1, Pdx-1 and insulin in IPC stage. We confirmed not only GFP positive cells were showed in PP stage but also, PDX1-GFP cells were co-stained with insulin and C-peptide in IPC. And also, we confirmed that insulin secretion of generated PDX1-GFP

IPC was significantly increased. We demonstrated that insulin-producing cell can be generated from human iPSCs enhanced PDX1-GFP. These results suggest that successful IPC were differentiated in 3D culture without matrigels. Therefore, this study showed that differentiated PDX-GFP IPC enable to application for cell tracking and the study of differentiation of IPC in clinical trial.

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NEURAL PRECURSOR CELLS/DOPAMINE NEURON DIRECT CONVERSION USING HYBRID NANOFIBER SCAFFOLDS

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The concept of cellular reprogramming was developed to generate induced neural precursor cells (iNPCs)/dopaminergic (iDA) neurons using diverse approaches. Here, we investigated the effects of various nanoscale scaffolds (fiber, dot, and line) on iNPC/iDA differentiation by direct reprogramming. The generation and maturation of iDA neurons (microtubule-associated protein 2-positive and tyrosine hydroxylase-positive) and iNPCs (NESTIN-positive and SOX2-positive) increased on fiber and dot scaffolds as compared to that of the flat (control) scaffold. This study demonstrates that nanotopographical environments are suitable for direct differentiation methods and may improve the differentiation efficiency.

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P-221

HOMOGENEOUS GENERATION OF IDA NEURONS WITH HIGH SIMILARITY TO BONA FIDE DA NEURONS USING A DRUG INDUCIBLE SYSTEM

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Recent work generating induced dopaminergic (iDA) neurons using direct lineage reprogramming potentially provides a novel platform for the study and treatment Parkinson's disease (PD). However, one of the most important issues for iDA-based applications is the degree to which iDA neurons resemble the molecular and functional properties of their endogenous DA neuron counterparts. Here we report that the homogeneity of the reprogramming gene expression system is critical for the generation of iDA neuron cultures that are highly similar to endogenous DA neurons. We employed an inducible system that carries iDA-inducing factors as defined transgenes for direct lineage reprogramming to iDA neurons. This system circumvents the need for viral transduction, enabling a more efficient and reproducible reprogramming process for the generation of genetically homogenous iDA neurons. We showed that this inducible system generates iDA neurons with high similarity to their bona fide in vivo counterparts in comparison to direct infection methods. Thus, our results suggest that homogenous expression of exogenous genes in direct lineage reprogramming is critical for the generation of high quality iDA neuron cultures, making such culture systems a valuable resource for iDA-based drug screening and, ultimately, potential therapeutic intervention in PD.

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ELECTROMAGNETIC FIELDS MEDIATE EFFICIENT CELL REPROGRAMMING INTO A PLURIPOTENT STATE

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Life on earth is constantly exposed to natural electromagnetic fields (EMF) and it is generally accepted that EMF may exert a variety of effects on biological systems. Particularly, extremely low frequency electromagnetic fields (EL-EMFs) affect biological processes such as cell development and differentiation, however, the fundamental mechanisms by which EMF influences these processes remain unclear. Here we show that that EMF exposure induces epigenetic changes that promote efficient somatic cell reprogramming to pluripotency. These epigenetic changes resulted from EMF-induced activation of the histone lysine methyltransferase Mll2. Remarkably, an EMF-free system that eliminates earth's naturally occurring magnetic field abrogates these epigenetic changes, resulting in a failure to undergo reprogramming. Therefore, our results reveal that EMF directly regulates dynamic epigenetic changes through Mll2, providing efficient tool for epigenetic reprogramming including the acquisition of pluripotency.

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P-223

IMPROVEMENT OF THREE-DIMENSIONAL DIRECT CARDIAC REPROGRAMMING BY ELECTRICAL STIMULATION USING MICROPILLAR ARRAY

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In recent years, direct reprogramming technology has attracted great interest for treating cardiac disease which is life-threatening. Direct reprogramming, which can convert somatic cells into other lineage cells without the use of stem cells, is relatively simple and time saving. However, there is still a need to improve reprogramming efficiency and maturity of reprogrammed cells. Herein, we chemically induced cardiac reprogramming from mouse fibroblasts in microwells to generate 3D cardiac spheroids. Induced 3D cardiac spheroids showed highly increased cardiac gene expressions as compared to 2D reprogrammed cardiomyocytes. In addition, we newly introduced a micropillar array to provide electrical stimulation directly into the interior of the spheroids undergoing reprogramming. We confirmed that the electrical stimulation provided by the micropillar device improved differentiation and maturation of the cardiac spheroids. Electrically stimulated cardiac spheroids showed autonomous beating, which was facilitated by isoproterenol. We developed a novel platform to directly provide electrical stimulation to 3D cellular structures. Our devices would be useful to produce functional tissue-like structures for a variety of applications such as drug screening and disease modeling.

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SIMPLIFIED SELECTION OF COLONY MORPHOLOGY FOR IMPROVING SUCCESS RATES OF ESTABLISHMENT AND MAINTENANCE IN PORCINE-INDUCED PLURIPOTENT STEM CELLS

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Reprogramming efficiency of porcine fibroblasts into induced pluripotent stem cells (iPSCs) is unfortunately poor (~ 0.1%). During reprogramming process, mixed populations of primary colonies become the major obstacle in iPSCs establishment. This barrier can be simply overcome by prescreening of primary iPSC colonies prior to further expansion. We examined the pluripotent characteristics between

colony morphologies at colony pick-up. The fibroblast cells were isolated from the tails of piglets and transfected with retroviral vectors expressing OCT4, SOX2, KLF4 and c-MYC. The iPS-like colonies were divided into compact colonies (n=10) and loose colonies (n=10) morphology at colony pick-up. The compact colonies contained with tightly packed cells with a distinct border between the colony and feeder cells, while colonies with irregular shape and border were classified as loose colony morphology. A total of 1,697 iPS-like colonies (2.34%) were observed. Although the two types of iPSC colonies, expressed alkaline phosphatase and OCT4 immunostaining and had normal karyotype, higher proportion of compact iPSC phenotype (5 of 10, 50%) could be maintained in an undifferentiated state for more than 50 passages compared to loose morphology (3 of 10, 30%). All iPS cell lines were expressed pluripotent genes and could form in vitro embryoid bodies. However, only compact iPSC colonies differentiated into embryonic structures of three germ-layers. In this study, we found that the cell lines from compact morphology can be maintained for longer time when compared with loose morphology. Higher rate of differentiation of loose iPS colony may also indicate that this type of colony may have different pluripotency signals or incomplete reprogramming compared with compact colony. In conclusion, selection of colony morphology at colony pick up is a simple procedure for prescreening of in vitro pluripotency of porcine iPS cell lines and thereby improving success rate of iPSC generation.

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P-225

RECONSTRUCTION OF DEVELOPMENTAL LANDSCAPES AND TRAJECTORIES FROM INTEGRATIVE ANALYSIS OF LARGE-SCALE SINGLE-CELL DATA

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Understanding the molecular programs that guide cell fate transition during development is a major goal of modern biology. Here, we developed novel experimental and computational methods for studying developmental time course single-cell data to infer ancestor- descendant fates and model the regulatory programs that underlie them. We demonstrated the power of these methods by applying them to around 1,000,000 scRNA-seq, scATAC-seq, and spatial transcriptomic profiles collected during 1) reprogramming of fibroblasts to pluripotency by the Yamanaka factors; 2) by different reprogramming cocktails; 3) reprogramming of fibroblasts to totipotency by a new cocktail; 4) mouse embryonic development from the pluripotent state to terminally differentiated state focusing on extraembryonic lineage development. We constructed high-resolution maps of mouse embryonic development and different reprogramming strategies that uncovers universal mechanisms of successful reprogramming; discovers new cell fates; predicts the origin and fate of any cell class; suggests cell-cell interactions, and implicates regulatory models in particular trajectories in development and reprogramming. Our approach provides the first large-scale, high resolution roadmaps of normal mouse embryonic development and reprogramming to pluripotency and totipotency using various reprogramming cocktails. It also provides a general framework for studying cell fate conversions in natural and induced settings.

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GENERATION AND CHARACTERIZATION OF INDUCED NEURAL STEM CELL AND OLIGODENDROCYTE PROGENITOR CELLS

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The generation of human neural stem cells (NSCs) and oligodendrocyte progenitor cells (OPCs) may be therapeutically valuable for human neurological diseases such as spinal cord injury and multiple sclerosis. However, it has been challenging to isolate NSCs and OPCs from human central nerve tissues, thus leading to induction of these cells by various methods in vitro. Here, we report, the direct reprogramming of human somatic cells into induced NSCs (iNSCs) and OPCs (iOPCs) using a combination of transcription factors and a small molecule cocktail. The iNSCs expressed neural progenitor markers including SOX1, SOX2, NESTIN, PAX6 and PLZF, while no pluripotency marker such as OCT4 and NANOG was observed. The iNSCs showed differentiation potential to astrocytes, oligodendrocytes, and functional neurons in vitro/in vivo. The iOPCs retain the ability to differentiate into MBP-positive mature oligodendrocytes, and engrafted iOPCs remyelinated the brains of adult shiverer mice and experimental autoimmune encephalomyelitis mice with MOG-induced 14 weeks after transplantation. Each transcriptome of iNSCs and iOPCs were similar to those of NSCs and OPCs analyzed by RNA-seq. Furthermore, ChIP-seq analysis revealed that putative OCT4-binding regions were detected in the promoters of CNS development-related genes. In conclusion, our study may contribute to the development of therapeutic approaches for neurological disorders.

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Tissue Stem Cells and the Niche

P-301

CONTRIBUTION OF INDIVIDUAL SATELLITE CELLS TO MUSCLE REGENERATION ASSESSED USING A CONFETTI MOUSE MODEL

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Insufficient regeneration is implicated in muscle pathologies, but much remains unknown about the contribution of individual satellite cells (SC). Recent work found that individual SCs contribute to regeneration of more than one muscle fiber, after severe full-muscle damage. We investigated whether SCs also contribute to more than one fiber after a moderate injury. We induced a gradient of injury severity, using a single cardiotoxin injection in the tibialis anterior. Lineage tracing using confetti fluorescence showed that adjacent regenerated fibers often expressed the same colors. A novel method of stochastic modeling, applied to the lineage tracing data, revealed that the observed color patches could not be explained statistically if SCs were only contributing myonuclei to a single fiber. Instead we conclude that satellite cells must have contributed myonuclei to multiple adjacent fibers. Interestingly, these results were similar across the severities of injury, suggesting that severe destruction is not required for fiber-crossing.

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A NOVEL BETA-CATENIN-INDEPENDENT ROLE OF WNT SIGNALING IN MUSCLE STEM CELLS

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WNT/β-catenin signaling has been implicated to play a critical role in skeletal muscle stem cells during muscle regeneration. Surprisingly, muscle stem cell-specific β-catenin (Ctnnb1) gene knockout or gain-of-function in mice does not result in a significant defect in muscle regeneration, raising an uncertainty about the actual role of WNT/β-catenin signaling in muscle stem cells. To address this uncertainty, we determine the role of the Lrp5 and Lrp6 receptor genes encoding two highly homologous WNT receptors essential for WNT/β-catenin signaling in muscle stem cells by using muscle stem cell-specific Lrp5 and Lrp6 gene knockout mice. Unlike in the Ctnnb1 gene knockout mice, the ablation of both the Lrp5 and Lrp6 genes significantly impairs muscle regeneration, suggesting the key role of LRP5 and LRP6 receptors in muscle stem cells. In primary muscle stem cells, activation of WNT/β-catenin signaling inhibits proliferation but promotes the onset of myogenic differentiation in a Lrp5 and Lrp6-dependent manner. Furthermore, we found the convincing evidence that WNT signal is transmitted via the mTOR-S6K signaling pathway and CyclinD3 plays a key role in regulating the onset of myogenic differentiation. Our study uncovers a novel signaling mechanism by which WNT signaling activation through the LRP5 and LRP6 regulates the function of muscle stem cells independent from the β-catenin function.

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FUNCTIONAL ROLE OF WNT SIGNALING REGULATOR, R-SPONDIN2, IN THE HOMEOSTASIS OF ADULT LUNG EPITHELIAL STEM AND PROGENITOR CELLS

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The lung has a remarkable ability to regenerate the damaged tissue caused by acute injury whereas it shows a low cellular turnover rate during homeostasis in the adult. Many lung diseases, especially chronic ones, are associated with a reduced or disrupted regeneration potential of the lung. Therefore, understanding the underlying mechanisms of the regenerative capacity of the lung offers potential in identifying novel therapeutic targets for these diseases. R-spondin2 (RSPO2) is a co-activator of WNT/β-catenin signaling and plays an important role in lung development. Mice lacking the Rspo2 gene exhibited lung hypoplasia and defects in the lung branching. However, the role of Rspo2 in lung homeostasis and regeneration process in the adult remains unknown. As a first step toward uncovering Rspo2 function, we examined Rspo2 expression in normal and regenerating lung tissues in adult mice. Robust Rspo2 expression was detected in different epithelial cells of lung airways assessed by RNA in situ hybridization and immunohistochemistry. Interestingly, Rspo2 expression decreases during the first week after naphthalene-induced injury and restored by 14 days after injury. To determine the role of RSPO2 on lung epithelial stem/progenitor cells, we treated recombinant RSPO2 protein on the EpCAM+ve epithelial stem/progenitor cell-derived lung organoids. We found that RSPO2 protein has a positive effect on both the colony-forming efficiency and colony size. Furthermore, mice lacking the Rspo2 gene showed defects in airways epithelial cell regeneration at 14-days after naphthalene-induced injury. Our results strongly suggest that RSPO2 may play a key role in the homeostasis and regeneration of adult lung epithelial stem/progenitor cells.

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P-304

SATELLITE CELL FUSION IS NOT REQUIRED FOR MUSCLE HYPERTROPHY INDUCED BY MYOSTATIN INHIBITION

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An increase in muscle mass is accompanied by a robust increase in the level of protein synthesis. However, whether this elevated muscle proteins during muscle hypertrophy results mainly from increased protein synthesis from existing myonuclei or addition of new nuclei by satellite cell fusion remains controversial. Here, we investigated whether myonuclear addition through satellite cell fusion is required for muscle hypertrophy induced by inhibition of myostatin (MSTN), a well-known negative regulator of muscle growth. More specifically, we examined the relationship between the number of myonuclei, satellite cells, and myofiber diameter in extensor digitorum longus (EDL) muscles of wt, Mstn null, and F66 mice overexpressing follistatin (FST), which strongly inhibits MSTN, its homolog GDF11, and activin A. EDL muscle weights were only mildly increased in both Mstn-/- and F66 mice compared to wt mice at 3 weeks of age, but were increased by 2-fold in Mstn-/- mice and close to 3-fold in F66 mice at both 10 weeks and 20 weeks of age, suggesting that significant hypertrophic change occurs after 3 weeks of age in these mice. It is noteworthy that increased muscle mass observed in Mstn-/- mice results from both hyperplasia and hypertrophy, while that observed in F66 mice results mostly from hypertrophy. As expected, fiber diameter was notably increased in Mstn-/- and F66 EDLs at 10 weeks of age (wt: 52 μm; Mstn-/-: 67 μm; F66: 87 μm) and at 20 weeks of age (wt: 64 μm; Mstn-/-: 79 μm; F66: 109 μm). However, there were no significant differences in the number of myonuclei per unit length of myofibers between all groups at both 10 weeks and 20 weeks of age (67-73 nuclei/ mm fiber length) when observed after staining nuclei with DAPI and satellite cells with Pax7 antibody. Likewise, there were no significant differences in the number of Pax7-positive satellite cells between all groups at both 10 weeks and 20 weeks of age (4-6 satellite cells/ fiber), indicating that muscle hypertrophy induced by MSTN blockade mainly results not from satellite cell fusion, but from myonuclear protein synthesis. Our findings not only elucidate the cellular mechanisms of muscle hypertrophy, but also suggest that inhibition of MSTN signaling pathway can be an effective therapeutic strategy to increase muscle mass even in patients with satellite cell dysfunction.

P-305

THE HYPOXIC MICROENVIRONMENT MAINTAIN DENTAL EPITHELIAL STEM CELLS VIA RHO- YAP/TAZ SIGNAL

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The microenvironmental oxygen level is closely associated with the cell fate decision of various tissue stem cells. However, the role of oxygen level on epithelial stem cells, and the molecular regulatory mechanism are largely unknown. In this study, we investigated the impact of oxygen level on the cell fate decision from slow-cycling dental epithelial stem cells (DESCs) to actively-proliferating transit-amplifying (TA) cells using continuously growing mouse incisors. First, we histologically found that blood vessels are localized out of touch with the labial cervical loop epithelium (DESCs) in incisor tooth germs. The number of mitochondria in DESCs was fewer and the size was smaller, compared with that in TA cells. The expression of pyruvate dehydrogenase (PDH) in DESCs were also lower than that of TA cells. The results suggested that DESCs were maintained in hypoxic environment. Next, when mouse incisors were cultured under hypoxic condition, the number of Ki67 positive cells decreased. Additionally, hypoxic condition induced activation of Rho signal in the area of DESCs. Conversely, inhibition of Rho signal using ROCK inhibitor promoted cell proliferation and translocation of Yap/Taz into nucleus concomitantly with loss of cortical actomyosin. Furthermore, knock down of cytoskeletal protein, Merlin, also induced cell proliferation and nuclear translocation of YAP / TAZ. Together, these results suggested that hypoxic condition maintained DESCs via RhoA-cytoskeleton-YAP / TAZ signal.

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P-306

CHARACTERIZATION OF THE SUBVENTRICULAR-THALAMO-CORTICAL CIRCUIT IN THE NIEMANN-PICK TYPE C MOUSE BRAIN, AND NEW INSIGHTS REGARDING TREATMENT

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Gliosis in Niemann-Pick type C (NP-C) disease is characterized by marked changes in microglia and astrocytes. However, the gliosis onset and progression in NP-C has not been systematically studied, nor has the mechanism underlying this finding. Here we found early gliosis in subventricular zone (SVZ) of NP-C mice. Neural progenitor damage by Npc1 mutation suppressed vascular endothelial growth factor (VEGF) expression and further induced microglia activation followed by astrogliosis. Interestingly, excessive astrogliosis in the SVZ induced neural progenitor retention/migration into thalamus via astrocyte-derived VEGF, resulting in acceleration of thalamic and cortical gliosis through thalamo-cortical pathways. Transplantation of VEGF-overexpressing neural stem cells into the SVZ improved whole-brain pathology of NP-C mice. Overall, our data provide new pathological perspective on NP-C neural pathology, revealing abnormalities in subventricular-thalamo-cortical circuit of NP-C mouse brain, and highlighting the importance of the SVZ microenvironment as a therapeutic target for NP-C disease.

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P-307

NEURONAL REGENERATION ELEVATED BY VASCULAR FACTORS IN MEMORY AND SOCIABILITY DEFICIENCY MODEL RATS

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Microenvironment of hippocampal subgranular zone contains specialized cells such as vascular pericytes and micro-vascular endothelium in addition to brain cells including neural precursor cells (NPCs) and astrocytes. We found NPCs are located at the branching sites of micro-vessels directly contacting to vascular cells. We investigated the factors released from brain vascular cells by RNA sequencing method and protein array. We found that many neurogenic factors were released from brain ECs and pericytes that mediate the interaction between NPCs and niche cells. We investigated whether these factors promotes neuronal regeneration in memory and sociability-deficient model rats. We used human mesenchymal stem cells (hMSCs) as a vector to deliver the factors using recombinant AAV2 into injury sites. As a result, memory and social behaviors and neuronal cell damages were recovered compared to saline injected memory-deficient group. These results proposes that neurogenic factors released from brain ECs and pericytes mediate the interaction between NPCs and niche cells and the neurogenesis is largely influenced by these factors in the NPC niche of the adult hippocampus.

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P-308

INVESTIGATION OF DENTAL PULP STEM CELLS WITH 3D IMAGING OF TOOTH AFTER TISSUE CLEARING

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To achieve a comprehensive understanding of tissue-resident dental stem cells and regeneration mechanism, we develop 3D imaging protocol for tooth using tissue clearing and light sheet microscopy. Mouse molars were partially decalcified in 10% EDTA (pH8.0) for 2 weeks with or without formic acid, followed by CUBIC or bone CLARITY tissue clearing protocol using X-CLARITY. Cleared molars were subjected to the primary antibody staining for 10 days and the secondary antibody staining for 10 days. After antibody staining, molars were stained with the hoechst 33342 for overnight and finally immersed in the refractive index matching solution (RIMS). Samples were imaged with ZEISS Lightsheet Z.1 microscope and 3D images were rendered using Arivis software. Addition of formic acid produced opaque area along dental pulp and tissue clearing with CUBIC protocol was not efficient, thus either protocol was not adopted. After 10% EDTA and bone CLARITY process, molars were divided half with razor and subjected to staining with antibodies of BMI1 or SOX2 among stem cell markers and β-actin for counterstaining. Followed by the corresponding secondary antibody staining and RIMS immersion, molars were imaged with light sheet microscope. The result showed the presence of dentin layer and adequate staining of beta-actin in dental pulp area, which suggests the successful penetration of antibody and light sources for imaging. Interestingly, both BMI1 and SOX2 staining were localized to root area with increasing signal to root apex and decreasing signal toward pulp chamber. These results suggest the higher stem cell activity at root canal in dental pulp in comparison with crown chamber. We established 3D imaging protocol for tooth using bone CLARITY tissue clearing and lightsheet microscope, which provides a platform for the analysis of cellular and molecular mechanism of dentin and pulp regeneration.

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P-309

EFFECTS OF ALPHA-LIPOIC ACID(ALA) AND GLUTATHIONE(GSH) ON HUMAN TURBINATE-DERIVED MESENCHYMAL STEM CELLS(TMSC): THEIR ROLE OF ANTI-OXIDATIVE EFFECTS

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Rapid and effective proliferation of mesenchymal stem cells is critical for their clinical application. It has been reported that reactive oxygen species(ROS) prevent the proliferation of various mesenchymal stem cells. But the effects of ROS to hTMSCs are not still known. The objective of this study is to evaluate the effect of the oxidative stress on hTMSCs and to evaluate the anti-oxidative effect of glutathione(GSH) and alpha-lipoic acid(ALA) on the proliferation of hTMSC. -MTT assays to evaluate cell viability activity of H2O2 on hTMSCs -MTT assays to evaluate cell viability activity of GSH and/or ALA in presence of H2O2 on hTMSCs -Flow cytometry for effect of GSH and ALA on hTMSC -Fluorescent staining for effect of GSH and ALA on hTMSC -Fluorescent staining of CD90, CD105 positive cells for effect of GSH and ALA on hTMSC - Chondrogenic differentiation. - hTMSCs-H2O2 cell viability activity In 0μM-500μM H2O2, cell viability activity of hTMSCs decreased as increasing concentrations of H2O2. - GSH/ALA-hTMSC cell viability activity To GSH 5mM, cell viability activity increased, but over GSH 5mM it decreased. To ALA 250uM cell viability activity increased but over ALA 250uM it decreased. - GSH/ALA-hTMSC cell viability activity in the treatment of H2O2 H2O2 suppressed cell viability and GSH and ALA reversed and combination of GSH and ALA revealed weak synergic effects. -Flow cytometry results with and without CD90/CD105 in treatment of ALA and/or GSH were corresponding to results of MTTs. -Fluorescent staining results in the treatment of ALA and/or GSH were corresponding to results of MTTs.

Glutathione and alpha-lipoic acid facilitate the proliferation of hTMSCs and have some synergic effects.

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THE WHITENING EFFECT OF EXTRACELLULAR VESICLES FROM ORBICULARIS OCULI MUSCLE-DERIVED STEM CELLS

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Skeletal muscle-derived stem cells possess potent therapeutic activities in the treatment of muscle-related disorders. In this study, we isolated and characterized stem cells from discarded tissues, namely orbicularis oculi muscle (ORM) that obtained from patients subjected to ocular surgery. We verified the stemness properties of the ORM-derived stem cells (ORM-SCs) through evaluation cell morphology, proliferation, the expression level of the surface markers, stemness-associated markers, tri-lineage differentiation capacities, and colony-forming capacity. Isolated ORM-SCs showed spindle-like morphology and positive expression of CD105, CD 90, and CD73, but they were negative in expression of CD45 and CD34. The ORM-SCs showed the capacity to osteogenic, adipogenic, and chondrogenic differentiation. Next, we prepared the natural extracellular vesicles (EVs) from the ORM-SCs and found that the ORM-SC-derived EVs (ORM-SC-EVs) have an apparent inhibitory effect on the melanin synthesis in B16F10 cells by blocking the tyrosinase activity, although ORM-SC-EVs treatment did not dramatically change the expression level of melanogenesis-related genes, such as, microphthalmia-associated transcription factors (MITF), tyrosinase(TYR), tyrosinase-related protein1(TYRP-1), and TYRP-2. Taken together, this study revealed the stem cell property of ORM-SCs and the suppressive effect of ORM-SC-EVs on melanogenesis, suggesting that ORM-SCs and ORM-SC-EVs can be used for a promising candidate not only in the stem cell therapy, but also in regulation of the melanogenesis and skin whitening, and further application in cosmetics.

P-311

INTRAVITAL LONG-TERM IMAGING OF DYNAMIC CELLULAR BEHAVIOR OF TRANSPLANTED HEMATOPOIETIC STEM CELLS IN CRANIAL BONE MARROW

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Bone marrow (BM) is soft and flexible tissue inside the bone, which houses hematopoietic stem cells with multi-potent differentiation capability. BM is the most important organ in maintaining homeostasis of circulatory and immune system. Bone marrow transplantation (BMT) is actively performed to treat patients with various hematologic disorders. However, despite significant medical applications, the underlying cellular dynamics in spatiotemporal distribution and engraftment of transplanted BM cells including hematopoietic stem and progenitor cells have not been fully identified yet. In this study, we utilized a custom-built intravital microscopy system with cranial BM window and stereotaxic mount for a repetitive longitudinal imaging and long-term time-lapse imaging of same BM area up to 7 days after BMT. Using various fluorescence expressing transgenic mice including H2B-GFP/β-actin-DsRed, CX3CR1-GFP/β-actin-DsRed and CSF1R-GFP mice as BMT donor or recipient, we successfully visualized various spatiotemporal cellular dynamics such as proliferation, migration, and differentiation into myeloid or lymphoid lineages and cell-cell interaction between transplanted BM cells and host macrophages in vivo. With a longitudinal wide-area intravital imaging during early engraftment, we identified 4 distinctive behaviors of BM cell group: Cell cluster formation from single cell; Formation of a large cell cluster from small clusters; Cluster dispersion into scattered cells; Scattered cell group not forming cluster. Long-term time-lapse in vivo imaging

for 20 hours was performed to analyze detailed cellular behaviors of 4 cell groups in regard to proliferation rate, migration velocity, gathering efficiency for cluster forming. Of note, active cellular interactions between BM resident host macrophages and transplanted BM cells are observed during early engraftment. In addition, we successfully monitored myeloid lineage clusters in a longitudinal manner, which revealed most of them maintained myeloid lineage during early engraftment. We believe in vivo imaging of transplanted BM cells in early BMT stage can be further extended to investigate unknown cellular mechanisms involved in pathological complications of BMT.

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P-312

NEURONAL DIFFERENTIATION OF HUMAN STEM CELLS FROM APICAL PAPILLA INDUCED BY EMBRYONIC CHICK AUDITORY BRAINSTEM SLICES CONDITIONED MEDIUM

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Sensorineural hearing loss is caused by the degeneration of the cochlear hair cells and spiral ganglion neurons (SGNs). The mammalian cochlear lacks the capacity to regenerate these cells. Currently, there are no biological therapies to rescue the dying sensorineural cells or regenerate these cells. Previous studies also showed the possibility of various types of stem cells to differentiate into auditory neurons. Recent studies reported the potential of the dental stem cells by using exogenous neurotrophins for neuronal differentiation into the spiral ganglion-like neurons. Therefore, dental stem cells are the best candidate for the restoration of hearing deficiencies through cell-based therapy. In the present study aim to investigate neuronal differentiation of the human dental stem cells from apical papilla (hSCAPs) by using conditioned medium from chick auditory brainstem slices (ABS-CM). Previous studies demonstrated that brain slice conditioned medium contains BDNF and GDNF that induce survival and differentiation into neurons of the boundary cap cell. The hSCAPs were isolated and characterized as the Mesenchymal stem cells (MSCs) through cell surface molecule analysis (CD73+, CD90+, CD105+, CD146+, CD34-), colony forming unit (CFU) and osteogenic, adipogenic differentiation. The embryonic chick auditory brainstem slices at embryonic day 12 (E12) were dissected and collected the conditioned medium. The experiment was performed the ABS-CM treatment for 1 and 2 weeks with 1:1 ratio of ABS-CM/αMEM 1% Penicillin/Streptomycin compared with control medium (αMEM 1% Penicillin/Streptomycin). The immunocytochemistry staining with the neurofilament-low type (NF-L) and DAPI (nuclei staining) showed the neuronal differentiation rate was highly detected in the ABS-CM at 2 weeks when compared to control group and 1 week treatment. Therefore, the preliminary results indicated that the ABS-CM could induce the neuronal differentiation of the hSCAPs.

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AN OPTIMIZED METHOD FOR MANUAL HUMAN ADIPOSE-DERIVED STEM CELLS ISOLATION

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Adipose-derived stem cells (ADSC) are a potential source of autologous mesenchymal stem cells in the growing field of regenerative medicine as they can be reliably obtained in large quantities by lipoaspiration under local anesthesia, with minimal discomfort. Here we describe an optimized method for manual ADSC isolation based on the mechanic and enzymatic digestion. Human adipose tissue was obtained by suction-assisted lipectomy and processed within

the next three hours. The lipoaspirate was extensively washed with PBS to remove contaminant oil and hematopoietic cells. The sample was disaggregated with a scalpel in a 100mm Petri dish to eliminate any larger fragments and then minced with scissors in a 50 ml conical tube until it acquires a homogenous appearance. The tissue was incubated with 0.01% collagenase solution at 37°C for 30 minutes. The upper layer was discarded and control media (CM, high glucose DMEM supplemented with 10% FBS), was added to the lower liquid phase to neutralize the enzyme activity. The mixture was centrifuged at 1200g for 10 minutes at room temperature (RT) to separate and discard the floating mature adipocytes from the pelleted stromal vascular fraction (SVF). The SVF pellet was resuspended in PBS, and red blood cells lysis was performed for 10 minutes at RT with 160 mM NH4Cl. After centrifugation, the SVF was resuspended in 5 ml of CM and filtered through a 100 μm nylon mesh filter to remove any remaining tissue fragments. Hemocytometer counting with trypan blue staining was performed to assess cellular density and viability, resulting in cell yield of 1.17E+06 ± 6.51E+05 viable cells per cubic centimeter of lipoaspirate (n=12). Cells were plated in tissue treated T-75 flasks and incubated in the humified atmosphere at 37 °C and 5% CO2 until 90% confluence was reached. Isolated ADSC were CD73(+), CD90(+), CD45(-), and CD34(-). Cellular plasticity was validated through in vitro chondrogenic differentiation, a high-density micromass pellet was observed after 12 days of micromass culture in the presence of lineage-specific induction factors. Furthermore, gene expression of the chondrogenic genes SOX9, COL1A1 and ACAN was evaluated at days 6, 12, and 18.

Funding Source: Conacyt.

P-314

BIOINSPIRED RGD-ENGINEERED BACTERIOPHAGE NANOFIBERS PROVIDE A VASCULAR NICHE WITH ANTIOXIDANT FUNCTION

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Instructive biomaterials provide a vascular niche and protect oxidative stress in injured tissue. Herein, we demonstrated that Arg-Gly- Asp (RGD)-engineered bacteriophage nanofibers provide a vascular and anti-oxidant niche, thereby having cytoprotective functions against cellular oxidative stress. These bio-inspired RGD-engineered bacteriophage nanofibers can serve as a novel therpeutic platform for ischemic diseases.

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ANGIO-PHAGEL: PHAGE BASED ANGIOGENIC ENTRACELLULAR MATRIX NICHE ENGINEERING

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Although stem cell niche plays a vital role in stem cell differentiation towards different lineages, an artificial stem cell niche achieved so far is not successful to fulfill the complex microenvironment of the stem cell. Here, we demonstrated engineered hybrid phage matrices that possess cell adhesive and angiogenic peptides with a suitable scaffold by formulating polyacrylamide hydrogel incorporating phage in different stiffness to guide adult stem cells (ASC) and could achieve higher stiffness favoring osteogenesis and lower stiffness favoring adipogenesis. In this study, we present a specific phage based angiogenic matrices by modulating physical and biochemical cues in differentiation of ASC, providing convenient artificial stem cell niche.

Funding Source: This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Korea (H16C1067).

P-316

MOLECULAR, SPATIAL, AND FUNCTIONAL SINGLE-CELL PROFILING OF THE MOUSE PLACENTA

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The placenta is an organ that is crucial for successful pregnancy and health of the developing fetus. However, the cellular architecture of placenta is poorly understood. Here, we developed experimental and computational methods to reconstruct the molecular identity, cis-regulatory networks, spatial organization, and function of distinct cell types at single-cell resolution. We integrated single-cell RNA- sequencing, single-cell ATAC-sequencing and single-cell spatial transcriptomics to create a molecularly annotated and spatially resolved single-cell atlas of the placenta. This serves as a foundation for further studies of placental development.

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A NOVEL IMMUNOMODULATORY MECHANISM DEPENDENT ON ACETYLCHOLINE SECRETED BY HUMAN BONE MARROW-DERIVED MESENCHYMAL STEM CELLS

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Mesenchymal stem cells (MSCs) are used to treat autoimmune or inflammatory diseases. Our aim was to determine the immunomodulatory mechanisms elicited by MSCs during inflammation. We cocultured MSCs with peripheral blood mononuclear cells for a mixed lymphocyte reaction or stimulated them by phytohemagglutinin. Morphological changes of MSCs and secretion of acetylcholine (ACh) from MSCs were measured. The effects of an ACh antagonist and ACh agonist on lymphocyte proliferation and proinflammatory-cytokine production were determined. The inflammatory milieu created by immune-cell activation caused MSCs to adopt a neuronlike phenotype and induced them to release ACh. Additionally, nicotinic acetylcholine receptors (nAChRs) were upregulated in activated peripheral blood mononuclear cells. We observed that ACh bound to nAChR on activated immune cells and led to the inhibition of lymphocyte proliferation and of proinflammatory-cytokine production. MSC-mediated immunosuppression through ACh activity was reversed by an ACh antagonist called alpha-bungarotoxin, and lymphocyte proliferation was inhibited by an ACh agonist, ACh chloride. Our findings point to a novel immunomodulatory mechanism in which ACh secreted by MSCs under inflammatory conditions might modulate immune cells. This study may provide a novel method for the treatment of autoimmune diseases by means of MSCs.

Stem Cell Metabolism and Homeostasis

P-401

ROLE OF SIRT2 TO MAINTAIN MUSCLE STEM CELL POPULATION AND SUSTAIN METABOLIC HOMEOSTASIS

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Sirtuins which contain seven species are known as NAD+-dependent protein deacetylases and their functions are related to numerous biological functions. Sirtuin2 (SIRT2), one of these sirtuins which is found in cytoplasm and nucleus, is associated with the regulation of numerous aged-related biological functions such as cellular metabolism and cell cycle. Also, SIRT2 is known to be a critical factor to sustain stemness of pluripotent cells. In pathological conditions in aging-related sarcopenia, the amount of lean muscle is decreased with the increased level of intramuscular adipose tissue. Skeletal muscle and liver functions with close relationship in metabolic regulation and aging is one of the important factor to dysregulate homeostasis of both organs. Damaged skeletal muscle regenerate new muscle from muscle stem cells, satellite cells, and to maintain the population of satellite cells is critical

for appropriate muscle regeneration. In this study we investigate the role of SIRT2 in the regulation of muscle stem cell and homeostasis of liver and muscle by analyzing SIRT2 knockout (SIRT2 KO) mice. Aged SIRT2 KO mice showed enhanced fat deposit in liver and reduced intermuscular adipocyte and intramyocellular lipid accumulation compared to WT mice. In addition aged SIRT2 KO muscle showed lower Pax7 expression suggesting reduced level of satellite cells. In conclusion, SIRT2 plays a role in the aging process via regulating homeostasis of liver and muscle and the population of satellite cell. Also, lower level of adipocyte accumulation of in SIRT2 KO muscle suggests abrogated metabolic process and reduced fibro adipogenic precursor cells in muscle.

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P-402

A NEW METHOD FOR EVALUATING STEM CELL QUALITY BY MEASURING CELLULAR REDOX CAPACITY WITH FRESHTRACER, A FLUORESCENCE PROBE FOR GLUTATHIONE

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Stem cells are located in the hypoxic niche and dependent on glycolysis, suggesting that redox homeostasis plays a critical role in the regulation of their fate: self-renewal, differentiation, and proliferation. Glutathione (GSH) is the most abundant antioxidant in cells, and thus intracellular GSH level has been used to assess the redox capacity. However, measurement of GSH level to estimate redox capacity in live cells is hindered due to the lack of proper imaging probe. Previously, we have developed FreSHtracerTM, which is a reversible and ratiometric fluorescence probe for GSH and showed that cellular GSH levels are dynamically changed in response to oxidative stress. In this study, we established the method for measuring GSH regeneration capacity (GRC) by monitoring the changes of GSH level in a single cell after exposure to H2O2 using confocal microscopy. Moreover, we also measured GSH mean level (GM) and GSH level heterogeneity (GH) in cell populations using flow cytometry. Using these three parameters, we could estimate and compare redox capacity between cell populations. This simple, rapid and quantitative method could be used not only to elucidate the mechanism of GSH homeostasis in stem cells but also to find more effective antioxidant agents.

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P-403

THE SIGNIFICANCE OF MITF IN FAR-INFRARED-PRECONDITIONED RAT BONE MARROW-DERIVED STEM CELLS FOR CELL SURVIVAL

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Bone marrow-derived stem cells (BMSCs) have been broadly investigated for treatment of ischemic heart diseases. However, there are many uncertainties that are prevailing against bench-to-bedside research related to BMSC-based therapy: like the optimal rout of cell transplantation, appropriate dosage, duration, safety, and efficacy of applications. Herein, we focus on a strategy for improving the low survival rate of BMSC after transplantation. To achieve this, we extend our previous study about the effects of preconditioning with far-infrared irradiation (FIR) on survival of BMSCs under condition of metabolic stress, such as oxidative stress, low temperature, and ischemic hypoxia condition. BMSCs were isolated and harvested from femur bone marrow of 6-week-old male Sprague-Dawley rat. To determine the effects of a FIR generator with an energy flux of 0.13 mW/cm2 on viability of rat BMSCs following metabolic stress, survival of BMSCs was measured by crystal violet staining and PI staining. FIR preconditioning

was observed to significantly increase BMSC survival against H2O2, low temperature, and ischemic hypoxia condition. Of note, qRT-PCR and Western blot analysis demonstrated that FIR induced microphthalmia-associated transcription factor (MITF), BCL2, HIF-1α, mTOR and CD63 at mRNA and protein levels. It is well known that the mTOR or MITF is a master regulator of various important cellular responses, including protein synthesis, cellular growth, proliferation, autophagy, lysosomal function, and cell metabolism. In agreement with these observations, MITF-depleted BMSCs or rapamycin-treated BMSCs is decreased proliferation and survival of preconditioned BMSC by FIR- associated with up-regulation of MITF and mTOR. Overall, our results demonstrated for the first time that preconditioning with FIR can open new insights to help therapeutic efficacy of BMSCs, and the expression of MITF and mTOR-mediated cellular response is a key to understanding its role in survival or death of BMSCs after transplantation.

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P-404

TREATMENT OF THE TELAGLENASTAT (CB-839) LED TO THE ENHANCED PROLIFERATION AND THE STEMNESS PROPERTIES OF HUMAN PLURIPOTENT STEM CELLS

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Pluripotent stem cells (PSCs) and cancer cells share similar proliferative and metabolic activities and also show quite different proliferation and metabolism properties dependent on the various environmental factors. In PSCs and cancer cells, glycolysis was reported to work as main metabolic pathway, in which the pyruvate is converted to lactate that recycles the nicotinamide adenine dinucleotide (NAD+) required for the rapid continuation of glycolysis. Moreover, in case of cancer cells, one of the major nutrients that promote the growth of many cancers was studied to be an amino acid, glutamine which is the most abundant amino acid in plasma. In our study, we checked the impact of the glutaminase 1 (GLS1) inhibitors, telaglenastat (CB-839), bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl) ethyl sulfide (BPTES), and 6-diazo-5-oxo-L-norleucine (DON) on the proliferation of various cancer cell lines, patient- derived cancer stem-like cells (CSLCs), and several stem cells, including Wharton's Jelly-derived MSCs (WJ-MSCs), adipose-derived MSCs (AD-MSCs), human embryonic stem cells (hESCs), and human induced PSCs (hiPSCs). Our data showed that CB-839, BPTES and DON significantly suppressed the proliferation of breast cancer cell line, MDA-MB231. Moreover, CB-839 treatment markedly inhibited the proliferation of breast cancer cell line MCF-7, CSLCs, and neuroblastoma, SH-SY5Y cell. CB-exposed MSCs did not show a significant alteration in cell proliferation or migration properties. Interestingly, treatment of CB-839, but not BPTES and DON resulted in apparent promotion of the proliferation of hESCs and hiPSCs and the CB-839-treated hiPSCs showed upregulation of the stemness markers and increase in alkaline phosphatase (ALP) and high number of the colony forming units. We also found that treatment of CB-839, but not BPTES and DON led to enhanced phosphorylation of extracellular signal-regulated kinase (ERK) and AKT. CB-839 treatment resulted in downregulation of the expression level of activating transcription factor 4 (ATF4), which was reported to induce apoptosis, cell-cycle arrest, and senescence. Taken together, we could find that CB-839 can be used for enhancing the proliferation and stemness properties of PSCs by GLS1-independent mechanism via modulating the kinase and ATF4 pathways.

