

 ISSCR

 | SÃO PAULO



FROM CONCEPT TO CLINIC:
ADVANCES IN
STEM CELL RESEARCH

22-24 September 2023
Ribeirão Preto, Brazil

SUPPORTED BY



UNIFESP

PROGRAM BOOK

Welcome

Dear Colleagues,

On behalf of the International Society for Stem Cell Research welcome to São Paulo and Ribeirão Preto, Brazil and the ISSCR's International Symposium, "From Concepts to Clinic: Advances in Stem Cell Research."

This unique symposium is designed to show the relentless progress in the continuum of stem cell science from early development into clinical applications, highlighting the dynamic nature of the stem cell field. Scientists from Latin America and across the globe will convene to share their latest stem cell research in areas such as pluripotency and early development and its adult counterpart, tissue stem cells and regeneration. Advances in understanding cellular plasticity through reprogramming and directed differentiation will be showcased as will the emerging field of tissue self-organization dynamics and innovative new technologies and tools that are driving the field forward. The program will be capped by sessions on the advances in modeling and treating disease and some of the ethical issues that scientists, and the public, are confronting.

The symposium serves as a capstone of the [São Paulo School of Advanced Science in Stem Cell Biology](#). Developed in partnership with the São Paulo Research Foundation (FAPESP) and The Federal University of São Paulo (UNIFESP), the School which precedes the symposium, brings together 100 undergraduate and graduate students, and post-doctoral scholars from across Brazil and the world to learn about stem cell research in a program modeled on the ISSCR's [Core Concepts in Stem Cell Biology: Syllabus and Learning Guide](#). The ISSCR is proud to support this course and make stem cell science accessible to students worldwide.

We hope you will enjoy the symposium and take advantage of the intimate setting of this symposium. We encourage you to spend time getting to know each other to foster new connections, build productive collaborations, and take the ideas and inspiration back to your own laboratories.

Sincerely,

Symposium Organizing Committee:

Alejandro Sánchez Alvarado, PhD, *Stowers Institute for Medical Research, USA*

Nissim Benvenisty, MD, PhD, *The Hebrew University of Jerusalem, Israel*

Lygia da Veiga Pereira, PhD, *Universidade de São Paulo, Brazil*

Fernando Pitossi, PhD, *Foundation Leloir Institute-University of Buenos Aires, Argentina*

Jose M. Polo, PhD, *Monash University and The University of Adelaide, Australia*

Marimélia Porcionatto, PhD, *Universidade de Federal São Paulo (UNIFESP), Brazil*

Iván Velasco, PhD, *National Autonomous University of Mexico, Mexico*



ABOUT THE ISSCR

The mission of the International Society for Stem Cell Research (ISSCR) is to promote excellence in stem cell science and applications to human health. The ISSCR is the largest society in the world dedicated to the advancement of responsible stem cell research – a field that strives to advance scientific understanding, treatments, and cures that better human health. We foster junior scientists, give voice and visibility to scientific advancement, and encourage a positive global environment for future discovery and treatment. Our promise is to help the field of stem cell research reach its potential.

Contact Us

The International Society
for Stem Cell Research
630 Davis St, Suite 200
Evanston, IL 60201 USA
+1-224-592-5700
www.isscr.org

GOLD SPONSOR



BRONZE SPONSOR



Table of Contents

EVENT INFORMATION..... 1

- Organizing Committee Welcome Letter1
- Meeting Information 5
- Meeting Policies 7
- Board of Directors, ISSCR Staff, and Organizers 8

PROGRAM SCHEDULE 10

- Friday, 22 September 10
- Saturday, 23 September 11
- Sunday, 24 September.....13

SPONSORS AND EXHIBITORS..... 16

INNOVATION SHOWCASE 17

SPEAKER ABSTRACTS 19

- Friday, 22 September19
- Saturday, 23 September21
- Sunday, 24 September..... 27

POSTER ABSTRACTS36

AUTHOR INDEX98



THE FUTURE

HERE
STARTS



**HAMBURG
GERMANY**
10-13 JULY 2024

PLEASE JOIN US FOR OUR
2024 ANNUAL MEETING AT
THE CONGRESS CENTER
HAMBURG

CO-SPONSORED BY:



#ISSCR2024 ISSCR2024.ORG



Meeting Information

ONSITE BADGE PICK UP

Pick-up your name badge in the **Lotus Pool Foyer area** of the Royal Tulip JP Ribeirão Preto Hotel during posted hours. Name badges are required for admission to all sessions, social events, and the Exhibit & Poster Hall. Badges may be picked up during the following times:

Friday, 22 September	9:30 AM – 7:00 PM
Saturday, 23 September	8:00 AM – 5:00 PM
Sunday, 24 September	8:00 AM – 4:00 PM

ISSCR DIGITAL PLATFORM

All registered attendees have access to the [Digital Platform](#). Click the link and login with your ISSCR credentials. Browse and add sessions to your agenda, live-stream the scientific sessions, interact with other attendees, and more!

Need Virtual Assistance? Email ISSCRdigital@isscr.org for troubleshooting.

SMOKING

Smoking or the use of e-cigarettes is prohibited at the Royal Tulip JP Ribeirão Preto Hotel, **unless you are in one of their designated smoking areas.**

LOST AND FOUND

Please bring found items to the ISSCR Registration Desk **Lotus Pool Foyer area** of the Royal Tulip JP Ribeirão Preto Hotel during posted hours. If you lost an item, stop by during registration hours for assistance.

PARKING

Parking for attendees that are also hotel guests is complimentary and guests can park in front of or close to their room. Parking for non-hotel guests is provided by an outside contractor, Rib Park, and non-hotel guests must arrange for parking on their own by contacting contato@ribpark.com.br. Attendees that are not hotel guests are responsible for their own parking fees.

POSTER INFORMATION

Each poster is presented during a 1- hour session in Room Jasmim/Primula. Poster presenters must adhere to the scheduled date and time of their poster display and presentation.

Friday, 22 September - Poster Session I - A and B

Poster Session A

Poster Session A Setup:
3:00 PM - 3:30 PM

Poster Session A Presentation start and end time:
5:15 PM - 6:15 PM

Poster Session A posters **MUST** be taken down by:
6:30 PM

Poster Session B

Poster Session B Setup:
6:15 PM - 6:30 PM

Poster Session B Presentation start and end time:
6:30 PM - 7:30 PM

Poster Session B posters **MUST** be taken down by:
7:45 PM

Saturday, 23 September - Poster Session II - A and B

Poster Session A and B


Poster Session Setup both categories A and B:
3:15 PM - 3:35 PM

Poster Session both categories
A and B Presentation start and end time:
5:30 PM - 6:30 PM

All posters **MUST** be removed by 6:30 PM
23 September.

Poster presenters are responsible for removing their posters upon completion of their presentation. Any posters that are not removed at the end of the session will be discarded.

FOR
SCIENTISTS.
BY
SCIENTISTS.

 **ISSCR**
INTERNATIONAL SOCIETY
FOR STEM CELL RESEARCH



- Open access, peer reviewed science.
- Articles and commentaries that challenge science & advance the field.
- Reduced publication fees for ISSCR members.

Your support of *Stem Cell Reports* enhances the ISSCR's global mission and outreach to promote, educate and advocate for stem cell research and its application.



STEM CELL REPORTS

@StemCellReports
#StemCellReports
stemcellreports.cell.com

ISSCR Meeting Policies

CODE OF CONDUCT

The ISSCR is committed to providing a safe and productive meeting environment that fosters open dialogue and discussion and the exchange of scientific ideas, while promoting respect and equal treatment for all participants, free of harassment and discrimination.

All participants are expected to treat others with respect and consideration, follow venue rules, and alert staff or security, if at an onsite meeting, of any dangerous situations or anyone in distress. Attendees are expected to uphold standards of scientific integrity and professional ethics.

These policies comprise the [Code of Conduct](#) for all ISSCR meetings and events and apply to all attendees, speakers, exhibitors, sponsors, staff, contractors, volunteers, media, and guests.

HARASSMENT POLICY

The ISSCR prohibits any form of harassment, sexual or otherwise. Incidents should immediately be reported to ISSCR meetings staff at isscr@isscr.org.

ABSTRACT CONTENT/ PRESENTATION EMBARGO POLICY

Abstract content may not be announced, publicized, or distributed before the presentation date and time in any way including in media stories, blogs, and social media. ISSCR does permit promotion of general topics, speakers, and presentation days and times. This embargo policy applies to all formats of abstract publication—including abstracts in electronic or print version of Meeting Program Books/Poster Abstract Books, online via the Program Planner and Poster Abstract PDFs, the society's website(s), and other publications.

MEDIA POLICY

The ISSCR invites science journalists to cover science presented at its meetings and events in adherence with the society's Media Policy. Still photography and video and/or audio taping of the sessions, presentations and posters at ISSCR meetings and events are strictly prohibited. Intent to communicate or disseminate results or discussion presented at ISSCR meetings and events is prohibited until the start of each individual presentation. For related questions about the ISSCR Media Policy, please contact Kym Kilbourne at media@isscr.org.

By registering for ISSCR meetings and events, all attendees agree that their image or recording may be used by the ISSCR for promotional purposes in the future.

HEALTH & SAFETY

By registering for an ISSCR in-person event, you agree to present proof of COVID-19 vaccination and/or a negative COVID-19 test if requested. You agree to release the ISSCR and its sponsors and exhibitors, and their employees and agents, from and against claims, liabilities and expenses arising from injury, sickness or death from contraction or spread of COVID-19 or other communicable disease due to travel to, or attendance at, an ISSCR event. You agree to take necessary precautions to ensure your own, and others,' safety. By registering, you agree not to attend any ISSCR event if you feel sick or have had recent exposure to COVID-19. If you have questions on our [health & safety policies](#), please contact isscr@isscr.org.

Board of Directors, ISSCR Staff, and Organizers

ISSCR BOARD OF DIRECTORS

Officers

President

Amander T. Clark
University of California,
Los Angeles, USA

President Elect

Valentina Greco
Yale Medical School, USA

Vice President

Hideyuki Okano
Keio University, Japan

Treasurer

Clive Svendsen
Cedars-Sinai Regenerative Medicine
Institute, USA

Clerk

Fiona Doetsch
Biozentrum at the University
of Basel, Switzerland

Past President

Haifan Lin
Yale University School of Medicine,
USA

Directors

Helen Blau

Stanford University School of
Medicine, USA

Melissa Carpenter

ElevateBio, USA

Shuibing Chen

Weill Cornell Medicine, USA

Michele De Luca

University of Modena & Reggio
Emilia, Italy

Jürgen Knoblich

Institute of Molecular
Biotechnology, Austria

Arnold R. Kriegstein

University of California,
San Francisco, USA

Jane S. Lebkowski

Patch Technologies, USA

Megan Munsie

University of Melbourne & Murdoch
Children's Research Institute,
Australia

Kathy Niakan

University of Cambridge, UK

Malin Parmar

Lund University, Sweden

Martin F. Pera

The Jackson Laboratory, USA

Lygia da Veiga Pereira

Universidade de São Paulo, Brazil

Mitinori Saitou

Kyoto University, Japan

Takanori Takebe

Cincinnati Children's Hospital
Medical Center, USA/Tokyo
Medical and Dental University and
Yokohama City University, Japan

Kenneth S. Zaret

University of Pennsylvania School of
Medicine, USA

Ex Officio Members

Melissa H. Little

Novo Nordisk Foundation Centre
for Stem Cell Medicine (reNEW),
Denmark / Murdoch Children's
Research Institute, Australia

Leonard I. Zon

Boston Children's Hospital, USA

Keith Alm

Chief Executive Officer, ISSCR

ISSCR STAFF

Meredith Albrecht

Senior Event Services Coordinator

Keith Alm

Chief Executive Officer

Chris Barry

Director of Strategic Partnerships

Liza Boscow

Event Services & Membership
Coordinator

Lynnea Brand

Senior Manager, Outreach and
Committees

Denise de Villa

Policy Manager

Dodie Dwyer

Administrative Coordinator

Yvonne Fischer

Managing Editor, Stem Cell Reports

Ayesha Khan

Program Coordinator

Kym Kilbourne

Director of Media and Strategic
Communications

Megan Koch

Marketing Manager

Erika Kowalczyk

Director of Global Events

Tyler Lamb

Director of Policy

Sarah McNamara

Meetings & Events Manager

Jack Mosher

Scientific Advisor

Vihar Patel

Director of Technology and Business
Operations

Andy Podczerwinski

Senior Business Development
Coordinator

Kendra Prutton

Science Communications and
Education Manager

Hunter Reed

Marketing Coordinator

Glori Rosenson

Director of Outreach

Liz Weislogel

Meeting Program Manager

ABSTRACT REVIEWERS

Ori Baru-Nur

Barak Blum

Antonio Carlos Campos de Carvalho

Juan Cruz Casabona

Joseph Chen

Lina Maria Delgado-Garcia

Kieren Harvey

Nayara Leite

Luciana Osaki

Jose Polo

Mahendra Rao

Nadav Sharon

Roberta Sessa

Stilhano Yamaguchi

Achia Urbach

Atilgan Yilmaz



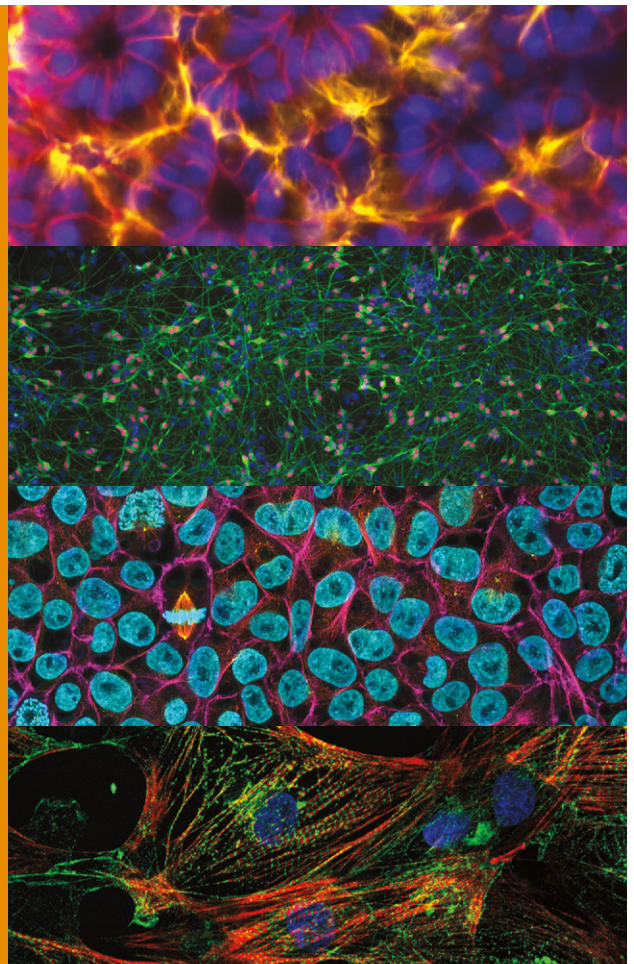
GET MORE FROM YOUR RESEARCH WITH OUR hPSC WORKFLOW

Source, expand, maintain, and differentiate high-quality cells with our reagents, protocols, and STEMdiff™ differentiation kits. At the core of our workflow is the TeSR™ family of feeder-free media, produced using pre-screened materials for batch-to-batch consistency and experimental reproducibility.



EXPLORE PRODUCTS FOR EACH STAGE OF YOUR WORKFLOW
www.stemcell.com/Pluripotent

Copyright © 2023 by STEMCELL Technologies Inc. All rights reserved including graphics and images. STEMCELL Technologies & Design, STEMCELL Shield Design, Scientists Helping Scientists, and STEMdiff™ are trademarks of STEMCELL Technologies Canada Inc. TeSR is a trademark of WARP. All other trademarks are the property of their respective holders.



“

The ISSCR promotes and stands by principles of **rigor, transparency, diversity, and equity** in all areas of stem cell research. I am proud of being an active member of this community that, through policy initiatives, is shaping the **future of stem cell research.**

”

Giorgia Quadrato, PhD
USC Keck School of Medicine, USA

Become a member today at ISSCR.org/membership

Program Schedule

Friday, 22 September

2023 São Paulo International Symposium | *From Concept to Clinic: Advances in Stem Cell Research*

Friday

22 September 2023

ALL TIMES ARE LISTED IN BRASÍLIA TIME (BRT)

1:35^{PM} – 3:00^{PM}

KEYNOTE SESSION

ROOM: Iris/Gerbera

Chair: **Marimélia Procionatto**, *Federal University of São Paulo (UNIFESP), Brazil*

1:35 PM – 1:40 PM

Welcome Remarks
Keith Alm, *ISSCR*

1:40 PM – 2:20 PM

Keynote Speaker
Malin Parmar, *Lund University, Sweden*
STEM CELL THERAPY AND CELL REPROGRAMMING IN PARKINSON'S DISEASE

2:20 PM – 3:00 PM

Keynote Speaker
Amander Clark, *University of California, USA*
MODELLING EMBRYO AND GERMLINE DEVELOPMENT WITH PLURIPOTENT STEM CELLS

3:00^{PM} – 3:30^{PM}

BREAK

ROOM: Jasmim/Primula

3:30^{PM} – 5:05^{PM}

PLURIPOTENCY AND REPROGRAMMING

ROOM: Iris/Gerbera

Chair: **Esteban Mazzoni**, *New York University (NYU), USA*

3:30 PM – 4:00 PM

Nissim Benvenisty, *The Hebrew University, Israel*
THE ESSENTIALS OF HUMAN DEVELOPMENT AND DISEASE

4:00 PM – 4:30 PM

Lygia da Veiga Pereira, *Universidade de São Paulo, Brazil*
INCREASING THE DIVERSITY OF HUMAN PLURIPOTENT STEM CELL LINES

4:30 PM – 4:50 PM

Eftychia Apostolou, *Weill Cornell Medical College, USA*
SINGLE-CELL ANALYSIS OF BIDIRECTIONAL REPROGRAMMING BETWEEN EARLY EMBRYONIC STATES REVEALS MECHANISMS OF DIFFERENTIAL LINEAGE PLASTICITIES

4:50 PM – 5:05 PM

Juliane Viegas, *The Hebrew University of Jerusalem, Israel*
RNA DEGRADATION ELIMINATES DEVELOPMENTAL TRANSCRIPTS DURING MURINE EMBRYONIC STEM CELL DIFFERENTIATION VIA CAPRIN1-XRN2

5:15^{PM} – 7:30^{PM}

WELCOME RECEPTION & POSTER NETWORKING SESSION I

ROOM: Jasmim/Primula

Saturday

23 September 2023

8:30 AM – 10:05 AM

DEVELOPMENT

ROOM: Iris/Gerbera

Chair: **Fiona Doetsch**, *University of Basel, Switzerland*

8:30 AM – 9:00 AM

Kieran Harvey, *Monash University and Peter MacCallum Cancer Centre, Australia*
**A NEW SUBCELLULAR LOCATION FOR HIPPO SIGNALLING AND ITS
 ROLE IN ORGANOGENESIS**

**Virtual Presenter*

9:00 AM – 9:30 AM

Dieter Egli, *Columbia University, USA*
A STRESSFUL BEGINNING - DNA REPLICATION IN THE MAMMALIAN EMBRYO

9:30 AM – 9:50 AM

Guillermo Lanuza, *Fundación Instituto Leloir, Argentina*
A DEVELOPMENTAL PERSPECTIVE OF ASTROCYTE DIVERSITY IN THE SPINAL CORD

9:50 AM – 10:05 AM

Zsuzsanna Izsvak, *Max-Delbrück-Center, Germany*
**A NEW HUMAN EMBRYONIC CELL TYPE ASSOCIATED WITH ACTIVITY OF YOUNG
 TRANSPOSABLE ELEMENTS ALLOWS DEFINITION OF THE INNER CELL MASS**

10:05 AM – 10:35 AM

BREAK

ROOM: Jasmim/Primula

10:35 AM – 12:10 PM

ADULT STEM CELLS AND REGENERATION

ROOM: Iris/Gerbera

Chair: **Fernando Pitossi**, *Fundación Instituto Leloir -CONICET, Argentina*

10:35 AM – 11:05 AM

Sophie Jarriault, *Institut de Génétique et de Biologie Moléculaire et Cellulaire,
 Development & Stem Cells Dept, CU Strasbourg, France*
**DIFFERENTIATION TRAJECTORY OF CELLS ABLE - OR NOT - TO LATER
 BE REPROGRAMMED**

11:05 AM – 11:35 AM

Fiona Doetsch, *University of Basel, Switzerland*
STEM CELLS IN THE ADULT BRAIN: REGULATION AND DIVERSITY

11:35 AM – 11:55 AM

Rosalía Mendez-Otero, *Federal University of Rio de Janeiro (UFRJ), Brazil*
TITLE NOT AVAILABLE AT TIME OF PRINT

11:55 AM – 12:10 PM

Carlos Da Silva Goncalves, *School of Pharmaceutical Sciences of the
 University of São Paulo, Brazil*
**REDUCED PROTEIN INTAKE AND AGING AFFECT THE SUSTAINMENT OF
 HEMATOPOIESIS BY IMPAIRING BONE MARROW MESENCHYMAL
 STEM CELLS IN MICE**

12:10 PM – 1:10 PM

LUNCH BREAK

*Bouganville Restaurant -
 Attendee badge is required.*

Program Schedule

Saturday, 23 September

1:10 PM – 3:15 PM**ROOM: Iris/Gerbera****DIRECTED DIFFERENTIATION AND TRANSDIFFERENTIATION**Chair: **Nissim Benvenisty**, *The Hebrew University of Jerusalem, Israel*

1:10 PM – 1:40 PM

Marius Wernig, *Stanford University School of Medicine, USA*
NEXT GENERATION CELL THERAPIES FOR THE BRAIN

1:40 PM – 2:10 PM

Esteban Mazzoni, *Instituto de Fisiologia Celular, Universidad Nacional Autonoma de Mexico, Mexico*
MODELING DIFFERENTIAL MOTOR NEURON ALS SENSITIVITY

2:10 PM – 2:30 PM

Ivan Velasco, *New York University (NYU), USA*
PLURIPOTENT STEM CELLS DIFFERENTIATED TO DOPAMINE NEURONS: STRATEGIES TO TREAT EXPERIMENTAL MODELS, AND TO UNDERSTAND PARKINSON DISEASE PATHOGENESIS

2:30 PM – 2:45 PM

Xi Wang, *Harvard University, USA*
CELLULAR FITNESS OF HUMAN STEM CELL-DERIVED BETA CELLS UNDER HYPOXIA

2:45 PM – 3:15 PM

Innovation Showcase Presented by **STEMCELL Technologies, Inc.**
Erin Knock, PhD, *STEMCELL Technologies Inc., Canada*
USING HUMAN STEM CELL-DERIVED NEURAL ORGANOIDs FOR MODELING DISEASE**3:15 PM – 3:35 PM****ROOM: Jasmim/Primula****BREAK****3:35 PM – 5:25 PM****ROOM: Iris/Gerbera****LEVERAGING TOOLS TO STUDY STEM CELL BIOLOGY**Chair: **Jose Polo**, *The University of Adelaide, Australia*

3:35 PM – 4:05 PM

Knut Woltjen, *Center for iPS Research and Application (CIRA), Kyoto, Japan*
GENOME AND EPIGENOME EDITING TOOLS FOR STEM CELL RESEARCH

4:05 PM – 4:35 PM

Stevens Rehen, *Federal University of Rio de Janeiro (UFRJ), Brazil*
EXPLORING THE USE OF HUMAN BRAIN ORGANOIDs TO STUDY PSYCHEDELICS

4:35 PM – 4:55 PM

Maria Marchetto, *University of California, San Diego, USA*
USING STEM CELLS TO STUDY EVOLUTION IN DIVERSE PRIMATE SPECIES

4:55 PM – 5:10 PM

Jian Shu, *Massachusetts General Hospital/Harvard University, USA*
MULTIMODAL DECODING OF CELL FATES THROUGH CHEMICAL IMAGING, SINGLE CELL OMICS, AND MACHINE LEARNING

5:10 PM – 5:25 PM

César Meléndez, *Universidad Nacional Autónoma de México (UNAM), Mexico*
CHARACTERIZATION OF EPIGENOMIC AND TRANSCRIPTOMIC PROFILES THROUGHOUT THE DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS INTO DOPAMINERGIC NEURONS**5:30 PM – 6:30 PM****ROOM: Jasmim/Primula****POSTER NETWORKING SESSION II**

Sunday

24 September 2023

8:30 AM – 10:00 AM*ROOM: Iris/Gerbera***SELF-ORGANIZATION OF STRUCTURES**Chair: **Amander Clark**, *University of California, Los Angeles, USA*

8:30 AM – 9:00 AM

Jose Polo, *The University of Adelaide, Australia***SELF-ORGANISATION OF REPROGRAMMING CELLS INTO IBLASTOIDS**

9:00 AM – 9:30 AM

Jun Wu, *University of Texas Southwestern Medical Center, USA***MODELING POST-IMPLANTATION STAGES OF HUMAN DEVELOPMENT INTO EARLY ORGANOGENESIS WITH STEM CELL-DERIVED PERI-GASTRULOIDS**

9:30 AM – 10:00 AM

Nicolas Rivron, *Institute of Molecular Biotechnology, Austrian Academy of Science, Austria***BLASTOIDS: MODELING MOUSE AND HUMAN BLASTOCYST DEVELOPMENT AND IMPLANTATION WITH STEM CELLS****Virtual Presenter***10:00 AM – 10:30 AM***ROOM: Jasmim/Primula***BREAK****10:30 AM – 12:20 PM***ROOM: Iris/Gerbera***DISEASE MODELING**Chair: **Iván Velasco**, *Universidad Nacional Autonoma de Mexico, Mexico*

10:30 AM – 11:00 AM

Alysson Muotri, *University of California, USA***APPLICATIONS OF HUMAN BRAIN ORGANOGENESIS**

11:00 AM – 11:30 AM

Marimelia Porcionatto, *Federal University of São Paulo (UNIFESP), Brazil***CURRENT CHALLENGES AND PROGRESS IN 3D MODELING OF THE BLOOD-BRAIN BARRIER IN NEUROLOGICAL DISORDERS**

11:30 AM – 11:50 AM

Verónica Palma, *Universidade de Chile, Chile***A TRIP BACK IN TIME TO UNDERSTAND THE ORIGIN OF SCHIZOPHRENIA**

11:50 AM – 12:05 PM

Thomas Hutschalik, *Ncardia Services B.V., Netherlands***3D HIPSC MODEL OF ATRIAL INFLAMMATION FINDS ACTIVATED MACROPHAGES AS CAUSE OF PRO-ARRHYTHMIC ELECTROPHYSIOLOGICAL AND STRUCTURAL CHANGES IN CARDIAC TISSUE**

12:05 PM – 12:20 PM

Andre Teles E. Silva, *Hospital Israelita Albert Einstein, Brazil***ELUCIDATING MECHANISMS OF OLIGOGENIC INHERITANCE IN AUTISM SPECTRUM DISORDER USING CORTICAL ORGANOID IN VITRO AND TRANSPLANTED INTO MICE BRAINS****12:20 PM – 1:35 PM***Bouganville Restaurant -
Attendee badge is required.***LUNCH BREAK**

ETHICS AND UNPROVEN STEM CELL THERAPIES IN SOUTH AND LATIN AMERICA

1:35 PM – 3:20 PM

ROOM: *Iris/Gerbera*

Chair: **Lygia da Veiga Pereira**, *Universidade de São Paulo, Brazil*

1:35 PM – 2:05 PM

Fernando Pitossi, *Fundación Instituto Leloir -CONICET, Argentina*
BIOMEDICAL ASPECTS OF UNPROVEN STEM CELL INTERVENTIONS AND INITIATIVES TO PREVENT THEM AND FACILITATE ETHICALLY SOUND CLINICAL TRANSLATION

2:05 PM – 2:35 PM

Maria Medina-Arellano, *Institute of Legal Research UNAM, Mexico*
ETHICAL GUIDELINES AND LEGAL LANDSCAPES OF THERAPIES IN LATIN AMERICA: UNDERSTANDING THE CIRCUITS OF USE TO PREVENT EXPLOITATION BY UNPROVEN STEM CELL THERAPY CLINICS

2:35 PM – 2:50 PM

Mairim Solis, *Gorgas Memorial Institute for Health Studies/National Bioethics Research Committee, Panama*
REGULATORY FRAMEWORK FOR BIOETHICAL AND LEGAL USE OF STEM CELL IN PANAMA: A SWOT ANALYSIS

2:50 PM – 3:20 PM

Megan Munsie, *University of Melbourne & Murdoch Children's Research Institute, Australia*
TURNING THE TIDE: HOW ADVOCACY AND POLICY REFORM CURBED THE BUSINESS OF SELLING STEM CELLS DOWN UNDER

3:20 PM – 3:40 PM

BREAK

ROOM: *Jasmim/Primula*

3:40 PM – 5:15 PM

CLINICAL APPLICATIONS OF STEM CELL BIOLOGY

ROOM: *Iris/Gerbera*

Chair: **Dieter Egli**, *Columbia University, USA*

3:40 PM – 4:10 PM

Mahendra Rao, *panCella/PLSX, USA*
MAKING CELL THERAPY AFFORDABLE BY OPTIMIZING LICENSING, MANUFACTURING AND DISTRIBUTION MODELS

4:10 PM – 4:40 PM

Antonio Carlos Carvalho, *Universidade Federal do Rio de Janeiro, Brazil*
MODELING CARDIAC DISEASE USING ISOGENIC hiPSC-DERIVED CARDIOMYOCYTES

4:40 PM – 5:00 PM

Nayara Leite, *Vertex Pharmaceuticals, USA*
PROTECTING SC-ISLETS FROM STRESS

5:00 PM – 5:15 PM

Quinn Peterson, *Mayo Clinic, USA*
BOTTOM-UP ISLET ENGINEERING: PROGRESS TOWARD DESIGNER ISLET ORGANOID FROM HUMAN PLURIPOTENT STEM CELLS

Standards for Human Stem Cell Use in Research



Quality standards and core principles for the laboratory culture and characterization of both adult and pluripotent human stem cells and in vitro model systems using them.



Supported by:



Sponsor and Exhibitor Directory

GOLD SPONSOR

STEMCELL TECHNOLOGIES

1618 Station St
Vancouver, BC V6A 1B6
Canada

www.stemcell.com



At STEMCELL, science is our foundation. Driven by our mission to advance research globally, we offer over 2,500 tools and services supporting discoveries in stem cell research, regenerative medicine, immunotherapy, and disease research. By providing access to innovative techniques like gene editing and, organoid cultures, we're helping scientists accelerate the pace of discovery. Inspired by knowledge, innovation, and quality, we are Scientists Helping Scientists.

BRONZE SPONSOR

QIAGEN BRASIL

Av. Roque Petroni Júnior, 850
Jardim das Acacias,
São Paulo - SP, 04707-000
Brazil

www.qiagen.com



At the heart of QIAGEN's business is a vision for making improvements in life possible. We deliver on this promise by providing differentiating Sample to Insight solutions that help our customers to unlock valuable molecular insights encoded in the building blocks of life. Scientists, doctors, lab directors and other professionals committed to answering life's questions look to QIAGEN for solutions in molecular testing.

EXHIBITORS

ANALÍTICA

Av. Fábio Eduardo Ramos Esquivel, 2100 - Centro
Diadema, São Paulo 09941-202
Brazil

+55 11 2162-8080

analitica@novanalitica.com.br

www.analiticaweb.com.br

Analítica was founded in 1992 by professionals with extensive experience in instrumental analytical chemistry, particularly in the chromatography and spectrometry segment, diversifying its activities in the research and development laboratory market, expanding its product line to serve the bioscience, and other segments.

The purpose of Analítica is to promote and guarantee customer satisfaction, meeting or exceeding expectations with the quality of products and services. For this, it seeks to supply the best equipment and accessories, manufactured by internationally renowned companies, and to offer technical and commercial support that meet the needs of each client.

BIOINVISION, INC.

781 Beta Dr. Ste E
Cleveland, OH 44143
216-373-1500

www.bioinvision.com

BioInVision, based in USA, offers imaging instrumentation and methodologies critical to preclinical studies. The unique CryoViz instrument, utilizes the patented hi-resolution blockface-imaging technology, to capture microscopical anatomical and molecular fluorescence imaging of laboratory small animals such as a mouse, rats or organs excised from them with single-cell sensitivity. With its sub-10-micron-scale imaging, CryoViz allows one to detect even single stem or cancer cells anywhere in a mouse. The technology is also offered as a service and is targeted to a variety of biomedical applications including stem cell homing and bio-distribution, cancer micro-metastasis, imaging agents, drug discovery, tissue engineering, mouse phenotyping etc.

Innovation Showcase

SATURDAY, 23 SEPTEMBER

2:35 PM – 3:15 PM
INNOVATION SHOWCASE

*Presented by: **STEMCELL Technologies, inc.***

**USING HUMAN STEM CELL-DERIVED NEURAL
ORGANOIDS FOR MODELING DISEASE**

Erin Knock, PhD

STEMCELL Technologies Inc., Canada

In this showcase talk, we will introduce the various types of neural organoids offered by STEMCELL Technologies. We will review how to generate cerebral, midbrain, and spinal cord organoids and demonstrate which cell types you can expect from each. Finally, we will show how each type of organoid can be used to model diseases such as microcephaly, epilepsy, Parkinson's disease and amyotrophic lateral sclerosis.



ISSCR

STEM CELL REPORTS



THE STEM CELL REPORT

A PODCAST WITH MARTIN PERA

SEASON 3 EPISODE 1

ORGANOIDS: MULTI-DIMENSIONAL STANDARDS FOR THREE DIMENSIONAL MODELS



KIM JENSON, PHD
NOVO NORDISK FOUNDATION CENTRE FOR
STEM CELL MEDICINE (RENEW), DENMARK



MELISSA H. LITTLE, PHD
NOVO NORDISK FOUNDATION CENTRE FOR STEM
CELL MEDICINE (RENEW), DENMARK & MURDOCH
CHILDREN'S RESEARCH INSTITUTE, AUSTRALIA

SEASON 3 EPISODE 2

SETTING THE STANDARDS FOR HUMAN STEM CELL RESEARCH

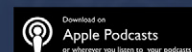
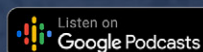


PETER ANDREWS, DPHIL
UNIVERSITY OF SHEFFIELD, UK



TENNEILLE E. LUDWIG, PHD
WICELL RESEARCH INSTITUTE, USA

SUBSCRIBE



<https://thestemcellreport.buzzsprout.com>

Photo: NIH

Speaker Abstracts

Friday, 22 September

FRIDAY, 22 SEPTEMBER

1:40 PM – 3:00 PM KEYNOTE SESSION

1:40 PM – 2:20 PM

STEM CELL THERAPY AND CELL REPROGRAMMING IN PARKINSON'S DISEASE

Parmar, Malin

Experimental Medical Science, Lund University, Lund, Sweden

Parkinson's Disease (PD) is a focal neurodegenerative disorder where clinical transplantation trials using stem cell-derived dopamine (DA) neurons are already on their way. In addition, patient derived DA neurons can be obtained via iPSc reprogramming or direct neural conversion. We have developed a highly efficient approach to generate functional, induced dopaminergic (DA) neurons from adult human dermal fibroblasts using a combination of neuronal conversion factors and DA fate determinants. The patient-derived neurons maintain age-related properties of the donor and exhibit disease related pathology in vitro. In this presentation, I will discuss how reprogrammed cells can be used to better understand the basis of heterogeneity of PD and important considerations for further developing reprogrammed cells for cell therapy or as a cellular model to diagnose disease and study pathological development.

Keywords: cell reprogramming, dopamine neurons, parkinson's disease

2:20 PM – 3:00 PM

MODELLING EMBRYO AND GERMLINE DEVELOPMENT WITH PLURIPOTENT STEM CELLS

Clark, Amander

Department of Molecular Cell and Developmental Biology, University of California, Los Angeles, CA, USA

Infertility is a disease with an estimated global lifetime prevalence of 17.4%. Infertility affects all genders, races and ethnicities, and is associated with multifaceted etiologies due to the complexity of the reproductive system, and the sensitivity of reproductive organs to damage from disease, infection and injury. Our lab is interested in the biology of the germline, the lineage responsible for the generation of gametes, as well as the formation and function of the ovary.

Using pluripotent stem cells, our lab generates models of the germline in order to understand the cell and molecular mechanisms involved in germ cell specification in the embryo, and more recently the interaction of immature germ cells with the ovarian niche through the self-assembly of ovarian organoids with pluripotent stem cell-derived germ cells. Our work shows that germ cell specification is a transcription factor-driven process involving the binding of key transcription factors to germ cell-specific regions of germline chromatin. Binding of these transcription factors upon germ cell specification is associated with epigenetic remodeling, and specifically the conversion of 5-methyl cytosine to 5-hydroxymethyl cytosine in DNA. Epigenetic remodeling during germ cell specification involves the ape-specific transposon family called Long Terminal Repeat 5 Human Specific (LTR5Hs). A family of transposons that are found in the DNA of apes and humans but not in other mammalian species including monkeys. CRISPRi to target LTR5Hs sequences in human DNA leads to a significant decrease in human germ cell specification in vitro suggesting that LTR5Hs sequences have been incorporated into the germ cell transcription factor network to ensure human fertility. Taken together, human pluripotent stem cells are a powerful tool to evaluate transcription factor binding and molecular pathways involved in developmental events that are evolutionary new and important to human biology.

Funding Source: R01HD079546

Keywords: pluripotency, germline, human

3:30 PM – 5:05 PM PLURIPOTENCY AND REPROGRAMMING

3:30 PM – 4:00 PM

THE ESSENTIALS OF HUMAN DEVELOPMENT AND DISEASE

Benvenisty, Nissim

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

Human pluripotent stem cells (hPSCs) have a major role in investigating human development, and in modeling genetic disorders. We have recently generated haploid hPSCs carrying only one set of chromosomes. 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. Furthermore, we demonstrated the superior utility of haploid hPSCs for loss-of-function genetic screening. To define the essentialome of hPSCs we generated a genome-wide loss-of-function library in the haploid cells utilizing CRISPR/Cas9 technology using about 180,000 guide RNAs, targeting virtually all coding genes. This library enabled us to define the genes essential for the normal growth and survival of undifferentiated hPSCs. We could also allude to an intrinsic bias of essentiality across cellular compartments, uncover two opposing roles for tumor suppressor genes and link autosomal-recessive disorders with growth retardation phenotypes to early embryogenesis. More recently, we set out to map the essential genes for the differentiation of hPSCs into ectoderm, mesoderm and endoderm, defining the essentialome of each germ layer separately and also identified commonly essential genes for the transition from pluripotency stage into differentiated cells. Our data also enabled analysis of all hereditary neurological disorders, uncovering essentiality of a significant fraction of microcephaly-causing genes during neuroectoderm development. In addition to defining the essentialome of early human development, we utilized the genome-wide screening to identify genes that can reverse the phenotype of imprinting and neurological disorders. We focused on chromatin-related genes that their mutation can induce a normal molecular phenotype, and validated their effect in diseased cells, as a way to suggest novel therapies. Overall, our work sheds light on the gene networks regulating pluripotency, early gastrulation, and human genetic disorders by utilizing genome-wide screening in hPSCs.

Keywords: Human pluripotent stem cells, Genome-wide screening, Human disorders

4:00 PM – 4:30 PM

INCREASING THE DIVERSITY OF HUMAN PLURIPOTENT STEM CELL LINES

Pereira, Lygia da Veiga

*Department of Genetics and Evolutionary Biology,
Universidade de São Paulo, Brazil*

Despite great advances in the use of hiPSCs for research, one important caveat has been the limited ethnic diversity of available lines of human pluripotent stem cells (hPSCs), mostly of European and Asian ethnicity. We established a collection of hPSC lines derived from the Brazilian population and show that, while lines derived from cryopreserved embryos are mostly of European genomic descent, hiPSCs derived from Brazilians contain different proportions of Indigenous, African and European

genomic ancestries. In addition, we looked at the local ancestry of 6 lines around genes from the CYP family, and identified great heterogeneity of ancestries in those regions. The availability of hiPSCs with diverse genetic backgrounds will allow investigations on the contribution of different haplotypes to cells', and thus patients', phenotypes, including differential drug response. The hiPSC lines described represent a significant advance in increasing the ethnic diversity and genetic admixture of the currently available hPSCs.

Funding Source: FAPESP

Keywords: genomic diversity, hiPSCs, drug response

4:30 PM – 4:50 PM

SINGLE-CELL ANALYSIS OF BIDIRECTIONAL REPROGRAMMING BETWEEN EARLY EMBRYONIC STATES REVEALS MECHANISMS OF DIFFERENTIAL LINEAGE PLASTICITIES

Apostolou, Eftychia

*Medicine, Division of Hematology and Medical Oncology,
Weill Cornell Medical College, New York, NY, USA*

Two distinct fates, pluripotent epiblast (EPI) and primitive (extra-embryonic) endoderm (PrE), arise from common progenitor cells, the inner cell mass (ICM), in mammalian embryos. To study how these sister identities are forged, we leveraged embryonic (ES) and eXtraembryonic ENdoderm (XEN) stem cells – in vitro counterparts of the EPI and PrE. Bidirectional reprogramming between ES and XEN coupled with single-cell RNA and ATAC-seq analyses uncovered distinct rates, efficiencies and trajectories of state conversions, identifying drivers and roadblocks of reciprocal conversions. While GATA4-mediated ES-to-iXEN conversion was rapid and nearly deterministic, OCT4, KLF4 and SOX2-induced XEN-to-iPS reprogramming progressed with diminished efficiency and kinetics. The dominant PrE transcriptional program, safeguarded by Gata4, and globally elevated chromatin accessibility of EPI underscored the differential plasticities of the two states. Mapping in vitro trajectories to embryos revealed reprogramming in either direction tracked along, and toggled between, EPI and PrE in vivo states without transitioning through the ICM.

Funding Source: NIH (1R01GM138635), STARR Tri-Institutional Stem Cell Initiative

Keywords: epiblast, primitive endoderm, ES cells, XEN cells, pluripotency, extra-embryonic endoderm, reprogramming, lineage plasticity, single-cell analysis, blastocyst

4:50 PM – 5:05 PM**RNA DEGRADATION ELIMINATES DEVELOPMENTAL TRANSCRIPTS DURING MURINE EMBRYONIC STEM CELL DIFFERENTIATION VIA CAPRIN1-XRN2****Viegas, Juliane O.**¹, Azad, Gajendra², Kaganovich, Daniel³, Lv, Yuan⁴, Pattabiraman, Sundararaghavan⁵, Park, Jung Eun⁶, Sze, Siu Kwan⁷, Esteban, Miguel A.⁴, Rabani, Michal¹ and Meshorer, Eran¹¹Genetics, The Hebrew University of Jerusalem, Israel,²Department of Zoology, Patna University, Patna, India,³Wren Therapeutics, Cambridge, UK, ⁴Laboratory of Integrative Biology, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou, China,⁵School of Biological Sciences, University of Southampton, UK, ⁶School of Biological Sciences, Nanyang Technological University, Nanyang Drive, Singapore,⁷Faculty of Applied Health Sciences, Brock University, St. Catharines, Canada

Embryonic stem cells (ESCs) are self-renewing and pluripotent. In recent years, factors that control pluripotency, mostly nuclear, have been identified. To identify non-nuclear regulators of ESCs, we screened an endogenously labeled fluorescent fusion-protein library in mouse ESCs. One of the more compelling hits was the cell-cycle-associated protein 1 (CAPRIN1). CAPRIN1 knockout had little effect in ESCs, but it significantly altered differentiation and gene expression programs. Using RIP-seq and SLAM-seq, we found that CAPRIN1 associates with, and promotes the degradation of, thousands of RNA transcripts. CAPRIN1 interactome identified XRN2 as the likely ribonuclease. Upon early ESC differentiation, XRN2 is located in the nucleus and colocalizes with CAPRIN1 in small RNA granules in a CAPRIN1-dependent manner. We propose that CAPRIN1 regulates an RNA degradation pathway operating during early ESC differentiation, thus eliminating undesired spuriously transcribed transcripts in ESCs.

Keywords: embryonic stem cells, differentiation, RNA degradation**SATURDAY, 23 SEPTEMBER****8:30 AM – 10:05 AM
DEVELOPMENT****8:30 AM – 9:00 AM****A NEW SUBCELLULAR LOCATION FOR HIPPO SIGNALLING AND ITS ROLE IN ORGANOGENESIS****Harvey, Kieran***Organogenesis and Cancer Program, Peter MacCallum Cancer Centre, Australia*

Adult organs grow from small pools of progenitor cells that proliferate and become more specialized as development progresses. Organ size is controlled by numerous factors including mechanical forces, which are mediated in part by the Hippo signalling pathway. In growing *Drosophila melanogaster* epithelial tissues, cytoskeletal tension influences Hippo signalling in progenitor cells by modulating the subcellular localisation of key pathway proteins in different apical domains, namely adherens junctions, the sub-apical region and the medial apical cortex. Here, using both electron and light microscopy, we have discovered the existence of new cell-cell adhesion complexes in *D. melanogaster* epithelial imaginal disc tissues – the basal spot junctions. These cell-cell junctions respond to morphogenetic forces and also regulate Hippo signalling. Like adherens junctions, the Warts kinase is recruited to basal spot junctions via the Ajuba and E-cadherin proteins, which prevent Warts activation by segregating it from upstream Hippo pathway proteins. Basal spot junctions are prominent when tissues undergo morphogenesis and are highly sensitive to fluctuations in cytoskeletal tension. Basal spot junctions are distinct from focal adhesions, but the latter profoundly influences the abundance of spot junctions by modulating the basal-medial actomyosin network and tension experienced by spot junctions. Thus, basal spot junctions are important for coupling morphogenetic forces and Hippo pathway activity during organogenesis.