P-405

INCREASED SREBP1 EXPRESSION BY PLACENTA-DERIVED MESENCHYMAL STEM CELLS ATTENUATE ADIPOGENESIS IN A RAT MODEL OF BILE DUCT LIGATION

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The liver modulates central lipid metabolism including phospholipid transport, fatty acid synthesis and oxidation. Impaired bile secretion, caused by liver damage, disrupts hepatic lipid metabolism. Placenta-derived mesenchymal stem cells (PD-MSCs) have their distinctive features such as ease of accessibility,

abundant cell numbers, multi-potency and strong immuno-suppressive properties. We reported that PD-MSCs rehabilitate hepatic lipid metabolism and mitochondrial function in a rat model of bile duct ligation (BDL). However, the mechanism by PD-MSCs in hepatic failure has not been elucidated. Therefore, our objectives of this study were to analyze the expression of key factors related to lipid metabolism and fatty acid oxidation through PD-MSCs transplantation in BDL rat model. Lipid accumulation through Oil Red O staining in rat liver tissues of BDL was alleviated in transplanted PD-MSCs (Tx) group compared with non-transplanted (NTx) group ($p<0.05$). The serum levels of triglycerides, total cholesterol, and LDL were decreased in Tx group versus NTx group, whereas HDL level significantly was increased in Tx group ($p<0.05$). While the expression of genes related to adipogenesis was drastically declined, the key factors of lipid metabolism and mitochondrial β-oxidation significantly increased in Tx group than NTx group ($p<0.05$). In vitro model treated oleic acid (OA) and palmitic acid (PA) in rat hepatocytes, the area of lipid droplets in cytoplasm and the expression of genes related to adipogenesis were significantly decreased in co-cultured PD-MSCs group compared to control ($p<0.05$). However, the mitochondrial β-oxidation was increased in PD-MSCs co-cultured group than control. These findings suggest that PD-MSCs attenuate lipid accumulation and enhance lipid metabolism as well as fatty acid oxidation in hepatic failure rat model.

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P-406

MATERNAL EXPOSURE TO ATMOSPHERICALLY RELEVANT PARTICULATE MATTER DURING PREGNANCY INDUCES PROGRESSIVE SENESCENCE OF HSCS UNDER PREFERENTIAL IMPAIRMENT OF THE BONE MARROW MICROENVIRONMENT

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The embryo, which has various fetal hematopoietic sites for primitive hematopoietic stem cells (HSCs), is susceptible to tiny amounts of external stress. We investigated how maternal exposure to atmospherically relevant particulate matter with a diameter less than 2.5 μm (PM2.5) affects HSCs during pregnancy. When pregnant mice inhale PM2.5, it induces oxidative stress and inflammation in the lungs of their fetuses. Maternal PM2.5-exposed offspring at 6 months exhibited HSC senescence-associated phenotypes via the ROS- p38 MAPK pathway including impairment of the bone marrow (BM) microenvironment and other age-related phenotypes. Furthermore, PM2.5-exposed offspring at 1 year of age were more likely to develop a myeloproliferative disease. The impairments caused by PM2.5 only occurred via maternal exposure, but not by adolescent exposure. This study demonstrates that maternal exposure to PM2.5 during pregnancy is much more harmful than exposure during adolescence, due to modulation of the BM microenvironment- associated HSCs.

P-407

SIRT1 ENHANCES THE SURVIVAL OF HUMAN EMBRYONIC STEM CELLS BY PROMOTING DNA REPAIR

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Human embryonic stem cells (hESCs) hold great promise for the treatment of many currently incurable

diseases through cell replacement therapy. Sirtuin1 (Sirt1), an NAD+-dependent class III histone deacetylase, is abundantly expressed in hESCs and has been reported to play a role in regulating early differentiation and telomere elongation. We found that blocking the function of Sirt1 in hESCs induced massive cell death, thus leading us to hypothesize that Sirt1 is required for hESC survival. Either blocking the function or decreasing the level of Sirt1 dramatically promoted cell death in hESCs, but not in differentiated cells such as fibroblasts. Sirt1 inhibition-mediated cell death was preceded by increased DNA damage. Our detailed mechanistic study showed that the increased DNA damage caused by Sirt1 down-regulation was at least partially due to decreased levels of DNA repair enzymes such as MSH2, MSH6, and Apex1. Furthermore, we observed p53 activation followed by the overexpression of PUMA and BAX, two pro-apoptotic p53 target genes, after Sirt1 inhibition. Owing to these events, apoptotic cell death was induced in hESCs in the absence of Sirt1, thus suggesting that Sirt1 acts as a guardian of pluripotent stem cells. Together, our results demonstrated that Sirt1 is required to maintain a high level of the DNA repair proteins MSH2, MSH6, and Apex1 and to prevent massive hESC death. This study provides valuable insights into the mechanism of Sirt1-mediated hESC survival and should contribute to the development of safe and effective cell replacement therapies.

Tissue Engineering and Organoids

P-501

IONIZED COLLAGEN HYDROGELS USED FOR LONG-TERM 3D SPHEROID CULTURE OF FUNCTIONAL INSULIN-PRODUCING CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Pancreatic islet transplantation is a fundamental treatment for insulin dependent diabetes. However the donor shortage is a big huddle to became a standard teatment. Insulin producing cells (IPCs) differentiated from stem cells or adult cells is actively developing as a new islet source but the physiological function of IPC is still not enough to control diabetics in vivo. In this study, to improve the differentiation function of IPC, we fabricate 3-dimension (3-D) IPC spheroids using concave microwell. The 3D structure was enhanced the differentiation efficiency through cell-cell interaction and to have a similar shape to the actual pancreatic islets. Also, we cultured 3D IPCs in ionized collagen hydrogel for long-term culture of functional IPCs. The iPSCs were expanded in vitro and differentiated to IPC throughout the differentiation process using small molecules. To improve the differentiation efficiency and cell viability, we fabricated 3-D IPC spheroids using concave microwell, which was possible to mass- produce spheroids of the desired size. The next day, IPC construced 3-D spheroid. For long-term culture we collected IPC spheroid from mold and cultured in various conditions, including suspension culture, collagen hydrogel culture, and laminin matrix hydrogel culture. Ionized collagen was prepared using the esterification technique of atellocollagen. Laminin matrix was used Cultrex Reduced Growth Factor Basement Membrane Matrix (BME Type2). We characterized physical function of hydrogels by Proteomics analysis and structure. In IPC spheroids in collagen hydrogel was detected more than 500 times insulin secretion than 2-D culture condition. All 3-D culture groups except 2-D culture group showed glucose stimulated insulin secretion. Beta cell related transcription factors and endocrine hormone gene expression was increased in 3-D culture groups. Interestingly, exocrine hormone and duct cells related genes was decreased in collagen hydrogel comparing to BME hydrogel. Although, laminin-based matrix is the most commonly used material for 3-D hydrogel culture, clinical application was difficult and main material, laminin, promoted duct differentiation. IPC spheroids in collagen hydrogel enhanced differentiation efficacy of IPCs in vitro.

P-502

TISSUE ENGINEERING AND ORGAN FUNCTION REGENERATION BY 3D SPHEROID CULTURE OF MESENCHYMAL STEM CELLS

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For cell physiology and behavior, cells in 3D spheroid culture system may better mimic conditions of our body than two-dimensional cultures. Though ongoing research is attempting to the applications of stem cells to the clinical trials in severe intractable diseases, further understanding on their efficacy and optimal condition for therapeutic effect are required. In the terms of tissue engineering, we used a combination of cells, materials engineering, and suitable biochemical and physicochemical factors to improve or replace biological functions of tissues or organs. Here, we developed 3D spheroid culture system of various cells on the hemispherical concave microwell chips homemade of polydimethylsiloxane for tissue regeneration and regenerative medicine. First, we reported that tonsil-derived mesenchymal stem cells are successfully differentiated into the target cells, releasing parathyroid hormone and demonstrating the therapeutic role in hypoparathyroidism. Second, we present therapeutic effect of placenta-derived mesenchymal stem cells on a rat model with ovary dysfunction, restoring ovary function through increased estrogen production as well as folliculogenesis by spheroid forms of stem cells. These findings suggest new insights into further understanding of stem cell-based therapeutic mechanisms for tissue and organ function regeneration and would demonstrate new avenues to develop more efficient therapies using biomaterials and new 3D organoid culture system.

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P-503

BIOMIMETIC HETEROGENEOUS TISSUE MODELS FABRICATED BY THREE-DIMENSIONAL BIOPRINTING TECHNOLOGY

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Human tissues are extremely heterogeneous and comprised of different cells. In particular, the hepatic lobule in the human liver tissue is a building block of the liver parenchyma, consisting of a portal triad, hepatocytes arranged in the liver cord within a sinusoid network, and a central vein. The cross-sectional diameter of a hepatic lobule is approximately 1 mm, while the diameter of the sinusoid is approximately several tens of micrometers, and the size of the whole liver is several tens of centimeters. Therefore, a printing technique for the liver tissue should be able to reproduce structures at a wide size range from the micrometer to centimeter scale. Current precision bioprinting methods such as inkjet-based printing and laser-assisted printing have a high resolution; however, their applicability to the fabrication of large organs is limited. By contrast, while extrusion-based printing can help fabricate human-scale tissues, its resolution is not sufficiently high to reproduce microscale structures such as the sinusoid. In this study, we developed a pre-set extrusion bioprinting method that allows for the in-situ fabrication of heterogeneous artificial tissue-like structures. Various structures such as the spinal cord, hepatic lobule, capillary, blood vessels, and even an 'S'-shaped object were fabricated heterogeneously to confirm the feasibility of pre-set extrusion bioprinting. Moreover, a tetramerous structure was fabricated by both pre-set extrusion and conventional bioprinting to compare cell viability with the two techniques. Further, endothelial cells (ECs) and HepG2 cells were heterogeneously co-printed by pre-set extrusion bioprinting, and the cell viability, proliferation, and enzyme activity of CYP3A4 in both groups (homogeneous or heterogeneous cell printing) were evaluated

in parallel.

P-504

ARTIFICIAL CORNEA FOR ULTRA-THIN DESCEMET'S STRIPPING ENDOTHELIAL KERATOPLASTY USING STEM CELLS

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Loss of corneal endothelium sometimes lead to incurable eye diseases including corneal edema, vision impairment, inflammation and pain. Although corneal transplantation is the ultimate treatment for the diseases, many patients are on the waiting lists due to the shortage of donor cornea. Moreover, Descemet's (DM) stripping endothelial keratoplaty have advantage of low risk of graft rejection than penetrating keratoplasty. Here, we produced bioengineered ultra-thin DM strips and endothelial layers using porcine cornea and human induced pluripotent stem cell–derived corneal endothelial-like cells (iPS-CEC). For producing ultra-thin DM strips from porcine cornea, decellularization of porcine cornea were done for 5 hours using 0.1% SDS and then manually separate DM strips. Finally, the strips were decellularized additional 5 hours, washed and sterilized. The thickness of the ultra-thin DM strips were less than 100μm. Next, we seeded 5x103 iPS-CEC/mm2 on the DM and the recellularized cornea were cultured. The xenogenic antigens of porcine cornea including galactose-alpha-1,3-galactose, swine leukocyte antigen and porcine endogenous retrovirus were disappeared but cornea derived extracellular matrices such as collagen I and IV were maintained. The reseeded cells were distributed over DM. Moreover, gene and specific marker expression were confirmed using RT-PCR and immuno-staining. Although future clinical studies are needed, engineered DM and endothelial tissues using stem cells will be useful tools for the treatment of incurable corneal diseases.

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P-505

INDUCED OSTEOGENESIS IN PLANTS DECELLULARIZED SCAFFOLDS

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Ectopic calcification occurs frequently in human body, yet it is not well defined about tropism and major determining factors. A three- dimensional (3D) culture system is essential to generate bone-like structures in vitro that accurately represent the in vivo microenvironment of a calcification. Nature's plants have 3D cellulose structure, plausible alternative scaffold for osteoblast culture and differentiation. In this study, we aimed to generate bone-like tissue using human induced pluripotent stem cell (hiPSCs) in nature's plant scaffold. We cultured and differentiated hiPSCs into osteoblasts inside of a decellularized various fruits scaffold. Mineralization was evaluated by osteogenic differentiation marker. Apple scaffold containing regular pores with diameter of 300μm was most calcification-friendly. This bone-like tissue was implantable in a calvarial defect rat model and helped to form mineralized tissue. We could generate mineralized bone tissue in simple nature's apple scaffold without sophisticated technique. Regularity and specific size of scaffold pore may affect bone formation and mineralization.

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P-506

MODELING OF GLIOBLASTOMA USING BRAIN ORGANOID

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Glioblastoma multiforme (GBM) is the most devastating cancer that represent 15% of brain tumors. With standard treatment, median survival for adults is approximately 12 to 15 months and less than 3% of patients are known to survive more than five years. Standard treatments for GBM include surgery, chemotherapy and radiotherapy. Although temozolomide (TMZ) is often used as part of chemotherapy, most patients eventually die within a short period of time. Currently there has not been much research done by a proper GBM model to imitate the human microenvironment. To address this problem, we have established a 3D brain organoid system as an in vitro model of GBM. Our GBM model is expected to provide a unique opportunity to study mechanistic details of etiopathophysiology of GBM and to screen and validate efficacy of drugs to treat patients suffering from GBM.

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P-507

CHARACTERIZATION OF NEURO-ORGANOID DERIVED FROM PIG EMBRYONIC STEM CELL LINE

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Although the human brain is an ideal model for studying human neuropathology, it is difficult to culture in in vitro that genetically engineer healthy or diseased brain tissue. Therefore, it is an inadequate model for studying molecular mechanisms responsible for neurological diseases. In this study, we established two porcine embryonic stem cell(pES) lines. These pES lines contained brain tumor-inducing genes and were confirmed by PCR. These pES lines were used to induce neural differentiation to produce the in vitro brain tumor model. pES were cultured using the SFEBq(serum-free floating culture of embryoid-body(EB)-like aggregates with quick reaggregation) method. The SFEBq culture method recreates the process of in situ generation by using the phenomenon (self-organization) that the cell group makes a spontaneous orderly structure. Neural organoids were formed through neural induction, neural patterning and neural expansion stages. They were cultured in vitro for up to 61 days. The expression of Dopaminergic Neuronal Marker(TH) and Mature Neuronal Marker(MAP2) was confirmed at the 61st day by PCR. Expression of MAP2 was also confirmed in neural cells. On day 61, the organoid was cryosectioned and immunostained. As a result, expression of Mature Neuronal Marker(-MAP2), Neural Stem Cell marker (PAX6), Neural Progenitor Markers (S100, SOX2) and Early Neuronal Markers (Nestin) was confirmed. In conclusion, we have formed neuronal organoids derived from porcine embryonic stem cells in in vitro. This protocol can be used as a tool to develop in vitro models for drug development, patient-specific chemotherapy, and human CNS disease studies.

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P-508

IN VITRO 3D LIVER MODEL UTILIZING HUMAN IPSC INDUCED HEPATOCYTES FOR DRUG TESTING

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Chronic liver diseases including liver cirrhosis have

been one of the highest leading causes of death in Hong Kong and in other developed countries for these decades, yet no cures have been found as the current drug discovery process relies on cells growing on petri dishes, which is lack of representativeness to their in vivo counterparts. Cells growing in monolayer lack the cell-interactions and cell-ECM interactions, which contributes to the high failure rate of preclinical and clinical studies. Our study aims at creating a novel vascularized 3D liver model using human induced pluripotent stem cells (iPSCs) induced hepatocytes and other non-parenchymal and stromal cell types, including hepatic stellate cells, mesenchymal stem cells and endothelial cells. Parenchymal and non-parenchymal cell types are firstly co-cultured in an organoid manner, after the induction of vascularization, organoids are then further fabricated into a large and functional "liver tissue" with the help of a novel biocompatible bioink. Liver models utilizing iPSC-derived cells allow drug screening which demonstrates patient-specificity, as well as future clinical applications, such as organ transplantation for chronic liver disease patients, with minimal level of immune response.

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P-509

CHARACTERIZATION AND ISOLATION OF BOVINE LGR5+ INTESTINE STEM CELLS TOWARD THREE-DIMENSIONAL (3D) ORGANOID

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Three-Dimensional (3D) organoid culture holds great promise for regenerative medicine as well as disease modeling based host-pathogen interactions and drug toxicity in livestock. However, very limited studies are available on characterization and isolation of bovine intestine stem cells compared to rodents or human. Recently, significant efforts have been made to establish an in vitro validation system for 3D organoid culture of bovine intestine stem cells for replacement of animal experiments and practical applications, including stem cell transplantation and nutrition physiology. In this study, Lgr5+ intestine stem cells from different bovine intestine in adult were characterized with several specific markers in vivo, and sequentially isolated. Furthermore, Lgr5+ intestine stem cells were cultivated and the expression of specific markers and functionality was maintained during several passages of culture. Collectively, these results first time demonstrate the efficient isolation and characterization of bovine Lgr5+ intestine stem cells and 3D organoid culture. Finally, culturing derived bovine Lgr5+ intestine stem cells in 3D has opened up new possibility for the exploration of nutrition physiology, toxicity and the system development of fatal disease modeling approaches in agriculture.

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P-510

GENERATION OF INSULIN-PRODUCING CELLS FROM MOUSE SMALL INTESTINE ORGANOIDS

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Organoids, a multi-cellular and organ-like structure cultured in vitro, can be used in a variety of fields such as disease modeling, drug discovery, or cell therapy development. Small intestinal organoids have become an important tool to study crypt homeostasis, cell fate dynamics and tissue biomechanics. The purpose of this study was to establish the organoid culture conditions in mouse small intestinal crypts and to generate of insulin-producing cells from intestinal organoids. Crypts were isolated from jejunum of C57BL/6 mouse. Two hundred crypts were cultured in organoid medium with either epidermal growth factor/Noggin/R-spondin1 (ENR). For generation of insulin-producing cells from intestinal organoids, we used adenoviruses vector carrying a construct Ngn3/Pdx1/MafA/mCherry (Ad-NPM). The intestinal organoids were transplanted under the kidney capsule of normoglycaemic C57BL/6 mice. Long-term culture conditions were established using the organoid culture technique in the small intestine of mouse. We observed the mRNA expression of intestinal markers in the small intestinal organoids using RT-PCR. And we identified the protein expres-

sion of Lgr5 (intestinal crypt stem cell marker) and chromogranin A (enteroendocrine cells marker) in the small intestinal organoids by immunofluorescent staining. In vivo study, we confirmed that insulin expression cells were observed in the transplanted graft over two and four weeks. Our results thus demonstrate that the intestine is an accessible and abundant source of insulin-producing cells.

P-511

MODELLING G2019S-LRRK2 SPORADIC PARKINSON'S DISEASE IN 3D MIDBRAIN ORGANOIDS

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Recent advances in generating 3 dimensional (3D) organoid systems from stem cells offer new possibilities for disease modeling and drug screening because organoids can recapitulate aspects of in vivo architecture and physiology. In this study, we generate isogenic 3D midbrain organoids with or without a Parkinson's disease-associated LRRK2 G2019S mutation to study the pathogenic mechanisms associated with LRRK2 mutation. We demonstrate that these organoids can recapitulate the 3D pathological hallmarks observed in patients with LRRK2-associated sporadic Parkinson's disease. Importantly, analysis of the protein-protein interaction network in mutant organoids revealed that TXNIP, a thiol-oxidoreductase, is functionally important in the development of LRRK2-associated Parkinson's disease in a 3D environment. These results provide proof-of-principle for the utility of 3D organoid-based modeling of sporadic Parkinson's disease in advancing therapeutic discovery.

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P-512

ENHANCED DIFFERENTIATION AND MYELINATION OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED OLIGODENDROCYTES ON ALIGNED BRAIN EXTRACELLULAR MATRIX

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Myelination by oligodendrocytes (OL) is a key developmental milestone in terms of the functions of the central nervous system (CNS). Thus, demyelination caused by defects in OL consider as a hallmark of several CNS-related disorders. Although a potential therapeutic strategy involves treatment with the myelin-forming cells, there is no readily available source of these cells. OL can be differentiated from pluripotent stem cells, however there is a lack of efficient culture systems that generate functional OL. In this study, we demonstrate biomimetic approaches to promote OL differentiation from human induced pluripotent stem cells (iPSCs) and to enhance the maturation and myelination capabilities of iPSC-derived OL (iPSC-OL). Our results indicated functionalization of culture substrates using brain extracellular matrix (BEM) derived from decellularized human brain tissue enhanced the differentiation of iPSCs into myelin-expressing OL. We also observed that co-culture of iPSC-OL with induced neuronal (iN) cells on BEM substrates, which closely mimics the in vivo brain microenvironment for myelinated neurons, not only enhanced myelination of iPSC-OL, but also improved electrophysiological function of iN cells. BEM-functionalized aligned electrospun nanofibrous scaffolds further promoted the maturation of iPSC-OL, enhanced the production of myelin sheath-like structures by the iPSC-OL, and enhanced the neurogenesis of iN cells. Thus, the biomimetic strategy presented here can generate functional OL from stem cells and facilitate myelination by providing brain-specific biochemical, biophysical, and structural signals. To conclude, our system comprising stem cells and brain tissue from human sources could help in the establishment of human demyelination disease models and the development of regenerative cell therapy for myelin disorders.

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P-513

DEVELOPMENT OF MATURE FOREBRAIN ORGANOIDS BY SPATIALLY AND TEMPORALLY CONTROLLING NEURODEVELOPMENTAL PATTERNING CUES

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The biggest challenge to study human brain is the lack of experimental systems that can precisely represent the complexity of human brain. Recently, with the advance of stem cell technologies, the efforts have been made to develop in vitro three-dimensional organ- like tissues called organoids, providing reliable tools to study human brain and neurological disorders. Current technology of developing brain organoids, however, is limited in that it fails to provide essential patterning cues and adequate signals to induce the proliferation of neural progenitors, which are two crucial factors for the development of normal brain. As a result, currently available brain organoids only recapitulate the early stage of human brain development, lacking laminar organization of mature cortical layers. Here, we generate human forebrain organoids that represent the mature six-layered cortical structure with increased size of each layer by spatially and temporally controlling three crucial patterning molecules, Hedgehog, Wnt, and Reelin. The pathway activities of Hedgehog and Wnt signaling are initially suppressed to induce the early lineage specification of forebrains. To expand the neural progenitor population at the later stage, the activities of the same pathways are pharmacologically increased, leading to the heightened proliferation of neural progenitors and the development of thicker cortical layers. Correct organization of developing cortex such as accurate positioning of neuronal layers is finally achieved by reconstituting brain organoids with tetracycline-inducible, Reelin expressing mesenchymal stem cells, which allow to spatially and temporally control its expression during the later stage of forebrain organoid development. Furthermore, we demonstrate the applicability of our platform to model the various neurodevelopmental disorders, such as schizophrenia, whose understanding of pathogenesis requires organoid system that is capable of representing mature characteristics of human brains including thick cortical layers and connectivity. Taken together, our work shows the effective strategies to develop mature human forebrain organoids, and will provide the strong foundation to understand various neurological disorders for the development of better therapeutic options.

P-514

SINGLE CELL TRANSCRIPTOMIC ANALYSIS OF HUMAN CEREBRAL ORGANOIDS REVEALS SKEWED NEURAL DIFFERENTIATION IN DOWN SYNDROME

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Down syndrome, caused by trisomy for chromosome 21 (T21), is the most common specific cause of intellectual disability with an incidence of approximately 1/750 births world-wide. Impaired cognition is the major disabling feature in Down syndrome and this is associated with gross regional and cellular brain abnormalities. The limited access to brain specimens have made neural derivatives from induced pluripotent stem cells with T21 an attractive in vitro model of Down syndrome. Stem cell-derived brain organoids recapitulate key stages of prenatal human cortex development, thus they make an in-depth analysis of the fetal Down Syndrome cortex possible. Single cell transcriptome analysis from T21 neural cultures and T21 organoids revealed an abnormal distribution of cell types. Radial glia populations appeared vastly under-represented in T21, in contrast vascular-leptomeningeal cells (VLMC), a meningeal cell type that forms protective membranes around the infiltrating vasculature of the brain, appeared vastly over-represented. The formation of VLMCs in organoids with and without T21 appears to be continuous and with a differentiation trajectory distinct from that of neuronal cells. We hypothesize that carrying an extra chromosome 21 induces a shift away from neural differentiation of cerebral organoids resulting in the generation of a higher proportion of vascular and leptomeningeal cells at the expense of radial glia. This could explain the reduced number and

density of cortical neurons found in the DS etiology.

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P-515

THREE-DIMENSIONAL RECONSTITUTION OF MINIATURE BLADDERS THAT STRUCTURALLY AND FUNCTIONALLY RECAPITULATE IN VIVO TISSUE REGENERATION AND CANCER

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Current organoid models are limited by their failure to account for factors, such as mature organ architecture and tissue microenvironment. Here, we reconstitute tissue stem cell-based, multilayered miniature bladders that structurally and functionally mimic mature mammalian urinary bladders. These mini-bladders recapitulate the in vivo tissue dynamics of the regenerative response to bacterial infection; heightened activity of signalling feedback between the urothelium and stroma and the associated increase in cell proliferation cause the regenerative portions of urothelium to arise from single cells through oligoclonal expansion. Further, using three-dimensional bioprinting technology, we developed multilayered tumor organoids with stroma that recapitulate the in vivo pathophysiology of patient-derived invasive urothelial carcinoma, including tumor–stroma interaction, slower drug response, immune cell infiltration and muscle invasion. Thus, our study provides a conceptual framework for the reconstitution of multilayered, functional organoids derived from tissue stem cells or tumor cells that mimic the biology of native tissues.

P-516

HIGH-THROUGHPUT MICROFLUIDIC PLATFORM FOR STUDYING VASCULARIZATION OF IPSC-DERIVED KIDNEY ORGANOIDS

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Kidney organoids derived from human induced pluripotent stem cells (iPSCs) represent a powerful in vitro model for studying kidney development, disease mechanisms and drug testing. Despite the great level of structural complexity reached in vitro, these kidney organoids are immature possibly due to the lack of a functional vascular system. Transplantation of kidney organoids under the kidney capsule of a mouse can significantly improve their maturation. However, alternative approaches are valuable for studying these processes in vitro. Microfluidic techniques show great potential in bridging the gap between 2D in vitro cultures and animal models. Here, we present the use of a high-throughput in vitro ‘grafting’ platform which allows co-culture of vessels with kidney organoids. One unit of the Mimetas Organoplate® Graft is made of two microfluidic channels in which endothelial cells can be patterned against ECM. Presence of a tissue chamber allows endothelial cell co-culture with 3D tissues. When kidney organoids are used, extensive vascular remodeling occurred with formation of a complex 3D network of angiogenic sprouts growing towards the tissue. Moreover, vessel stabilization can be monitored overtime by real time imaging and perfusion with 150 kDa Dextran. The established kidney organoid-on-a-chip system provides a promising platform for drug testing and disease modeling.

Stem Cells in Cancer and Aging

P-601

TRANSPLANTATION OF WHARTON JELLY MESENCHYMAL STEM CELLS (WJ MSC) AND NEURAL PROGENITOR CELLS (NPC) IN ALZHEIMER'S DISEASE (AD)

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Since there is no cure or effective treatment for Alzheimer's disease (AD), stem cell transplantation therapy may be able to replace the damaged neurons during the course of the disease. Previous studies have shown that Wharton jelly mesenchymal stem cells (WJ- MSCs) release factors and anti-inflammatory cytokines following transplantation, making them less vulnerable to rejection by host. However, the evidence for WJ- MSCs acquiring the phenotypes of neuro-ectodermic cells has yet to be elucidated. Neural progenitor cells (NPCs) can give rise to central nervous system (CNS)'s cell types such as neurons, astrocytes and oligodendrocytes. In the present study, WJ-MSCs (1x105) are injected into the lateral ventricles of AD mouse model mice and then 7 days later NPCs (1x105) are transplanted into the hippocampus. 4 weeks after transplantation, animal group which received both WJ MSCs and NPCs displayed higher number of alternations in Y-maze test than those which with WJ MSC or NPC transplants only. We conclude that a combinatory transplant of WJ MSCs and NPCs has therapeutic effects in AD model.

P-602

ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS INHIBIT THE GROWTH OF HEPATOCELLULAR CARCINOMA CELLS THROUGH IFN-BETA-MEDIATED JAK/STAT1 PATHWAY IN VITRO

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Adipose tissue-derived mesenchymal stem cells (ASCs) are emerging as promising anti-agents to inhibit tumor growth. However, it was not investigated that ASCs inhibit hepatocellular carcinoma cells through IFN-beta and TRAIL in vitro. In order to test the hypothesis, Huh7 human hepatocellular carcinoma cells were indirectly co-cultured with ASCs for 2 days. After indirect co-culture with ASCs, the Huh7 cells were examined for cell viability using MTT assay or trypan blue assay. Huh7 cells also were individually treated with concentrated media (CM) obtained from supernatant of cultured ASCs for 2 days with or without neutralizing antibody to IFN-beta or TRAIL. In order to investigate unveiled mechanism by IFN-beta or TRAIL, cell lysates were examined for PARP, PCNA, p53, p21, STAT1 and pSTAT1 via Western blot. Additionally, cell cycle was analyzed through flow cytometry in order to observe cell cycle arrest. Results of experiments showed that cell viability by MTT assay of Huh7 cells indirectly co-cultured with ASCs was decreased, but not apoptosis, cell viability compared to control group. Western blot analysis showed increased pSTAT1 in cell lysates of Huh7 co- cultured with ASCs as compared with untreated group. This results promoted us to make a hypothesis that IFN-beta secreted by ASC suppress cell viability through activation of STAT1 pathway in Huh7 cells. In order to test the hypothesis, firstly Huh7 cells were individually treated with concentrated media (CM) obtained from supernatant of cultured ASCs for 2 days with or without neutralizing antibody to IFN-beta and/or TRAIL. It was observed that cell viability of the Huh7 cells treated with ASC-CM alone was decreased as compared with control group. Huh7 cells co-treated with ASC-CM and neutralizing antibody to IFN-beta, but not TRAIL, showed increased cell viability com-

pared to Huh7 cells treated with ASC-CM alone. To further investigate IFN-beta-mediated mechanism, it was hypothesized that IFN-β decreases cell viability via pSTAT1-mediated p53/p21. Huh7 cells were treated with ASC-CM with or without JAK1/2 inhibitors, and it was found that ASC-CM treated Huh7 cells given JAK1/2 inhibitors showed increased cell viability with reduced p53/p21 compared with Huh7 cells given ASC-CM alone.

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P-603

ELUCIDATING THE DEVELOPMETAL DEFECT IN WERNER SYNDROME USING HUMAN EMBRYOINC STEM CELLS

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Werner Syndrome (WS) is an autosomal recessive genetic disorder characterized by premature aging. This disease is caused by mutations in the WRN gene. The first sign of WS is short stature. Individuals with WS have an abnormally slow growth rate, and growth stops at puberty. As a result, affected individuals have short stature. However, the mechanism is still not clear. To find out the cause of short stature in WS, we reprogrammed the WS patients fibroblasts and the isogenic normal control into mesenchymal stem cells and compared the transcriptome by RNA-Seq. Among the top 10 candidates, SHOX (short stature homeobox) was noted. SHOX plays an important role in chondrogenesis. SHOX deficiency is a frequent cause of short stature. So, what is the role of SHOX in WS pathogenesis? To answer this question, we induced hESCs towards chondrocytes. We analyzed the WRN and SHOX expression during chondrogenesis and noticed that the expression of both genes increased gradually, indicating a high correlation during chondrogenesis. Then, we induced the chondrocytes by the same protocol in WRN knockout (WRN-/-) or SHOX knockdown cells. We noticed that WRN loss led to the decrease of SHOX during chondrogenesis. However, SHOX knockdown has no effect on WRN, suggesting that WRN may regulate SHOX as an upstream target. To further assess the role of WRN and SHOX during chondrogenesis, we immunostained the cells for specific chondrogenic markes, SOX9 and COL2A1. Again, we noticed that WRN knockout or SHOX knockdown suppressed SOX9 and COL2A1 expression. We conclude that the loss of WRN and SHOX blocked the chondrocyte differentiation. We next asked how WRN regulated SHOX. It is reported that the WRN helicase is able to recognize G-quadruplex (G4) motif and thus altering gene transcription. So we did immunostaining to detect the prevalence of G4 in vitro. Interestingly, we found that the signals of G4 staining in WRN-/- cells was much stronger than WRN+/+ cells, indicating that WRN is able to unwind some of the G4. Then, binding of WRN to G4-containing SHOX promoter was confirmed by ChIP, whereas luciferase assays suggested that WRN could unwind SHOX G4 complex and promoted its expression. In summary, we found that the downregulation of SHOX in WS inhibited chondrogenesis at the early developmental stage, which may account for the short stature.

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P-604

A MOLECULAR SUBTYPE OF CANCER ORIGINATING FROM ADULT STEM CELLS DURING REGENERATION IS DRIVEN BY DUX TRANSCRIPTION FACTORS

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Rhabdomyosarcoma (RMS) is a rare and aggressive childhood cancer and the most common soft-tissue sarcoma in children and adolescents. Rhabdomyosarcomas are generally thought of as skeletal muscle tumors, in large part because they typically arise in or near muscle beds and show features of myogenic differentiation. However, as for many cancer types, a long standing open question is what the cellular origin of RMS is and what the driven genes. The hypothesis has been fueled that muscle progenitors or muscle stem cells could be a cellular origin of RMS. The

idea has been put forward that a somatic mutation in a physiologically healthy stem cell would give rise to a tumor propagating cell that essentially would be the source of a respective tumor. Nevertheless, purification of tumor stem cell also helps to identify novel driven genes. Employing lineage tracing and skeletal muscle regeneration as a paradigm, here we show that regeneration-based loss of muscle stem cell quiescence is necessary to elicit spontaneous acquisition of oncogenic copy number amplifications in p53 deficient stem cells resulting in 100% penetrance of rhabdomyosarcoma formation. Through genomic analyses of purified, lineage-traced tumor cells we discovered discrete oncogenomic amplifications driving tumorigenesis including, but not limited to, the homeobox transcription factor Duxbl. We show that Dux transcription factors driving embryonic/zygotic gene signatures define a molecular subtype of a broad range of human cancers. We found that Duxbl initiates tumorigenesis by enforcing a mesenchymal-to-epithelial like transition and demonstrate that targeted inactivation of Duxbl specifically in Duxbl expressing tumor cells abolishes tumor expansion. These findings suggest that a subtype of RMS is driven by Dux transcription factors.

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P-605

TOWARDS A MECHANISTIC UNDERSTANDING OF THE TUMOR SUPPRESSOR FUNCTION OF WISKOTT-ALDRICH SYNDROME PROTEIN

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Wiskott-Aldrich syndrome (WAS) is a rare pediatric disorder caused by mutations in the WAS gene. The biological features of this disease include thrombocytopenia, eczema, complex immunodeficiency, and malignancy. WAS protein (WASP), encoded by the WAS gene, is a classical actin nucleation-promoting factor. Yet, the well-known functions of WASP fail to fully explain the high rate (13%~ 22%) of cancer in children with WAS. Recently, WASP was identified as a tumor suppressor by Chiarle's group; however, the mechanism of its tumor suppressor function is not clear. Mounting evidence has already demonstrated that the ribosomal DNA (rDNA) gene inside the nucleolus is critical for genome stability, chromatin structure, and cancer pathogenesis. In addition, the perinucleolar heterochromatin shows structural alterations in cancer cells. Here, we use induced pluripotent stem cells (iPSCs) from patients with WAS (WAS-iPSC), isogenic gene-corrected cells (cWAS-iPSC), WASP knock out iPSCs, and B lymphoblastoid cell lines to study the mechanisms of WAS pathogenesis. Our results show that WASP interacts physically with partners inside the nucleolus and binds to the rDNA. Mutation cells undergo 5S rDNA copy number amplification and 45S rDNA array loss. WASP deficiency results in a higher proliferation rate, and abnormal perinucleolar heterochromatin. Taken together, our results show that WASP is important for genome stability, revealing its tumor suppressor mechanisms in blood cells.

P-606

STEM CELL SECRETORY PROTEIN MFG-E8 PROMOTES PROLIFERATION AND MIGRATION OF HUMAN HEPATOMA CELLS

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Milk fat globule-EGF factor 8 (MFG-E8) is a glycoprotein, which is secreted from various cell types, including mesenchymal stem cells, and mediates a wide spectrum of biological processes such as phagocytic clearance of apoptotic cells. MFG-E8 has also been known to be associated with different types of cancer. Although therapeutic effects of MFG-E8 in liver fibrosis has been recently revealed, roles of MFG-E8 in liver cancer invasion and progression remain largely unknown. The aim of this study was to elucidate the biological role of MFG-E8 in human hepatocellular carcinoma cells. To this end, three kinds of well-known human hepatocellular carcinoma (HCC) lines (HepG2, Hep3B, and Huh7) were investigated through gain-of-function and loss-of-function studies for MFG-E8 in vitro. Overexpression of MFG-E8 by lentiviral transfection induced EMT processes and increased the proliferation of HCCs, as determined by RT-PCR, Western blot, and MTT analyses. The enhanced proliferative capacity was abolished by the treatment with anti-MFG-E8 neutralizing antibodies. Wound healing assay showed that the migration ability of HCCs was also significantly promoted by MFG-E8 overexpression. In consistent with data obtained from overexpression study, knock-down of MFG-E8 gene by RNA interference profoundly reduced both migration and proliferation of HCCs in vitro, compared with control groups. The reduction in both migration and proliferation was rescued by the treatment with recombinant MFG-E8 protein in HCCs knocked down for MFG-E8 in vitro. These results suggest that MFG-E8 plays a critical role in liver cancer progression and may provide opportunities for the further studies of liver cancer treatment.

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P-607

EFFECT OF ASTAXANTHIN ON ENHANCED CELL PROLIFERATION AND PROTECTIVE RESPONSE AGAINST OXIDATIVE DAMAGE IN UMBILICAL CORD DERIVED MESENCHYMAL STEM CELLS VIA FOXO3 / SIRT1 AXIS

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A lipid-soluble xanthophyll carotenoid, astaxanthin (ASX) is a highly potent antioxidant compared with many vitamins and other polyphenol compounds. As a promising nutraceutical resource that is synthesized by many marine organisms, ASX has been studied on various cellular activities including anti-inflammation and anti-cancer. It has been reported that ASX could improve the proliferation of neural stem cells as well as promote neural stem cell differentiation. However, influence of ASX on human mesenchymal stem cell has not been investigated. The purpose of this recent study is to clarify the effect of ASX on umbilical cord derived mesenchymal stem cells (UC-MSCs) and to examine the underlying mechanism of ASX against H2O2 induced oxidative damage. By using real time cell analysis (RTCA) under the electrical impedance monitoring to quantify cell division, treatment of UC-MSCs with ASX exhibited a significant increase in cell proliferation index while H2O2 inhibited MSCs growth during the period 24h to 48h. Interestingly, ASX greatly improved the viability of UC-MSCs induced oxidative damage by H2O2 exposure. Furthermore, ASX treatment also led to marked activation of FOXO3 and induced co-localization of FOXO3 and SIRT1 protein under immunofluorescence assay. This indicated that ASX exerted an anti-oxidative activity under FOXO3/SIRT1 in mesenchymal stem cells.

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P-608

AN ABILITY OF PROGRAMIN 2 TO REVERSE SENESCENCE OF HUMAN BONE MARROW DERIVED MESENCHYMAL STEM CELLS

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Human mesenchymal stem cells (MSCs) are multipotent connective tissue cells that are one of the most attractive stem cell source for tissue engineering and cell therapy. MSCs possess a potential to self-renew and differentiate into various types of cells such as osteocytes, adipocytes, chondrocytes and neurons. Compared to the pluripotent stem cells these cells are also easier to harvest and free from ethical complications. MSCs have also good immunosuppressive capacity and they do not form teratomas in vivo. MSCs proliferate well in vitro but old patients' MSCs enter senescence a lot faster compared to the young patients' MSCs. This limits their use in tissue engineering and therapy since old patients are usually in higher demand of treatments. Therefore, it is crucial to enhance the proliferation capacity of these cells. In this project we tested an ability of the small molecule Programin 2 to reverse the senescence of bone marrow derived mesenchymal stem cells (BM-MSCs). Level of various proliferation markers such as phosphorylated histone H3 (PH3) and senescence associated beta-galactosidase staining were observed. In addition, an expression of proliferation associated genes such as Ki67 and SIRT1 and the level of senescence associated genes such as p21 and p16 were investigated after the Programin 2 treatment. Also the morphological changes were detected after the treatment. The results of this project have showed evidences that small molecule Programin 2 has potential to re-activate the proliferation in senescent BM-MSCs.

P-609

COMMOM AND DISEASE-SPECIFIC ALTERATION OF BONE MARROW MESENCHYMAL STORMAL CELLS IN MYELODYSPLASTIC SYNDROME AND MULTIPLE MYELOMA

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The objective of this study was to explore characteristics of bone marrow mesenchymal stromal cells (BM-MSCs) derived from patients with myelodysplastic syndrome (MDS) and multiple myeloma (MM). BM-MSCs failed in colony formation in 35.0% of patients and half of isolated BM-MSCs stopped to proliferate before passage 6. Gene expression profiles by microarray analysis demonstrated that premature senescence occurred via common molecular mechanisms including activation of ERK1 and ERK2 cascade. Patient's BM-MSCs also showed impaired hemotopoietic support, decreased osteogenesis, and increased angiogenesis. Disease-specific alterations including tendency of neurogenesis in MDS-MSCs, decreased adipogenesis, and tendency of cardiogenesis in MM-MSCs were identified. These results were validated through in vitro differentiation and ex-vivo coculture experiments. We firstly demonstrated that BM microenvironment was altered variously in each patient. Specifically, the value of CDKN2A expression in BM-MSCs measured by reverse transcription quantitative PCR was correlated with the degree of impaired proliferation activity. Further immunohistochemistry on BM biopsy showed that CDKN2A was intensely accumulated in perivascular cells of BM that showed failure in colony formation. These results collectively indicate that MDS-MSCs and MM-MSCs have common and different alterations at various degrees. Hence, it is necessary to evaluate their alteration status using representative markers such as CDKN2A expression.

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P-610

HYPOXIC HUMAN UMBILICAL CORD-DERIVED MESENCHYMAL STEM CELLS EXERT ENHANCED ANTI-CANCER EFFECTS ON HUMAN CERVICAL CANCER CELLS

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The aim of this study was to investigate whether hypoxic human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) showed anti-cancer effects on human cervical cancer cells (HeLa cells) compared with normoxic hUC-MSCs. CM from hypoxic hUC-MSCs (H-CM) strongly suppressed cell viability and induced apoptosis of HeLa cells compared with conditioned medium (CM) from normoxic hUC-MSCs (N-CM). Furthermore, H-CM increased caspase-3/7 activity, decreased mitochondrial membrane potential (MMP), and induced cell cycle arrest including increased G0/G1 phase, decreased S phase and decreased G2/M phase in HeLa cells. However, cell viability, apoptosis, and MMP of human dermal fibroblast (hDFs) were not significantly changed between treatment of N-CM and H-CM, whereas caspase-3/7 activity was decreased by H-CM. According to protein antibody array, activin A, Beta IG-H3, TIMP-2, RET, and IGFBP-3 were upregulated in H-CM compared with N-CM. In Gene Ontology (GO) analysis on intracellular signaling of HeLa with H-CM compared with N-CM, upregulated intracellular proteins in HeLa with H-CM represented apoptosis and cell cycle arrest terms of biological processes, whereas upregulated intracellular proteins in hDFs with H-CM represented negative regulation of apoptosis. In Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, upregulated intracellular proteins in HeLa cells with H-CM compared with N-CM represented cell cycle as a term with highest −log10 p value, whereas upregulated intracellular proteins in hDFs cells with H-CM represented PI3K-Akt signaling pathway as a term with highest −log10 p value. The most common protein in biological process of GO and KEGG pathways of upregulated proteins in HeLa cells with H-CM was p53 (Acetyl-Lys386). In conclusion, H-CM with upregulation of activin A, Beta IG-H3, TIMP-2, RET, and IGFBP-3 showed enhanced anti-cancer effects on HeLa cells but did not influenced on cell survival of hDFs, and induced intracellular pathway related with apoptosis in HeLa cells whereas cell survival in hDFs.

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Disease Modeling and Drug Screening

P-701

BISPHENOL-A AFFECTS TO ELECTROPHYSIOLOGY IN HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

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Bisphenol-A (BPA) is an endocrine disrupting chemical which is used on a wide range in industry. This compound has been used in the manufacture of polycarbonate plastics and epoxy resins. Although BPA is widely used in industry, it is reported taht exposure to BPA is effect on brain, behavior of animal, prostate and breast cancer, miscarriage, birth defects, diabetes and obesity in animal study. Thus, BPA safety issues was concerned. Epidemiological studies have shown positive correlations between endocrine disrupting chemical exposure and coronary artery disease, hypertension, atherosclerosis, and myocardial infarction. Recently, human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs)-based assay is increasingly used as a new model of cardiac risk assessment. Thus, we investigates the effects of BPA on cardiac risk by electrophysiological method using hiPSC-CMs. Initially, we study the effect of BPA on cytotoxicity was no effect on any concentrations up to 100 μM. Next, we investigate whether BPA affects cardiac field potential with electrophysiological parameters including field potential duration (FPD),

sodium spike amplitude (SSA), and beat per minute (BPM) in Multi-electrode array (MEA). This result from treatment of BPA with chronic showed that FPD, sodium spike amplitude and BPM were decreased in a concentration dependent manner whereas acute treatment of BPA was decreased only sodium spike amplitude. In conclusion, BPA exerted not only long-term effects but also short-term effects on cardiac field potential of hiPSC-CMs. Therefore, we suggested that BPA affects cardiac risk such as arrhythmia.

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P-702

DIFFERENTIAL EXPRESSION OF MITOCHONDRIAL MATRIX AND OXIDATIVE STRESS IN TROPHOBLAST DIFFERENTIATION OF PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELLS FROM PREECLAMPTIC PREGNANCY

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Preeclampsia is thought to begin in inadequate trophoblast invasion and deficient remodeling of uterine spiral arteries. A substantial reduction in placental perfusion provokes an ischemic placental microenvironment due to oscillations in oxygen delivery to the placenta and fetus, which results in oxidative stress. Our aim was to induce trophoblast differentiation from iPS cells and investigate candidate genes associated with possible mechanism of preeclampsia.
We generated patient-specific induced pluripotent stem (iPS) cells from human amniotic epithelial (HAE) cells of women with normal pregnancy and preeclampsia. In order to induce trophoblast, we performed feeder free method, iPS cells were directly transferred on Matrigel-coated dishes, and cultured with BMP4. Patient-specific trophoblast from iPS cells were isolated and total mRNA from each cells were prepared. We performed transcriptome analysis to investigate the candidate genes associated with the possible pathophysiology of preeclampsia. Functional annotation clustering was analyzed to be significantly up or down regulated from the trophoblasts of patients with preeclampsia using Database for Annotation, Visualization and Integrated Discovery (DAVID) software. These analyses were checked with enrichment score of >1.7 for the data set analyzed. These iPS cells were similar to human embryonic stem (ES) cells in morphology, expressed alkaline phosphatase and expression of the ES markers. Expression of trophoblast marker (KRT7) and morphology like trophoblast were confirmed. Functional annotation clustering showed down regulated clustering genes that included those involved in oxidation-reduction process (biological processes), oxidoreductase activity (molecular function) and mitochondrial matrix (cellular component). Oxidation reduction process and oxidoreductase activity-related genes, such as DHRS2, FAR2P1, PNPO, PDPR and TXNRD2, were down-regulated. Mitochondrial matrix related gene, such as NME4, also was down-regulated. Especially, we confirmed NME4 gene was down-regulated in induced trophoblasts of preeclampsia and upregulated apoptototic related genes. A mitochondrial defect triggers the impairment of differentiation and invasion of the trophoblast that leads to this disorder. Thus, we suggest that these mitochondrial matrix related gene, such as NME4, down-regulated expression may play a role in a pathogenic mediator of oxidative stress in preeclampsia.

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P-703

EVALUATION SYSTEM ON QUANTIFICATION OF HUMAN STEM CELLS ENGRAFTED INTO TARGET TISSUES OF ANIMAL MODELS BASED ON REAL TIME-PCR ANALYSIS

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Stem cell-based cell therapy is a promising tool for the treatment of various degenerative diseases, such as liver cirrhosis, neurological diseases. Their therapeutic effects are correlated to mobilization of stem cells engrafted into target tissues of animal models with diseases. Also, in vivo tracing of migrated, dispersed, and survived cells are needed to define the safety of clinical applications. Biodistribution studies were performed meaningfully in a safety study, with in vivo imaging analysis, histological analysis and real-time quantitative PCR analysis. Real-time PCR is considered as a gold standard for cell biodistribution assay. However, evaluation technology for detecting stem cells engrafted into target tissues or whole body still in needs to be developed. Therefore, the objectives of the present study are to design a set of highly sensitive specific primers and probes for real-time PCR methods that distinguish human and rodent cells, compare their expression patterns for human Alu sequences and GAPDH, and evaluate their sensitivity and specificity for quantification of human stem cells engrafted into target tissues of animal diseases model after stem cells transplantation. We designed primers for human specific Alu sequences and GAPDH and performed real-time PCR with genomic DNA from each whole organ rather than random spots for more accurate detecting of human cells, after administering into NOD/SCID mice via local or intravenous systemic injection. The biodistribution of human cells could be traced in the mouse organs and blood for at least 1 week in our system. Based on our data, the cells were clearly found at injected target organ and could not be found in any other organs. Our findings indicate that our primers and probe sets are applicable to quantitatively detect the tiny amounts of human cells xeno-transplanted in rodents. Therefore, our company-designed real-time PCR assays provide an established tool for assessing the safety and efficacy of cell therapies on preclinical examination.

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P-704

ALZHEIMER-LIKE PATHOLOGY IN TRISOMY 21 CEREBRAL ORGANOIDS REVEALS BACE2 AS A GENE-DOSE-SENSITIVE AD-SUPPRESSOR IN HUMAN BRAIN

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A rare triplication of the APP gene alone (DupAPP) imposes a 100% risk of Alzheimer's dementia (AD) by age 60, but triplication of APP as part of triso-

my 21 (T21, or Down syndrome (DS), >6 million people worldwide) causes dementia in only 70% of patients by age 60, suggesting the presence of unknown AD-suppressing genes on chromosome-21. We aimed to utilise the cellular models of T21 to identify genes and mechanisms that can modulate AD pathogenesis. Cerebral organoids were grown in vitro from isogenic T21 and normal induced-pluripotent-stem-cells (iPSCs), as well as a DupAPP patient. Trisomy of chromosome-21 gene BACE2 was corrected to disomy by CRISPR/Cas9 editing. Organoids were analysed histologically and profiled for β-amyloid secretion by immunoprecipitation-mass spectrometry (IP-MS). Tissue-quantity-independent peptide ratios were compared between organoid conditioned media (CM) and cerebrospinal fluid (CSF) from DS and age-matched euploid individuals. We found that T21, but not DupAPP, organoids secrete increased proportions of putative BACE2-θ-secretase (Aβ1-19) and BACE2-Aβ-degrading protease (AβDP or Aβ-clearance) products (Aβ1-20 and Aβ1-34) compared to isogenic normal controls. Increased ratios of BACE2-related to BACE2-unrelated anti-amyloidogenic cleavages were reproduced in CSF of people with DS, mirroring organoid secretions. We showed that BACE2 AβDP-cleavage at Leu34-Met35 is cross-inhibited by a clinically-trialled BACE1-inhibitor and detect its products intra-neuronally, and in large extra-cellular aggregates in AD-brain. Finally, CRISPR/SpCas9-HF1-reduction of BACE2 (3 to 2 copies) in T21-iPSC significantly decreased AβDP/amyloidogenic ratio and triggered early and accelerated AD-like pathology in T21 organoids. The pathology consisted of insoluble amyloid plaque-like deposits, fibrillar aggregates, pathologically altered Tau, and premature neuronal loss. Our combined data demonstrate the role of BACE2 as a genetic-dose-dependent AD-suppressor in human brain, and organoid technology as a potential assay for screening for other protective genes and disease-preventive drugs.

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P-705

AN IPS-BASED APPROACH TO STUDY THE TRANSCRIPTIONAL AND EPIGENETIC CONSEQUENCES OF X-CHROMOSOME ANEUPLOIDIES

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Klinefelter Syndrome (KS) is a multisystemic disorder associated with a plethora of phenotypic features including cardiac abnormalities, osteoporosis, infertility, gynecomastia, psychiatric disorders, diabetes and increased cancer risk. KS is the most common aneuploidy in humans (prevalence of 1:500-1:1000 born males) and is characterized by one or more supernumerary X-chromosomes (47-XXY, 48-XXXY and 49-XXXXY karyotypes). In female cells, the second X chromosome is physiologically repressed through a mechanism called X-chromosome inactivation (XCI), a process that equalizes X-linked gene dosage between the two sexes. However, few genes called "escape genes", elude XCI mechanism and are actively transcribed from both chromosomes. While in female cells the expression of the escape genes from both X chromosomes is associated to a healthy phenotype, in males affected by KS the over-dosage of the escape genes is considered the molecular landscape of the phenotypic features of the disease. Using a mRNA-based and integration-free reprogramming approach, we generated a large cohort of induced pluripotent stem cells (iPSCs) from seven KS patients' and two healthy donors' fibroblasts. Notably, the healthy controls are direct relatives of one of the KS patients, thus mitigating the transcriptional variability associated with different genetic backgrounds. Our findings indicate that the transcriptomic dysregulation associated to supernumerary X chromosomes in KS patients is already detectable at the pluripotent stage. Our iPSCs cohort overcomes the scarcity of adequate KS human models and provides an unprecedented cellular platform to study the transcriptional and developmental consequences of the escape gene overdosage, the expression of the genes located at the pseudoautosomal regions (PAR) and the parental origin of the supernumerary X-chromosome.

P-706

MOLECULAR SIGNATURES OF BIPOLAR DISORDER: A STUDY USING PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELL CULTURES

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Bipolar disorder (BPD) is a chronic psychiatric condition characterized by alternating episodes of depression and mania. The heritability of BPD is estimated at 70–85%, and genetic susceptibility loci are emerging despite each with small effects. Dysfunctions in neurotransmitter systems, several intracellular signaling and circadian rhythm, have been proposed to be associate with the onset and symptoms of BPD. To gain insights into molecular and cellular signatures of BPD neurons, we established induced pluripotent stem cell (iPSC) lines from BPD patients and produced cerebral cortical neuron-like cultures from them. We then compared genome-wide RNA expression profiles in cultured neurons derived from patients and control individuals. Subsequent gene enrichment analysis (GEA) on differentially expressed genes (DEGs) between groups revealed that gene transcripts involved in organization of cytoskeleton, neural development, movement disorders and neurodegeneration. In addition, we identified a set of DEGs responsive to valproate (VPA), which are widely used as a mood stabilizer. It should be noted that key genes constituting circadian molecular clock exhibited differential expression in neurons originated from BPD patients in well accordance with their expression patterns in postmortem human prefrontal cortex. Taken together, the present studies suggests that patient-derived neuronal cultures may provide valuable model tools to understand the etiology of BPD and thereby to develop new diagnostic and therapeutic strategies.

P-707

IN VITRO CELLULAR MODELING OF THE INDUCED NEURAL STEM CELLS CONVERTED FROM DISEASE FIBROBLASTS

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder. The motor dysfunction is primarily involved in loss of γ-aminobutyric acid (GABA) medium spiny neurons (MSN) in the basal ganglia, which is caused by the abnormal expansion of cytosine-adenine-guanine (CAG) repeats in the genome that lead to mutant Huntingtin proteins. In this study, we generated the induced neural stem cells (iNSCs) from patient fibroblasts, containing the expanded CAG repeats, of Huntington's disease, which called as HD iNSCs. The CAG repeats within the genome of iNSCs were corrected by CRISPR/Cas9 system. The iNSCs were differentiated into the mature GABAergic neurons with high efficiency of 84% by using our in vitro differentiation protocol. They expressed neuronal markers such as MAP2 and TUJ1 and the GABAergic neuron markers such as GAD65/67 and GABA. Interestingly, the HD iNSCs highly increased the apoptosis genes such as BCL-2, caspase3, p53 and annexinV compared to the gene corrected iNSCs during the early in vitro differentiation process. Taken together, these results suggest that the HD iNSCs may be used as a cellular model for pathogenesis and drug screening of Huntington's disease, and the gene corrected iNSCs could be used for the treatments of Huntington's disease by cell-based therapy in the future.

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P-708

IMPAIRED OSTEOGENESIS OF COSTELLO SYNDROME IPSCS-DERIVED MESENCHYMAL STEM CELLS

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Costello syndrome (CS) is caused by HRAS mutations in RAS/MAPK signaling pathway. Approximately 90% of CS patients have bone abnormalities such as craniofacial malformation, kyphoscoliosis, osteoporosis, and short stature. However, molecular mechanisms about how HRAS mutation accounts for aberrant bone development are poorly understood. iPSCs derived from fibroblasts of a CS patient (CS-iPSCs) normally differentiated into mesenchymal stem cells (MSCs) that expressed MSC-positive markers such as CD73+, CD90+, and CD105+. Intriguingly, CS-MSCs exhibited decreased alkaline phosphatase (ALP) activity and mineralization during osteogenic differentiation. Hyperactive HRAS was observed in CS-MSCs and CS-osteoblasts, and resulted in elevated p-ERK activity. Treatment with a farnesyl transferase inhibitor led to increments of ALP activity and mineralization in CS-osteoblasts. The results demonstrate that hyperactive HRAS cause impaired osteogenesis in CS-MSCs.

P-709

PATIENT SPECIFIC IPSC DERIVED DOPAMINERGIC NEURONS; PHYSIOLOGICAL ANALYSIS

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Parkinson's disease (PD) is the second most common neurodegenerative disease, a slow-progressing, that results from the degeneration of neuro-melanin(N-M)-containing dopamine neurons(DA) in the substantia nigra par compacta. There are many PD disease models, but there are limited in vitro models that make it difficult to imitate PD. Here, we observed the clinical potential of iPSCs derived from PD patients to dopaminergic neurons. The first data showed differentiation of Dopaminergic neurons from patient specific iPSCs. Differentiation markers were confirmed at each differentiation steps. Also, we detected dose dependent dopamine releasing result using high sensitive ELISA. Dopamine secretion increased with 100μM of L-DOPA, but there was no significant change at higher concentration of L-DOPA. The electrophysiological data by Patch-clamp technique in normal human derived cells is comparable and important result. Action-potential was detected at –57mV. The frequency decreased at 10 uM of L-Dopa but two action potentials appeared simultaneously, 30 μM L-Dopa increased the frequency compared to the action potential of the control in the current clamp mode. In the voltage clamp mode, the frequency of dopaminergic neurons was increased and the amplitude of L-Dopa increased at 30uM. However, the 60uM L-dopa increased the frequency compare to 10uM L-dopa but decreased level than 30uM L-dopa. Further experimentation is required but we expect these pattern to be derived from patient cells. The following steps will be the clue whether these dopaminergic neurons derived from patient-specific iPSCs could become in-vitro models. As we showed, iPSC derived dopaminergic neurons showed recovery from L-dopa therapy, so patient-specific iPSC could be a potential of useful tool for modeling Parkinson's disease.

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P-710

HYPERACTIVE NOTCH SIGNALING IN MELAS NEURAL CELLS INHIBIT NEURONAL DIFFERENTIATION

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Mitochondrial encephalomyopathy, lactic acidosis, and stroke like episodes (MELAS) syndrome is a disorder caused by mutation in mitochondria DNA. One of the most common genetic mutations of MELAS is the m.A3243G mutation that occurs in MT-TL1 gene encoding tRNALeu(UUR). It is postulated that this

mutation affects mitochondrial protein synthesis and ultimately oxidative phosphorylation. Unsurprisingly, organ systems that utilize mitochondrial respiration as their main source of energy production are most affected. These include the central nervous system and skeletal muscles. MELAS patients usually suffer from symptoms common to motor neurons diseases (MNDs) such as muscular weakness, difficulties in speech and swallowing. While the neuromuscular unit appears to be affected in MELAS, research on motor neuron pathology has not been extensively covered. Here we show that spinal organoids derived from MELAS patients show severe neurogenesis defect that is attributed to hyperactive Notch signaling, which prevented neurogenesis. In addition, we also found that inhibition of mitochondrial Complex I recapitulated the MELAS phenotype and led to increased Notch signaling. Lastly, we report that treatment with a gamma-secretase inhibitor could reverse the neurogenesis defects in MELAS organoids.

P-711

DECIPHERING THE CONTRIBUTIONS OF PERIPHERAL SENSORY NEURONS AND SPINAL INTERNEURONS TO MOTOR NEURON DEATH IN ALS

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The incidence of motor neuron disorders (MNDs) has been progressively increasing in recent years. Amongst the most commonly occurring MNDs, amyotrophic lateral sclerosis (ALS) is regarded as the most debilitating as it leads to the loss of both upper and lower motor neurons, resulting in severe motor disabilities and eventual fatality. While the dysfunction and death of motor neurons in ALS is well-documented, the specific neuronal populations and their independent contributions to this phenotype remain unclear. In this study, we look at the individual contributions of peripheral sensory neurons, dorsal interneurons and ventral interneurons to motor neuron death in ALS. Using both co-culture as well as organoid models, we will attempt to identify neuronal populations which might accelerate the onset of ALS more by analyzing neuronal morphology, numbers, calcium signals, deficits in circuitry and synapse formation. By classifying the contributions of each of the neuronal populations, we hope to gain a more holistic understanding of disease progression in ALS and also identify neuronal subtypes for advanced therapeutic targeting.

P-712

DIFFERENTIATION AND CHARACTERIZATION OF OLIGODENDROCYTES FROM X-LINKED ADRENOLEUKODYSTROPHY PATIENTS

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X-linked Adrenoleukodystrophy (X-ALD) is an inherited peroxisomal metabolic neurodegenerative disorder caused by mutations in a gene encoding the ATP-binding cassette transporter (ABCD1), also known as adrenoleukodystrophy protein (ALDP). One of the most representative pathological features in X-ALD is an inflammatory demyelination in the cerebral cortex. Oligodendrocytes are glial cells that provide a metabolic support and myelination to neuronal axons, therefore the loss of oligodendrocytes leads to various neurological symptoms such as motor impairment and mental retardation in X-ALD. Even though mouse genetic models have provided insights into pathological mechanisms, they neither faithfully recapitulate the symptoms seen in human patients, nor provide a promising in vitro model for studying the loss of oligodendrocytes. To establish a cellular model for X-ALD, we generated iPSC lines and oligodendrocytes from healthy subject and individuals with X-ALD. Overexpression of transcription factor SOX10 in iPSC-derived oligodendrocyte precursor cells produced >10% O4+ oligodendrocytes in less than 2 weeks, and magnetic-beads mediated cell sorting enriched O4+ cells by ~80% among total cells. We found that there was no obvious defect in differentiation potential toward oligodendrocytes in X-ALD patient-derived iPSCs. In addition, oligodendrocytes derived from X-ALD patients showed high levels of very-long chain fatty acid compared to the ones derived from healthy subject, which is consistent with the previous reports. We performed RNA-seq analysis with the differentiated O4+ oligodendrocytes, and are currently investigating disease-specific phenotypes and potential molecular target(s) specific for the death of oligodendrocytes derived from X-ALD patients.

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P-713

ASTROCYTES DERIVED FROM X-LINKED ADRENOLEUKODYSTROPHY PATIENTS EXHIBIT MOLECULAR SIGNATURES OF NEUROINFLAMMATION

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Astrocytes have long been thought to play supportive roles for cells in the central nervous system. Recent researches, however, begin to provide evidence that astrocytes are active players in regulation of metabolism and immune response in CNS, and that dysfunction of astrocytes is highly associated with several neurological diseases involving neuroinflammation, such as Parkinson's disease, and Alzheimer's disease. In the present study, we hypothesized that astrocytes could also play a key role in X-linked leukodystrophy (X-ALD), a rare neuro-metabolic disease, of which pathophysiology is largely unknown, and that they might contribute initiation and/or propagation of neuroinflammation in this disease. To test this hypothesis, we established a method for differentiation of astrocytes on a specific substrate through a forced expression of the transcription factor nuclear factor I B (NFIB) in hPSC-derived neural precursors. Our differentiation method generated more than 80% GFAP+/S100B+ astrocytes from iPSCs derived from healthy subjects and individuals with X-ALD in 2 weeks. More importantly, X-ALD patient-derived astrocytes exhibited molecular signatures of neuroinflammation including one that has not been associated with pathophysiology of X-ALD. Since our culture system does not involve microglia, a cell type in charge of initiation of immune response in CNS, this result provides a clue that astrocytes may be the one that initiates neuroinflammation in the context of X-ALD.

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P-714

SCREENING THERAPEUTIC TARGETS FOR RECOVERING ABERRANT OSTEOGENESIS IN CFC SYNDROME-IPSCS

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Cardiofaciocutaneous (CFC) syndrome is a rare genetic disorder with consistently active ERK signaling, which is mainly caused by the germline mutations in BRAF gene. Among various symptoms, skeletal defects such as short stature, bone growth delay, and low bone mineral density can be easily observed in the majority of CFC patients. We previously established CFC-induced pluripotent stem cells (CFC-iPSCs) from CFC patient dermal fibroblasts and reported that the osteogenic differentiation potency of these cell lines are impaired. CFC-mesenchymal stem cells (CFC-MSCs) differentiated from CFC-iPSCs represented aberrant alkaline phosphatase (ALP) activity and mineralization during osteogenic differentiation in vitro. In addition, activated SMAD2 signaling and downregulated SMAD1 signaling in CFC-MSCs was responsible for this phenotype. Based on these results, we screened potential therapeutic targets to rescue defective osteogenic differentiation of CFC-MSCs using para-Nitrophenylphosphate (pNPP) assay on MSCs-plated 384- wells. Among 2261 clinical compounds (provided by Korea Chemical Bank), 10 chemicals that alleviated ALP activity of CFC-MSCs were selected. These chemicals also revealed pharmacological effects on the recovery of defective osteogenesis in CFC-MSCs in larger scale. Our findings provide novel insights on the pathological mechanism and therapeutic targets in CFC syndrome.

P-715

RECAPITULATION OF METHOTREXATE HEPATOTOXICITY WITH INDUCED PLURIPOTENT STEM CELL-DERIVED HEPATOCYTES FROM PATIENTS WITH RHEUMATOID ARTHRITIS

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Methotrexate (MTX) is widely used for the treatment of rheumatoid arthritis (RA). The drug is cost-effective, but sometimes causes hepatotoxicity, requiring a physician's attention. In this study, we simulated hepatotoxicity by treating hepatocytes derived from RA patient–derived induced pluripotent stem cells (RA-iPSCs) with MTX. RA-iPSCs and healthy control iPSCs (HC-iPSCs) were established successfully. RA-iPSCs were differentiated into hepatocytes in two-dimensional (2D) monolayers and three-dimensional (3D) hepatocyte spheroid cultures; this process required growth factors such as BMP4, bFGF, HGF, and OSM. Immunofluorescence staining and flow cytometry were performed to confirm that the mature hepatocytes expressed cytokeratin 18, anti–alpha-1 antitrypsin, and albumin. MTX toxicity was evaluated via monitoring of cell viability, alanine aminotransferase, and mitochondrial status after MTX treatment in 2D and 3D cultures. RA-iPSCs generated from three RA patients suffering from MTX-induced hepatotoxicity differentiated into the endoderm lineage, hepatoblasts, and hepatocytes. In 2D culture, RA-iPSC-derived hepatocytes were more sensitive to MTX than healthy controls. A 3D culture system using hepatocyte spheroids also successfully recapitulated MTX-induced hepatotoxicity. The 3D culture system had several advantages, including longer culture periods under more complex conditions. A patient-derived iPSC platform could recapitulate MTX toxicity. Simulation of drug toxicity in vitro may help clinicians choose safer drugs or less toxic doses.

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P-716

TRANSCRIPTOMIC CHANGES DURING INDUCED PLURIPOTENT STEM CELL-DERIVED NEURAL CREST CELL DIFFERENTIATION HIGHLIGHT GENES INVOLVED IN ENDOCARDIAL CUSHION AND OUTFLOW TRACT DEVELOPMENT

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Neural crest cells (NCCs) play a critical role in normal cardiac development, and defects in NCCs likely cause congenital heart disease (CHD). NCCs are multipotent migratory stem cells that give rise to diverse tissues, including cardiac structures such as the smooth muscles of the great arteries and semilunar valves. Induced pluripotent stem cells (iPSC) can be differentiated into NCCs, demonstrated by expression of NCC markers NGFR and HNK1. To better define iPSC-NCCs and to better understand the progression of iPSCs to NCCs, we have compared the transcriptomes of iPSC and iPSC-NCCs. PGP1 iPSCs were differentiated to NCCs and RNA was collected at 0, 5, 10, and 15 days of differentiation. RNAseq analysis showed that by day 15, 6483 genes were upregulated in NCC vs iPSCs, 6406 downregulated, and 6715 unchanged (FDR 5%). Enrichment analysis for the top 500 upregulated genes showed 4 cardiac-related gene ontology (GO) terms in the top 10, including 'endocardial cushion morphogenesis' (fold enrichment 11.85, $p = 0.012$). Notably, of 45 genes under GO term 'endocardial cushion development', 38 (84%) differentially expressed in NCCs by day 15 (FDR 5%). Next, we compared this RNAseq data with that of iPSC-derived cardiomyocyte (CM) differentiation in a set of 253 genes previously implicated in CHD. Of these, 143 genes were differentially expressed in both iPSC-CMs and iPSC-NCCs. However, 27 genes (10.6%) including MYH6, PITX2, and TBX5 were uniquely upregulated in iPSC-CMs, while 65 genes (25.7%) including CHD4 and NOTCH1 were uniquely upregulated in iPSC-NCCs. In conclusion, transcriptomic changes during iPSC-NCC differentiation include upregulation of genes involved in cardiac development, particularly endocardial cushions and the outflow tract. Importantly, a subset of genes implicated in CHD are altered during iPSC-NCC differentiation but not in iPSC-CM

differentiation. Thus, iPSC-NCCs offer a new model with which to investigate the pathogenesis and mechanisms of CHD.

P-717

AUTOIMMUNE DISEASE MODELING IN VITRO WITH COMBINATION OF IPSC-DERIVED CARDIOMYOCYTES AND DISEASE ACTIVE SERUM

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Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that usually affects young women, including women of childbearing age. SLE complicates many organs such as skin, joints, muscles, kidneys, lungs and heart. The most common cause of death in SLE patients is cardiovascular complication. Rarity of disease and complexity of cardiovascular system prevents from investigating SLE cardiovascular complications thoroughly. Application of patient-derived induced pluripotent stem cells (iPSCs) becomes a new strategy for drug screening and pathophysiologic studies of various diseases. We intended to generate and study in vitro cardiomyocytes from iPSCs in SLE patients. 5 Patients-derived iPSCs were generated from primary cells of SLE patients. SLE patient-specific iPSCs were differentiated into cardiomyocytes using a small molecule-based monolayer and 3D differentiation protocol. Differentiated cardiomyocytes were treated with serum of active SLE patients. Cardiomyocytes dysfunction was revealed by electrophysiological study and proliferation/apoptosis assay. Serum of higher SLE disease activity suppressed cardiomyocyte function more vigorously. We propose a combination of disease specific cardiomyocyte with disease serum can be better “disease in a dish” platform.

P-718

EFFECTS OF ARGINYL-DIOSGENIN ANALOG ON THE MOUSE HIPPOCAMPAL NEUROGENESIS IN LPS-INDUCED NEUROINFLAMMATION

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Microglia-mediated neuroinflammatory responses are well known to inhibit neurogenesis in the dentate gyrus (DG) of the adult hippocampus, and growing evidence indicates that therapeutic intervention to suppress microglial activation could be an effective strategy for restoring the impaired neurogenesis and memory performance. In the present study, we investigated the effects of water- soluble arginyl-diosgenin analog (Arg-DG) on the adult hippocampal neurogenesis using a central LPS-induced inflammatory mice model, along with the fundamental mechanisms in vivo and in vitro using LPS-stimulated microglial BV2 cells. Arg-DG (0.6 mg/kg) attenuates LPS-impaired neurogenesis by ameliorating the proliferation and differentiation of neural stem cells (NSCs), and prolonging their survival. The impaired neurogenesis in the hippocampal DG triggered the cognitive function, and that treatment of Arg-DG led to the recovery of cognitive decline. Arg-DG also suppressed the production of LPS-induced pro-inflammatory cytokines in hippocampal DG by blocking microglial activation. In in vitro study, Arg-DG inhibited the production of nitric oxide (NO), nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) expression, and prostaglandin D2 production (PGD2), as well as the pro-inflammatory cytokines, such as interleukin (IL)-6, IL-1β, and tumor necrosis factor alpha (TNF-α). The anti-inflammatory effect of Arg-DG was regulated by NF-κB and MAPK JNK signaling both in vivo, and in LPS-stimulated microglial BV2 cells. Taken together, these results suggest that Arg-DG might have the potential to treat various neurodegenerative disorders resulting from microglia-mediated neuroinflammation.

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P-719

EQUIVALENT THERAPEUTIC EFFICACY OF THE FROZEN HUMAN CLONAL MESENCHYMAL STEM CELLS WITH ITS FRESH TYPE IN RAT MODEL OF SEVERE ACUTE PANCREATITIS

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Mesenchymal stem cells are a promising treatment for acute pancreatitis (AP). However, the long culture time requirement for mesenchymal stem cell proliferation limited the use in patients with severe AP, who need intensive care promptly after abdominal pain onset. We compared the therapeutic efficacy of the frozen human clonal mesenchymal stem cell (hcMSC) with its fresh type in severe AP model. Severe AP was induced in Sprague-Dawley rats by intra-pancreatic duct injection of 3% sodium taurocholate solution. The frozen and fresh hcMSCs were administered to rats (each n=15, 2x10^6 cells/head) through the tail vein after 4 hours from the induction. The normal and vehicle groups (each n=15) were administered a vehicle instead. Serum amylase and lipase level were measured at 2, 4, 12, 24, 48, 72 hours after the induction. Serum cytokine level and pancreatic myeloperoxidase were measured at 72 hours after the induction. Histology scoring of the pancreas was also performed at 72 hours after the induction. All of the hcMSC groups (the frozen and fresh types) lowered serum amylase and lipase at all time points, respectively from 12 hours and from 24 hours, compared with the vehicle group. The cytokine level measurement revealed lowered IL-6, TNF-alpha, and IFN-gamma and increased IL-10 in all of the hcMSC groups. Both hcMSC groups also showed lowered myeloperoxidase level and improved histological score in all of the parameters (edema, acinar necrosis, hemorrhage, fat necrosis, and inflammatory infiltration). The infusion of frozen hcMSCs, as well as its fresh type, lowered pro-inflammatory cytokines and serum enzyme levels related to the severity of AP and lead to the fast recovery of the pancreatic tissue. The equivalent therapeutic efficacy of the frozen hcMSC can proceed to the development of cell therapy for severe AP in humans.

P-720

MSX2 MEDIATE ABNORMAL OSTEOGENESIS AND IS INFLUENCED BY PHYSICAL, INFLAMMATORY STIMULATION IN ANKYLOSING SPONDYLITIS

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Ankylosing spondylitis (AS) is an autoimmune disease that primarily affects the axial skeleton, sacroiliac joints and enthesis. Patients with AS develop osteogenesis in the tendon or enthesis but osteoporosis in the bone body. Well-known genetic causes include HLA-B27 gene or ERAP1 mutation. Recent studies have shown that MSX2 is highly expressed in AS patients. MSX2 is known to promote bone formation in osteoblasts, but to suppress bone formation in fibroblast or myoblast. Based on this, MSX2 is expected to play a role in regulating osteogenesis and osteoporosis. To identify the feature of the disease, PBMCs from healthy and AS patients were isolated and induced into iPSC, and the difference was compared by osteoblast differentiation using the iPSCs. As a result of the experiment, there was no difference between the phenotypes of each group. However, BMP2 and RUNX2 expressions were increased in osteoblasts differentiated by AS iPSC, and OCN and OPN, which are osteoblast markers, were also increased to a higher level. Interestingly, MSX2 gene was greatly increased. Through various studies, MSX2 has been known to play a role in promoting or inhibiting osteogenesis depending on the different type of cells and the external environment. Therefore, MSX2 was thought to be deeply related to AS and the experiment was conducted. Treatment with TNFα to induce an inflammatory environment resulted in less osteogenic differentiation and reduced the expression of MSX2. In order to investigate the effect of mechanical stress on osteoblasts, compressive, stretching, and shear stress stimulation were performed as well. BMP2, MSX2 and RUNX2 were found to have increased expression levels when shaking stress was given. But, MSX2 and osteogenic markers were found to have decreased expression levels when compressive and stretching stress. To support the above results, MSX2 knockdown experiment

was conducted. When the expression level of MSX2 was decreased, it was confirmed that other osteoblast markers were decreased. Thus, inhibition of bone morphogenesis by inflammation and physical stress may be evidence of association with MSX2. Through this research, it was confirmed that MSX2 upregulation in AS patients has abnormal osteogenic potential and is stimulated by physical stress.

P-721

IPSC-BASED MODELING OF EARLY OSTEOARTHRITIS PATIENT

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Osteoarthritis (OA) is the most common type of arthritis. It already affects millions of people worldwide, and tends to increase as our population age as well. Other than aging, it is also thought to increase along with the increase in obesity seen at all ages. While it is usually known to be related with aging or obesity, we were able to observe a unique pattern of OA in one patient. The patient already showed OA symptoms in her late 20's and the cartilage tissue in her finger joint were all worn out in her late 30's. However, these symptoms were not observed in the cartilage tissue of the sibling. In this study, we generated induced pluripotent stem cells using peripheral blood mononuclear cells of the two sisters. The generated iPSCs were then differentiated into chondrogenic pellets. Chondrogenic pellets were characterized and vacuole-like morphologies were observed in the early OA patient-derived pellets. The chondrocytes isolated from the pellets were analyzed by microarray and we screened a possible target that can be responsible for these symptoms. This study shows another case of disease modeling using iPSCs, and suggests a possible factor that can be considered as a target for early OA.

P-722

CHARACTERIZATION OF ORTHOTOPIC GLIOBLASTMAL MODEL FOR THE GENE- AND CELL THERAPY

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Intrinsic heterogeneous and infiltrative nature of glioblastoma cells to the adjacent normal brain parenchyma are the main obstacles to the treatment of glioblastoma multiforme (GBM). The infiltrative nature of GBM increases following various treatments, which often leads to tumor recurrence. U87 cell line, a commonly used as a GBM cell line till recently, has appeared inappropriate for GBM model. Thus, we established an orthotopic GBM model using another cell line, LN229. Here, we discuss the infiltrative nature of LN229 in the mouse brain and their interaction with adjacent brain microenvironment. We also show that the LN229-driven brain tumor model is appropriate for the study of mesenchymal stem cell-mediated gene therapy using deliver a bacterial suicide gene, cytosine deaminase (CD).

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P-723

THYROID HORMONES AND THEIR DERIVATIVES PROMOTE DOPAMINE NEURON DIFFERENTIATION

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Parkinson's disease (PD) is a neurodegenerative disease characterized by progressive movement disorders caused by the selective loss of dopamine (DA) neurons in the substantia nigra. Despite the identification of the causal mechanisms underlying the pathogenesis of PD, effective treatments remain elusive. In this study, we observed that a low level of fetal bovine serum (FBS) effectively induced DA neurons in rat

neural precursor cells (NPCs) by enhancing nuclear receptor related 1 protein (NURR1) expression. Among the different components of FBS, the thyroid hormones triiodothyronine (T3) and thyroxine (T4) were identified as key factors for the induction of DA neurons. Since an overdose of thyroid hormones can cause hyperthyroidism, we synthesized several thyroid hormone derivatives that can partially activate thyroid hormone receptors but completely induce the differentiation of DA neurons from NPCs. Two derivatives (#3 and #9) showed positive effects in the induction and maturation of DA neurons without showing significant affinity to the thyroid hormone receptor. They also effectively protected and restored DA neurons from neurotoxic insults. Taken together, these observations demonstrate that thyroid hormone derivatives can strongly induce DA neuron differentiation while avoiding excessive thyroid stimulation and might therefore be useful candidates for PD treatment.

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P-724

THE PROTECTIVE EFFECT OF SUBSTANCE-P ON BAPN-INDUCED AORTIC DISSECTION

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This study is aimed to explore the protective effects of Substance-P (SP) on development of aortic dissection (AD). To create an aortic dissection preclinical model, BAPN was administered to SD rats orally for 4 weeks and SP was injected intravenously, concurrently with BAPN treatment, twice a week for 4 weeks. BAPN treatment caused the dilation of aorta with infiltration of monocyte and elevation of pro-inflammatory cytokine within 1 week-post treatment. However, SP treatment inhibited dilation of aorta and mitigated highly activated inflammatory responses due to monocyte infiltration and pro-inflammatory cytokines-enriched environment, which eventually prevented dissection of aorta. Intriguingly, SP injection also blocked the BAPN-induced-VCAM-1 expression to protect endothelium and this function might contribute to the decline in infiltration of monocytes and immune suppression from the early stage of AD. Collectively, this study supports that SP blocks AD development by modulating endothelial dysfunction and immune response, simultaneously.

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P-725

EVALUATING THE EFFECT OF MONOMERIC AMYLOID B ON HEALTH OF FOREBRAIN NEURONS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

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There is an increase in effort and interest from many laboratories around the world for developing an appropriate disease models for neurodegenerative diseases including Alzheimer's disease (AD). Despite such awareness, most of the in vitro AD models do not resemble the AD patient brain environment, and this is partly due to poor understanding of amyloid beta (Aβ) peptide and pathophysiological changes of affected neurons. To address this critical issue, we treated human embryonic stem cells (hESCs)-derived neurons with monomeric Aβ (1-42) at pathophysiologically more relevant concentrations for longer period compared to other conventional AD studies. Since the most common aetiology observed from post-mortem brains of AD patients is loss of synapses, synaptic health of monomeric Aβ (1-42) treated neurons were examined in this study. To generate forebrain neurons from pluripotent stem cells (PSCs) we used a combination of small molecules for the first 9 days of differentiation. The small molecules include inhibitors of SMAD signalling (LDN193189 and SB431542; LSB) and a Wnt signalling pathway inhibitor (XAV939) to derive central nervous system lineages, followed by other small molecules (PD0325901, SU5402 and DAPT; PSD) to derive forebrain neurons. The immunocytochemistry using different neuronal and synapse markers confirmed that the neurons produced by the current protocol are forebrain glutamatergic neurons. The Aβ (1-42) monomer treatment of these neurons initiated on day 20 of differentiation for 15 days and the monomer

concentrations ranged from 0 μM to 5 μM. Immunocytochemistry demonstrated that there is a reduction in both thickness and length of axons under treatment with Aβ (1-42) monomers. This illustrates that low concentrations of Aβ (1-42) monomers damages axons hence synapses over time. Currently, changes in neurotransmitter glutamate levels and inflammatory cytokines in culture media collected during the treatment are under investigation. Overall, these results suggest that the combination of current differentiation protocol and extended Aβ (1-42) treatment successfully produces a more disease relevant model for AD. Future studies will involve further quantitative analyses of the impacts of monomeric Aβ (1-42) on synaptic health of hESC-derived forebrain neurons.