9:00 AM – 9:30 AM**A STRESSFUL BEGINNING - DNA REPLICATION IN THE MAMMALIAN EMBRYO****Egli, Dieter***Pediatrics, Columbia University, New York, USA*

Human reproduction is extremely inefficient – most fertilized eggs fail to develop beyond the day5 of development. We have recently shown that human embryos inefficiently repair a Cas9-induced double strand break (DSB) at the EYS gene, resulting in frequent chromosomal aneuploidies, including both whole chromosome aneuploidies as well as of chromosomal arms. The inefficient repair of DSB also applies to spontaneous breaks arising during cell cycle progression. We examined the timing of formation and repair of spontaneous DSBs in the early human embryo and show frequent markers of DNA breakage and repair occurring from the first cell cycle. Spontaneous DNA breaks occur at and near long neuronal genes, resulting in frequent losses of chromosome arms. We furthermore show that the origin of these breaks is DNA replication stress in the first cell cycle after fertilization. These findings suggest that inefficient DSB repair account for frequent genetic change in the human embryo, aneuploidies, and developmental failure. We will discuss the relevance of these findings in the context of understanding both normal as well as disease-causing genetic variation in the mammalian genome, as well as the causes of replication stress. Furthermore, we will discuss how these findings inform future attempts for gene correction in the human germ line.

Keywords: embryonic development, DNA replication stress, gene editing, DNA damage, aneuploidy

9:30 AM – 9:50 AM**A DEVELOPMENTAL PERSPECTIVE OF ASTROCYTE DIVERSITY IN THE SPINAL CORD****Lanua, Guillermo***Developmental Neurobiology Lab, Fundación Instituto Leloir, Buenos Aires, Argentina*

Significant progress has been made in elucidating the basic principles that govern neuronal subtype specification in the developing central nervous system. In contrast, much less is known how developmental origin influences astrocytic diversity. Here we demonstrate that a restricted pool of progenitors in the mouse spinal cord, expressing the transcription factor Dbx1, produces a subset of astrocytes, in addition to pre-motor interneurons. Ventral p0-derived astrocytes (vA0 cells) exclusively populate the intermediate regions of the spinal cord with extraordinary precision and reproducibility. Postnatal vA0 population comprises gray matter protoplasmic, white matter fibrous

astrocytes and a group of cells with strict radial morphology contacting the pia. vA0 cells in the lateral funiculus are distinguished by the expression of reelin and Kcnmb4. We show an increased production of vA0 cells in the absence of Dbx1, which are generated at the expense of p0-derived interneurons. Manipulation of the Notch pathway, together with the alteration in their ligands seen in Dbx1 mutants, indicate that Dbx1 controls neuron-glia balance by modulating Notch-dependent cell interactions. This study highlights that progenitor transcriptional programs highly influence glial fate, and that positional identity is instrumental in creating astrocyte diversity, with restricted dorsal-ventral progenitors producing region-specific astrocytic subgroups.

Keywords: gliogenesis, mice, specification, positional identity, spinal cord

9:50 AM – 10:05 AM**A NEW HUMAN EMBRYONIC CELL TYPE ASSOCIATED WITH ACTIVITY OF YOUNG TRANSPOSABLE ELEMENTS ALLOWS DEFINITION OF THE INNER CELL MASS****Izsvak, Zsuzsanna**¹, Garcia-Perez, Jose², Hurst, Laurence³, Kondrashkina, Aleksandra⁴ and Singh, Manvendra⁵¹Mobile DNA, Max-Delbrück-Center, Berlin, Germany,²Centre for Genomics and Oncological Research, GENYO, Granada, Spain, ³Life Sciences, Milner Centre for Evolution,⁴Mobile DNA, Max-Delbrück-Center for Molecular Medicine in the Helmholtz Society, Berlin, Germany, ⁵Clinical Neuroscience, Max Planck Institute of Multidisciplinary Sciences, Gottingen, Germany

There remains much that we do not understand about the earliest stages of human development. On a gross level, there is evidence for apoptosis, but the nature of the affected cell types is unknown. Perhaps most importantly, the inner cell mass (ICM), from which the foetus is derived and hence of interest in reproductive health and regenerative medicine, has proven hard to define. Here we provide a multi-method analysis of the early human embryo to resolve these issues. Single-cell analysis (on multiple independent data sets), supported by embryo visualization, uncovers a common previously uncharacterised class of cells lacking commitment markers that segregates after embryonic gene activation and shortly after undergo apoptosis. The discovery of this cell type allows us to clearly define their viable ontogenetic sisters, these being the cells of the inner cell mass (ICM). While ICM is characterized by the activity of an Old non-transposing endogenous retrovirus (HERVH) that acts to suppress Young transposable elements, the new cell type, by contrast, expresses transpositionally-

competent Young elements and DNA-damage response genes. As the Young elements are RetroElements and the cells are excluded from the developmental process, we dub these REject cells. With these and ICM being characterised by differential mobile element activities, the human embryo may be a “selection arena” in which one group of cells selectively die, while other less damaged cells persist.

Keywords: Human endogenous retrovirus H, Inner cell mass, apoptosis

10:35 AM – 12:10 AM Adult Stem Cell and Regeneration

10:35 AM – 11:05 AM
DIFFERENTIATION TRAJECTORY OF CELLS ABLE -
OR NOT - TO LATER BE REPROGRAMMED

Jarriault, Sophie

*Institut de Génétique et de Biologie Moléculaire et Cellulaire,
Development & Stem Cells Dept, CU Strasbourg, France*

Abstract not available at time of print

11:05 AM – 11:35 AM
STEM CELLS IN THE ADULT BRAIN: REGULATION
AND DIVERSITY

Doetsch, Fiona

Biozentrum, University of Basel, Switzerland

Neural stem cells reside in specialized niches in the adult mammalian brain. Adult neural stem cells dynamically integrate intrinsic and extrinsic signals to either maintain the quiescent state or to become activated to divide and generate neurons and glia. I will present our recent findings highlighting adult neural stem cell heterogeneity, including the identification of novel gliogenic domains and cell types, and the key roles of physiological states and long-range signals in the regulation of regionally distinct pools of adult neural stem cells.

Keywords: brain, niche, mice

11:35 AM – 11:55 AM
TITLE NOT AVAILABLE AT TIME OF PRINT

Mendez-Otero, Rosalia

Federal University of Rio de Janeiro (UFRJ), Brazil

Abstract not available at time of print

11:55 AM – 12:10 PM
REDUCED PROTEIN INTAKE AND AGING AFFECT THE
SUSTAINMENT OF HEMATOPOIESIS BY IMPAIRING
BONE MARROW MESENCHYMAL STEM CELLS IN MICE

Da Silva Goncalves, Carlos E., da Silva, Renaira, Hastreiter, Araceli, Vivian, Gabriela, Makiyama, Edson, Borelli, Primavera and Fock, Ricardo

*Department of Clinical and Toxicological Analyses, School of
Pharmaceutical Sciences of the University of São Paulo, Brazil*

Bone marrow (BM) mesenchymal stem cells (MSCs) regulate hematopoiesis by interacting with hematopoietic and non-hematopoietic populations of cells that reside within the BM. These interactions, however, are known to be impaired in certain conditions, like in aging and in protein malnutrition (PM). Despite PM being common among the elderly, how aging and PM affect hematopoiesis through BM MSCs remains poorly understood. Thus, the aim of this study was to evaluate how PM influences the hematopoietic regulatory function of BM MSCs when associated with aging. Young and aged C57BL/6J mice were fed with a normoproteic or hypoproteic (12 or 2% of protein, respectively) diet and had their nutritional, biochemical and hematological parameters evaluated. BM MSCs were characterized following the recommendations of the International Society for Cellular Therapy and had their secretome, gene expression, autophagy, production of reactive oxygen species (ROS) and double-stranded breaks (DSBs) evaluated. Additionally, the regulatory function of MSCs over hematopoiesis was evaluated in vitro with the C1498 murine acute myeloid leukemia cell lineage (TIB-49™, ATCC®). Lastly, mice from all groups were challenged with these leukemic cells to measure BM invasiveness and to estimate mice survival during a 12-day period. This study was performed following its approval by the institutional animal care committee (CEUA-FCF Protocol #544). Aging and PM altered the biochemical and hematological parameters of mice. BM MSCs had their autophagy affected by aging both in the presence or absence of PM. The production of ROS and frequency of DSBs were most heavily affected in aged MSCs. Both the secretome and gene expression of BM MSCs were also affected by aging as well as by PM. Additionally, aging and PM affected the expression of genes related to the maintenance of pluripotency of C1498 cells, while aging also impaired the differentiation of these malignant cells. Young and aged malnourished mice displayed a higher frequency of C1498 cells in the BM following their injection, thus showing a higher invasiveness. Lastly, malnourished aged mice had the lowest percentage of survival when compared with all other groups. In conclusion, aging and PM affect hematopoiesis, and this can be at least be partially induced by impaired BM MSCs.

Funding Source: National Research Council (CNPq) and FAPERJ

Keywords: CNPq and FAPESP

1:10 PM – 3:15 PM DIRECTED AND DIFFERENTIATION AND TRANSDIFFERENTIATION

1:10 PM – 1:40 PM

NEXT GENERATION CELL THERAPIES FOR THE BRAIN

Wernig, Marius

Stanford University School of Medicine, CA, USA

Abstract not available at time of print

1:40 PM – 2:10 PM

MODELING DIFFERENTIAL MOTOR NEURON
ALS SENSITIVITY

Mazzoni, Esteban O.

Cell Biology, New York University, NY, USA

Despite their clear therapeutic potential, the mechanisms that confer differential neuronal sensitivity are poorly understood. During Amyotrophic Lateral Sclerosis (ALS), sensitive spinal motor neurons (SpMN) die while a subset of rostral cranial motor neurons (CrMN) survive. We optimized a protocol to differentiate CrMNs and SpMNs from human induced pluripotent stem cells (iPSCs) by direct programming and positional patterning. Human iCrMNs are more resistant than iSpMNs to proteotoxic stress and rely on the proteasome to maintain proteostasis. iCrMNs better prevent mislocalization of TDP43 from the nucleus, a hallmark of ALS progression. iSpMNs contain more splicing defects than iCrMNs in response to ALS-related stress with genes involved in splicing and proteostasis maintenance. Therefore, iCrMNs resist ALS at two levels, preventing protein accumulation and reducing splicing defects in response to TDP43 nuclear depletion. Thus, ALS-sensitive iSpMNs appear to enter a downward spiral compromising their ability to maintain proteostasis and splicing.

Funding Source: NIH/NIA R21 AG067174, CZI 2020-222014 (5022)

Keywords: neurodegeneration, iPSCs, Neuronal differentiation, motor neuron

2:10 PM – 2:30 PM

PLURIPOTENT STEM CELLS DIFFERENTIATED TO
DOPAMINE NEURONS: STRATEGIES TO TREAT
EXPERIMENTAL MODELS, AND TO UNDERSTAND
PARKINSON DISEASE PATHOGENESIS

Velasco, Ivan

*Neural development, Instituto de Fisiologia Celular,
Universidad Nacional Autonoma de Mexico, Mexico*

Neural differentiation of pluripotent stem cells can produce midbrain neurons that produce and release dopamine, upon appropriate stimuli. We have engineered mouse ES cells to produce constantly glial cell-line derived neurotrophic factor (GDNF), which resulted in enhanced dopaminergic differentiation and increased survival after grafting in hemiparkinsonian rats. Human ES cells were induced to commit to dopamine neurons; these neurons were placed on rat organotypic cultures, that lack endogenous dopaminergic cells, and stimulated to extend axons through the release of Semaphorin 3C by a hydrogel. This strategy modified the striatal circuits, that were dominated by a group of neurons after dopamine depletion. Human dopamine neurons grafted into the brain of parkinsonian monkeys had a positive impact on biochemical, imaging and behavioral parameters. Human iPSC cells from Mexican patients were generated to perform metabolomic studies and microglial differentiation. These later cells will be stimulated to investigate if they can produce dopaminergic neurodegeneration. These approaches highlight the relevance of pluripotent stem cells in understanding different aspects of development and its potential use in translational science.

Funding Source: Supported by PAPIIT IN219122, DGAPA, UNAM; Conacyt CF-2019-64382 and CF-2023-I-1668.

2:30 PM – 2:45 PM

CELLULAR FITNESS OF HUMAN STEM CELL-DERIVED
BETA CELLS UNDER HYPOXIA

Wang, Xi, Brielle, Shlomi, Melton, Douglas

*Stem Cell and Regenerative Medicine, Harvard University,
Cambridge, MA, USA*

Stem cell-derived β (SC- β) cell therapy holds immense potential for the treatment of Type 1 Diabetes. However, low oxygen supply often leads to substantial loss of cells post-transplantation, especially in subcutaneous spaces and encapsulation devices. The response of β cells to hypoxia and effective strategies to alleviate its detrimental effects remain poorly understood. Therefore, this study aimed to investigate the impact of hypoxia on SC- β cells and elucidate the underlying mechanisms. We performed

transcriptional profiling of human stem cell-derived islets exposed to hypoxic conditions, and profile the transcriptomic and epigenetic profiles of single cells. Our comprehensive findings demonstrate that SC- β cells gradually undergo a decline in their cell identity and metabolic function in response to hypoxia. Our analysis revealed that the loss of immediate early genes, specifically EGR1, FOS, and JUN, resulted in the down-regulation of key transcription factors (TFs) that are essential for maintaining SC- β cell identity under hypoxic conditions. By comparing SC- β cells under both low and high oxygen conditions, we successfully identified key genes that play a pivotal role in maintaining the fitness of SC- β cells within a low-oxygen environment. Notably, we demonstrated that the over-expression of EDN3, potent vasoconstrictor gene that is enriched in pancreatic β cells, significantly aids in preserving β cell identity under hypoxic conditions. Remarkably, elevated expression of EDN3 in SC- β cells effectively mitigated the deleterious effects of hypoxia by modulating genes involved in SC- β maturation including genes associated with glucose sensing insulin. These valuable insights provide crucial advancements in understanding and enhancing the fitness of SC- β cells under hypoxic conditions, offering substantial potential for future clinical applications in the treatment of Type 1 Diabetes.

Funding Source: This work was supported by grants from the Harvard Stem Cell Institute (DP-0180-18-02), JDRF (5-COE-2020-967-M-N) and the JPB Foundation (award no. 1094).

Keywords: stem cell-derived β cells, hypoxia, Type 1 Diabetes

2:45 PM – 3:15 PM INNOVATION SHOWCASE

2:45 PM – 3:15 PM

USING HUMAN STEM CELL-DERIVED NEURAL ORGANIDS FOR MODELING DISEASE

Knock, Erin

STEMCELL Technologies Inc., Canada

In this showcase talk, we will introduce the various types of neural organoids offered by STEMCELL Technologies. We will review how to generate cerebral, midbrain, and spinal cord organoids and demonstrate which cell types you can expect from each. Finally, we will show how each type of organoid can be used to model diseases such as microcephaly, epilepsy, Parkinson's disease and amyotrophic lateral sclerosis.

3:35 PM – 5:25 PM LEVERAGING TOOLS TO STUDY STEM CELL BIOLOGY

3:35 PM – 4:05 PM

GENOME AND EPIGENOME EDITING TOOLS FOR STEM CELL RESEARCH

Woltjen, Knut

Life Science Frontiers, Center for iPS Research and Application (CiRA), Kyoto, Japan

Establishing control over the genome and epigenome is desirable for the study of human health and aging as well as the development of reliable cellular therapeutics. To achieve accurate disease models, we have focused on predicting DNA repair outcomes following gene editing in human induced pluripotent stem (iPS) cells with CRISPR-Cas9. In this way we have established an isogenic allelic series for single nucleotide variants, provided a genome-wide resource for creating deletion mutations, and are exploring the design requirements for cutting edge methods such as Prime Editing. In an alternative strategy, we are employing epigenome editing for cell engineering without permanent genetic modification. Examples of partial reprogramming of somatic cells for biological age reversal and targeted gene repression for the creation of 'universal' iPS cells will be presented. The current challenges of using epigenome editing for cellular rejuvenation and cell therapies will be discussed.

4:05 PM – 4:35 PM

EXPLORING THE USE OF HUMAN BRAIN ORGANIDS TO STUDY PSYCHEDELICS

Rehen, Stevens K.

D'OR Institute for Research and Education, Rio de Janeiro, Brazil

We leveraged the potential of human induced pluripotent stem cells to explore the impacts of various psychedelics, including 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT), psilocin, lysergic acid diethylamide (LSD), and harmine, a beta-carboline present in Ayahuasca, on specific neural cell populations. We conducted a proteomic analysis which revealed that exposure to these compounds triggered considerable alterations in proteins integral to neural plasticity and inflammation in human brain organoids. LSD, specifically, was linked with the

Speaker Abstracts

Saturday, 23 September

augmentation of the mTOR pathway. In contrast, 5-MeO-DMT and psilocin appeared to regulate inflammatory processes. Notably, harmine demonstrated inhibitory activity on DYRK1A-dependent axonal transport of APP in neurons, thereby indicating potential relevance for neurodegenerative diseases. Our research, facilitated by the use of human brain cells, provides novel insights into the effects of psychedelics and contributes to a deeper understanding of the molecular underpinnings of altered states of consciousness.

Funding Source: D'Or Institute for Research and Education, Beckley Foundation, Promega Corporation

Keywords: astrocytes, inflammation, plasticity

4:35 PM – 4:55 PM

USING STEM CELLS TO STUDY EVOLUTION IN DIVERSE PRIMATE SPECIES

Marchetto, Maria Carolina

Anthropology, University of California, San Diego, CA, USA

Since the split of *Homo sapiens* from the last common nonhuman primate (NHP) ancestor, the human brain has substantially altered its size, structure and connectivity. The human brain has a larger mass with respect to body weight, increased cortical neurons with respect to size, an expanded proliferative zone, and unique connectivity patterns. Human-specific neurodevelopment is not only marked by physical differences, but also by temporal changes. Human neurons, during both prenatal neurodevelopment and adult neurogenesis, exhibit an exceptionally delayed time course, a characteristic termed neoteny. It is hypothesized that this longer developmental period plays a role in the aforementioned structural and connectivity differences. It has long been proposed that the phenotypic differences between closely related species may be driven, in part, by divergent transcriptional regulation rather than by novel protein-coding sequence. However, how these regulatory mechanisms play a role in the protracted maturation process in human neurons remains largely unknown. Signatures of human-specific neoteny have been observed and reproduced across different systems including induced pluripotent stem cell (iPSC) and brain organoids models. To examine the evolutionary constraints on the rate of neuronal maturation, we compared neurogenesis across iPSC-

derived cells from five primate species - *Macaca mulatta* (rhesus), *Gorilla gorilla* (gorilla), *Pan paniscus* (bonobo), *Pan troglodytes* (chimpanzee), and *Homo sapiens* (human) - and assessed the differences in transcriptional dynamics. We found that transcriptional differences increased between all species throughout neuronal differentiation and maturation. We identified a pioneer transcription factor with binding in human accelerated regions that exhibited elevated neuronal expression only in humans; and which down-regulation increased the rate of physiological maturity in human neurons. Our results indicate that the species-specific rate of physiological maturity is cell intrinsic and can be modulated by perturbing a single, conserved transcription factor. These findings provide evidence for the divergence of gene regulation as a contributor to human neoteny.

Funding Source: The Brinson Foundation

Keywords: Neural stem cells, nonhuman primate, evolution, neoteny, transcription factor

4:55 PM – 5:10 PM

MULTIMODAL DECODING OF CELL FATES THROUGH CHEMICAL IMAGING, SINGLE CELL OMICS, AND MACHINE LEARNING

Shu, Jian

Cutaneous Biology Research Center, Massachusetts General Hospital / Harvard University, Boston, USA

Reconstructing the circuits that control how cells adopt specific fates and engineering these circuits to reprogram cellular functions are major challenges in biology. I will introduce a series of experimental and computational frameworks such as “Waddington-OT (optimal transport)”, “Raman2RNA” for reconstructing molecular dynamics over time and in live cells through single-cell genomics and label-free chemical imaging. I will introduce how we can use these approaches to decode the cellular and molecular mechanisms governing reprogramming and development at a scalable and high-resolution level.

Keywords: machine learning, multimodal, imaging, single-cell omics, reprogramming

5:10 PM – 5:25 PM**CHARACTERIZATION OF EPIGENOMIC AND TRANSCRIPTOMIC PROFILES THROUGHOUT THE DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS INTO DOPAMINERGIC NEURONS****Meléndez, César D¹**, Cuevas, Raquel², del Moral, Aylin³, Giacomani, Mayela², Soto, Ernesto³ and Velasco, Iván¹¹*Instituto de Fisiología Celular, UNAM, Mexico City, Mexico,*²*Escuela de Medicina Tec de Monterrey, Instituto Tecnológico y de Estudios Superiores de Monterrey, Mexico,* ³*Universidad Autónoma Metropolitana Xochimilco, Universidad Autónoma Metropolitana, Mexico City, Mexico*

Human pluripotent stem cells (hPSCs) have been successfully induced into midbrain dopaminergic (mDA) neurons using a floor-plate based strategy which mimics midbrain DA neuron development. The viability and functionality of the resulting DA neurons have been demonstrated. Nevertheless, a comprehensive epigenomic and transcriptomic characterization is needed to understand the regulatory mechanisms driving DA neuron cell specification. Gene expression is driven by an interplay of transcription factors, promoters and epigenetic regulatory elements like enhancers. It has been demonstrated that histone modifications and chromatin accessibility are related to the activation status of promoters and enhancers. To understand how cell-specific programs of gene expression are achieved, the ENCODE Project Consortium and the Roadmap Epigenomics Project have generated epigenomic signatures of diverse human tissues and cell lineages. However, to the best of our knowledge, the epigenomics of human induced DA neurons has not been addressed. To gain insight into the regulatory mechanisms involved in DA neuron cell specification, we used the aforementioned protocol to obtain mDA expression profiles (RNA-Seq) and chromatin accessibility maps (ATAC-Seq). After data analysis we found significant changes in gene expression and chromatin accessibility. To further characterize open chromatin regions, we performed histone modification (H3K27ac and H3K4me1) ChIP-seq on in vitro generated mDA. The integration of this epigenomic information allowed us to obtain putative active enhancers driving dopaminergic induction and we designed an experimental system to validate these enhancer candidates.

Funding Source: PAPIIT-UNAM IN213719, IN219122**Keywords:** enhancers, neuronal, differentiation**SUNDAY, 24 SEPTEMBER****8:30 AM – 10:00 AM****SELF-ORGANIZATION OF STRUCTURES****8:30 AM – 9:00 AM****SELF-ORGANISATION OF REPROGRAMMING CELLS INTO IBLASTOIDS****Polo, Jose***Adelaide Centre for Epigenetics, The University of Adelaide, Australia*

Human pluripotent and trophoblast stem cells have been essential alternatives to blastocysts for understanding early human development. However, these simple culture systems lack the complexity to adequately model the spatial-temporal cellular and molecular dynamics occurring during early embryonic development. Recently, we and others have reported different in vitro integrated 3D models of the human blastocyst obtained by reprogramming of fibroblasts, termed iBlastoids, or via the differentiation of pluripotent stem cells, termed blastoids. Initial characterization of iBlastoids and blastoids demonstrated that they model well the overall architecture of blastocysts, presenting an inner cell mass-like structure, with epiblast (EPI)- and primitive endoderm (PE)-like cells, a blastocoel-like cavity and a trophectoderm (TE)-like outer layer of cells. Single-cell RNA sequencing (sc-RNAseq) further confirmed the presence of EPI, PE, and TE-like cells. However, how well these different sources of blastocyst-like structure compare is still not clear. In order to shed some light into this, we integrated the sc-RNAseq data of all these different blastoids and iBlastoids as well as pre- and post- implantation blastocysts. This integrative analysis confirmed that the majority of these in vitro generated blastocyst-like structures represent the preimplantation blastocyst. Strikingly these analyses also showed how similar blastoids and iBlastoids are, regardless of the origin and methodology in which they were derived. In summary, we have compared the different in vitro models of the human blastocyst, confirming their capacity to model the blastocyst stage and determine the similarities and differences among them. We hope that this will provide guidance and facilitate the selection of models for the study of early human development.

Keywords: reprogramming, blastoids, blastocyst-like structures, throphoblast

9:00 AM – 9:30 AM**MODELING POST-IMPLANTATION STAGES OF HUMAN DEVELOPMENT INTO EARLY ORGANOGENESIS WITH STEM CELL-DERIVED PERI-GASTRULOIDS****Wu, Jun***Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX, USA*

In vitro stem cell models that replicate human gastrulation have been generated, but they lack the essential extraembryonic cells needed for embryonic development, morphogenesis, and patterning. Here, we describe a robust and efficient method that prompts human extended pluripotent stem cells to self-organize into embryo-like structures, termed peri-gastruloids, which encompass both embryonic (epiblast) and extraembryonic (hypoblast) tissues. Although peri-gastruloids are not viable due to the exclusion of trophoblasts, they recapitulate critical stages of human peri-gastrulation development, such as forming amniotic and yolk sac cavities, developing bilaminar and trilaminar embryonic discs, specifying primordial germ cells, initiating gastrulation, and undergoing early neurulation and organogenesis. Single-cell RNA sequencing unveiled transcriptomic similarities between advanced human peri-gastruloids and primary peri-gastrulation cell types found in humans and non-human primates. This peri-gastruloid platform allows for further exploration beyond gastrulation and may potentially aid in the development of human fetal tissues for use in regenerative medicine.

Funding Source: New York Stem Cell Foundation (NYSCF) and Discovery and Innovation Grant from the American Society for Reproductive Medicine (ASRM) Research Institute.

Keywords: peri-gastruloids, stem cell derived integrated embryo models, early post-implantation human development, gastrulation, primitive streak formation, neurulation, early organogenesis

9:30 AM – 10:00 AM**BLASTOIDS: MODELING MOUSE AND HUMAN BLASTOCYST DEVELOPMENT AND IMPLANTATION WITH STEM CELLS****Rivron, Nicolas***Laboratory for Blastoid Development and Implantation, Institute of Molecular Biotechnology, Austrian Academy of Science, Vienna, Austria*

The blastocyst is the early mammalian organism before implantation. We have figured out how to promote the self-organization of stem cells into models of the mouse and human blastocysts, which we have named blastoids. Blastoids are morphologically and transcriptionally similar to

the blastocyst and contain analogs of all three cell types that would eventually develop into the complete organism (embryonic and extraembryonic tissues). Because blastoids are complete and model the preimplantation stage, they can be introduced into the uterus (mouse model) or combined in vitro with uterine cells (human model) to recapitulate aspects of the normally hidden implantation processes. Unlike blastocysts, blastoids come in large numbers and facilitate the more systematic modulation and analysis of the impact of cell numbers, states, and communication mechanisms on development. As such, they represent both a scientific and ethical alternative to the use of embryos for research. Using this approach, we are investigating the rules of multicellular interaction underlying species-specific aspects of blastocyst development and implantation, with the long-term goal of contributing to solving the global health problems of fertility decline, family planning, and developmental origin of health and disease.

Funding Source: European Research Council

Keywords: blastocyst, blastoid, implantation, uterus

10:30 AM – 12:20 PM**DISEASE MODELING****10:30 AM – 11:00 AM****APPLICATIONS OF HUMAN BRAIN ORGANOGENESIS****Muotri, Alysso R.***Pediatrics/Cellular and Molecular Medicine, University of California, San Diego, CA, USA*

The complexity of the human brain, with thousands of neuronal types, permits the development of sophisticated behavioral repertoires, such as language, tool use, self-awareness, symbolic thought, cultural learning, and consciousness. Understanding what produces neuronal diversification during brain development has been a longstanding challenge for neuroscientists and may bring insights into the evolution of human cognition. Human pluripotent stem cells can be achieved from living individuals by reprogramming somatic cells that would capture their entire genome in a pluripotent state. From this pluripotent state, it is possible to generate models of the human brain, such as brain organoids. The reconstruction of human neural network activity in a dish can help to understand how neural network oscillations vary between normal and disease states. Our findings suggest a potential bridge to the gap between the microscale in vitro neural networks electrophysiology and a non-invasive electroencephalogram.

Keywords: brain organoid, organogenesis, brain evolution, gene therapy

11:00 AM – 11:30 AM**CURRENT CHALLENGES AND PROGRESS IN 3D MODELING OF THE BLOOD-BRAIN BARRIER IN NEUROLOGICAL DISORDERS****Porcionatto, Marimelia A.***Biochemistry / EPM, Federal University of São Paulo (UNIFESP), São Paulo, Brazil*

A neurological disorder is any disorder that affects the peripheral or the central nervous system. Some neurological disorders occur due to impaired neurodevelopment, for example, autism spectrum disorder and schizophrenia. Other disorders appear later in life due to infection, cerebrovascular impairment, and neurodegeneration, resulting in disabilities and death. Neurological disorders are the leading cause of disability and the second cause of death of adults worldwide. Amongst neurological disorders, stroke has ranked first since the 1990s and Alzheimer's disease has moved to second place in the past twenty years. Numerous *in vivo* and *in vitro* neurological disease models exist, but reproducing the complexity of the disorders as they occur in humans is still challenging. Brain organoids are excellent models for neurodevelopmental disorders, whereas lab animals have best modeled neurodegenerative and acute injuries to the central nervous system. More recently, new technologies such as 3D bioprinting and microfluidics, combined with human iPSCs, have been proposed to build new *in vitro* models for neurological diseases. Our lab is interested in developing *in vitro* blood-brain barrier models to study cellular and molecular mechanisms of late-onset Alzheimer's disease, ischemic stroke, and subarachnoid hemorrhage. Combining microfluidics and 3D bioprinting of human iPSC-derived endothelial cells, astrocytes, and neurons, we aim to model these three neurological disorders. We focus on identifying the optimal biomaterials, bioprinting conditions, and in-house development of a chip to create all compartments of the blood-brain barrier. We aim to recreate the neurovascular unit microenvironment representing cellular and biochemical parameters disrupted in neurological disorders' progression.

Funding Source: FAPESP 2018/12605-8, 2021/02795-7, 2021/14327-8, and 2022/08664-4; CNPq 311026/2022-2 and 406258/2022-8 (INCT Model3D); CAPES Financial Code 001.

Keywords: hiPSC, disease modeling, brain, blood-brain barrier, 3D bioprinting, microfluidics, chip

11:30 AM – 11:50 AM**A TRIP BACK IN TIME TO UNDERSTAND THE ORIGIN OF SCHIZOPHRENIA****Palma, Verónica***Biology, Universidade de Chile, Santiago, Chile*

Schizophrenia (SZ) is a chronic debilitating neuropsychiatric disorder affecting around 1% of the population worldwide. SZ has no cure, and more than one-third of patients describe little to no progress after treatment, for which an expanded knowledge and comprehension of SZ physiopathology is imperative. Increasing evidence is tracing SZ's origin to embryonic neurodevelopment, where the brain is formed concomitantly with a vast and complex vascular network, structuring the neurovascular unit (NVU). We have worked in the modeling of early neurovascular interactions in SZ using induced pluripotent stem cell (hiPSC) reprogrammed from healthy controls (HC) and SZ patients (SZP) into the main cellular components of the NVU: Neurons, astrocytes and brain endothelial cells (BEC). We have studied their functional and molecular alterations and the effect of their secretomes on angiogenesis, brain barrier formation, and neural activity. SZP- hiPSC derived BEC show a decreased response to angiogenic stimuli and present alterations in their barrier capacities and secretome. SZP hiPSC-derived astrocytes reveal a chronic inflammatory profile with broad effects on their secretome resulting in vascular alterations when assayed both *in vitro* and *in vivo*. Finally, our results show changes in functional connectivity dynamics of SZP hiPSC-derived long-term neuronal cultures suggesting that alterations in neuronal communicational dynamics are already present during early development in SZ and may contribute to brain functional connectivity anomalies described in SZ. Our findings indicate the presence of inherent deficiencies in both neural and vascular components resulting in defective crosstalk in the formation of the NVU that possibly contributes to the phenotype described in SZP. Current investigations aim to correlate these results with clinical research on HC versus SZP for the development of novel therapeutics.

Funding Source: Fondecyt grant N° 1190083 and 1221522

Keywords: hiPSC, schizophrenia, neurovascular unit

11:50 AM – 12:05 PM**3D HIPSC MODEL OF ATRIAL INFLAMMATION FINDS ACTIVATED MACROPHAGES AS CAUSE OF PRO-ARRHYTHMIC ELECTROPHYSIOLOGICAL AND STRUCTURAL CHANGES IN CARDIAC TISSUE****Hutschalik, Thomas¹**, Schotten, Ulrich² and Matsa, Elena³*¹Discovery Technology, Ncardia Services B.V., Leiden, Netherlands, ²Physiology / Cardiology, Cardiovascular Research Institute Maastricht / Maastricht University Medical Center, Maastricht, Netherlands, ³Cell Therapy Research, Cellistic, Mont-Saint-Guilbert, Belgium*

Inflammation is known to play a role in various cardiac pathophysiologies, among them the most common arrhythmia, atrial fibrillation (AF). In correlation, AF patients show increased amounts of pro-inflammatory (M1) macrophages. However, the causative relationship of inflammation, AF and M1 is poorly understood. This study investigated the structural, functional and electrophysiological effect of M1-caused inflammation in a 3D human iPSC-derived model. Atrial cardiomyocytes (aCM), cardiac fibroblasts (cfb) and macrophages (M ϕ), were each differentiated from 3 healthy donor lines and combined in an isogenic 3D tissue model. 70,000 cells (56% aCM, 24% cfb, 20% M ϕ) were seeded in a pillar mould after 3 days of pre-culture. Pillar devices were designed and produced by Philips Research. M ϕ were activated 6 days after seeding. Videos of tissue contractions, whole tissue calcium transients and sharp electrode traces were recorded at d1 after activation. Whole tissues were fluorescently labeled for collagen 1 deposition at d8 after activation. All experiments included control tissues with non-activated M ϕ and activated tissues without M ϕ . Activated M1 influenced tissue structure, seen as a significant reduction of collagen compared to controls (19.9%, $P < 0.05$). Further, M1 affected tissue functionally by decreasing contraction amplitudes (18.1% reduction, $P < 0.05$) and congruently reduced calcium transient amplitude by 22.6% ($P < 0.01$). M1 caused significant increase in arrhythmia, demonstrated as an 18.2% increase in calcium peak to peak time variance ($P < 0.001$), while video contraction analysis showed an 153.1% increase in contraction irregularity ($P < 0.05$) compared to controls. Sharp electrode recordings of single cell action potentials in the tissue revealed a significant reduction of upstroke velocity (36.4%, $P < 0.01$) and further confirmed the pro-arrhythmic effect of M1 shown by an increase in action potential variance (78.7%, $P < 0.01$). The presented 3D model of atrial inflammation revealed macrophages as being pro-arrhythmic by influencing aCM electrophysiology on a cellular level. M1 further caused

functional and structural changes, suggesting them as a contributor to cardiac pathophysiology in general and AF in particular.

Funding Source: This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 860974.

Keywords: inflammation, 3D engineered heart tissue, atrial arrhythmia

12:05 PM – 12:20 PM**ELUCIDATING MECHANISMS OF OLIGOGENIC INHERITANCE IN AUTISM SPECTRUM DISORDER USING CORTICAL ORGANOID IN VITRO AND TRANSPLANTED INTO MICE BRAINS****Teles E. Silva, Andre L.¹**, Yokota Moreno, Bruno¹, Nobrega, Isabella¹, Branquinho, Mariana¹, Griesi-Oliveira, Karina¹, Souza, Thiago¹, Lerner, Bruno¹, Torres, Davi¹, Salles, Geisa², Porcionatto, Marimélia³, Passos Bueno, Maria Rita⁴, Gamarra Contreras, Lionel¹ and Sertié, Andrea¹*¹Instituto Israelita de Ensino E Pesquisa Albert Einstein, Hospital Israelita Albert Einstein, São Paulo, Brazil, ²Department of Biochemistry, Escola Paulista de Medicina, Universidade Federal de São Paulo, Brazil, ³Universidade Federal de São Paulo, Department of Biochemistry, São Paulo, Brazil, ⁴Universidade de São Paulo, Centro de Estudos do Genoma Humano e Células Tronco, São Paulo, Brazil*

One of the greatest challenges in autism spectrum disorder (ASD) research is to identify the combinations of genetic variants required for determining disease causality and understand how the risk variants interact and converge on causative neurobiological pathways. Recently, we identified in one Brazilian proband with ASD and macrocephaly rare compound heterozygous missense variants in the Reelin gene (RELN) and a rare de novo splicing variant in the Cav3.2 calcium channel gene (CACNA1H). Using neural progenitor cells (NPCs) derived from induced pluripotent stem cells (iPSCs) of this individual, we showed that the variants in RELN and CACNA1H are deleterious and interact via the mTORC1 pathway. Furthermore, analysis of sequencing data from two large ASD cohorts revealed a significant increased burden of simultaneous risk variants in both alleles of Reelin pathway genes and in one allele of calcium channel genes in ASD. Nevertheless, the specific mechanisms by which these variants disrupt brain development and contribute to oligogenic ASD remain elusive. To investigate the impact of RELN and CACNA1H variants on specific cell types and cellular phenotypes in the brain using gene editing technologies, 2D neuronal

cultures, and 3D brain organoids. Our findings indicate hyperactivation of the mTORC1 pathway in patient-derived neurons in 2D cultures, resulting in impaired neuronal differentiation. Patient-derived cortical organoids exhibited increased size, upregulated expression of NPC markers, disrupted ventricular zone-like structures, and aberrant extracellular electrical recordings. Additionally, we are currently using gene editing to correct the RELN and CACNA1H pathogenic variants in patient-derived iPSCs to generate isogenic engineered cell lines, and also optimizing the process of transplantation of cortical organoids into the brain of immunosuppressed mice to study human-derived cortical cells within in vivo circuits. Finally, analysis of publicly available single-cell RNA sequencing databases allowed us to identify cell populations and target regions exhibiting elevated co-expression of RELN and CACNA1H. This study integrates risk variant identification and functional genomics, shedding light on genetic interactions and oligogenicity in ASD.

Funding Source: This project is funded by FAPESP in the Regular Research Grant modality, Process Number 2021/14491-2, effective from 07/01/2022 to 06/30/2024.

Keywords: autism spectrum disorder, oligogenic inheritance, cortical organoids

1:35 PM – 3:20 PM ETHICS AND UNPROVEN STEM CELL THERAPIES IN SOUTH AND LATIN AMERICA

1:35 PM – 2:05 PM

BIOMEDICAL ASPECTS OF UNPROVEN STEM CELL INTERVENTIONS AND INITIATIVES TO PREVENT THEM AND FACILITATE ETHICALLY SOUND CLINICAL TRANSLATION

Pitossi, Fernando

Fundación Instituto Leloir, Fundación Instituto Leloir -CONICET, Argentina

Therapies based on pluripotent stem cells hold a great promise for numerous patients suffering from diseases with inadequate or no cure. Clinical translation of scientific discoveries to stem cell-based treatments is regulated worldwide with basic similar criteria and some country-specific variations. However, taking advantage of desperate patients and families, numerous unproven stem cell interventions are marketed globally. In this talk, I will present evidence of the biomedical consequences of carrying out unproven stem cell interventions. In addition, I will describe the available international initiatives to aid ethically sound clinical translation including harmonizing standards for basic and pre-clinical research, the ISSCR Guidelines for Stem Cell Research and Clinical Translation and global initiatives

such as the Haplobank. Finally, I will discuss present efforts to bring closer relevant information to patients and families to help them in the decision making process when a stem cell treatment is offered.

Keywords: therapies, clinical translation, ethics

2:05 PM – 2:35 PM

ETHICAL GUIDELINES AND LEGAL LANDSCAPES OF THERAPIES IN LATIN AMERICA: UNDERSTANDING THE CIRCUITS OF USE TO PREVENT EXPLOITATION BY UNPROVEN STEM CELL THERAPY CLINICS

Medina-Arellano, Maria de Jesús

Institute of Legal Research UNAM, Mexico

The rampant exploitation of vulnerable patients seeking unproven stem cell therapies in Latin America necessitates a comprehensive approach to prevention. Understanding circuits of use is crucial for effective measures. I highlight mapping circuits and the value of ISSCR guidelines for ethical practices and patient protection. Circuits of use involve private clinics, patients, and intermediaries. Unregulated clinics target desperate patients with risky therapies. Patients, driven by limited options, turn to these clinics. Intermediaries facilitate access to exploit demand. To combat exploitation, comprehensive measures within circuits are needed. Governments must implement strict regulations and oversight to hold clinics accountable. ISSCR guidelines ensure ethical, evidence-based practices and patient safety, curbing unproven therapies. Patient education is vital. Accurate information on risks and regulated options empowers informed decisions and avoids victimization. Collaboration among regulatory bodies, healthcare professionals, advocacy groups, and the scientific community is essential. Awareness campaigns, education, and support networks reduce intermediaries' influence and empower patients. Leveraging ISSCR guidelines enhances patient protection. Governments should incorporate guidelines, aligning with international best practices. Understanding circuits is vital for targeted prevention. Mapping interactions identifies intervention areas. Integration of ISSCR guidelines strengthens regulations and promotes patient welfare. In conclusion, preventing exploitation requires understanding circuits of use. Tailored measures acknowledge clinics, patients, and intermediaries. ISSCR guidelines promote ethical practices, patient safety, and limit unproven therapies. Collaboration, education, and regulations safeguard patients in Latin America.

Funding Source: I'm grateful to the Committee for the NIH grant, enabling me to present at this Symposium. Their invaluable support has made my participation possible.

Keywords: circuits of use, stem cell therapies, unproven interventions, Latin America, ISSCR guidelines

2:35 PM – 2:50 PM**REGULATORY FRAMEWORK FOR BIOETHICAL AND LEGAL USE OF STEM CELL IN PANAMA: A SWOT ANALYSIS****Solis, Mairim Alexandra**¹, Herrera, Luis² and Quintana, Magalys³

¹*Stem Cell Research Group, Department of Research in Sexual and Reproductive Health, Gorgas Memorial Institute for Health Studies/National Bioethics Research Committee in Panama, Panama,* ²*Universidad Santa Maria la Antigua, National Committee of Bioethics Research in Panama, Panama,* ³*Secretaría Nacional de Ciencia, Tecnología, e Innovación, National Committee of Bioethics Research in Panama, Panama*

Human stem cell research raises serious ethical and regulatory controversies that forces the need to encounter an environment for appropriate research and medical interventions that will improve human health. Many jurisdictions around the world have legislations with substantial gaps governing these issues. Latin America must restructure itself to face the challenges posed by stem cell research through the presence of a critical mass of researchers to halt stem cell tourism and gain intellectual sovereignty. As an effort to develop a regulatory framework for the use of stem cells, Panama approved the Executive Decree No. 179 of June 8, 2018. Through this Decree an important improvement has been made in regulating research with human tissues and cells in Panama, stating that clinical trials must be evaluated by the National Committee of Bioethics in Research of Panama, with the advisory role of the National Transplant Committee regarding safety and protection; that trials must be conducted in centers with licenses granted for the extraction and transplantation of anatomical component; and that related research must follow the regulatory process of an investigational new drug by the National Directorate of Pharmacy and Drugs of the Ministry of Health, among other policies. The Institutional Committee of Regenerative Medicine and Advanced Therapies of the Ministry of Health was created by means of the Resolution No. 1477 of October 30, 2018, with the purpose to establish, coordinate, and update related policies in Panama. Five years since the N°179 decree approval, makes the appropriate timing for a critical SWOT analysis of the progress, setbacks, opportunities, strengths, and weaknesses that this decree has made to solve this issue. The purpose of this talk is to provide these insights as well as the difficulties we are now facing from other sectors that seek to abolish current regulatory policies. The collective efforts in Panama to strengthen legislations regarding stem cell research and its clinical translation seeks to halt premature commercialization of unapproved stem cell treatments and

exemplifies future actions that other countries can take in regulatory formulations. An urgent call-to-action is needed to reinforce regional collaborations that may collectively strengthen legislations in Latin America.