P-726

CARDIOPROTECTION WITH NOVEL SPIDER VENOM PEPTIDE

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Cardiovascular medicine would profoundly benefit from novel therapeutics that improve the ischemic tolerance of cardiac tissue. During ischemia, altered metabolism leads to tissue acidification which exacerbates tissue injury. We identified acid sensing ion channel 1a, ASIC1a, as a proton-gated ion channel that initiates cell death pathways during acidosis. To assess a functional role for ASIC1a in cardiac ischemia, we used ASIC1a genetic knockout hearts exposed to global ischemia/reperfusion (I/R) injury in mouse ex vivo Langendorff perfusions. ASIC1a KO hearts showed significantly improved functional recovery post I/R with increased left ventricular developed pressure and decreased end diastolic pressure compared to controls. Using human pluripotent stem cell (hPSC) derived cardiomyocytes, we have shown that ASIC1a translocates to the sarcolemma during severe acidosis. Based on this, we tested a therapeutic strategy for cardiac ischemia using a double-knot peptide, Hi1a, a potent and specific inhibitor of ASIC1a derived from the venom of the funnel web spider, Hadronyche infensa. Treatment with Hi1a in hPSC-CMs does not acutely alter cardiac electro- mechanical coupling but significantly improves survival during severe acidosis and ischemia in vitro. Using a monomer of Hi1a, PcTx1, derived from Psalmopoeus cambridgei tarantula spider venom, we validated the cardioprotective effect of ASIC1a inhibition that was lost with an inactive variant of PcTx1 (R27A/V32A). Additionally, hPSC-CM treatment with ASIC1a agonist, MiTx1, causes cell death in the absence of severe acidosis. We used global I/R injury to test efficacy in whole organs and showed that Hi1a and PcTx1 do not alter baseline contractility, but significantly improve recovery post I/R compared to vehicle controls and inactive PcTx1 treated hearts. To determine a role for Hi1a in a clinical model of heart transplant, we used an isolated working rat heart model of donor heart preservation. Hi1a supplementation in clinical heart preservation solution, Celsior, significantly improved heart function following prolonged ischemia. These data reveal ASIC1a as a novel mediator of injury during cardiac ischemia and identify ASIC1a inhibition through the spider-venom peptides Hi1a and PcTx1 as a therapeutic cardioprotective strategy.

P-727

Withdrawn

P-728

MODELING OF FRONTOTEMPORAL DEMENTIA USING IPSC TECHNOLOGY

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Frontotemporal dementia (FTD) is caused by the progressive degeneration of the temporal and frontal

lobes of the brain. FTD is pathologically characterized by abnormal expression of tau, TDP-43 (TAR DNA-binding protein 43) and FUS (Fused in Sarcoma) proteins. However, its pathological mechanisms are largely unknown. In this study, we have generated and characterized induced pluripotent stem cell (iPSC) lines from one normal control and three sporadic bvFTD (behavioral variant FTD) patients. Firstly, we examined the expression of stemness markers using immunocytochemistry and real-time PCR. Secondly, we differentiated each iPSC line into post-mitotic neurons, which were positive for typical neuronal markers, such as Tuj1 and MAP2. At the same time, we also differentiated two patient derived-iPSC lines carrying MAPT or PGRN mutations that are known as FTD-related mutation, in order to compare them with sporadic patient-derived iPSC lines. As a result, we observed that the expression of p-Tau and TDP-43 C-terminal fragments were increased in both of neurons carrying mutation and some of bvFTD patient-derived iPSC lines. Also, FUS proteins were decreased in all of FTD patient iPSC-derived neurons, compared with control iPSC-derived neurons. We also found that the expression of cleaved caspase-3, a general cell death marker, was surprisingly increased only in the sporadic patient iPSC-derived neurons with treatment of staurosporine, a cell death-inducing agent. Furthermore, we observed that cell death-related proteins, such as Bax and Cytochrome C were also increased in some of bvFTD patient iPSC-derived neurons. Taken together, this study demonstrates that the typical phenotypes of sporadic bvFTD can be recapitulated by iPSC technology, which will be useful for studying FTD pathogenesis, as well as drug screening.

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P-729

MITOCHONDRIAL DYSFUNCTION AND IMPAIRED AUTOPHAGY IN ALZHEIMER'S DISEASE PATIENT IPSC-DERIVED NEURONS

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Alzheimer's disease (AD) is a neurodegenerative disease which is characterized by the formation of amyloid-beta (Aβ) plaques and neurofibrillary tangles. In this study, we generated an induced pluripotent stem cell (iPSC) line from familial AD patient carrying presenilin-1 (PS1)-S170F mutation, and then differentiated them into cortical neurons. Extracellular and intracellular of Aβ levels were dramatically increased in the neurons, compare with the control group. Furthermore, the AD iPSC-derived neurons exhibited high expression levels of phosphorylated tau, especially in AT8 (Ser202/Thr205), which was also detected in the soma and neurites by immunocytochemistry. We next investigated the mitochondrial dynamics in AD iPSC-derived neurons, which exhibited abnormal patterns of mitochondria velocity using Mito-tracker. We also found that the levels of Mfn1 (membrane proteins mitofusin 1) was reduced and Drp1 (Dynamin related protein 1) was increased in AD iPSC-derived neurons. We also observed that LC3b and ubiquitin is highly increased in AD iPSC-derived neurons, indicating that the autophagy system is also defective. Taken together, we have characterized the pathological features of AD patients carrying mutations for PS1-S170F using iPSC technology for the first time, which will serve as useful resources for studying AD pathogenesis and drug screening in the future.

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P-730

THE FIRST GENERATION OF IPSC LINE FROM A KOREAN ALZHEIMER'S DISEASE PATIENT CARRYING APP-V715M MUTATION EXHIBITS A DISTINCT MITOCHONDRIAL DYSFUNCTION

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Alzheimer's Disease (AD) is a progressive neurodegenerative disease, which is pathologically defined by the accumulation of amyloid plaques and hyper-phosphorylated tau aggregates in the brain. Mitochondrial dysfunction is also a prominent feature in AD, and the extracellular Aβ and phosphorylated tau result in the impaired mitochondrial dynamics. In this study, we generated an induced pluripotent stem cell (iPSC) line from an AD patient with amyloid precursor protein (APP) mutation (Val715Met; APP-V715M) for the first time. We demonstrated that both extracellular and intracellular levels of Aβ were dramatically increased in the APP-V715M iPSC- derived neurons. Furthermore, the APP-V715M iPSC-derived neurons exhibited high expression levels of phosphorylated tau (AT8), which was also detected in the soma and neurites by immunocytochemistry. We next investigated mitochondrial dynamics in the iPSC- derived neurons using Mito-tracker, which showed a significant decrease of anterograde and retrograde velocity in the APP-V715M iPSC-derived neurons. We also found that as the Aβ and tau pathology accumulates, fusion-related protein Mfn1 was decreased, whereas fission-related protein DRP1 was increased in the APP-V715M iPSC-derived neurons, compared with the control group. We also demonstrated that APP-V715M iPSC-derived neurons showed significant increase in Cytochrome C and Caspase3-dependent cell death, and also investigated that APP-V715M mutation iPSC-derived neurons are sensitive to oxidative stress-dependent cell death. Taken together, we established the first iPSC line derived from an AD patient carrying APP-V715M mutation and showed that this iPSC-derived neurons exhibited typical AD pathological features including a distinct mitochondrial dysfunction, which will serve as useful resources for studying AD pathogenesis and drug screening in the future.

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POSTSYNAPTIC DENSITY PROTEIN DLG2 HAS A NOVEL ROLE IN CORTICAL NEURONAL DEVELOPMENT RELEVANT TO SCHIZOPHRENIA AETIOLOGY

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Discs large homologue 2 (DLG2) is a membrane associated guanylate kinase protein with an established role as a scaffold in the postsynaptic density (PSD) where it regulates receptor clustering and signal transduction, contributing to higher cognition. Recurrent de novo deletions of DLG2 have been identified in schizophrenic individuals. Although DLG2 mRNA expression has been reported in human embryonic brain and human embryonic stem cell (hESC)-derived neural precursors (NPCs), its functional role during neural development has yet to be studied. Studying cortical differentiation of hESCs we uncovered a novel role for DLG2 in neuronal cell-fate determination, migration

and maturation. Although a similar amount of postmitotic neurons were generated in DLG2 knockout (KO) and wildtype cultures, neurons lacking DLG2 showed simplified neuronal morphology and altered cortical layer identity including the expression of TBR1 and CTIP2. RNA-seq analysis revealed many protein-coding genes to be differentially expressed in DLG2 KO cells across developmental stages, peaking during early neurogenesis, long before PSD formation. Gene set enrichment analyses of differentially expressed genes indicated significant dysregulation of NPC proliferation, adhesion, neuronal migration and neuronal electrical properties, all of which were confirmed by experimental assays. Analysis of human genetic data revealed that neurodevelopmental gene expression programmes dysregulated in DLG2 KO lines were highly enriched for genetic variants conferring risk of schizophrenia. Specifically, genes downregulated in DLG2 KO cells during the transition from NPCs to immature neurons were highly enriched for schizophrenia common variant association. Further analysis revealed this enrichment to be greatest amongst genes underlying active neuronal properties (e.g. action potential generation and voltage-gated sodium channel activity). This study unveils a novel role for DLG2 in cortical development, shedding light on neurodevelopmental processes contributing to schizophrenia pathophysiology.

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P-732

GENERATION OF AN IMMORTALISED ERYTHROID CELL LINE FROM HAEMATOPOIETIC STEM CELLS FROM A HAEMOGLOBIN E/BETA -THALASSEMIA PATIENT

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Beta thalassemia is a common genetic disorder with high prevalence in areas where malaria is (or was) highly endemic. It is caused by mutations in and around the β-globin gene, resulting in reduced or absent β-globin synthesis, a component of haemoglobin in red blood cells (RBC) required for oxygen delivery in the body. A particular subtype of β-thalassemia, HbE/β-thalassemia has extremely high frequency in many countries in Asia including Thailand, the HbE mutation resulting in both reduced β-globin levels and a structural change. In all cases of β-thalassemia aberrant expression of β-globin results in impaired RBC production, along with instability and hemolysis of those RBCs in circulation. At present, treatment for most thalassemia patients is limited to blood transfusion therapy to maintain RBC levels, with resultant consequences such as iron overload and immune response to blood products from frequent transfusion. There are presently no drugs available, with human model cellular systems for this disease required both for further delineating underlying molecular mechanisms, and as screening platforms for the effect and efficacy of potential new drugs and reagents, facilitating development of new therapeutic strategies. In this study, we have generated an immortalised erythroid cell line from stem cells collected from the blood of

a patient with haemoglobin E/β-thalassemia. The cell line has unlimited self-renewal property with their ability to re-establish after freezing confirmed. The cells have now been proliferating continuously for over 100 days, with a mean doubling time of 17 hours. The morphology of the cells is similar to that of pro-erythroblasts and basophilic erythroblasts. Importantly, on transfer to our 2-stage erythroid culture system the cells differentiate efficiently along the erythroid pathway, becoming orthochromatic erythroblasts and reticulocytes. The differentiated cells express 29.2% HbF, 9% HbA and 61.8% HbE which was comparable to 24.4% HbF, 5.9% HbA and 69.7% HbE in patient's red blood cells. Further characterisation of the line is presently on going. This is the first β-thalassemia cell line, or model cellular system for β- thalassemia, created and provides a valuable resource for both researchers in the field and pharmaceutical companies for drug development programmes.

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P-733

GENE-EDITED PLURIPOTENT STEM CELLS TO MODEL NEUROPATHY AND INTELLECTUAL DISABILITY

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Induced pluripotent stem cells (iPSCs) and genome editing have enabled disease modeling in vitro of otherwise inaccessible human neurons. We are using these methods to generate isogenic knock-in mutants in wild-type iPSCs using CRISPR/Cas9 ribonucleoprotein complexes. We have introduced several disease-associated mutations on MCM3AP (minichromosome maintenance complex component 3 associated protein, encoding GANP), which we recently identified to underlie early-onset sensorimotor neuropathy and intellectual disability. GANP is a scaffold protein in the TRanscription-EXport-2 (TREX-2) complex that interacts with the nuclear pore. It has been suggested to play a role in exporting selective mature mRNAs from the nucleus or in regulating export of stress-related transcripts. GANP has an mRNA-binding Sac3 (suppressor of actin) domain, where homozygous patient mutations cluster. Compound heterozygous mutations outside of the Sac3 domain lead to a partial loss of GANP protein in patient fibroblasts. Earlier onset and a more severe disease course are typically associated with the compound heterozygous mutations, whereas a more benign disease course is associated with the homozygous Sac3 mutations. By generating mutations within the Sac3, as well as near the C-terminus of GANP in iPSCs, we will investigate neuronal phenotypes caused by the different mutations. We will differentiate and phenotype sensory and motor neurons from knock-in iPSCs to dissect the effect of GANP mutations on axonal mRNA transport contributing to axon degeneration. Furthermore, we will investigate the role of TDP-43 in the pathogenesis. We previously identified Drosophila GANP (Xmas-2) in a genetic screen to eliminate the toxicity of TDP-43 overexpression in fly motor neurons, and in SH- SY5Y cells GANP knockdown rescues TDP-43 toxicity. We are now utilizing genetically engineered iPSCs differentiated into sensory and motor neurons to further investigate the role of GANP in health and neuronal disease, and the link between GANP and TDP-43.

Funding Source: Instrumentarium Science Foundation, Doctoral Programme Brain & Mind, Lastentautien tutkimussäätiö.

P-734

ESTABLISHMENT OF IPS CELL LINES FROM THE MARMOSETS WITH MECP2 HETEROZYGOUS MUTATION

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Rett syndrome is a neurodevelopmental disease presenting almost exclusively in girls. Mutations of the methyl-CpG-binding protein 2 (MECP2) gene located on the X chromosome are known to cause this disease. Although some genetically modified murine models

were reported, differences in the brain structure and functions between humans and mice have been major obstacles for clarifying the pathological mechanism of Rett syndrome. To overcome these issues, we previously generated a heterozygous MECP2 mutant common marmoset (Callithrix jacchus) using the zinc finger nuclease technology, and this marmoset exhibits a microcephaly-like phenotype (Kishi et al., in preparation). In this study, to further characterize the pathophysiology of this marmoset, we attempted to establish an in vitro model for Rett syndrome by generating transgene-free iPS cells using episomal vectors encoding reprogramming factors, from somatic fibroblasts of this marmoset. We initially attempted to use a conventional method for establishing human iPS cells, but marmoset iPS cells could not be derived. Next, we attempted to convert the fibroblasts into intermediate cells by using a novel set of small molecules supplemented in an induction medium, and succeeded in establishing iPS cells from the intermediate cells. The obtained iPS cells showed an endogenous expression of PSC markers, an in vitro differentiation potential into all three germ layers, and transgene excisions. Interestingly, we discovered that the intermediate cells had neural stem cell-like characteristics. Furthermore, we assessed whether the status of X chromosome inactivation is sustained in the iPS cells. We performed MECP2 transcript analyses to confirm the X chromosome inactivation status. As a result, we succeeded in establishing iPS cells which expressed each of the wild type MECP2 or the mutant MECP2, but there was a biased tendency towards the wild type one. This result indicated that iPS cells maintained the X chromosome inactivation status and the mutant MECP2 may have an adverse effect on deriving iPS cells. In summary, we succeeded in generating transgene-free iPS cells by the novel reprogramming method from marmosets for the first time.

P-735

LYSOSOMAL DYSFUNCTION ACCELERATES PATHOLOGIC PROGRESSION IN THE SPORADIC ALZHEIMER'S DISEASE PATIENT-DERIVED IPSC LINES

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Alzheimer's disease (AD) is the most common dementia which is characterized by the extracellular amyloid-beta (Aβ) plaques and neurofibrillary tangles (NFT). Recently, many recent studies have been focused on the sporadic AD cases, since no drugs targeting pathological pathway of current hypotheses based on the familial AD have been successful in clinical trials as yet. AD brains exhibit dysfunctions in autophagy-lysosomal pathway and endo-lysosomal network, resulting in abnormal lysosomal enzymatic activity and accumulation of autophagosomes and autolysosomes in the dystrophic neurites. In this study, we have classified sporadic AD patients into rapid decliner (RD) and slow decliner (SD) groups, based on their scores in the Mini-Mental State Examination (MMSE). Next, we generated and characterized induced pluripotent stem cell (iPSC) lines from a total of 14 sporadic AD cases, including 2 RD controls, 5 RD patients, 2 SD controls, and 5 SD patients. We then differentiated them into cortical neurons and characterized their phenotypes using cortical layer makers, such as CTIP2 and TBR1. Cortical neurons derived from both SD and RD showed a dramatically increased pattern of extracellular secretion of Aβ levels, as well as phosphorylated tau, especially at the sites of AT180 (Thr231) and S396 (Ser396). Interestingly, the level of secreted Aβ was significantly higher in RD than in SD. Moreover, we observed several tau species with low molecular weights less than 65kDa and increased phosphorylated tau species (S396) with high molecular weight in RD, suggesting that both SD and RD produce tau fragments of low molecular weights which may cause tau oligomerization by its hyperphosphorylation. Since the background information regarding the genetics of corresponding patients have been validated by sequencing analy-

sis, we targeted the possibility that the breakdown of protein clearance system can be caused by abnormal protein productions. Therefore, we have analyzed the autophagy-lysosomal pathway and endo-lysosomal pathway using western blot and co- localization analysis by immunochemistry assay in order to prove our hypothesis. Taken together, our results strongly suggest that defective lysosome may play a crucial role in the disease progression of sporadic AD.

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Genome Editing and Stem Cell Technologies

P-801

IMPROVING HDR/NHEJ RATIOS FOR PRECISE GENOME EDITING IN HUMAN IPS CELLS

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Precise gene editing aims at correcting or recreating pathogenic mutations, to study underlying disease mechanisms and advance drug discovery. The CRISPR-Cas9 system is a powerful tool that catalyzes DNA double-strand breaks (DSBs) at target genomic loci defined by programmable Cas9-associated gRNA sequences. DSBs are resolved by endogenous DNA damage repair pathways resulting in imprecise insertion/deletion (indel) mutations through mutagenic end-joining (MutEJ), including non-homologous end-joining (NHEJ), or in precise editing through template-mediated homology-directed repair (HDR). In human induced pluripotent stem cells (hiPSCs), the frequency of HDR-mediated repair outcomes is typically low (<1%) and MutEJ outcomes predominate, obstructing precise genome editing. We established a fluorescent DNA repair assay based on GFP to BFP conversion in hiPSCs that are heterozygously or homozygously targeted with a GFP reporter to the AAVS1 locus. Targeting GFP with CRISPR-Cas9 either results in 1) no modification and retained GFP fluorescence, 2) GFP knock-out through MutEJ and loss of GFP fluorescence, or 3) GFP to BFP conversion through HDR when co-transfecting a BFP donor template, resulting in BFP fluorescence. The latter HDR repair outcomes are desired for precise genome editing. This fluorescent DNA repair assay allows visualization and quantification of HDR and MutEJ repair outcomes with single-cell resolution in FACS-based analysis. Using this assay, we identified conditions favoring precise monoallelic and biallelic repair outcomes when modulating cell cycle and DNA repair pathway components. Finally, we apply these findings to recreate heterozygous compound mutations in endogenous genes, for example involved in cardiac channelopathies and cardiac sudden death.

P-802

NEW CRISPR INTERFERENCE SYSTEM REGULATES HLA PRESENTATION ON HUMAN CELLS

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CRISPR interference (CRISPRi), which employs a deactivated version of Cas9 (dCas9), is known to efficiently knock-down gene expression by blocking transcription. Moreover, it was shown that a tetracycline-inducible (Tet-ON) system enabled CRISPRi to be both reversible and tunable. Beyond Tet-ON CRISPRi, we developed a new reversed system using Tet-OFF to control dCas9-KRAB expression. We observed that Tet-OFF CRISPRi could silence beta-2 microglobulin (B2M), the common subunit of human leukocyte antigen class I (HLA cI), in a dox-dependent and reversible manner. Additionally, we performed a titration of doxycycline to confirm the lowest concentration sufficient to repress CRISPRi, corresponding to a recovery of B2M target gene expression. We expect that this minimum concentration of doxycycline could facilitate in vivo applications by reducing doxycycline

dosage requirements. Next, we established a new iPSC line with one copy of the B2M sgRNA in the AAVS1 locus, a known genetic "safe harbour." This stable cell line showed similar behaviour to B2M knockdown cell lines previously established using sgRNA expression from multi-copy piggyBac transposons. In hematopoietic progenitor cells, B2M knockdown was similarly achieved in the absence of doxycycline. But, doxycycline treatment could not recover HLA class I presentation, despite the lack of dCas9-KRAB. These data indicate epigenetic modification introduced at the pluripotent state cannot be removed in differentiated cells. This suggests an altered ability to remove repressive epigenetic marks in pluripotent versus differentiated cells and shed fresh light on CRISPRi reversibility using dCas9-KRAB.

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P-803

A SIMPLE ROBUST EXVIVO MICROFLUIDIC PLATFORM FO STEM CELL ENGINEERING

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As CRISPR/Cas9 systems, and stem cell technologies have matured, so too have the possibilities for their combined use. However efficient delivery of CRISPR systems ex vivo and in vivo remains challenging and serves as a barrier to the progress of these two powerful technologies. Here we present a simple, robust ex Vivo microfluidic platform to overcome these limitations by delivery large CRISPR payloads in a variety of stem cells of up to 90% efficiency with high cell viability. Our method can be scaled up from the lab for industrial purposes thereby making it of great utility. Finally, although we currently do not show data for the invivo editing of stem cells we believe are platform can be tailored for in vivo applications with modifications.

Funding Source: Laguna ventures fund.

P-804

A FLUORESCENT CHEMICAL PROBE CDY9 SELECTIVELY STAINS AND ENABLES THE ISOLATION OF LIVE NAÏVE MOUSE EMBRYONIC STEM CELLS

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Human and mouse embryonic stem cells (ESCs) differ in terms of their pluripotency status, i.e., naïve vs. primed. This affects various biological properties and leads to several technical hurdles for future clinical applications, such as difficulties in chimera formation, single-cell passaging, and gene editing. In terms of generating functional human tissues and organs via mammalian interspecies chimerism, a fluorescent chemical probe that specifically labels naïve ESCs would help to isolate these cells and monitor their conversion. This study demonstrates that the fluorescent chemical probe compound of designation yellow 9 (CDy9) selectively stains naïve, but not primed, mouse ESCs (mESCs). CDy9 entered cells via Slc13a5, a highly expressed membrane transporter in naïve mESCs. Fluorescence-based cell sorting based on CDy9 staining successfully separated naïve mESCs from primed mESCs. Mice generated using CDy9+ cells isolated during the conversion of mouse epiblast stem cells into naïve mESCs exhibited coat color chimerism. Furthermore, CDy9 specifically stained cells in the inner cell mass of mouse embryos. These findings suggest that CDy9 is a useful tool to isolate functional naïve mESCs.

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P-805

IN VIVO TRACKING OF 14C THYMIDINE LABELED MESENCHYMAL STEM CELLS USING ULTRA-SENSITIVE ACCELERATOR MASS SPECTROMETRY

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Mesenchymal stem cells (MSCs) offer high potential in regenerative medicine and are used as therapeutic cell sources in clinical trials. However, even with a therapeutic efficacy, there is an inconsistent effect when injected into the in vivo model, and there is still a limit to the trace associated with the survival amount of injected cells and the location of cells depending on the migration in body. In order to improve the therapeutic effect of MSCs in vivo, a bio-distribution screening of implanted cells using a stable and long-term tracking method is required. Herein, we labeled adipose derived-mesenchymal stem cells (AD-MSCs) using carbon radioisotope labeling thymidine (14C Thymidine), which is able to incorporated into new DNA during cell replication. After the labeling with different concentrations of 14C Thymidine, the radioactive emission and biological characteristics of AD-MSCs were measured. As a result, AD-MSCs labeled with 5 nCi possessed high radioactivity and there was no change in the proliferation compared to the control group, but higher concentrations (10 nCi, 25 nCi) affected the cell proliferation rate. The biological characteristics (i.e. morphology, MSCs specific markers, tri-lineage differentiation into osteoblasts, adipocytes, and chondroblasts) showed no significant changes after labeling. In addition, 14C-labled AD-MSCs were injected into tail vein of nude mice and the organs were collected at hours 4, 12, 24, 48, and 168 after injection to investigate survival rate and cell distribution of MSCs in vivo. The 14C-labled AD-MSCs distributed in each organ were analyzed not only by liquid scintillator counter, which is a conventional analyzer, but also by using Ultra-Sensitive Accelerator Mass Spectrometry (AMS) which can detect less than 10 cells through low radioactivity measurement. Overall, 14C Thymidine and AMS-based technique allows bio-distribution tracking of small amounts of cells in vivo, suggesting that it may be usefully used to reduce the error of results in stem cell therapy and to improve mechanism research of MSCs.

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P-806

MODULATING HUMAN EMBRYONIC STEM CELLS FATE BY THE DIFFERENTIAL EXPRESSION LEVEL OF MIR-5739

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The microRNA is composed of small nucleotides and acts as a transcription factor that inhibits the expression of target genes by binding 3 'untranslated regions. We have constructed a human embryonic stem cells derived knockout cell line to analyze the function and properties of the novel microRNA miR-5739, which has been shown to play an important role in mesoderm lineage development. In particular, this study showed that miR-5739 knockout significantly inhibited the differentiation of endothelial cells and cardiomyocytes, suggesting that miR-5739 gene plays an important regulatory role in muscle and vessel formation during embryonic development.

P-807

ESTABLISHMENT AND VALIDATION OF EGFP KNOCK-IN HIPSCS TO MONITOR DIFFERENTIATION INTO NEURAL PROGENITOR CELLS AND BETA CELLS USING THE CRISPR/CAS9 SYSTEM

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Nestin and SOX1 are specific markers known to be expressed in neural progenitor cells, and PDX1 plays a crucial role in pancreatic development and β-cell maturation. In this study, we introduced an EGFP reporter into the C-terminus of the gene of interest

(NES, SOX1, and PDX1) via homology-directed repair (HDR) using the CRISPR/Cas9 nuclease and established three reporter cell lines (Nestin-EGFP, SOX1-EGFP, and PDX1-EGFP). Successfully edited cell lines were validated by PCR and sequencing analysis to ensure that the EGFP protein and downstream endogenous genes were within the frame. They had a normal karyotype and a typical human pluripotent stem cell-like morphology. They also expressed several pluripotency markers and exhibited differentiation potential into three germ layers. Further, we confirmed the co-localization of the target proteins (Nestin, SOX1, and PDX1) and EGFP by directed differentiation. Taken together, Nestin-EGFP hiPSCs and SOX1-EGFP hiPSCs will provide a useful model for neural differentiation and PDX1-EGFP hiPSCs can be a powerful tool for studies involving pancreatic development and β-cell differentiation. These cell lines are available and registered at the National Stem Cell Bank, Korea National Institute of Health.

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P-808

GENERATION OF THE ENDOSOMAL TOLL-LIKE RECEPTORS KNOCKOUT HUMAN INDUCED PLURIPOTENT STEM CELL LINES USING CRISPR/CAS9 GENOME EDITING SYSTEM

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Till date, thirteen mammalian TLR members (TLR1-13) have been identified, ten in human and thirteen in mice. The human TLRs are further divided into two subgroups: six surface bound (TLR 1, 2, 4, 5, 6 and 10) and four within the intracellular endosomal compartment (TLR 3, 7, 8, and 9) of TLR-expressing cells. TLR3 recognizes double-stranded RNA (dsRNA), TLR7 and TLR8 recognize single-stranded RNA (ssRNA) and TLR9 recognizes DNA. Activation of nucleic acid-sensing TLRs triggers downstream signaling and activation of transcription factors such as NF-κB and IRFs, enhancing pro-inflammatory and anti-viral responses, respectively. Furthermore, the endosomal Toll-like receptors have been implicated in the pathogenesis of autoimmune diseases. Here, we generated TLR3, TLR7, TLR8, and TLR9 knockout human induced pluripotent cell lines (iPSCs) using the CRISPR/Cas9 genome editing method. The TLRs knockout iPSCs showed pluripotent characteristics such as teratoma formation, EB differentiation, pluripotent markers expressions. Further, we differentiated TLR7, TLR8 knockout iPSCs into human macrophage- like cells and confirmed depleted TLR protein expression. These lines are valuable laboratory resources to offer insight into the roles of endosomal TLRs in inflammation and related diseases.

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P-809

NEURONAL ACTIVATION CAN MODULATE ENHANCER ACTIVITY THROUGH DE NOVO DNA METHYLATION

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Stem cells alter their epigenome upon differentiation, acquiring new and stable gene expression pattern. Interestingly, contrary to the notion that terminally differentiated cells exhibit low epigenetic dynamics, recent studies have shown that neuronal activation can induce local DNA demethylation changes without cell fate switching. Here we show that neuronal activity-dependent DNA methylation can even induce de novo DNA methylation in sequence-specific manners. In order to examine the degree to which DNA methylation changes upon neuronal stimulation, we conducted DNA methylome analysis using post-bisulfite adapter-tagging method for primary cultured neurons pre-treated with bicuculline/4-aminopyridine that induces hyperactivation. We identified 826 and 1,075 genomic loci where methylation and demethylation respectively occurred. Using publicly available ChIP-seq data for H3K4me1, we observed accumulation of H3K4me1 at the differentially methylated regions, suggesting that not only DNA demethylation but also

DNA methylation occur at a set of enhancer regions to regulate downstream gene expressions. We tried to confirm the enhancer activity of selected differentially methylated regions using a reporter assay system with a CpG-free luciferase. As expected, the test DNA fragment enhanced the reporter activity and DNA methylation at the differentially methylated regions impaired its enhancer activity. Moreover, supply of a DNMT inhibitor, RG108, or simultaneous knockdown of Dnmt1 and Dnmt3a, reversed the DNA methylation status from hyper-methylated to hypo-methylated in sequence-specific manners. Our results strongly suggest that a set of enhancer regions can be targeted by neuronal activity-dependent rapid de novo DNA methylation through machinery including DNMT1 and DNMT3a. Exploring the mechanisms of this de novo DNA methylation as well as demethylation in terminally differentiated neurons would have a considerable impact on manipulating stem cell epigenetic control.

P-810

UNIVERSAL CORRECTION OF BLOOD COAGULATION FACTOR VIII IN PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELLS USING CRISPR/CAS9

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Hemophilia A (HA) is caused by genetic mutations in the blood coagulation factor VIII (FVIII). Genome editing approaches can be used to target the mutated site itself in patient-derived induced pluripotent stem cells (iPSCs). However, these approaches can be hampered by difficulty preparing thousands of editing platforms for each corresponding variant found in HA patients. Here, we report a universal approach to correcting various mutations in HA patient iPSCs by the targeted insertion of the FVIII gene into the human H11 site via CRISPR/Cas9. We derived corrected clones from two types of patient iPSCs with frequencies of up to 64% and 66%, respectively, without detectable unwanted off-target mutations. Moreover, we demonstrated that endothelial cells differentiated from the corrected iPSCs successfully secreted functional protein in vitro and functionally rescued the disease phenotype in vivo. This strategy may provide a universal therapeutic method for correcting all genetic variants found in HA patients.

P-811

RESTORATION OF OSTEOGENESIS BY CRISPR/CAS9 GENOME EDITING OF THE MUTATED COL1A1 GENE IN OSTEOGENESIS IMPERFECTA

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Osteogenesis imperfecta (OI) is a genetic disease characterized by bone fragility and repeated fractures. The bone fragility associated with OI is caused by a defect in collagen formation due to mutation of COL1A1 or COL1A2. Current strategies for treating OI are not curative. In this study, we generated induced pluripotent stem cell (iPSCs) from OI patients harboring a mutation in the COL1A1 gene. OI-iPSCs exhibited abnormal collagen triple-helix structure and defective mineralization during osteogenesis in vitro. Gene-corrected OI-iPSCs exhibited recovery from the "untwisted" to "twisted" collagen triple-helix structure. In addition, gene correction restored osteogenic potential.

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P-812

GENERATION AND VALIDATION OF GENE-SPECIFIC KNOCK-IN REPORTER LINES OF HUMAN IPS CELLS

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Directed differentiation of human iPS cells toward various cell types has been important as regenerative

medicine, disease modeling, drug development as well as basic biology. cell type-specific reporter iPS cell lines must provide better technological platforms in establishing directed differentiation methods and maintaining and purifying relatively homogenous cell population from human iPS cells. We are attempting to generate knock-in human iPS cell lines for various types of lineage-specific genes. Here we introduce the knock-in lines with a self-renewal marker, OCT4, an early neural marker, PAX6, and early endodermal markers, MIXL1, and SOX17. In order to generate knock-in reporter cell lines CRISPR-Cas9 technology with homologous donor arms is used to generate homologous recombination. Fluorescent proteins are expressed with each gene as separated by 2A-peptide inserted before the stop codon of each gene. Drug resistant genes for selecting knock-in iPS cells are also usually inserted with constitutive active promoters. After drug selection, single cell colony-derived human iPS clones have been isolated. We evaluate these iPSC clones by flow cytometric analysis and genotyping. We believe that our gene-specific knock-in reporter lines of human iPS cells will be widely used in research community.

P-813

STRUCTURE-ACTIVITY RELATIONSHIP ANALYSIS OF YM155 FOR INDUCING SELECTIVE CELL DEATH OF HUMAN PLURIPOTENT STEM CELLS

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Despite great potential for regenerative medicine, the high tumorigenic potential of human pluripotent stem cells (hPSCs) to form undesirable teratoma is an important technical hurdle preventing safe cell therapy. Various small molecules that induce the complete elimination of undifferentiated hPSCs, referred to as 'stem-toxics', have been developed to facilitate tumor-free cell therapy, including the Survivin inhibitor YM155. In the present work, based on the chemical structure of YM155, total 26 analogs were synthesized and tested for stem-toxic activity toward human embryonic stem cells (hESCs) and induced PSCs (iPSCs). We found that a hydrogen bond acceptor in the pyrazine ring of YM155 derivatives is critical for stem-toxic activity, which is completely lost in hESCs lacking SLC35F2 encoding a solute carrier protein. These results suggest that hydrogen bonding interactions between the nitrogens of the pyrazine ring and the SLC35F2 protein are critical for entry of YM155 into hPSCs, and hence stem-toxic activity .

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P-814

FUNCTIONAL EXPRESSION OF NEURONAL DIFFERENTIATION-SPECIFIC SURFACE ANTIGEN

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Cell therapeutic agents for treating degenerative brain diseases using neural stem cells are actively being developed. However, few systems have been developed to monitor in real time whether the transplanted neural stem cells are actually differentiated into neurons. Therefore, it is necessary to develop a technology capable of specifically monitoring neuronal differentiation in vivo. In this study, we established a system that expresses cell membrane-targeting red fluorescent protein under control of the Synapsin promoter in order to specifically monitor differentiation from neural stem cells into neurons. In order to overcome the weak expression level of the tissue-specific promoter system, the partial 5' UTR sequence of Creb was added for efficient expression of the cell surface-specific antigen. This system was able to track functional neu-

ronal differentiation of neural stem cells transplanted in vivo, which will help improve stem cell therapies. This research was supported by a grant from KHIDI: HI16C1013.

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P-815

Withdrawn

P-816

CRISPR/CAS9 NUCLEASE-MEDIATED GENE KNOCK-IN IN BOVINE-INDUCED PLURIPOTENT CELLS

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Efficient and precise genetic engineering in livestock such as cattle holds great promise in agriculture and biomedicine. However, techniques that generate pluripotent stem cells, as well as reliable tools for gene targeting in livestock, are still inefficient, and thus not routinely used. Here, we report highly efficient gene targeting in the bovine genome using bovine pluripotent cells and clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9 nuclease. First, we generate induced pluripotent stem cells (iPSCs) from bovine somatic fibroblasts by the ectopic expression of yamanaka factors and GSK3b and MEK inhibitor (2i) treatment. We observed that these bovine iPSCs are highly similar to naı¨ve pluripotent stem cells with regard to gene expression and developmental potential in teratomas. Moreover, CRISPR/Cas9 nuclease, which was specific for the bovine NANOG locus, showed highly efficient editing of the bovine genome in bovine iPSCs and embryos. To conclude, CRISPR/Cas9 nuclease-mediated homologous recombination targeting in bovine pluripotent cells is an efficient gene editing method that can be used to generate transgenic livestock in the future.

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P-817

RESTORATION OF FVIII EXPRESSION BY TARGETED GENE INSERTION IN THE FVIII LOCUS IN HEMOPHILIA A PATIENT-DERIVED IPSCS

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Target-specific genome editing, using engineered nucleases zinc finger nuclease (ZFN), transcription activator-like effector nuclease (TALEN), and type II clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPRassociated protein 9 (Cas9), is considered a promising approach to correct disease-causing mutations in various human diseases. In particular, hemophilia A can be considered an ideal target for gene modification via engineered nucleases because it is a monogenic disease caused by a mutation in coagulation factor VIII (FVIII), and a mild restoration of FVIII levels in plasma can prevent disease symptoms in patients with severe hemophilia A. In this study, we describe a universal genome correction strategy to restore FVIII expression in induced pluripotent stem cells (iPSCs) derived from a patient with hemophilia A by the human elongation factor 1 alpha (EF1α)-mediated normal FVIII gene expression in the FVIII locus of the patient. We used the CRISPR/Cas9-mediated homology-directed repair (HDR) system to insert the B-domain deleted from the FVIII gene with the human EF1α promoter. After gene targeting, the FVIII gene was correctly inserted into iPSC lines at a high frequency (81.81%), and these cell lines retained pluripotency after knock-in and neomycin resistance cassette removal. More importantly, we confirmed that endothelial cells from the gene-corrected iPSCs could generate functionally active FVIII protein from the inserted FVIII gene. This is the first demonstration that the FVIII locus is a suitable site for integration of the normal FVIII gene and can restore FVIII expression by the EF1α promoter in endothelial cells differentiated from the hemophilia A patient-derived genecorrected iPSCs.

P-818

SPT4 GENE-EDITED STEM CELL THERAPY IN HUNTINGTON'S DISEASE: TRANSPLANTATION OF SPT4 KO HD PATIENT IPSC-DERIVED NPCS RESCUES NEURONAL DYSFUNCTION IN THE YAC128 MODEL OF HD

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Induced pluripotent stem cell technology has provided the possibility that patient-specific iPSCs can be generated and utilized for autologous cell therapy without the concern of immune rejection. However, when iPSCs are developed from patients carrying a genetic mutation(s), the resulting iPSCs will still carry such mutation(s). Spt4 is a translation elongation factor involved in the expansion of CAG repeats. In this study, we first knock-outed the Spt4 gene in the Q57 HD-iPSC-derived neural precursor cells using CRISPR/Cas9 technology, which showed 80~90% indel efficiency. We next transplanted this Spt4-knockout Q57 HD iPSC-NPC, together with unedited Q57 HD iPSC-NPC and media, into the striatum of 6 month-old YAC128 transgenic mouse model of HD. Human-specific nuclei (hNu) antibody staining indicated that transplanted cells were detected in all groups at 12week post- transplantation. Interestingly, transplanted unedited cells showed the expression of mutant huntingtin protein. However, EM48-positive cells were not detected in the transplanted Spt4-knockout cells, suggesting that the gene editing of Spt4 resulted in the removal of mutant huntingtin proteins in Q57 HD iPSC-NPCs. We also observed the same result in the differentiated cells after transplantation. Transplantation of unedited cells exhibited abnormal functions. Especially, astrocytes differentiated from the unedited cells following transplantation showed decreased level of glutamate transporter. We also observed that Spt4 knockout group showed behavioral improvements. From two months after transplantation, Spt4 knockout group showed improvement of motor functions, judged by rotarod and grip strength tests. Improvement of emotional functions, judged by elevated plus maze test, was observed from three months after transplantation. These results strongly suggest that ex vivo editing of Spt4 gene can remove EM48 expression and improve behavioral deficits in YAC128 HD mice, providing a new possibility of silencing the mutation of huntingtin gene, in order to develop autologous cell therapy using HD patient's own iPSC. Given the nature of Spt4 gene, this approach can be generally applied to any types of HD iPSC lines.

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P-819

IMPROPER CONTROL OF IMPRINTING ON MEST/PEG1 PROMOTER IN THE PATIENTS OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a progressive, neurodegenerative disorder leading to dementia and behavioral changes. Extracellular deposition of the amyloid plaques (Aβ) and intracellular deposition of neurofibrillary tangles in neurons are the major pathogenicities of AD; nonetheless, the drugs targeting the above therapeutic targets developed by big pharmaceutical companies are not effective. Therefore, searching for conceptually alternative, novel targets for the treatment of AD is a matter of urgency. Previously, we have shown that Mest/Peg1 inhibits Wnt signaling by the regulation of LRP6 glycosylation. The expression of Mest/Peg1 is regulated by genomic imprinting, only the paternal allele is active for transcription. Interestingly, we found hypermethylation on the promoter of Mest/Peg1, which led to the reduction of Mest/Peg1 mRNA level and the activation of Wnt signaling, in the blood and brain tissues of AD patients. Consistently, the activation of Wnt signaling using BIO and LiCl caused neurodegeneration in cortical and hippocampal neurons. Mest/Peg1 knockout using CRIPSR/Cas9 in mouse embryonic stem cells and P19 embryonic carcinoma cells resulted in neuronal differentiation arrest. The depletion of Mest/Peg1 in primary hippocampal neurons via lentivirus expressing shMest/Peg1 caused neurodegeneration. Overall, our data suggest that improper control of imprinting on Mest/Peg1 during aging may cause or facilitate the progression of AD

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P-820

"OFF-THE-SHELF" HUMAN PLURIPOTENT STEM CELLS FOR IMMUNE-COMPATIBLE CELL THERAPY

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Human pluripotent stem cells (hPSC) provide unique opportunities for cell therapeutic approaches to treat a variety of incurable diseases. One of the major hurdles to overcome before application of hPSC-derived cells for allogeneic cell therapy is immune-incompatibility issue. Human Leukocyte Antigens (HLAs), especially HLA-A, B, and DRB1, existing on the surface of cells play a central role in allogeneic rejection via the activation of T cells. Therefore, establishment of "off-the shelf" hPSC lines that are HLA-compatible with a large number of people is of great benefit. In this study, we designed an easy and efficient method to generate homozygote-like hPSC lines and provided some results about whether these lines could be used for immune-compatible cell therapy or not. This technique would open a new door to efficient generation of "off-the-shelf" hPSC bank that allows most people to receive immune-compatible cell therapy in the near future.

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P-821

SCARLESS ENRICHED SELECTION OF GENOME EDITED HUMAN PLURIPOTENT STEM CELLS USING INDUCED DRUG RESISTANCE

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An efficient gene editing technique for use in human pluripotent stem cells (hPSCs) would have great potential value in regenerative medicine, as well as in drug discovery based on isogenic human disease models. However, the extremely low efficiency of gene editing in hPSCs is a major technical hurdle that remains to be resolved. Previously, we demonstrated that YM155, a survivin inhibitor developed as an anti-cancer drug, induces highly selective cell death in undifferentiated hPSCs. In this study, we demonstrated that the high cytotoxicity of YM155 in hPSCs, which is mediated by selective cellular uptake of the drug, is due to high expression of SLC35F2 in these cells. Consistent with this, knockout of SLC35F2 with CRISPR-Cas9 or depletion with siRNAs made hPSCs highly resistant to YM155. Simultaneous gene editing of a gene of interest and transient knockdown of SLC35F2 following YM155 treatment enabled genome-edited hPSCs to survive because YM155 resistance was temporarily induced, thereby achieving enriched selection of genome-edited clonal populations. This precise and efficient genome editing approach took as little as 3 weeks without cell sorting or introduction of additional genes.

Stem Cell Applications and Regenerative Medicine

P-901

HUMAN NASAL TURBINATE-DERIVED STEM CELLS ATTENUATE RHEUMATOID INFLAMMATION IN MICE

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Multipotent mesenchymal stem cells (MSCs) are widely being studied as a cell-based treatment for inflammatory disorders. The therapeutic effects, however, are sometimes inconsistent and unpredictable. In this study, we investigated the therapeutic effect of human nasal turbinate-derived stem cells (hNTSCs) in collagen-induced arthritis (CIA), the prototype animal model of rheumatoid arthritis (RA). We also compare the therapeutic effects of hNTSCs with those of other sources of MSCs including bone marrow (BM)-derived MSCs or adipose (AD)-derived MSC. hNTSCs can be easily isolated from turbinate tissue discarded during turbinate surgery and possess multipotent capacity and exhibit immunoregulatory properties. Human turbinate-, bone marrow (BM)- or adipose (Ad)-derived mesenchymal stem cells (1 x 106 cells/100µl/mouse) were intravenously injected three times between 5th and 6th weeks after 1st immunization. Human CD4+CD25-T cells are co-cultured with each kinds of MSC and the degree of T cell proliferation was determined using Cell trace violet dye. Human NTSCs attenuated the clinical and histologic features of inflammatory arthritis in CIA mice like BM-MSCs and Ad-MSCs. hNTSCs treated CIA mice showed decreased levels of serum anti-CII IgG2a. The alleviation of arthritis was accompanied by the increase of regulatory T cells. In addition, hNTSC suppressed human T cell proliferation through increase of indoleamine-2,3 dioxygenase. Our findings suggest that hNTSCs ameliorated inflammatory arthritis in mice. They could be a useful cell source for cell-based treatment for RA.

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P-902

TRANSPLANTATION OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES IMPROVES MYOCARDIAL FUNCTION IN INFARCTED RAT HEARTS

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The objective of this study was to evaluate the ability of human iPSC-CMs transplantation to improve myocardial function in the rat myocardial infarction (MI) model. Eight-week old male Sprague-Dawley rats were randomized to receive intramyocardial injection of 5% albumin solution with or without 1x107 human iPSC-CMs at 10 days after left anterior descending coronary artery (LAD) ligation. Cyclosporine A and methylprednisolone were used to prevent graft rejection before iPSC-CMs injection until the rats were killed. Cardiac function was evaluated by echocardiography. The survival of grafted cardiomyocytes was confirmed by labeling with the fluorescent cell tracer Vybrant™ CM-DiI, tagging with enhanced green fluorescent protein (eGFP) expression and polymerase chain reaction (PCR) for human mitochondria DNA. Sirius red stain was used to evaluate the fibrosis ratio. Hematoxylin-eosin (HE)-staining was used to observe the formation of teratoma. During 4 weeks after iPSC-CMs intramyocardial injection, the rats undergoing iPSC-CMs transplantation had a low mortality compared with control group. Functionally, animals injected with 5% albumin solution demonstrated significant left ventricular (LV) functional deterioration, whereas grafting of iPSC-CMs attenuated this remodeling process. Post-MI, ejection fraction deteriorated by 10.11% (from 46.36% to 41.67%), fractional shortening deteriorated by 9.23% (from 24.37% to 22.12%) in control group. While ejection fraction improving by 18.86% (from 44.09% to 52.41%), fractional shorten-

ing improving by 23.69% (from 23.08% to 28.54%) in iPSC- CMs transplantation group. A number of labeling, tracking and molecular biological techniques indicated the grafted cardiomyocytes survived in the rat heart after one month of iPSC-CMs therapy. Sirius red stain indicated that fibrosis decreased in the cell therapy group, but there was no statistical significance. Transplantation of human iPSC-CMs improves myocardial function in infarcted rat hearts and grafted cardiomyocytes survived in the rat heart after one month of transplantation, which highlight the potential of iPSC-CMs for myocardial cell therapy strategies.

P-903

TARGETING SOX9 EXPRESSION IN HUMAN NEURAL STEM CELLS IS A THERAPEUTIC STRATEGY FOR SPINAL CORD INJURY

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Neural stem cells (NSCs) exist in both embryonic and adult neural tissues and are characterized by their self-renewal capacity and multipotency that contribute to the formation of neurons, oligodendrocytes and astrocytes in the vertebrate central nervous system (CNS). The tremendous therapeutic potential of NSCs to treat CNS diseases and injuries has provoked intensive study in the regulation of their formation and differentiation into specific neural lineage. Previous studies showed that SOX9 is crucial for the formation and maintenance of NSCs as well as inhibition of neuronal differentiation in both mouse and chick CNS. Whether SOX9 plays similar roles in human NSCs (hNSCs) remains unknown. Here, we demonstrate that high SOX9 expression is associated with acquisition of hNSC fate. Targeting SOX9 expression by shRNA does not affect hNSCs formation, but promotes precocious differentiation into motor neurons compared to scramble control. Gene expression profiling studies in SOX9 knockdown (SOX9 KD) hNSCs reveal upregulated expression of sonic hedgehog and its downstream effectors that are known to promote spinal neuron formation. Most importantly, transplantation of SOX9 KD hNSCs into the injured rodent spinal cord results in robust differentiation into motor neurons without forming glial scar at the lesion site and also improved locomotion capabilities. Altogether, our findings reveal a distinct, but conserved, role of SOX9 in NSCs formation and the onset of neuronal differentiation between human and other vertebrate species, respectively. Targeting SOX9 expression in hNSCs could be a viable therapeutic strategy for spinal cord injury.

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P-904

HIGH EXPRESSION OF SSEA4 IN HUMAN ADIPOSE STEM CELLS HAS POTENTIAL FOR INDUCING TO DEFINITE ENDODERM

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Human adipose stem cells (hASCs) have the advantages of easiness to collect large amounts of the cells that maintain the characteristics of stem cells regardless of age. In vitro, hASCs are possible to proliferate, and differentiate to chondrocyte, adipocyte, osteocyte with differentiation condition. We have isolated hASCs from adipose tissues of various donors with surgical operations in Inha University Hospital [INHAUH 2015-09-004]. Then, hASCs were maintained in an α-MEM supplemented with Human platelet lysate (HPL). Growth potential of hASC was measured, and surface antigen expression of hASCs was analyzed for surface marker of mesenchymal stem cells (MSCs) (CD105, CD90, and CD73), Hematopoietic stem cell/ leukocyte (CD34 and CD45), MHC class (HLA- ABC and HLA-DR), and embryonic stem cell (SSEA4) by flow cytometry at passage 5. The stemness of cells were evaluated by colony-forming unit (CFU) assay at passage 5. Multi-lineage-differentiation ability was also investigated in differentiation condition and with staining for each lineage. As a result, their surface antigen expression was positive CD105, CD73, CD90 and negative CD34, CD45, HLA-DR. Although there was variation depending on donors, they were similar with the cells in culture media supplemented with FBS in terms of CFU, and multi-lineage-differentiation ability. We investigated experiments that induced definite en-

doderm in SSEA4 gene expression groups with more than 60% and less than 60%. Induction of definite endoderm cells was examined for gene expression level of FOXA2, CER1, CXCR4, and SOX17, endodermal markers by RT-PCR. Compared with more than 60 percent of the SSEA4 gene expression groups, they were expressed significantly higher definite endodermal markers level than the lower group. In conclusion, this study shows that SSEA4 gene has potential to induce definite endoderm in hASC.

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P-905

COMPARISON OF HUMAN PLATELET LYSATE WITH FETAL BOVINE SERUM FOR PROLIFERATION POTENTIAL OF LIVER-DERIVED CELLS

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Fetal bovine serum (FBS) is one of serum supplements that have been commonly used in cultivation of mammalian cells. However, requirement for alternative of the supplement has been recently emerging because of safety issues such as viral contamination as well as its toxic impact on mesenchymal stem cells (MSCs) when infused in human. Moreover, needs for large amounts of cells when applied to cell therapy requires FBS replacement to grow the MSCs as much as possible as well as to be able to escape from the safety issues in culture medium. Human platelet lysate (HPL) was originated from human serum and is well-known for replaceable supplement of FBS in culture medium for its advantages in growth potential and safety. We compared HPL with FBS as supplements in growth medium and investigated the characteristics of liver-derived cells, such as gene and protein expression, surface antigen expression, osteogenic potential, growth potential. As the result, cells in HPL-based medium showed an outstanding proliferative capacity than those in FBS-based medium in terms of doubling time and cumulative population doubling level (CPDL). Average doubling time from passage 2 to 10 for liver-derived cells in FBS-based medium and HPL-based medium was 83.7 ± 1.3 and 36.2 ± 1.6 hrs, and average CDPL at passage 10 was 27.9 ± 0.6 and 15.5 ± 1.3, respectively. They both expressed hepatocyte specific maker, albumin and an endodermal maker, SOX17. Liver-derived cells in HPL-based medium were positive for CD73, CD90, and CD105, as the typical MSCs Markers, and negative for CD34 and CD45, heamatopoetic stem cells markers. Moreover, liver-derived cells cultured in HPL-based medium showed the similar osteogenic potential compared with cells in FBS-based medium Based on these results, HPL is a superior xeno-free media supplement and an alternative to FBS in large-scale culture of liver-derived cells for cell therapy.

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P-906

EXPANSION OF HUMAN LIVER-DERIVED CELLS IN BIONOC CARRIERS

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Human mesenchymal stem cells (hMSCs) are considered as a primary candidate in cell therapy because of their proliferation capacity, multi-differentiation potential, and secretion of growth factors an cytokines. For clinical application, large quantities of therapeutically competent cells are required that cannot be generated in conventional cell culture in plastic dishes. Two dimensional culture has also many limitations, such as the disturbance of interactions between the cellular and extracellular environments, changes in cell morphology, polarity, and method of division. Unlike the more 2D monolayer culture, BioNOC carriers provide to cells with 3D matrix as in vivo microenvironment which may influence cell function, morphology and gene expression. In this study, we investigated the massive expansion of liver-derived cells including the procedures to monitor the proliferation, metabolic status and phenotype of cells during cultivation in CelCradle system. In the results, we could obtain maximum of 4.2x10^8cells with one CelCradle and expected to achieve enough cell numbers required for cell therapy with 5 CelCradles. Liver-derived cells showed osteogenic differentiation potency and they were positive for the antigens of MSC markers (CD44, CD73, and CD90) In conclusion, our results suggest CelCradle is suitable for cGMP compliant culture conditions enabling the clinical grade production of liver-derived

cells.

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P-907

ESTABLISHMENT OF FUNCTIONAL MACROPHAGE DIFFERENTIATION FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Monocytic lineage cells, such as monocytes, macrophages and dendritic cells (DCs), are central to immune responses and play key regulator in various pathological conditions. Erythro-myeloid progenitors (EMPs) can give rise to yolk sac-derived primitive macrophages, then embryonic macrophages were completed cellular differentiation and specialization within the final resident tissues. Aside from providing the first line of defense against invading pathogens, tissue-resident macrophages have a fundamental role in maintaining tissue integrity and homeostasis. For this reason, development of tissue-resident macrophage cells from human induced pluripotent stem cells (hiPSCs) is of particular interest because it provides an unlimited cell source in various research fields. Here, we demonstrated efficient generation of functional macrophages from hiPSCs. Hemangioblast-like hematopoietic cells were induced by sequential addition of BMP4 and the combination of bFGF, VEGF and SCF. To induced macrophage, we generated CD45+ hematopoietic cells and CD14+ monocytes with defined cytokines. These cells were cultured with FBS and M-CSF for differentiation into macrophages. hiPSC-derived macrophages (mPs) were identified by the cell surface marker expression at each step. Also, these cells were polarized into distinct inflammatory (M1) or anti-inflammatory (M2) subtypes with exposure to LPS or IL-4. These subtypes showed specific gene and cell surface protein expression, respectively. hiPSC-mPs demonstrated phagocytic capability, as determined by the uptake of beads. We asked whether hiPSC-mPs could become liver-resident macrophages. Therefore, hiPSC-mPs co-cultured with hiPSC-derived hepatocyte-like cells (hiPSC-HLCs) and then induced inflammatory response. High-magnification imaging showed a direct physical interaction between hiPSC-HLCs and mPs. hiPSC-mPs secreted TNF-α, which is kupffer cell-specific inflammatory cytokine by LPS stimulation. Also, expression of CD163 surface marker that associated with hepatic inflammation. In conclusion, we generated functional hiPSC-mP and these cells have potential to give rise tissue-resident macrophages that can be used for pathophysiological and toxicological studies.

P-908

LONGITUDINAL INTRAVITAL IMAGING OF TRANSPLANTED MESENCHYMAL STEM CELLS ELUCIDATES THEIR FUNCTIONAL INTEGRATION AND THERAPEUTIC POTENCY IN AN ANIMAL MODEL OF INTERSTITIAL CYSTITIS

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Mesenchymal stem cell (MSC) therapy may be a novel approach to improve interstitial cystitis/bladder pain syndrome (IC/BPS), an intractable disease characterized by severe pelvic pain and urinary frequency. Unfortunately, the properties of transplanted stem cells have not been directly analyzed in vivo, which hampers elucidation of the therapeutic mechanisms of these cells and optimization of transplantation protocols. Here, we monitored the behaviors of multipotent stem cells (M-MSCs) derived from human embryonic stem cells (hESCs) in real time using a novel combination of in vivo confocal endoscopic and microscopic imaging and demonstrated their improved therapeutic potency in a chronic IC/BPS animal model. Ten-week-old female Sprague-Dawley rats were instilled with 10 mg of protamine sulfate followed by 750 μg of lipopolysaccharide weekly for 5 weeks. The sham group was instilled with phosphate-buffered saline (PBS). Thereafter, the indicated dose (0.1, 0.25, 0.5, and 1×106 cells) of M-MSCs or PBS was injected once into the outer layer of the bladder. The distribution, perivascular integration, and therapeutic effects of M-MSCs were monitored by in vivo endoscopic and confocal microscopic imaging, awake cystome-

try, and histological and gene expression analyses. A novel combination of longitudinal intravital confocal fluorescence imaging and microcystoscopy in living animals, together with immunofluorescence analysis of bladder tissues, demonstrated that transplanted M-MSCs engrafted following differentiation into multiple cell types and gradually integrated into a perivascular-like structure until 30 days after transplantation. The beneficial effects of transplanted M-MSCs on bladder voiding function and the pathological characteristics of the bladder were efficient and long-lasting due to the stable engraftment of these cells.

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P-909

COMPARISON OF BONE MARROW-DERIVED MESENCHYMAL STEM CELLS (BMSCS) ISOLATED FROM NORMAL AND CIRRHOTIC PATIENTS

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Mesenchymal stem cells (MSCs) have been actively studied for use as regenerative medicine for various intractable diseases including cirrhosis. In particular, autologous or allogenic MSCs isolated from bone marrow (BM) and umbilical cord have been applied in clinical trials. Nevertheless, none of the autologous MSCs of the cirrhotic patient and normal MSCs have been shown to be effective in the treatment of cirrhosis. The aim of this study is to identify the BM-derived MSCs (BMSCs) suitable for the treatment of cirrhosis by comparing the characteristics of the BMSCs isolated from the BM of liver cirrhosis patient and normal BM. Mesenchymal stem cells from liver cirrhosis patients (BpMSCs) were obtained from Pharmicell. Normal MSCs (BcMSCs) were isolated and cultured from normal BM mononuclear cells in Lonza. Potentials of proliferation and differentiation, cell surface antigen expression, immunosuppressive activity and mitochondrial activity of BMSCs were compared and analyzed. In the early passage, the population doubling time of BpMSCs was 68 hours, 10 hours shorter than that of BcMSCs, when the BMSCs were cultured until 150 hours of population doubling time. But the number of cells finally obtained was higher in BcMSCs. Adipocyte differentiation was slightly better in BcMSCs and cell surface antigen expression (MFI) of CD90 and CD105 was higher in BcMSCs. The inhibition of active IL-1beta secretion in macrophages was also higher and the activity of mitochondria was slightly higher in BcMSCs. The proliferative capacity of BMSCs in the early stage of subculture was high in BpMSCs, but the differentiation potential, cell surface antigen expression, immunosuppressive activity and mitochondrial activity were higher in BcMSCs. However, there was no statistical significance. Therefore, a larger number of BMSCs need to be compared and analyzed, and there is a need to analyze the therapeutic effect in an animal model of liver cirrhosis. Therefore, it is necessary to comparatively analyze a larger number of BMSCs, and it is necessary to directly analyze the therapeutic effect in an animal model of liver cirrhosis.

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P-910

STEM CELL THERAPY FOR THE TREATMENT OF SEVERE TISSUE DAMAGE AFTER RADIATION EXPOSURE

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The late adverse effects of pelvic radiotherapy concern 5 to 10% of them, which could be life threatening. However, a clear medical consensus concerning the clinical management of such healthy tissue sequelae does not exist. Our group has demonstrated in preclinical animal models that systemic MSC injection is a promise approach for the medical management of gastrointestinal disorder after irradiation. We have shown that MSC migrate to damaged tissues and restore gut functions after irradiation. The clinical status of four first patients suffering from severe pelvic side effects resulting from an over-dosage was improved following MSC injection in a compassional situation. A quantity of 2x106 - 6x106 MSC /kg were infused intravenously to the patients. Pain, hemorrhage, frequency of diarrheas and fistulisation as well as the lymphocyte subsets in peripheral blood were evaluated before MSC therapy and during the follow-up. Two patients revealed a substantiated clinical response for pain and hemorrhage after MSC therapy. In one patient pain reappeared after 6 months and again substantially responded on a second MSC infusion. A beginning fistulisation process could be stopped in one patient resulting in a stable remission for more than 3 years of follow-up. The frequency of painful diarrhea diminished from an average of 6/d to 3/d after the first and 2/d after the 2nd MSC injection in one patient. In all patients, prostate cancer remained in stable complete remission. A modulation of the lymphocyte subsets towards a regulatory pattern and diminution of activated T cells accompanies the clinical response in refractory irradiation-induced colitis. No toxicity occurred. MSC therapy was safe and effective on pain, diarrhea, haemorrhage, inflammation, fibrosis and limited fistulisation. For patients with refractory chronic inflammatory and fistulising bowel diseases, systemic MSC injections represent a safe option for salvage therapy. A clinical phase II trial will start in 2019.

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P-911

DOPAMINE TRANSPORTER NEUROIMAGING ACCURATELY ASSESSES THE MATURATION OF DOPAMINE NEURONS IN A PRECLINICAL MODEL OF PARKINSON'S DISEASE

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Significant developments in stem cell therapy for Parkinson's disease (PD) have already been achieved, however, methods for reliable assessment of dopamine neuron maturation in vivo are lacking. Establishing the efficacy of new cellular therapies using non-invasive methodologies will be critical for future regulatory approval and application. To examine the utility of neuroimaging to characterise the in vivo maturation, innervation and functional dopamine release of transplanted human embryonic stem cell-derived midbrain dopaminergic neurons (hESC-mDAs) in a preclinical model of PD. Female NIH RNu rats received a unilateral stereotaxic injection of 6-OHDA in to the left medial forebrain bundle to create the PD lesion. hESC-mDA cell and sham transplantations were carried out 1 month post-lesion, with treated animals receiving approximately 4 x 105 cells per transplantation. Behavioural analysis, [18F]FBCTT and [18F]Fallypride microPET/CT was conducted at 1, 3 and 6 months post-transplantation and compared with histological characterisation at 6 months. PET imaging revealed transplant survival and maturation into functional dopaminergic neurons. [18F]FBCTT-PET/CT dopamine transporter (DAT) imaging demonstrated pre-synaptic restoration and [18F]Fallypride-PET/CT indicated functional dopamine release, while amphetamine-induced rotation showed significant behavioural recovery. Moreover, histology revealed that the grafted cells matured differently in vivo producing high-TH and low-TH expressing cohorts, and only [18F]FBCTT uptake was well correlated with TH differentiation. This study provides further evidence for the value of in vivo functional imaging for the assessment of cell therapies and highlights the utility of DAT imaging for the determination of early post-transplant cell matura-

tion and differentiation of hESC-mDAs.

P-912

DIFFERENTIATION OF HUMAN LIVER-DERIVED STEM CELLS INTO HEPATIC LINEAGE FOR THERAPEUTIC LIVER RECONSTITUTION

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In patients with end-stage liver disease, liver transplantation is the only available curative approach. But due to organ shortages and long latencies, stem cell therapy is being proposed as a promising tool to extend the latency. We here present modified two-step differentiation methods for adult stem cells into the hepatic lineage, especially using resourceful human liver-derived stem cells (hLD-SC) which present mesenchymal stem cell markers (CD90 and CD105). Fadusil and 5-azacytidine were supplemented with Nicotinamide, FGF, and HGF to initiate two-step hepatocyte differentiation. The protocol produced a robust differentiation of stem cells into hepatic fate in hLD-SC by days 7 through 21. Especially, cells with 7-day differentiation showed most advanced mitochondrial functions. During in vivo cell transplantation, hLD-SC migrated into the liver within 2 hours. All stem cells and cells on differentiation settled in the liver over 2 weeks and acted as functional cells in TAA-induced liver injury mice. Those results cumulatively support that our two-step differentiation protocol on hLD-SC is a promising source for cell therapy with unique regenerative potential in liver reconstruction for patients with severe liver diseases.

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P-913

REPAIRING THE CELL TRANSPLANT PARADIGM FOR PARKINSON'S DISEASE: SCIENCE AND ETHICS

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Parkinson' Disease (PD) has proven to be both very difficult to address through cell transplantation and yet still beckons as putatively low-hanging fruit in the cell transplantation world. Fetal ventral mesencephalic (FVM) cell transplants were successful in reducing symptoms in a small number of cases, but only in the hands of a small number of clinician-scientists whose results have been difficult to replicate. Possible explanations include variation in the quality (for clinical purposes) of the fetal tissues used, variation in mode, site, and mechanism of delivery, and the number of cells / size of sample delivered; additionally, there were concerns about the methods for measuring success, questions about the mode of action, and issues associated with inclusion and exclusion criteria for participants. Concerns on many of these fronts needed to be alleviated in order to proceed with the cell transplant paradigm for treating PD. FVM cell transplant research dwindled, but the cell transplant paradigm was rescued nearly twenty years ago when there was much excitement about the prospect of cultivating hPSCs for transplantation. Using cells derived from hPSC research would address the first of the possible explanations of the demise of FVM transplants: the clinical quality of the cells to be transplanted. No longer would surgeons be transplanting gross fetal tissue that might vary from fetus to fetus (as from surgery to surgery); instead, hPSC scientists imagined creating clinical grade cells for transplantation. Were everyone to use the same calls, or well-characterized variants thereof, rapid clinical progress might be achieved on the path to cell-based treatments for PD. And yet another two decades have passed without a successful

cell-transplant treatment for PD. One possible explanation is that research has been hampered by ethical restrictions on deriving and studying hPSCs from human embryos. A more probable explanation is that the almost-exclusive (and reductionistic) focus on the cellular material to be transplanted has led to the neglect of all of the other issues originally identified in FVM research. As we move into a new round of attempts in the cell-transplantation paradigm, it is imperative that we step back and systematically explore more systemic issues to make sure we do not repeat past mistakes.

P-914

RADIOGRAPHIC AND HISTOPATHOLOGIC ANALYSIS OF BONE REGENERATION BY EARLY OSTEOGENIC INDUCED ADIPOSE-DERIVED STEM CELLS IN IMPROVE METHOD ON BIODEGRADABLE SCAFFOLD

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Bone fracture and defect are life-threatening injuries because of the slow nature of healing. The objective of this study was to investigated the regeneration capacity of adipose-derived mesenchymal stem cells (ADMSCs) in improve method on biodegradable scaffold in canine femoral segmental defeat model by X-ray, microCT and histopathology. This analysis provided information on the correct positioning of the scaffold and on the quantification of bone regeneration by measuring bone mineral density (BMD). We induced canine femoral segment defect model and apply canine ADMSCs (cADMSCs)cultured in CEFOgro™ cADMSC on scaffolds in defect lesion. During 12 weeks, animals were taken X-ray at interval of 4 weeks. After 12 weeks, animal were sacrificed and femur were collected. Isolated femurs were evaluated by microCT and undecalcified histopathologic analysis to quantify the bone regeneration such as increasing BMD and changing cellular morphology. During the periods, Body weight and serum biochemistry showed that canine ADMSCs cultured in CEFOgro™ cADMSCs did not cause toxicity in canine bone defect model. The radiological and histological finding confirmed that canine ADMSCs cultured in CEFOgro™ cADMSCs were biocompatible and does not induced any inflammatory reaction. In addition, areas in the defect sites in the cADMSCs treatment group was almost recovered as normal tissue. In conclusion, the results of the present study demonstrate that transplantation of canine ADMSCs cultured in CEFOgro™ cADMSC show improve new bone formation efficiency in canine segmental bone defects. Further research is necessary to investigate application of CEFOgro™ cADMSC cultured ADMSCs in various disease models.

P-915

INVESTIGATING THE EFFICACY OF MESENCHYMAL STEM CELLS ADMINISTERED INTO A MOUSE MODEL OF VASCULAR COGNITIVE IMPAIRMENT

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Like Alzheimer's disease (AD), effective options to treat vascular cognitive impairment (VCI) are limited. Recently, mesenchymal stem cells have emerged as a potential candidate to treat a wide range of diseases including AD. Very few studies have looked into the effects of MSCs as a treatment option for VCI. Thus, the objective of this study was to examine the therapeutic effects of MSCs in a mouse chronic hypoperfusion mouse model. By referring to a recently reported method, a microcoil and ameroid constrictor were used to gradually constrict the left and right common carotid arteries of C57BL6/J mice. Ten-eleven days after inducing VCI, human mesenchymal stem cells or MEMα1x media (control) were transplanted bilaterally into the lateral ventricles of the mice. Behavioral performance was assessed prior to stem cell transplantation and before sacrificing the mice. MR images were acquired weekly up to the sacrifice time point. Mice were sacrificed 10-11 days after stem cells were injected. Mixed pathologies were observed from the mice. Some mice showed hippocampal neuronal loss

or subcortical infarcts and others showed both. Compared to the MEM group, the MSC group showed no significant difference in motor and memory function. Furthermore, a striking difference in both microglia (Iba-1) and hippocampal neuronal density (NeuN) were not noted when comparing the MSC group to the MEM group. Further study is warranted. The optimal time point to administer stem cells and reproducibility of the mouse models must be accounted for to closely examine the therapeutic application of MSCs for the treatment of VCI.

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P-916

FEEDER–FREE, CHEMICALLY DEFINED SYSTEM FOR LONG TERM HUMAN PLURIPOTENT STEM CELL CULTURE

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Human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) have conventionally been cultured using chemically undefined media on feeder dependent culture systems that restrict their applications in regenerative medicine. Development of feeder-free hPSCs culture systems has been an important focus of hPSCs research that abide by GMP guidelines for applications in regenerative medicine. Recently, researchers have established numerous culture systems using defined combinations of xeno-free matrices and media that support the growth and differentiation of hPSCs. Here we describe a detailed and updated hPSCs feeder-free culture method using rhVitronetin and TeSR-E8 medium that includes a supplement of bioactive lysophospholipid that promote the properties of hPSCs like proliferation and maintaining stemness. Enhanced synthetic sphingolipid, cP1P (O-cyclic Phytosphingosine-1-phosphate), has a potential role in increment of proliferation while decreasing apoptosis and/or modulates the self-renewal potential of the hPSCs during long-term culture.

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P-917

ENHANCEMENT OF HOMING PHENOMENON IN SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLE LABELED MESENCHYMAL STEM CELL USING MAGNETIC ATTRACTION

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Optimal delivery of the stem cell to the desired organ or the target area is essential for improving the benefits of stem cell based therapies. However, the manipulation of the stem cell migration to the target site still remains as a trial matter. In this study we have applied the superparamagnetic iron oxide nanoparticle with the mesenchymal stem cell to determine the manipulation of stem cell migration with the use of magnet attached within the target area. Our results showed the at MSC have remained in the area near the magnet. Thus, magnetic manipulation of the polymeric iron oxide nanoparticle labeled mesenchymal stem cell can be used as an option for the efficient and optimal delivery of the stem cell to the target site.

P-918

TESTING THE NEUROTROPHIC SUPPORT OF MESENCHYMAL STEM CELLS IN A MOUSE MODEL OF ALS

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Amyotrophic lateral sclerosis (ALS) is a terminal neurodegenerative disease that results in a loss of motor neurons in the brain and spinal cord, leading to a deterioration of motor function and ultimately culminating in death. Currently there are no effective therapies for ALS. Our goal is to develop novel therapeutic strategies that prevent the degeneration of upper motor neurons (UMNs) in the motor cortex of the brain. Our brain-centric approach is based on the postulated dying forward model, which suggests that UMN pathology precedes lower motor neuron (LMN) loss in the brainstem and spinal cord. Our hypothesis is that targeting ALS disease pathology in UMNs will delay or even prevent the progression of ALS to LMNs. For this purpose, we use hSOD1G93A transgenic mice, an ALS model. We have delivered a proprietary population of mesenchymal stem cells (MSC-NTFs) that secrete neurotropic factors (NurOwn®; Brainstorm Cell Therapeutics) into hSOD1G93A pre-symptomatic mice at week 12, targeting two brain sites into the motor cortex and striatum. Therapeutic efficacy was then monitored using a suite of motor behavioural assays and histology. Preliminary data suggests that the delivery of MSC-NTFs in the striatum of hSOD1G93A transgenic mice may delay disease onset, whereas delivery into the motor cortex was not efficacious in this experiment. Future studies are now directed at testing whether we have injected the optimal cell number, at further testing the striatum as a novel target for therapeutic delivery, and at characterizing the phenotype of MSC-NTFs post-transplant. Ultimately our goal is to evaluate the therapeutic potential of MSC-NTFs directly delivered within specific brain regions in ALS.

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P-919

Withdrawn

P-920

ALLOGENEIC UMBILICAL CORD BLOOD THERAPY COMBINED WITH ERYTHROPOIETIN FOR CHILDREN WITH CEREBRAL PALSY: A DOUBLE BLIND, 2X2 FACTORIAL, RANDOMIZED PLACEBO-CONTROLLED TRIAL

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Children with cerebral palsy (CP) treated by allogeneic umbilical cord blood (UCB) with erythropoietin (EPO) showed therapeutic efficacy in our previous report, yet it lacked identification of synergistic effect of UCB and EPO combination therapy by not including UCB alone treated group. This randomized placebo-controlled trial aimed to identify individual and synergistic efficacies of UCB and EPO in children with CP. Children with diagnosis of CP whose age were between 10 months and 6 years were randomly assigned into four groups: A) UCB + EPO, B) UCB + placebo EPO (pEPO), C) placebo UCB (pUCB) + EPO, and D) pUCB + pEPO. A single infusion of allogenic UCB or pUCB was delivered intravenously and 500 IU/kg of EPO or pEPO were injected subcutaneously for 6 times twice per week. Gross motor function measure (GMFM), gross motor performance measure (GMPM), Bayley scales of infant development-II (BSID-II) were assessed at baseline, 1, 3, 6

and 12 months post-intervention. Also brain images including diffusion tensor tractography and electroencephalography were followed up at 12 months post-intervention. All adverse events were monitored during 12 months. Total 124 children with CP were screened and 92 children were enrolled, and finally 88 completed the study (n=22, 24, 20, and 20 for group A, B, C, and D, respectively). There were no significant differences in baseline characteristics among four groups. Group A showed meaningful improvement in GMPM score at 1 and 12 months post-therapy compared to group D ($p=0.016$). In group A, subjects who received more HLA-matched UCB and more number of cell presented better outcomes in GMFM scores ($p<0.05$). Diffusion tensor image showed biggest increment of fractional anisotropy value at right anterior thalamocortical radiation in group A compared to the other 3 groups ($p<0.05$). Also, brain waves showed more mature pattern in the intervention groups after 12 months, whereas it did not in group. During a year, 10 serious adverse events were reported without difference of distribution among the groups and the events were all resolved. Also other adverse events were not different among the groups. These results suggest that the allogeneic UCB and EPO combination therapy is effective for neurological recovery in children with CP without side effects.

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P-921

BIOACTIVE LYSOPHOSPHOLIPID CP1P ENHANCES DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS INTO CARDIOMYOCYTES

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Pluripotent Stem Cells (PSCs) possess the properties of self-renewal and pluripotency, the ability to differentiate to cells from the three germ layers. Therefore, hPSCs have been suggested as potential therapeutic targets for numerous diseases. Using a modified protocol for differentiation of hPSCs into cardiomyocytes, we examined whether bioactive lipid cP1P could enhance cardiomyocyte differentiation by checking its effect at various stages which were validated confirmed by qRT-PCR, western blotting and immunofluorescence. We focused on the SMAD pathway to study its effects in cardiac differentiation and we examined the potential roles of SMAD in the upregulation of cardiac related genes by cP1P which were confirmed by western blotting. We showed that treatment of cP1P during differentiation resulted in the upregulation of cardiac related genes. These findings demonstrate that although the mechanism responsible for cardiac development has not been completely elucidated, cP1P appears to augment the differentiation of hPSCs into cardiomyocytes. Moreover, cP1P has also been shown to regulate SMAD signaling and expedite the up-regulation of cardiac related genes.

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P-922

POTENTIAL APPLICATION OF HUMAN NEURAL CREST-DERIVED NASAL TURBINATE STEM CELLS IN MODELS OF ALZHEIMER'S DISEASE AS A CLINICALLY APPLICABLE THERAPY

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Stem cell transplantation is a promising therapeutic strategy for the treatment of many neurological disorders; however, the efficacy and safety of stem cell therapy depend on the cell type used in therapeutic and translational applications. Human neural crest-derived nasal turbinate stem cells (hNTSCs) are an excellent alternative source of adult stem cells for clinical use because they can be obtained easily by minimally invasive collection procedures and expanded rapidly ex vivo for transplantation. Moreover, hNTSCs show neurogenic properties in culture, which appear to be

clinically promising candidates for stem cell therapy in neurodegenerative disease. In the present study, we investigated its potential for treatment of Alzheimer's disease (AD) in comparison with human bone marrow-derived mesenchymal stem cells (hBMSCs), which is the most commonly used cell type for treatment of neurological disorders. Here, co-culture of hNTSCs with human neural stem cells (hNSCs) in the presence of amyloid-beta 42 (Abeta42) showed much greater cell viability compared with hNSCs treated with only Abeta42, similar result was also seen for hBMSCs. Likewise, in a mouse model of AD, transplantation of hNTSCs markedly reduces the levels of Abeta42 and plaque formation, microglial activation in the brain of 5xFAD transgenic AD mouse model, concomitant with increased survival of hippocampal and cortex neurons when compared with transplantation of hBMSCs. Moreover, transplantation of hNTSCs led to greater increase of Abeta-degradading enzyme neprilysin (NEP) expression and autophagy signals than hBMSCs. Notably, transplantation of hNTSCs greatly improved cognitive impairments, which could be explained by increase of TIMP2 expression, which are necessary for spatial memory in young mice. The clinical relevance of these results is underlined by the potential of treatment for patients with AD transplanted with hNTSCs.

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P-923

SCRAPER-FREE DETACHMENT METHOD USING EDTA WITHOUT TRYPSIN FOR HUMAN INDUCED PLURIPOTENT STEM CELLS CULTURED ON LAMININ-511E8

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Recombinant E8 fragment of laminin-511 (LM511E8) is used in the culturing of human pluripotent stem cells (hPSCs) because it sustains long-term pluripotency, an undifferentiated state, and single cell passaging. LM511E8 is a truncated form of laminin-511, and its ability to offer these features comes from its binding activity with integrin α6β1, an isoform predominantly expressed on hPSCs. However, because of their strong interaction with each other, scraping following trypsinization is recommended to harvest the cultured cells. Nevertheless, cell scraping causes mechanical damage to the cells, reducing cell viability. In addition, cell scraping cannot be used when cells are cultured on multi-layer flasks or in automated cell culture systems. In this study we aim to develop a scraper-free cell detachment method for human induced pluripotent stem cells (hiPSCs) cultured on LM511E8. By referring to protocols available to date, we examined whether incubation of hiPSCs with 5 mM EDTA/PBS(-) at 37˚C for 15 min enabled the detachment of cells with high efficiency. The inclusion of trypsin to EDTA/PBS(-) was also tested to see if there were any additional gain to cell detachment efficiency. We found that more than 95% of hiPSCs were detached without compromising cell viability when they were incubated with 5 mM EDTA/PBS(-) alone. Surprisingly, the addition of trypsin remarkably decreased the detachment efficiency. This decrease was rescued when enough trypsin inhibitor was added to neutralize the enzyme. Quantification of the protease activity confirmed that the lowered detachment rate was dependent on the trypsin activity, suggesting an ill-defined mechanism operating in hiPSCs to render them less susceptible for cell detachment by depletion of divalent cations.

P-924

THE EFFICACY OF THE INDUCED NEURAL STEM CELL TRANSPLANTATION ON MOTOR FUNCTION RECOVERY IN HUNTINGTON'S DISEASE MODEL ANIMALS TREATED WITH QUINOLINIC ACIDS

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Huntington's disease is an inherited neurodegenerative disease characterized by chorea, depression and dementia caused by progressive nerve cell degeneration.

Since there is no therapeutic agents for this disease, the cell-based therapies that replace lost neurons can be a new therapeutic approach. In this study, we observed the therapeutic effects of induced neural stem cells (iNSCs), which were directly converted from human somatic cells, in Huntington's disease model animal rats. iNSCs expressed the NSC markers, Sox1, Sox2, and Nestin, and differentiated into neuronal and glial cells in vitro that expressed neuronal and glial markers such as Tuj1, MAP2 and GFAP. For Huntington's disease model animals, SD rats were injected with 200nmol/ul Quinolinic acids, one of the neurotoxins, as injecting into right striatum of brain. A week after the lesion was induced, the iNSCs were transplanted into striatum of rat brain. The degree of recovery was then measured every two weeks through the rotarod, balance beam, and apomorphine-induced tests. In the experimental group transplanted with the iNSCs, the rats were stayed in the rota-rod for a longer time, and passed the Balance beam faster, and significantly decreased the number of rotations in apomorphin-induced test compared to the control group injected with PBS. The efficacy was appeared two weeks after transplantation, and it was sustained up to 12 weeks. In our immunostaining assays of rat brain slices, the transplanted iNSCs were differentiated into GABAergic neurons and astrocytes, which were positive against Tuj1, MAP2, GAD65/67 and GFAP antibodies. These results show that transplantation of iNSCs improved the motor functions in Huntington disease model animal rats, suggesting that the iNSCs could be used as a therapeutic cell source for the treatments of Huntingtons's disease and other neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease and Amyotrophic lateral sclerosis in the future.