Funding Source: Secretaría Nacional de Ciencia, Tecnología e Innovación**Keywords:** bioethics, stem cells, Panama**2:50 PM – 3:20 PM****TURNING THE TIDE: HOW ADVOCACY AND POLICY REFORM CURBED THE BUSINESS OF SELLING STEM CELLS DOWN UNDER****Munsie, Megan**

Department of Medicine, University of Melbourne and Murdoch Children's Research Institute, Australia

For many patients and their families, stem cell science and regenerative medicine is synonymous with hope for a brighter future. While there are increasing opportunities for eligible candidates to participate in clinical research, these places are limited, and to date few stem cell medicines have received regulatory approval. In Australia, like many other countries across the globe, businesses claiming to already be able to offer 'stem cell' treatments have stepped in over the last decade to cater for the unmet demand. Until recently, these for-profit enterprises have been able to exploit a regulatory loophole that placed their services outside the remit of the appropriate Australian regulator. However, in response to concerns raised by the scientific and community groups, the Australian Government recently introduced a ban on advertising of unproven cell therapies and increased regulatory oversight to provide better safeguards for Australian consumers. This presentation will explore how Australian patients and their carers understood and experienced these interventions, and in particular how their presumptions about autologous stem cells, the ethics of medical practice, and the regulatory environment in Australia made them more receptive to unproven treatments. We will also examine how sustained calls for policy reform has impacted the Australian marketplace and the challenge of implementing these changes in the context of high community hopes, conflicting information and scientific uncertainties.

Funding Source: Megan Munsie receives funding from the Australian Government Medical Research Futures Fund and the Novo Nordisk Foundation through reNEW, the Novo Nordisk Foundation Center for Stem Cell Medicine.**Keywords:** regulation, stem cell tourism, professional ethics

3:40 PM – 5:15 PM CLINICAL APPLICATIONS OF STEM CELL BIOLOGY

3:40 PM – 4:10 PM

BIOMEDICAL ASPECTS OF UNPROVEN STEM CELL INTERVENTIONS AND INITIATIVES TO PREVENT THEM AND FACILITATE ETHICALLY SOUND CLINICAL TRANSLATION

Rao, Mahendra S.

Stem Cells, panCella/PLSX, Timonium, MD, USA

Currently the cost of cell therapy is beyond the reach of most individuals. This is due to a combination of factors that include regulatory issues, licensing issues, manufacturing issues as well as the ongoing expenses of developing a new therapy. In my talk I will provide case studies of how cost can be reduced by careful consideration of the applicable licenses required right from the earliest step on the clinical development pathway, careful selection of manufacturing modalities and assessment of autologous versus allogenic sourcing.

Funding Source: Private Funds

Keywords: stemcell, manufacturing, regulations, autologous, allogeneic

4:10 PM – 4:40 PM

MODELING CARDIAC DISEASE USING ISOGENIC HIPSC-DERIVED CARDIOMYOCYTES

Carvalho, Antonio Carlos C.

Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Brazil

Congenital long QT syndrome (LQTS) is a cardiac disorder characterized by ECG prolongation of the QT interval. Most LQTS are induced by variants in ion channels and may lead to sudden cardiac death episodes. LQTS may display incomplete penetrance, suggesting that modifier genes may influence its pathophysiology. In this context, the identification of both pathogenic variants and modifier genes constitutes a challenge for better understanding this syndrome and earlier identification of subjects at risk. We have generated iPSC cell lines from patients presenting with LQTS types 1 and 2 and have either introduced the variants present in these patients in control iPSC cells or corrected the variants in the patient's iPSC cells using

CRISPR-Cas9. iPSC generated by Sendai virus containing the Yamanaka factors presented normal morphology, karyotype and pluripotent markers and were efficiently differentiated into cardiomyocytes. Electrophysiological analyzes demonstrated a prolonged action potential duration (APD) in LQTS iPSC-derived cardiomyocytes (iPSC-CM) when compared to asymptomatic carriers or control iPSC-CMs. Introduction or correction of the variants by CRISPR-Cas either increased or decreased APD. Furthermore, these isogenic iPSC lines allowed us to envisage the cellular mechanisms involved in the LQTS and in the incomplete penetrance in patients presenting the same variant but discordant phenotypes.

Funding Source: National Research Council (CNPq) and FAPERJ

Keywords: iPSC; cardiomyocytes; arrhythmias; heart; penetrance

4:40 PM – 5:00 PM

PROTECTING SC-ISLETS FROM STRESS

Leite, Nayara

Stem Cell Research, Vertex Pharmaceuticals, Massachusetts, MA, USA

The in vitro production of stem-cell-derived islets (SC-islets) has brought forth the potential of transplanting these cells to restore glycemic control in people with diabetes. Nonetheless, alloimmune and autoimmune responses remain considerable challenges for a broad clinical implementation of β -cell replacement therapies. β -cell stress has been implicated in the onset of β -cell immunogenicity and death and is likely to contribute to β -cell failure following transplantation. We show that inducing stress and/or administering cytokines causes SC-islet apoptosis, cellular dysfunction, and an increased expression of β -cell stress- and immune-interaction-related genes. We then demonstrate that manipulating some of these genes results in enhanced protection of SC-islets from apoptosis in vitro.

Funding Source: Harvard Stem Cell Institute American Diabetes Association Juvenile Diabetes Research Foundation JPB Foundation

Keywords: β -cell stress, Cell replacement therapy, T1D disease modeling

5:00 PM – 5:15 PM**BOTTOM-UP ISLET ENGINEERING: PROGRESS TOWARD DESIGNER ISLET ORGANOID FROM HUMAN PLURIPOTENT STEM CELLS****Peterson, Quinn***Department of Physiology and Biomedical Engineering,
Mayo Clinic, Rochester, MN, USA*

Diabetes Mellitus results from dysfunction of pancreatic islets leading to elevation of blood glucose levels and an increase in morbidity and mortality. In type 1 diabetics, the precipitating event is the loss of insulin producing pancreatic beta cells through autoimmune attack. As such, the in vitro production of human beta cells from stem cells for use as a cell transplantation therapy has been a major focus of type 1 diabetes research. However, it is unlikely that beta cells by themselves will recapitulate the complex biology involved in islet function. Indeed, the three major approaches proposed by the field to regain glycemic control in diabetic patients (bionic pancreas, transplantation of in vitro derived beta cells, and production of beta cells in vivo through replication or reprogramming) fail to fully account for the complexity of islet endocrine function and focus almost exclusively on the function of the beta cell. To this end, we seek to generate human islet organoids from component parts using a bottom-up tissue engineering approach. Here we report the development of separate protocols for generating stem cell-derived alpha, beta and delta cells from pluripotent sources and subsequently combine these cell types to create islet organoids of defined composition. Stem cell-derived alpha, beta and delta cells exhibit many of the characteristics of their bona fide counterparts including gene expression, hormone secretion, ultrastructure and in vivo function. We also find that combination of stem cell-derived alpha, beta and delta cells in designer islet organoids exhibit improved function in vitro and in vivo as compared to beta cells alone. These results demonstrate the clinical utility of a designer islet organoid and the need for translational development of these therapies.

Keywords: type 1 diabetes, islet, cell replacement therapy

Upcoming International Symposia

**ELUCIDATING PRINCIPLES OF
DEVELOPMENT WITH STEM CELLS**
4-6 December 2023 | Vienna, Austria

IS | VIENNA

SPEAKERS INCLUDE



Barbara Treutlein, PhD
ETH Zürich, Switzerland



Alexander van Oudenaarden, PhD
Hubrecht Institute-KNAW &
University Medical Center,
The Netherlands

CINCINNATI INTERNATIONAL SYMPOSIUM
4-5 April 2024 | Cincinnati, USA
In partnership with Center for Stem Cell &
Organoid Medicine (CuSTOM) at Cincinnati
Children's Hospital.



COPENHAGEN INTERNATIONAL SYMPOSIUM
October 2024 | Copenhagen, Denmark
In partnership with Novo Nordisk



SINGAPORE INTERNATIONAL SYMPOSIUM
11-13 December 2024 | Singapore
In partnership with Stem Cell Society
Singapore (SCSS)



Learn more at isscr.org/upcoming-programs

Poster Abstracts

FRIDAY, 22 SEPTEMBER

Session Times are in BRT, Brasília Time Zone
(São Paulo, Brazil)

Poster Session I - A 5:15 PM – 6:15 PM

Poster Session I - B 6:30 PM - 7:30 PM

TRACK: ADULT STEM CELL AND REGENERATION

101 - A

ANALYSIS OF THE IN VIVO FREQUENCY AND THE IN VITRO OSTEOGENIC DIFFERENTIATION OF IMMUNOPHENOTIPICALLY DEFINED HUMAN SKELETAL STEM AND PROGENITOR CELL SUBPOPULATIONS

Costa, Isabela de Souza, Dias, Rhayra, Bonfim, Danielle
Institute of Biomedical Sciences, Federal University of Rio de Janeiro, Brazil

Skeletal stem and progenitor cells are responsible for bone maintenance and regeneration. Recently, a more specific set of phenotypic markers was described to identify the multipotential stem cell population (SSC, PDPN+ CD146-CD164+ CD73+), which gives rise to a PDPN+ CD146+ Bone, Cartilage and Stromal Progenitor (BCSP), which in turn originates a PDPN- CD146+ osteoprogenitor (OP) or a PDPN+ CD146- chondroprogenitor (CP). However, little is known about the frequency of these cell populations in bones of distinct anatomical locations, whether these phenotypic markers are stable enough to be used as distinctive markers during in vitro expansion, and whether these populations differ in osteogenic potential, three relevant questions for the development of characterized and effective Advanced Medicinal Therapeutic Cellular Products (AMTCPs) for application in innovative therapies for bone diseases. Therefore, the objective of this study was to investigate the frequency of SSCs and its progeny in the bone marrow collected from different bone sources and the stability of their immunophenotypic profile following in vitro expansion by FACS, their osteogenic differentiation potential in vitro, and how these profiles

correlate with that of unfractionated Bone Marrow Stromal Cells (BMSCs). Following approval, human hip (N=19) and humerus (N=5) samples were collected. In hip samples, SSCs accounted for $0,13\% \pm 0,20\%$ of total nucleated non-hematopoietic cells, BCSPs were $4,78\% \pm 10,47\%$, CPs were $7,38\% \pm 14,12\%$, and OPs were $9,94\% \pm 12,20\%$. In humerus, SSCs and CPs were only detected in one sample ($0,2\%$ e 12% , respectively). BCSPs were $0,3\% \pm 0,15\%$ and a higher frequency of OPs ($25,05\% \pm 5,30\%$, $p = 0,04$) was detected in comparison to hip samples. Following PDPN-CD146+ (OP) and PDPN+ CD146- (SSCs and CPs) sorting, it was observed that their immunophenotypic profiles converged during expansion to PDPN+ CD146+, becoming similar to BMSCs. However, the expanded subpopulations differed in osteogenic capacity, with the PDPN+ CD146- (SSCs and CPs) producing a lower mineralized matrix area revealed by Alizarin Red staining than the BMSCs and OPs. Further in vivo functional assays will be performed to confirm these findings, that may lay the foundation to produce better AMTCPs.

Funding Source: This study was funded by Carlos Chagas Filho Foundation for Research Support in the State of Rio de Janeiro (FAPERJ) and the National Council for Scientific and Technological Development (CNPq).

Keywords: human skeletal stem and progenitor cells, bone marrow niche, bone, bone engineering, cell therapy

101 - B

ASSOCIATION OF MESENCHYMAL STEM-CELL THERAPY TO CURRENT TREATMENT AS A STRATEGY TO PROMOTE PODOCYTE PROTECTION AND HALT DIABETIC KIDNEY DISEASE PROGRESSION

Beyerstedt, Stephany, Franco, Marcella, Carlos, Alanah, Rangel, Érika

Albert Einstein Israeli Institute of Teaching and Research, Hospital Albert Einstein, Santo André, Brazil

Introduction: Diabetic kidney disease is a complication of Diabetes Mellitus and is associated to disruption of the glomerular filtration barrier due to podocyte injury. Hyperglycemia, its main etiology, causes oxidative stress and disbalanced activation of renin-angiotensin-aldosterone system (RAAS), leading to decreased protective action of its ACE2/Angiotensin (1-7)/Mas axis and overactivation of the deleterious ACE/Angiotensin II/AT1R axis and, that way, leading to structural disorganization of the podocyte and downregulation of the renoprotective protein Klotho. Current therapy for

Poster Abstracts

DKD consists in using different strategies, for example, changing eating habits and drug therapy, such as inhibitors of the sodium–glucose cotransporter 2 (SGLT2), but it does not promote structural regeneration. In that regard, mesenchymal stem cells create a favorable microenvironment for regeneration, therefore, are a potential complementary approach for DKD. Methodology: we used the BTBRob/ob, a murine model of DKD that develops key changes seen in the human disease, such as hyperglycemia, podocyte loss and mesangial expansion. We evaluated the effect of drug therapy with the SGLT2 Empagliflozin in combination or not with calorie restriction and administration of mesenchymal stem cells from bone marrow (BM-MSc) intraperitoneally. Results: we obtained a reduction in fasting capillary blood glucose with BM-MSc combined therapy (197 ± 66 mg/dL) compared to monotherapy with SGLT2 (343 ± 72 mg/dL) and BTBRob/ob (464 ± 104 mg/dL) ($p < 0.05$), in addition to a decrease in the progression of mesangial expansion (13% reduction vs BTBRob/ob). Moreover, we observed increased gene expression of ACE2 in combined therapy (5.17 ± 2.28) in comparison to monotherapy (2.78 ± 0.52) and BTBRob/ob (2.34 ± 1.09) ($p < 0.05$), along with higher levels of gene expression of Klotho and podocyte cytoskeleton markers. Conclusion: the strategy of associating mesenchymal stem cells to current therapy for DKD has a greater renoprotective potential, with preservation of podocyte structure and reduced deleterious activation of RAAS.

Funding Source: FAPESP 2021/02216-7

Keywords: bone marrow mesenchymal stem cell, diabetic kidney disease, Renin-angiotensin-aldosterone system

102 - A

BIOACTIVE DECELLULARIZED EXTRACELLULAR MATRIX-BASED HYDROGEL SUPPORTS MESENCHYMAL STEM CELL MAINTENANCE AND FIBROCARILAGE PHENOTYPE

Josino, Raphaella, Stimamiglio, Marco

Laboratory of Stem Cell Basic Biology, Carlos Chagas Institute, Curitiba, Brazil

Articular cartilage is a highly specialized tissue whose capacity for spontaneous regeneration is restricted. The limitations of recovery reflect the features of its structure, such as the low mitotic activity of the chondrocytes. Mesenchymal stem cells (MSCs) are multipotent cells with immunomodulatory action and potential for differentiation into chondrocytes. As a strategy to recapitulate the

complexity of the extracellular matrix of native cartilage and promote the recovery of damaged cartilaginous tissue, hydrogels based on the extracellular matrix of decellularized cartilage (dECM) combined with MSCs have been employed. In order to investigate how MSCs behave in a cell culture on dECM hydrogel or when printed with a dECM bioink, we used MatriXpec™ cartilage (TissueLabs, Brazil). Human adipose-derived MSCs were cultured on or 3D bioprinted with MatriXpec™. Microscopic and proteomic analysis demonstrated that MatriXpec™ has a nanofibrous structure and is mainly composed by collagenous proteins. When seeded or bioprinted on MatriXpec™, MSCs demonstrate maintenance of their basic biological functions, surviving and distributing throughout the hydrogel. Electron and fluorescence microscopy analyzes demonstrated that the dynamics of growth and morphology of MSCs is altered when they are cultured on MatriXpec™, adopting a fusiform organization when compared to cells plated on standard culture plastic. When bioprinted, the MSCs adopted a star-shaped morphology, emitting protrusions in different focal planes of the biomaterial, evidencing their ability to respond to the three-dimensional information represented by the bioprinting microenvironment. Through the quantification of glycosaminoglycans (GAGs) and the analysis of gene expression by RTqPCR, we showed that cell culture on MatriXpec™ favored the deposition of GAGs, as well as the expression of the master transcription factor in the cartilage formation process, SOX9, with statistical difference in relation to the two-dimensional culture. Furthermore, cell culture on MatriXpec™ increased the expression of genes related to the fibrocartilage phenotype, such as MMP13, COLX10 and COL1, indicating that the fibrous environment represented by MatriXpec™ is capable of leading MSCs to a path of differentiation to fibrocartilage phenotype.

Funding Source: This research work is funded by the National Council for Scientific and Technological Development (CNPq) – Brazil.

We thank all the staff of Carlos Chagas Institute - FIOCRUZ/PR.

Keywords: 3D bioprinting, decellularized extracellular matrix, tissue engineering

Poster Abstracts

102 - B

BIOINK CONTAINING DECELLULARIZED SPINAL CORD TISSUE AND MESENCHYMAL STEM CELLS DIMINISHES INFLAMMATORY RESPONSE IN A 3D NEUROINFLAMMATION MODEL

Dos Santos, Marcelo Garrido¹, Pereira, Fernanda², Barros, Julia², dos Santos, Natália K. V.³, Gasparotto, Mariana², Czarnabay, Debora², Sperling, Laura², Pranke, Patricia⁴

¹Physiology Post Graduate Program, UFRGS, Porto Alegre, Brazil, ²Stem Cells Laboratory, UFRGS, Porto Alegre, Brazil, ³Inorganic Chemistry Department, UFRGS, Porto Alegre, Brazil, ⁴Stem Cells Research Institute, UFRGS, Porto Alegre, Brazil

Lately, 3D bioprinting has emerged as a promising approach in the field of regenerative medicine. This technique allows for the production of 3D scaffolds that support cell transplantation due to their ability to mimic the extracellular environment. This feature is fundamental to support functional tissue regeneration. Counterbalance inflammatory response after a traumatic injury is also fundamental to improve the outcome. The aim of this study was to evaluate the antiinflammatory activity of a bioink containing rat Decellularized Spinal Cord Tissue (DSCT) and mesenchymal stem cells (MSC) in a 3D neuroinflammation model (3DNM). The hydrogel used as base of the bioink was produced combining 1.5% DSCT, 3% gelatin and 4% sodium alginate. The material was exposed to RAW 264.7 macrophages previously stressed with H₂O₂ and inflammatory markers were quantified by ELISA and flow cytometry. The biomaterial induced macrophage polarization into an anti-inflammatory phenotype (M₂), evidenced by IL-10 increased levels and CD206, a classical M₂ marker, higher expression. The bioink was prepared by adding PEDOT:PSS, an electro conductive polymer, at a concentration of 0.1 mg/mL and 7.5x10⁵ MSC/mL. Rheological characterization was performed using a rheometer with the Peltier equipment and SEM images were acquired to visualize the internal structure. Rheological analyses indicated that material presents a shear thinning behavior and SEM images showed a highly porous 3D structure. The bioink was bioprinted and was exposed to the 3DNM model stressed with LPS. The model consisted of a hydrogel with 4% gelatin and 5% alginate and 2x10⁵ PC12 cells (neuron model), 4x10⁵ BV2 cells (microglia) and 8x10⁵ C6 cells (astrocyte model). Cytokines IL-1 β , IL-6 and IL-10 were quantified by ELISA. Reactive oxygen species (ROS) production was measured by 2-7-dichlorofluorescein diacetate oxidation and antioxidant defenses were evaluated by quantifying sulfhydryl

compounds. The bioink decrease IL-1 β , IL-6 and ROS production on the 3DNM. On the other hand, IL-10 and sulfhydryl compounds presented higher levels when exposed to the bioink. The data mentioned above showed that the bioink decreases inflammatory response in both macrophages and a more complex 3DNM and is an optimal candidate for neural tissue engineering.

Keywords: 3D bioprinting, decellularized tissue, immune modulation

103 - A

BMP4 NEUROPROTECTIVE AND ASTROGLOGENIC EFFECTS IN VITRO OXYGEN AND GLUCOSE DEPRIVATION MODEL

Ahmed, Ahmed Khalid Mohamed Albashir, Isaksen, Toke Jost, Yamashita, Toshihide, Nakagawa, Hiroshi

Graduate School of Medicine, Molecular Neuroscience Department, Osaka University, Suita, Japan

CNS ischemia is one of the leading causes of death and disability world-wide. Limitations of therapeutic modalities for ischemic stroke have necessitated research in other possible treatment options for improved outcomes of CNS ischemia. Recently, the focus on stem cells therapeutic potential have been increasing due to their ability to proliferate and differentiate into other cell types. Neural stem cells (NSCs) that exist in the adult brain exhibit the ability to migrate into ischemic regions, differentiate into neural cells and integrate in the ischemic brain tissue. Several factors have been identified to manipulate the NSCs behavior/responses. However, in this study we focus on Bone Morphogenetic Protein 4 (BMP4), a member of the transforming growth factor- β (TGF- β) superfamily of signaling ligands known to play roles in NSCs proliferation and differentiation. We performed Oxygen and glucose deprivation (OGD) for cultured NSCs to mimic CNS ischemia in vitro. We observed that total absence of Oxygen and glucose results in significant cell death that can be prevented by BMP4 pre-treatment. Furthermore, exposure of NSCs cultured under OGD to BMP4 enhanced their astrogliogenic differentiation but inhibited neuronal differentiation. These findings further prompt us to investigate the mechanisms to optimize the NSCs potential to enhance recovery outcomes following neural ischemia.

Keywords: adult neural stem cells, ischemia, astrogliogenesis

Poster Abstracts

103 - B

CHARACTERISTICS OF SSEA-4+ SUBPOPULATION ISOLATED FROM WHARTON JELLY MESENCHYMAL STEM/STROMAL CELLS

Smolinska, Agnieszka¹, Chodkowska, Magdalena¹, Janiec, Jakub², Kominek, Agata², Piwocka, Katarzyna², Sarnowska, Anna¹

¹*Translational Platform of Regenerative Medicine, Mossakowski Medical Research Institute of Polish Academy of Sciences, Warsaw, Poland, ²Laboratory of Cytometry, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland*

Stage-specific embryonic antigen 4 (SSEA-4) is a surface molecule found during embryonic development. However, SSEA-4 has also been identified in a population of mesenchymal stem/stromal cells (MSCs) - a promising group of stem cells widely used in regenerative medicine. In the presented study, the characteristics of SSEA-4+ MSCs were described in order to find out whether such a population is prominent from a therapeutic point of view. SSEA-4 was identified on the neonatal source of MSCs – Wharton Jelly, a stroma of the umbilical cord. Two methods of cell separation were considered: Magnetic Activated Cell Sorting (MACS) and Fluorescence Activated Cell Sorting (FACS). The following properties were analyzed for the positive population (WJ-MSC-SSEA-4+): colony forming unit capacity, population doubling time, expression of stemness related genes and ability to form spheres under 3D culture conditions. The results obtained were compared with a negative population (WJ-MSCSSEA-4-) and an unsorted population (unsorted WJ-MSC). SSEA-4 expression was dependent on the environmental factors used, such as the human platelet lysate used in the culture medium. FACS was chosen for the selection of WJ-MSC SSEA-4+ cells because of the better cell recovery observed. FACS allowed the enrichment of SSEA-4 in the WJ-MSC population up to the 6th passage after sorting. WJ-MSC-SSEA-4+ were characterized by higher expression of stemness related genes (Nanog, Oct4, Sox2), but did not differ in clonogenicity and proliferation ratio. 3D spheres formed with WJ-MSC-SSEA-4+ reached smaller diameters and were characterized by better survival. The study showed that WJ-MSC-SSEA-4+ cells sorted by FACS differed in stemness related genes expression and sphere forming ability. However, there was no change in the physiological activity of the described cells in comparison to negative and initial populations.

Funding Source: National Science Centre, Poland, Preludium 21 2022/45/N/NZ3/02564; National Science Centre, Poland, OPUS NCN 2018/31/B/NZ4/03172; ESF, POWR.03.02.00-00-1028/17-00

Keywords: SSEA-4, Mesenchymal stem/stromal cells, FACS

104 - A

CHARACTERIZATION AND CRYOPRESERVATION OF DENTAL PULP STEM CELL SPHEROIDS FOR TISSUE ENGINEERING APPLICATIONS

Zuliani, Carolina Coli¹, Teixeira, Larissa², Cunha, Jéssica¹, Moraes, Angela², Coimbra, Ibsen¹

¹*School of Medical Sciences, University of Campinas (UNICAMP), Brazil, ²School of Chemical Engineering, University of Campinas (UNICAMP), Brazil*

Cryopreservation allows long-term preservation of cells. Although the procedures for cell freezing are already well established, techniques applied to cell spheroids require improvement of freezing and thawing rates, as well as the penetration capacity of the cryoprotective solution. The objective of this work was to characterize spheroids of dental pulp stem cells (DPSCs) and evaluate techniques for their cryopreservation. Spheroids of two different sizes were evaluated. Cell spheroids were produced by depositing either 0.5.106 or 1.106 DPSCs in a non-adhesive mold followed by incubation for five days. Then, the spheroids were removed from the mold and characterized regarding their diameter, circularity, sphericity, solidity, and roundness. Histological and immunohistochemical characterization detected collagen II and aggrecans was conducted. 5-day spheroids were gradually frozen using a container with isopropanol (-1°C.min-1 freezing rate) before the cryotubes were stored in liquid nitrogen. Two different cryopreservation solutions were evaluated: (1) alpha-MEM/FBS/DMSO, and (2) FBS/DMSO. After thawing, the spheroids were washed at least 3 times with culture medium and placed in a multi-well plate for reactivation of cellular metabolism. The cryoprotectant efficiency was assessed through cellular viability and by fusion activity. As a positive control, non-cryopreserved spheroids were used. Spheroids of 0.5 and 1.106 cells showed similar characteristics based on the morphological parameters. Histological analysis revealed that larger cellular aggregates produced more extracellular matrix, which could act as an auxiliary cryoprotectant, in which collagen II and aggrecans were detected. This hypothesis was confirmed by the LIVE/DEAD assay, as the images demonstrated a thicker layer of dead cells in the outer region of the 0.5.106 thawed cell spheroids. Cryoprotective solution (2) yielded the highest cell viability, but even in this situation, the fusion capacity of the spheroids was reduced. Thus, it can be concluded that freezing DPSC spheroids can be performed to optimize the production of cellular aggregates for tissue engineering applications, but there is room for improvement in this process.

Funding Source: São Paulo Research Foundation (FAPESP 2022/12970-3; 2021/14738-8) National Council for Scientific and Technological Development (CNPq 314724/2021-4) Coordination for Improvement of Higher Education Personnel (CAPES) Brazil

Keywords: spheroid, tissue engineering, cryopreservation

Poster Abstracts

104 - B

CHARACTERIZATION OF CYTOCHALASIN B-INDUCED MEMBRANE VESICLES EXTRACTED FROM DENTAL PULP STROMAL CELLS

Morais, Gabriel S.¹, Fracaro, Letícia¹, Correa, Alejandro², Marcon, Bruna³, de Souza, Ingrid Larissa², Brofman, Paulo Roberto¹

¹Core for Cell Technology - School of Medicine and Health Sciences, Pontifical Catholic University of Parana, Curitiba, Brazil, ²Laboratory of Basic Biology of Stem Cells, Carlos Chagas Institute - FIOCRUZ/PR, Curitiba, Brazil, ³Confocal and Electronic Microscopy Facility, RPT⁰⁷C, Carlos Chagas Institute - FIOCRUZ/PR, Curitiba, Brazil

Mesenchymal Stromal Cells (MSCs) are adult cells with self-renewal capacity, the ability to differentiate into several cell types, and extensive paracrine and immunomodulatory activity. Dental tissue has several sources of MSCs, including Dental Pulp Stromal Cells (DPSC). Cell-based therapies show promising results, but there is interest in cell-derived material for therapeutic purposes. Extracellular Vesicles (EVs) have an advantage in regenerative medicine because they are safer when compared to MSCs. EVs are crucial in cell communication, transporting biomolecules that can influence the microenvironment. Considering the limited quantity of EVs, inducing the release of membrane vesicles from the surface of cells presents an alternative. To explore this therapeutic potential, better isolation methods need to be tested first, as well as testing different techniques. This study reports the characterization of Cytochalasin B-induced membrane vesicles (CIMVs) extracted from DPSC. Cells were isolated and cultured until the fourth passage and were immunophenotypically characterized by flow cytometry. Cells were cultured serum-free for 24 and 72 hours before CIMV extraction for comparison. CIMVs were extracted and subsequently characterized. Their size and concentration were evaluated using Nano Tracking Analysis (NTA), protein concentration by fluorescence assay, and Transmission Electron Microscopy (TEM). DPSCs exhibited morphology and surface markers expression according to the International Society for Cell and Gene Therapy. NTA analysis indicated CIMVs' dimensions averaging $169 \pm 4,6\text{nm}$ (24h) and $173,3 \pm 2\text{nm}$ (72h). As for the concentration, 24h CIMVs exhibited an average of $2,47 \times 10^{10}$ particles/mL whereas 72h CIMVs demonstrated an average of $4,28 \times 10^{10}$ particles/mL. CIMVs showed average protein concentrations of $143 \pm 5,3 \mu\text{g/mL}$ (24h) and $200,2 \pm 1,9 \mu\text{g/mL}$ (72h). TEM analysis confirmed the morphology and size commonly seen in EVs. These findings demonstrate the effectiveness of the isolation protocol for CIMVs

extracted from DPSCs. The results support the potential of CIMVs as an alternative to regenerative medicine and emphasize the importance of optimizing isolation protocols and culture conditions to maximize CIMV production and therapeutic efficacy.

Funding Source: CNPq Process 465656/2014-5 (INCT-REGENERA)

Keywords: extracellular vesicles, cell-based therapies, isolation methods

105 - A

CHARACTERIZATION OF PSC-DERIVED MESENCHYMAL-LIKE STEM CELLS AND PSC-DERIVED EXTRACELLULAR VESICLES: INQUIRY OF POTENTIAL IN REGENERATIVE MEDICINE

de Matos, Bruno M., Josino, Raphaella, Robert, Anny, Stimamiglio, Marco, Correa, Alejandro

Laboratory of Basic Biology of Stem Cells, Carlos Chagas Institute/FIOCRUZ-PR, Curitiba, Brazil

Mesenchymal stem cells (MSCs) can promote increased activity and viability of many cell types through paracrine communication, including through mechanisms mediated by extracellular vesicles (EVs). Aiming to avoid the limitations of primary MSC isolates, such as heterogeneous populations and early cellular senescence, this project proposes the derivation and characterization of MSC-like cells regarding their functional and molecular properties, as well as the isolation and characterization of EVs from pluripotent stem cell (PSC) lineages, as strategies for new potential treatments in regenerative medicine. Among six methodologies tested for cell derivation, one generated cells with morphology and phenotypic profile consistent with MSC-like. Multipotent differentiation experiments showed that MSC-like have osteogenic potential comparable to primary MSCs, but no adipogenic potential. Chondrogenesis assays will further enlighten the multipotent potential of MSCs-like. Regarding the isolation of EVs from PSCs lineages, the protocol was consistently more profitable than the standard yield for EVs of primary MSC cultures. The EV-PSCs showed uniform morphology and size in TEM and nanotracking experiments, and protein markers of extracellular vesicles were identified by western blot. Analysis such as LC/MS and in vitro assays will be performed to unveil the molecular and functional characteristics of EV-PSC.

Funding Source: Carlos Chagas Institute/FIOCRUZ-PR; Araucaria Foundation; INOVA-FIOCRUZ; CAPES; Cnpq;

Keywords: regenerative medicine, MSC-like, EV-PSC

Poster Abstracts

105 - B

DEVELOPMENT OF THE CRISPR-CAS13D SYSTEM FOR DOWNREGULATION OF THE PHD2 ISOFORM OF THE HIF ENZYME PROLYL 4-HYDROXYLASE 2 TO STIMULATE ANGIOGENESIS IN VITRO**Terra, Sara Carolina Vieira**¹, Ivanov, Giovana²¹*Santa Casa de São Paulo School of Medical Sciences, São Paulo, Brazil,* ²*Biochemistry, Santa Casa de São Paulo School of Medical Sciences, Santo André, Brazil*

Hypoxia-inducible factor (HIF) is a crucial player in coordinating the cellular response to hypoxia, and HIF prolyl hydroxylase domain enzymes (PHDs) play a pivotal role in HIF α degradation. The CRISPR-Cas13d system, known for its RNase activity, offers the potential for targeted gene downregulation or even silencing. Therefore, this study aimed to develop a CRISPR-Cas13d system for PHD2 downregulation to stimulate angiogenesis in HEK-293T and HUVEC under hypoxic and normoxic conditions. Using the Cas13 Design Tool, RNA guides were designed to target the EGLN1 gene, encoding PHD2 (gRNA 1), along with a non-targeting genome control (gRNA 2). Cloning of the gRNAs into the Cas13d vector was performed. Bacterial transformation, plasmid extraction, and lentiviral production were carried out. HEK-293T and HUVEC were transduced with lentivirus carrying Cas13d + gRNA 1 or 2 and selected using puromycin. Meanwhile, high molecular weight (HMW) and low molecular weight (LMW) alginates were oxidized (0% or 1%). MDSCs (muscle-derived stem cells) were obtained and encapsulated in cross-linked alginate microgels modified with RGD peptide. Cells were then cultivated in DM (differentiation media), PM (proliferation media), and EGM (endothelial growth media). Live and Dead assay with calcein and 7AAD was performed to evaluate viability. As a result, both gRNAs were successfully cloned into the Cas13d backbone, according to sequencing results. Lentivirus was collected and HEK-293T and HUVEC were transduced, selected, and cell lines 1 and 2 were established. In parallel, MDSC cells underwent alginate encapsulation and exhibited a 60% viability for the formulations 0% PM, 0% DM and EGM 1%. In conclusion, successful cloning of gRNAs into the Cas13d backbone and lentiviral vector construction was achieved. Transduction and establishment of HEK-293T and HUVEC cell lines were accomplished. Encapsulation of MDSCs 2D cultures in alginate hydrogel demonstrated that cell survival depends on formulation and culture media. Finally, future experiments will explore the interaction between transduced cells carrying Cas13d + gRNA and stem cells, with or without hydrogels, to enhance angiogenesis promotion.

Funding Source: FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo)**Keywords:** Angiogenesis, Gene therapy, Cell therapy

106 - A

DNA DAMAGE SIGNALLING ACTIVATION IN PLACENTA-DERIVED MESENCHYMAL STEM CELLS AFTER IN VITRO SARS-COV-2 INFECTION**Guerrero, Erika N.**¹, Fu, Cindy², López, Sandra², Solis, Erika³¹*Regenerative Medicine Department, Gorgas Memorial Institute for Health Studies, Panama,* ²*Virology, Instituto Comemorativo Gorgas, Panama,* ³*Regenerative Medicine, Instituto Comemorativo Gorgas, Panama*

The human placenta has been considered attractive alternative source for non-invasive isolation of human mesenchymal stem cells (PDMSCs). However, donors age affects PDMSCs regenerative and immunomodulatory potential. Previously, we reported a correlation in proliferative capacity and telomere shortening in PDMSCs of 5 maternal donor age groups. Short telomeres activate the DNA damage signalling. Dysregulation in DNA repair pathways can predispose MSCs to senescence, apoptosis and reduced stemness and self-renewal. In this study, we isolated PDMSCs from COVID19 positive donors between the age group of 33-35 and compared the gene expression of DNA damage response and repair pathways. We performed in vitro infection of SARS-CoV-2 in PDMSCs and COVID19-PDMSCs to analyse and compare gene expression using RT2 profiler array. Data showed 28 genes with more than ~ 2 fold up-regulation involved in nucleotide excision repair, base excision repair, mismatch repair, double-strand break repair and other DNA repair genes altered expressions in infected PDMSCs and infected COVID-19 PDMSCS when compared to un-infected controls. Interestingly, genes involved in ATM/ATR signalling pathway were more than ~ 2 fold down regulated in infected PDMSCs, but stably expressed infected COVID19-PDMSCs. Activating DNA damage checkpoint help maintain MSC function including regenerative secretome release to activate tissue repairing mechanisms. Further, we performed RT-PCR of the 28 genes with altered expression and western blots to confirm the RT2 profiler array results. It is known that viral infection induces DNA damage via different mechanism such as, host cell replication stress, however it has been shown that SARS-CoV-2 is not able to infect MSCs due to the lack of ACE2 receptor. The regenerative capacity and the evasion of SARS-CoV-2 infection of PDMSCs conferred the potential for the treatment of COVID19 and the decrease of de inflammatory cascade.

Keywords: DNA repair, DNA damage signaling, in vitro infection

Poster Abstracts

106 - B

EFFECT OF HUMAN PLATELET LYSATE ON HUMAN MESENCHYMAL STEM CELL SUPPLEMENTATION AND APPLICATION IN ACUTE NEURAL INJURY MODEL: STAB WOUND

Silva, Karina Ribeiro Da¹, Almeida, Danilo², Câmara, Niels³, Longo, Beatriz⁴, Romariz, Simone⁴

¹Medicine, São Paulo, Brazil, ²Nephrology, Federal University of São Paulo, Brazil, ³Immunology, University of São Paulo, Brazil, ⁴Physiology, Federal University of São Paulo, Brazil

Traumatic brain injury (TBI) affects millions of people worldwide, resulting in severe impact on life-quality. Currently, treatments for TBI are still quite limited, and Multipotent mesenchymal stromal cell (hMSCs) therapy is considered a promising strategy. Thus, this study evaluated the impact of human Platelet Lysate (hPL) supplementation on hMSCs culture and its application in an experimental model of TBI. hMSCs were cultured or with 10% of fetal bovine serum (FBS_hMSCs) or with highly concentrated 10% hPL (hPL_MSCs). Subsequently, both cell populations were characterized “in vitro” according to their immunophenotypic panel; mesodermal and neuronal differentiation capacity and by proliferation and oxidative stress markers. The therapeutic potential “in vivo” was investigated by histopathological analyses and gene expression profile. Both subsets of hMSCs presented a fibroblastoid morphology with a phenotypic profile characteristic of hMSCs. Both hMSCs showed mesodermal differentiation potential, but it was lower in cells cultured with hPL. Neuronal morphological features were identified only in the hPL_MSCs cultures, which had positive regulation of neuronal markers. When compared to FBS_hMSCs cultures, hPL_MSCs also showed lower cellular mitochondrial stress and lack of proliferative potential. Additionally, in hMSCs-infused brain tissues, the neurorepair potential (DCX+ cells) was higher in the hPL_MSCs-treated neuronal sections, which also showed a low presence of inflammatory cells (Iba+ and GFAP+). We observed lower cytochrome C and GFAP expression in the cortex of hPL_MSCs-infused animals in comparison to FBS_hMSCs. In the hippocampus of hPL_MSCs-treated animals, it was detected low expression of IL-4 and neural function/plasticity markers (AMPA-1, NMDA-1, Cap-23, Gap-43, PSD-95) when compared to FBS_hMSCs group. Finally, we found elevated presence of BDNF in hPL_MSC culture supernatants and in serum from hPL_MSC-transplanted animals. In summary, our results suggest that hPL_MSCs exhibit classic neuronal characteristics “in vitro” with “in vivo” neuroprotective potential, indicating that hPL is a promising inducer of neuron-like cells with limited plasticity, serving as an alternative and potential tool for neuronal-based cellular therapy.

Keywords: human mesenchymal stem cell, human platelet lysate (hPL), neuroregeneration

107 - A

EFFECT OF THE USE OF AUTOLOGOUS CELLS OVER DIFFERENT SCAFFOLDS FOR THE TREATMENT OF SECOND AND THIRD DEGREE BURNS IN HUMANS

Serrano, Grecia Vanessa¹, Díaz, Emmanuel¹, Miranda, Ariel², Hernández, Oscar³, Hernández, Claudia⁴, Mendoza, Luis¹

¹Biotecnología Médica y Farmacéutica, CIATEJ, Guadalajara, Mexico, ²Atención a Niños con Quemaduras Graves, Nuevo Hospital Civil De Guadalajara – Dr. Juan I. Menchaca and Guadalajara, Mexico, ³Departamento de Fisiología, Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad de México, Mexico, ⁴Instituto Nacional de Enfermedades Respiratorias, Secretaría de Salud, Guadalajara, Mexico

My thesis project deals with the treatment of deep second and third-degree burns in children. Various deep burns are difficult to treat because the extracellular matrix is destroyed; and the new skin is never as elastic as it used to be before the injury. The elasticity of the skin is especially important in children due to the growth and development of the tissue, in addition to it, the skin is subject to stretching. To address this problem we propose the use of autologous cells combined with the most innovative skin substitutes based on biocellulose and polylactic acid; both of these will be able to regenerate more elastic and aesthetic skin than the dermal substitutes currently used. Before the treatment application, a cell culture method will be standardized on the dermal substitutes with laboratory-generated fibroblast cell lines. After cell culture, the viability and proliferation of the cells will be tested. The autologous cells will be obtained from a biopsy of the patients and will be cultured in the laboratory under aseptic methods and conditions. Then, the treatment will be applied to patients and they will be followed up to compare results in the physical characteristics of the skin and inflammation with the proposed treatment, and standard treatments. All procedures will be under the guidelines of the Helsinki Declaration.

Keywords: stem cells, burns, scaffolds

Poster Abstracts

107 - B

EFFECT OF TRYPANOSOMA CRUZI PARASITISM ON THE PARACRINE REGENERATIVE POTENTIAL OF HUMAN MESENCHYMAL STEM CELLS

Garcez, Emanuella Melgaco¹, Moraes, Aline², Silva-Carvalho, Amandda³, De Alencar-Silva, Thuany¹, Barcelos, Stefhani², Victória Souto, Maria², Saraiva, Rivaldo¹, Pogue, Robert¹, Hagstrom Bex, Luciana², Saldanha-Araujo, Felipe³, Hecht, Mariana², Lott Carvalho, Juliana²

¹Genomic Sciences and Biotechnology, Catholic University of Brasília, Brazil, ²Multidisciplinary Laboratory of Biosciences, Faculty of Medicine, University of Brasília, Brazil, ³Hematology and Stem Cells Laboratory, Health Sciences Department, University of Brasília, Brazil

Chagas disease is a parasitic infection caused by the *Trypanosoma cruzi* (Tc). The disease affects several tissues, such as the bone marrow and adipose tissue, in which Mesenchymal Stem Cells (MSCs) can be found. Even though it is known that Tc is capable of infecting most nucleated cell types, the consequences of MSC infection by the parasite have not yet been described. In the present study, we aimed to investigate the influence of Tc infection on the paracrine regenerative potential of human adipose tissue-derived MSCs. To do so, MSCs were isolated from the adipose tissue of healthy donors and characterized according to the minimal criteria established by the International Society for Cell Therapy. The immunological and regenerative signature of MSCs was established by evaluating the basal expression of immunomodulatory genes IL-10, TSG-6, IDO and TGF- β , as well as the basal expression of genes related to tissue regeneration (FGF and VEGF). Following their characterization, the MSCs were infected with the Colombian strain of *T. cruzi* at a 1:1 ratio (MSCs:parasites). Parasite load was verified by detecting and quantifying the Tc40 gene. The conditioned medium collected on the 7th day post-MSc infection was used to treat primary cultures of fibroblasts plated for wound scratch assays. Our results show that the isolated MSCs presented the expected immunophenotypic profile, as determined by the ISCT, as well as the capacity to differentiate into osteoblasts and adipocytes. The immunological signature of the infected MSCs showed that they presented high basal expression of IL-10, IDO, and TSG-6, which were 4, 2 and 1.8 times superior to GAPDH respectively, while the basal expression of FGF and VEGF was approximately 5 and 20 times superior to GAPDH, respectively. At 7 days post-infection, MSC cultures presented signs of cellular toxicity. The Tc-infected MSC secretome obtained at 7d post infection significantly promoted fibroblast migration

compared to the negative control of the assay (untreated cells; $p < 0.05$), while the secretome of non-infected control cells was limited to promoting a trend towards increased cellular migration. Taken together, our results demonstrate that MSCs can be parasitized by *T. cruzi* and that the infection enhances the regenerative potential of infected MSCs in the conditions tested.

Funding Source: FAP/DF, Capes

Keywords: *Trypanosoma cruzi*, Chagas disease, Mesenchymal stem cells

108 - A

EFFECTS OF HMSCS PRECONDITIONING WITH CANNABIS SATIVA CHEMOTYPES I AND III ON CHAPERONES' HSP-70 AND HSP-90 SIGNALING PATHWAYS

Fontecilla Escobar, Javiera, Flores Montero, Karina, Ruete, Maria Celeste

IHEM, CONICET, Mendoza, Argentina

Mesenchymal stem cells must maintain their biological functions for application in regenerative medicine. One approach to this challenge is the preconditioning of MSCs to safeguard their stemness, ensuring their suitability for subsequent regenerative medicine interventions. Cannabis is known for its antiapoptotic and antioxidant properties. Molecular chaperones, including Hsp90 and Hsp70, are involved in protein homeostasis, subcellular trafficking and play a crucial role in maintaining pluripotency. However, their specific functions in stem cells remain relatively unexplored compared to differentiated cells. This study investigates the effects of preconditioning MSCs with two chemotypes of *Cannabis sativa*, type III (high in CBD) and type I (high in THC), on signaling pathways associated with cell proliferation, stemness, and chaperone expression. Additionally, the role of caspase-3 in the apoptosis pathway will be explored. We demonstrate that preconditioning MSCs with both chemotypes induces the phosphorylation of pSTAT3, p-ERK, and p-AKT, indicating the activation of proliferation pathways. Furthermore, the upregulation of pSTAT3 suggests that Cannabis activates this proliferation pathway through Hsp90. Interestingly, the expression of Hsp90 increases with higher doses of type I and III, which generally has a beneficial effect as an antiapoptotic heat shock protein. In contrast, the levels of Hsp70 remain unchanged under the different treatments. Hsp70 is known to be highly expressed in MSCs compared to differentiated cells. Therefore, the lack of change in Hsp70 expression suggests that MSCs retain their stemness after preconditioning with the two chemotypes of *Cannabis sativa*. In conclusion, this study provides insights into the effects of

Poster Abstracts

two *Cannabis sativa* chemotypes on MSCs, highlighting their impact on signaling pathways related to proliferation and the expression of chaperones. Understanding these mechanisms can contribute to developing therapeutic strategies involving MSCs and Cannabis-based treatments for various applications, including regenerative medicine.