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P-925

ENDOCYTIC TRAFFICKING OF POLYMERIC CLUSTERED SPIO NANOPARTICLES IN MESENCHYMAL STEM CELLS

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Study of regarding cellular uptake of nanoparticles to the stem cells is important for future biomedical applications. In most studies, nanoparticle-labeled stem cells could enable an effective pharmacotherapy area to delivery tiny space. To address these issues, we used mesenchymal stem cells as carriers for nanoparticles. In this study, we investigated the interaction of iron oxide nanoparticles with mesenchymal stem cells, their uptake mechanism, and their subcellular localization. The study of internalization routes is surely needed to know, because they regulate advantage cellular molecules in stem cells. The use of metal-containing nanoparticles also raise concerns regarding their distribution and toxicity, because they are quickly removed from the blood by the reticuloendothelial system and remain in organs such as the liver and spleen for extended periods of time. Under our experimental conditions, nanoparticles were readily internalized into cells and the presence of nanoparticles at specific concentrations and for specific time periods did not affect cell viability or differentiation. We determined the potential internalization mechanism of nanoparticles into cells using two types of endocytosis inhibitors: Pistop2 and Dynasore. Furthermore, we investigated the trafficking of nanoparticles through the endocytic pathway at different time points, using antibodies against cellular organelles, and observed that the final destination was the lysosome. These results indicate that nanoparticles are internalized without significant harmful effects to the stem cells, and that stem cells can be used as carriers for nano-therapy applications.

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P-926

THE EFFECTS OF HOLOTHURIA SCABRA EXTRACTS ON NEURONAL DIFFERENTIATION OF HUMAN PLACENTA-DERIVED MESENCHYMAL STEM CELLS

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Neuronal differentiation potential of human stem cells can be valuable for treatment of neurodegenerative diseases. Holothuria scabra, a sea cucumber, has the ability to regenerate its own body after injury. Preliminary study revealed that epidermal growth factor and nerve growth factor were expressed in body wall and visceral organ of H. scabra. This study focused on the effects of H. scabra extracts on the proliferation and neuronal differentiation of human mesenchymal stem cells (PL-MSCs). H. scabra crude extracts were prepared from the body wall (BW), viscera (VI), and radial nerve cord (RN) using 0.1M PBS. MSCs were isolated from placenta (PL-MSCs) and cultured in DMEM supplemented with 10%FBS. The expression of MSC markers was studied using flow cytometry. The proliferation of PL-MSCs after treated with different doses of H. scabra extracts were examined. The neuronal differentiation potential was also determined by immunofluorescence and quantitative real time-PCR. The results demonstrated that spindle-shaped PL-MSCs were positive for MSC markers (CD73, CD90, and CD105), and negative for hematopoietic markers (CD34 and CD45). These MSCs could differentiated into osteoblasts and adipocytes. The proliferation of PL-MSCs could enhance after treatment with 0.1 and 1 μg/ml of H. scabra extracts. After neuronal induction, PL-MSCs could differentiate into neuronal-like cells as characterized by cell morphology and the presence of neuronal markers including MAP-2, nestin and β-tubulin III. In addition, quantitative real time-PCR revealed that PL-MSCs treated with H. scabra extracts had a higher neuronal gene expression compared with control. The data obtained might provide a new insight of using H. scabra extracts to treat neurodegenerative diseases in the future.

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P-927

CORD BLOOD MONONUCLEAR CELLS UP-REGULATE ANGIOGENIC GROWTH FACTORS VIA IL-8-MEDIATED PATHWAY IN A MOUSE MODEL OF NEONATAL HYPOXIC-ISCHEMIC BRAIN INJURY

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In our previous clinical trial of cerebral palsy, the level of plasma interleukin-8 (IL-8) was observed to increase significantly in correlation with motor function improvement after human umbilical cord blood mononuclear cell (hUCB) administration. This study aimed to determine whether hUCB promotes angiogenesis, a known IL-8-mediated cellular process, as the potential mechanism of action in a mouse model of hypoxic-ischemic brain injury (HI) mimicking cerebral palsy. Postnatal day 7 mice were exposed to HI and then received an intraperitoneal hUCB injection at post-HI day 7. In HI mice brain, hUCB administration upregulated the mRNA level of the mouse IL-8 homologue Cxcl2 and increased the expression of its receptor CXCR2. Sequentially, the downstream p-p38/p-MAPKAPK2 signaling axis and NFκB activation, and the protein levels of VEGF, PDGF, bFGF, and vessel marker CD31 which were downregulated by HI, were restored by hUCB administration in vivo. Mouse brain endothelial b.End3 cells transfected with siRNA for CXCR2 knockdown showed downregulation of angiogenic upregulation by IL-8 administration in IL-8 downstream molecules. Additionally, p38 inhibition downregulated previously increased angiogenic signaling molecules both in vivo and in vitro. Immunohistochemistry analysis of HI mice treated with hUCB or IL-8 revealed increased expression of VEGF and CD31 in striatum. In conclusion, as a response from the host, hUCB administration in mice promoted Cxcl2 upregulation which led to activation of the IL-8-mediated p-p38 signaling pathway. Upregulation of its downstream pathway and angiogenic growth factors via NFκB could be inferred to be the potential

action mechanism of hUCB.

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P-928

ALLOGENIC UMBILLICAL CORD BLOOD THERAPY COMBINED WITH ERYTHROPOIETIN IN CHILDREN WITH CEREBRAL PALSY: A RANDOMIZED PLACEBO CONTROLLED TRIAL

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Recently, stem cell therapy has been highlighted as a new treatment option for cerebral palsy (CP). In our previous study, children with CP treated by allogeneic UCB with erythropoietin (EPO) showed higher improvements in motor and cognitive aspects, yet it lacked to identify individual and synergistic efficacies of UCB and EPO. This factorial designed study aimed to identify individual and synergistic efficacies of UCB and EPO in children with CP. Children with 1) a diagnosis of CP, 2) age between 10 months and 6 years, 3) appropriate UCB units, 4) written informed consents from parents were included as study candidates. Participants were randomly assigned into four groups: A) UCB + EPO, B) UCB + placebo EPO (pEPO), C) placebo UCB (pUCB) + EPO), and D) pUCB + pEPO groups. Allogeneic UCB units were selected including at least 3 × 107/kg total nucleated cells, matched for at least 4 of 6 human leukocyte antigen (HLA) types A, B, and DRB1. A single infusion of UCB or pUCB was delivered intravenously and 500 IU/kg of EPO or pEPO were injected subcutaneously for 6 times every 3 days. Group A and B received oral cyclosporine. Primary outcomes such as gross motor function measure (GMFM), gross motor performance measure (GMPM), Bayley scales of infant development II (BSID-II) were assessed at baseline, 1, 3, 6 and 12 months post intervention. Baseline and post-intervention MRI and PET/CT were also acquired. All adverse events were monitored during 12 months. Eighty-eight children with CP were included as final subjects in this study (n=22, 24, 20, and 20 for Group A, B, C, and D, respectively). There were no significant differences of baseline characteristics among four groups. Group A showed meaningful improvement in the ratio of GMPM change at 12 months post-therapy compared to group D (p=0.021). Most of the parameters in four groups showed improvements in primary outcomes, although the changes were not significant. More HLA-matched UCB presented better enhancement in change of GMFM at 1 month (p=0.036) and 3 months (p=0.050) post-intervention. Ten serious adverse events were reported, although these cases were all resolved and the distribution of events did not differ among four groups. These results suggest that UCB therapy is safe and effective treatment for children with CP, and its combination with EPO can bring more synergistic effects.

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P-929

COMBINING HUMAN UMBILICAL CORD BLOOD CELLS WITH ERYTHROPOIETIN ENHANCES ANGIOGENESIS/NEUROGENESIS AND BEHAVIORAL RECOVERY AFTER STROKE

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Disruption of blood flow in the brain induces stroke, the leading cause of death and disability worldwide. However, so far the therapeutic options are limited. Thus, the therapeutic efficacy of cell-based approaches has been investigated to develop a potential strategy to overcome stroke-induced disability. Human umbilical cord blood cells (hUCBCs) and erythropoietin (EPO) both have angiogenic and neurogenic properties in the injured brain, and their combined administration may exert synergistic effects during neurological recovery following stroke. We investigated the therapeutic potential of hUCBC and EPO combination treatment by

comparing its efficacy to those of hUCBC and EPO alone. Adult male Sprague-Dawley rats underwent transient middle cerebral artery occlusion (MCAO). Experimental groups were as follows: saline (injected once with saline 7 d after MCAO); hUCBC (1.2×107 total nucleated cells, injected once via the tail vein 7 d after MCAO); EPO (500 IU/kg, injected intraperitoneally for five days from 7 d after MCAO); and combination of hUCBC and EPO (hUCBC+EPO). Behavioral measures (Modified Neurological Severity Score [mNSS] and cylinder test) were recorded to assess neurological outcomes. Four weeks after MCAO, brains were harvested to analyze the status of neurogenesis and angiogenesis. In vitro assays were also conducted using neural stem and endothelial cells in the oxygen-glucose deprivation condition. Performance on the mNSS and cylinder test showed the most improvement in the hUCBC+EPO group, while hUCBC- and EPO-alone treatments showed superior outcomes relative to the saline group. Neurogenesis and angiogenesis in the cortical region was the most enhanced in the hUCBC+EPO group, while the findings in the hUCBC and EPO treatment alone groups were better than those in the saline group. Astrogliosis in the brain tissue was reduced by hUCBC and EPO treatment. The reduction was largest in the hUCBC+EPO group. These results were consistent with in vitro assessments that showed the strongest neurogenic and angiogenic effect with hUCBC+EPO treatment. This study demonstrates that combination therapy is more effective than single therapy with either hUCBC or EPO for neurological recovery from subacute stroke. The common pathway underlying hUCBC and EPO treatment requires further study.

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P-930

IN VIVO PRIMING HUMAN MESENCHYMAL STEM CELLS WITH HEPATOCYTE GROWTH FACTOR ENGINEERED MESENCHYMAL STEM CELLS PROMOTE THERAPEUTIC POTENTIALS FOR CARDIAC REPAIR

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A major challenge of the therapeutic use of stem cells for cardiac repair is poor cell engraftment in vivo after transplantation. 3D cell printing technology can make cardiac patches rapidly similar to cardiac niche by depositing various cells with a printable heart-decellularized extracellular matrix (hdECM). These cardiac patches will provide long-term cell survival and sustained paracrine effects of mesenchymal stem cells (MSCs) and more potentiate the therapeutic effects together with high-performance engineering MSCs such as a hepatocyte growth factor (HGF-eMSCs). First, we demonstrated that BM-MSCs/HGF-eMSCs had favorable tube-forming capacity and strong expression of angiogenic factors in vitro. We developed 3 types of 3D printed cardiac patches using hdECM bioink, embedded by 1) BM-MSCs only (total 1x106), 2) HGF-eMSCs only (total 1x106), and 3) BM-MSCs/HGF-eMSCs (5x105 in each number) and transplanted on the epicardium of infarcted rat heart. Serial echocardiography showed that three groups (BM-MSCs/HGF-eMSCs, BM-MSCs only and HGF-eMSCs only) had higher ejection fractions than the control at 8 weeks (Control 35.34±3.73 vs BM-MSCs 33.93±4.32 vs HGF-eMSCs 34.20±6.27 vs BM-MSCs/HFG-eMSCs 43.72±2.52, $p<0.01$, respectively). Interestingly, BM-MSCs/HGF-eMSCs group maintained cardiac functions for up to 8 weeks, although BM-MSCs only and HGF-eMSCs only groups showed dramatically reduction of cardiac functions during that time. BM-MSCs/HGF-eMSCs group showed significantly

higher in capillary density and percent fibrosis than single cell cardiac patches and MI control. Confocal microscope examination showed that Dil labelled BM-MSCs in 3D printed cardiac patch migrated and incorporated into host capillary network in the infarcted myocardium. This study demonstrated that highly tunable 3D printed cardiac patch embedded by BM-MSCs/HGF-eMSCs enhances the paracrine effects for angiogenesis and also de novo capillary formation after post-MI. Therefore, this 3D cell printing technology with BM-MSCs/HGF-eMSCs and hdECM bioink can develop into a novel option for treating MI.

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P-931

THERAPEUTIC EFFICACIES OF HUMAN EMBRYONIC STEM CELL-DERIVED MESENCHYMAL STEM CELL AND SYNERGISTIC POTENTIATION OF IT BY COMBINED THERAPY OF ERYTHROPOIETIN IN STROKE MODEL

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Despite it being the leading cause of death and disability worldwide, stroke has very limited therapeutic options. hMSC transplantation has been reported to induce neurological recovery after brain injury and many molecular mechanisms of the treatment have also been reported. While hMSC therapy has shown promising results, its limited source causing lack of cell numbers, as well as donor- dependent variability, restrains clinical utility. Human embryonic stem cell-derived MSC (hES-MSC) may overcome the restrictions of other MSCs by enabling to supply virtually unlimited quantities of allogeneic cells. However, yet efficacy issue has not been solved so far in clinical applications of stem cells. And combined use of growth factors such as erythropoietin (EPO) may potentiate its therapeutic efficacy. In this study, stroke model was prepared by conducting middle cerebral artery occlusion (MCAO) and reperfusion injury efficacy in male SD rats. One week after the MCAO, the representative model of subacute stage stroke, hES-MSCs were administrated intravenously to monitor its therapeutic effects. To observe effects of cell dose, low, medium, and high dosage amounts of hES-MSCs were administered. Administration of EPO was simultaneously conducted with intraperitoneal injection for 5 consecutive days. The efficacy was assessed with neurobehavioral tests (mNSS and cylinder test) on 7, 14, 21, and 28 days after ES-MSC administration. And we compared the results from different cell doses of ES-MSCs and analyzed for synergistic enhancement by co- administration of EPO. According to comparison of cell-dose effect, performance on the mNSS and cylinder test showed the biggest improvement in high-dose hES-MSC group, and as for EPO combination effect, medium-dose hES-MSC and EPO treatments showed superior outcomes compared to the saline group. The results show that the hES-MSCs improve neurological behavior in a dose- dependent manner, with even further improvements when treated simultaneously with EPO in the MCAO model. This study suggests that combination therapy can be more effective than single therapy with either hES-MSCs or EPO for neurological recovery from subacute strokes. The common pathway underlying hES-MSCs and EPO treatment requires further study.

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P-932

DEVELOPMENT OF A NOVEL THERAPY FOR CHRONIC TOPHACEOUS GOUT USING URICASE PRODUCING MSCS

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Chronic tophaceous gout is a debilitating condition that is characterized by chronic inflammation and sustained pain in the affected area and can result in the subsequent joint destruction and dysfunction. To date, the treatment for chronic tophaceous gout mainly stays in using urate lowering treatment until it slowly dissolves the tophi over several years. In selected cases, surgery can be considered. Uricase such as pegloticase is also used for severe cases. However, it is currently not available in Korea and frequent infusion reaction discourages the active application of the drug. Mesenchymal stem cell (MSC) is known to have anti-inflammatory property and has been implicated as a therapeutic option for inflammatory autoimmune disease including rheumatoid arthritis and lupus. As known, the initiation of acute gout inflammation is associated with monosodium urate (MSU) crystal induced IL-1B production by monocyte/macrophage. Therefore, we hypothesized that MSCs could suppress MSU induced acute gout inflammation. Based on these, we tried to develop a novel therapy for gout using uricase producing MSCs. This uricase producing MSCs may be locally injected to the tophi site. The concept is that a locally acting uricase may dissolve the tophi more efficiently with less adverse event and at the same time MSCs may exert anti-inflammatory effect to prevent flare up during the rapid dissolution. Minicircle vector was chosen because its non-integration into genomic DNA seems safe. Minicircles were transfected to MSCs to temporarily produce uricases when delivered in vivo. We observed that MSCs transfected with minicircle vector coding uricase produce the functional uricase which reduces uric acid level in vitro.. Currently we are working to set up a chronic tophi model to investigate the efficacy of the eMSCs to provide more direct evidence of reducing the size of tophi. We hope this cell therapy will introduce a novel way to treat chronic tophaceous gout.

P-933

MESENCHYMAL STEM CELLS-CONDITIONED MEDIUM REGENERATE CUTANEOUS BURN WOUND THROUGH INCREASED VASCULARIZATION AND EXPRESSION OF TGF-B

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Mesenchymal stem cells are known to promote tissue regeneration based on their paracrine effect by secreting a variety of growth factors (GFs) and cytokines. These GFs also found in their cultured/ conditioned media (CM-MSC). Burn wound is complex and may cause a devastated effect in both functional and cosmetically. TGF-β play important role in all phases of wound healing that lead the process of epithelialization, neovascularization and granulation tissue formation. Although many researches have shown the benefit of CM-MSC in various tissue regeneration, its underlying mechanism remains to be elucidated. In this study we investigated the effect and possible mechanism of CM-MSC in burn wound healing. We used male Sprague Dawley rats which randomly divided into control (C) and CM groups. Burn wounds were created using preheated metal plat and placed it on body site of prepared rats which received daily local application of normal saline and CM respectively. Skin wound tissues were collected and processed for histological slides and genes expression. We evaluated wound closure (visitrak), neovascular density (immunohistochemistry) and expression of TGF- β and VEGF (RT-PCR). CM-MSC demonstrated to enhance skin regeneration by promoting wound closure earlier compared to control ($p<0.05$). Vascular density which represent as neovascularization in granulation tissue were also higher in CM-MSC compared to control ($p<0.05$), followed by increased of TGF- β ($p<0.05$) and VEGF expression in wound tissue. Our findings indicate the benefits of CM-MSC in burn wound tissue regeneration through wound closure acceleration, promoting neovascularization and expression of TGF-β and VEGF. Therefore local application of CM-MSC may provide a novel cell-free therapeutic approach for

cutaneous wound healing that hold promising tool to avoid the ethical and rejection issues of cell transplantation.

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P-934

ESTABLISHMENT OF A COMPLEX SKIN STRUCTURE VIA LAYERED CO-CULTURE OF KERATINOCYTES AND FIBROBLASTS DERIVED FROM INDUCED PLURIPOTENT STEM CELLS

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Skin is an organ that plays an important role as a physical barrier and has many other complex functions. Skin mimetics may be useful for studying the pathophysiology of diseases in vitro and for repairing lesions in vivo. Cord blood mononuclear cells (CBMCs) have emerged as a potential cell source for regenerative medicine. Human induced pluripotent stem cell derived from CBMC has great potential of allogenic regenerative medicine. Further study is needed on skin differentiation using CBMC-iPSCs. Human iPSCs were generated from CBMCs by Sendai virus. CBMC iPSCs were differentiated to fibroblasts and keratinocytes using embryonic bodies (EB) formation. For generating of CBMC iPSC-derived 3D skin organoid, CBMC iPSC-derived fibroblasts (iPSC-F) were added into insert of a Transwell plate and CBMC iPSC-derived keratinocytes (iPSC-K) were seeded onto the fibroblast layer. Transplantation of 3D skin organoid was performed by tie-over dressing method. Epidermal and dermal layers were developed using keratinocytes and fibroblasts differentiated from cord blood-derived human induced pluripotent stem cells, respectively. A complex 3D skin organoid was generated by overlaying the epidermal layer onto the dermal layer. A humanized skin model was generated by transplanting this human skin organoid into SCID mice and effectively healed skin lesions. This study reveals that a human skin organoid generated using CBMC-iPSCs is a novel tool for in vitro and in vivo dermatologic research.

P-935

DIRECT REPROGRAMMING TO HUMAN INDUCED DOPAMINERGIC NEURONAL PROGENITORS FOR PARKINSON'S DISEASE THERAPY

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Parkinson's disease (PD) is the second most neurodegenerative disorder and associated with selective loss of midbrain dopaminergic neurons (DNs). Previous clinical studies of transplanting human fetal mesencephalon tissue have shown the long-term improvement of motor symptoms in some PD patients. However, the ethical issue, the limited amount, and the heterogeneity of aborted fetal tissue hinder further development and require alternative methods to obtain optimal transplantable cells for PD. To overcome these drawbacks, pluripotent stem cell (PSC)-derived dopaminergic neuronal progenitors (DPs) are considered in several clinical studies, although the tumorigenicity of undifferentiated PSC still remains. Thus, we sought to generate DPs via another reprogramming technology, i.e., direct reprogramming or transdifferentiation. We successfully changed human fibroblasts into induced DPs (hiDPs) using pluripotency factor-mediated direct reprogramming. The hiDP shows epigenetically open chromatin structure and specific expression pattern of the key genes for nervous and midbrain development. Although we used the pluripotency factors,

we confirmed the absence of PSC-like cells during hiDP reprogramming process. The hiDPs efficiently generate functional DNs showing A9 specific marker expressions, dopamine release, and DN-specific electrophysiological signature. Surprisingly, hiDPs are highly expandable and highly pure cell population of expressing DP markers such as CORIN and FOXA2 compared to differentiated DPs from human PSCs. In addition, when differentiated, about 90 % efficiency of DN over total neurons without serotonergic neurons which can cause dyskinesia. When we transplanted the hiDPs to PD mouse model, the movement defect was recovered and ectopic cell growth was not observed. Thus, we expect that hiDPs are notably competitive cells compared to other cell sources currently under development for regenerative PD therapy.

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P-936

STUDY ON QUALITY CONTROL MARKERS OF CARDIOMYOCYTES DERIVED FROM HUMAN INDUCIBLE PLURIPOTENT STEM CELLS

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Induced pluripotent stem cells (iPSCs) are expected to yield novel therapies with the potential to solve many issues involving incurable diseases, drug development, because iPSCs are free for the ethical and immunological problems that have obstructed the clinical applications of embryonic stem cells. However, as iPSCs research has progressed, new problems have emerged that need to be solved for clinical application of iPSCs. For example, iPSCs have defects on genetic instability caused by integration of reprogramming transgenes and accumulation of mutations during the reprogramming process and cell passaging. Thus, that is required that a comprehensive understanding of characterization and quality control about differentiation from iPSCs. In this study The cardiomyocytes differentiation method was established using induced pluripotent stem cells. Using the marigel and feeder free culture method, the differentiation of cardiomyocytes was induced for 43 days (about six weeks). Each stage of differentiation was investigated by qPCR, Western blot, immunocytochemistry and flowcytometry with iPSC markers (OCT4, nanog, SOX2, and SSEA4), mesoderm markers (MSX-1 and ISLET1), and cardiomyocyte markers (cTnT, MYL7, MYH7, and TBX5). During differentiation expression of iPSC markers was decreased but cardiomyocyte markers were increased. Mesoderm markers have the highest expression in middle stage of differentiation. Further analysis will be conducted to present the cardiomyocyte markers as a quality control of differentiated cardiomyocytes.

Funding Source: Ministry Of Food And Drug Safety.

P-937

CHARACTERISATION OF SECRETOME FROM BONE MARROW DERIVED MESENCHYMAL STEM CELLS DURING OSTEOBLAST DIFFERENTIATION USING NANO LC-MS/MS

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The mesenchymal stem cells(MSCs) are highly used for stem cell treatment due to the diversity of tissues to be harvested. But it is difficult to evaluate the quality and generalization because they are separated from various tissues. Recently, it has been reported that the secretome, paracrine factors, from MSCs are more effective for therapeutic treatment than the transplantation function of MSC. Secretome has potential to be used as an alternative treatment to complement limitations such as immune side effects. Hence, many researches have been carried out to clarify the characteristics of secretome through proteomic analysis. In this study, we investigated Xeno-free conditioned MSC secretome analysis to supplement the limitations of MSC characteristic evaluation index through conventional target-based analysis by LC-MS/MS. we

induced differentiation from mesenchymal stem cells to osteoblasts, and recovered cell culture medium and subjected to proteomic analysis using LC-MS/MS. We identified several indicators that differed in expression level before and after differentiation. Moreover, we confirmed the possibility of evaluating stem cell characteristics using cell culture medium. Further studies should be conducted to establish a validated method for quality evaluation of stem cell treatment drugs.

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P-938

DEVELOPMENT OF A NEW CELL CULTURE MEDIUM FOR ES/IPS CELLS AND A CULTURING SYSTEM

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Cell therapy using human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) is focused as a breakthrough treatment substitute for conventional drug therapies. In order to achieve cell therapy, it is necessary to produce ES/iPSCs on a large-scale, but there are some problems especially related to culturing costs and techniques. To resolve these problems, we developed a new ES/iPSCs culturing medium and method. First, to reduce cell culturing costs, we acquired a new cell culture medium for ES/iPSCs which is chemically defined with low concentration of growth factors. And, we developed a novel 3D spheroid cell culture system with deacylated gellan gum polymer FP001 which has the ability to suspend cell spheroids uniformly without agitation to achieve large-scale cell production. Finally, to culture ES/iPSCs with a constant quality and easy technique, we developed culturing tools consist of a scalable and gas-permeable cell culture bag, a sphere collecting module and a sphere splitting module. By using these tools, we can easily culture ES/iPSCs without contamination risk because these tools are all single use and completely closed system. And then, this system is applicable from small lab-scale to large-scale cell production.

P-939

TRANSCRIPTIONAL AND EPIGENETIC REGULATION OF HUMAN TYROSINE HYDROXYLASE GENE EXPRESSION VIA COOPERATIVE FUNCTION OF TFII-I ISOFORMS AND NURR1

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Our previous study showed that Nurr1 actively represses human tyrosine hydroxylase (hTH) transcription in precursor cells, while it activates hTH expression in dopaminergic (DA) neuronal cells. TFII-I was identified as an interacting partner of Nurr1. Here we report that two alternative splicing forms of TFII-I results in switch from repression to activation of hTH gene expression. While precursor cell expresses TFII-IΔ, DA neuronal cells highly do TFII-Iγ. TFII-IΔ preferentially interacts with Nurr1 in F3, but TFII-Iγ in SH-SY5Y cells. In addition, each TFII-I isoform majorly occupies hTH promoter in each cells. The transcriptional outcome of each isoform is totally different; repression for TFII-I and activation for TFII-Iγ. Next, to investigate if SUMOylation of TFII-IΔ represses Nurr1 transcriptional activity via synergy control motif, we mutated SUMO sites of TFII-I isoforms as K221R and K240R. While de-SUMOylation did not change nuclear localization, it loses transcriptional repression activities in F3 cells. In addition, oligoprecipitation experiment indicated that sumoylation deficient TFII-IΔ forms has higher DNA-binding activity on hTH gene promoter than wild-type. TFII-IΔ preferentially interact with bivalent chromatin marks H3K4/K27me3 in precursor cells while TFII-Iγ with H3K27ac in dopaminergic cells that might responsible for hTH gene expression during development. In addition, based on mutations of binding sites for TFII-I, E-box and Inr, TFII-IΔ mediates transcriptional repression via Inr site. All our results demonstrated that two alternative splicing forms of TFII-I gene may play an important role in fine tuning of hTH gene expression during DA neurogenesis.

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P-940

HUMAN MESENCHYMAL STEM CELLS REDUCE LIVER FIBROSIS BY MFG-E8-MEDIATED REGULATION OF TGFB SIGNALING AND TARGETING OF COLLAGEN FOR PHAGOCYTIC CLEARANCE

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Mesenchymal stem cells (MSCs) have been developing as cellular therapeutics for diverse diseases, including liver fibrosis. It has recently been shown that grafted MSCs improved the function of fibrotic liver tissues by paracrine actions. Here, we show that the cell- free secretome of umbilical cord-derived MSCs (UCMSCs) and UCMSCs primed with hepatotropic growth factors (hpUCMSCs) had powerful anti-fibrotic effects in mice with liver fibrosis. We found that this anti-fibrotic effect was involved with the reduction of activated hepatic stellate cells (HSCs) via inhibiting TGFβ-SMAD2 signaling in vitro. We identified 32 potential anti-fibrotic proteins, which were more abundant in hpUCMSC secretome that showed more powerful anti-fibrotic effects than UCMSCs. Among the 32 potential proteins, a glycoprotein MFG-E8 strongly downregulated the HSC activation and reduced fibrotic progression by inhibiting TGFβ- SMAD2 signaling in vitro and in vivo. We also found that MFG-E8 has ability to directly bind to collagen through C1 and C2 domain, regulating macrophage-mediated collagen uptake. MFG-E8-treated macrophages exhibited a greater degree of collagen uptake in vitro, proposing an additional anti-fibrotic action mechanism of MFG-E8, along with the reduction of activated HSCs. These findings suggest that MFG-E8, a key anti-fibrotic protein secreted from MSCs, could be developed as a promising protein drug against liver fibrosis.

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P-941

MELATONIN IMPROVES THERAPEUTIC POTENTIAL OF MESENCHYMAL STEM CELLS-DERIVED EXOSOMES AGAINST RENAL ISCHEMIA-REPERFUSION INJURY IN RATS

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Renal ischemia-reperfusion injury (RIRI) is an overwhelming renal disorder that usually leads to acute then chronic kidney injury. Several previous studies failed to find an appropriate therapy for RIRI. However, therapies using mesenchymal stem cells (MSCs) or their exosomes could be used as effective treatment for AKI. Therefore, this study was conducted to test the hypothesis that exosomes derived from MSCs preconditioned with Mel may offer superior protection against RIRI compared to therapy by MSCs or exosomes from non-preconditioned MSCs. Our data showed a significant improvement. To the best of my knowledge, this may be the first study to report that administration of exosomes (250 μg) derived from MSCs preconditioned with melatonin (Exo + Mel group) give better ameliorative effect against RIRI, induced by bilateral clamping of renal arteries, than using bone marrow derived MSCs (1 × 106) or their exosomes without precondition. Our overall conclusion was based on the following evidences that favor the best effect of Exo + Mel group over that of other treated groups: 1) restored renal function as indicated by reduced blood levels of kidney damage markers BUN and creatinine; 2) declined oxidative stress as revealed by decreased MDA content and HIF1α mRNA level, and NOX2 protein expression; 3) increased anti-oxidants status as indicated by higher activities of SOD, CAT, GPX and upregulated HO1

gene expression; 4) decreased apoptosis as evidenced by decreased activity and gene expression of caspase 3, and declined mRNA levels of PARP1, Bax and increased mRNA level of anti-apoptotic marker Bcl2; 5) inhibited inflammation as showed by decreased MPO activity and mRNA levels of ICAM1, IL1β, NFκB and increased IL10 mRNA level; 6) increased tissue repair and regeneration as noticed by decreased kidney injury histopathological score; improved expression of regeneration-related proteins bFGF, HGF and SOX9; and 7) enhanced angiogenesis as judged by increased expression of angiogenesis-related VEGF gene. These data indicate that treatment with exosomes derived from MSCs preconditioned with melatonin gave best protective effect against renal ischemia-reperfusion injury as compared to therapy by non-preconditioned MSCs or their exosomes.

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P-942

THE POTENTIAL OF CONDITIONED MEDIUM DERIVED FROM HUMAN ADIPOSE STEM CELLS ON FETAL DEVELOPMENT AND GENE EXPRESSION IN REPRODUCTIVE ORGANS OF AGED MICE

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Aging decreases fertility and increases the possibility of miscarriage, associated with reactive oxygen species and antioxidant levels. Adipose-derived stem cell conditioned medium (ASC-CM) contain immunomodulatory and regenerative factors secreted from ASC which have shown the capacity to reduce oxidative stress in various reports. The purpose of this study is to investigate the effect of human ASC-CM on aged mice fertility regarding apoptosis and antioxidant related gene expression. Intravenous injection of hASC-CM was conducted to the tail vein of 4-month-old and 6-month-old ICR mice respectively in three groups: Control, three times in eight days interval (3T-8D) and six times in four days interval (6T-4D). The hASC-CM used in this experiment was provided from R Bio Stem Cell Research Center, Seoul, Korea. In case of control group, phosphate buffer saline was intravenously injected. The amount of single dose was determined by the weight of each mouse (1ul/g). After the last hASC-CM injection, mice in three groups were mated with intact male ICR mice according to their estrous cycle. Mice were sacrifized and abdominal section was conducted 6 days post plug examination. The number of fetus were evaluated and the expression of apoptosis related genes (Bax, Bcl2) and antioxidant related genes (SOD1, SOD2) were analyzed from ovary and uterus using real-time polymerase chain reaction. The expression of genes was quantified relative to that of housekeeping gene 18S. As a result, the number of fetus significantly increased in 6-month-old 6T-4D group compared to the control but no difference in 4-month-old groups. The antioxidant SOD2 expressed significantly higher in ovaries and uterus of CM treated groups than the control in 4-month-old mice. Furthermore, Bax/Bcl2 ratio of 6T-4D group was significantly lower than the control in ovaries of 4-month-old mice. In case of 6-month-old mice ovaries, Bcl2 and SOD2 were expressed significantly higher of 3T-8D group than the control. In conclusion, the administration of hASC-CM can be applied for improving aged mice fetal development and bring about beneficial effects on reproduction system.

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P-943

ASSESSMENT OF TUMOR-INITIATING CELL FREQUENCY AND TUMOR GROWTH KINETICS OF PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELLS

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Residual undifferentiated cells in human induced pluripotent stem cells (iPSCs)-derived products have the ability to develop teratoma and induce malignant transformation, in which the continued growth and propagation of the tumor depends on tumor-initiating cells (TIC). We aimed to determine the TIC frequency and tumor growth kinetics of an iPSC line derived from patients with peripheral artery disease. We performed a limiting dilution cell transplantation assay. The iPSC line PAD 3-8 was subcutaneously injected into female. NSG mice at serial dilutions ranging from 102 to 106 cells, mixing the cells with Matrigel. Tumor formation was examined by observation and palpation for 40 weeks. The time to tumor formation after injection of a dose of 1 x 10^4, 1 x 10^5 and 1 x 10^6 cells ranged from 56 - 182 days, 25 -189 days, and 42 - 115 days, respectively. No tumor formation detected following injection of 1 x 10^2 and 1 x 10^3 cells. The TIC frequency values at 6, 11, and 16 weeks of transplantation were 1/863,012 (95% confidence intervals (CI) = 1/2,376,015 - 1/313,461), 1/370,233 (95% CI = 1/974,769 - 1/140,620), and 1/27,954 (95% CI = 1/66,014 - 1/11,837), respectively. These results provide evidence supporting the idea that patient-derived iPSCs contain a subpopulation of cells that are capable of initiating tumor formation in NSG mice. TIC frequency should be estimated depending on the observation time and the variation in the latency period for tumor. Our method can serve as a reference for tumorigenicity evaluation of patient-derived iPSCs.

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P-944

IDENTIFICATION OF CELL SURFACE MARKERS FOR OSTEOBLASTS FROM BONE MARROW-MESENCHYMAL STEM CELLS BY MONOCLONAL ANTIBODIES

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Human bone marrow-derived mesenchymal stem cells (BM-MSCs) have long been considered promising candidates for bone formation and repair due to the differentiation potential of BM-MSCs into osteoblasts and osteocytes. However, little is known about definitive surface markers for osteoblasts. In the previous study, we generated a panel of murine monoclonal antibodies (MAbs) against surface molecules on transforming growth factor β1 (TGF-β1)-treated A549 cells by a modified decoy immunization strategy and selected TGF-β1-induced surface molecules by the MAbs because TGF-β1 promotes osteoblast proliferation and induces the early stages of differentiation of osteoblasts. Among 20 surface molecules induced by TGF-β1 treatment, 4 surface molecules were downregulated in RUNX2 knockdown osteosarcoma cells while 3 surface molecules were upregulated. The expression of the 4 surface molecules were increased on TGF-β1/BMP-2-treated BM-MSCs and further increased during osteogenic differentiation of BM-MSCs, suggesting that they may be surface marker candidates for osteoblasts. Immunoprecipitation and LC-MS/MS identified that two MAbs recognized integrin α3 and αV, respectively. To demonstrate whether the integrin-positive cells are associated with osteogenic potential, the integrin-positive and negative BM-MSCs were sorted with the MAbs and differentiated into osteocytes. The expression of osteogenic markers were drastically increased in the integrin-positive cells, as compared with them in the integrin-negative cells, suggesting that integrin 3 and αV are cell surface markers for osteoblasts. The MAbs will be useful for the identification and isolation of osteoblasts from heterogeneous BM-MSCs.

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P-945

IMPROVED ISOLATION AND CULTURE OF THE URINE-DERIVED STEM CELLS (USCS) AND ENHANCED IMMUNE CELL DIFFERENTIATION FROM THE USC-DERIVED INDUCED PLURIPOTENT STEM CELLS

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Autologous urine-derived stem cells (USCs) can be non-invasively obtained from urines and the USCs and the USC-derived induced pluripotent stem cells (U-iPSCs) are thought to be ideal for applications in stem cell research and regenerative medicine. Here, we developed a method for efficient isolation and culture of USCs using Matrigel, skim milk, and an antagonist of the rho-associated protein kinase (ROCK) signaling pathway. The prepared USCs showed significantly enhanced migration, colony forming capacity, and differentiation into osteogenic or chondrogenic lineage. The USCs were also used for efficient production of human U-iPSCs and we could prepare the U-iPSCs-derived kidney organoid and hematopoietic stem cells (HSCs). The U-iPSC-derived HSCs were subsequently differentiated to CFU. Moreover, using a flavonoid molecule which enhanced mesodermal differentiation efficiency and we could increase the production of HSCs from the U-iPSCs. Taken together, our data present a method for the improved isolation and culture of the USCs and enhanced HSC differentiation from the U-iPSCs, which can be successfully used for production of high-quality USCs, U-iPSCs, and U-iPSC-derived HSCs for stem cell research and further application in regenerative stem cell-based therapies.

P-946

KUF11 TREATMENT LED TO ENHANCED HEMATOPOIETIC STEM CELL DIFFERENTIATION FROM HUMAN PLURIPOTENT STEM CELLS

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Efficient maintenance of the undifferentiated status of human pluripotent stem cells (hPSCs) may be crucial for producing a high- quality cell source that could be successfully applied in stem cell research and therapy. Here, we tried to screen various natural compounds to find materials enhancing the quality of hPSCs. Among the tested compounds, treatment of KUF11 led to significant increase in cell proliferation and expression of naïve stemness markers, and decrease in dissociation-induced apoptosis of hPSCs. Of note, KUF11-exposed hPSCs showed upregulation in the level of intracellular glutathione (GSH) and KUF11 treatment resulted in increase in percentage of GSH-high cells when measured with FreSHtracer system. hPSCs treated with KUF11 showed enhanced mesodermal differentiation and, particularly, increased differentiation into hematopoietic stem cells (HSC) and natural killer (NK) cells. Taken together, our data demonstrate a novel natural compound that can be used to improve the proliferation and quality of hPSCs and to increase the efficiency of HSC and NKC production from hPSCs.

P-947

THE COMPARISON STUDY OF CDHS CHARACTERISTICS BY AGE IN MICE AND HUMANS

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Due to a shortage of organ donors, liver transplantation being the only choice of treatment for end-stage liver disease is problematic. Therefore, researchers are now leaning towards stem cells as a solution. Interestingly, clinical feature analysis revealed age to be

the most important factor for the generation of CdH. We recently reported the differentiation of human hepatocytes into human chemically derived hepatic progenitors (hCdHs) through a cocktail of three small molecules (A83-01, CHIR99021, HGF). We firstly examined the effect of age in mouse liver progenitor cells isolated from E16.5 hepatoblast, young mice (6-8 weeks old) and old mice (72-96 weeks old) hepatocytes. Mouse hepatocytes of different ages were stably reprogrammed. In particular, the generation efficiency of mCdHs was different in hepatocytes according to aging. The best of them were liver precursor cells (EmCdHs) made from E16.5 hepatoblasts. These various age-matched mCdHs expressed progenitor cell markers, and they also had the ability to differentiate into functional hepatocytes. Next, we transplanted mCdHs of various ages into Fah- / - mice and obtained significant survival results in the mCdH transplantation group compared to non- transplanted mice. Hepatocytes of various ages were reprogrammed as hepatic progenitor cells. Especially, the generation efficiency of the youngest E16.5 hepatoblast-derived mCdHs (EmCdHs) identified a significant difference when compared to adult hepatocytes. The efficiency of hepatic differentiation and function also showed better results with age. Similar results were also observed in mice transplanted with mCdHs. We are in the process of studying to see if the results obtained from mice are the same in humans. In particular, human hepatoblasts (14.2 weeks old) showed the same generation efficiency as old hepatocytes (75 years old) similarly to the mice model. In the future, our study will reveal the reprogramming mechanism of hepatocytes according to age and then these studies support the new contribute to the cell therapy for customized patients based on stem cell technology.

P-948

A REGENERATIVE THERAPY FOR MUSCULOSKELETAL SYSTEM BY THE EQUINE AMNIOTIC FLUID STEM CELLS

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The therapeutic ability of stem cells known to promising treatment in health care field for humans as well as companion animals. A branch of fetal stem cells, the amniotic fluid derived mesenchymal stem cells (AF-MSCs) are recently reported that promising therapeutic sources for clinical applications of degenerative diseases. Because the amniotic fluid is easily isolated right after mother's delivery, low immune reactive, and acceptable ethical issue. And it can be valuable for curing degenerative damages due to its therapeutic potential of tissue regeneration and differentiating into multiple lineages. The aim of this study is to suggest the competence of AF-MSCs obtained from equine (eAF-MSCs) as stem cell therapeutics for curing musculoskeletal injuries. The protocol for current research was approved in accordance with the conditions of 'The Guide for the Care and Use of Laboratory Animals' by the Ethical Committee of the Chungnam National University. The amniotic fluid samples used for this study were obtained from 8 Thoroughbred mature mares without any invasive surgery. After isolated and culture process, the eAF-MSCs adhered to plastic ware, expanded in shape of a fibroblast and showed positive staining and gene expression after tri-lineage (adipogenic, chondrogenic and osteogenic) differentiation. Also eAF-MSCs expressed MSC specific antigen markers, even ESC specific gene, OCT4, Klf4 and c-Myc, which assumed that the cell has a pluripotent characteristic, those are characteristics of stem cell. And we evaluated cell quality measured by doubling time and colony formation unit assay. After identified its capacity as multipotent stem cells, the cells were transplanted into fifteen horses whose tendons or ligaments had injured. And we monitored before and after the area by repeated ultrasonic diagnosis. In a few weeks, the injected area was improved without adverse reactions, even severe lesions had become better. Our in-situ results showed the cells are competent to be used for horse regenerative medicine. In conclusion, we have demonstrated that the amniotic fluid is an efficient source in terms of being obtained via non-invasive methods and the AF-MSCs can be useful for the cell therapy and tissue repair in future.