Funding Source: PICT 2018 - 00668 PROJECT 4A - MINCYT

Keywords: human mesenchymal stem cell, cannabis sativa, chaperones

108 - B**ENDOCANNABINOID SIGNALING REGULATES EPENDYMOGLIAL STEM CELL BEHAVIOUR IN THE REGENERATING AXOLOTL SPINAL CORD**

Carlone, Robert Leo, Tolentino, Michael, Foran, Gregory, Necalov, Aleksandar, Spencer, Gaynor

Biological Sciences, Brock University, St. Catharines, ON, Canada

The endocannabinoid system (ECS) is a neuromodulatory system conserved throughout the animal kingdom. Most studies on the ECS have been conducted on mammals, where it contributes to important cellular processes during central nervous system (CNS) development. These processes are thought to be conserved and/or reactivated during regeneration of the nervous system, though most mammals have a limited capacity for CNS regeneration. The Mexican axolotl however, does have the capacity for CNS regeneration and is one of the few vertebrates that can regenerate their spinal cord. As there is currently little known about the ECS in a regeneration-competent species, we investigated the role of the ECS in the pro-regenerative response of the axolotl spinal cord. We provide evidence that the predominant ECS receptor in the CNS, (CB1), is upregulated in the regenerating caudal spinal cord and tail tissues of larval axolotls within 4 hours post amputation (pa), and that this upregulation persists for 14 days. CB1 expression was found in the ependymal radial glial (ERG) cells (a stem cell population within the axolotl spinal cord), as well as in the subependymal layer. A second ECS receptor, CB2, primarily localized to the subependymal layer, was upregulated later than CB1, but also persisted for at least 14-days pa. Treatment with either the CB1 inverse agonist, AM251, or the CB2 inverse agonist, AM630, significantly inhibited caudal tail and spinal cord regrowth. Inverse agonist treatments also revealed that both CB1 and CB2 signaling were necessary for the proliferation of ERGs during the first 7 dpa, while only CB1 was required during that period for the differentiation of these cells into immature neurons. To further investigate the role of the

ECS in this system, we have developed both CB1 and CB2 molecular sensors that allow for the real time dynamic measurement of ECS activity within stable human cell lines and transgenic axolotls. These will allow us to precisely map the spatio-temporal pattern of receptor activation by endogenous cannabinoids during spinal cord regeneration. These studies are the first to examine the role of the ECS during spinal cord regeneration in a regeneration-competent vertebrate and contribute to our understanding of the molecular pathways underlying successful vertebrate CNS regeneration.

Funding Source: Natural Sciences and Engineering Research Council Discovery Grant (RGPIN 2019 06380) to R. Carlone

Keywords: endocannabinoid, ependymoglia, regeneration

109 - A**EVALUATION OF THE POTENTIAL OF MURINE MESENCHYMAL STEM CELLS THERAPY, ASSOCIATED TO EMPAGLIFLOZIN, FOR THE MODULATION OF AUTOPHAGY AND MTOR PATHWAYS, ALONGSIDE PODOCYTES' PRESERVATION, IN BTBR OBOB MICE**

Franco, Marcella L., Beyerstedt, Stephany, Carlos, Alanah, Rangel, Érika

Experimental Research / Jewish Institute of Research and Education at Albert Einstein Hospital, Hospital Israelita Albert Einstein, São Paulo, Brazil

Diabetic kidney disease (DKD) is the leading cause of end-stage renal disease worldwide and it is characterized especially by podocytes' damage, which often leads to proteinuria. Dysregulation of autophagy and mTOR pathways is one of DKD's pathophysiology mechanisms. In this context, the synergism between Empagliflozin and Mesenchymal Stem Cells (MSCs) may be an interesting therapeutic strategy for this disease. Thus, the aim of the study is the evaluation of the modulation of autophagy and mTOR pathways, alongside podocytes' cytoskeleton, by the proposed therapeutic approach. Male mice from BTBR ob/ob strain in 14-15 weeks and 18-20 weeks of age were used. These leptin-knockout animals are obese and develop diabetes mellitus and DKD. Animals were treated with Empagliflozin, Calorie Restriction (CR) and MSCs. We evaluated animals' glycemia and body weight. We also made RT-qPCR and Western Blotting analysis. This project was approved by the Institutional Animal Care and Use Committees of the Jewish Institute of Research and Education Albert Einstein, SP, Brazil (N°4845-21). MSCs' therapy significantly reduced glycemia (206,08 mg/dL ± 33,33) in relation to the control group (408,87 mg/dL ± 39,09)

Poster Abstracts

in 14 weeks. Animals remained obese in all groups, though. When combined, Empagliflozin, CR and MSCs seem to modulate autophagy pathway, as observed through higher LC3-II/LC3-I ratio ($1,384 \pm 0,373$) comparatively to the control group. In regard to mTORC1, data suggest high levels of Raptor in all groups, including MSCs group ($2,414 \pm 0, 017$) comparatively to wild-type (WT) animals. Concerning mTORC2, results suggest that both Empagliflozin ($9,685 \pm 3,512$) and MSCs ($5, 582 \pm 1,800$) might modulate this pathway, as seen through higher expression of Rictor in relation to WT animals. Concerning mesangial expansion and glomerular hypertrophy, in turn, we have not detected differences by the proposed therapy so far. In short, the evaluated pathways seem to be modulated by MSCs in our model, and when combined to Empagliflozin and CR, cell therapy is able to reduce glycemia in a time-dependent pattern. It is important to mention, though, that future experiments are still necessary.

Funding Source: FAPESP (2021/02216-7)

Keywords: diabetic kidney disease, mesenchymal stem cells, autophagy

109 - B

EVALUATION OF THE REGENERATIVE POTENTIAL OF EXTRACELLULAR VESICLES DERIVED FROM BOVINE MESENCHYMAL STEM CELLS ON OVARIAN STROMAL CELLS

Barcelos, Stefhani M.¹, Rosa, Paola Maria², Moura, Ana Beatriz², Villarroel, Carla³, Bridi, Alessandra², Bispo, Elizabete⁴, Garcez, Emãnuella⁵, Malard, Patricia³, Peixer, Maurício³, Saldanha-Araujo, Fellipe⁴, Dallago, Bruno⁶, Silveira, Juliano², Pogue, Robert⁵, Perecin, Felipe², Carvalho, Juliana Lott⁶

¹Faculty of Medicine, University of Brasília, Brazil,

²Department of Veterinary Medicine, Faculty of Animal Sciences and Food Engineering, University of São Paulo, Pirassununga, Brazil, ³Cell Therapy, Bio Cell - Cell Therapy, Brasília, Brazil, ⁴Department of Pharmacy, Faculty of Health Sciences, University of Brasília, Brazil, ⁵Genomic Sciences and Biotechnology Program, Catholic University of Brasília, Brazil, ⁶Multidisciplinary Laboratory of Biosciences, Faculty of Medicine, University of Brasília, Brazil

Extracellular vesicles (EVs) are secreted by cells in various contexts and can be found in biological fluids and culture media. Commonly used isolation methods for EVs include ultrafiltration (UF) and ultracentrifugation (UC).

Mesenchymal stem cell-derived EVs (MSC-EVs) have

demonstrated promising effects in regenerative medicine, influencing cell proliferation, migration, and apoptosis protection through the transfer of genetic material, proteins, and lipids. This study aimed to compare the regenerative potential of MSC-EVs obtained by UF and UC for future therapeutic application for ovarian lesions. First, we compared EV isolation methods with regard to EV yield and diameter. Then, the MSC-EVs obtained through UF and UC were assessed in relation to their capacity to promote the migration and proliferation of bovine stromal cells. For this, MSCs were obtained from healthy cows and characterized. The wound scratch assay was performed to determine the migratory behavior of bovine ovarian stromal cells treated with MSC-EVs processed by UF and UC. Furthermore, treated cells had their proliferative potential determined by calculating cell doubling time. Analysis of the size distribution and concentration of EVs isolated by UF and UC revealed that most vesicles had a diameter of approximately 200 nm. UF exhibited significantly higher yield than UC ($p < 0.01$), with mean particle concentrations of $7.91 \times 10^{11} \pm 2.05 \times 10^{11}$ for UF and $1.52 \times 10^{11} \pm 1.62 \times 10^{10}$ for UC. Both treatments promoted significantly higher cellular migration ($p < 0.05$) as well as reduced doubling time ($p < 0.05$), compared to non-treated cells. Furthermore, no significant differences were observed between EVs obtained by UF and UC in terms of their capacity to promote migration and proliferation of stromal cells derived from bovine ovarian tissue. These findings suggest that both isolation methods are equally effective in inducing cellular migration and proliferation, which are essential processes involved in tissue regeneration. Based on the comparative analysis of UF and UC for EV isolation, ultrafiltration emerges as an advantageous methodology with higher yield and potential for large-scale replication in a bovine preclinical model, facilitating future applications for treatment of ovarian lesions and the promotion of female fertility.

Funding Source: This work was funded by the Brazilian National Research Council, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Fundação de Amparo à Pesquisa do Distrito Federal, and Fundação de Amparo à Pesquisa do Estado de São Paulo.

Keywords: bovine mesenchymal stem cells, extracellular vesicles, regeneration

Poster Abstracts

110 - A

EXTRACELLULAR VESICLES OF NEURAL STEM CELLS MEDIATE AXONAL REGENERATION IN RESPONSE TO ISCHEMIA**H. Real, Fernando¹**, Tovar y Romo, Luis²¹*Molecular Neuropathology / Institute of Cellular Physiology, Mexico City, Mexico*, ²*Molecular Neuropathology, UNAM, Mexico City, Mexico*

Ischemic stroke is a leading cause of mortality and disability. Various molecular factors are triggered in response to stroke to promote tissue recovery, including the proliferation of neural precursors, mainly observed in murine models. Neural stem cells (NSCs) release extracellular vesicles (EVs) that contain factors capable of promoting neural recovery. This study investigated whether EVs shed from NSC isolated from the subventricular zone can enhance axonal regeneration after brain ischemia. To address this question, we conducted a series of experiments in vitro where we established a model for assessing axonal growth following ischemia in microfluidic chips with cultured mouse hippocampal neurons exposed to NSC-EVs, and found that the administration of EVs isolated under normoxic conditions promote axonal recovery, using kymography as a proxy to axon functionality of the regenerated axons. We also monitored neuronal viability with flow cytometry and found that treating neurons with EVs isolated from NSC cultured under normoxic conditions reduces the number of apoptotic and necrotic cells after ischemia. Interestingly, EVs obtained after oxygen and glucose deprivation exhibited no protection, despite proteomic data indicating the presence of recovery-related proteins. These results indicate that it is plausible that some alternative signaling molecules, such as regulatory RNAs, mediate these EVs' protective effect. These findings are of great significance as EVs represent a novel therapeutic approach, and our data highlight their potential to promote post-ischemic recovery. Subsequently, our future research will focus on deciphering the underlying mechanisms orchestrating the effects of EVs on axonal regeneration.

Funding Source: This project was supported by DGAPA-PAPIIT IN214723**Keywords:** extracellular vesicles, neural stem cell, ischemia

110 - B

HUMAN IMMATURE DENTAL PULP STEM CELLS (hDPSC) INDUCE IMMUNOMODULATORY PHENOTYPE IN MONOCYTES AND MACROPHAGES**Da Costa, Vitor Rodrigues¹**, Souza, Olívia², Teixeira, Michelli Ramires³, Alievi, Anderson³, Oliveira, Melissa⁴, Orikaza, Cristina², Popi, Ana Flávia², Kerkis, Irina³, Araldi, Rodrigo¹¹*Departamento de Morfologia e Genética, Universidade Federal de São Paulo (UNIFESP), Brazil*, ²*Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de São Paulo (UNIFESP), Brazil*, ³*Laboratório de Genética, Instituto Butantan, São Paulo, Brazil*, ⁴*Instituto de Ciências Biomédicas, Universidade de São Paulo (USP), Brazil*

Mesenchymal stem cells (MSCs) have been explored as an attractive tool for the treatment of inflammatory disorders. Once that monocytes and macrophages are the main players in orchestrating inflammation, the aim of this study was to evaluate the immunomodulatory potential of human immature dental pulp stem cells (hDPSCs) on monocytes and macrophages. For this, we co-cultured two different cell lines of monocytes (THP-1 and U-937) and their derived macrophages with hDPSCs and evaluated the secreted cytokines and immunophenotype of immune cells. Our results showed that the co-culture of hDPSCs does not affect the cell viability of both immune and stem cells. As expected, hDPSCs produce large quantities of interleukin (IL)-6, and after exposure to monocytes and macrophages, hDPSCs strongly increase the secretion of IL-6, while IL-6 expression was poorly modified in immune cells. In contrast, the co-culture of macrophages with hDPSCs decreased TNF secretion while increasing the secretion of IL-10 by macrophages. In the same line, the monocyte-hDPSCs co-culture turned monocytes into an IL-10-producing cells. Although changes in secreted cytokines, macrophage immunophenotypic changes of the molecular markers CD64 and CD163 were not detected after co-culture with hDPSCs. In conclusion, these results demonstrated that hDPSCs have immunomodulatory properties in macrophages and monocytes.

Keywords: Immunomodulation, Monocytes and Macrophages, human Immature Dental Pulp Stem Cells (hDPSC)

Poster Abstracts

111 - A

KIDNEY-DERIVED C-KIT+ PROGENITOR/STEM CELLS CONTRIBUTE TO TISSUE REGENERATION IN A MOUSE MODEL OF DIABETIC KIDNEY DISEASE

Beyerstedt, Stephany¹, Barbosa, Bruno¹, Franco, Marcella¹, Seidler, Barbara², Saur, Dieter³, Gomes, Samirah⁴, Hare, Joshua⁵, Rangel, Érika¹

¹Albert Einstein Israeli Institute of Teaching and Research, Hospital Albert Einstein, Santo André, Brazil, ²School of Medicine, Technische Universität München, Munich, Germany, ³Department of Internal Medicine, Technische Universität München, Munich, Germany, ⁴Faculty of Medicine, University of São Paulo, Brazil, ⁵Interdisciplinary Stem Cell Institute, University of Miami Miller School of Medicine, Miami, FL, USA

Kidney-derived c-Kit⁺ is a population of stem cells, as they possess clonogenicity, self-renewal and multipotential differentiation capacities. These cells exhibit therapeutic properties in acute kidney disease. Therefore, we hypothesize that this population exerts biological implications and therapeutic potential for chronic kidney injuries, such as diabetic kidney disease (DKD). We crossed inducible c-Kit reporter mice (c-KitCreERT2/+) with mT/mG and BTBR ob/ob, a murine model of DKD. Through tamoxifen administration, c-Kit cells and their progeny were specifically labeled with green fluorescent protein (EGFP). Thus, we evaluated its spatial distribution and expression of different markers in 20-week-old male and female heterozygous (HT) and ob/ob mice using confocal microscopy and Z-stack analysis of colocalization. We observed c-Kit expression in the glomerulus and in cortical and medullary tubules, and mainly higher percentage of cortical positive tubules in female BTBR ob/ob (12.7 ± 3.6%) in comparison with female HT (7.7 ± 3.4%) (p < 0.05). Moreover, we detected c-Kit colocalization with markers of distal tubules (calbindin and SCL12A3), collecting ducts (calbindin and aquaporin-2), podocytes (α-actinin-4), mesangial cells (PDGF-R), and endothelium (isolectin). Additionally, in male BTBR ob/ob, we observed higher percentage of colocalization (colocalization area/EGFP area) of α-actinin-4 (39 ± 4.7% vs 25.3 ± 7.3% vs male HT; p < 0.05), PDGF-R (51.1 ± 11.3% vs 26.6 ± 12.7% male HT; p < 0.05) and isolectin (61.3 ± 10.7% vs 40.9 ± 14.2% male HT; p < 0.05). Kidney-derived c-Kit⁺ progenitor/stem cells undergo dynamic modulation throughout the progression of DKD and hold great promise as a therapeutic approach for kidney regeneration.

Funding Source: FAPESP 2021/02216-7

Keywords: adult stem cell, tissue regeneration, diabetic kidney disease

111 - B

MESENCHYMAL STEM CELLS PRIMING WITH CANNABIS SATIVA CHEMOVAR II EXTRACT IMPROVES ITS REGENERATIVE POTENTIAL

Frontini Lopez, Yesica Romina Romina¹, Gambarte Tudela, Julián², Frontini Lopez, Yesica Romina², Ruete, María Celeste²

¹IHEM (UNCuyo-CONICET), Mendoza, Argentina,

²Laboratorio de Biomedicina de Fitocannabinoides, IHEM (UNCuyo-CONICET), Mendoza, Argentina

Herbal remedies, extracts, and ointments have been used for centuries to tackle several diseases and conditions. Although various plant derivatives or purified components have been used to improve Mesenchymal Stem Cells' properties (MSCs), priming with Cannabis sativa has been little studied. Evidence employing C. sativa has been generated from studies using synthetic or purified cannabinoids, disregarding the role of terpenes and phenols present in full-spectrum extracts. In this sense, a growing line of thought hypothesizes that the therapeutic efficiency of the complete extracts is more significant due to the synergy of the compounds. Under specific physiological conditions, MSCs contribute to their niche with their paracrine action promoting tissue regeneration and wound healing. Research on the effects of C. sativa extracts on MSCs' properties and their paracrine action can improve their application in regenerative medicine. In this study, we intended to evaluate MSCs viability, proliferation, migration, apoptosis, and activation of signaling pathways primed with C. sativa chemovar II extract. Oil-based extracts (3% w/v) from C. sativa chemovar II inflorescences were obtained. MSCs were primed with different concentrations of the oil-based extract or its vehicle as a control. The two lower concentrations of the extract (150 and 450 ng/mL) increased cell migration and inhibited apoptosis induced by oxidative stress (H₂O₂) and staurosporine. The higher dose (900 ng/mL) of the extract caused the activation of pPDGFRA and pSTAT3 proliferation and immunomodulatory signaling pathways. Since PDGF regulates proliferation and survival of MSCs by activating PI3K/Akt/MAPK/Erk signaling pathway, we assessed this cascade at different levels. We found that the conditioned media of Cannabis-primed MSCs increased phosphorylation levels of pPDGFRA, pAkt, and pErk of MSCs, indicating that a paracrine modulatory program was boosted by Cannabis preconditioning. Our results suggest the ability of C. sativa chemovar II extract to enhance several MSCs' regenerative properties and tip the scales towards a novel therapeutic combined strategy.

Funding Source: Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT), Argentina (PICT-2018-00668).

Keywords: mesenchymal stem cells, cannabis sativa, full-spectrum chemovar II extract

Poster Abstracts

112 - A

SECRETOME DERIVED FROM NON-PRIMED HUMAN UMBILICAL CORD-MESENCHYMAL CELLS ENHANCES NUCLEUS PULPOSUS CELLS EXTRACELLULAR MATRIX CATABOLISM AND INFLAMMATION IN VITRO

Martins Oliveira, Natássia¹, Martins, Júlia¹, Maza, Rafael², Cebinelli, Guilherme³, Oliveira, Alexandre⁴, Vo, Nam⁵, Antonioli, Eliane¹, Lenza, Mario¹, Pohl, Pedro⁵

¹Department of Orthopedics, Hospital Israelita Albert Einstein, São Paulo, Brazil, ²Faculty of Chemical Engineering, Universidade Estadual de Campinas, Brazil, ³Albert Einstein Teaching and Research center, Hospital Israelita Albert Einstein, São Paulo, Brazil, ⁴Institute of Biology, Universidade Estadual de Campinas, Brazil, ⁵Department of Orthopaedic Surgery, University of Pittsburgh, PA, USA

Intervertebral disc degeneration (IDD) has a multifactorial etiology that ultimately leads to progressive loss of nucleus pulposus, imbalance between extracellular matrix composition, excessive oxidative stress, and inflammation in the disc. Mesenchymal stromal cell (MSC) has emerged as a promising therapy for IDD, especially mediated by the secretion of soluble paracrine factors and extracellular vesicles, collectively defined as their secretome. In this study, we aimed to evaluate the effect of umbilical-cord MSC (UC-MSC) secretome through its extracellular vesicles (EVs) and supernatant (SUP) counterpart over human nucleus pulposus (NP) cells extracellular matrix metabolism in vitro. At passage 5/6, characterized UC-MSCs (n=4) were stained with a lipophilic fluorescent dye (Dil) and serum deprived for 60h. The supernatant from four donors either from unstained and Dil-stained UC-MSCs were collected and pooled for differential centrifugation. EVs and SUP fractions obtained after ultracentrifugation were quantified for protein concentration (Bradford). EV characterization was performed by Flow Cytometry (FC), Nanoparticle Tracking Analysis (NTA) and MET. In parallel, NP cells were obtained from intervertebral disc tissues of three patients undergoing discectomy due to disc herniation. At passage 2, NP cells were treated with EV or SUP portion from pooled UC-MSC for 24h to evaluate EV uptake, and for 72h to access anabolic, catabolic, and inflammatory-related gene expression. Confocal imaging showed EVs uptake by NP cells, which was confirmed by flow cytometry with more than 95% of Dil staining from the cells treated with EVs-Dil, compared to NPs treated with EVs unstained. RT-qPCR analysis demonstrated significant upregulation of inflammatory marker IL6, and catabolic markers MMP3 and MMP13 for SUP, compared to non-

treated control (NTC). Concurrently, downregulation of anabolic markers ACAN and COL2A1 was significantly observed for SUP and only COL2A1 for EV, in relation to NTC. SUP fraction, particularly, enhanced nucleus pulposus cells extracellular matrix catabolism and inflammation at mRNA levels. As this is an ongoing project, further evaluation of secretome originated from primed-MSCs to meet the context-specific for IDD, could benefit modulation of key gene expression markers.

Funding Source: This project is financially supported by AO Spine for Dr. Pedro Pohl (AOS-Startup-21-039).

Keywords: intervertebral disc degeneration, mesenchymal stromal cells, secretome

112 - B

THERAPEUTIC POTENTIAL OF HUMAN DENTAL PULP STEM CELLS IN AN ANIMAL MODEL OF PARKINSON'S DISEASE: INSIGHTS INTO NEUROPROTECTION AND FUNCTIONAL RECOVERY

Azevedo, Evellyn M.¹, Fracaro, Letícia², Hochuli, Agner³, Bail, Ellen¹, Ilkiw, Jéssica¹, Lisboa, Mateus³, Brofman, Paulo³, Senegaglia, Alexandra³, Lima, Marcelo¹

¹Physiology, Universidade Federal do Paraná (UFPR), Curitiba, Brazil, ²School of Medicine and Health Sciences, Pontifícia Universidade Católica do Paraná (PUCPR), Curitiba, Brazil

Parkinson's disease (PD) is the second most prevalent neurodegenerative disease worldwide, affecting 2-3% of individuals aged 65 and above. PD is characterized by the death of dopaminergic neurons in the substantia nigra pars compacta (SNpc), leading to motor and non-motor symptoms that impact the patient's quality of life. Current treatments are mainly symptomatic, making dental pulp stem cells (DPSC) a possible and promising option due to their neuroprotective/regenerative properties. This study aimed to evaluate the effects of intranigral infusion of DPSC (differentiated and undifferentiated) on cell survival and the expression of key proteins related to the dopaminergic system in an animal model of PD. This project was approved by the Local Research Ethics Committee (CAAE 28861419.2.3001.0102)/ CEUA 1372). DPSCs were isolated from third molars by enzymatic digestion and characterized by flow cytometry. The classical cytogenetics technique (G-banding) was carried out for the genetic stability of DPSC before inducing neuronal differentiation. Subsequently, the DPSC (both differentiated and undifferentiated) were transplanted into SNpc of rats with PD previously induced by 6-hydroxydopamine. After seven days, an open field test was conducted to evaluate the locomotor behavior, and

Poster Abstracts

subsequently, the animals were euthanized. Immunohistochemistry for tyrosine hydroxylase and western blot for dopaminergic system-related proteins were performed. Our findings demonstrated that isolated DPSC exhibited characteristics of mesenchymal stem cells according to the International Society for Cell and Gene Therapy (ISCT) guidelines, and cells showed normal karyotypes. In the animal model, infusion of DPSCs significantly reversed motor impairment caused by the neurotoxin ($P < 0.05$) associated with the survival of dopaminergic neurons in the SNpc ($P < 0.01$). Within the SNpc, we observed reduced GFAP levels ($P < 0.05$) and p-Thr 34 DARPP-32 levels ($P < 0.05$). Additionally, in the striatum, there was a decrease in JUN expression ($P < 0.05$) and an increase in p-Thr 75 DARPP-32 levels ($P < 0.05$). Collectively, our results suggest that DPSC could significantly impact PD's key proteins and possess potential neuroprotective effects that warrant further exploration in future studies.

Funding Source: CNPq Process: 465656/2014-5 (INCT-REGENERA)

Keywords: dental pulp stem cell, Parkinson Disease, neuroprotection

TRACK: CLINICAL APPLICATIONS OF STEM CELL BIOLOGY

201 - A

COMPARATIVE ANALYSIS OF SARS-COV-2 INFECTION IN INDUCED PLURIPOTENT STEM CELL-DERIVED ASTROCYTES AND NEURONS FROM RECOVERED CENTENARIANS AND SEVERE CASES

de Castro, Mateus V.¹, Assoni, Amanda², de Oliveira, Danyllo², Silva, Monize Valéria², Chianca, Fernanda², Costa, André², Kuriki, Patricia², Malaquias, Vanessa³, Hirata, Mario³ de Castro-Amarante, Maria Fernanda⁴, Ferreira, Luís Carlos⁴, Zatz, Mayana²

¹Institute of Biology, State University of Campinas, Brazil, ²Human Genome and Stem Cell Research Center, University of São Paulo, Brazil, ³Department of Clinical & Toxicological Analyses, School of Pharmaceutical Sciences, University of São Paulo, Brazil, ⁴Laboratório de Desenvolvimento de Vacinas, Instituto de Ciências Biomédicas, University of São Paulo, Brazil

Understanding the great variability associated with SARS-CoV-2 infection, ranging from lethal outcomes to asymptomatic cases has been a great challenge. It is

known that the oldest individuals and people with comorbidities were at the highest risk to develop severe cases of COVID-19. However, our group has identified a cohort of centenarians recovered from COVID-19 or remained asymptomatic, before vaccination (the resilient group). To enhance our understanding of the cellular and molecular mechanisms of resistance and/or susceptibility to infection by the new coronavirus SARS-CoV-2 and the great clinical variability of COVID-19, we have generated astrocytes and neurons from induced pluripotent stem cells from two discordant groups: 5 resilient centenarians versus 5 younger volunteers who had a severe form of COVID-19 which required hospitalization. The cell lines were infected with SARS-CoV-2 (Wuhan strain) at MOI 1.0 for 48h. The experiments were performed in a biosafety level 3 laboratory. In agreement with previous data, we observed that astrocytes were permissive to SARS-CoV-2 infection. Interestingly, our preliminary data showed that the rate of cell infection was higher in the young-adults group (mean = 8%) than in the resilient centenarians (mean = 3%) under the same conditions. In addition, measures of cytokine production after in vitro infections and cell viability assays are currently being evaluated and the data will be present during the ISSCR meeting. The study was approved by the committee for ethics in Research of the Institute of Biosciences at the University of São Paulo (CAAE 34786620.2.0000.5464).

Funding Source: Support by the São Paulo Research Foundation (grant numbers 2013/08028-1, 2014/50931-3, and 2020/09702-1), and the National Council for Scientific and Technological Development (grant numbers 465355/2014-5 and 404134/2020-3).

Keywords: COVID-19, centenarians, SARS-CoV-2

201 - B

DEVELOPMENT OF 3D BIOPATCHES CONTAINING NEUROPROGENITORS FOR CENTRAL NERVOUS SYSTEM INJURY REPAIR

Paskevicius, Gabriela de Oliveira, Zamproni, Laura, Machado, Lucas, Porcionatto, Marimelia

Biochemistry and Molecular Biology, Universidade Federal de São Paulo, (UNIFESP), Brazil

Neural progenitor cells (NPCs) derived from induced pluripotent stem cells (iPSCs) have great potential for tissue repair due to their ability to differentiate and mature into neural cell types. Three-dimensional (3D) bioprinting has the potential for tissue repair, and the combination with human NPCs (hNPCs) suggests new possibilities for therapeutic approaches to repair neural tissue. Three-D bioprinted constructs enable a more precise anchorage for

Poster Abstracts

hNPCs and in vivo intracortical incorporation as a biopatch combining NPCs with matrix-like material to aid regeneration after injury. The main objective of this project is to fabricate and study cellular and molecular characteristics of 3D constructs containing hNPCs or biopatches, in a traumatic brain injury (TBI) mouse model. Mice were submitted to the TBI model and divided into five groups: G1 - sham; G2 – TBI, no treatment; G3 – TBI + 3D biopatches; G4 – TBI + constructs without cells; G5 – isolated hNPCs. To fabricate the 3D biopatches, hNPCs at P7 were bioprinted at a density of 12-15x10⁶ cells/mL and cultured in neural expansion medium (NEM). The biopatches were cultured for seven days in NEM. We observed the proliferation of hNPCs, self-organization, and the formation of extensions within the construct, confirming the viability of the cells in the construct. The biopatches were implanted at the moment of surgery and kept in the mice for 15 days. hNPCs migration and differentiation were analyzed by immunofluorescence, and CXCL12, PROK2, and VEGF expression were quantified by qPCR. Mice that received constructs and biopatches had remnants of the constructs in the craniotomy area, indicating possible degradation of the constructs/biopatches. In addition, the confirmation of the lesion was observed by viewing the depression at the insertion site of the metal rod associated with a “whitish” expansion area. The approximate size of the lesion area, i.e., depression + tissue expansion, in all animals, is 2mm in diameter. The results obtained in this project will be used as a basis for the study of other aspects involving this cell type applied to tissue engineering in the biopatch and the subsequent development of a new therapeutic approach to use this scientific basis in different animal models to develop and evolve a tissue repair method that can be used in humans.

Funding Source: Grants and fellowships 2018/12605-8, 2022/16047-5 and 2021/14327-8 from FAPESP; 406258/2022-8 from CNPq. Attendance at this symposium was also supported by São Paulo School of Advanced Science on Stem Cell Biology.

Keywords: biopatches, bioprinting 3D, neuroprogenitors

202 - A

GENERATION OF CAR-T CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS (iPSC) USING A FEEDER FREE METHODOLOGY

Correia Croda Catharino, Luana¹, do Amaral Costa Ribeiro, Paula¹, Coeli Menezes Evangelista, Gabriela², Ribeiro Almeida, Rafael¹, Rocha, Vanderson¹, Gremen Mimary Oliveira, Theo¹, Nalio Ramos, Rodrigo¹, Alexandre Marzagão Barbutto, José²

¹Department of Hematology and Cell Therapy, Laboratory of Medical Investigation in Pathogenesis and Directed Therapy in Onco-Immuno-Hematology (LIM-3¹) / Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Brazil, ²Department of Immunology, Institute of Biomedical Sciences / University of São Paulo, Brazil

Among the immunotherapies, the CAR-T cell therapy has been standing out in the past few years, given the promising results obtained for hematologic malignancies. The insertion of a CAR (Chimeric Antigen Receptor) molecule in the T cells provides an antigen specific and MHC (Major Histocompatibility Complex) independent antigen recognition. The CAR-T cell products currently approved for clinical use are obtained from autologous sources. However, the use of allogeneic sources could overcome many manufacturing difficulties, such as the high cost and extensive production time, and the presence of dysfunctional T cells and limited number of lymphocytes obtained from the patients. The use of human iPSCs (Induced Pluripotent Stem Cells) for the generation of the CAR-T cells is an interesting alternative, since they can be obtained by the reprogramming of different somatic cells, can proliferate with indefinite potential and also have good susceptibility to genetic manipulation. For the iPSCs differentiation to T cells, various methodologies have been described in the literature. However, limitations such as the need to use murine feeder-cells restrict the future translational potential of these approaches. Therefore, this project aims to generate CAR-T cells from iPSC using a feeder-free methodology. So far, an iPSC clone has been expanded and differentiated to CD34⁺ hematopoietic progenitor cells through the formation of embryoid bodies (EBs), using an ultra-low attachment plate and StemPro-34 SFM medium, supplemented with GlutaMAX, 2-mercaptoethanol, NEAA and L-Ascorbic acid. The cytokines BMP4, FGF2, VEGF, SCF, FLT3L and IL3 were added in the culture in different days throughout the differentiation process. At differentiation day 9, the EBs were dissociated and the resultant cells were evaluated by flow cytometry. Our preliminary data showed that this differentiation process led to the generation of about 7,86%

Poster Abstracts

(\pm 3,41) of CD34+ cells. In future steps, these CD34+ cells will be FACS-sorted, expanded and used for further differentiation to T cells. This methodology will also be reproduced with iPSCs already transduced and expressing the CAR molecule, aiming to generate CAR-T cells in the end of the differentiation process.

Funding Source: CNPq 130036/2023-4, PRONON 25000.027785/2021-21, CNPq 442676/2020-4, FAPESP 20/07055-9, FAPESP 13/08135-2

Keywords: human induced Pluripotent Stem Cells (iPSC), CAR-T cells, immunotherapy

202 - B**GENERATION OF DENDRITIC CELLS EXPRESSING CAR FROM INDUCED PLURIPOTENT CELLS: AN ALTERNATIVE TREATMENT FOR CANCER**

Costa Ribeiro, Paula Do Amaral¹, Coeli Menezes Evangelista, Gabriela¹, Correia Croda Catharino, Luana¹, Gremen Mimary de Oliveira, Théo², Nalio Ramos, Rodrigo², Ribeiro Almeida, Rafael², Rocha, Vanderson²

¹*Department of Immunology, Institute of Biomedical Sciences of University of São Paulo (ICB-USP), Brazil,*

²*Department of Hematology and Cell Therapy, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Brazil*

Cell therapy is a branch of immunotherapy that has been growing in recent decades and shows to be a promising strategy to treat tumors. The cell therapy modality that has grown the most in recent years is therapy based on T cells expressing Chimeric Antigen Receptor (CAR-T). Six products of CAR-T have been approved by the FDA (Food and Drug Administration, USA), all for hematological tumors. Despite the success of CAR-T therapy in hematological tumors, the same is not reported in solid tumors. To increase the antitumor response in solid tumors other immune cells are being explored to receive CAR. Dendritic cells (DC), are a heterogeneous population specialized in the antigen presentation, with great migratory capacity and that promote the stimulation of the adaptive immune response, being good candidates to receive the CAR. Among the subtypes, there are: cDC1 and cDC3, which can, respectively, promote cross-presentation of antigens and induce a resident memory phenotype by stimulating antitumor CD8+ T lymphocytes. However, due to their scarcity in peripheral blood and their low proliferative potential, an “off-the-shelf” source is necessary to enable the use of these cells in cell therapy. The use of induced pluripotent cells (iPSC) as a source of dendritic cells can overcome these obstacles, as they have an indefinite potential for self-renewal and proliferation, in

addition to being easily genetically modified. Therefore, our project's main objective is to generate a platform to produce cDC1 and cDC3 cells expressing CAR from iPSC cells with the aim of developing a new cell therapy strategy aimed, in the long term, at the clinical use of these cells in patients with cancer. We are validating the iPSC transfection protocols, our data suggest that the electroporation have a better transfection efficacy than the lipofections.

Funding Source: PRONON, grant number NUP#: 25000.027785/2021-21, Conselho Nacional de Desenvolvimento Científico e Tecnológico, grant number 442686/2020-4 and São Paulo Research Foundation (FAPESP) grant #20/07055-9 and #13/08135-2

Keywords: dendritic cells, chimeric antigen receptor (CAR), induced pluripotent cells (iPSC)

203 - A**HIGH FREQUENCY OF LEUKEMIC STEM CELLS ARE ASSOCIATED WITH UNFAVORABLE PROGNOSIS IN A BRAZILIAN COHORT OF ACUTE MYELOID LEUKEMIA WITH NORMAL KARYOTYPE**

Medeiros, Mariana¹, Marani, Leticia², Costa, Amanda Fernandes¹, Garcia, Camila Araujo¹, Jacovelli, Gustavo², Silva, Fernanda², Madeira, Maria Isabel², Scheucher, Priscila², Lima, Ana Silvia², Pagnano, Katia³, Kerbauy, Fabio⁴, Feliciano, João Vitor⁵, Higashi, Marcia⁶, Nunes, Elenaide⁷, Glória, Ana Beatriz⁸, Rego, Eduardo⁹, Traina, Fabíola², Figueiredo-Pontes, Lorena²

¹*Hematology Division, Department of Medical Images, Hematology, and Clinical Oncology, University of São Paulo, Ribeirão Preto, Brazil,* ²*Hematology, Hemotherapy and Cellular Therapy Division, Department of Medical Imaging, Hematology, and Clinical Oncology, University of São Paulo, Ribeirão Preto, Brazil,* ³*Hemocentro, University of Campinas, Brazil,* ⁴*Hematology Division, Federal University of São Paulo, Brazil,* ⁵*Bone Marrow Transplant Center, Hospital de Base, Sao José do Rio Preto, Brazil,* ⁶*Amaral Carvalho Hospital, Jaú, Brazil,* ⁷*Hematology Division, University Hospital of the Federal University of Paraná, Curitiba, Brazil,* ⁸*Hematology Division, Hospital das Clínicas da Universidade Federal de Minas Gerais, Belo Horizonte, Brazil,* ⁹*Internal Medicine, Medical School of the University of São Paulo, Brazil*

Normal karyotype in Acute Myeloid Leukemia (NK-AML) is present in 40-50% of patients. Prognosis is less predictable for these patients, thus prognostic markers in NK-AML could improve disease assessment and therapeutic approaches. Since leukemic stem cells (LSCs) have been associated with AML initiation and persistence, we aimed

Poster Abstracts

to correlate the frequency of LSCs by multiparametric flow cytometry at diagnosis with known prognostic factors and to assess its impact on overall survival (OS). Clinical and laboratorial data, ELN2017-based risk stratification (excluding TP53 and ASXL1), and OS data were collected from 54 bone marrow samples of NK-AML from 7 Brazilian centers (Feb-2015 to Jan-2020). FLT3 and NPM1 mutations were assessed by RT-qPCR, karyotype by classical cytogenetics. LSCs were considered as CD34+/CD38-/low/CD123+ cells among total blast cells. Statistical analysis were performed by GraphPad Prism v.8.0 ($p < 0.05$ as significant). Patients were classified into two groups according to their percentage of LSCs at diagnosis. A percentage of LSCs $> 0.1\%$ was associated with higher WBC ($49.2 \times 10^3/\mu\text{L}$ vs. $13.6 \times 10^3/\mu\text{L}$, $p=0.02$), with NPM1mutFLT3mut ($n=13$; 88.9% vs. 11.1%, $p < 0.001$), and with high FLT3-ITD allelic ratio ($n=6$; 44.4% vs. 4.4%, $p < 0.001$). The NPM1wtFLT3wt status was associated with LSC $< 0.1\%$ (66.7% vs. 0%). FLT3 was associated with LSC $> 0.1\%$ ($p=0.0174$) and NPM1 with LSC $< 0.1\%$ ($p=0.0028$). LSCs showed intensified CD123 MFI when compared to bulk ($p=0.004$) and blasts ($p=0.0016$). Frequency of LSC and CD123 MFI independently were higher in NPM1mut ($p < 0.0001$ and 0.0038) and FLT3mut ($p < 0.0001$ and 0.0018) patients. Combined NPM1 and FLT3-ITD mutation patients showed CD123 MFI higher than all other genotypes ($p < 0.0001$). The complete remission rate was 63%: 69% ($n=31$) in the LSC $< 0.1\%$ group and 34% ($n=3$) in the LSC $> 0.1\%$ group. The LSC $> 0.1\%$ group had 6.3 months OS, while a 24.2 months OS in the LSC $< 0.1\%$ group. Among intermediate-risk patients ($n=31$), those with LSC $> 0.1\%$ had 7.1 months OS, whereas the LSC $< 0.1\%$ group had a 23.9 months OS. Among adverse-risk patients, LSC $> 0.1\%$ group had 3 months OS and LSC $< 0.1\%$ group had 2.3 months OS. The Log Rank (Mantel-Cox) demonstrated equality of survival distributions between LSC $< 0.1\%$ or LSC $> 0.1\%$ groups within the total cohort and within each risk group. Our findings showed that a higher frequency of LSCs at diagnosis was associated with higher leukocytosis, NPM1mutFLT3mut status, and higher FLT3-ITD allelic ratio, reinforce the potential value of LSC quantification as an easily appraisable prognostic factor at diagnosis that may be further evaluated in AML measurable residual disease time points and help with therapeutic decisions.

Keywords: Acute Myeloid Leukemia, CD123, Leukemic Stem Cells

203 - B

IN VIVO AND EX VIVO CHARACTERIZATION OF TRANSPLANTED HUMAN iPSC-DERIVED DOPAMINE NEURONS FOR THE TREATMENT OF PARKINSON'S DISEASE

Paladini, Carlos Antonio

Neurology, BlueRock Therapeutics, New York City, NY, USA

Parkinson's Disease (PD) is marked by loss of dopaminergic cells in the ventral midbrain that results in pathophysiological motor and cognitive deficiencies. Over the last decade, cell therapies have aimed to replace the lost dopamine (DA)-producing cells to reverse the pathophysiological symptoms of PD. Induced pluripotent stem cell (iPSC)-derived progenitor DA neurons transplanted into the striatum of PD model animals show great promise as an effective treatment for PD. To become an effective therapy, the transplanted cells must contain a sufficient number of DA-producing neurons that can properly integrate into the host brain. An important part of this requires transplanted cells to make synaptic connections with the host brain for optimally timed DA release during motor initiation. This functional integration is critical for long-term functional efficacy. Here, we characterize a research-grade human iPSC-derived DA neuron cell therapy product, DA02, applying behavioral, molecular, and functional approaches during cell engraftment and maturation. We found that DA02-transplanted rats demonstrate complete rescue of amphetamine-induced rotations in 6-OHDA lesioned rats at four-month timepoint post-engraftment. Using gene expression approaches, we show that DA02 cells contain mature markers of dopaminergic neurons. Furthermore, we observe no evidence of off-target neurons such as serotonergic or GABAergic cells. Using patch clamp electrophysiology, we observe spontaneous synaptic events that indicate proper graft-host interactions. Features of intrinsic excitability such as action potential waveform are also similar to those of canonical DA neurons. In summary, these data provide important evidence of proof-of-concept for feasibility of an iPSC-derived DA cell therapy product for the treatment of PD.

Keywords: dopamine, Parkinson's Disease, iPSC

Poster Abstracts

204 - A

PLASMA-DERIVED EXTRACELLULAR VESICLES DELAY STEM CELL DERIVED HUMAN BETA CELL TRANSPLANT REJECTION IN IMMUNOCOMPETENT C57BL/B6 MICE**Peterson, Quinn, Khashim, Zenith***Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, MN, USA*

Islet transplantation is a promising approach to treating type 1 diabetes. Recent advances in the development of stem cell-derived beta cells enable the production of consistent cell products for transplantation. Despite these advances, graft immune rejection remains a hurdle. Extracellular vesicles (EVs) have been hypothesized to possess immunomodulatory properties that may enhance immunotolerance, particularly in the context of islet transplantation. Plasma-derived extracellular vesicles (PEV) are readily obtainable from blood and represents a promising source for biomedical investigations. Here we report the immunoregulatory characteristics of PEVs in both a transplant model of stem cell-derived β cell (SC- β cells) and in human peripheral blood mononuclear cells (PBMCs). This study investigated the effects of PEVs on SC- β cells transplanted into the kidney capsule of immunocompetent mice and PBMCs. SC-beta cells were transplanted into the left kidney capsule of C57BL/6 mice alone or with PEVs to assess the effect of PEV on graft tolerance. Blood samples were collected from mice at various time points to monitor the presence of the transplanted graft, and immune cell infiltration and inhibition was evaluated using IF and qPCR. The impact of PEV on immune cell infiltration was assessed in xenogeneic, syngeneic, and allogeneic animal models and the impact of PEV on human PBMCs was also characterized. Treatment with PEV prolonged the survival and secretion of human insulin from transplanted SC- β cells. The analysis of cellular and gene expression revealed that PEV affected the recruitment and infiltration of several different immune cells, including T cells, CD45, macrophages, and natural killer (NK) cells. PEV exhibited a potent inhibitory effect on NK cells suggesting a role for dampened NK cells in graft survival. We demonstrate the immunoprotective properties of PEV, delaying SC- β cell graft rejection and hypothesize a role for NK cell and other immune cells inhibition in graft survival. Collectively, these findings enhance our understanding of immune cell behavior in SC- β cell transplantation and offer valuable insights for the development of more effective tolerogenic treatments for cell replacement therapies.