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P-949

DEVELOPMENT OF FUSED THERAPEUTICS COMBINING TRANSPLANTATION OF HUMAN MESENCHYMAL STEM CELLS AND TREATMENT OF REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION IN PARKINSONIAN ANIMAL MODEL

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Repetitive transcranial magnetic stimulation (rTMS) has been used to treat neurological diseases, such as stroke and Parkinson's disease (PD), even if the underlying mechanism remains unclear. Transplantation of mesenchymal stem cells may help to recover the PD functional deficit by replacing damaged dopaminergic neurons or through other mechanisms. Combining rTMS with transplantation of human mesenchymal stem cells (hMSCs) in an animal model, we attempted to establish a novel synergistic therapy for PD and to clarify the synergistic effect and therapeutic mechanism of such combined therapy. The neuroprotective effect in nigral dopamine neurons, neurotrophic/growth factors and anti-/pro-inflammatory cytokine regulation, and functional recovery were assessed in a 6-hydroxydopamine (6OHDA)-induced PD animal model upon administration of hMSCs and rTMS. Transplanted hMSCs were identified in the substantia nigra (SN), striatum (ST) and subventricular zone (SVZ). The survival rate of SN dopamine (DA) neurons and expression of the tyrosine hydroxylase (TH) protein upon rTMS treatment and/or hMSC transplantation were somewhat greater than that of untreated group. In particular, the highest survival rate and TH protein expression were recorded in the rTMS+hMSC group. The expression levels of brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), nerve growth factor (NGF) and platelet-derived growth factor (PDGF) were significantly elevated upon rTMS treatment and/or hMSC transplantation. Similarly, the highest growth factors expression was recorded in the rTMS+hMSC group. In addition, anti-/pro-inflammatory cytokine were significantly elevated (IL-10) or decreased (IFN-γ, TNF-α) in the rTMS treatment and/or hMSC transplantation groups in a synergistic manner. The treadmill locomotion test (TLT) score was significantly improved by the rTMS treatment and/or hMSC transplantation, revealing that motor function was improved, with synergy observed in the rTMS+hMSC treatment. Our findings demonstrate that rTMS treatment and/or hMSC transplantation could synergistically create a favorable microenvironment for cell survival within the PD rat brain, through alteration of soluble factors such as neurotrophic/growth factors and anti-/proinflammatory

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P-950

Withdrawn

P-951

ESTABLISHING AND CHARATERIZING THE CLINICAL SIMULATION OF TESTICLE TORSION IN RAT MODEL

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Testicular torsion is a urological emergency in which misdiagnosis and inappropriate treatment can lead to testicular atrophy and male infertility. To date, there is no effective method for restoration of spermatogenesis after testicular torsion. Animal models provide insights into the pathogenesis of testicular torsion and may aid in the development of new promising therapeutics. Although many experimental studies have been preceded, no standard animal model yet exists for testicular torsion based on ischemic-reperfusion injury. Therefore, we established a rat model of testicular torsion and confirmed its characterization. Six to eight-week-

old Sprague–Dawley rats received surgical 720 degree torsion (ischemia) for 1, 2, and 3 hours, followed by 4 hours and 30 days detorsion (reperfusion) on the left testis. In 4 hours reperfusion group, malondialdehyde (MDA) and superoxide dismutase (SOD) level were analyzed by ELISA to detect oxidative stress (MDA) and anti-oxidant function (SOD) in both testes. Histopathological analysis including Johnsen`s scoring for spermatogenesis were examined in 4 hours and 30 days reperfusion group. In 4 hours reperfusion group, histopathological exam revealed increased hemorrhage, congestion and edema in ipsilateral testis. Mean relative MDA and SOD level (left to right ratio) was elevated in ischemia time dependent manner in 4 hours reperfusion group. In 30 days reperfusion group, mean size of ipsilateral testis was decreased in ischemia time dependent manner. Johnsens's score of 30 days reperfusion group also gradually deteriorated over time. (1 hour ischemia: 9/10, 2 hour ischemia: 5/10, 3 hour ischemia: 3/10). In this study, we identified aggravation of testicular damage in ischemia time dependent manner and the serious deterioration of spermatogenic activity starting from 3 hours ischemia in a rat model of testicular torsion. This model may be useful in the assessment of novel therapeutics in testicular torsion.

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P-952

MULTILINEAGE DIFFERENTIATION POTENTIAL OF HEMATOENDOTHELIAL PROGENITOR CELLS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS (HIPSCS)

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Human iPSCs offer a renewable source of cells for generation of hematopoietic cells for regenerative medicine, disease modeling and drug screening. To date, many serum/feeder-free protocols have been reported including step-wise monolayer and embryoid body (EB)-based approaches with varying efficiencies. However, these protocols involve either the use of various cytokines, which makes the process very costly or the generation of EBs, which are heterogeneous and labor-intensive. Here, we report a simple feeder/serum-free monolayer protocol for efficient generation of hematoendothelial progenitor cells from hiPSCs. The differentiation was initiated by inhibiting GSK3 signaling for 2 days followed by addition of VEGF and FGF2 for 3 days. This condition significantly enhanced the number of CD34+ CD31+ KDR+ hemogenic endothelial (HE) cells on day 5. Further culture of HE cells in angiogenic condition promoted formation of mature endothelial cells, which expressed CD34, CD31, CD144, vWF and ICAM-1, and had the ability to form vascular-like network. By inhibiting TGF-β signaling after HE induction, we observed emergence of hematopoietic stem/progenitor cells (HSPCs) expressing high levels of CD34 and CD43 on day 8. These HSPCs formed robust myeloid and erythroid colonies in CFU assay, and gave rise to CD45+ CD5+ CD7+ CD3+ TCRαβ+ CD4+ CD8+ T lymphocytes when co-cultured with OP9-DL1 cells

for 4 weeks. Continuous culture of the remaining adherent cells on day 8 for 4 days resulted in the second wave of floating HSPCs. We cultured these HSPCs in the 3-stage erythroid liquid culture system. The cells were expanded up to 40-fold and converted to cells with morphology of proerythroblasts, basophilic erythroblasts, orthochromatic erythroblasts and reticulocytes over the period of 11 days. These erythroblasts expressed erythroid-related genes such as GPA, Band 3 and hemoglobin. Our study demonstrated that hiPSC-derived HE population is capable of hematopoietic and endothelial differentiation. Additionally, the resulting HSPCs can give rise to various blood cell lineages. This protocol offers an efficient and simple approach for generation of hematoendothelial progenitor cells and could be adapted to generate desired blood cells in large numbers for applications in regenerative medicine.

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SELECTION OF INTERSTICIAL CYSTITIS INDUCING SUBSTNACE AND INJECRION ROUT OF DIFFERENT STEM CELLS FOR IC TREATMENT

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Interstitial cystitis (IC) causes serous urinary symptoms, and there is no fundamental therapy yet. This study was to establish the optimal IC animal model, stem cell source and injection route for effective treatment of IC. To establish an animal model, SD rats were treated 5 substances; HCl, acetic acid, cyclophosphamide, lipopolysaccharide or uroplakin (UPK) II. For selection of effective stem cell source, urothelial stem cell (USC), adipose-derived stem cell (ADSC), bone marrow derived stem cell (BMSC), and amniotic fluid-derived stem cell (AFSC) were injected into the established IC model. In order to establish the optimal cell injection route, stem cells were injected into the bladder mucosa directly, intravascularly through the tail vein or transurethrally. The functional and morphological comparisons were performed by cystometry, histological and PCR analysis. In compare of IC induces substances, LPS and UPKII group showed significant shorter voiding interval compared to the other groups. In histologic analysis, UPKII group showed epithelial cell detachment, increased infiltration of mast cells, tissue fibrosis, and expression of IL-1β, -6, MPO, MCP 1, and TLR 2 and 4 compared with the other groups. With these results, uroplakin II was selected as the IC induces substance. In compare of stem cell efficacy, USC showed significantly increased voiding interval, and decreased the inflammatory reaction and the fibrosis. In compare of cell injection route, direction injection into bladder mucosal was prolonged the voiding interval and inhibited morphologic regeneration and inflammation compared to injection via tail vein or urethra. Subcutaneous injection of UPK II was the most effective substance to induce IC, USC was the effective stem cell source for treat of IC, and direct injection into the bladder mucosa showed functional recovery, inflammatory inhibition and histological regeneration on IC model.

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P-954

THE EFFECT OF OSTEOPOROSIS ON ADSCS ACTIVITY

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Osteoporosis is a chronic decrease with decreased bone mass, leading to fracture. Stem cells are expected to be a good candidate to cure osteoporosis. Bone marrow-derived stem cells (BMSCs) are applied for critical diseases but its application is confined in osteoporosis, due to low activity and the risk for isolation procedure. Now, adipose-derived stem cells (ADSCs) have emerged as an alternative to BMSCs. Comparing to BMSC, ADSCs is more easily obtained and

has the bone-forming ability, inferring the possibility of ADSCs therapy for bone regeneration. This study explored the effect of osteoporosis on ADSCs activity. ADSCs was isolated from a rat at 12 weeks post-ovariectomy (OVX). We have compared the proliferation, cellular activity, paracrine potential and differentiation activity of ADSCs from sham or OVX. Osteoporosis reduced proliferation rate of ADSCs with increased population doubling time. Intriguingly, ADSCs from OVX rat produced higher VEGF and TNF-α than sham. Differentiation assay showed that ADSCs from OVX preferred adipogenic differentiation, rather than osteogenesis. Taken together, these results suggest osteoporotic environments can influence on ADSCs function by altering cell cycle, cytokine profile or differentiation capacity. These should be considered for ADSCs transplantation.

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P-955

HUMAN MESENCHYMAL STEM CELLS PRIMED WITH ETP8 ACCELERATES PROLIFERATION AND MIGRATION

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Mesenchymal stem cells (MSCs) have self-renewal and differentiation ability. So it can be a useful source for cell therapy in numerous degenerative diseases. Studies on MSCs therapy have focused on cell replacement through transdifferentiation or damaged cells treatment by the paracrine factors secreted by MSCs. MSCs secrete various cytokines which promote anti-inflammation, anti-oxidant activities, neuroprotection, and neurogenesis in the brain. In spite of these effects, limitations are present due to the poor survival rate of MSCs and their migration towards injury sites in-vivo. In this study, we screened a library of FDA-approved compounds by using the high-throughput screening system. We identified ETP8, a potential therapeutic compound which accelerated the proliferation and migration of human MSCs. After ETP8 priming, MSCs exhibited a significant increase in proliferation and survival signaling was increased. The expression levels of phospho-Akt and phospho-ERK were enhanced after ETP8 treatment. Furthermore, ETP8-primed MSCs showed enhanced migration ability and gene expression of migration related chemokine receptors and their ligands, such as C-X-C motif chemokine receptor 4, C-X-C motif chemokine receptor 7 and C-X-C motif chemokine ligand 12. ETP8-primed MSCs also promoted the secretion of paracrine factors such as neurotrophic factors and anti-oxidants. We confirmed that MSCs primed with ETP8 has increased cell survival compared to naïve MSCs in vivo. These results suggest that enhancing MSC function by ETP8 priming represents a promising strategy to maximize the effectiveness of MSCs-based therapies.

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Withdrawn

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MICRORNA PROFILING BY HUMAN PLACENTA-DERIVED MESENCHYMAL STEM CELLS FOR HEPATIC REGENERATION IN A RAT MODEL WITH HEPATIC FAILURE

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Placenta-derived mesenchymal stem cells (PD-MSCs) have the promising cell-based therapeutic source in several degenerative diseases. MicroRNAs (miRNAs) regulate biological stem cell behaviors and potentials in regenerative medicine. However, miRNA-mediated liver regeneration by phosphatase of regenerating liver-1, which is involved in liver regeneration, overexpressing (PRL-1+) PD-MSCs in bile duct ligation (BDL) rat model remains unclear,

Hence, we investigate patterns of miRNA expression for hepatic regeneration about stem cell homing and vascular remodeling by PRL-1+ PD-MSCs. We validated miRNA candidates in invaded naïve PD-MSCs under hypoxic condition and cirrhotic rat liver tissue according to PD-MSC transplantation. Invaded PRL-1+ PD-MSCs under hypoxic condition increased integrin-dependent migration ability compared to naïve through Rho family targeted miRNA expressions (e.g. hsa-miR-30a-5p, 340-5p, 146a-3p). rno-miR-30a-5p, 340-5p downregulated engraftment into injured rat liver by PRL-1+ PD-MSC transplantation compared to naïve through integrin family. Moreover, rno-miR-27a-3p targeted platelet derived growth factor receptor alpha (PDGFRA) regulated vascular remodeling by PRL-1+ PD-MSC in cirrhotic rat liver. For hepatic regeneration, PRL-1+ PD-MSCs increased hepatic proliferation through interleukin-6 (IL-6)/signal transducer and activator of transcription 3 (STAT3) signaling by repressing miRNAs (e.g. rno-miR-21-5p, 122-5p). Taken together, these findings suggest that dynamic expressions of miRNA patterns by PRL-1+ PD-MSCs could be monitoring to confirm therapeutic effects in degenerative disease for regenerative medicine.

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PLACENTA DERIVED MESENCHYMAL STEM CELLS ENHANCED INVASION ABILITY OF TROPHOBLAST VIA DYNAMIC MITOCHONDRIAL FUNCTION

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Trophoblast is a major cell for responsible placental development and embryo implantation through the process of invasion. We have reported that placenta-derived mesenchymal stem cells (PD-MSCs) have several potentials for differentiation, immunomodulation and higher self-renewal as well as PD-MSCs enhancing the invasion ability of trophoblast through immunomodulation. However, the mitochondrial function of invasiveness trophoblast via PD-MSCs co-cultivation is still unclear. Therefore, the objectives of this study is to analyze the activity of trophoblast by mitochondrial function. The expression of markers related to the invasion of trophoblast accompanied by PD-MSCs co-cultivation was analyzed by qRT-PCR. The mitochondrial functions including ROS levels, ATP production, Ca+ channel, mitochondrial DNA copy number and mitochondrial autophagy were analyzed by qRT-PCR and XF analysis. As the results, the invasiveness of trophoblast with PD-MSCs co-cultivation was increased by upregulated MMP-2/-9 activities and Rho family (e.g., FAK, Rho A, Rock and Rac1) ($p<0.05$). Also, the HO-1/-2 mRNA expressions related to ROS protective function of mitochondria were increased in trophoblast with PD-MSCs co-cultivation ($p<0.05$). Interestingly, the ATP production was remarkably decreased ($p<0.05$), and the mRNA expressions related to Ca+ channel such as IP3R and MCU were significantly increased in trophoblast with PD-MSCs co-cultivation ($p<0.05$). In addition, glycolysis ability was increased in the the invading trophoblast with co-cultured PD-MSCs. Otherwise, there was no difference in the respiration rate of mitochondria. However, mtDNA was significantly decreased in trophoblast with PD-MSCs co-cultivation. The mRNA expressions related to mitophagy such as PARKIN and PINK1 were significantly increased in the invading trophoblast with co-cultured PD-MSCs ($p<0.05$). Taken together, PD-MSCs could regulate trophoblast invasion by dynamic mitochondrial functions via balanced mitophagy mechanism between PARKIN and PINK1 expression. These results support that the fundamental mechanisms of mitochondria function contribute to trophoblast invasion and suggest new therapeutic strategy in infertility.

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P-959

MORPHOLOGICAL CLASSIFICATION OF HIPS-DERIVED HINDGUT SPHEROIDS BY USING NOVEL LABEL-FREE IMAGING

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In cellular medical studies such as regenerative medicine and cancer research, the importance of quality control protocols are increased. However, it is difficult to establish a defined criteria for them, because the cellular regulations include complex status. Though the current cellular studies need mainly the fluorescent imaging assay to find out the morphological character, we think that a non-invasive quality control becomes essential to adopt the morphological analysis in cellular medicine. We aim to develop a novel procedures of morphological analysis with non-invasive methods to establish a quality control procedure for cellular medicine in this study. Especially, we have developed a unique imaging technology which uses an artificial intelligence. Firstly, we induced hindgut from hiPS cells and observed it with our cellular imaging system "Cell3iMager duos". Secondly, using morphological features of cells, expression of CDX2 and nuclear staining, we obtained learning results with deep learning. Finally, we acquired efficiency of induction and area of hindgut spheroids by morphological classification of differentiated cells based on the learning results. It is suggested that our system will be useful to develop the non-invasive quality control in cellular analyses.

P-960

PRECLINICAL DOSE ESCALATION STUDY OF ADULT HUMAN MULTIPOTENT NEURAL CELLS FOR SPINAL CORD INJURY

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Stem cells could be the next generation therapeutic option for neurodegenerative diseases including spinal cord injury (SCI). However, several critical factors such as transplantation dose should be determined before their clinical applications. In this study, we investigated optimal dose of stroke patient-derived NSCs (ST-NSC) for SCI using a SCI animal model. Dose range was from 300,000 (low) to 3,000,000 (high). Cell engraftment, differentiation, lineage-specific migration, and recovery of locomotor function were determined at 5 weeks post transplantation. ST-NSCs promoted locomotor recovery at all transplantation groups compared to the control group. Especially, transplantation of 1 × 106 (medium dose) or 3 × 106 (high dose) ST-NSCs resulted in significant locomotor function recovery, decreased tissue loss, increased migration of host glial cells on the periphery of injury sites. However, those factors were not different between the medium and high dose group. The data suggest that optimal dose of ST-NSC for SCI would be 1 × 106 (medium dose) in the animal model. This result could be translated to calculate treatment dose of ST-NSCs for SCI patients in clinical trials.

P-961

ANTI-INFLAMMATORY ACTIVITY OF ANGPTL4 FACILITATES MACROPHAGE POLARIZATION TO INDUCE CARDIAC REPAIR

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Mesenchymal stem cells (MSCs) can suppress pathological inflammation. However, the mechanisms underlying the association between MSCs and inflammation remain unclear. Under coculture condition with macrophages, MSCs highly expressed angiopoietin-like 4 (ANGPTL4) to blunt the polarization of macrophages toward anti-inflammatory phenotype. Angptl4-deficient mice derived MSCs failed to inhibit the inflammatory macrophage phenotype. In inflammation-related animal models, the injection of cocultured media or ANGPTL4 protein increased the anti-inflammatory macrophages in both peritonitis and myocardial infarction. In particular, cardiac function and pathology were markedly improved by ANGPTL4 treatment. We found that retinoic acid-related orphan receptor a (RORa) was increased by inflammatory mediators such as interleukin-1b and bound to ANGPTL4 promoter in MSCs. Collectively, RORa-mediated ANGPTL4 induction was shown to contribute to the anti-inflammatory activity of MSCs

against macrophages under pathological conditions. This study suggests that the capability of ANGPTL4 to induce tissue repair is safe and promising opportunity for stem cell-free regeneration therapy from a translational perspective.

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Withdrawn

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THERAPEUTIC EFFECTS OF MESENCHYMAL STEM CELL-DERIVED EXOSOMES ON DERMATITIS

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Exosomes are nano-sized (30 – 200 nm) membrane-bound vesicles actively secreted by almost all cell types and contain various cargo such as microRNAs and proteins. The ability of exosomes to deliver their cargo, from originating cells to recipient cells, makes them a promising cell-free therapy option to treat various diseases. In particular, exosomes derived from mesenchymal stem cells (MSCs) were shown to facilitate tissue repair and regeneration, and reduce inflammation. Atopic dermatitis (AD) is a chronic, relapsing, and highly pruritic inflammatory skin disease that can significantly reduce the quality of life. Current evidence suggests that the pathogenesis of AD is attributed to both epidermal barrier dysfunction and Th2/Th22-deviated immune reactions within the skin. In the present study, we investigated whether human adipose tissue-derived mesenchymal stem cell-derived exosomes (ASC- Exosomes) can ameliorate AD in two distinct animal models: 1) As a skin barrier disruption model, dermatitis was induced by topical application of a chemical irritant; and 2) As an inflammatory model, AD-like skin lesions were induced in mice by treatment with a house dust mite antigen. ASC-Exosomes were found to attenuate AD-like symptoms 1) by improving skin barrier function as evidenced by reducing the trans-epidermal water loss (TEWL), enhancing skin hydration, and increasing the amount of lipids in skin lesions; 2) by reducing inflammatory reactions as evidenced by reducing the levels of serum IgE and inhibiting both mRNAs and proteins for pro-inflammatory cytokines. Taken together, our findings suggest ASC-Exosomes as a potential cell-free therapeutic option for AD and related dermatitis.

P-964

PRECONDITIONING WITH ALLERGIC INFLAMMATION IMPROVES THE THERAPEUTIC POTENTIAL OF HUMAN MESENCHYMAL STEM CELLS FOR ATOPIC DERMATITIS

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Although human mesenchymal stem cells (hMSCs) hold considerable promise as an alternative therapeutic reagent for allergic disorders including atopic dermatitis (AD), the strategy for enhancing hMSC-based therapy remains challenging. We sought to investigate whether preconditioning with mast cell (MC) granules could enhance the therapeutic efficiency of human umbilical cord blood-derived MSCs (hUCB-MSCs) against AD. Pretreatment of MC granules enhanced the therapeutic effects of hUCB-MSCs by attenuating the symptoms of AD in an experimental animal model. MC granule-primed cells suppressed the activation of major disease-inducing cells, MCs and B lymphocytes more efficiently than naïve cells both in vitro and in vivo. Histamine-mediated upregulation of the COX-2 signaling pathway was shown to play a crucial role in suppression of the allergic immune response by MC-pretreated hUCB-MSCs. Moreover, MC pretreatment improved the wound healing ability of hUCB-MSCs. Our findings indicate that pre-exposure to MC granules improved the therapeutic effect of hUCB-MSCs on experimental AD by resolving the allergic immune reaction and accelerating the tissue regeneration process more efficiently than naïve cells, suggesting a potential enhancement strategy for stem cell-based therapy.

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P-965

NOVEL NEUROPROTECTIVE EFFECTS OF MELANIN-CONCENTRATING HORMONE IN PARKINSON'S DISEASE

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Acupuncture has shown the therapeutic effect on various neurodegenerative disorders including Parkinson's disease (PD). While investigating the neuroprotective mechanism of acupuncture, we firstly found the novel function of melanin-concentrating hormone (MCH) as a potent neuroprotective candidate. Here, we explored whether hypothalamic MCH mediates the neuroprotective action of acupuncture. In addition, we aimed at evaluating the neuroprotective effects of MCH and elucidating underlying mechanism in vitro and in vivo PD models. First, we tested whether hypothalamic MCH mediates the neuroprotective effects of acupuncture by challenging MCH-R1 antagonist (i.p.) in mice PD model. We also investigated whether MCH has a beneficial role in dopaminergic neuronal protection in vitro primary midbrain and human neuronal cultures and in vivo MPTP-induced, Pitx3−/− , and A53T mutant mice PD models. Transcriptomics followed by quantitative PCR and western blot analyses were performed to reveal the neuroprotective mechanism of MCH. We first found that hypothalamic MCH biosynthesis was directly activated by acupuncture treatment and that administration of an MCH-R1 antagonist reverses the neuroprotective effects of acupuncture. A novel finding is that MCH showed a beneficial role in dopaminergic neuron protection via downstream pathways related to neuronal survival. This is the first study to suggest the novel neuroprotective action of MCH as well as the involvement of hypothalamic MCH in the acupuncture effects in PD, which holds great promise for the application of MCH in the therapy of neurodegenerative diseases.

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P-966

WOUND HEALING PROMOTING ACTIVITY OF TONSIL-DERIVED STEM CELLS ON 5-FLUOROURASIL-INDUCED ORAL MUCOSITIS

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Oral mucositis (OM) is a severe and frequent adverse effect that occurs during chemotherapy or radiotherapy for cancer treatment. Recently, stem cells have received increased attention because of their potential for use in tissue engineering and clinical applications. The human palatine tonsils are lymphoepithelial tissues that are located in the oropharynx and have been proposed as an alternative source of adult stem cells. Furthermore, tonsil-derived MSCs have been reported to be beneficial for the treatment and prevention of various diseases, including liver fibrosis, peripheral nerve injury, allergic rhinitis, and osteoradionecrosis. Therapeutic effects of spheroid gingiva-derived MSCs (GMSCs) in a mouse model of OM induced by 5-fluorourasil (FU) have been reported, however, this study discussed systemic injection of spheroid GMSCs, but did not focus on topical/lesional delivery. In this study, we first determined the efficacy of lesional injection of tonsil-derived MSCs for the treatment of 5-FU-induced OM. OM was induced in hamsters by administration of 5-FU (day 0, 2, 4) followed by mechanical trauma (MT) (day 1, 2, 4). The experimental groups included MT, 5-FU+MT, TMSC, DEXA, and saline. On day 10, gross and histologic analyses showed that nearly complete healing and epithelialization of the cheek mucosa of the TMSC group, whereas the other groups showed definite ulcerative lesions. Compared with the MT and DEXA groups, CD31 expression was greater in the TMSC group on days 10 and 14. These results indicate that tonsil-derived MSCs can improve wound repair by promoting neovascularization, and tonsil-derived MSC-mediated angiogenesis seems superior to angiogenesis induced by normal mucositis. In addition, enhanced neovascularization could accelerate wound healing in 5-FU-induced OM. On day 10, the TMSC group showed the highest expression of TGF-β and NOX4, which indicated effective wound contraction of OM of the hamster cheek mucosa. In conclusion, intralesional administration of

tonsil-derived MSCs may accelerate wound healing of 5-FU-induced OM by upregulating neovascularization and effective wound contraction.

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CREB1 IS A POTENT MASTER REGULATOR OF GLUTATHIONE LEVEL IN HUMAN MESENCHYMAL STROMAL/STEM CELL

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Continuous exposure to oxidative stress during the expansion of mesenchymal stromal/stem cells (MSCs) based on traditional culture techniques results in a progressive loss in proliferative and differentiation potential of MSCs. To prevent these reactive oxygen species (ROS) mediated damages, it is required to understand the regulation network of glutathione (GSH), a major anti-oxidant in living cells. Recently, we reported a new method to real-time monitor intracellular GSH level employing a newly synthesized fluorescent probe (FreSHtracer; Stem Cell Reports. 2018;10(2):600-614). Here, we fractionized human MSCs into GSH-high and -low cells based on FreSHtracer and compared their transcriptomes. As results, several genes related to DNA metabolism and repair were highly expressed in GSH-high MSCs. Furthermore, MSCs with high level of GSH showed increased expression and transcription activity of cyclic AMP-responsive element-binding protein 1 (CREB1). In this regard, CREB1 enhanced the recovery capacity of GSH after exposure to oxidative stress. Accordingly, CREB1 positively modulated core functions of MSCs including self-renewal, chemoattraction to growth factors, and angiogenesis activities. Taken together, these results demonstrate that CREB1 is a master regulator of GSH dynamics in MSCs, thus it will be a potent target to improve the therapeutic efficacy of MSCs.

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AMELIORATING LIVER FIBROSIS IN AN ANIMAL MODEL USING THE SECRETOME RELEASED FROM MIR-122-TRANSFECTED ADIPOSE-DERIVED STEM CELLS

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AIM Recently, the exclusive use of mesenchymal stem cell (MSC)-secreted molecules, called secretome, rather than cells, has been evaluated for overcoming the limitations of cell-based therapy, while maintaining its advantages. In this study, we aimed to determine the therapeutic potential of the secretome released from miR-122-transfected adipose-derived stromal cells (ASCs). METHODS We collected secretory materials released from ASCs that had been transfected with antifibrotic miR-122 (MCM) and compared their antifibrotic effects with those of the naïve secretome (CM). MCM and CM were intravenously administered to the mouse model of thioacetamide-induced liver fibrosis, and their therapeutic potentials were compared. RESULTS MCM infusion provided higher therapeutic potential in terms of (a) reducing collagen content in the liver, (b) inhibiting proinflammatory cytokines, and (c) reducing abnormally elevated liver enzymes than the infusion of the naïve secretome. The proteomic analysis of MCM also indicated that the contents of antifibrotic proteins were significantly elevated compared to those in the naïve secretome. CONCLUSION We could, thus, conclude that the secretome released from miR-122-transfected ASCs has higher antifibrotic and anti-inflammatory properties than the naïve secretome. Because miR-122 transfection into ASCs provides a specific way of potentiating the antifibrotic properties of ASC secretome, it could be considered as an enhanced method for reinforcing secretome effectiveness.

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IS LOW-DOSE IONIZING RADIATION A "FOUNTAIN OF YOUTH" FOR HUMAN STEM CELLS?

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Adult tissue stem cells (SC) are multipotent, capable of self-renewal, proliferation and differentiation into mature cellular subtypes. While very promising, the use of stem cells for therapy is thwarted by the challenge of obtaining sufficient numbers of donor-derived SCs and their ex vivo expansion leads to premature aging, loss of stemness and decreased functional and regenerative capacity. To address this challenge and based on our previous scientific reports that acute exposures to low-dose ionising radiation delays aging of fibroblasts in vitro and mice in vivo, we decided to investigate the effects of low-dose radiation (LDR) on cord-blood derived mesenchymal stem and progenitor cells (MSPCs) and endothelial colony forming cells (ECFCs). Young, passage 4 stem cells were acutely irradiated with 10, 50 and 100 mGy ɣ-rays. Both SC types were aged in culture and their proliferation was monitored using Incucyte. Aging was defined as a decline in cell proliferative capacity and function. MSC function was evaluated based on the extent of chondrogenic differentiation. ECFC function was assessed by migration capacity in a scratch wound assay. Results of this study demonstrated that in vitro cultured non-irradiated MSCs and ECFCs aged significantly with cell doubling time increasing ~3.0 fold over 14 passages for MSCs (~45-55 days) and 5-9 passages for ECFCs (20-30 days). However, all doses of LDR treatment at early passage provided lasting effects and delayed aging and improved proliferative capacity by 1.5-2.2 fold. Similarly, chondrogenic differentiation of aged MSCs decreased ~2 fold but was restored back to level for young cell control with 50 and 100 mGy treatment. Interestingly, a 10 mGy exposure led to 50% further improvement exceeding the levels for chondrogenesis of young controls. The migratory capacity of aged ECFCs decreased ~2 fold in comparison to young cells and was improved by ~30% with LDR exposure. Thus, this report provides the first evidence of delayed aging and improved functional capacity of in vitro expanded mesenchymal and endothelial stem cells.

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THERAPEUTIC POTENTIAL OF STEM CELL CONDITIONED MEDIUM ON CHRONIC SKIN ULCER: A HUMAN PILOT STUDY

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Chronic wounds still represents a heavy burden to many patients and health care institution. Despite the most recent advances in wound management, up to 50% of chronic wounds still fail to heal. Conventional treatment of chronic wounds frequently fail in most of the cases anddepletes enormous amount of health cost and time.Because of that, it is important to develop novel strategies for treating chronic wounds. Previous studies have reported stem cells ability in tissue regenerations due mainly to its secreted paracrine factors, rather than its differentiation ability to become new cells. Secretomes, microvesicles, and exosomes can be found in the medium where the cells are growing,which is known as conditioned medium (CM).During the last decade, mesenchymal stem cells (MSCs) have been studied and emerge as a promising therapy for chronic wounds through wound healing enhancement.To the best of our knowledge, after conducted a pilot study using animal model to gain the preliminary data for the ulcer healing potential, this is our first clinical study to see the therapeutic potentials of stem cellconditioned medium as an additional growth factors in chronic skin ulcer healing and to compare the results of chronic ulcer healing in patients who underwent CM treatment and standard approach.We have examined the therapeutic effect of human wharton jelly-MSC-CM in wound healing on 18 patients with chronic skin ulcer (diabetic, vascular and pressure) and significant improvement was found in CM groups. Stem cell conditioned medium might become a promising therapy for chronic skin wounds.

P-971

PRE-CLINICAL EVALUATION OF UMBILICAL CORD-DERIVED HUMAN MESENCHYMAL STEM CELL SECRETOME TOWARDS SKIN ULCER IN DIABETIC RATS

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Diabetic prevalence in Indonesia, according to World Health Organization (WHO), was 6.3% per 2017 and contributed as one of top five mortality causes in this country. Since Diabetes is associated with so much complications through certain pathways, so, there are more patients develop serious condition because of it. One of the complications which often occurs is skin ulcer. Insufficient blood supply to the limbs causes Diabetic Skin Ulcer (DSU) patients to have vascular and peripheral neuritis complications and abnormal collagen, which lead to skin wounds that are refractory and which often ulcerate. Mesenchymal Stem Cell is well known of its wound healing rich properties since most of the microenvironment for tissue regeneration contained in Mesenchymal Stem Cell (MSC) secretome such as growth factor, cell migration factor, and collagen. So, the aim of this study is to observe the clinical therapeutic effect of secretome derived from human Umbilical Cord Mesenchymal Stem Cells (S-hUCMSC) on its wound healing capability. Passage 3 UCMSCs were cultured in MEM alpha supplemented with 10% Fetal Bovine Serum and 1% antibiotic until passage 6 to obtain number of cells needed. When passage 6 UCMSC reached 70%-80% confluency, the nutritious medium was replaced with basal medium and incubated for 72 hours in 5%CO2 and 5%O2 to collect the secretome. Animals, Sprague Dawley (SD) rats, were grouped as healthy group and diabetic induced group were rendered ulcer wounds and given treatment according as follows: non- treatment (NT), antibiotic topical (AB), and intracutaneous injection of S-hUCMSC. The wound size of each treatment was measured at day 7 and day 14. At endpoint, the wounds sites were histological analyzed for re-epithelialization and collagen formation. This study suggested that S-hUCMSC increased the collagen formation especially in diabetic rats thus showed greater effect in promoting wound healing compared with the healthy rats. In conclusion of our study, the S-hUCMSC significantly enhanced diabetic wound healing.

P-972

WHARTON'S JELLY-DERIVED MESENCHYMAL STEM CELLS CULTURED ON MCR2 PEPTIDE-COATED CULTURE PLATE SHOWED IMPROVED PROLIFERATION, DIFFERENTIATION, AND OSTEOARTHRITIS MITIGATION EFFECT

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Mesenchymal stem cells (MSCs) are potent tools for tissue repair in regenerative medicine and the production of MSCs with high proliferative and differentiative potentials is important for further application in tissue regeneration. We revealed the capacity of MCR2 peptide in promoting proliferation and the differentiation of the Wharton's jelly-derived MSCs (WJ-MSCs) that isolated from placental tissue. WJ-MSCs cultured on MCR2 peptide-coated culture plate showed significantly enhanced proliferation, expression of stemness markers, and colony-forming capacity. Interestingly, WJ-MSCs cultured on MCR2 peptide-coated plate and then subjected to the three-dimensional culture (3D) showed spheroids with bigger sized and higher number compared to WJ-MSCs without peptide coating. Moreover, MCR2 peptide-cultured WJ-MSCs showed a marked promotion of the osteogenic differentiation, which verified with the high expression of the osteogenic or chondrogenic differentiation-associated markers and high calcium mineralization by alizarin red staining. The chondrogenic differentiation was also prompted in WJ-MSCs cultured on MCR2 peptide-coated plate, which evidenced by the high expression level of chondrogenic differentiation-related markers and the positive alcian blue staining. In addition, upon injection of the MCR2 peptide-cultured WJ-MSCs resulted in alleviation of arthritis-related symptoms in in vivo experimental osteoarthritis mouse model (EOA) and significantly suppressed the expression of arthritis-related genes. Taken together, our study revealed a novel peptide that markedly enhanced the proliferation as well as the osteogenic and chondrogenic differentiation of isolated

WJ-MSCs and significantly improved the quality of MSCs for further application in the regeneration of bone injury and treatment of arthritis.

P-973

HUMAN EMBRYONIC STEM CELL DERIVED CARDIOVASCULAR PROGENITORS FOR HEART REGENERATION IN PRECLINICAL MODELS

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In recent years, stem cell-based therapies have been considered a potential solution for myocardial infarction (MI). However, cell therapy trials using adult stem cells have been deemed futile, pluripotent stem cell (hPSC) derived cardiomyocytes (CM) have led to arrhythmia (Romagnuolo et al., 2019), inconsistencies in protocols and poor reproducibility and sadly large-scale falsifications of data on adult heart stem cells have caused a crisis in the field of regenerative cardiology. Cardiovascular progenitors (CVPs) are considered a promising source that have shown improvements in animal models of MI and some early-phase clinical trials. To address these challenges, we have recently published a highly reproducible differentiation protocol to generate CVPs from hESCs in a fully-defined laminin-based differentiation system. We demonstrated that LN-221 induces specific biological effects in hESCs by downregulating genes involved in pluripotency and teratoma development, while upregulating genes for cardiac development. We have also compared the transcriptomic gene expression from day 0 to day 120 of differentiation in two different hESC lines and shown that the genes highly corelated (R=0.9) at all time points. Following transplantation of CVPs into a MI injury model in mice CVPs matured and formed well organized of human muscle fiber bundles with sarcomeres and gap junctions. Transplanted hearts also showed improved cardiac function by echocardiogram analysis. In order to develop the CVPs further, we intracardially transplant the cells into MI pig hearts. Yorkshire swines are subjected to permanent ligation of the first branch of left coronary artery and first branch of left circumflex. The CVPs are injected into the infarcted myocardium at several points. Cardiac function is measured by MRI at 1 and 4 weeks post-treatment assessing the left ventricular ejection fraction, regional wall motion and thickness and infarct size. At week 4, hearts are harvested for histology analysis using human nuclei antibody, human cardiac markers (cTNT, alpha-actinin) to visualize human cells retention and organisation. The results of this pig study will be presented.

P-974

Withdrawn

P-975

GENERATION OF INDUCED SECRETOME FROM ADIPOSE-DERIVED STEM CELLS SPECIALIZED FOR DISEASE-SPECIFIC TREATMENT: AN EXPERIMENTAL MOUSE MODEL

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Recently, the exclusive use of mesenchymal stem cell (MSC)-secreted molecules, named as the secretome, rather than cells has been evaluated for overcoming the limitations of cell-based therapy while maintaining its advantages. The goal of this study was to improve cell-free therapy by adding disease-specificity through stimulation of MSCs using disease-causing materials. We collected the secretory materials (named as inducers) released from AML12 hepatocytes that had been pretreated with thioacetamide (TAA) and generated the TAA-induced secretome (TAA-isecretome) after stimulating adipose-derived stem cells (ASCs) with the inducers. The TAA-isecretome was intravenously administered to mice with TAA-induced hepatic failure and those with partial hepatectomy. TAA-isecretome infusion showed higher therapeutic potential in terms of (a) restoring disorganized hepatic tissue to normal tissue, (b) inhibiting proinflammatory cytokines (interleukin-6 and tumor necrosis factor-α), and (c) reducing abnormally elevated liver enzymes (aspartate aminotransferase and alanine aminotrans-

ferase) compared to the naïve secretome infusion in mice with TAA-induced hepatic failure. However, the TAA-isecretome showed inferior therapeutic potential for restoring hepatic function in partially hepatectomized mice. Our results suggest that appropriate stimulation of MSCs with disease-causing agents leads to the production of a secretome specialized for treating a specific disease. Additionally, isecretome therapy is expected to open a new way of developing various specific therapeutics based on the high plasticity and responsiveness of MSCs.

Funding Source: We would like to thank Drug & Disease Target Team (Ochang), Korea Basic Science Institute.

P-976

MULTIFUNCTIONAL BIOSENSING PLATFORM FOR STEM CELL APPLICATIONS AND ANTI-CANCER DRUG SCREENING

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To date, electrochemical assessment have exhibited great potential as a sophisticated tool for wide range of scientific applications including biomedical field, such as monitoring stem cell pluripotency and investigating anti-cancer drug effects on proliferation of cancer cells. In this work, we report a high density gold nanostructure (HDGN) platform that is modified with different types of extracellular matrix (ECM) materials, which is effective for multidisciplinary studies as follows: i) precise label-free electrochemical detection of human embryonic stem cell (hESC) to prevent teratoma formation after transplantation and ii) rapid and sensitive electrochemical detection of anti-cancer effects of curcumin on human glioblastoma (U87MG) cells. The HDGN platforms were generated by surface modification of indium tin oxide (ITO) glass with gold nanoparticles as the electrocatalytic material, in various densities. For the sake of hESC growth, matrigel was used in combination with HDGN platform while a cysteine-containing branched arginyl-glycyl-aspartic acid (RGD-MAP-C) peptide was functionalized to increase U87MG cell adhesion. We found that hESC with the amount of ~12,500 cells were successfully detected through differential pulse voltammetry (DPV) using HDGN/matrigel platfrom and therefore, concluded that the developed hybrid platform could be useful to assure safety of hPSC-derived therapeutic products. On the other hand, the electrical signals of U87MG cells were also successfully detected under cyclic voltammetry, showing an excellent linearity (R2 = 0.99) within the detection range of 20,000-60,000 cells. This indicates that HDGN/peptide-modified platform is promising for screening various natural/synthetic compounds that might be effective for cancer treatments. Remarkably, the developed platform was completely free from a possible signal interference and, also enabled rapid (detection time < 3 min) and precise detection of the viability of target cells (e.g. hESCs, U87MG). Hence, we can conclude that the platform is highly promising for both stem cell and cancer cell studies, which are favorable for clinical therapy and drugs discovery.