Keywords: type 1 diabetes, transplant, islet

204 - B

POSSIBLE TUMOUR TROPISM AND TARGETING OF TRIPLE NEGATIVE BREAST CANCER STEM CELLS USING HEMATOPOIETIC STEM CELLS AND CONDITIONED MEDIA IN VITRO**Mallick, Sumit, Bose, Bipasha, Shenoy, Sudheer***Stem Cell and Regenerative Medicine Centre, Yenepoya Research Centre, Yenepoya (Deemed to be University), Mangalore, India*

Hematopoietic stem cells (HSCs) are indispensable for life. They are responsible to maintain the blood cells pool in our body. Their unique features are that they can migrate to inflammatory sites and other body organs during any injuries, and release various cytokines which leads to reduce the inflammation and tissue injuries and helps in wound healing process. From this characteristic, we have hypothesized that hematopoietic stem cells can show tumour tropism and can change the metabolic alteration in cancer and cancer stem cells focusing on Triple Negative Breast Cancer Stem Cells (TNBC CSCs). TNBC is a subtype of breast cancer that does not express estrogen receptor (ER), progesterone receptor (PR), or HER2/neu. Therefore, it is difficult to treat with traditional hormone therapies or targeted therapies. TNBC-SCs are a subpopulation of cancer cells responsible for tumour initiation, maintenance, and relapse in our body. Our study indicating that haematopoietic stem cells derived conditioned media cytokines are interacting with triple-negative breast cancer stem cells by involving with various molecular pathways of TNBC cells. Haematopoietic stem cells derived conditioned media is downregulating the cell cycle synthesis phase and triple negative breast cancer stem cells proliferation. We have used bioinformatics tools such as Cytoscape and the string database for pathway analysis; and for in vitro studies, we have sorted CD34+/CD45+ stem cells from haematological cell line and performed conditioned media culture and co-culture assays. Further we have performed metabolomics studied to understand the cytokines-cytokines interaction between hematopoietic stem cells and TNBC cells to reveals the specific molecular pathway which can be further helpful for targeted drug delivery.

Funding Source: Yenepoya (Deemed to be University) Indian Council of Medical Research**Keywords:** hematopoietic stem cells, triple negative breast cancer stem cells, metabolomics

Poster Abstracts

205 - A

TREATMENT OF ACUTE RESPIRATORY DISTRESS SYNDROME CAUSED BY COVID-19 WITH HUMAN UMBILICAL CORD MESENCHYMAL STEM CELLS

Shablii, Volodymyr¹, Bukreieva, Tetiana², Svitina, Hanna², Nikulina, Viktoriia², Vega, Alyona³, Chybisov, Oleksii⁴, Shablii, Iuliia⁵, Ustymenko, Alina⁶, Nemtinov, Petro², Lobyntseva, Galyna², Skrypkina, Inessa⁵

¹Centre of Science, Institute of Cell Therapy, Kyiv, Ukraine, ²Placenta Stem Cell Laboratory, Centre of Science, Institute of Cell Therapy, Kyiv, Ukraine, ³Department of Infectious Diseases, Shupyk National Healthcare University of Ukraine, Kyiv, Ukraine, ⁴Endoscopic Unit, CNE Kyiv City Clinical Hospital # 4, Kyiv, Ukraine, ⁵Laboratory of Biosynthesis of Nucleic Acids, Institute of Molecular Biology and Genetics of NAS of Ukraine, Kyiv, Ukraine, ⁶Laboratory of Cell and Tissue Cultures, Department of Cell and Tissue Technologies, Institute of Genetic and Regenerative Medicine, Kyiv, Ukraine

This study aimed to identify the impact of mesenchymal stem cell transplantation on the safety and clinical outcomes of patients with severe COVID-19. This research focused on how lung functional status, miRNA, and cytokine levels changed following mesenchymal stem cell transplantation in patients with severe COVID-19 pneumonia and their correlation with fibrotic changes in the lung. This study involved 15 patients following conventional anti-viral treatment (Control group) and 13 patients after three consecutive doses of combined treatment with MSC transplantation (MCS group). We confirmed that triple MSC transplantation in individuals with severe COVID-19 was safe and did not cause severe adverse reactions. The total score of lung CT between patients from the Control and MSC groups did not differ significantly on weeks 2, 8, and 24 after the beginning of hospitalization. However, on week 48, the CT total score was 12 times lower in patients in the MSC group ($p \leq 0.05$) compared to the Control group. In the MSC group, this parameter gradually decreased from week 2 to week 48 of observation, whereas in the Control group, a significant drop was observed up to week 24 and remained unchanged afterward. In our study, MSC therapy improved lymphocyte recovery. The percentage of banded neutrophils in the MSC group was significantly lower in comparison with control patients on day 14. Inflammatory markers such as ESR and CRP decreased more rapidly in the MSC group in comparison to the Control group. The plasma levels of surfactant D, a marker of alveocyte type II damage, decreased after MSC transplantation for four weeks in contrast to patients in the Control group, in whom slight elevations were observed. We first showed that MSC

transplantation in severe COVID-19 patients led to the elevation of the plasma levels of IP-10, MIP-1 α , G-CSF, and IL-10. MSC transplantation had no impact on the relative expression levels of miR-146a, miR-27a, miR-126, miR-221, miR-21, miR-133, miR-92a-3p, miR-124, and miR-424. In vitro, UC-MSC exhibited an immunomodulatory impact on PBMC, increasing neutrophil activation, phagocytosis, and leukocyte movement, activating early T cell markers, and decreasing effector and senescent effector T cell maturation.

Funding Source: This study was funded by the NRFU 2020.01/0246 "Study of the state of respiratory, cardiovascular and immune systems in patients with COVID-19 pneumonia after transplantation of cryopreserved allogeneic mesenchymal stem cells."

Keywords: COVID-19, acute respiratory distress syndrome, mesenchymal stem cells

TRACK: DEVELOPMENT

301 - A

BASAL CELL EXTRUSION PRIMES PLURIPOTENT CELLS FOR DIFFERENTIATION

Rosa, Viviane¹, Sato, Nanami¹, Makhlof, Aly¹, Kretzmer, Helene², Kumar, Abhishek², Grosswendt, Stefanie², Mattei, Alexandra², Courbot, Olivia³, Wolf, Steffen¹, Langevin, Frederic⁴, Wiacek, Michal⁵, Karpinski, Daniel⁵, Elosegui-Artola, Alberto³, Meissner, Alexander², Zernicka-Goetz, Magdalena⁶, Shahbazi, Marta¹

¹Cell Biology, MRC - Laboratory of Molecular Biology, Cambridge, UK, ²Max Planck Institute for Molecular Genetics, Berlin, Germany, ³Cell and Tissue Mechanobiology Laboratory, The Francis Crick Institute, London, UK, ⁴Transgenic Facilities, MRC - Laboratory of Molecular Biology, Cambridge, UK, ⁵Biological Services Group, MRC - Laboratory of Molecular Biology, Cambridge, UK, ⁶University of Cambridge, Cambridge, UK

Mouse gastrulation starts after implantation at embryonic day 6.5. In response to high levels of Wnt, Bmp, and Nodal, epiblast cells undergo apical constriction followed by basal extrusion, and ingress through the primitive streak, giving rise to mesoderm and endoderm progenitors. The mechanisms underlying the coordination between differentiation and loss of epithelial integrity at gastrulation remain poorly understood due to the complexity of dissecting these events in vivo. To fill this knowledge gap, we established a new embryonic stem cell culture condition that maintains epiblast stem cells in a self-

Poster Abstracts

renewing 3D epithelial state, recapitulating the transcriptional and architectural features of the gastrulating epiblast. Single-cell transcriptomics and image analyses revealed that approximately 5% of cells express primitive streak markers and are basally extruded, despite the presence of homogenous culture conditions. Functional analyses in combination with microfabrication and in vivo experiments showed that upon proliferation-mediated crowding, cells that express high levels of the apical polarity protein aPKC undergo basal cell extrusion. This morphological transition primes pluripotent cells for primitive streak fate acquisition in a Wnt-dependent manner, indicating that extrusion decreases the signalling threshold required to activate this pathway. Therefore, the coupling between shape and fate via modulation of signalling safeguards differentiation at the primitive streak.

Keywords: pluripotency, gastrulation, differentiation

301 - B

CEREBRAL ORGANOID CRISPR SCREEN OF HUMAN AND PRIMATE SPECIFIC GENES IDENTIFIES A NEURODEVELOPMENTAL ROLE FOR SERF1A/B

Trabish, Sophie, Neely, Greg, Moreno, Cesar

School of Life and Environmental Sciences, University of Sydney, New South Wales, Australia

The molecular orchestration of human and non-human primate brain development is complex and not completely understood. In this study, we conducted a loss-of-function screen of 40 previously uncharacterized human and primate unique genes to identify new candidates involved in primate neurodevelopment. To that end, we combined CRISPR/Cas9-based approach that incorporates molecular barcodes amenable to screening and stem cell-derived 3D cortical forebrain organoids. Using next-generation sequencing of pooled brain organoids collected at various developmental stages, we identified nine candidate genes with potential to disrupt normal human forebrain development. One of these genes, SEFF1A/B is a primate-specific gene duplication, encoding a protein of unknown function that is predicted to be involved in nervous system development. Its conserved ancestral copy was previously shown to be involved in protein binding and destabilization of disease associated amyloidogenic proteins and in amyloid fibril formation and proteotoxicity. Our screen found that depletion of SERF1A/B coincides with neuroepithelium expansion. We tested this hypothesis by generating loss-of-function SERF1A/B brain organoids in two SERF1A/B KD iPSC lines which led to the production of smaller cerebral organoids. Mechanistically, a reduction of

SERF1A/B leads to decreased expression of the transcription factor SOX2 and upregulation of the WNT/ β -catenin signaling pathway following neural induction, which might account for their diminished size. Moreover, SERF1A/B depletion leads to an increased NEUN expression indicating premature neuronal differentiation and maturation. Overall, these results support a mechanism by which SERF1A/B activity contributes to the temporal regulation of brain maturation that is characteristic of primates and humans.

Keywords: Human cerebral organoids, CRISPR/Cas9 screen, Neurodevelopment

302 - A

ESTABLISHMENT OF NOVEL NAIVE HUMAN EMBRYONIC STEM CELL LINES TO INVESTIGATE X CHROMOSOME INACTIVATION IN HUMAN DEVELOPMENT

Felissimo, Daniel Minatti¹, Macedo, Taiany², Tófoli, Fabiano¹, Orsi, Ana Elisa¹, Martins, Karina², Lorenzon, Aline², Pereira, Lygia¹

¹Department of Genetics and Evolutive Biology / Institute of Biosciences, University of São Paulo, Brazil, ²Research Department, Huntington Medicina Reprodutiva—Eugin Group, Brazil

Human development has long intrigued the scientific community, posing significant challenges due to the absence of accurate models capable of representing the complexities of living human organisms, including intricate cell signaling and organization dynamics. The advent of pluripotent stem cells (PSCs) has improved our possibilities to study human development and diseases, but still a major hurdle remains in studying the process of human X chromosome dosage compensation (XC-DC) in female embryos, which is triggered during early preimplantation development. Murine PSCs are found in a pre-XC-DC state and undergo random X chromosome inactivation (XCI) upon in vitro differentiation. In contrast, human PSCs in general have one inactive X (iX), or a reactivated iX (eroded iX, eX), which is compatible with a primed state of pluripotency. These primed hPSCs can be reprogrammed into a developmentally earlier naive stage, similar to mPSCs, where both X chromosomes become active. Nevertheless, upon differentiation these cells tend to inactivate the original iX, indicating the maintenance of epigenetic marks of XCI in the iX. Our group and others have shown that in humans the process of XC-DC (either by X chromosome dampening or XCI) is triggered between the morula and early blastocyst stages. Therefore, in order to develop an in vitro model to study human XC-DC, we

Poster Abstracts

propose the establishment of naive hPSCs lines directly from morulas, which have not initiated any kind of XC-DC. In collaboration with an IVF clinic, we have been able to extract cells from human embryos at the E4 stage (morulas) and placed them in different human naive PSCs culture medium. Our first test in feeder-free conditions has shown that clumped blastomeres were able to adhere and form dome-shaped-like colonies, but did not proliferate as expected. Different culture conditions will be tested next, and once established, the female naive hESCs lines will be characterized for the state of X-linked gene activity.

Funding Source: I would like to acknowledge the São Paulo Research Foundation (FAPESP) and the Coordination for the Improvement of Higher Education Personnel (CAPES) for the financial support to the execution of this research project.

Keywords: naive human embryonic stem cells, X chromosome dosage compensation, epigenetics

302 - B

EXAMINING HOW DIFFERENT MEDICATIONS AND ELECTROLYTES AFFECT PLURIPOTENT STEM CELL TO CARDIOMYOCYTE DIFFERENTIATION

Ganguly, Mohuli Roy¹, Parga, Elizabeth²

¹CARLOW Center for Medical Innovation, La Salle High School, Pasadena, CA, USA, ²Science Department, CARLOW Center for Medical Innovation, Pasadena, CA, USA

Induced pluripotent stem cells (iPSCs) are reprogrammed embryonic cells that can be differentiated into other cell types. For this experiment, HiPSCs were differentiated into cardiomyocytes to observe its development. Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) can be used to model cardiac pathophysiology and personalize medical treatment on a case-by-case basis to yield better patient outcomes. However, during differentiation, the hiPSC-CMs fail to reach an adult stage and remain as fetal cardiomyocytes that cannot be used for patient treatment or for research. Thus, this experiment observed how three naturally occurring electrolytes [Potassium, Magnesium, and Sodium] and three commonly prescribed medications [Metoprolol, Aspirin, and Digoxin] impacted differentiation. All supplements were diluted into 0.5mM, 5mM, and 10mM concentrations and, then added to the differentiation medium. Incorporating the supplements during differentiation allowed for the active observation of cardiomyocytes responding to the varying supplements and eventual confirmation of their positive influence on the robustness of the developed cells. My results showed that

the cells that had Metoprolol added at a 10mM concentration and, separately, MgCl added at 10mM, had the most differentiation of mature cardiomyocytes. This was measured by immunolabeling each well and staining them with TBX5. TBX5 is a key transcription factor necessary for the development of cardiomyocytes and the formation of the cardiac conduction system as the cells mature. By measuring the expression of TBX5 during the induction of HiPSCs into cardiomyocytes, we can track the effects of various treatments on the ability to allow for the formation of mature cardiomyocytes from HiPSCs. Furthermore, Metoprolol and magnesium-chloride significantly increased mature cardiomyocyte yield as compared to the control of the experiment. Thus, it can be concluded that the medication Metoprolol and electrolyte Magnesium could potentially allow for larger yields of developed cardiomyocytes. This experiment added to research on how electrolytes and medications impact the heart muscles at their most basic, cellular level.

Funding Source: CARLOW Center for Medical Innovation at La Salle College Preparatory

Keywords: cardiomyocytes, differentiation, electrolytes/medications

303 - A

EXPRESSION PROFILE OF A POTENTIAL PREDICTOR OF CONGENITAL SYNDROME ENDOPHENOTYPE NAMED NUCLEAR DISTRIBUTION ELEMENT ASSOCIATED GENES IN BRAIN ORGANIDS INFECTED BY ZIKA VIRUS AND EFFECTS OF TREATMENT

Hayashi, Mirian¹, Garcez, Patricia², Krenn, Veronica³, Nani, João⁴

¹Pharmacology, EPM/, Universidade Federal de São Paulo (UNIFESP), São Paulo, Brazil, ²Institute of Biomedical Sciences, UFRJ, RJ, Brazil, ³Department of Biotechnology and Bioscience, University of Milan-Bicocca, Milan, Italy, ⁴Pharmacology, Universidade Federal de São Paulo (UNIFESP), São Paulo, Brazil

Maternal infections are among the main risk factors for cognitive impairments and fetal brain malformation in the offspring. The iPSCs have emerged as a powerful tool in research, offering new avenues to study disorders at a cellular and molecular level. One of the significant advantages of iPSCs is their ability to differentiate into various cell types, including neurons. Brain organoids, also referred to as cerebral organoids, have emerged as a promising tool for modeling neurodevelopmental disorders

Poster Abstracts

in the laboratory, as they recapitulate the cell composition and 3D environment of the embryonic human brain. These three-dimensional structures are derived from pluripotent stem cells and offers a more physiologically relevant environment compared to conventional cell culture models, and brain organoids provide a unique opportunity to study the developmental and functional abnormalities associated with neurotropic virus infections, such as those by zika virus (ZIKV) which can led to the congenital syndrome of ZIKV (CSZ) or microcephaly. In fact, it was showed that viruses can efficiently replicate in brain organoids and attenuate their growth, also allowing to demonstrate by transcriptional profiling the distinct cellular responses for ZIKV compared with another virus. In addition, the brain organoids growth attenuation could be also rescued by distinct type I interferons (Krenn et al. 2021, Cell Stem Cell). Considering the several important roles widely recognized for the cytoskeleton proteins, such as the NDE1 (Nuclear Distribution Element 1, also known as NudE) and NDEL1 (NDE-Like 1, also known as NudEL) that are encoded by mammalian paralog genes showing important and at least partially overlapping roles for brain development, we present here the RNA expression profile of these genes and correlated ligands aiming to understand the pathways involved in microcephaly phenotype induced by ZIKV infection in brain organoids, also considering the response to the treatment. We show here that brain organoids can provide a valuable platform for studying neurospecific effects of ZIKV infections in the laboratory settings due to their ability to replicate many aspects of human brain development, offering clear advantages over neuroblastoma and iPSC derived neurons.

Funding Source: This work was supported by FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo), CAPES and CNPq. Work in V.K.'s laboratory is funded by the Human Technopole Early Career Fellowship Programme (HT-ECF Programme).

Keywords: zika virus, nuclear distribution element, brain organoids

303 - B

GMP-COMPLIANT PROCESS FOR ISOLATION AND EXPANSION OF HUMAN WHARTON'S JELLY MESENCHYMAL STROMAL CELLS TO ENABLE CLINICAL TRANSLATION IN COLOMBIA

Quintero, Carolina¹, Halpert, Karolynn², Rendón, Kelly³, Sanchez, Vanesa¹, Jaraba, Wendy³, Ortega, Hector¹, López, Lucas²

¹Cell bank, BioXtech, Medellin, Colombia, ²BioXcellerator, Medellin, Colombia, ³BioXscience Research and Innovation Hub, BioXcellerator, Medellin, Colombia

Mesenchymal stromal cell manufacturing must adhere to Good Manufacturing Practices (GMP) to ensure compliance with quality standards and achieve successful transition from preclinical research to the development of cell-based therapies for preclinical and clinical use. In the world, GMP regulatory system is in the process of strengthening and harmonization to facilitate the adoption by competent authorities so that manufacturers may achieve marketing authorization in countries where there is no regulatory framework, such as Colombia and other Latin American countries. Because adherence to GMP guidelines in stem cell culture is crucial for ensuring the safety, consistency, and efficacy of therapies, our objective was to establish a GMP strategy to guarantee the isolation and expansion of umbilical cord Wharton's Jelly MSC (WJ-MSC). Based on a previous experience in a small-scale cell bank, the set-up of a GMP-compliant laboratory for clinical-grade expansion of WJ-MSC began in 2020. Upgrading the facilities, hiring, and training qualified personnel, standardizing operating procedures, ensuring traceable validated materials and equipment maintenance and thorough quality control measures were necessary to achieve the GMP conditions in which thirty-one clinical-grade MSC cultures were completed in 2 years of operation. WJ-MSC primary cultures were obtained using OptiMEM® medium and expanded in culture medium supplemented with human Platelet Lysate until passage 7. WJ-MSC expressed MSC markers CD105, CD73 and CD90 greater than 95% and hematopoietic markers in less than 2.0%, in vitro differentiation to mesodermal lineage and microbiological tests were also conducted. Additional quality attributes were evaluated, such as senescence, differentiation to ectodermal lineage (confirmed by positive expression of neural markers) and a preliminary exosome characterization (showing up to 34 exosome exclusive proteins). The results obtained suggest that large clinical-scale expansion of WJ-MSC under GMP conditions allowed the obtention of a high-quality and homogeneous cellular product with reproducible characteristics and the potential to be used in preclinical and clinical translation protocols.

Keywords: good manufacturing practice, Wharton's Jelly mesenchymal stromal cells, cell manufacturing

Poster Abstracts

304 - A

HUMAN ERYTHROPOIETIN PHARMACEUTICALS PRODUCTS POTENCY: AN IN VITRO ANALYTICAL METHOD IMPLEMENTATION

Paes de Camargo, Bruno T.¹, Brunoro, Giselle², Lopes, Patricia³, Andreo-Filho, Newton³, Leite-Silva, Vania³

¹*Pesquisa e Desenvolvimento Biotecnológico, Universidade Federal de São Paulo (UNIFESP), Crotia, Brazil*, ²*Pesquisa e Desenvolvimento Biotecnológico, Blau Farmacêutica, Cotia, Brazil*, ³*Ciências Farmacêuticas, Universidade Federal de São Paulo (UNIFESP), Diadema, Brazil*

Human Erythropoietin (EPO) acts on erythrocyte predecessor cells by stimulating their proliferation, differentiation, maturation and inhibiting apoptosis, thus increasing erythrocyte production. EPO is used clinically for the treatment of anemia associated with chronic kidney failure, treatment of anemia associated with cancer, HIV infection, pre and postoperative, rheumatoid arthritis and bone marrow transplantation. The potency test (biological activity) of EPO is still described today, in official compendiums, as an in vivo test, but with the increasing demands of ethical care in the use of animals in experiments and the variability of results, it becomes interesting an alternative method (in vitro) that directly impacts ethical, financial and analytical issues, bringing safety and quality to potency tests. In order to address these issues, the development of an in vitro analytical method for application in quality control within the pharmaceutical industry was initiated. First, the development of the methodology consisted of evaluating the proliferation of the TF-1 cell line (derived from human erythroleukemia) according to the stimulus received from the EPO, using as reference a primary pharmacopeial pattern. The results of the analytical development demonstrated that TF-1 is able to respond to the EPO stimulus, showing dose-response curves between 0.15 IU and 5.00 IU with $r^2=0.99$. The next proposal for continuation of the work will be to carry out the EPO biological potency test using pluripotent cells and thus try to find suitable results by evaluating cells that can respond properly to the EPO stimulus and present more evident cell differentiation. The implementation of this method will considerably reduce the use of animals in the industry, generating adequate product quality control regarding identity, purity and potency using an in vitro method, leading to a transition to animal-free testing and more relevant results for humans.

Keywords: biological potency, development, in vitro

304 - B

INTERACTION BETWEEN FORM AND FATE IN HUMAN CEREBRAL ORGANIDS

Chiaradia, Ilaria¹, Imaz-Rosshandler, Ivan¹, Nilges, Benedikt², Boulanger, Jerome¹, Pellegrini, Laura¹, Das, Richa³, Kashikar, Nachiket², Lancaster, Madeliney¹

¹*Cell Biology, MRC Laboratory of Molecular Biology, Cambridge, UK*, ²*European Spatial Biology Centre, Leuven, Belgium*, ³*Resolve Biosciences, Monheim am Rhein, Germany*

Organs are the ensemble of different cell types in a complex architectural milieu. It is well-known that progression through fate decisions sets up the complex cellular makeup and architecture of an organ, but how that same architecture may impact on cell fate is less clear. We sought to examine this by taking advantage of the unique capabilities of organoids as a tractable in vitro model to interrogate how fate and form interact during organ development. We developed a semi-automated pipeline to quantify organoid morphological features from brightfield pictures. We screened for protocol-to-protocol variations that could explain the observed differences in organoid morphology, often overlooked. We demonstrated that patterning early on and exposure to exogenous extracellular matrix affect morphology. We next analysed the transcriptional profile of organoids displaying different morphology scores and organoids with a controlled morphology. This resulted in a change in the transcriptome consistent with the morphological score, further supporting the link between tissue shape and fate. Here we suggest that scoring based on morphological features appears to be a better predictor of organoid quality and reliability in mimicking in vivo human fetal brain development than the specific protocol used. We next sought to investigate the role of cytoarchitecture in cell fate determination. We perturbed organoid cytoarchitecture by mechanical dissociation and reaggregation and analysed the transcriptome as a whole and in its tissue context, through the integration of snRNA-seq and spatial transcriptomics. Mechanically redistributing cells in a random spatial conformation resulted in profound changes in tissue identity. This suggests an interdependency of tissue structure and tissue identity. Organoid with poor tissue architecture displayed aberrant developmental trajectories and bivalent progenitor/neuron cell fate. Cells that lack proper spatial coordinates also fail to undergo the proper temporal progression of events. In poor morphology organoids, neural progenitors and neuronal markers were intermingled in time and space. Overall, cells sense their surroundings to interpret developmental clock. This reveals the strong interplay between space and time during development.

Keywords: morphology, transcriptome, organoid

Poster Abstracts

305 - A

LATE NEURAL PROGENITORS HINGE ON SOX9 FOR CELL FATE DETERMINATION IN MOUSE SPINAL CORD

Berti, Joselina, Campetella, Carla, Lanuza, Guillermo
Leloir Institute, Ciudad Autónoma de Buenos Aires, Argentina

Neural progenitors give rise to both neurons and glial cells during distinct stages of central nervous system development. Therefore, their transcriptional programs must be altered in order to generate such different cell types. Among the many mechanisms involved in the neuro-to-glial switch lies Sox9 expression, necessary for glial specification. Even though the separate timing of neuro and gliogenesis was a well-established principle, the mouse spinal cord cerebrospinal fluid-contacting neurons (CSF-cNs) are an exception. These peculiar neurons arise at late developmental stages along with astrocytes, oligodendrocytes, and ependymocytes. In this work, we demonstrate that the lack of Sox9 leads to an increased production of neurons expressing the ion channel Pkd2l1, characteristic of CSF-cNs. These cells follow the classical differentiation program of CSF-cNs, initiated by the proneural protein Ascl1 and continued by the Gata3/2 transcription factors in their postmitotic specification. We show that neuronal overproduction in mutants originates from the p2/pOL progenitor domain. We also suggest that the change in potency of neural progenitors is delayed, leading to improper timing of glial development, shown by a reduction of Nfia+;Sox2+ cells. Altogether, our results demonstrate that Sox9 promotes progenitor determination towards glial fates, and its absence triggers differentiation of CSF-cNs during late spinal cord development.

Keywords: neurogenesis, gliogenesis, Neural progenitors

305 - B

PARP1 IS DIFFERENTIALLY EXPRESSED IN BCR-ABL P190+ ALL PATIENT SAMPLES AND TARGETING PARP INHIBITION INDUCES CELL DEATH COMPARABLE TO THAT OF TYROSINE-KINASE GOLDEN STANDARD IN PRE-CLINICAL MODELS

Machado, Caio Bezerra¹, Silva, Emerson¹, Ferreira, Wallax², Pessoa, Flávia¹, Quadros, Andreza³, Fantacini, Dianne³, Furtado, Izadora³, Rossetti, Rafaela³, Silveira, Roberta³, Lima, Sarah³, Mello-Júnior, Fernando⁴, Seabra, Aline⁴, Moreira, Edith⁵, Moraes-Filho, Manoel¹, Moraes, Maria¹, Montenegro, Raquel¹, Ribeiro, Rodrigo⁵, Khayat, André¹, Oliveira, Edivaldo², Souza, Lucas³, Moreira-Nunes, Caroline¹

¹Núcleo de Pesquisa e Desenvolvimento de Medicamentos, Universidade Federal do Ceará, Fortaleza, Brazil, ²Seção do Meio Ambiente, Instituto Evandro

Chagas, Ananindeua, Brazil, ³Hemocentro de Ribeirão Preto, Instituto Butantan, Ribeirão Preto, Brazil, ⁴Laboratório de Biologia Molecular, Hospital Ophir Loyola, Belém, Brazil, ⁵Instituto de Estudos em Saúde e Biológicas, Universidade Federal do Sul e Sudeste do Pará, Marabá, Brazil, ⁵Departamento de Hematologia, Hospital Geral de Fortaleza, Brazil

BCR-ABL1 p190 translocation represents a worse prognosis for acute lymphoblastic leukemia (ALL) patients, and development of new therapeutic approaches is of utmost importance to the oncologic routine. This study aims to evaluate the cytotoxic effect of inhibiting poly-ADP-ribose (PARP) in an ALL Philadelphia positive (Ph+) cell model and validate biomarker differential expression in patient samples. Cell lines SUP-B15, Raji and Namalwa were screened for PARP1 expression through qPCR. Non-neoplastic cell line MCR5 was used as a control and ACTB was chosen as the housekeeping gene. Data was compared through one-way ANOVA followed by Bonferroni's post-test. Cells were then treated with either AZD2461, Imatinib or Doxorubicin through serial dilutions and, after 72 hours of treatment, cells were incubated with Alamar Blue and read for fluorescence. Viability results were analyzed through non-linear regression. Finally, samples from 31 p190+ ALL patients were quantified for PARP1 expression and compared to 10 healthy controls. The study was approved by the Ethics Committee of the Federal University of Ceará (approval number: 5.207.521) and of the Ophir Loyola Hospital (approval number: 9611320.0.0000.5550), informed written consent was obtained from the patient, being brought into the study only after accepting the terms, and all methods were carried out in accordance with Helsinki guidelines and regulations. Statistical comparison went as previously described for qPCR analysis. All statistical analysis were made using GraphPad Prism (v. 5.01). SUP-B15 considerably overexpresses PARP1 in comparison to both Raji and Namalwa, as well as to the control MRC5 ($p < 0.0001$), and treatment with AZD2461 showed cytotoxic activity comparable to that of Imatinib in SUP-B15, represented by an IC50 at 72 hours of treatment of 344.3 nM and 329.2 nM, respectively, while showing little to no efficacy when treating either of the remaining cell lines. Validation of biomarker in patient samples reveals an approximate 2-fold increase in PARP1 levels compared to healthy controls ($p < 0.01$). The use of PARPis shows great effect over the viability of ALL Ph+ models and PARP1 represents a potential new target for therapeutic of BCR-ABL1 positive ALL. However, further in vivo and clinical studies are necessary to support this hypothesis.

Funding Source: This study was supported by Brazilian funding agencies: CAPES, CNPq, FUNCAP and FAPESP.

Keywords: Poly(ADP-ribose) Polymerase Inhibitors, acute lymphoid leukemia, molecular targeted therapy

Poster Abstracts

306 - A

PHENOTYPIC CHARACTERIZATION OF NULL ALLELES GENERATED IN iPSC-DERIVED PRIMITIVE SYNCYTIUM

Alcoforado Diniz, Juliana¹, Mitchell, Joshua², Taggar, Arti¹, Ning, Gang³, Zheng, Shujian², Thappa, Maheshwor², Skarnes, Bill³, Li, Shuzhao², Robson, Paul¹

¹Robson Lab, The Jackson Laboratory for Genomic Medicine, Farmington, CT, USA, ²Li Lab, The Jackson Laboratory for Genomic Medicine, Farmington, CT, USA, ³Skarnes Lab, The Jackson Laboratory for Genomic Medicine, Farmington, CT, USA

The extra-embryonic primitive syncytium (PrSyn) is a multinucleated cell type that invades the endometrium following implantation. Its main functions include mediating attachment, invasion, and endocrine control of the maternal system as the blastocyst implants. Herein, we aim to characterize genes specifically expressed in the PrSyn as part of the Molecular Phenotypes of Null Alleles in Cells (MorPhiC) initiative. We chose the PrSyn as it is 1) evolutionary divergent, 2) it can be derived quickly from human induced pluripotent stem cells (hiPSCs), and 3) it is responsible for many biological processes. Initially, CRISPR-Cas9- engineered null allele generation strategies were used to compare a premature termination codon +1 base frame shift (PTC+1) strategy with a comprehensive coding sequence deletion (KO) and critical exons (CE) in a well-characterized, stable hiPSC line, KOLF2.2. Following a 6-day differentiation protocol the cells underwent regulatory and biochemical phenotyping. Among the transcription factors tested were the oxygen-sensing EPAS1, FOSB, GHRL1 and POU2F3. Bulk RNA-seq at Day 6 of wildtype, PTC+1, CE and KO lines was used to analyze gene expression differences by editing biallelic PTC, exon and gene deletion alleles. For genes encoding other protein classes we employed a biochemical phenotyping approach utilizing unbiased metabolomics and lipidomics. Initial efforts to optimize media conditions led us to define 15 micronutrients that individually lead to enhanced biological activity in iPSC-derived PrSyn thus favoring our phenotyping approach on null alleles. As a proof of concept, the supplementation with prolactin in a basal media for 24h was tested for metabolomic analysis on the cells and supernatant. Samples were analyzed by ultra-high-performance LC-MS and pathway analysis was performed using Mummichog. The metabolomic profiling indicated a PrSyn response to addition of prolactin, and pathway enrichment suggested biological roles for these metabolites which our PRLR KO will validate. Taken together, this MorPhiC pipeline will through the characterization of 125 genes uncover the rich, primate-specific biology of human implantation relevant to understanding infertility and, through pleiotropy, gene function relevant to many other aspects of human biology.

Funding Source: We would like to acknowledge the MorPhiC consortium, 1UM1HG012651-01, for the financial support.

Keywords: developmental biology, extra-embryonic lineage, primate-specific

306 - B

PLACENTAL STEM CELLS: A PROMISING APPROACH TO CONTROL DIFFERENT TYPES OF CANCER

Oropeza, Anderson¹, Miglino, Maria Angelica¹, Carreira, Ana Claudia Oliveira²

¹Departamento de Anatomia, Universidade de São Paulo, Brazil, ²Departamento de Anatomia, Universidade de São Paulo and Universidade Federal do ABC, São Paulo, Brazil

Addressing drug-resistant cancers while sparing healthy tissues is a pressing challenge. Placental stem cells hold great potential in modulating tumor progression due to their unique biological properties. This study focused on using spheroid from canine mammary ductal carcinoma (CMC) cells and canine amniotic membrane-derived cells (AMSC) to explore their therapeutic effectiveness. Optimization of spheroid formation for CMC and AMSC revealed positive expression of CD105 and vimentin in CMCs, highlighting their identity. Intriguingly, in a cocultivation system, direct contact between AMSC spheroids and CMC monolayers triggered a remarkable response as direct contact between AMSC spheroids and CMC monolayers prompted a distancing response in CMC cells at 1 hour. Subsequently, over the course of the following hours, a substantial increase in cell death was observed. Conversely, CMC spheroids cocultured with fibroblasts showed morphological changes. Furthermore, the conditioned medium (CM) derived from AMSCs exhibited the ability to inhibit CMC cell migration. Remarkably, wound healing assays demonstrated that CMCs cultured with AMSC-CM lost their migratory capacity and instead entered apoptosis, which was not observed when CMCs were cultured in high glucose DMEM medium. These preliminary results suggest that AMSCs secrete elements that possess selective anticancer effects against CMC cells, paving the way for potential therapeutic applications. Similar experiments with human cells and placentas yielded promising results. Future investigation will focus on evaluating the chemical composition of placental stem cell-derived CM to uncover the responsible elements. This study unlocks the potential of placental stem cells, offering a novel approach to combat drug-resistant cancers. Harnessing their unique characteristics, these cells hold promise for personalized therapeutic interventions, revolutionizing cancer treatment.

Funding Source: CAPES

Keywords: placental stem cells, cancer, small extracellular vesicles

Poster Abstracts

307 - A**SELF-ORGANIZED MORPHOGENESIS ON THE HUMAN NEURAL TUBE****Polanco, Angel**, Karzbrun, Eyal*Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel*

Investigating the intricate process of human organ development is a scientific puzzle that holds medical significance. Recent advancements utilizing three-dimensional stem cell cultures and chip-based culture systems have shed light on the differentiation of human cells through the self-organization of micropatterned stem cells into precise three-dimensional cell-fate patterns and organ structures. Combining human stem-cell micropatterning with neural induction drove the formation of a millimeter-long in-vitro neural tube covered with surface ectoderm, a critical developmental milestone. Here, we studied the dynamics of in vitro neural tube formation using immunostaining and 3D confocal imaging on different micropattern geometries. We systematically quantified the number of closed neural tubes and the number of samples with open neural tissue. We applied this approach to study the effect of pharmaceutical perturbations on neural tube closure. This platform will allow us to establish an in-vitro model for studying neural tube defects, a severe and prevalent birth defect. Overall, the micropatterned stem-cell system opens a new window into studying human organogenesis in health and disease.

Keywords: neural tube, self-organization, micropatterning**307 - B****THE ROLE OF PROLACTIN IN MOUSE PLURIPOTENCY AND CORTICAL NEURON DEVELOPMENT****Martinez-Alarcon, Omar**¹, Colin-Lagos, Daniela¹, Ramirez-Meza, Ximena¹, Castillo-Villalon, Madai¹, Avila-Gonzalez, Daniela¹, Garcia-Lopez, Guadalupe¹, Molina-Hernandez, Anayansi¹, Portillo, Wendy², Diaz, Nestor F.¹*¹Physiology and Developmental Cell, Instituto Nacional de Perinatologia, Mexico, ²Sexual Behavior and Neural Plasticity, Instituto de Neurobiologia, Juriquilla, Mexico*

Prolactin (PRL) has over 300 physiological functions, including maternal behavior and neurogenesis. However, its possible role in early development remains unknown. This work focuses on determining the effect of PRL during pluripotency and cortical neuron differentiation in a mouse embryonic stem cell platform. As a first approach, we

evaluated the presence of the PRL receptor under two undifferentiated conditions: 1) serum + LIF and 2) 2i + LIF. We observed a significant presence of the receptor under the second condition compared to the first. Also, when we withdrew the two inhibitors plus LIF, we observed a reduction in the receptor after 48 and 72 hours, along with a redistribution of the marker from cytoplasmic to nuclear regions after the switch-off. In another series of experiments, PRL treatment (0.2, 0.4, 2, 6, and 20nM) for five days did not change the number of Oct4 and Sox2 positive cells in the experimental groups compared to the control. However, the number of Nanog-positive cells diminished in all hormone-treated groups. Then, we evaluated the long isoform of the PRL receptor during the differentiation of mESC to cortical neurons through PCR. We did not find constitutive expression of the PRL receptor (it was not expressed on days 1, 7, 12, 14 and 18). Nonetheless, we detected the emergence of Nestin/PRL receptor double-positive cells through immunofluorescence on day 12. Finally, we explored the effect of PRL (6nM) during the proliferation phase of our neural protocol; but no effect on the number of Nestin/EdU double-positive cells on day 14 was observed. Our study provides insights into the effects of PRL on the differentiation of mouse ESCs into cortical neurons, shedding light on the possible involvement of PRL and its receptor in pluripotency and corticogenesis.

Funding Source: INPer 212250-3230-21214-03-16**Keywords:** prolactin receptor, embryonic stem cells, cerebral cortex**308 - A****THREE DIMENSIONAL (3D) BIOPRINTED CONSTRUCT RECAPITULATES EARLY NEUROGENESIS FEATURES****Bim, Larissa V.**, Machado, Lucas, Porcionatto, Marimelia*Biochemistry, UNIFESP, São Paulo, Brazil*

Brain organoids are the main three-dimensional (3D) in vitro model to study early neurodevelopment. Essential genes and molecular mechanisms involved in normal and pathological brain development were described using brain organoids generated from human iPSCs. The main downside of organoids is batch-to-batch heterogeneity, and much work is underway to solve or minimize it. Three-D bioprinting has the potential for developing in vitro models that show higher complexity when compared to a bi-dimensional (2D) culture and may overcome issues that arise from the self-organization of organoids. We present the generation of complex 3D structures formed by human

Poster Abstracts

neural progenitor cells (hNPCs) bioprinted in Geltrex® and Gelatin methacryloyl (GelMA) bioink. Bioprinted hNPCs maintained in a neural expansion medium (NEM) for two weeks formed large clusters of cells connected by several broad processes. When 3D bioprinted hNPCs were cultured in a neural maturation medium (NMM) containing ascorbic acid, db-cAMP, BDNF, and GDNF, we observed smaller and more compact clusters with thinner and longer processes connecting the clusters. Cells with neuron-like processes scattered around the construct were also observed. Cells in constructs maintained in both media (NEM and NMM) showed increased NESTIN, PAX6, FOXG1, and TBR2 expression. Immunofluorescence at day 28 post-printing showed GFAP and TUBB3 expression by cells in the clusters. In contrast, neuron-like scattered cells were negative for GFAP and positive for tubulin beta III. Preliminary data on patch clamp analysis showed cells with resting membrane potential around -50mV that undergo hyperpolarization but do not depolarize when stimulated. Immunostaining of tubulin beta III and GFAP with the absence of MAP2 proteins evidence the formation of early neural structures, which is in accordance with the increasing expressions of FOXG1 and TBR2 genes. We suggest that 3D bioprinting of hNPCs may be a good model for studying cellular and molecular mechanisms involved in neural proliferation, migration, and differentiation during early neurogenesis.

Funding Source: Financial support: FAPESP (2018/12605-8); CNPq (406258/2022-8; 311026/2022-2); CAPES (Financial Code 001).

Keywords: 3D-bioprinting, neurodevelopment, hNPC

TRACK: DIRECTED AND DIFFERENTIATION AND TRANSDIFFERENTIATION

401 - A

ANALYSIS OF THE EFFECTS OF TOPOISOMERASE 2 INHIBITORS ON IN VITRO HEPATIC DIFFERENTIATION

Pacheco, Lara, Komatsu, Sabrina, Telles-Silva, Kayque, Goulart, Ernesto, Caires, Luiz, Zatz, Mayana

Department of Genetics and Evolutionary Biology, Institute of Biosciences of University of São Paulo, Brazil

Liver diseases are a major global health problem, having different etiologies (e.g. alcohol, viruses, drugs, and chronic inflammation), and resulting in two million deaths annually worldwide. In the event of liver failure, transplantation is the only therapy, but donor shortage and immunosuppressive therapy hamper its efficacy. Bioengineering (e.g. bioprinting, organoid development, and decellularization) represents an alternative for traditional transplant, but it requires proliferative and functional cell sources. Primary hepatocytes (PHH) are the gold standard, but they rapidly lose their phenotype in vitro. Human-induced pluripotent stem cell (hiPSC)-derived hepatocyte-like cells (HLCs) are easily handled in vitro and can differentiate in almost every tissue, but their production is expensive, time-consuming, and inefficient, as HLCs present a fetal phenotype. We found that a drug that inhibits topoisomerase II (1.iTOP2) improves the differentiation of HLCs in vitro. We differentiated iPSCs in HLCs and characterized them by RT-qPCR and flow cytometry. We investigated the effect of other drugs related to TOPII: a TOP1 inhibitor (iTOP1), another TOP2 inhibitor (2.iTOP2) and an apoptosis inducer (aAPOP) on the differentiation of hiPSCs into hepatocytes. We showed that 1.iTOP2 treatment (positive control) increased ALB expression by 200-fold through RT-qPCR. The treatments with either iTOP1, 2.iTOP2 or aAPOP did not significantly alter ALB expression. We also inhibited the aryl hydrocarbon receptor (AhR) after 1.iTOP2 treatment and showed the reduction of ALB levels by RT-qPCR for pre-1.iTOP2 treatment level. Therefore, we indicated that the inhibition of topoisomerase II by 1.iTOP2 contributes to the maturation of HLCs and this effect is mediated by the AhR pathway.

Funding Source: This research project was supported by São Paulo Research Foundation (FAPESP).

Keywords: hiPSCs, topoisomerase, hepatocytes

Poster Abstracts

401 - B

A METHOD FOR THE GENERATION OF HUMAN STEM CELL-DERIVED PANCREATIC DELTA CELLS**Peterson, Quinn,** Belame-Shivakumar, Sharath*Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, MN, USA*

Cell-based therapies hold great promise in treating the increasing burden of Type 1 diabetes. These therapies require human pancreatic beta cells that can properly respond to and secrete insulin in response to glucose levels in the body. Although islet transplantation has been effective, a shortage of donor islets restricts its use in cell therapy. An alternative approach is the use of stem cell-derived beta cells (SC-beta) for Type 1 diabetes treatment. However, for proper functioning within islets, endocrine cells experience both paracrine and autocrine regulation, which is not present in single cells generated using stem cells. To improve treatment efficacy to that of donor islets, SC-beta cells can be combined with other stem cell-derived pancreatic endocrine cells, but this is limited by a lack of precise methods for generating these cell types. While SC-beta and stem cell-derived alpha cells (SC-alpha) have been successfully generated, other endocrine cell types are not yet accomplished. Here we report the first protocol for generating human stem cell derived delta cells (SC-delta) from pluripotent cell sources. Using several small molecule screening approaches, we identified small molecules that could efficiently convert human embryonic stem cells into pancreatic delta cells. The protocol results in somatostatin expression in nearly 60% of the cell population, with 17% of the cells expressing only somatostatin as monohormonal SC-delta cells. The resulting SC-delta cells expressed key delta cell-specific markers such as Hhex, Ptch1, and Pax6, and could secrete somatostatin in response to glucose challenge. Furthermore, SC-delta cells inhibit the secretion of glucagon in SC-alpha cells. The identification of these small molecules has led to the development of a novel protocol that produces functional human SC-delta cells. This not only advances our understanding of human delta cells but also provides a basis for drug screening, disease modeling, and the development of designer islets, which has the potential to refine and improve current cell therapeutic regimens.

Keywords: type 1 diabetes, islet, pancreas

402 - A

ANALYSIS OF TOPOISOMERASE INHIBITOR ON IN VITRO CARDIAC DIFFERENTIATION**Kadowaki Komatsu, Sabrina Kaori,** Caires, Luiz, Goulart, Ernesto, Pacheco, Lara, Telles-Silva, Kayque, Zatz, Mayana
Department of Genetics and Evolutionary Biology, Institute of Biosciences of University of São Paulo, Brazil,

The heart - the core of the circulatory system - distributes hormones, nutrients, and gasses throughout the body, maintaining homeostasis. Transplantation is the only therapeutic option for severe heart diseases. The limited number of donors, incompatibility between donor and recipient, and immunosuppression hampers the traditional transplantation methods. Bioengineering techniques (e.g. de-recellularization, 3D bioprinting) represent potential alternatives for organ transplantation. However, the lack of functional recellularization protocols and the incapacity to generate mature cardiomyocytes (CM) in vitro hinders the application of bioengineering technologies. We demonstrated that hiPSC-derived hepatocytes (HCs) treated with a topoisomerase 2 inhibitor (TOP2i) showed higher liver-specific gene expression and protein profile. Due to the interdependence between the liver and the heart during embryonic development, we evaluated the effect of TOP2i in the maturation of iPSC-derived CMs. We differentiated iPSCs in CMs and characterized them by RT-qPCR, immunofluorescence and flow cytometry. The hiPSC-CMs was treated with TOP2i, resulting in a 2x increase in the expression of mature CM-specific genes (TNNT3, MYH7, and HK2). To further evaluate the effect of other topoisomerase isoforms inhibitors on CM maturation, we treated CMs with a topoisomerase 1 inhibitor (TOP1i). Through gene expression analysis using RT-qPCR, we observed that TOP1i had a more pronounced effect on the maturation of hiPSC-CMs compared to TOP2i. These findings suggest that topoisomerase isoforms exert tissue-specific influences during fetal development, shedding light on their potential significance in cardiac development and regenerative medicine.

Funding Source: This research project was supported by São Paulo Research Foundation (FAPESP).**Keywords:** hiPSC, cardiomyocytes, topoisomerase

Poster Abstracts

402 - B

ENHANCING SPECIALIZATION AND REDUCING ARRHYTHMOGENIC POTENTIAL OF iPSC-CMS THROUGH NOTCH1 PATHWAY MODULATION: A PROMISING APPROACH TO LEVERAGE CARDIAC REGENERATION

Ferreira, Nikolas Dresch¹, Valadão, Luri¹, Lima, Vanessa², Roman-Campos, Danilo³, Neri, Elida⁴, Krieger, José⁴

¹Department of Cardiopneumology / Heart Institute, University of São Paulo Medical School, São Paulo, Brazil,

²Cardiovascular bioengineering, Institut National de la Santé et de la Recherche Médicale, Paris, France,

³Department of Biophysics, Federal University of São Paulo, Brazil, ⁴Department of Cardiopneumology,

University of São Paulo Medical School, São Paulo, Brazil

Induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) hold great promise as platforms for human disease modeling and cell therapy in cardiac regeneration field. However, the occurrence of arrhythmogenic events associated with iPSC-CM transplantation limit their therapeutic application. We previously demonstrated that Notch1 is a central signaling pathway in the crosstalk between fibroblasts and cardiomyocytes (CMs) for the induction of CM maturation into fast-conducting fibers. In this study, we aimed to investigate whether modulating the Notch1 pathway during the differentiation of iPSC-CMs could enhance maturation and minimize its arrhythmogenic potential. To achieve this, we employed an inhibitor of Notch1 activation (DAPT, 10 μ M) during the differentiation process of human iPSC-CMs. Specifically, we administered DAPT for three days after the formation of cardiac progenitor cells (days 5-8). Our findings reveal that inhibiting Notch1 activation led to a decrease in the expression of critical genes (Gja5, Gjc1, Cntn2, Hcn4, and Cav1.3) and proteins (Cx40 and Cntn2) that are typically associated with specialized conductive cells. Conversely, it enhanced the expression of Cx43, the primary connexin type found in working CMs, and resulted in structural changes characterized by larger CMs with more organized contractile structures. Additionally, functional changes were observed, including a decrease in automaticity and alterations in the action potential such as increased amplitude, MaxRiseSlope, and duration at 30%, 50%, and 90% repolarization (APD30, APD50, APD90), which are typical of working CMs. Immunofluorescence analysis confirmed a higher prevalence of ventricular CMs, as indicated by the APD90/50 ratio. Importantly, the transient inhibition of the Notch1 pathway during the early stages of differentiation was sufficient to induce these molecular, structural, and functional changes, ultimately influencing

cell specialization. Taken together, our findings provide compelling evidence that targeting the Notch1 pathway promotes the differentiation of human iPSC-CMs towards a ventricular working-like phenotype. This approach holds significant implications for the development of safe and effective cell therapies for cardiac regeneration.

Funding Source: This project was supported by FAPESP and CNPq

Keywords: iPSC, cardiomyocyte, Notch1

403 - A

SINGLE CELL FATE MAPPING DURING IN VITRO DIFFERENTIATION OF HUMAN PANCREATIC BETA CELLS

Shlomi, Shlomo, Wang, Xi, Pop, Ramona, Melton, Doug

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

Insulin-producing, stem cell-derived β cells are a promising course of long-term treatment for patients with Type 1 diabetes. However, cells demonstrate considerable heterogeneity in the lineage of both differentiated and undifferentiated cells. The underlying mechanism for this variability is not well understood. To address this, we implemented a clonal lineage tracing strategy where we barcoded cells, allowed them to divide, and then sampled clones at early and late stages of differentiation into β cells. Enabling us to link the transcriptional profile of progenitor cells with their final cell fate. Differential gene expression between clones with distinct fates allowed us to observe early transcriptional biases and identify subpopulations of endocrine-committed progenitor cells. We identified and isolated a subpopulation of endoderm origin that exhibits remarkable potential for differentiating into the endocrine lineage. Moreover, these investigations have provided valuable insights into the early processes governing cell fate decisions leading to the commitment of cells to the β cell lineage.

Funding Source: HHMI, JRDF, HSCI

Keywords: stem-cell-derived β cells, lineage tracing, type 1 diabetes

Poster Abstracts

403 - B

SLC30A8 LOSS-OF-FUNCTION ALLELE P. ARG138* AND FOXA2 ENHANCER PRIMING IN HESC-DERIVED PANCREATIC PROGENITORS

Alvarez, Carla Barragan¹, Ávila, Daniela², Jave, Luis³, García, Guadalupe²

¹Medical and Pharmaceutical Biotechnology Unit, CIATEJ, Guadalajara, Mexico, ²Díaz, Fabián - Departamento de Biología Celular, Instituto Nacional de Perinatología, Ciudad de México, Mexico, ³Departamento de Biología Celular, Instituto Nacional de Perinatología, Mexico City, Mexico, ³División de Inmunología, Centro de Investigación Biomédica de Occidente, Guadalajara, Mexico

Zinc transporter 8 (ZnT8) is a zinc ion antiporter that mediates the zinc influx into insulin granules. Genome-wide association studies (GWAS) have shown an association between ZnT8 variants and type 2 diabetes mellitus (T2DM); however, there is not clear evidence that explains the involved molecular mechanisms. Using the tool Islet Regulome Browser, we predicted and validated with ChIP-qPCR binding sites for the transcription factor FOXA2 in hESC-derived pancreatic progenitors. These binding sites are found inside and close to exon 3 of SLC30A8 where the loss of function (LOF) variant pArg138* is located. During pancreas embryogenesis, enhancer activity plays a crucial role in lineage determination. Multiple enhancers are poised and prepared from endocrine progenitors, in genes associated with vital islet-specific cellular properties, such as hormone biosynthesis and insulin secretion. It has been demonstrated that the pioneer transcription factor (TF) FOXA2 is required for proper chromatin opening and the establishment of enhancer marks during pancreatic fate specification. Our data revealed a possible enhancer activity of the pArg138* variant that needs to be further investigated.

Keywords: zinc transporter 8, loss-of-function, enhancer

404 - A

THE GLUCOSE PUZZLE: EXPLORING TRANSDIFFERENTIATION OF MESENCHYMAL STEM CELLS TO PANCREATIC PROGENITORS

Jaén Alvarado, Yanelys Lourdes, Guerrero, Erika, Solis, Mairim

Stem Cell Research Group, Department of Research in Sexual and Reproductive Health, Gorgas Memorial Institute for Health Studies, Panama

Pancreatic cell development is regulated in a dose-dependent manner by glucose, making it crucial for the correct development of pancreatic β cells. As increased glucose concentrations results in cell dysfunction and low concentrations in a stable environment, finding the appropriate glucose concentration for in-vitro differentiation into pancreatic cells has been of global interest. This study focused on evaluating the in-vitro capacity of placenta-derived mesenchymal stem cells (PDMSC) for optimal transdifferentiation into pancreatic progenitor cells utilizing different glucose concentrations, 2, 5, and 20mM, and the effect it exerts in the phenotypic characteristics and the expression of multipotency and pancreatic-related markers. Results from this study demonstrated that an increase in glucose concentration in the culture medium caused a decrease in cellular size, proliferation, and expression of pluripotent markers in PDMSCs. However, an increase in the expression of multipotent markers was observed. Interestingly, the adjustments of glucose in the culture medium didn't affect DNA integrity nor the glycolytic metabolism of PDMSCs, showing that glucose adjustments maintained the characteristics of PDMSCs. PDMSCs differentiated at 2mM glucose resulted in a pancreatic-like morphology and a 10-fold increases in the expression of SOX17 at the definitive endoderm phase, a 20-fold increase of HNF6 in the primitive gut phase, and up to a 30-fold increase in pancreatic progenitor markers, NKX6.1 and PDX1. A bioenergetic switch occurred in differentiated cells, with a gradual decrease in glycolytic-related proteins observed during the different phases of pancreatic differentiation. Taken together, 2mM glucose seems to be an appropriate concentration to be utilized with our formulated pancreatic differentiation cocktail for inducing differentiation. Results from this study contributes to the global seek of the optimal glucose concentration for inducing pancreatic differentiation that may assist in future applications in regenerative medicine for diabetes treatment.

Keywords: mesenchymal stem cells, transdifferentiation, pancreatic progenitor

Poster Abstracts

TRACK: DISEASE MODELING**501 - A****3D PRECLINICAL MODELS AS PLATFORMS TO STUDY THE PRION PROTEIN IN THE INVASIVENESS OF GLIOBLASTOMA STEM CELLS**

da Silva Souza, Maria Clara Da Silva¹, Coelho, Barbara¹, Fernandes, Camila¹, Masotti, Cibele², Passos Bueno, Maria Rita dos Santos³, Prado, Mariana¹, Santos, Tiago Góss dos⁴, Sousa, Breno¹, Lopes, Marilene Hohmuth¹

¹Department of Cellular Biology and Development, Institute of Biomedical Science, University of São Paulo, Brazil, ²Molecular Oncology Center, Hospital Sirio Libanês, São Paulo, Brazil, ³Genetics and Evolutionary Biology, University of São Paulo, Brazil, ⁴International Research Center, Hospital A.C. Camargo Cancer Center, São Paulo, Brazil

Glioblastoma (GBM), sustained by a subpopulation of stem cells (GSCs), stands among the most lethal tumors. GSCs are increasingly being considered responsible for the malignant features of this cancer, including increased recurrence rates and resistance to therapy. Its aggressive and invasive behavior reduces patient survival, making complete surgical resection impossible. Due to its cellular and molecular heterogeneity, GBM remains difficult to model, so efforts have been employed to understand the mechanisms underlying GSCs biology. Our group has previously proposed the cellular prion protein (PrPC), encoded by the PRNP gene, as a scaffold protein that integrates signaling platforms involved in GSCs maintenance. Therefore, a deeper understanding of the intracellular pathways modulated by PrPC is essential to identify novel targets for GBM treatment. We analyzed publicly available bulk-RNA sequencing (RNA-seq) data from patient-derived xenografts (PDX) and established in vitro preclinical experimental models. RNA-seq analyses identified differentially expressed genes associated with adhesion, invasion, and migration signaling pathways in PDX cells with high PRNP expression (PRNP-HIGH), such as ITGA6 and B1, YAP1, STAT3, CD44, RHOC, and MMP7. Functionally, these findings were corroborated by assays demonstrating impaired invasion and migration capabilities in PrPC knockout GBM cell lines using the CRISPR-Cas9 system. We also established 3D emerging models to study the PrPC in invasive behavior: glioblastoma organoids (GBO) from patients' surgically resected GBM tumors and brain organoids derived from human induced pluripotent stem cells. We noticed a morphological and molecular reproducibility of the cytoarchitecture of brain organoids and GBO, as described in the literature, indicating consistency in 3D structures and cellular markers, despite

patient variability. Furthermore, brain organoids have been co-cultured with GSCs, providing platforms for studying the interaction between tumor cells and brain parenchyma. Establishing these models will help to improve our comprehension of the roles of PrPC in invasive behavior and GSCs maintenance providing additional tools to understand GBM progression and response to treatment.

Funding Source: Project supported by FAPESP (18/155574; 2021/13070-3) and CNPq (409941/2021-2).

Keywords: glioblastoma stem-cells, prion, 3D models

501 - B**A HIGH EFFICIENCY, SINGLE CELL DIFFERENTIATION METHOD FOR THE GENERATION OF FIBROBLASTS, ENDOTHELIAL AND MULTIPLE MYELOID CELL TYPES FROM HUMAN INDUCED PLURIPOTENT STEM CELLS FOR DISEASE MODELLING**

Rizal, Rizal, Hannan, Nicholas, Ocana, Sara, Zuniga, Carlos
Translational Medical Sciences, The University of Nottingham, UK

Macrophages (MΦ), dendritic cells (DCs), fibroblasts (FBs), and endothelial cells (ECs) are present in all tissues and play important role in tissue homeostasis, repair, immunity, and angiogenesis. MΦ-FBs-ECs interactions contribute to the development of diseases such as fibrosis, cancer, and chronic inflammation. Recruited MΦ and ECs secrete cytokines and profibrotic mediators which activate FBs into MyoFBs to produce more extracellular matrix (ECM). Robust methods for generating these cell types will be important for developing more complex stem-cell-based disease and developmental models. Currently protocols to produce haematopoietic cells rely on either formation of EBs or differentiation using clumps of stem cells as the starting material. As a result differentiation is often inefficient, has low reproducibility and requires undefined media components that limit clinical application. To address this issue, we have optimised a chemically defined, xeno-free differentiation platform that can generate MΦ, DCs, FBs, and ECs from the same starting population of human induced pluripotent stem cells (hiPSCs). Starting with a minimal seeding density we induce mesoderm differentiation over 2 days and then by changing growth factor composition can drive the mesoderm towards fibroblasts, vascular endothelial cells or haemogenic endothelial cells and monocytic cells. At each stage, conversion of cells to the desired cell type is >80% limiting the need for enrichment steps throughout the protocol. We have validated the resulting cell types via mRNA-seq as well as using cell type specific functional assays including polarisation of MΦ (iMΦ) into M1 and M2 cells endocytosis assay and their cytokines release is comparable with the

Poster Abstracts

primary M Φ . DCs phagocytic activity using beads uptake assay. Activation of FBs into MyoFBs using TGF- β stimulation and tube formation assay of hiPSC derived ECs. In summary, we generated isogenic M Φ , FBs and ECs in a chemically defined, xeno free, single cell differentiation protocol that have gene expression and functional profiles similar to their primary equivalents. These cells will be an important step towards development of more complex in-vitro developmental and disease models and could also be applicable to translational clinical applications

Funding Source: UKRI and Ipdp

Keywords: macrophages, fibroblasts, endothelial cells, dendritic cells, tissue-specific, hiPSCs

502 - A**ALTERED AUTOPHAGY RESPONSE IN HUNTINGTON'S DISEASE (HD) HUMAN NEURAL STEM CELLS IS CONTEXT-DEPENDENT**

Pitossi, Fernando¹, Casabona, Juan Cruz¹, Farias, Isabel¹, Gorojod, Roxana², Porte Alarcon, Soledad², Kiselev, Sergey³, Kotler, Monica², Gatto, Emilia⁴

¹Laboratorio de Terapias Regenerativas y Protectoras del Sistema Nervioso Central, Fundacion Instituto Leloir - IIBBA - CONICET, CABA, Argentina, ²Laboratorio de Disfunción Celular en Enfermedades Neurodegenerativas y Nanomedicina, Universidad de Buenos Aires- IQUIBICEN- CONICET, CABA, Argentina, ³Vavilov Institute of General Genetics - Russian Academy of Sciences - Moscow, Russia, ⁴Area de Enfermedad de Parkinson y Movimientos Anormales, INEBA, CABA, Argentina

To evaluate the effect of sphingosine 1-phosphate receptor (S1Pr) activation in human induced pluripotent stem cells (hiPSc)-derived neural stem cells (NSC) from HD patients on apoptosis, mitochondrial network and autophagy. Although S1P signaling has been involved in several pathways of neurodegenerative diseases such as HD, data regarding a protective or detrimental role S1Pr stimulation still remains controversial. In this sense, the NSC from HD patients retain several characteristics of HD and have been proposed as a promising strategy to test the functional role of S1Pr. hiPS from controls or HD patients were generated from fibroblasts and differentiated to NSC. >95% of the NSCs expressed Sox1, Sox2, Pax6 and Nestin markers, as expected for bona fide NSCs. One NSC line from each group were incubated with or without growth factors for 24 h and treated with different dilutions of a S1Pr modulator (BAF312) or media. Under these conditions, cell viability was studied by MTS assay, apoptosis was evaluated by measuring Cleaved Caspase 3 and DAPI-stained apoptotic nuclei counting and autophagy activity was analyzed by LC3B immunofluorescence. Changes in morphology of the

mitochondrial network were studied by staining with MitoTracker Red CMXRos. The incubation of cells with BAF312 showed no changes in cell viability, apoptosis or on mitochondrial network in any cell line used, with or without growth factors deprivation. Interestingly, growth factors deprivation elicited autophagy downregulation in control NSC, but not in HD NSC. In the presence of growth factors, S1Pr stimulation at 1/10000 dilution increased autophagy in control NSC, but not in HD NSC. At different dilutions (1/1000 and 1/100.000) this treatment increased autophagy in both cell types. In HD NSCs autophagy depends on the context, dose of S1Pr modulator and growth factors presence. These variables add to the ones previously reported (compartment and cell-type) to affect S1Pr effects, keeping the controversy open.

Funding Source: This project was funded by Novartis. JC Casabona, I Farias, R Gojorod, SP Alarcon, M Kotler and F Pitossi belong to CONICET.

Keywords: Huntington's disease, BAF312 S1P Receptor Modulator, autophagy

502 - B**ALZHEIMER'S DISEASE BRAIN ENDOTHELIAL-LIKE CELLS REVEAL DIFFERENTIAL DRUG TRANSPORTER EXPRESSION AND MODULATION BY POTENTIALLY THERAPEUTIC FOCUSED ULTRASOUND**

da Silva Chaves, Juliana Cristina

Cellular and Molecular Neurodegeneration, Queensland Medical Research Institute, Lutwychee, Australia

The blood-brain barrier (BBB) has a key function in maintaining homeostasis in the brain, partly modulated by transporters, which are highly expressed in brain endothelial cells (BECs). Transporters mediate the uptake or efflux of compounds to and from the brain and they can also challenge the delivery of drugs for the treatment of Alzheimer's disease (AD). Currently there is a limited understanding of changes in BBB transporters in AD. To investigate this, we generated brain endothelial-like cells (iBECs) from induced pluripotent stem cells (iPSCs) with familial AD Presenilin 1 (PSEN1) mutation and identified AD-specific differences in transporter expression compared to control (ctrl) iBECs. We first characterized the expression levels of 12 BBB transporters in AD-, Ctrl-, and isogenic iBECs to identify any AD specific differences. We then exposed the cells to focused ultrasound, in the absence (FUSonly) or presence of microbubbles (FUS+MB), to examine whether the expression or activity of key transporters can be modulated by therapeutic ultrasound, a novel technique allowing for transient BBB opening. Our findings demonstrate that PSEN1 mutant AD-iBECs possess

Poster Abstracts

phenotypical differences compared to PSEN1 corrected and unrelated control iBECs in BBB transporter expression and function. Additionally, we show that FUSonly and FUS+MB can modulate BBB transporter expression and functional activity in iBECs, having potential implications on drug penetration and amyloid clearance. These findings highlight the differential responses of patient cells to FUS treatment, with patient-derived models likely providing an important tool for modelling of therapeutic effects of FUS.

Funding Source: This research was supported by QUT Postgraduate Research Award (QUTPRA) and QUT Higher Degree Research Tuition Fee Scholarship 2020; QIMR Berghofer SEED grant; NHMRC Project grants; NHMRC Senior Research Fellowship.

Keywords: Alzheimer's disease, blood-brain barrier, induced pluripotent stem cells, transporters, ultrasound therapy, drug transport

503 - A

CELLULAR EFFECTS OF VAPB MUTATION IN IPS-DERIVED MOTOR NEURONS FROM ALS8 PATIENTS

Horianski, Romina Soledad, Ferrari, Merari

Department of Genetics and Evolutionary Biology / Biosciences Institute, University of São Paulo, Brazil

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease presented as sporadic or familial form, caused by the death of motor neurons, and characterized by progressive muscular atrophy, progressive paralysis and respiratory burden, leading to patient's death in 2 to 5 years from the onset. VAPBP56S, a causative gene for type-8 ALS, is a mutated form of the VAPB gene from which protein is located in the endoplasmic reticulum membrane, as an integral protein which participates in membrane contact sites (MCS), including mitochondria-ER contact sites (MERCS), by binding to the mitochondrial PTPIP51 protein. The VAPBP56S mutation affects its MSP (major sperm protein) protein-interacting domain, leading to a loss of the cellular homeostasis, ultimately resulting in apoptotic death. However, in the current literature, in vitro cellular models don't use patient-derived cells and are based on the VAPBP56SS overexpression, VAPB knockout or VAPBP56S knockin, leading to results that are little representative of the diseased cellular phenotype. To address this issue, this project uses iPS-derived motor neurons from ALS8 patients, as well as its RNA-seq public dataset, to elucidate the VAPBP56S effects on the cellular homeostasis, starting with (i) an in silico analysis of VAPB-interacting DEGs between patient and control group through Protein-Protein Interaction software STRING followed by (ii) in vitro experiments of VAPB dependent processes. Bottom-up

and to-down in silico analysis showed altered expression profiles of autophagy related genes, such as ATG16L1 (up-regulated) and UBQLN2 (down-regulated), indicating a potential disturbance in this process. Additionally, iPS cells were differentiated to motor neurons, with subsequent maturation of 2/10 and 5/10 days, identifying the most appropriate day to 2, followed by vectors-dependent co-transfection with Split-GFP-based contact site sensor to detect MERCS and subsequently quantify these through FIJI software, obtaining a standardized protocol for the iPS-based cellular model. With a patient-based model approach, we argue that our results, focusing on the autophagy levels and ER-Mitochondria interactions, have the potential to elucidate the underlying mechanisms associated with the disease and direct future research in the field.

Funding Source: FAPESP 2013/08028-1

Keywords: ipsc, neurodegeneration, ALS

503 - B

CEREBRAL ORGANIDS: A PLATFORM FOR THE STUDY OF DRUG-INDUCED MITOTOXICITY

Munteanu, Raluca S., Herland, Anna, Rogal, Julia
Neuroscience, Royal Institute of Technology (KTH), Sollentuna, Sweden

Recent decades have seen a surge in interest in the role mitochondrial dysfunction plays in the development of neurodegenerative diseases, including Parkinson's disease, Alzheimer's and amyotrophic lateral sclerosis (ALS), which are all known leading causes of disability worldwide. Simultaneously, there has been an increase in the number of reports indicating mitotoxicity as a common outcome of in vitro drug toxicity screening assays. Thus, the study of mitotoxicity, mitochondrial dysfunction and the development of a resultant disordered state needs to be investigated. In the past, the study of mitotoxicity has been complicated by the lack of appropriate models, with in vivo models presenting translational challenges, and conventional in vitro models falling short of the complex physiological system. Here we present a human induced pluripotent stem cell (hiPSC)- derived cerebral organoid model aiming to more accurately emulate the brain cellular environment, architecture and functionality, and investigate its potential applications in mitotoxicity studies by treating it with a known mitotoxic compound. Following from the study, it is apparent that cerebral organoids are a good platform for the investigation of mitochondrial injury, though further development and optimisation of mitotoxicity assessment methods, as well as further characterisation of organoid cellular composition may be required.

Keywords: cerebral organoids, hiPSC, mitotoxicity

Poster Abstracts

504 - A

CO-CULTURING OF NEURONS AND ASTROCYTES INDIVIDUALLY DIFFERENTIATED BY NOVEL METHODS FROM HUMAN iPSC ELICITED ALZHEIMER'S DISEASE-LIKE PHENOTYPES

Supakul, Sopak¹, Murakami, Rei¹, Oyama, Chisato², Hatakeyama, Yuki¹, Maeda, Sumihiro¹, Okano, Hideyuki¹

¹Physiology, Keio University School of Medicine, Tokyo, Japan, ²Electrical Engineering and Bioscience, Waseda University, Tokyo, Japan

Induced Pluripotent Stem Cell (iPSC) technology enabled disease modeling using patients' cells. However, most of the cellular disease models from iPSCs composed of only a single cell type, albeit several cell types co-exist and interact with each other in brains. Here, we demonstrated that the newly developed co-culture system of neurons and astrocytes individually differentiated from iPSCs is a useful tool to recapitulate disease phenotypes observed in the brains of Alzheimer's disease patients. The neurons were induced from iPSCs using the dual SMAD inhibition, and the astrocytes were induced from iPSCs via the formation of embryoid bodies and neurospheres. In the co-culture model, several cellular changes in both cell types were observed. For astrocytes, the co-culturing increased the branching of astrocyte processes and the number of GFAP-positive astrocytes. For neurons, Ca²⁺ imaging visualized higher neuronal activities. Electron microscopy revealed an increase in the number of synapses and the density of pre-synaptic vesicles. In addition, the tripartite synapse structure formation in the co-culture system was confirmed by immunoelectron microscopy, and the treatment with EAAT 1,2 inhibitors led to an increase in neuronal hyperexcitability. These indicated that the co-culture model enhanced the formation of functional tripartite synapses. In the co-culture model, familial AD (APP V717L mutation) iPSCs showed an astrogliosis-like phenotype and astrocytic hypertrophy. Furthermore, treatment with 17 β -estradiol ameliorated the astrogliosis-like phenotype. The co-culture system is a useful tool for the disease modeling of neurodegenerative diseases and the confirmation of the intervention effects in vitro.

Keywords: induced pluripotent stem cells, co-culture system, Alzheimer's disease

504 - B

DEVELOPMENT OF AN IN VITRO HUMAN MODEL OF OPIOID TOLERANCE

Marconi, Pedro Batista¹, Pijuan, Teresa¹, Abrantes, Paulo², Rehen, Stevens¹, Guimarães, Marilia²

¹D'OR Institute for Research and Education, Rio de Janeiro, Brazil, ²Biomedical Sciences Institute, Federal University of Rio de Janeiro, Brazil

Opioid substances are efficacious analgesics for the management of severe pain that nevertheless present serious side effects. Among the undesirable effects is the tolerance, which refers to the decrease in drug effectiveness over continuous use. Attempts to dissociate the analgesic effects of opioids from their undesirable effects were not successful. Therefore, more research on the mechanisms of opioid tolerance are necessary to solve this problem of great impact on healthcare. The role of neuroinflammation on cellular tolerance also needs to be further clarified as glial activation and upregulation of inflammatory mediators are present in long-term opioid usage. In recent years, human in vitro modeling techniques have emerged as powerful tools to investigate the mechanisms underlying this condition and explore potential therapeutic interventions, some promising models are the three-dimensional cell cultures such as neurospheres and brain organoids. They recapitulate the architecture and cellular interactions that occur in the central nervous system, ultimately providing a more physiologically relevant environment for studying opioid tolerance. With the aim to establish this model, we are generating brain organoids, neurospheres and astrocytes derived from human induced Pluripotent Stem Cells (hiPSC) and treating them with opioid compounds such as morphine and fentanyl acutely and chronically, subsequently testing the model for functional and biochemical markers of cellular tolerance. Our preliminary results showed that neurospheres, brain organoids and astrocytes express opioid receptors. In addition, we observed a reduction in spontaneous spikes measured with a Multi-electrode array following the exposure to morphine in neurospheres. Morphine was also able to induce increased expression of TLR4 and TNF- α in neurospheres acutely treated. Collectively, these findings suggest that our model holds significant promise for investigating the cellular changes that occur in the nervous system during opioid exposure/tolerance and for assessing alterations induced by new potential treatments.

Funding Source: FAPERJ - Rio de Janeiro State Research Foundation CAPES - Coordination of Superior Level Staff Improvement CNPq - National Council for Scientific and Technological Development FME - Maria Emília Foundation

Keywords: human induced Pluripotent Stem Cells, drug dependence, neuroinflammation

Poster Abstracts

505 - A

DEVELOPMENT OF HUMAN PLURIPOTENT STEM CELL MODEL FOR THE STUDY OF EPILEPTIC ENCEPHALOPATHY CAUSED BY CYFIP2 R87C VARIANT AND PHENOTYPIC EFFECTS IN THE NEURONAL CONTEXT

Zaboroski Silva, Isabelle L.¹, Ventura Biembengut, Ísis², Gomes Junior, Rubens¹, Abud, Ana Paula¹, Brenha, Bianca³, Landry, Curran³, Vaz, Isadora⁴, Jamur, Valderez⁴, Souza, Bruno Solano de Freitas⁵, De Souza, Tatiana Brasil⁶, Miranda, Helen³, Shigunov, Patricia¹

¹Stem Cell Basic Biology Lab, Carlos Chagas Institute, Oswaldo Cruz Foundation (Fiocruz PR), Curitiba, Brazil, ²Proteomics and Protein Engineering Lab, Carlos Chagas Institute, Oswaldo Cruz Foundation (Fiocruz PR), Curitiba, Brazil, ³Department of Genetics and Genome Sciences, Case Western Reserve University (CWRU), Cleveland, OH, USA, ⁴Cellular Therapy Center, Pontifical Catholic University of Paraná (PUCPR), Curitiba, Brazil, ⁵Tissue Engineering and Immunopharmacology Lab, Gonçalo Muniz Institute, Oswaldo Cruz Foundation (Fiocruz BA), Salvador, Brazil, ⁶Proteomics and Protein Engineering Lab, Carlos Chagas Institute, Oswaldo Cruz Foundation (Fiocruz PR), Curitiba, Brazil

Mutations in the CYFIP2 gene are associated with the neurological disorder epileptic encephalopathy. One of the variants associated with the disease is the R87C, which, compared to previously described variants, is associated with more severe symptoms. There is a single animal model with the CYFIP2 R87C variant, however, this model does not fully recapitulate disease pathogenesis. Thus, establishing a human model that can evaluate the impacts of the mutation in the neuronal context is crucial to understanding the variant's molecular mechanisms and enabling new therapeutic discoveries. This study aims to develop a pluripotent stem cell (PSC) model to study the effect of the CYFIP2 R87C variant in neural cells. Thus, we will differentiate PSCs into cortical neurons and cortical organoids and investigate the impact of CYFIP2 R87C to neuronal morphology and electrophysiological function. For that purpose, we initially reprogrammed urine cells from a patient with the R87C variant and generated iPSC CYFIP2WT/R87C cells. The iPSCs were characterized by their pluripotent markers. These cells were then differentiated into neural progenitor cells (NPCs), and it was possible to observe that CYFIP2 was expressed as cytoplasmic granules in the CYFIP2WT/R87C cells compared to controls. Additionally, we edited ESCs using CRISPR/Cas9, to generate CYFIP2 knockout and CYFIP2R87C/R87C isogenic cell lines. The CYFIP2R87C/R87C cortical neurons have similar differentiation efficiency and mean firing rates compared to the wild-type cells in the early stages of differentiation. However, the CYFIP2R87C/R87C cortical organoids showed an increase in size, a decrease of CYFIP2 protein levels, and no SOX2-positive cells in the neural rosettes compared to the wild-type organoids on day 30 of

differentiation. Other molecular and electrophysiology assays are being conducted in the cortical organoids to verify other phenotypic effects of the R87C variant. Lastly, these models will be used in a drug screening with compounds predicted in silico to selectively bind the mutant CYFIP2 (R87C).

Funding Source: CNPq/Instituto Carlos Chagas N° 15/2019 - PROEP/ICC - 442324/2019-7; CAPES PDPG-FAP N° 18/2020 - 88887.629870/2021-00; CAPES PDSE N° 10/2022 - 88881.690048/2022-01; NIH/NINDS R01 NS121374.

Keywords: CYFIP2, induced pluripotent stem cell, disease modeling

505 - B

GENERATION OF A HIPSC-DERIVED 3D MICROTISSUE MODEL FOR THE STUDY OF THE CARDIAC PHENOTYPES IN MARFAN SYNDROME

Butenas, Alexandre B., Borsoi, Juliana, Pereira, Lygia, Sarafian, Raquel

Genetics and Evolutionary Biology, Instituto de Biociências da Universidade, São Paulo, Brazil

Marfan Syndrome (MFS) is an autosomal dominant disorder of the connective tissue caused by mutations in the FBN1 gene encoding fibrillin-1, a major structural component of microfibrils and, thus, of the elastic fiber. Clinical manifestations of MFS affect skeletal, ocular and cardiovascular systems. Our group has produced isogenic hiPSCs with null and dominant-negative mutations in the FBN1 gene to develop in vitro models of the disease. In particular, we are interested in understanding the molecular mechanisms involved in dilated cardiomyopathy, present in 20-25% of MFS patients. Although hiPSCs can be efficiently differentiated into cardiomyocytes in 2D cultures, these do not express FBN1 hampering their use in modeling the disease in vitro. Use of 3D cardiac microtissues (MTs) composed of hiPSC-derived cardiac fibroblasts, cardiomyocytes and endothelial cells might prove valuable models for the study of heart phenotypes of MFS, since fibroblasts express FBN1, they enable higher maturation state of hiPSC-derived cardiomyocytes, and MTs better represent the organ. We have effectively differentiated all four hiPSC lines used in the study into the three cell types of interest. Cells were characterized by flow cytometry and immunocytochemistry assays for cTNNT (cardiomyocytes), CD31 and CD144 (endothelial cells), DDR2 and COL1A1 (cardiac fibroblasts). We generated MTs by co-culturing the three cell types, and characterized them by immunofluorescence with markers for CD31, cTNNT1 and COL1A1, in order to assess if all cell types were present in the model as well as their organization level. The MTs with different mutations in the FBN1 gene will be deeply phenotyped in order to understand if they can model dilated cardiomyopathy and other cardiac phenotypes in MFS.

Funding Source: Funding for this research was provided by CAPES.

Keywords: Marfan Syndrome, dilated cardiomyopathy, cardiac microtissues

Poster Abstracts

506 - A

EFFECTS OF ISCHEMIA ON HIPSC-DERIVED VENTRICULAR CARDIOMYOCYTES CARRYING A MUTATION CAUSATIVE OF ADVANCED AGE SYNDROME (HUTCHINSON-GILFORD PROGERIA SYNDROME)

Monteiro Da Rocha, Andre¹, Yudelman, Micaela¹, Monge, Evan², Laimon, Erinn¹, Herron, Todd¹

¹FCVC Cell Regeneration Core, University of Michigan, Ann Arbor, MI, USA, ²Internal Medicine Cardiology, University of Michigan, Ann Arbor, MI, USA

Children living with Hutchinson-Gilford Progeria Syndrome (HGPS), an advanced age syndrome caused by a LMNA mutation (Exon 11 - c.1824C>T), experience ischemic heart disease and heart attacks like older adults. Understanding the impact of HGPS on cardiomyocyte function and resilience to ischemia is extremely important to tailor therapeutic interventions improving cardiac function of HGPS patients and older adults. We generated matured functional syncytia of hiPSC-derived ventricular cardiomyocytes (VCM) from a HGPS patient (HGPS-VCM) and a genetically related control (GRC-VCM). VCMs were cultured in control medium (CM), or ischemic medium (IM) to induce an ischemic heart disease phenotype prior to high throughput optical mapping to assess functional changes in action potential (AP) and/or intracellular calcium transients (CaT). Our results indicate that HGPS-VCM were bradycardic in relation to GRC-VCM, and that IM increased spontaneous beat rate only in GRC-VCMs. As expected, increase in beat rate in IM treated GRC-CMs resulted in significant reduction of Fridericia corrected AP duration at 80% of repolarization (fAPD80%). Conversely, HGPS-VCMs had prolonged fAPD80% in relation to GRC, and treatment with IM did not alter fAPD80%. HGPS-VCMs in CM presented signs of intracellular calcium overload characterized by smaller amplitudes of CaT, higher diastolic calcium levels, and prolonged CaTD80%. GRC-VCMs treated with IM also presented intracellular calcium overload similar to HGPS hiPSC-VCM in CM. Treatment of HGPS-VCMs with IM further aggravated the state of calcium overload. Overexpression of sarcoplasmic/endoplasmic reticulum Ca²⁺ ATPase 2 (SERCA2a) rescued the intracellular calcium overload phenotype. Our results indicate that the LMNA mutation causative of advanced age syndrome induces electrophysiology and intracellular calcium handling changes that recapitulate bradycardia and diastolic dysfunction observed in children living with HGPS and older adults. Additionally, HGPS decreased the resilience to IM, but overexpression of SERCA2a reverted the diastolic dysfunction in HGPS hiPSC-VCMs and partially protected cardiomyocytes

against the effects of ischemia indicating the potential use of small molecule activation of SERCA2a to improve cardiac function in HGPS and older adults.

Funding Source: Claude D. Pepper Older Americans Independence Center grant number AG024824 Health Research (MiCHR) grant number UL1TR002240 Michigan Biology of Cardiovascular Aging Program NIH - R01HL148068

Keywords: hiPSC-derived cardiomyocytes, Hutchinson-Gilford Progeria Syndrome, cardiac aging

506 - B

EFFICIENT CO-CULTURE OF MICROGLIA AND MESENCEPHALIC ORGANIDS FOR THE STUDY OF NEUROIMMUNE INTERACTIONS

Santillan Cigales, Juan Jair¹, Velasco Velázquez, Ivan²

¹Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Ciudad de México, Mexico, ²Neurodevelopment, Universidad Nacional Autónoma de México, Mexico City, Mexico

Most Parkinson disease cases are sporadic. Different factors might play a role in the dopaminergic degeneration causing the motor alterations in these patients. Microglial cells have been suggested to participate in both the development and the degeneration of dopamine neurons. In this work, we aimed to develop a system to study the soluble and cellular interactions of microglia, differentiated from human pluripotent cells, on the development and viability of dopaminergic neurons, growing on mesencephalic organoids. Pluripotent cells were induced for hematopoietic differentiation to obtain microglial precursors, which were allowed to mature, showing some of the relevant markers for this cell type. On separate cultures, midbrain organoids were formed after aggregation of progenitor cells on neural-inducing conditions. We evaluated different co-cultures of microglia-like cells and mesencephalic organoids, where microglia-like cells came from different cell lines, including human iPSCs from Mexican patients. We found that the influence of microglia is of great importance for the development of dopaminergic neurons. These interactions occur through cell-cell communication and secretion of diverse molecules. Thus, microglial cells have effects on the viability of dopaminergic neurons in this 3D model, which results in a new tool for the study of neuroimmune interactions. This approach represents a novel strategy for the study of diverse pathologies, such as Parkinson's disease.

Funding Source: Papiit-DGAPA IN219122.

Keywords: microglia, Parkinson's Disease, organoids

Poster Abstracts

507 - A

EMPLOYING iPSC-DERIVED MODELS TO INVESTIGATE THE NEUROBIOLOGICAL MECHANISMS CAUSING SCHIZOPHRENIA

Almeida, Valéria¹, Hausruckingner, Anna², Raabe, Florian³, Gagliardi, Miriam³, Galinski, Sabrina³, Rossner, Moritz³, Ziller, Michael⁴

¹Department of Neurobiology/LMU and Department of Psychiatry/University of Münster, LMU Munich and WWU Münster, Munich, Germany, ²Lab for Genomics of Complex Diseases, Max Planck Institute of Psychiatry, Munich, Germany, ³Department of Psychiatry and Psychotherapy, LMU Munich, Germany, ⁴Department of Psychiatry, University of Münster, Germany

Schizophrenia (SCZ) is a severe mental disorder that affects 1% of the worldwide population. The pathophysiology of SCZ is highly complex and involves several neurotransmission systems. The advent of human-derived iPSC technology combined with the differentiation of iPSCs into neuronal or glial cell types is a potential model to study SCZ. It is believed that these technologies will push the borders of translational psychiatry. Therefore, we have generated the iPSC lines from 38 individuals with schizophrenia, and 38 healthy controls (Ethikkommission München, Projekt-Nr.: 17-880). As a follow-up of our iPSC case-control cohort study, we aim to generate excitatory cortical neurons to contribute to the knowledge about the molecular pathways and neurobiology of SCZ. Despite some studies that have shown neuronal dysfunction in SCZ using iPSCs, data on more homogeneous excitatory neuron culture is still incomplete. Thus, we aim to produce large-scale glutamatergic-induced neurons from iPSCs, using the overexpression of transcription factors. The overexpression of the neurogenin-2 (NGN2) transcription factor in iPSC lines, in combination with small molecules, allows the generation of stable cell lines that can be differentiated into glutamatergic excitatory neurons. Currently, we perform multi-omic and morphological studies to investigate the molecular basis and neuronal phenotype of SCZ compared to controls. So far, no studies have performed the same analysis with the same number of samples. Therefore, with these approaches, we aim to provide relevant data on features of excitatory cortical neuron neurons obtained from our large SCZ cohort.

Keywords: schizophrenia iPSC cohort, cortical glutamatergic neurons, neurogenin-2 (Ngn2)

507 - B

FUNCTIONAL AND STRUCTURAL BASIS OF TDP-43 DISRUPTION MEDIATED BY RRM2 MUTATION ASSOCIATED WITH AMYOTROPHIC LATERAL SCLEROSIS

Julio, Alison¹, Cambraia, Amanda², Carvalho, Lian¹, Martins, Robertta¹, Leal, João Victor¹, Melo, Lucas³, Junior, Mario², Zembrzuski, Verônica², Trindade, Pablo⁴, Vasques, Juliana³, Gubert, Fernanda³, Araujo, Helena³, Otero, Rosalia¹

¹Biophysics Institute, Carlos Chagas Filho, Federal University of Rio de Janeiro, Brazil, ²Oswaldo Cruz Institute, Fiocruz, Rio de Janeiro, Brazil, ³Institute of Biomedical Science, Federal University of Rio de Janeiro, Brazil, ⁴Department of Clinical and Toxicological Analysis, Federal University of Rio de Janeiro, Brazil

Amyotrophic Lateral Sclerosis (ALS) is a multifactorial neurodegenerative disease mainly affecting motor neurons. ALS shows a high heterogeneity of genes and alleles based on the geographical distribution of the patients. Our team found a new patient-specific allele of the TARDBP gene, containing the substitution p.(Phe194Leu) surrounding the RRM2 motif; however, whether it is causative of ALS is unknown. To address this question, we present the proof-of-concept of the platform Fly2Human, which combines in silico protein structure with different genetic engineering technologies and screening approaches to explore Single Nucleotide Variants potentially associated with rare diseases. Using protein modeling, we found that p.(Phe194Leu) potentially disrupted TDP-43:RNA interaction. We generated a series of expression vectors to further explore this new allele, including TDP-43-F194L-YFP, for heterologous expression in iPSC-derived astrocytes. In contrast to the wild-type and a known C-terminal mutation p.(Gly335Ala), associated with ALS, which show nuclear localization, immunocytochemistry revealed that the p.(Phe194Leu) shows cytoplasm accumulation in a time-dependent manner as a strong indication of TDP-43 malfunction. Next, we employed gene editing in human iPSCs to generate an in-locus TDP-43 edition into the RRM2. We were able to generate, isolate, and bank two independent iPSC clones containing a biallelic modification in TARDBP surrounding the target Phe194 in the RRM2, for which we are currently performing functional assays. To explore in vivo the p.(Phe194Leu), we have developed a series of novel constructs driven by UAS promoter for the expression of human TARDBP in transgenic Drosophila lines. Currently, we are performing the genetic screening for these transgenic lines, which should allow the

Poster Abstracts

expression of TARDBP allelic variants conditionally. Using the Fly2Human platform, our results suggest that p.(Phe194Leu) promotes disruption of the TDP-43:RNA interface and modification of TDP-43 subcellular localization. Therefore, indicate the potential of this interdisciplinary approach to accelerate the modeling, discovery, and delivery of potential translational therapies applied to rare human genetic diseases such as ALS.