Funding Source: National Research Foundation of Korea (NRF) (Grant No. 2019R1C1C1007633) and the Nano Material Technology Development Program through the NRF of Korea funded by the Korea Government (MSIP) (NRF-2014M3A7B4051907).

P-977

COMPARATIVE ANALYSIS OF FBS CONTAINING MEDIA AND SERUM FREE DEFINED MEDIA FOR ADIPOSE DERIVED STEM CELLS PRODUCTION

Lee, Joo Youn, Kang, Min Hee, Lee, Myungseok, Choi, Ji Yong, Chun, Yuri, Jang, Ji Eun, Yang, Yuyeong, Lee, Jeong Eon, Kim, Seung Ki, Park, Sang Gyu

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The core of regenerative medicine is cell-based therapy, including stem cells. For the successful development of effective stem cell therapy, mass production of consistent quality cells is required. Excellent cell culture medium is important in mass production of quality of cells. Classically, FBS has been used as culture supplement for MSCs. However, due to the undefined and heterologous composition of FBS, researchers have prefered serum-free defined media (SFDM). However, there has not been a comprehensive analysis of the cells cultured in FBS containing

media and SFDM. In this study, we aimed to investigate and compare the stem cell characteristics cultured in both media as well as media performance tests. Growth media performance was compared with cell growth rate, accumulated cell number, population homogeneity and cell viability. Comparative analysis of cultured adipose derived mesenchymal stem cells (ADSCs) was performed based on surface marker expression using FACS, differentiation potency, genetic stability using cytokinesis block micronucleus assay, senescence analysis using β-galactosidase staining. Culture using SFDM provided more stable population doubling time, more homogenous cell population, and more cells production in a shorter time compared to FBS containing media. Adipogenic differentiation capability was similar in both media groups, but chondrogenic and osteogenic differentiation capabilities of ADSCs cultured in SFDM were higher than those cultured in FBS containing media. ADSCs cultured in FBS containing media showed a significant increase in the formation frequency of nucleoplasmic bridges comparable to those cultured in SFDM, suggesting that culture using SFDM is more genetically stable. SFDM can provide higher-speed cell production rate than the classical FBS containing culture media. SFDM enables the maintenance of higher population homogeneity, genetic stability, and excellent differentiation potency. Taken together, these results suggest that culture using SFDM provides various advantages through which it is possible to obtain safer, stable, and larger amounts of ADSCs.

P-978

CLINICAL AND NEUROLOGICAL IMPROVEMENT IN TRAUMATIC SPINAL CORD INJURY TREATED WITH AUTOLOGOUS BONE MARROW-DERIVED MESENCHYMAL STEM CELLS AT SARDJITO GENERAL HOSPITAL, YOGYAKARTA, INDONESIA

Sakti, Yudha[1], Admagusta, Rizky[1], Dwianingsih, Ery[2], Panggabean, Andre[3], Rhamadianti, Aulia[3], Filza, Muhammad[1], Yudiyanta, Yudiyanta[3], Rukmoyo, Tedjo[1], Malueka, Rusdy[3]

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Spinal cord injury (SCI) often lead to a severely debilitating condition which renders the patients to be wheelchair-bound or bedridden due to paralysis. Amidst limited results of current therapeutic measures, stem cell therapy has been thought to hold promise in promoting neurological improvements and functional recovery in these patients. Of all cell sources, mesenchymal stem cells (MSCs) has been shown to be an attractive candidate. They are easily obtainable and demonstrate a broad degree of differentiation capacity. Here, we presented 12 months evaluation results of 5 patients with SCI who received 3 series of bone marrow-derived MSCs transplantations with a total dose of 70-80x106 cells for each patient. After obtaining a written informed consent, bone marrow aspiration was conducted, and a volume of 60-80 mL aspirate was then cultured in a Good Manufacturing Practice-compliant facility. Following a strict quality control testing for each production batch, the isolated MSCs were injected back to the patients at the lesion site by minimally invasive surgical technique. Additional injections at one segment above and below the lesion were administered intrathecally. Measurement of changes in American Spinal Injury Association impairment scale (AIS), Barthel Index and Karnofsky Performance Status (KPS) scale were performed prior to and at 12 months after the injection procedure. After 12 months of last injections, there were improvement in AIS grade by at least one grade in 3 patients, improvement in motor and sensory function in three patients, improvement in urinary tract and bowel function in four patients, and improvement in erection ability in two patients. There were improvements in motor, sensory, autonomic, Barthel Index and KPS after 12 months, although these were not significant ($p < 0,05$). There were no adverse events reported by patients, or significant changes from laboratory or imaging examination using MRI. These results highlight the potential application of MSCs in promoting neurological recovery in patients with SCI. Further studies with larger sample size and longer follow-up period are needed to determine the significance and efficacy of this innovative therapeutic strategy in supporting neural regeneration.

P-979

THERAPEUTIC EFFECTS OF REPETITIVE MAGNETIC STIMULATION IN CELL MODELS OF ISCHEMIA/REPERFUSION INJURY

Baek, Ahreum[1], Jo, Seongmoon[2], Yu, Ji Hea[2], Seo, Jung Hwa[2], Shin, Yoon-kyum[2], Wi·Soohyun[2], Song, Suk-young[2], Nam, Bae-geun[2], Pyo, Soonil[2], Cho, Eunju[2], Huh, Jeonghyun[2], Kim, Ji Hyun[1], Kim, Sung Hoon[1], Cho, Sung-Rae[2]

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Repetitive transcranial magnetic stimulation (rTMS) has been used to treat a variety of serious neurological disorders. However, the neurobiological mechanisms underlying the effects of rTMS remain unclear. Therefore, this study examined the therapeutic effects of repetitive magnetic stimulation (rMS) in cell models of ischemia/reperfusion (I/R) injury, depending on its frequencies. Mouse neuroblastoma cells and astrocytes were used for in this study. Cells were divided into three groups—sham, low-frequency, and high-frequency—and were stimulated over three days. Then, low-frequency and high-frequency groups for each cell were characterized by RNA-seq transcriptome analysis. Among several pathways, long-term potentiation pathway and Janus kinase–signal transducer and activator of transcription (JAK–STAT) pathway are significant pathways for neuroblastoma cells and astrocytes. These pathways were further validated in cell models of I/R injury. To establish an I/R injured cell model, neuroblastoma cells were differentiated with retinoic acid and placed under oxygen and glucose deprivation (OGD) for 3 h. Likewise, mouse astrocytes were exposed to OGD condition with ZnCl2 for 3 h. Then the injured cells were replaced with growth medium and stimulated with rMS on day 1. I/R injured cells were also divided into the three groups. High-frequency stimulation increases cell proliferation and inhibits apoptosis in OGD/R injured neuronal cells. Then, high-frequency stimulation increases calcium/calmodulin-dependent protein kinase II-cAMP-response element binding protein pathway, further leading to the alternation of brain-derived neurotrophic factor expression and synaptic plasticity. Furthermore, reactive astrocytes showed activation of JAK–STAT inflammatory pathway and reactive astrogliosis. rMS stimulation prevented reactive astrogliosis and negatively regulated STAT-mediated inflammatory responses. Taken together, these studies will provide a better understanding of the therapeutic mechanisms of rMS and help cure other neurological disorders as a way of regenerative medicine. Moreover, these mechanisms could be applicable in neural stem cells and patient-derived induced pluripotent stem cells for future studies.

Funding Source: Yonsei University Wonju Campus Future-Leading Research Initiative of 2018-62-0056, the National Research Foundation (NRF- 2018R1A6A3A01013415, NRF-2018R1D-1A1B07048239, NRF-2018R1D1A1B07050957, NRF-2019R1I1A1A01041292), and the Ministry of Science and Technology, Korea, the Korean Health Technology R&D Project (HI16C1012).

P-980

BILE DUCT SEEDED WITH CHOLANGIOCYTES DERIVED FROM HUMAN HEPATIC PROGENITOR CELLS

Buisson, Elina Maria[1], Kang, Kyojin[1], Kim, Yohan[1], Yoon, Sangtae[1], Lee·Changhee[1], Yim, Jihye[2], Jeong·Jaemin[2], Choi, Dongho[2]

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Cholangiopathies are diseases of the bile duct which have no method of treatment other than whole liver transplantation. This is problematic as the need for transplantation is soaring and the number of organs available does not match. Furthermore, there is no clinically available drug to treat cholangopathies. Cholangiopathies also target more than one system in the body making it life threatening. Papers have already been published about the differentiation of induced pluripotent stem cells and embryonic stem cells into cholangiocytes. However, this method has several disadvantages. Bioengineering has been used widely nowadays to treat several diseases in terms of making prosthetics, visual aids or scaffolds and so on... Through a previously published method, we nare able to generate human chemically derived progenitor cells (hCdH) from patient hepatocytes and differentiate them into cholangiocytes. Furthermore, we were able to seed them into artificial bile ducts as well as micro and non fibered scaffolds. These cholangiocytes

were able to retain cholangiocyte functionality which was seen through RT-qPCR and immunohistochemistry. In conclusion, this method can open new doors for regenerative medicine by decreasing the need of whole liver transplantation as well as the advantage of it being patient specific.

P-982

THE DEVELOPMENT OF A NEW TREATMENT STRATEGY FOR OSTEOARTHRITIS: INTRA-ARTICULAR INJECTION OF MICROSCOPIC CHONDROGENIC PELLETS DERIVED FROM HIPSCS FOR CARTILAGE REGENERATION

Nam, Yoojun, Rim, Yeri Alice, Park, Narae, Lee, Jennifer, Lee, Kijun, Ang, Jaewoo, Jung, Hyerin, Kim, Yena, Choi, Jinhyuk, Kim, Jang Woon, Kim, Juryun, Won, Pureun, Ju, Ji Hyeon

Catholic iPSCs Center, Seoul, Korea

The defected articular cartilage does not naturally heal and result in loss of ultimate joint function. In order to treat the deficient cartilage area, tissue engineered stem cell-based cartilage are commonly used. Scaffold-free tissue engineering using three-dimensional spheroids is presented as an alternative approach to the delivery of cartilage structures. In this paper, human induced pluripotent stem cells (hiPSCs) were used to create tiny scaffoldless hyaline cartilage with a diameter of about 200uM that can be passed through a syringe for use in patients' joints. The generated microscopic scaffoldless hyaline cartilages showed high expression of chondrogenic genetic markers such as COL2A1, ACAN and SOX9. The production of extracellular matrix (ECM) proteins was confirmed by alcian blue, safranin O and toluidine blue staining. Chondrogenic pellets showed low expression of fibrotic (COL1A1) and hypertrophic (COL10A1) cartilage marker. Expression of collagen type II and aggrecan was detected in the accumulated ECM by immunohistological staining. We confirmed that pluripotent markers decreased in microscopic hyaline cartilage and that there was no toxicity in nude rat and scid mouse. In addition, the damaged area of the rat and rabbit cartilage was healed by the microscopic scaffoldless hyaline cartilage via intra articular injection. This study reveals the potential of microscopic scaffoldless hyaline cartilage derived from hiPSCs as a promising candidate for cartilage regeneration via injection method.

Funding Source: Supported by a grant of the Korea Healthcare Technology R&D Project, Korea (HI16C2177).

Stem Cell Ethics & Policies

P-1001

Withdrawn

P-1002

NATIONAL PRIMATE INFRASTRUCTURE FOR BIOMEDICAL AND BASIC SCIENCE

An, Ju-hyun[1], Lee, Jong-hee[2], Park, Sang-je[2], Kim, Young-hyun[2], Huh, Jae-won[3], Jin, Yeung Bae[2]

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Primates are valuable resources for different research fields including genetics, evolutionary biology, biomedical research, neuroscience, regenerative research, microbiology, vaccine development and pharmacology. Because primates have more biological and behavioral similarities and closer genetic relationship to humans than other animal models. However, primate resources are limited to access for individual researchers. In 2005, Republic of Korea government established National Primate Research Center (NPRC). First purpose of NPRC is production and supply of specific pathogen free (SPF) primate in Republic of Korea. Second purpose is supporting the regenerative medicine (bio organ transplantation, stem cell and gene therapy). The last one is supporting the basic biomedical research and basic science. Recently, NPRC established primate resource bank with various primate samples (Tissue deoxyribose nucleic acid (DNA), Blood DNA, ribose nucleic acid (RNA), cDNA, paraffin blocks (brain), etc) from crab-eating monkey, marmoset monkey, rhesus monkey, African

green monkey, and squirrel monkey. And also, we established cutting edge medical imaging technique using 3 Tesla magnetic resonance imaging (3T-MRI), positron emission tomographic-computed tomographic (PET-CT), micro PET-CT, and angiography imaging system. Therefore, researchers who want to access the primate resources and use the imaging analysis with primate for research purpose could get various national primate infra service, easily (http://portal.kribb.re.kr/primate).

P-1003

TENSIONS OF GENOMIC DATA SHARING IN GENOME RESEARCH AND PLURIPOTENT STEM CELL RESEARCH

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Genome research and pluripotent stem cell research are increasingly advancing for clinical uses. This translation from basic research to clinical care is gradually required to discuss the nature of sharing genomic data as well as other types of data for further understanding of genetic variants and mutations nationally and internationally. While this need of genomic data sharing is pressed by scientific and medical advances, the ethical and legal consideration of research participants who have provided or will provide their genomic data with research communities is questioned. One of the outstanding examples on this issue can be seen in the 2016 enactment of the European General Data Protection Regulation, often called GDPR, in which this regulation clearly binds genomic data with personal information. As is the case with the EU countries, Japan has also followed this change, and subsequently revised the Act on the Protection of Personal Information (APPI) in 2015. Prompted by this revision of the APPI, the nature of governmental ethical guidelines which cover genome and medical research was reviewed, and thereby these guidelines were revised to precisely follow the revised APPI in 2017. Since genomic data is strongly connected with personal information under the APPI and the guidelines as well, the nature of genomic data sharing is revisited in research communities. In this context, the coming wave of open science to share data freely and the broad utilization of cloud computing raise further tensions between data use and data protection. This study will refer to several key issues of ethical and legal regulations regarding the use of genomic data in Japan which include the latest revision process of ethical guidelines started in 2018, and consider the nature of genomic data sharing with different sharing methods such as open access and registered access, managed/controlled access for the international debate.

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ISSCR INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH

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6d	P	x	2F	x	x	F	repeat	
5d	P	F	2F	x	x	repeat		
3d/4d	P	F	x	P	2F	x	x	repeat
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P = Passage; F = Single Feed; 2F = Double Feed

Sustained pH and Stabilized Components, Including FGF2

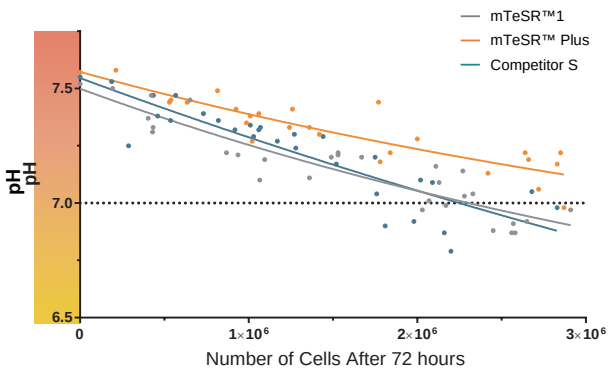

Figure 1. mTeSR™ Plus Maintains Optimal pH Levels Throughout a Weekend-Free Protocol

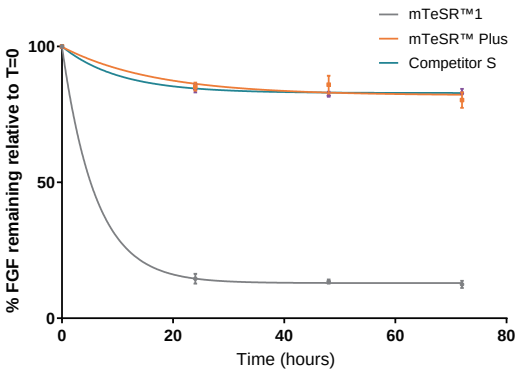

Figure 2. mTeSR™ Plus Maintains Consistent Levels of FGF2 Throughout a Weekend-Free Protocol

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Target
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Stage
IND filed for Phase 1/2

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Thermo Fisher Scientific은 다양한 종류의 줄기세포 연구를 지원 하고 있으며, 줄기 세포의 기초연구부터 임상연구까지 workflow에 따른 모든 단계마다 다양한 솔루션을 제공 드리기 위한 제품과 서비스 개발에도 많은 노력을 기울이고 있습니다.

Disease modeling kit

자가 재생(Self renewal)이 가능하고 다양한 세포로 분화능력을 가지고 있는 인간의 줄기세포는 생물학과 질병 모델, 세포치료 개발에 있어 무한한 가능성을 가지고 있기 때문에 많은 관심과 지원 속에 활발한 연구가 진행되고 있습니다. 그중 환자-특이적 체세포로부터 유도된 인간 만능 줄기 세포(induced pluripotent stem cells, iPSCs)의 유용성은 특정 질환에 대한 기존 동물 모델의 한계를 극복하고 약물 스크리닝에서도 새로운 가능성을 나타내며 두각을 보이고 있습니다.
이에 Thermo Fisher Scientific은 iPSC Disease Modeling 연구를 쉽고 경제적으로 제작하실 수 있는 Full Kit를 제공하여 효과적으로 연구하실 수있도록 도움드리고자합니다.

1 Reprogramming

CytoTune iPS Sendai Reprogramming Kits

DNA
Episomal vectors Epi5 vectors

2 Culture

Culture media
Essential 8 Medium
Essential 8 Flex
StemFlex Medium
KnockOut Serum Replacement–Multi-Species

Supplements
RevitaCell Supplement

Matrices
Vitronectin
Geltrex matrix
rhLaminin-521

3 Characterization

TaqMan hPSC Scorecard Panel
Immunocytochemistry and live staining kits

PSC characterization service
Karyotyping Service (KaryoStat, Array-based)
Pluripotency Assay Service (PluriTest, Array-based)
3 germ-layer Differentiation Potential Assay Service (Scorecard, qPCR-based)

4 Differentiation

Ectoderm
PSC Neural Induction Medium

PSC Dopaminergic Neuron Differentiation Kit

CultureOne Supplement B-27 Supplement

Mesoderm
PSC Cardiomyocyte Differentiation Medium

Endoderm
PSC Definitive Endoderm Induction Medium Kit

5 Cryopreservation

Freeze medium
Cryopreservation Kit

2개 Work Flow이상 구매 시, 10% 할인 해 드립니다.

3개 Work Flow이상 구매 시, 20% 할인 해 드립니다.

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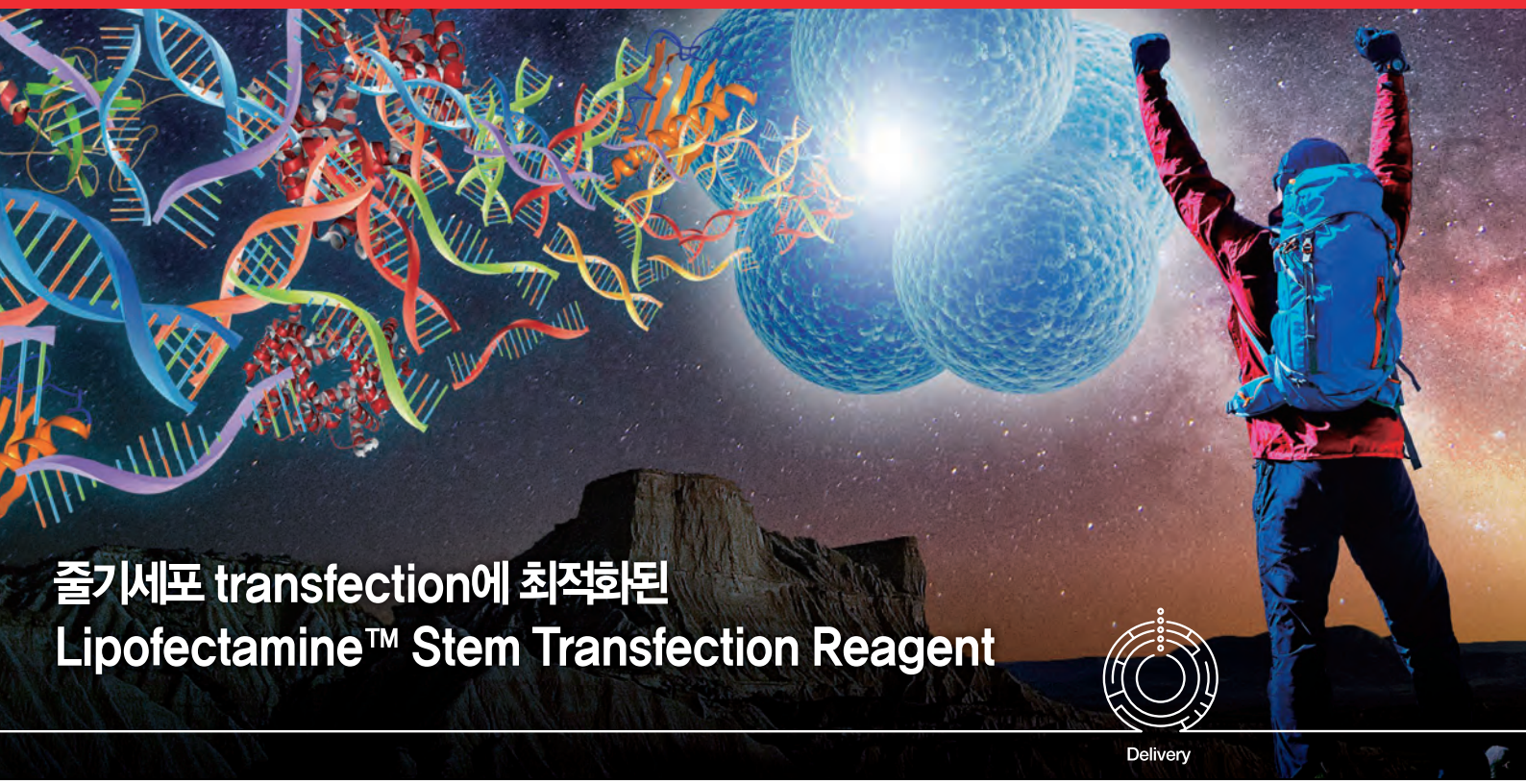
invitrogen



줄기세포 transfection에 최적화된 Lipofectamine™ Stem Transfection Reagent

줄기세포 transfection 실험 시 낮은 효율 때문에 고민이신가요? 지금 연구하고 계신 줄기세포에 적용해 보세요!

Induced Pluripotent Stem Cells (iPSCs)	Embryonic Stem Cells (ESCs)	Neural Stem Cells (NSCs)	Mesenchymal Stem Cells (MSCs)	Hematopoietic Stem Cells (HSCs)
↓	↓	↓	↓	↓
Lipofectamine Stem Reagent				Neon Transfection System

- 우수한 효율성–PSC 및 NSC에서 최대 80 %, MSC에서 최대 45 %의 transfection 효율 낮은 세포독성
- DNA (최대 11kb), RNA 및 Cas9 단백질 복합체 co-transfection 가능
- 부착세포와 부유세포에서 모두 transfection 가능

Order information

Product	Size	Cat. No.
Lipofectamine™ Stem Transfection Reagent	0.1 ml	STEM00001
Lipofectamine™ Stem Transfection Reagent	0.3 ml	STEM00003
Lipofectamine™ Stem Transfection Reagent	0.75 ml	STEM00008
Lipofectamine™ Stem Transfection Reagent	1.5 ml	STEM00015

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The Next Generation Media

Animal-Origin Free (AOF)

Weekend-Free Feeding

Single-Cell Expansion

One Bottle Composition

Animal-Origin Free

Minimize risk of virus contamination and lot-to-lot variation with animal-origin free formula.

Weekend-Free Feeding

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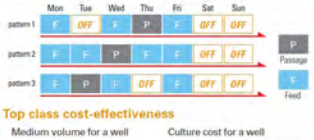
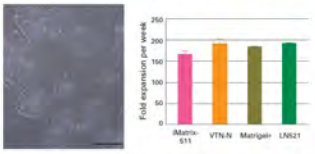


Top class cost-effectiveness

Single-Cell Expansion

Experience superior expansion with single-cell passaging and consistent performance with various matrices.



hiPSCs (1210B2) were cultured with StemFit Basic04 on respective cell adhesion substrates. The graph shows accumulated fold expansion.

One Bottle Composition

Achieve sterilization by simplifying the procedure with ready to use packaging.

Lineup

For Basic Research	For Clinical Research	Frozen form, easy to use
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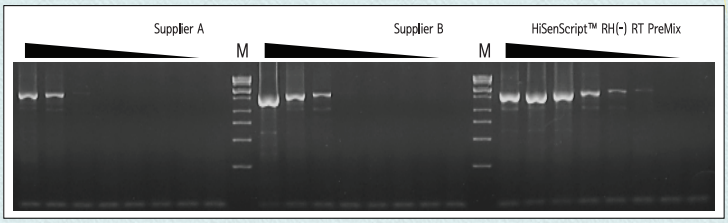
HiSenScript Series

최강의 sensitive!!

HiSenScript™ RH(-) RT PreMix Kit

Cat.No. 25087 / 96 Tubes

HiSenScript™ RH(-) RT PreMix Kit에 적용된 RevoScript™ RH(-) RTase는 RT 반응의 처음 단계인 first-strand cDNA 합성하는 과정에 생성되는 RNA/DNA hybrid을 잘라내어 cDNA의 합성을 방해하는 RNase H 활성이 없어 다른 RTase와 비교하여 좀 더 뛰어난 활성을 보여주어 first-strand cDNA 합성에 높은 민감도를 보유하고 있습니다. 또한 HiSenScript™ RH(-) RT PreMix Kit은 최적 RT 반응 조성으로 조성되어 있어 최소 1 pg의 total RNA, 또는 10 fg의 poly(A) tail mRNA까지도 first-strand cDNA 합성이 가능한 높은 민감도를 보여주고 있습니다.

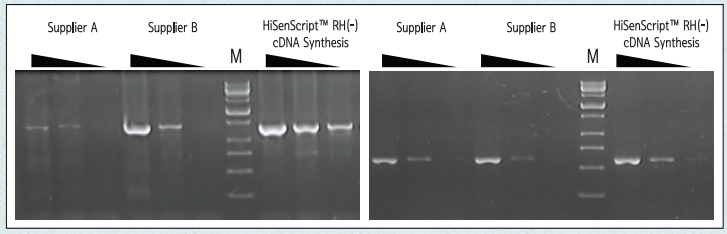


Human total RNA serially diluted from 10 ng to 10 fg (10-fold serial dilution), RT reaction temperature at 42°C at 1 hr, After cDNA synthesis, 1.6kb (18s rRNA) of DNA fragment was amplified with the Maxime PCR PreMix Kit (i-Taq) (iNtRON. Cat. 21131)

HiSenScript™ RH(-) cDNA Synthesis Kit

Cat.No. 25014 / 50 rxn.

HiSenScript™ RH(-) cDNA Synthesis Kit은 뛰어난 민감도뿐만 아니라 total RNA와 같은 다양한 종류의 RNA들이 혼재해 있는 template에서도 어려움 없이 모든 RNA들에게서 first-strand cDNA 합성이 가능합니다. 이는 RevoScript™ RH(-) RTase의 자체적으로 보유한 온도안정성에 추가적으로 유전공학적 기술로 RNase H 활성 감소가 이를 가능하게 하였으며, RT반응에 필요한 buffer 조성들이 최적화되어 사용자들의 optimization을 최소화하였으며 후속 실험 분석에 바로 사용이 가능하도록 준비되어 있습니다. 이를 바탕으로 RNA 유형에 구애 받지 않고 cDNA합성이 가능하여 RNA 발현 확인 및 cDNA library 확보, 분자진단 등의 다양한 분석 실험에 적용이 가능합니다.



Human total RNA serially diluted from 1 ng to 10 pg (10-fold serial dilution), RT reaction temperature at 42°C at 30min, After cDNA synthesis, the 1.3kb(left; 18s rRNA) and 575 bp(right; GAPDH) of DNA fragment was amplified with the Maxime PCR PreMix Kit (i-Taq) (iNtRON. Cat. 21131)

HiSenScript™ RH(-) RT-PCR PreMix

Cat.No. 25135 / 96 Tubes

HiSenScript™ RH(-) RT-PCR PreMix와 타 A, B, C, D 社의 1-step RT-PCR 제품들과 민감도 증폭 효율을 비교 확인하였습니다. 본 제품은 타사 제품과 비교하여 높은 민감도를 보여 주고 있으며 원하는 target RNA만 증폭하는 특이성도 뛰어난 제품입니다.

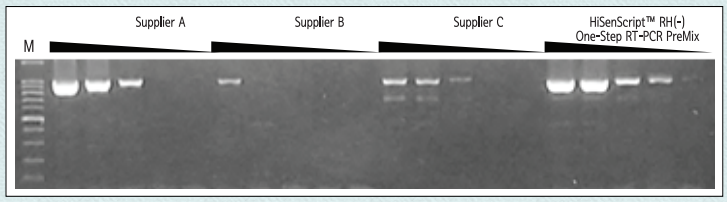


Fig. 2. Comparison of sensitivity RT-PCR with other competitors Lane M : 100bp ladder, Template: SNU-1 cell total RNA 10ng, 1ng, 100pg, 10pg, 1pg Target: GAPDH specific primer Product size: 570 bb

HiSenScript™ RH(-) One-step RT-PCR Kit

Cat.No. 25104 / 50 Tests

HiSenScript™ RH(-) One-step RT-PCR Kit과 타 A, B, C, D 社의 One-step RT-PCR 제품들과 민감도 증폭 효율을 비교 확인하였습니다. 본 제품은 타사 제품과 비교하여 높은 민감도를 보여 주고 있으며 원하는 target RNA만 증폭하는 특이성도 뛰어난 제품입니다.

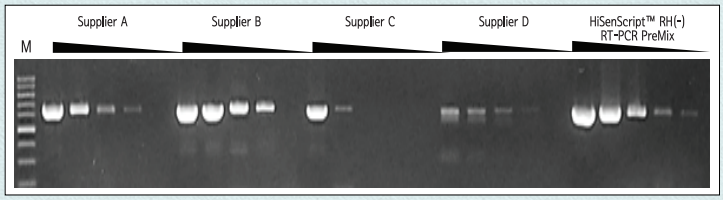


Fig. 2. Comparison of sensitivity RT-PCR with other competitors Lane M : 100bp ladder, Template: SNU-1 cell total RNA 10ng, 1ng, 100pg, 10pg, 1pg Target: β-Actin specific primer Product size:1 kb

HiSenScript™ RH(-) One-step qRT-PCR Kit

Cat.No. 26010 / 50 Tests

HiSenScript™ RH(-) One-step qRT-PCR Kit과 타 A, B 社의 One-step RT-PCR 제품들과 민감도 증폭 효율을 비교 확인하였습니다. 본 제품은 타사 제품과 비교하여 높은 민감도를 보여 주고 있으며 원하는 target RNA만 증폭하는 특이성도 뛰어난 제품입니다.

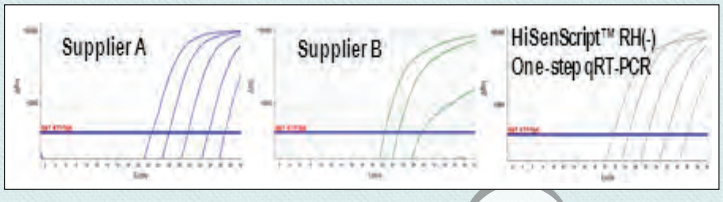


Fig. 2. Suitability of HiSenScript™ RH(-) One-step qRT-PCR Kit for real-time RT-PCR, the RNase P gene was amplified with specific primers and probes using an ABI 7500 real-time PCR system analyzer.. Template: SNU-1 cell total RNA 10 fold diluted 100ng, 10ng, 1ng, 100pg, 10pg Target: Rnase P primer/probe

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e-mail : mjw@mcbt.co.kr 연락주세요!

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Level 1	14	28
Level 0	7	12

DUR of 1.25. Dose rates subject to change.

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- 한 쌍 또는 그 이상의 PCR Primer를 사용하여 복제수가 증가된 유전자 또는 기타 관심부위의 염기서열을 효과적으로 분석
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- 다양한 유전자 연구에 쉽게 적용할 수 있는 신속하고도 신뢰성 높은 경제적인 Sequencing 접근법

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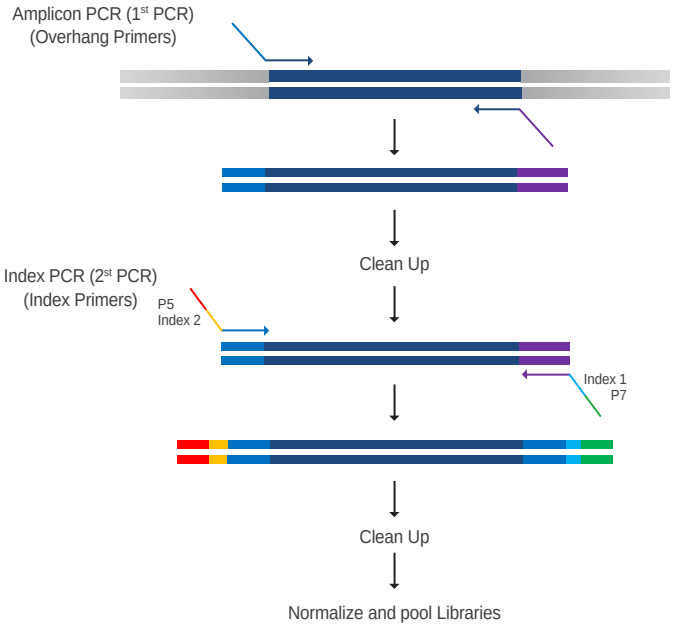
- Organelle Genome Analysis
- Bacterial Genome Analysis
- GenoAmp™ 16S Metagenome Sequencing Service
- NewGAS™ for Bacterial Genome Annotation

Two-Step Dual Index PCR



GenoTech PCR-TAS Service Workflow

Consultation / Order	Primers Design & Synthesis	Construct Library (PCR products preparation & adapters incorporation)		NGS Run	Analyze Data
서비스 종류 선택 라이브러리 제작 방식 선택 :	GenOlio™	Two-Step Dual Index PCR	1st PCR using Overhang Primers → 2st PCR using GenoAmp™ Index Primer Set	MiSeq	CLC CD-hit QiiMe
			1st PCR using Overhang Primers / 2nd PCR using GenoAmp™ Index Primer Set		

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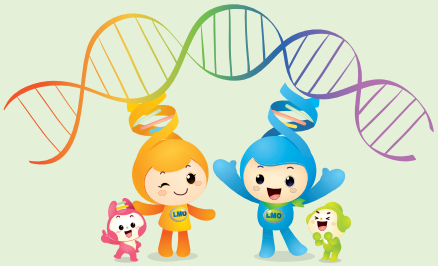

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	3·4등급 연구시설(환경위해성)	허 가	
LMO 수입·수출·경유	LMO 수입	사전신고	
	LMO 수출	사전통보	
	시험·연구용 LMO 경유	사전신고	
LMO 개발·실험	환경방출실험	승 인	

※ 3·4등급 연구시설 중, 인체위해성은 보건복지부장관에게 허가

신청방법

온라인 접수 : 시험·연구용 LMO 온라인신고시스템 http://report.lmosafety.or.kr

벌칙조항

- 신고를 하지 않고 LMO 연구시설 설치·운영 시 2년 이하의 징역 또는 3천만원 이하의 벌금
- 신고를 하지 않고 시험·연구용 LMO 수입 시 2년 이하의 징역 또는 3천만원 이하의 벌금
- 승인을 받지 않고 LMO 개발·실험 시 3년 이하의 징역 또는 5천만원 이하의 벌금

국가연구안전관리본부는 ....

검색창에 국가연구안전관리본부를 검색하세요

유전자변형생물체의 국가간 이동 등에 관한 법률(유전자변형생물체법)에 따라 과학기술정보통신부의 시험·연구용 LMO 안전관리 업무를 수행합니다. LMO 연구시설 안전성 확보, 위해관리 인프라 구축, 안전문화 확산, 법·제도 이행 효율화 등을 통해 안전한 LMO 연구개발 환경 조성과 LMO 안전문화를 선도하는 전문 연구기관으로 생명공학 발전에 기여하고자 합니다.

시험·연구용 LMO 온라인신고시스템(http://report.lmosafety.or.kr)을 통해 LMO 연구시설 및 수출·입 등에 관한 온라인 신고와 시험·연구용 LMO 정보시스템(http://www.lmosafety.or.kr)을 통해 LMO 동향 및 관련 정보 등을 확인할 수 있습니다.

과학기술정보통신부 국가연구안전관리본부

NanoEnTek

Automatic cell counter

ADAM-MC2 (PI Staining)

- 자동화된 이미지 분석
- 정확한 결과
- 정밀한 CMOS 감지 프로세스
- 자동 초점 기능
- 세포치료제 품질 관리 (QC)
- Stem cell / CAR-T cell / CAR-NK cell
  Adipose-derived stem cell / PBMCs / etc…

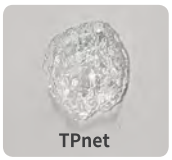
EVE™ Plus (Trypan blue staining)

- 1초 내에 측정 완료 (manual focus)
- 쉬운 작동법
- 하드웨어, 알고리즘 개선으로 정확성 향상

DaNAgreen

Life Science Research Lab

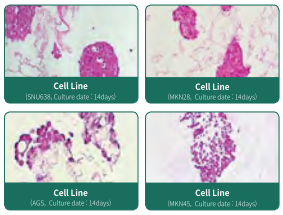
3D cell culture를 간단하게!! *in vivo* 환경과 유사한 3차원적 배양 환경 구현



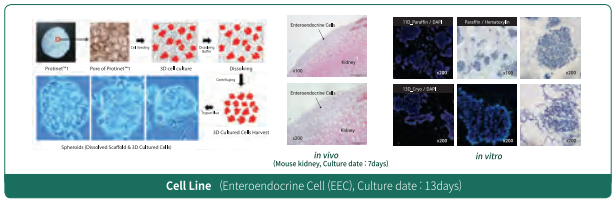
[3D cell culture를 위한 지지체]

Comparison with Human Tissue

Available 3D cell culture in various cell lines



3D Enteroendocrine Cell Culture (FBS free)

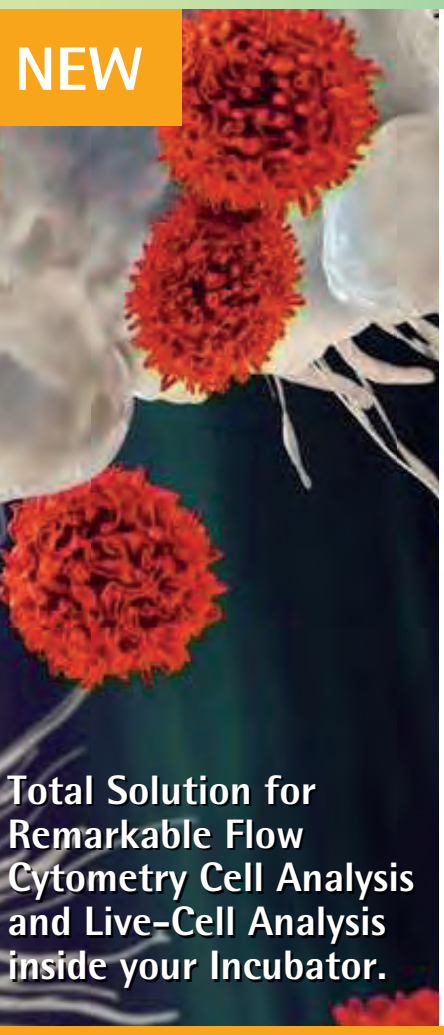


상담 및 구입문의
코스모진텍 학술지원팀

E support@cosmogenetech.com
H www.cosmogenetech.com

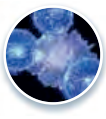
T (02) 465-6217
F (02) 499-3084

cosmo GENETECH

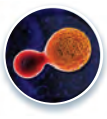


iQue3

Well plate를 기반으로 최소 1ul의 샘플을 사용하여 96-well 5분, 384-well 20분으로 샘플 측정 및 분석이 가능합니다. 또한, Cell과 Bead를 한 well에서 동시에 분석 할 수 있습니다.

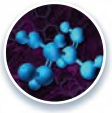


Immunophenotyping



Adoptive Cell Therapy

Antibody Discovery & Development

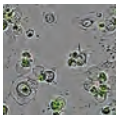


Small Molecule Screening

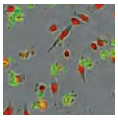
- 진보된 형태의 유세포 분석기입니다.
- Plate로 샘플을 주입합니다.
- 장비의 자동화 운영이 가능합니다.

IncuCyte® S3

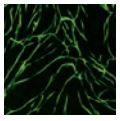
Microscope를 CO_2 Incubator안에 장착 함으로써, 효율적인 Long-Term, Kinetic Live-Cell Imaging 을 가능하게 합니다.



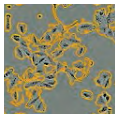
Apoptosis



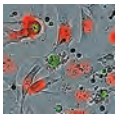
Cytotoxicity



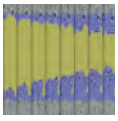
Angiogenesis



Proliferation



Immune-cell killing



Cell Migration and Invasion

- 사용 중인 Incubator에 적용 가능합니다.
- 실시간으로 세포 이미지를 분석합니다.
- 다양한 연구를 지원합니다.
- 세포 분석을 위한 total solution을 제공합니다.

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