Funding Source: Cnpq FAPERJ

Keywords: Amyotrophic Lateral Sclerosis, iPSC-derived astrocytes, TDP-43

508 - A**GENERATING PITUITARY CELLS FROM IPSCS OF PATIENTS WITH CHILD ONSET CONGENITAL HYPOPITUITARISM HARBORING PROP1 PATHOGENIC ALLELIC VARIANTS**

Marques, Juliana Moreira¹, Fattahi, Faranak², Carvalho, Luciani³

¹Department of Developmental Endocrinology, FMUSP, São Paulo, Brazil, ²Cellular and Molecular Pharmacology, UCSF, San Francisco, CA, USA, ³Developmental Endocrinology, FMUSP, São Paulo, Brazil

Reduced or absent levels of pituitary hormones due to genetic or organic causes is known as combined pituitary hormone deficiency (CPHD) and PROP1 is its most frequent cause, but more than 85% of the cases are without molecular diagnosis. Novel techniques such as induced pluripotent stem cells (iPSC), can be used to generate patient's specific pituitary cells for disease modeling. Thus, the aim of this study is to generate iPSC from CO-CH patients' peripheral blood mononuclear cells (PBMC) and differentiate them into pituitary cells. Peripheral blood was collected from 2 siblings (HC1 and HC2) harboring compound heterozygous PROP1 allelic variants and their parents (HC3 and HC4). PBMCs were isolated and expanded with MNC medium prior to nucleoporation and transfection with plasmids containing pluripotency genes (OCT4 and SOX2; cMYC and KLF). After 48h, transfected cells were transferred to Geltrex coated plates and fed with E8, NaB and bFGF until colonies were formed. iPSCs were differentiated into cranial placode cells using pituitary placode conditioned medium (PPCM) until day 15. For cell maturation, PPCM without SB431542 was used until day 30. qPCR and flow cytometry using the Kit BD Stemflow (BD Biosciences) for stem cells and the eBioscience™ Foxp3 / Transcription Factor Fixation/Permeabilization (Thermo Fisher Scientific)

for the placode and pituitary markers were used. Pituitary hormonal levels from cell supernatant were measured at the Laboratory of Hormones and Molecular Genetics - HCFMUSP. We generated iPSCs from patient and control blood cells. iPSCs were more than 90% positive for the pluripotency markers SOX2, NANOG, OCT3/4. Pituitary cells were generated with positivity for the placode marker SIX1 (58%) and pituitary markers, as LHX3 (56%) and PROP1 (59%) by day 15. Control cell lineages expressed higher levels of pituitary hormones at days 15 and 30 at RNA and protein levels while patient cells failed to induce hormone-producing cells properly. Pituitary hormonal release was impaired in patients pituitary cells. In conclusion, CO-CH patients' PBMC were reprogrammed and able to generate pituitary cells. Patient cells mimicked human phenotype. These cells can be used to study CO-CH basis in patients specific background, develop pituitary disease modeling as well as test possible new treatments.

Funding Source: Fundação de Amparo a Pesquisa do Estado de São Paulo (2020/06792-0, 2020/03299-0, 2021/10483-5)

Keywords: pituitary development, CPHD, PROP1

508 - B**GENERATION AND CHARACTERIZATION OF RETINAL PIGMENT EPITHELIUM CELLS FROM INDUCED PLURIPOTENT STEM CELLS IN A MODEL OF RETINITIS PIGMENTOSA CAUSED BY A MFRP MUTATION**

Nava, Jessica¹, Zenteno Ruiz, Juan Carlos², Mora Roldán, German Atzin², Gazarian, Karlen³, López Ramírez, Omar²

¹Faculty of Medicine, UNAM, Mexico City, Mexico, ²Genetics, Institute of Ophthalmology Conde de Valenciana, Mexico City, Mexico, ³Genomic Medicine and Environmental Toxicology, Institute of Biomedical Research at the National Autonomous University of Mexico, Mexico City, Mexico

Retinitis pigmentosa (RP), is a hereditary disease characterized by progressive degeneration of photoreceptor cells (PR) and retinal pigmented epithelium (RPE). MFRP mutations cause recessive forms of RP affecting mostly the RPE adjacent to PR in the eye and critical for normal retinal functioning, occasionally includes other anomalies such as nanophthalmos and foveoschisis, a syndromic disease initially described by our group. The cause of why some MFRP mutations cause isolated RP while other mutations cause a nanophthalmos-

Poster Abstracts

RP complex phenotype is still unknown. Induced pluripotent stem cells (iPSCs) made possible to model retinal diseases for in vitro recapitulation. iPSCs were generated from dermal fibroblast from a subject with RP due to the homozygous variant c.498_499insC (p. P165fsX198) in MFRP, using the Sendai virus method. iPSCs were characterized by the expression of pluripotency transcription factors. Differentiation to RPE was performed using nicotinamide and ketomine. Cells were evaluated by early expression markers of RPE precursors (OTX2, PAX6, MITF) and mature RPE markers (MERTK, RPE65, BEST1), using qPCR, RT-PCR and immunofluorescence at different days of differentiation. At day 10, clear OTX2 and PAX6 expression was observed in MFRP iPSC cell line and H9 cells; while at day 20 a reduction of OTX2 expression, maintenance of PAX6 expression and low MITF expression were observed in both lines; by day 30, a reduction in the expression of the early marker OTX2 and a higher expression of MITF was observed in both lines. This expression changes are consistent with that described during in vivo differentiation. These results indicate that the generated model allows RPE retinal precursors recapitulation from somatic cells with the variant in the MFRP gene for the study of this disabling disease. This project will continue further with the determination of expression markers differences, identification of changes in the regulation of the Wnt/B-catenin pathway in RPE, and the functional characterization describing structural and electrophysiological alterations of PR in retinal organoids differentiated from iPSCs. Our group is the first in Mexico to generate a RP model based on iPSC from patients, this will allow us to extrapolate different forms of RP due to genetic variants in Mexican population.

Funding Source: funded by CONACYT 568454

Keywords: retinosis pigmentaria, retinal pigment epithelium, Induced pluripotent stem cells

509 - A

HOXD1 INHIBITS TUMOR PROGRESSION BY PROMOTING GLIOBLASTOMA STEM CELL DIFFERENTIATION

Fernández, Edelmira A.¹, Gajardo, Felipe¹, Gaete Ramírez, Belén², Varas Godoy, Manuel², Armisen, Ricardo³, Allende, Miguel⁴, Palma, Veronica⁵

¹Department of Biology, Universidad de Chile, Santiago, Chile, ²Center for Cell Biology and Biomedicine, Universidad San Sebastián, Santiago, Chile, ³Cancer Functional Genomics Lab, Universidad del Desarrollo, Santiago, Chile, ⁴Center for Genome Regulation, Universidad de Chile, Santiago, Chile, ⁵Laboratory of Stem Cells and Developmental Biology, Universidad de Chile, Santiago, Chile

Glioblastoma is the most aggressive, invasive, and undifferentiated brain tumor among gliomas, designated Grade IV by the WHO. Despite advances in combined radiation and chemotherapy treatments following surgical resection, the life prognosis for patients remains discouraging. Cancer stem cells (CSCs), present in this type of tumor, constitute a promising therapeutic target due to their relationship with tumor occurrence, progression, and recurrence. HOX genes encode a broad family of transcription factors that, in addition to playing a central role during embryonic development, have been associated with the development and progression of different types of cancer, including glioblastoma. The alteration of the HOX gene expression profile suggests their role as tumor modulators, potentially acting as tumor suppressors genes or having an oncogenic role that may be associated with the acquisition of a CSC phenotype. Our hypothesis proposes that the activation of tumor suppressor HOX gene expression could induce CSC differentiation, thereby preventing the progression of glioblastoma. We performed different bioinformatics approaches based on transcriptomic and clinical data from glioblastoma databases. We identified HOXD1 as a potential tumor suppressor gene associated with a differentiated phenotype. Our in vitro analyses, which involved evaluating differentiation and stemness in various glioblastoma cell lines, are consistent with the findings from the bioinformatics analysis and indicate that the increase of HOXD1 expression leads to the differentiation of CSC in glioblastoma. We are currently engaged in validating the role of HOXD1 as a tumor suppressor gene in glioblastoma through in vivo experiments. The results of our ongoing research will enhance our comprehension of the underlying mechanisms responsible for the initiation and progression of glioblastoma and facilitate the identification of novel prognostic biomarkers.

Funding Source: ANID National Doctorate Scholarship 21181605 Millennium Institute Center for Genome Regulation ICN2021_044

Keywords: cancer stem cells, HOX genes, glioblastoma

Poster Abstracts

509 - B

HUMAN GASTROINTESTINAL ORGANOID MODEL INNATE IMMUNE RESPONSE AND INFECTION**de Sousa Vieira, Afonso***Biotechnology Department, Technische Universität, Berlin, Germany*

The intestinal pathogen Enteropathogenic Escherichia coli (EPEC) has been studied using mouse models and cell lines. However, with mice not being a natural host for EPEC and cell lines often lacking the complexity required to accurately model infections, human intestinal organoids appear as an alternative in the field. In our research group, we work with patient-derived organoids. These are primary cells obtained from patients which recapitulate patient variability, cell types, and are representative of different segments of the gastrointestinal (GI) tract. In order to further characterize EPEC infection, we use monolayers derived from human intestinal organoids. Upon infection with GFP-EPEC and consequent imaging under fluorescent microscope, the monolayers exhibit microcolonies, a hallmark of infection, as well as actin pedestals and intimate attachment. Moreover, our model allows us to use fluorescent-activated cell sorting and imaging to monitor infection across different patients and regions of the GI tract. We observe an increasing number of infected cells, depending on the multiplicity of infection (MOI). Notably, EPEC causes particularly strong pathology in young children but not in adults. Leveraging our organoid biobank, which includes organoids derived from individuals at a very early stage of life, we have a unique opportunity to investigate EPEC infection in both neonate and adult-derived organoids. This approach holds promise for gaining insights into the age-dependent outcomes of EPEC infection.

Keywords: infection, intestinal organoids, enteropathogenic E. coli

510 - A

IDENTIFICATION OF A NEW CHALCONE DERIVATIVE AS POTENT ABCG2 INHIBITOR, NONCYTOTOXIC AND CAPABLE TO REVERSE CHEMORESISTANCE PHENOTYPE

Ruginsk, Bruna Estelita¹, de Fátima Zattoni, Ingrid¹, Da Silva Zanzarini, Isadora¹, Rotuno Moure, Vivian¹, Boumendje, Ahcène², Valdameri, Glaucio¹

¹*Pharmaceutical Sciences Graduate Program, Universidade Federal do Paraná, Curitiba, Brazil,*

²*Medicinal Chemistry School, Université Grenoble Alpes, Grenoble, France*

Cancer is a leading cause of death worldwide and the pharmacological treatment failure is due to multidrug resistance (MDR), mainly caused by overexpression of ABC transporters. In cancer stem cells, a subpopulation identified as its ability to export Hoechst dye driven by increased expression of two ABC transporters, named P-glycoprotein (P-gp) and ABCG2. Among the strategies to reverse MDR, the use of ABC transporters inhibitors is the most promising. In contrast to P-gp, for which inhibitors are already in clinical trials, there are very few potent and noncytotoxic ABCG2 inhibitors described. Thus, this work targeted the identification of new ABCG2 inhibitors based on chalcone scaffold. The inhibition capacity was assessed by flow cytometry using stably transfected HEK293 cells to overexpress ABCG2 (HEK293-ABCG2). The assay relies on quantification of intracellular levels of a fluorescent dye called Hoechst 33342, a ABCG2 substrate. As control, the reference inhibitor Ko143 was used. 22 new chalcone derivatives (named as CH1 - CH22) were tested at 1 and 10 μ M. The inhibition percentage was obtained by comparing the results of Ko143. 12 chalcones showed at least 50% inhibition at 10 μ M and were eligible to determine the IC₅₀ values (concentration that produces half of maximal inhibition). The most potent chalcone CH10 showed a IC₅₀ value of 0.34 μ M. The cytotoxic effect was further evaluated by MTT assay in HEK293 and HEK293-ABCG2 cells. Both cell lines were treated with a range of 0.1 - 50 μ M for 72 h. Interestingly, no decrease in cell viability was detected, becoming impossible to determine the IG50 (concentration that reduces cell viability in 50%). Thus, the therapeutical ratio (IG50/IC₅₀) was estimated to >146. To evaluate the capacity of this chalcone towards the challenge of reversing the chemoresistance phenotype, a chemotherapeutic substrate of ABCG2 was used (SN38) in a non-tumoral (HEK293 and HEK293-ABCG2) and a tumoral model (H460 and H460/MX20), and cell viability was quantified by MTT. In both cases, CH10 was able to reverse resistance in overexpressing ABCG2 cells, with cell death similar to wildtype cell lines. Together, these results demonstrated a very promising chalcone derivative as ABCG2 inhibitor for future preclinical studies.

Keywords: Multidrug resistance, Cancer, ABCG2

Poster Abstracts

510 - B

IMMUNOMODULATORY EFFECT OF HUMAN MILK EXTRACELLULAR VESICLES IN NEONATAL NF-KB REPORTER INTESTINAL ORGANIDS

Pagotto, Romina, Céspedes, Paula, Giribaldi, Fiorella, Bollati-Fogolin, Mariela

Cell Biology Unit, Institut Pasteur Montevideo, Uruguay

Breast milk reduces the risk of necrotizing enterocolitis in very low birth weight preterm infants. However, its protective role against inflammatory intestinal diseases in older ages remains controversial. Milk extracellular vesicles (EVs), including exosomes, may have a protective effect. NF- κ B is a transcription factor with key functions in intestinal inflammation. To explore milk EVs' potential immunomodulatory effect on neonatal and adult intestinal epithelium, EVs from human breast milk were isolated and characterized. Furthermore, neonatal intestinal organoids were generated from a transgenic mice reporter for NF- κ B activation and challenged with TNF- α as pro-inflammatory trigger stimuli. Human milk EVs were isolated using ultracentrifugation and size exclusion chromatography. Characterization was performed through transmission electron microscopy (TEM), dynamic light scattering (DLS), flow cytometry (FC), and western blot analysis. Neonatal intestinal organoids, generated by isolating crypts from 9-day-old transgenic mice, were exposed to TNF α (10-100 ng/mL). Luciferase reporter activity was compared in both differentiated and non-differentiated conditions. Human breast milk EVs were isolated from 8 donors. Size analysis revealed the presence of particles ranging around 200 nm (FC), with an average size diameter of 270 nm (DLS). TEM images confirmed the presence of smaller vesicles exhibiting the exosome characteristic cup-shaped morphology. Enriched EVs fractions express tetraspanins CD63, CD9, and CD81 (detected by FC), as well as CD9 and TSG101 (evidenced by Western blot). When incubated in differentiation medium before stimulation, reporter organoids displayed a concentration-dependent response to the stimulus. No significant differences were observed between male and female organoids. Our results confirm the presence of small EVs in human milk samples and establish the NF- κ B reporter assay using neonatal intestinal organoids. Ongoing studies focus on assessing the immunomodulatory effects of isolated EVs in neonatal- and adult-derived reporter organoids. These studies will contribute to the characterization of the biological impact of milk EVs on intestinal cells and the identification of bioactive agents with anti-inflammatory properties.

Funding Source: This project was funded by MEC (FVF_2021_176). MB-F and RP are members of the SNI (National Research System, Uruguay) and PEDECIBA.

Keywords: milk extracellular vesicles, intestinal organoids, inflammation

511 - A

IN VITRO INTERACTION OF CANINE ADIPOSE-DERIVED MESENCHYMAL STEM CELLS WITH CANID ALPHAHERPESVIRUS: INFLUENCE ON CELL DIFFERENTIATION GENES AND VARIATIONS OF VIRAL GENOME

Prišlin, Marina¹, Zorec, Tomaž Mark², Vlahović, Dunja³, Naletilić, Šimun⁴, Ljolje, Ivana⁵, Kostešić, Petar⁶, Kunić, Valentina¹, Turk, Nenad⁷, Brnić, Dragan¹

¹*Virology Department, Croatian Veterinary Institute, Zagreb, Croatia*, ²*Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Slovenia*, ³*Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Zagreb, Croatia*, ⁴*Department for Pathological Morphology, Croatian Veterinary Institute, Zagreb, Croatia*, ⁵*Veterinary Clinic for Small Animals Buba, Zagreb, Croatia*, ⁶*Surgery, Orthopaedics and Ophthalmology Clinic, Faculty of Veterinary Medicine, University of Zagreb, Croatia*, ⁷*Department of Microbiology and Infectious Diseases with Clinic, Faculty of Veterinary Medicine, University of Zagreb, Croatia*

Canine adipose-derived mesenchymal stem cells (cASCs) have therapeutic potential, as they can differentiate into multiple cell types and release immunomodulatory molecules. However, in vitro culturing of cASCs can impact their differentiation (DI) capacity, and there is a risk of viral infection, such as with Canid alphaherpesvirus-1 (CHV), which can establish latency. This study aimed to assess the impact of CHV infection on the genetic profiles of cASCs' DI processes and investigate potential alterations in the CHV genome during passaging in cASCs. Abdominal adipose tissue from 12 young female dogs was used to extract cASCs. Immunophenotyping and three-lineage in vitro DI were carried out in the third passage (P3). Following RNA integrity testing, 24 cASCs DI genes were examined using an RT-qPCR array, for both the uninfected and CHV-infected cASCs (multiplicity of infection; MOI 0.1). Viral DNA was extracted after three CHV passages (MOI 0.1) in cASCs and Madin-Darby canine kidney (MDCK) cells and variations were searched using the NextSeq 550 platform. The results confirmed a conserved stemness immunophenotype and DI potential of cASCs. Array results showed 20.83% gene alterations due to in vitro CHV infection, including significant upregulation of ADIPOQ, promoter of adipoDI and osteoDI, and significant increases in expression of GDF5 and GDF7,

Poster Abstracts

key promoters of cartilage production. FGF10, involved in organ development and injury repair, and CEBPD, a promoter of adipoDI, exhibited no significant downregulation. The CHV passaging resulted in similar mutational stability between MDCK and cASCs cultures with minor variations emerging from the 1st passage (consensus-level and quasispecies-level mutations). In general, our research revealed significant changes in the expression of DI genes in female cASCs due to in vitro CHV infection. Preliminary results of the CHV passage on cASCs showed insignificant mutations, confirming cASCs' suitability as a primary cell culture for studying viral fitness. To validate these findings, future studies should involve a larger donor pool, including gender diversity. The preservation of DI potential is crucial to producing top-notch canine stem cell products, emphasizing the need for routine CHV testing before therapeutic use.

Funding Source: This research was funded by Croatian Science Foundation (HRZZ) Installation Research Project (UIP-2019-04-2178) "Revealing transcriptome and secretome of mesenchymal stem cells".

Keywords: canine adipose-derived mesenchymal stem cell, differentiation gene expression, canid alphaherpesvirus 1

SATURDAY, 23 SEPTEMBER

Session Times are in BRT, Brasília Time Zone (São Paulo, Brazil)

Poster Session II - A and B 5:30 PM - 6:30 PM

511 - B

INTERACTION BETWEEN LECTINS AND GLYCANS IN THE VIRAL ACTIVATION OF PLATELET-DERIVED GROWTH FACTOR RECEPTOR-A (PDGFR-A)

Gutierrez, Patricia Noemi¹, Gambarte Tudela, Julian¹, García, Alfredo¹, Bannoud, Nadia¹, Abba, Martín², Naipauer, Julian³, Coso, Omar⁴, Lacunza, Ezequiel⁵, Rabinovich, Gabriel⁶, Croci Russo, Diego¹

¹Institute of Histology and Embryology of Mendoza, CONICET, Mendoza, Argentina, ²Centro de Investigaciones Inmunológicas Básicas y Aplicadas, CONICET, Buenos Aires, Argentina, ³Institute of Physiology, Molecular Biology and Neurosciences, CONICET, Buenos Aires, Argentina, ⁴Instituto de Fisiología, Biología Molecular y Neurociencias, CONICET, Buenos Aires, Argentina, ⁵Centro de Investigaciones Inmunológicas Básicas y Aplicadas, Facultad de Ciencias Médicas, Universidad Nacional de La Plata, CONICET, La Plata, Argentina, ⁶Instituto de Biología y Medicina Experimental, CONICET, Buenos Aires, Argentina

At present, at least ten biological agents defined as carcinogenic to humans are known, one of them being Kaposi's sarcoma-associated herpesvirus (KSHV). KSHV causes Kaposi's sarcoma (KS), one of the most common cancers among HIV-1/AIDS patients. During the lytic reactivation phase, expression of the viral G protein-coupled receptor (vGPCR) oncogene promotes the secretion of galectin-1 (Gal-1) and growth factors such as platelet-derived growth factor (PDGF). Thus, the expression of vGPCR in infected cells is decisive during the sarcomagenesis process. Gal-1 is a lectin overexpressed in various cancers and contributes to tumor progression. The study of their interactions with N-glycans allows us to reveal the functional consequences generated by the fluctuating changes in the cellular glycome. PDGFRA is a highly glycosylated receptor tyrosine kinase responsible for driving KSHV-mediated sarcomagenesis. In this study, we hypothesized that the expression of vGPCR induces a change in the cellular glycome of bone marrow-derived mesenchymal stem cells (BM-MSCs), which favors the interaction

Poster Abstracts

between Gal-1 and PDGFRA-N-glycans promoting its oncogenic activation. Initially, we established a protocol for isolating mouse BM-MSCs, a cell lineage proposed as a possible origin of SK, and carried out its immuno- and glyco-phenotypic characterization by flow cytometry. BM-MSC was transfected with an expression vector encoding vGPCR viral oncogene. Modifications that increase the availability of glycoepitopes recognizable by Gal-1 were observed in these cells. Interestingly, upon cellular stimulation with Gal-1 and PDGF, activation of PDGFRA occurred in “patch-like” structures. In this sense, Gal-1 activates its signaling pathway regardless of its classical ligand. Therefore, we suggest that Gal-1-N-glycans interactions in PDGFRA receptor function as a potential non-canonical ligand-independent mechanism of receptor activation. Our data provide empirical evidence on the relevance of understanding and investigating the cellular glycome in normal and pathological conditions by studying glycan-binding proteins.

Funding Source: National Institutes of Health - Project U54CA221208-01

Keywords: glycosylation, bone marrow-mesenchymal stem cells, virus-associated cancer

512 - A

INVESTIGATION OF THE EFFECTS OF AGING IN MACROPHAGE AND SKELETAL STEM/PROGENITOR CELLS POPULATIONS DURING FRACTURE HEALING

Frade, Bianca Braga¹, Dias, Rhayra², Müller, Leonardo³, Santos, Caio⁴, Souza, Marcos², Bonfim, Danielle²

¹Cellular Biology and Parasitology/Carlos Chagas Filho Biophysics Institute, Federal University of Rio de Janeiro, Brazil, ²Biomedical Sciences Institute, UFRJ, Rio de Janeiro, Brazil, ³Surgery Sciences, UFRJ, Rio de Janeiro, Brazil, ⁴Alberto Luiz Coimbra Institute for Graduate Studies and Research in Engineering, UFRJ, Rio de Janeiro, Brazil

Bones can fully repair after a fracture, but with ageing, consolidation failures become frequent. Therefore, innovative treatments to promote fracture healing in the elderly are urgently needed, what demands a better understanding of the key events driving this process. While much attention has been directed to the role of Skeletal Stem and Progenitor Cells (SSPCs) in fracture

healing, recent evidence now highlights the important role of macrophages in SSPCs instruction. However, little is known about the molecular mechanisms used by macrophages to control SSPCs function and whether ageing impacts this crosstalk. To start depicting this relationship in young vs elderly individuals, in this study we aimed at: (i) evaluating the frequency of SSPCs in control and fractured bones of patients over 40 years, to determine whether ageing impacted SSPCs expansion immediately after injury; (ii) mapping the immunophenotype of the macrophage populations over the course of fracture healing, in control and middle-aged mice, as this analysis cannot be performed in human fracture samples. Following approval (nº 21768719.0.0000.5257), n = 15 control hip samples and n = 3 fractured bones were collected from patients undergoing primary arthroplasty surgery and FACS was used to determine the frequency of SSPCs in the bone marrow. For macrophage analysis, we established a femur fracture model in young (10-12 weeks) and middle-aged mice (48-50 weeks) (CEUA 101/22), and evaluated their immunophenotypic profile at days 7 and 21 after injury. Our preliminary results showed that SSPCs still expanded in the elderly bone marrow following a fracture (0,13% ± 0,20% in control bones vs 14,95% ± 8,44% in fractured bones), suggesting that the proliferative capacity of these cells is not totally compromised by ageing. We also observed an increase in the number of F4/80+ Mac2+ CD206+ macrophages along time in both groups, which suggests a preponderant role of a transitional (M1 to M2) population in the process of bone consolidation, which could either control inflammation and the activity of SSPCs. Further functional and transcriptomic analysis will be performed to determine whether ageing impacts the molecular mechanisms used in macrophage-SSPCs crosstalk, and, consequently, in their function in the healing process.

Funding Source: Carlos Chagas Filho Research Support Foundation of the State of Rio de Janeiro (FAPERJ) and National Council for Scientific and Technological Development (CNPQ).

Keywords: skeletal stem/progenitor cells, macrophages, bone regeneration

Poster Abstracts

512 - B

INVESTIGATION OF THE NEUROPROTECTIVE AND ANTI-INFLAMMATORY POTENTIAL OF MARINE SPONGE SPECIES EXTRACTS

Nunes, Catarina de Jesus¹, Santos, Cinthia¹, Souza, Luciano², Lanna, Emilio², Batista, Ronan², Nascimento, Ravena¹, Costa, Silvia¹

¹Department of Biochemistry and Biophysics, Institute of Science and Health-Federal University of Bahia, Salvador, Brazil, ²Department of Organic Chemistry, Institute of Chemistry, Federal University of Bahia, Salvador, Brazil

Neurodegenerative diseases are characterized by the progressive loss of neurons, leading to disability and death. The search for new therapies aims to investigate substances with anti-inflammatory potential in the CNS. Sponges are among the most prolific marine organisms for new substances, and in vitro studies have demonstrated anti-inflammatory activity of extracts or purified compounds from some species. Evaluation of cytotoxicity and the neuroprotective potential of extracts of marine sponge species against in vitro inflammatory damage associated with modulation of microglia response. Cultures of PC12 cells were treated with 20 extracts (0.1 to 200 µg/mL) obtained with DCM, EtOAc and MeOH solvents from marine sponge species of the genera *Aplysina*, *Cladocroce*, *Condrilla*, *Callyspongia* and *Haliclona*. Cell viability was determined after 72 h of treatment by the MTT test. PC12 cells were subjected to inflammatory damage with LPS for 12 h and treated for 24 h with *A. fulva* extract (0.1, 1 µg/mL), or with its purified compound AF-H1 (1.10 µM), and the cell viability assessed by Trypan Blue and Propidium Iodide. Microglia from the cerebral cortex of newborn Wistar rats were treated for 24 h with conditioned medium of PC12 cells under these conditions and the phenotype of these and PC12 cells were evaluated by phase contrast microscopy, Rosenfeld panchromatic staining and immunocytochemistry for the marker structural Iba-1. Most extracts showed toxicity at concentrations of 100 and 200 µg/mL, except for Ac-EtO extracts which were from 10 µg/mL. The MeOH extract of *A. fulva* and its AF-H1 compound were not toxic at the tested concentrations. PC12 cells subjected to damage with LPS showed contracted cell bodies, an effect that was not observed in cultures treated with AF-MeOH and AF-H1 extracts at the adopted concentrations. Microglia treated with conditioned medium from cultures of PC12 subjected to LPS showed an amoeboid shape; in contrast, microglia subjected to conditioned medium from PC12 cells treated with LPS and AF-MeOH or LPS and AF-H1 showed a more branched phenotype, similar to that of microglia under control conditions. Marine sponge extracts and isolated substances are non-toxic. Treatment with these compounds contributed to a possible reversal of the morphology of PC12 cells and microglia when exposed to damage.

Funding Source: SUPPORT: CNPq; CAPES

Keywords: neuroinflammation, neuroprotection, neurodegenerative diseases, anti-inflammatory marine sponges

513 - A

LYSOPHOSPHATIDIC ACID PROMOTES STEMNESS OF GLIOMA CELLS VIA SOX2 AND OCT4

Bello, Claudia¹, González-Arenas, Aliesha¹, Velasco-Velázquez, Marco A.²

¹Institute of Biomedical Research, Universidad Nacional Autónoma de México, Ciudad de México, México,

²Departamento de Farmacología, Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad de México, México

Glioblastomas (GB) are the most common and deadly primary brain tumors in adults. The therapy of choice for these patients has not changed in over 10 years and offers a survival rate of approximately 12 months. Lysophosphatidic acid (LPA) is a bioactive lipid that regulates a wide range of signals, including Akt, MAPKs, and PKC activation through interaction with six G protein-coupled receptors (LPA1-6). The role of LPA in cancer has been extensively studied and has been shown to induce the expression of transcription factors associated with cancer cell stemness, such as SOX2 and OCT4. However, there is no data on the involvement of LPA in stemness signaling pathways in GB cells. In this study, we evaluated the effect of LPA on the sphere culture of U251, U87, and C6 cell lines derived from human and murine glioblastoma, respectively. First, we isolated glioblastoma stem cells through sphere culture of the U251, U87, and C6 glioma-derived cell lines. We then established the distribution of the spheres by grouping them into three size ranges: 25-60 µm, 75-100 µm, and greater than 100 µm. Treatment with LPA increased the sphere-forming efficiency (SFE) in all three cell lines and all sizes, while treatment with Ki16425 (Ki) (LPA 1-3 inhibitor) decreased the SFE by greater than 100 µm. Interestingly, the decrease and increase in SFE were accompanied by decreased and increased expression of the transcription factors SOX2 and OCT4. The comparison between Ki (LPA1-3 inhibitor) and AM095 (AM) (LPA1 inhibitor) treatments suggests that the type of receptor involved in the LPA effect (LPA1, 2, or 3) is determined by the cellular context. The results of this work suggest that LPA increases the stemness of glioma-derived cells by increasing the expression of SOX2 and OCT4.

Keywords: glioblastoma, stem cell, lysophosphatidic acid

Poster Abstracts

513 - B

MATERNAL SARS-COV-2 INFECTION ELICITS AN IMMUNE RESPONSE AND A DISTINCT MULTIPOTENT CHARACTERISTIC IN PLACENTA-DERIVED MESENCHYMAL STEM CELLS

Solis, Mairim Alexandra¹, López-Vergès, Sandra², Fu Barrios, Cindy¹, Guerrero, Erika¹, Jaen-Alvarado, Yanelys¹, Gaitan, Melissa², Chen-German, Maria², Arauz, Dimelza², Sanchez, Jaime³, Villalobos, Rodrigo⁴, Vigil-De Gracia, Paulino⁵, Vega, Shantal¹, Saenz, Lisseth², Quintana, Hedley⁶, Delgado, Julio¹

¹*Stem Cell Research Group, Department of Research in Sexual and Reproductive Health, Gorgas Memorial Institute for Health Studies/National Bioethics Research Committee in Panama, Panama,* ²*Department of Research in Virology and Biotechnology, Gorgas Memorial Institute for Health Studies, Panama,* ³*Department of Gynecology and Obstetrics, Hospital Santo Tomas, Panama,* ⁴*Department of Pathology, Hospital Santo Tomas, Panama,* ⁵*Gynecology and Obstetrics Division, Complejo Hospitalario Metropolitano Doctor Arnulfo Arias Madrid, Panama,* ⁶*Department of Health Technology and Assessment, Gorgas Memorial Institute for Health Studies, Panama*

Maternal SARS-CoV-2 infection elicits placental immune activation even in the absence of virus in the placenta, inducing alterations in the placental environment and contributing to several pregnancy outcomes in individuals with coronavirus disease 2019 (COVID-19). Whether these outcomes alter the function of cells residing in the placenta remains poorly understood. We have previously reported SARS-CoV-2 detected in the placenta of COVID-19-positive pregnant women, with virus localized in chorionic villi endothelial cells. Although, SARS-CoV-2 detection in the placenta is not frequent, it remained to be elucidated whether COVID-19 exerts any changes in the biological function of placenta-derived mesenchymal stem cells (PDMSCs). In this study, we characterized the multipotent capacity of PDMSCs derived from women with a median of 6-days SARS-CoV-2-positive nasopharyngeal test (C-PDMSC). Even if the virus was not detectable, we found that early-passage C-PDMSC (C-PDMSC-EPs) presented an average 57% higher growth rate and up to a fourfold increase in the expression of multipotent markers, CD105 and CD73, when compared to non-COVID-19-derived PDMSCs at early passage (NC-PDMSC-EP). Higher expression of the differentiation markers involved in chondrogenesis (ACAN, SOX9), adipogenesis (ALP, FEB4, CEPB), and osteogenesis (COL1, OPN, RUNX2, OCN) were present in C-PDMSC-EPs, however these expressions seemed to be at least ten times lower at later passages. A sixfold increase in the expression of proinflammatory genes (IL-6, IDO) was found in immune-stimulated C-PDMSC-EPs. In-vitro infection of SARS-Cov-2 at 1 and 10 multiplicities of

infection into NC-PDMSC-EP, reduced cell viability and led to a threefold reduction in CD105 and CD73 expression when compared to C-PDMSC-EPs. We report that maternal SARS-CoV-2 infection induces a proinflammatory state and enhances an early stemness phenotype that is changed in long-term passaged C-PDMSCs. Our study underscores the biological impact of COVID-19 in PDMSCs of active SARS-CoV-2-positive pregnant women, highlighting the necessity in understanding its effect in placental and fetal development.

Funding Source: L'Óreal UNESCO for Women in Science Panama National Prize 2020, British Embassy in Panama City, Panamanian Secretaria Nacional de Ciencia Tecnología e Innovación, US National Institute of Health and Sistema Nacional de Investigación

Keywords: SARS-CoV-2, placenta, mesenchymal stem cells

514 - A

MODELING CHILDHOOD EPILEPSIES USING HUMAN NEURAL ORGANIDS

Hsieh, Jenny¹, Nieto-Estevez, Vanesa¹, Varma, Parul¹, Diaz-Guerra, Eva¹, Mirsadeghi, Sara¹, Caballero, Jimena¹, Gamero Alameda, Sergio¹, Goswami, Sonal¹, Lybrand, Zane², Navara, Christopher¹

¹*Neuroscience, Developmental and Regenerative Biology, The University of Texas at San Antonio, TX, USA,* ²*Biology, Texas Woman's University, Denton, TX, USA*

Mutations in ARX, an X-linked gene, are implicated in a wide spectrum of neurological disorders including patients who have intellectual disability and epilepsy. Mouse models have shown that Arx is critical for cortical development and interneuron migration, however they do not recapitulate the full phenotype observed in patients. Moreover, the epilepsy in patients with poly-alanine tract expansion (PAE) mutations in ARX show pharmacoresistance, emphasizing the need to develop new treatments. Here, we used human neural organoid models to study the consequences of PAE mutations, one of the most prevalent mutations in ARX. We found that PAE mutations result in an early increase in radial glia cells and premature differentiation leading to a loss of cortical neurons at later timepoints. We also found a cell autonomously enhanced interneuron migration, that likely results in an altered cortical neuronal circuitry. These data provide novel insights to the pathogenesis of these and likely related human neurological disorders and identifies a critical window for therapeutic interventions.

Funding Source: This work was supported by NIH grants (U01DA054170, R01NS093992, and R01NS113516) and the Robert J. Kleberg, Jr. and Helen C. Kleberg Foundation and the Semmes Foundation (to J.H.).

Keywords: brain development, epilepsy, organoids

Poster Abstracts

514 - B

MODELLING NEURON-ASTROCYTE INTERACTION IN ALZHEIMER'S DISEASE BY BIOPRINTING HUMAN IPSCS-DERIVED ASTROCYTES AND NEURON-LIKE CELLS WITH AMYLOID BETA

Salles, Geisa Rodrigues¹, Ferreira, Paula¹, Campos, Henrique², Ferreira, Natalia¹, Soares, Cristina³, Monteiro, Beatriz², Porcionatto, Marimelia¹

¹Biochemistry, Federal University of São Paulo, Taubaté, Brazil, ²Physiology, Federal University of São Paulo, Brazil, ³Cell Biology, Univap, São José dos Campos, Brazil

Alzheimer's disease (AD), a debilitating neurodegenerative disorder with progressive cognitive decline, is mainly characterized by amyloid beta (A β) accumulation between neurons. However, astrocytes are also highly involved in A β pathogenesis. Despite extensive research, understanding the complex role of neuron-astrocyte interactions and their mechanisms in an AD environment remains challenging. Recently, three-dimensional (3D) cell culture models have emerged as valuable tools for studying AD and exploring potential pathophysiological strategies. The bioprinting technique enables to control the organization of cells within a bioink, mimicking the spatial complexity of brain tissue. This study aims to bioprint a 3D model of AD with neurons from differentiated human neuroblastoma cells (SH-SY5Y), astrocytes derived from human induced pluripotent stem cells (hiPSC), and A β oligomers. Constructs were produced with a bioink composed of 6% alginate + 4% gelatin containing or not 1 μ M A β , cross-linked with 2% calcium chloride. Morphological aspects and cytoviability were evaluated by scanning electron microscopy and resazurin assay, respectively. Interestingly, 24 h after bioprinting, the cytoviability in the constructs containing A β was statistically reduced ($p < 0.001$) compared with constructs without A β , and the rugosity of the construct surface was modified by the presence of A β . The produced construct has the potential to provide valuable insights into the complex interplay between neurons, astrocytes, and A β . Moreover, it can serve as a platform for testing therapeutic strategies, including drug candidates and personalized and translational approaches, using hiPSC from patients. We are further using the construct in studies to elucidate critical pathological features of AD, including cell differentiation, neuroinflammation, and oxidative stress, building a more relevant alternative to traditional 2D culture and an interactive model of AD.

Funding Source: SP Research Foundation (FAPESP): 2022/08664-4 and 2018/12605-8. National Council for Scientific and Technological Development (CNPq): 406258/2022-8. Coordination of Superior Level Staff Improvement (CAPES) Financial Code 001.

Keywords: bioprint, Alzheimer's Disease, 3D model

515 - A

OPTIMIZED HIGH-EFFICIENCY PROTOCOL FOR CHOLINERGIC NEURON GENERATION AS A MODEL FOR INVESTIGATING ALZHEIMER'S DISEASE

Pinheiro, Luisa Machado, Ferrari, Merari, Tófoli, Fabiano

Department of Genetics and Evolutionary Biology, Institute of Biosciences, University of São Paulo, Brazil

Alzheimer's Disease (AD) has been extensively studied, utilizing models like post-mortem brain samples, cell lines, and animal models. However, it is vital to acknowledge the limitations of these models, as they can impact research outcomes. In this regard, the emergence of induced pluripotent stem (iPS) cells has revolutionized AD research by generating patient-specific neurons for more specific investigations into molecular and cellular mechanisms, which is complementary to other study models of AD. However, differentiating iPS cells into cholinergic neurons, crucial for cognition and affected in AD, is still a challenge and further research is warranted to develop cholinergic differentiation protocols that can enhance our understanding of AD pathology. Considering the aforementioned, the aim of this study is to optimize and direct an existing neuron differentiation protocol towards the generation of cholinergic neurons, seeking to achieve the development of a specialized cellular model for investigating AD, contributing to a more comprehensive understanding of the initial molecular aspects that underlie this disease, while also facilitating the identification of novel therapeutic targets. In order to achieve this goal, our model directs the differentiation towards cholinergic neurons by adding neurotrophins BDNF, GDNF, and NT3 at a concentration of 10ng/ml during the final 15 days of differentiation. To assess the effectiveness of our approach, the main analysis was performed using Fiji software and consisted of cell counting. Additionally, the characterization of neurons was performed through immunofluorescence staining for choline acetyltransferase (ChoAC) and MAP-2 (neuron marker), these images analysis showed an 78.2% efficiency in iPSC differentiation into cholinergic neurons expressing MAP-2 and ChoAC markers. Based on these findings, we conclude that the presented protocol exhibits high efficiency in generating cholinergic neurons, making it suitable as a model for studying cellular and molecular aspects of Alzheimer's disease pathology.

Funding Source: CAPES 88887.816560/2023-00 FAPESP 2013/08028-1

Keywords: Alzheimer's Disease, hiPSC-derived cholinergic neuron, differentiation protocol

Poster Abstracts

515 - B

PHENOTYPE FEATURES AND PROLIFERATIVE POTENTIAL OF INTIMA CELLS IN AORTIC ANEURYSM

Markin, Alexander¹, Zotova, Ulyana¹, Gantsova, Elena², Cherednichenko, Vadim¹, Kiseleva, Diana¹, Markina, Yuliya¹, Chakal, Deyyara³, Breshenkov, Denis³, Charchyan, Eduard³, Postnov, Anton¹, Orekhov, Alexander¹

¹Laboratory of Molecular and Cellular Pathology of Cardiovascular System, Petrovsky National Research Center of Surgery, Moscow, Russia, ²Research Institute of Molecular and Cellular Medicine, Peoples' Friendship University of Russia (RUDN University), Moscow, Russia, ³Department of Reconstructive Cardiovascular Surgery, Petrovsky National Research Center of Surgery, Moscow, Russia

The cellular composition of the aortic intima is extremely diverse. It is the cells of this layer that are the first to encounter damaging factors and react to their effects. Loss of ability to withstand negative influences leads to the development of various vascular diseases. In addition, initial genetic features can affect cell function, which may be the cause of the development of an aortic aneurysm. The aim of our work was to study various cell populations of the primary cell culture of the subendothelial layer of the human aortic intima, the ability of these cells to phagocytosis and LDL uptake, and their proliferative potential. The primary cells were isolated from human aortic samples, which were collected in accordance with the principles of medical ethics. The obtained samples were treated with a cocktail of proteolytic enzymes. In the course of the work, experiments were carried out to study the phagocytic activity of cells by co-incubation with atherogenic LDL and latex beads. The isolated cells were subjected to an immunophenotyping procedure. Immunofluorescence study showed that from 15 to 30% of single-passage cells isolated from the subendothelial layer of the aortic intima carry pericytic and MSC markers: CD146, CD73, CD105, CD13, NG2, CD140b. In the analysis of freshly isolated cells without passing, the number of cells with such a set of markers was up to 5%. Analysis of the ability to absorb latex beads and LDL showed that these functions of these cells are comparable to the properties of professional human phagocytes. It was also found that in comparison with cells isolated from the human aorta without an aneurysm, the cells of the intima of the aneurysmal aorta are able to multiply for a long time in culture up to 20 passages. While conditionally normal cells stop proliferating after 5 passages. Our data indicates that the cells of the subendothelial layer are in dynamic equilibrium, which can change under the influence of damaging factors and lead to the formation of pathological changes in the form of various lesions. Our further work will consist in the specific sorting and

immortalization of isolated cells. This will make it possible to obtain a cell model for studying the processes that initiate the development of aneurysms and other vascular changes, such as atherosclerosis.

Funding Source: This work was supported by the Russian Science Foundation (Petrovsky National Research Center of Surgery Grant N°22-65-00089).

Keywords: aneurysm, pericytes, cell proliferation

516 - A

PHOTOACTIVATED ALUMINIUM PHTHALOCYANINE ANTICANCER AGENT INDUCES OXIDATIVE STRESS AND POTENTIATE CASPASE 3/7 DEPENDENT APOPTOSIS IN HUMAN OESOPHAGEAL CANCER STEM CELLS

Didamson, Onyisi Christiana, Abrahamse, Heidi, Chandran, Rahul

Laser Research Centre, University of Johannesburg, South Africa

Oesophageal cancer is the eighth most commonly diagnosed malignancy and cancer-associated mortalities globally. Studies have shown that cancer stem cells (CSCs) are the main drivers for poor prognosis, cancer relapse, metastasis, and treatment resistance in oesophageal cancer. Photodynamic therapy (PDT), a light-based treatment strategy, has demonstrated treatment efficiency in several cancers. However, the therapeutic effect of aluminium phthalocyanine (AIPcS4Cl) mediated PDT in inducing reactive oxygen species (ROS) production and potentiating the apoptotic activity in oesophageal CSCs is limited. Therefore, this study seeks to examine the anticancer effects of aluminium phthalocyanine in generating ROS and identifying possible apoptotic cell death pathways in HKESC-1 human oesophageal CSCs. The CSCs were isolated from HKESC-1 cells. The CSCs were categorized into control and treatment groups. The CSCs treatment group were exposed to AIPcS4Cl-PDT irradiation at 5J/cm². Twenty-four hours after PDT, the anticancer actions of AIPcS4Cl on oesophageal CSCs in promoting ROS production and cell death were evaluated. The DCFDA/H₂DCFDA-cellular ROS assay was used to determine the intracellular ROS production, Rhodamine-123 fluorometric analysis for mitochondria membrane potential, Annexin V-FITC/PI double staining flow cytometry analysis for cell death mechanism, and Caspase-Glo 3/7 fluorometric assay for caspase activities. All treatment and control cells were conducted in three biological replicates (n=3). GraphPad Prism (v5) was used to collate the results and perform statistical analysis. The treatment groups were compared relative to the control cells. One-way ANOVA was used, and a p-value < 0.05 was indicated as

Poster Abstracts

statistically significant. Findings from our study showed that AIPcS4Cl-PDT with 5 J/cm² irradiation could significantly increase intracellular ROS, alter the integrity of mitochondria membrane potential, and induce apoptosis in the oesophageal CSCs. Also, our study showed that AIPcS4Cl-mediated PDT triggers the intrinsic apoptosis pathway via upregulating caspase-3 and caspase-7 activities. In conclusion, this study demonstrates the promising use of AIPcS4Cl-PDT in eradicating oesophageal CSCs and improving prognosis.

Funding Source: This research was funded by the South African Research Chairs Initiative of the Department of Science and Technology and the National Research Foundation of South Africa and the University of Johannesburg GES 4.0 Doctoral Scholarship.

Keywords: photodynamic therapy, oesophageal cancer stem cell, apoptosis and oxidative stress.

516 - B

PHYSIOLOGICAL AND MORPHOLOGICAL CHANGES ASSOCIATED TO THE INTERACTION BETWEEN SPIKE PROTEIN AND HUMAN CARDIOMYOCYTES DERIVED FROM PLURIPOTENT STEM CELLS

Salazar, Alejandro, Hernández, Arturo

Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Mexico City, Mexico

Cardiomyocytes are specialized cells that comprise the heart muscle and are responsible for their electrical activity and contraction. SARS-CoV-2 infects human cardiomyocytes in vitro, impairing their electrical and mechanical function. The spike protein present on the surface of the SARS-CoV-2 virus plays a critical role in its ability to infect human cells. There is limited research on the direct interactions between the soluble spike protein alone and cardiomyocytes. However, recent findings suggest that the spike protein may directly damage the heart cells affecting their contractility and electrical activity. One possible interaction is that the spike protein binds to ACE2 receptors on the surface of cardiomyocytes, interfering with the receptor's function and affecting the cardiomyocytes' physiological processes, leading to inflammation, oxidative stress, and cell damage. To address this issue, human embryonic stem cells were differentiated into cardiomyocytes. Once these cells were purified and mature, we characterized their electrophysiology, intracellular Ca²⁺ dynamics, and morphology. Then we exposed them in vitro to various concentrations of the spike protein and different incubation times from 3 to 72 hrs to investigate the possible morphological and physiological changes that result from this interaction. As a

control, we exposed the cells to the denatured spike protein. Exposure of cardiomyocytes to the spike protein after 24-48 hrs alters their normal spontaneous Ca²⁺ fluctuations. They become disorganized, desynchronized, and with slower frequency than control cells. We will compare these effects with those of exposing the cardiomyocytes to a lentivirus with the protein spike in its membrane (designed by us) to investigate if the union and membrane fusion of the virus with the cardiomyocyte and the internalization of the viral spike protein is necessary to alter the cardiomyocyte function.

Keywords: cardiomyocytes, spike, dysfunction

517 - A

PRPC AS A MAJOR PLAYER IN GLIOBLASTOMA STEM-LIKE CELLS: ITS IMPACT ON CD44 CELLULAR WHEREABOUTS

Alves, Rodrigo Nunes¹, Coelho, Bárbara¹, Iglesia, Rebeca, Lopes, Marilene, Prado, Mariana

Departamento de Biologia Celular e Tecidual, ICB-USP, São Paulo, Brazil

As the most aggressive type of glioma (grade IV/IV), Glioblastoma (or simply GBM) is a highly heterogeneous tumor with a diversity of cellular subpopulations. Amongst these cells our focus goes towards glioblastoma stem-like cells (or GSC's) which are essential to GBM maintenance, proliferation and differentiation. One of its main players is the cellular prion protein (PrPC), a very interactive protein that creates a signaling platform in membrane microdomains named lipid rafts by binding to ligands with intracellular domains, such as CD44. CD44 is a stem-cell and also a tumor biomarker that plays an important role on cellular events such as adhesion and migration, hence in GBM's aggressiveness. Thus, my goal is to identify the role of PrPC and CD44 in the phenotype regulation of GSC's, focusing on CD44 intracellular trafficking and in the endocytic pathway through loss of function studies. On that account, qPCR, Western Blotting and Immunohistochemistry assays were utilized to compare our genes and proteins of interest among two commercial GBM cell lines U87 and U251, cultivated either in monolayer or in 3D models, in which the culture of neurospheres enrich the rate of GSC's. Statistical analysis was performed in the GraphPad Prism software using Two Way ANOVA. Early results pointed out that in the absence of PrPC, CD44 is being modulated in its expression as well as in its subcellular localization. In the GBM PrPC K.O. model CD44 is not only downregulated but also appears to form intracellular aggregates instead of appearing exclusively in the cellular membrane. Following these data we also investigated the expression profile of subcellular

Poster Abstracts

compartment markers of the endocytic pathway. Proteins such as EEA1 (early endosome marker), CD63 (multivesicular body marker), LAMP1 (lysosome marker) and also CD71 and RAB11 (recycling endosome markers) appear to be altered without PRPC, indicating a possible disruption of the endocytic pathway. Further experiments need to be done in order to pinpoint whether this accumulation is prone to recycling or to protein degradation (and whether through lysosomes or the Ubiquitin-proteasome system). These results highlight the role of PrPC on CD44 processing in GSC biology and, in the future, can help in the development of new treatments and improve the quality of life of GBM patients.

Funding Source: Funding source for this project is mainly due to FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo).

Keywords: GSC, PRPC, CD44

517 - B

RAPID ASSESSMENT OF VIABILITY IN PATIENT-DERIVED COLORECTAL CANCER ORGANOIDS

Tavormina, Penny¹, Olsen, Cathy², Macha, Prathyushakrishna², Lim, Angeline², Greenow, Kirsty³, Sirenko, Oksana²

¹Distribution, Molecular Devices LLC, Downingtown, PA, USA, ²Marketing, Molecular Devices LLC, San Jose, CA, USA, ³Research and Development, Molecular Devices LLC, Cardiff, UK

Cancer cell lines grown in 2D have long served as experimental surrogates for cancers. In recent years, the 3D culture of cancer cells, often alongside other cell types in formats where they can form multi-layered structures, is enabling new models for cancer research that are considered more biologically relevant. Cancer organoids derived from patient tissue offer researchers a highly relevant disease model system, as these organoids and the patients from which they were derived have been shown to respond similarly to drugs. Recently, these models have become more widely utilized, thanks to standardization and scalability that have made available large numbers of uniformly sized and highly viable organoids. The characterization of organoid responses to candidate drug treatment is a powerful research tool that provides a wealth of detailed information, but screening numerous compounds requires significant effort and hands-on time. We demonstrate here methods for analyzing organoid viability that allow rapid identification of effective drug candidates and can be combined with more complex downstream image analysis.

Keywords: cancer organoids, colorectal cancer, high content imaging

518 - A

UNVEILING ENDOTHELIAL DYSFUNCTION IN MARFAN SYNDROME: INSIGHTS FROM A HUMAN IPSC-DERIVED MODEL REVEALING DEFECTIVE VASCULAR CELL PHENOTYPE

Nolasco, Patricia¹, Veiga Pereira, Lygia¹, Laurindo, Francisco²

¹Instituto de Biologia - Departamento de Genética, USP, São Paulo, Brazil, ²Biologia Vascular, InCor-HC-FMUSP, São Paulo, Brazil

Marfan Syndrome (MFS) is an autosomal dominant disease associated with the development of aortic aneurysms, leading to significant morbidity and mortality. It is caused by mutations in the fibrillin-1 gene (FBN1), and the relationship between genotype and phenotype remains unclear due to the clinical variability observed. One potential factor contributing to this variability is the classification of mutations as Haploinsufficient (HI) or Dominant-negative (DN), which has been shown to correlate with the severity of MFS. Emerging evidence suggests that endothelial dysfunction may play a role in aneurysm development in MFS, although it is not yet clear whether it is a cause or a consequence of aortic dilation. To investigate these mechanisms further, we have utilized an isogenic model in which we genetically modified human induced pluripotent stem cells (hiPSCs) derived from a healthy patient individual using the CRISPR/Cas9 gene editing system. This allowed us to generate distinct hiPSC lineages carrying HI or DN mutations in the FBN1 gene. Subsequently, we differentiated and characterized these hiPSC lineages into endothelial cells (iECs) and smooth muscle cells (iSMCs). We evaluated protein and mRNA expression using Western blotting and quantitative PCR (qPCR), assessed morphology through immunofluorescence and tube formation assays, and performed shear stress analyses to examine the profile of nitric oxide (NO) production. Validation of our model revealed the expression of fibrillin-1 in both iECs and iSMCs, with iSMCs showing a higher expression compared to iECs. Furthermore, the DN and HI models exhibited distinct expression patterns. Additionally, preliminary data indicated significant phenotype changes in MFS-iECs, including decreased gene expression of key phenotypic markers and impaired tube formation, as well as reduced NO production. These findings suggest that MFS is associated with significant deregulation of both iEC and iSMC phenotypes, potentially leading to the development of endothelial dysfunction and exacerbating aneurysm formation in the aorta.

Funding Source: Projeto FAPESP 2019/26007-8

Keywords: Marfan Syndrome, iPSC, aneurysm

Poster Abstracts

518 - B

SAXITOXIN POTENTIATES ZIKA VIRUS-INDUCED CELL DEATH IN HUMAN NEURONS BUT NOT IN NEURAL PROGENITORS AND ASTROCYTES

Guimaraes, Marilia Zaluar¹, Souza, Leticia¹, Pedrosa, Carolina¹, Pijuan, Teresa¹, Santos, Camila¹, Vitoria, Gabriela¹, Higa, Luiza², Tanuri, Amilcar², Rehen, Stevens¹

¹IDOR, Instituto D'Or de Pesquisa e Ensino, Rio de Janeiro, Brazil, ²Departamento de Genética, Universidade Federal do Rio de Janeiro, Brazil

The Zika virus (ZIKV) outbreak in Brazil between 2015 and 2016 was associated with increased prevalence of severe congenital malformations, including microcephaly. Notably, the distribution of microcephaly cases was not uniform across the country, with a disproportionately higher incidence recorded in the Northeast region (NE). Our previous work demonstrated that saxitoxin (STX), a toxin ubiquitously present in the NE's drinking water reservoirs, exacerbated the damaging effects of ZIKV on the developing brain. In the present study, we hypothesized that STX's impact might vary among different neural cell types. First, we treated human organoids for 13 days with STX immediately following ZIKV infection. Next, we isolated the different neural populations using an isotropic fractionating technique and observed a decrease of 5.6x and 2.9x in the percentage of immature (TBR1+) and mature neurons (NeuN+), respectively. The remaining populations were not significantly affected by STX, even though ZIKV infection had impact on astrocytes and neural stem cells (NSCs). Then we carried out similar experiments with 2D preparations of cortical neurons, sensory neurons, NSCs and astrocytes. The cortical neurons had a decrease in viability of 20% when comparing ZIKV+STX with ZIKV alone, and the presence of STX increased the number of infected cells (NS1+) by 2.7x. Similar results were observed with sensory neurons, whereas isolated NSCs and astrocytes were not affected by STX, both regarding cell viability and percentage of ZIKV-infected cells. These findings suggest that STX exacerbates the harmful effects of ZIKV on neurons, thereby providing a plausible explanation for the heightened severity of ZIKV-induced congenital malformations observed in Brazil's NE. This study underscores the importance of understanding the interactive effects of environmental toxins and infectious pathogens on neural development, with potential implications for public health policies and interventions.

Funding Source: FAPERJ, CAPES, CNPq, and intramural grant from IDOR.

Keywords: induced pluripotent stem cells, microcephaly, environmental toxins

519 - A

STEMNESS SIGNALLING ACTIVATION IN TRIPLE NEGATIVE BREAST CANCER ORGANIDS

Carreira, Ana Claudia¹, Fernandes, Letícia²

¹Center of Natural and Health Human Sciences, Federal University of ABC, University of São Paulo, Brazil,

²Department of Surgery, University of São Paulo, Brazil

Breast cancer (BC) is one of the leading causes of cancer-related death in women, despite major advances, some subtypes present poor responses and resistance to available therapies. To understand possible mechanisms behind these behaviors, several data demonstrate cancer-cells subpopulations with stem cells phenotype, such as self-renewing, differentiation potential and protein expression. Recently, our group demonstrated that CD90, a stem-cell marker, presented positive expression correlated with malignancy degree in triple-negative breast cancer (TNBC). Based on that, this work aims to evaluate the influence of CD90 knockdown in stemness-associated proteins in TNBC cell lines alone and with endothelial cells and fibroblast to investigate the molecular mechanisms involved in this cell model. A CD90 knockdown Hs578T (shCD90) and the parental Hs578T (WT) cell line was cultured, alone or associated with normal cells, using three-dimensional (3D) culture model. For 3D co-culture were used parental progenitor endothelial cell from canine yolk sac (SV) compared to SV transduced with VEGF (SV-VEGF), and murine fibroblasts (3T3). Controls and co-culture were analyzed by Scanning Electron Microscopic (SEM). The CD105 and β -catenin stemness-related markers were investigated by immunofluorescence assay, and the intensity quantification was made by ImageJ software. The increased expression of both stemness-related markers was observed when cancer cells were associated with progenitor cells in the 3D model. However, there was no significant changes when fibroblasts were associated. In both cases this behavior was independent of CD90 knockdown. Also, it was possible to observe that organoids cancer cells associated with progenitor cells presented different morphology compared to fibroblasts integration in the SEM. These findings suggest that stromal interactions, represented by fibroblasts together with breast cancer cells, does not activated stemness signaling, in contrast of their interaction with progenitor cells that upregulated CD105 and β -catenin. Possibly this data could imply that, in a cancer microenvironment, when stem cells are present could be activation of stemness associated with higher malignant phenotype, once our cell line represents the most aggressive breast cancer subtype.

Funding Source: Brazilian Federal and State research agencies: Coordination for Improvement of Higher Education Personnel (CAPES) (Leticia Alves Fernandes PhD fellow: Grant no. 88887.666887/2022-00).

Keywords: breast cancer cells, CD90/Thy-1, microenvironment

Poster Abstracts

519 - B

THE APPLICATION OF 3D-MICROTUMORS IN FUNCTIONAL PRECISION ONCOLOGY TO SUPPORT TREATMENT DECISIONS IN PATIENTS WITH GASTROINTESTINAL TUMORS

Martins, Vilma Regina¹, Osaki, Luciana², Santos, Tiago³, Clerice, Stefano³, Mello, Celso⁴, Felismino, Tiago⁴, dos Santos, Gabriel⁵, Silva, Virgilio⁴, Tabuada, Rodrigo⁴, Pelosof, Adriane⁶, Aguiar, Samuel⁷, Coimbra, Felipe⁷, Chojniak, Rubens⁸, Barbosa, Paula⁸, Portilho, Adrhyann², Dias, Queila², Gama, Patricia¹, Riechelmann, Rachel⁴, Martins, Vilma¹

¹Cell Biology and Development, University of São Paulo, Brazil, ²Research and Development, Aljava Biotech, São Paulo, Brazil, ³International Center for Research, A.C.Camargo Cancer Center, São Paulo, Brazil, ⁴Clinical Oncology, A.C.Camargo Cancer Center, São Paulo, Brazil, ⁵Pathology, A.C.Camargo Cancer Center, São Paulo, Brazil, ⁶Endoscopy, A.C.Camargo Cancer Center, São Paulo, Brazil, ⁷Surgical Oncology, A.C.Camargo Cancer Center, São Paulo, Brazil, ⁸Diagnostic Imaging, A.C.Camargo Cancer Center, São Paulo, Brazil

One of the major limitations in cancer treatment in advanced/metastatic tumors is the intrinsic or acquired resistance to (standard-of-care) drugs. About 70% of patients with primary or metastatic disease are candidates for chemotherapy however, 50-60% show little or no response to treatment. The absence of predictive markers for chemotherapy response leads to ineffective treatments, unnecessary toxicity, loss of quality of life, years of life lost, and high costs to the healthcare system. Precision functional oncology emerged in this context, where the vulnerability of living 3D-microtumors (self-renewing sources of tumor cells that maintain most of the morphological and molecular characteristics of the tumor tissue) to antitumor agents is tested ex-vivo to predict the best personalized therapy. However, the technology still needs standardization at the clinical level and prospective studies that establish its clinical value. We are conducting a proof-of-concept study (CEP- 3260/22) to develop 3D-microtumors from patients with gastrointestinal tumors to evaluate the correlation between the ex-vivo response to stand-of-care treatments and the patients' pathological or clinical response. Our preliminary results from 17 tissue samples

from biopsies or surgeries showed that 3D-microtumors are generated from tissue samples at an efficiency of 43% (3/7) for gastric, 83% (5/6) for rectum, and 75% (3/4) for colon adenocarcinomas. The immunostaining of 3D-microtumors with epithelial markers CDX2 and CK20 matches with the original tumors. Treatment of 3D-microtumors in dose-response curves were recently performed with standard-of-care chemotherapy with every single drug alone or in combination. 5-FU + Oxaliplatin (FOLFOX) or 5-FU+irinotecan (FOLFIRI) for colon and rectal tumors or 5-FU+Docetaxel+Oxaliplatin (FLOT) for gastric tumors. The 3D-microtumors from patients showed different chemosensitivity and will be compared with the patient's treatment response, which usually occurs in two or three months. From our understanding, this is the first study to validate the use of 3D-microtumors to predict therapeutic responses in cancer patients in Brazil. Positive results will allow the methodology integration into therapeutic decisions, leading to better treatment and less toxicity for patients.

Funding Source: Supported by Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP), MKM Venture Science Biotech, National Institute for Science and Technology in Modeling Human Complex Diseases with 3D Platforms.

Keywords: 3D-microtumors, functional precision oncology, predictive markers for chemotherapy response

520 - A

THE IMPACT OF CHRONIC MANGANESE ON GLUTAMATE EXCITOTOXICITY IN HUMAN IPSC-DERIVED CORTICAL MODEL OF ALZHEIMER'S DISEASE

Kim, Hyunjin¹, Bowman, Aaron¹, Harrison, Fiona², Strom, Madeleine¹, Tukker, Anke¹

¹School of Health Sciences, Purdue University, West Lafayette, IN, USA, ²Department of Medicine, Vanderbilt University Medical Center, Nashville, TN, USA

Manganese (Mn) is an essential metal widely present in the environment, but in excess is a neurotoxicant with broad impacts on neurological systems. Alzheimer's disease (AD) is a chronic multifactorial neurodegenerative disorder wherein the vast majority of AD cases cannot solely be attributed to familial inheritance and involve contributions from environmental

Poster Abstracts

risk factors. An important overlapping pathological feature shared by Mn neurotoxicity and AD is perturbed glutamate neurobiology. Enhanced neuronal excitability has been reported in AD patients and animal models and studies have shown that acute exposure to high levels of Mn can inhibit synaptic glutamate uptake. However, the effects of chronic exposure to physiologically relevant levels of Mn and its implication in AD etiology remain unknown. Hence, we hypothesized that chronic Mn exposure increases susceptibility to glutamate excitotoxicity in a manner that is altered by an individual's genetic risk for AD. Here, we utilize cortical neurons and astrocytes generated from induced pluripotent stem cells derived from neurotypical and AD patients. Cells were cultured for approximately 100 days to ensure neuronal maturation and astrogliogenesis and subsequently exposed to Mn (vehicle, 0.5 or 5 μ M) for up to 40 days. Alterations in glutamate uptake were quantified using 14 C-labeled glutamate wherein we observed a significant 30~40% decrease in glutamate uptake in AD patient-derived neurons/astrocytes, an effect that was not seen in the neurotypical controls. To investigate cell type-specific transcriptomic alterations that may, at least in part, contribute to the observed differential pathophysiology, we performed single cell RNA-sequencing following a 43-day Mn exposure. Bioinformatic analyses revealed several significantly altered pathways that share at least partial overlapping pathology in Mn and AD such as 14-3-3, EIF2, glutamate receptor, and mTOR signaling. Finally, preliminary analyses of functional micro-electrode array recordings showed a significant increase in mean spike rate following 5 μ M Mn exposure. In summary, we provide valuable insight into discerning the transcriptomic and functional alterations caused by chronic Mn and interrogating how an individual's genetic predisposition to AD may alter this pathophysiology.

Funding Source: NIH/NIEHS R01 ES031401 (FEH ABB)

Keywords: manganese exposure, Alzheimer's disease, glutamate excitotoxicity

TRACK: LEVERAGING TOOLS TO STUDY STEM CELL BIOLOGY

601 - A

A SYSTEMS BIOLOGY APPROACH IDENTIFIES A MODULE OF CO-EXPRESSED GENES THAT ARE DISTINCTIVE OF HUMAN LONG-TERM HEMATOPOIETIC STEM CELLS

Griesi Oliveira, Karina

Instituto Israelita de Ensino e Pesquisa, Albert Einstein Hospital, São Paulo, Brazil

Long-term hematopoietic stem cells (LT-HSCs) possess the unique ability to rebuild the bone marrow and produce all blood cell lineages, making them crucial in bone marrow transplants. However, due to their rarity, expanding such cells in vitro has always been a goal in Medicine. Reproducing conditions that allow these cells to expand to desirable levels while maintaining their potential has proven challenging though. To optimize the expansion protocols, evaluating both the quantity and functionality of LT-HSCs is therefore crucial. Although some surface markers of cultured LT-HSCs have been identified, their reliability is highly dependent on cultivation conditions, necessitating the use of xenotransplantation assays to assess cell functionality after protocol modifications. To address this limitation, our hypothesis was that utilizing a systems biology approach that considers regulatory networks of gene expression would enable us to find more resilient markers for LT-HSCs, which would retain validity regardless culture conditions. We used weighted gene co-expression network analysis (WGCNA) to inspect six different publicly available transcriptome datasets, comprising fresh (2) and cultivated (4) hematopoietic stem cells. We evaluated eigengene values (first principal component) associated with each module of co-expressed genes found to identify those exhibiting higher expression levels in sorted fresh LT-HSC populations (compared to progenitors), or in cultivated cell populations that were subjected to conditions resulting in an increased number of LT-HSCs. Consistently, we found at least one module highly correlated with LT-HSC across all datasets, with significant overlap among them. Overlying the LT-HSC associated modules from the six studies, we found 4 genes present in all of them, and 50 genes present in at least four. Notably, some of these genes, such as HLF and EPCR, are already known markers for LT-HSC. Our results support the notion that this approach effectively captures the central genes that characterize LT-HSC. As further steps, we plan to validate this set of genes as LT-HSC markers using in silico and in vitro approaches to determine if their combined expression levels can reliably predict the proportions of LT-HSC under different culture conditions.

Funding Source: Fundação de Amparo a Pesquisa do Estado de São Paulo - FAPESP

Keywords: hematopoietic stem cells, WGCNA, stem cell markers

Poster Abstracts

601 - B

BIOBANKS SCENARIO IN BRAZIL AND THEIR ROLE IN PUBLIC HEALTH

Zych Nadolny, Jaiesa¹, Gonçalves dos Santos Feres, Mariana¹, Colla, Débora Cristina², Rezende, Iram¹, Zych Nadolny, Jaiesa¹

¹Industrial Health Board, Paraná Institute of Technology (Tecpar), Curitiba, Brazil, ²Technology and Innovation Board, Paraná Institute of Technology (Tecpar), Curitiba, Brazil

Biobanks are important structures for the collection, storage, and supply of human biological materials used for scientific research and therapies. Cell Processing Centers (CPC) are health establishments responsible for selecting donors, collecting, transporting, evaluating, processing, and packaging, as well as storing and making available cells of human origin for therapeutic use. With the exponential growth of regenerative medicine and research using human cells, biobanks and CPCs' roles are increasing. The goal of this study was to conduct a survey on biobanks, and cellular processing centers in Brazil and highlight some advantages of establishing a public cell bank. Brazilian Health Regulatory Agency (Anvisa), Ministry of Health, Federal Revenue of Brazil, and Pubmed websites and databases were consulted. In Brazil, 485 companies have the CNAE (National Classification of Economic Activities) for "Services of banks of human cells and tissues". When the CNAE "Research and experimental development in natural sciences and physical engineering" is included, the number reduces to 63 companies. For research purposes, 88 biobanks were registered in Brazil until March 2023. There are 32 CPCs for Hematopoietic Progenitor Cells from Umbilical Cord and Placental Blood and 67 CPCs for Hematopoietic Progenitor Cells from Bone Marrow and Peripheral Blood. In both cases, more than half are private. These units require investment for the maintenance of the material stored. In contrast to private biobanks, with limited use of the samples, public biobanks can optimize the use of the stored material, ensuring the quality of the projects and therapies. So, having a public institution with a well-trained staff and appropriate infrastructure to meet the country's needs regarding the demand for stem cells for research and advanced therapies would be a differentiating factor. Providing donors and patients in need with a cost-free, high-quality service that extends nationwide.

Keywords: biobank, brazilian health scenario, public vs private

602 - A

COMBINING TAGMENTATION-PCR AND NANOPORE SEQUENCING FOR MULTIPLEXED SEQUENCING OF TARGETED GENOMIC LOCI

Chao, George¹, Mintzer, Esther¹, Gutierrez, Clair², Church, George¹

¹Genetics, Harvard Medical School, Boston, MA, USA, ²Chemical Biology, Massachusetts Institute of Technology, Cambridge, MA, USA

With the recent development of powerful tools for integrating synthetic DNA into the genome, such as lentivirus, transposons, and programmable DNA cutters, building genetically edited cell lines has become common practice. For instance, many transcription factor (TF) based differentiation approaches rely on the integrative delivery of TF expression cassettes to create cells such as pluripotent stem cells (iPSCs) or neurons (iNGNs). However, random integrative approaches create genetically heterogeneous populations, leading to high variability in cell behavior, and even site-specific strategies can lead to off-target integrations, underlining the importance of validating insertion events across the genome. Even with next-generation sequencing, this can be cost-prohibitive, especially across multiple cell lines, each with a different set of integration loci. By combining two revolutionary technologies, tagmentation-PCR and nanopore sequencing, it is now possible to significantly reduce the cost of validating integration sites by enriching the sequences of interest through PCR and barcoding to allow multiplexed sequencing of multiple samples on a single flow cell. Additionally, due to the long-read nature of nanopore sequencing and the absence of a ligation step in the library preparation, synthetic-genomic DNA junctions can be validated with high confidence. We demonstrate the utility of this approach by characterizing the integration location of monoclonal lines built through lipid nanoparticle delivery of Cas9 ribonucleoprotein complex and donor DNA and validate our findings through sanger sequencing of junction PCRs. We believe this approach will significantly lower the difficulty and cost of validating genetic integrations in cells and enable researchers to elucidate the cause of the variability observed when engineering cell lines with genomic integrations.

Keywords: genomic insertion, long-read sequencing, technology development

Poster Abstracts

602 - B

DEVELOPMENT OF FELINE HEPATOCYTES ORGANOIDS ASSOCIATED WITH ADULT MESENCHYMAL STEM CELL AIMING A 3D LIVER CULTURE IN VITRO MODEL**Carreira, Ana Claudia**¹, Chucair, Soraya²¹Center of Natural and Health Human Sciences, Federal University of ABC, University of São Paulo, Brazil, ²Surgery Department, Faculty of Veterinary and Animal Science, University of São Paulo, Brazil

The liver is an organ with multiple functions. It is also an organ affected by several pathologies. Understanding the physiology, pathophysiology of liver diseases and treatment strategies is essential, however, the use of animals is less accepted for these purposes. Although liver tissue contains a large number of hepatocytes, it is known that other cells make up the liver parenchyma and these are organized into complex structures. Mimicking this unique tissue architecture is essential for validating studies. The creation of organoids from 3D cell co-culture appears as a promising alternative in research. The integration of these organoids to microfluidic devices creates an environment that simulates the organic conditions. The objective of this study was to establish a protocol for the development of liver organoids associating hepatocytes and adipose tissue mesenchymal stem cells (MSC) from domestic cat (*Felis catus*) for future integration in on-a-chip devices. Domestic cats were donated after their deaths from natural causes. Liver and abdominal adipose tissue fragments were collected and immediately taken to cell culture. For adipose tissue, a digestion using collagenase I (1 mg/mL) was carried out. Afterwards, both tissues were filtered through 70 µm cell filters, centrifuged, and the supernatant was discarded. Cells were transferred to a culture plate with DMEM with 10% fetal bovine serum and 1% of penicillin/streptomycin solution, and maintained in an incubator at 37°C, 5% CO₂. Cells were identified by their morphological characteristics through Scanning Electron Microscopic (SEM), stem-cell marker and their behavior in culture. In a 12-well plate, an agarose base was made in each well from a 3D mold with 786 microwells printed on PLA. The cells were distributed in the following proportions in each well: 3.5 x 10⁵ hepatocyte; 3.5 x 10⁵ MSC; 1.75 x 10⁵ hepatocyte + 1.75 x 10⁵ MSC cells. The organoids were analyzed by live and dead test and SEM microscopy after 24 and 48 hours, demonstrating no significant cell death in any conditions. Also, all organoids remained morphologically intact until the end of the experiment. These findings demonstrated that both 3D co-culture provided a viable in vitro model to study liver properties targeting their possible integration in microfluidic devices.

Funding Source: Brazilian Federal and State research agencies: Coordination for Improvement of Higher Education Personnel (CAPES) (Soraya Chucair ME fellow: Grant no. 88887.715601/2022-00)

Keywords: 3D culture, mesenchymal cells, liver organoid

603 - A

EXPLORING SPATIAL ORGANIZATION OF NEURAL STEM CELLS IN ADULT ZEBRAFISH BRAIN USING GRAPH-BASED IMAGE ANALYSIS**Araña, Matheus C.**¹, Bonnet, Valentin², Mancini, Laure³, Dray, Nicolas³, Bally-Cuif, Laure³, Baroud, Charles²¹Biomedical Engineering, Institut Pasteur and École Polytechnique de Paris, France, ²Charles Baroud Lab, Institut Pasteur and École Polytechnique de Paris, France, ³Zebrafish Neurogenetics Lab, Institut Pasteur, Paris, France

The maintenance of neural stem cells (NSCs) in the adult brain depends on their activation frequency and division mode, but it remains largely unknown how these decisions are coordinated in time and space to sustain their long-term homeostasis. To address this question, we used the dorsal telencephalon (pallium) of the adult zebrafish as a model. This region displays a large pool of mostly quiescent neural stem cells (qNSCs) that can divide (aNSCs) and differentiate into neural progenitors (NPs). The Notch and BMP signaling pathways have been shown to be key in regulating the quiescence of NSCs. Recently, our laboratory has shown that the apical area of an NSC predicts its activation propensity and that the expression of deltaA, a ligand involved in Notch signaling, predicts fate acquisition upon NSC division. To explore how NSCs can regulate each other, we have designed a new method in which we convert cell segmentation and expression of certain molecular markers into a graph-based representation. This enables the analysis of spatial statistics of the NSCs and their interconnectivity. The tissue is found to follow the well-established laws that relate cell area with the number of connected cells (Aboav's and Lewis's laws). The analysis however highlights differences between the dorso-medial and the dorso-anterior regions of the brain, both in the statistics of the expression of deltaA and in the size distributions of cells: The deltaA expressing cells are less frequent in the dorso-anterior pallium, where the cells also have a larger mean apical area. In contrast, the distribution of the number of neighbors is maintained between the two regions. The link between these different cellular measures and their impact on cell fate is currently being explored. This work highlights the effectiveness of graph-based image analysis as a powerful tool for examining cell-cell interactions in the broader context of tissue homeostasis. Looking ahead, we aim to enhance our study by incorporating temporal data and investigating other key players within the Notch signaling pathway. This approach will allow us to further unravel the complex dynamics of NSC maintenance in the adult vertebrate brain.

Keywords: neural stem cells, graph-based image analysis, long-term homeostasis

Poster Abstracts

603 - B

EXPLORING THE INTERPLAY BETWEEN AUTOPHAGY, P53, AND CANCER STEM CELLS IN A KERATINOCYTE CELL LINE OVEREXPRESSING SPHINGOSINE KINASE 2**More Milan, Thais,** da Silva, Gabriel, Machado Leopoldino, Andréia*Department of Clinical Analyses, Toxicology and Food Sciences, School of Pharmaceutical Sciences of Ribeirão Preto /University of São Paulo, Ribeirão Preto, Brazil*

The sphingosine kinase 2 (SK2) has been reported as deregulated in cancer and related to autophagy and chemoresistance. Our research group recently identified a potential association between SK2 and cancer stem cells (CSC). Autophagy plays an anti-tumoral role in the initial cancer development, while in an advanced stage, autophagy presents pro-tumorigenic properties. Moreover, p53, the most mutated gene in cancer can be suppressed by autophagy. Here, we aimed to explore how the knockdown of ATG12, an autophagy protein, using two different sequences (7391 and 7393), and the overexpression of p53-HIS could impact cancer stem cells in a keratinocyte cell line with overexpression of SK2 (NOK-SK2). First, we performed a bioinformatic analysis using a data set of miRNAs differentially expressed in NOK-SK2 compared to control to identify deregulated pathways. Spheres formation assay was adopted to identify the CSC population, and adhesion and invasion spheres assay was selected to determine the metastatic characteristics of the CSC, and Western Blot to evaluate protein status. We observed 133 miRNAs deregulated in the NOK-SK2, in which many transcription factors related to autophagy (SMAD2, SMAD4, and CREB) were enriched, as well as TP53 gene. Knockdown of ATG12 in NOK-SK2 cells, in both 2D and 3D cultures, was confirmed, and it was accompanied by a decrease in vimentin. In 3D culture, we observed a decrease in the protein levels of OCT4 and ALDH in the NOK-SK2 cells with ATG12 silencing. The spheres' formation and adhesion were decreased in the NOK-SK2 with the knockdown of ATG12. Besides, NOK-SK2 with the overexpression of p53-HIS showed a decrease in the formation of the spheres and in the length of the invaded spheres. In conclusion, our results showed that blocking autophagy and increasing p53 could be a potential strategy to reduce cancer stem cell subpopulation in tumors with SK2 deregulation.

Funding Source: Capes, Fapesp and CNPQ**Keywords:** cancer stem cells, sphingosine kinase 2, autophagy

604 - A

RAPID STEM CELL 3D BIODISTRIBUTION ANALYSIS IN WHOLE MOUSE WITH DEEP LEARNING U-NET CONVOLUTIONAL NEURAL NETWORKS AND CRYOVIZ IMAGING**Roy, Debashish¹,** Gargasha, Madhusudhana², Scott, Bryan², Tilton, John³, Tomcik, Kristylen³*¹Research and Development, BioInVision, Cleveland, OH, USA, ²BioInVision, Cleveland, OH, USA, ³Nutrition, Case Western Reserve University, Cleveland, OH, USA*

One of the most effective ways to study stem cell biology in small animal models is to do a complete biodistribution study in an entire specimen. Traditional methods lack sensitivity and quantification over large volumes such as whole rodents. CryoViz imaging (BioInVision) acquires 3D microscopic anatomical color and molecular fluorescence volumes from whole rodents by serial-sectioning and imaging frozen tissue block face followed by counting of fluorescently tagged stem cells. In a multi-sample large study, the challenge is slow throughput of analysis due to laborious and time-consuming manual segmentation. We have developed a tool for fully automated, high-throughput whole-mouse 3D biodistribution quantification, which exploits deep-learning (DL) Convolutional Neural Networks (CNNs) for multi-organ segmentation. Color anatomical block-face images were processed using a 3D stitching algorithm to create 3D volumes, on which a multi-level U-net CNN segmented liver, lung, spleen, brain, spinal cord, thymus, heart, stomach, kidneys, and bladder. CNN models were derived from extensive offline training on existing vast repository of image data. Machine-learning stem cell detection was applied on fluorescence data constrained within CNN segmented organs. Unlike manual segmentation which is prone to human errors especially at the organ periphery/boundary, DL CNN segmentation is very consistent across datasets (due to data augmentation during training), a feature that is critical in mouse studies where a few cells home to organs and any missed cells due to inaccurate manual segmentation can potentially affect findings. Fully automated DL CNN segmentation, in one study, segmented lungs, liver, and spleen, locating homing sites of intravenously injected NK cells in whole mice. In another study, spinal cord segmentation and subsequent cell detection aided in making an important finding in the context of HIV therapy - modifying immune cells to overexpress a chemokine receptor stimulated migration to the periphery of the spine. Segmentation time/mouse was greatly reduced [2h (manual) to 5min (DL CNN) - a 95% savings]. A high segmentation accuracy was indicated by a Dice overlap of ~0.9 w.r.t human ground truth. DL CNN multi-organ segmentation enabled accurate inferences in stem cell imaging studies.

Keywords: block-face imaging, deep learning neural network, stem cell biodistribution

Poster Abstracts

604 - B

USE OF MACHINE LEARNING APPROACHES TO IDENTIFY AND VALIDATE BIOMATERIALS FOR THE PRODUCTION OF 3D NEURAL MODELS**Manzini, Bruna M.**, Porcionatto, Marimélia*Department of Biochemistry, Federal University of São Paulo, Paulínia, Brazil*

Neurodegenerative diseases are multifactorial disorders which pathophysiology is not entirely elucidated. Three-dimensional (3D) neural models can provide information about these processes as they mimic the development of the neural tissue. Concerning this, biomaterials are essential for 3D models to reflect neural development accurately. Machine learning can be used to predict material properties and their interaction with the biological components of the construct, to optimize the costs and time involved in the biomaterial selection and testing processes. Thus, this study aimed to identify and validate biomaterials for 3D neural models' fabrication using computational methods of machine learning. The methodology involves three stages: proof of concept, predictive modeling, and validation. A dataset was created based on indexed research articles. The variables considered were the type of biomaterial, 3D bioprinting parameters (temperature and speed), and cell aspects (type, density, percentage of viability, culture time, and viability analysis method). The RStudio software was used to perform machine learning for predicting cell viability. The models used were classification (random forest, logistic regression, and support vector machines - SVM) and supervised regression (support vector, linear, and random forest). The random forest and SVM algorithms achieved accuracies of 0.9375 and 0.8750, respectively. The database is continually being updated. Subsequently, regression algorithms will be used to predict the percentage of viability based on the biomaterial, and validation will be performed.

Funding Source: São Paulo Research Foundation: 2022/15909-3 and 2018/12605-8; Brazilian National Council for Scientific and Technological Development: 406258/2022-8; Coordination of Superior Level Staff Improvement: Financial Code 001.

Keywords: machine learning, 3D neural models, biomaterials

TRACK: LEVERAGING TOOLS TO STUDY STEM CELL BIOLOGY

701 - A

ASTROCYTES AND NEURONS IPSCS-DERIVED MODELS FOR CHARACTERIZATION OF NEURONAL INVOLVEMENT IN FABRY DISEASE**Nicolicht-Amorim, Priscila**¹, Varela-Calais, Patrícia², Pesquero, João Bosco¹¹*Department of Biochemistry, UNIFESP, São Paulo, Brazil,*²*Department of Genetic Medicine, Johns Hopkins Medicine, Baltimore, MD, USA*

Fabry disease (FD) is a rare X-linked glycosphingolipidosis caused by mutations in GLA, the gene responsible for encoding α -galactosidase A (α -Gal A), an enzyme required for degradation of glycosphingolipids, mainly globotriaosylceramide (Gb3), leading to progressive multisystem diseases. α -Gal A is present in all tissues, thus its deficiency covers a wide clinical phenotypic spectrum, such as cardiac, renal, gastrointestinal and cerebrovascular symptoms. Psychiatric illnesses, especially depression, are frequently reported in FD patients, however the pathophysiological bases of FD in the central nervous system are poorly understood and it is a matter of debate whether psychiatric symptoms are caused by the disease or by causality. Therefore, to understand if Gb3 accumulation could produce neuronal alterations leading to psychiatric symptoms, we are developing a FD model in iPSCs-derived neurons and iPSCs-derived astrocytes. The GLA gene in iPSCs was edited using the CRISPR/Cas9 technology, being produced four different mutations, one missense and three deletions. The mutated iPSCs were characterized accordingly to the absence of off-targets and pluripotency potential, as well as for the deficiency of the enzymatic activity of α -Gal A. GLA wild type and mutated iPSCs were induced into Neural Progenitor Cells (NPCs). iPSCs-derived NPCs morphology was characterized through light microscopy during cell culture, immunostained to OCT4, SSEA4, NANOG, SOX2, PAX6 and NESTIN and validated when stained only for SOX2, PAX6 and NESTIN. Transcriptomics and proteomics analysis will be performed in astrocytes and neurons differentiated from these NPCs, in order to evaluate the impact of α -Gal A deficiency on the cerebrovascular and neuropsychiatric symptoms observed in FD patients.

Funding Source: This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

Keywords: rare lysosomal storage disease, cellular and molecular biology, Induced pluripotent stem cells

Poster Abstracts

701 - B

CHARACTERIZATION OF CD44+ CANCER STEM CELLS AND PLURIPOTENCY MODULATION IN DOXORUBICIN-TREATED CSC-ENRICHED TRIPLE-NEGATIVE BREAST CANCER SPHEROIDS IN VITRO

Barros, Barbara Avelar Ferreira¹, Almada, Daniele², Borges, Natalia², Anjos, Bianca³, Moreira, Milene², Queiroz, Fabio⁴, Silva, Luciana²

¹Department of Genetics/ICB, Federal University of Minas Gerais/Ezequiel Dias Foundation, Contagem, Brazil, ²Cell Biology Service, Ezequiel Dias Foundation/University of Minas Gerais, Belo Horizonte, Brazil, ³Department of Genetics, University of Minas Gerais/Ezequiel Dias Foundation, Belo Horizonte, Brazil, ⁴Department of Oncology, Mario Penna Institute, Belo Horizonte, Brazil

Triple-negative breast cancer (TNBC) accounts for 10-20% of all breast cancer diagnoses worldwide. It is considered more aggressive than other forms of invasive breast tumors due to its ER-/PR-/HER2- phenotype, which gives patients few treatment alternatives besides chemotherapy and poor prognosis. Cancer stem cells (CSCs) are a small subpopulation of the Tumor Microenvironment (TME), involved in differentiation, migration, self-renewal and proliferation. They are resistant to chemotherapy and tend to expand after treatment, leading to recurrence, metastasis and a more aggressive tumor phenotype. The aim of this project is to establish how CSCs operate in the TME by growing TNBC cell lines Hs578T, MDA-MB-231 and BT-549 in 3D culture for the formation of CSC-enriched tumor spheroids for analysis of gene expression of different signaling pathways via qPCR, miRNAs by NGS, morphology by Confocal Laser Microscopy, cell viability analysis by MTT and immunophenotyping through flow cytometry. Confocal Laser Microscopy of the MDA-MB-231 grown in CSC-specific medium and marked for actin filaments, nucleus, lysosomes, and CD44 showed heterogeneity that could be caused by epithelial-mesenchymal transition (EMT). Cells marked positive for CD44, a CSC trait, exhibited a larger amount of microvesicles (MVs) when compared to the CD44- population, which could influence modulation inside the TME and drug resistance. ZetaSizer analysis of MDA-MB-231 spheroid's extracellular vesicles (EVs) showed elevated presence of MV-type particles (150-1300nm) while the existence of exosomes (30-150nm) was low. Furthermore, CSCs tended to stay close to each other

compared to their counterparts, often leading to the formation of clusters. A qPCR assay performed on DOX-treated BT-549 spheroids displayed that genes related to pluripotency, proliferation and spheroid growth, POU5F1, AKT1 and AKT3 were upregulated, while other pluripotency targets KLF4 and MYC were downregulated. Another qPCR performed on DOX-treated MDA-MB-231 spheroids with the same targets revealed that KLF4, MYC, POU5F1, AKT1, AKT3 and SOX4 were negatively regulated. Further studies are being conducted. We believe that uncovering the main pathways that assist in CSC survival and growth is the key to developing alternative, more effective treatments for TNBC.

Keywords: cancer stem cells, triple-negative breast cancer, pluripotency

702 - A

COMPARISON OF CELL CULTURE SUBSTRATES AND MEDIUMS FOR HUMAN ES AND IPS CELL LINES

Kovacs, Gergo, Natarajan, Kedar Nath

DTU Bioengineering, Technical University of Denmark, Kgs. Lyngby, Denmark

Human embryonic (hESCs) and induced pluripotent stem cells (hiPSCs) have a huge potential in regenerative medicine and they increasingly utilized in drug development and gene therapies. A variety of cell culture media and coating substrates including feeders have been developed for culturing different human embryonic (hES) and induced pluripotent stem cells (iPS). While a combination of feeder-free coating substrates and xeno-free cell culture medium have been tested and adapted by different labs and companies, it is unclear whether they provide comparable pluripotency phenotypes across different hESC and hiPSC lines. Here I propose to compare 3 stem cell maintenance mediums (StemFit Basic, Essential 8 Flex, mTeSR Plus), 4 coating substrates (Matrigel, Geltrex, Vitronectin XF, iMatrix-511 laminin) on 4 different hESC and hiPSC lines. Upon culturing cells across 12 different resulting combinations of mediums and substrates, I will compare growth rates, assess pluripotency markers and spontaneous differentiation rates using commonly used pluripotency and differentiation markers. Our preliminary analysis captures differences in morphology and pluripotency marker levels, suggesting that stem cell maintenance for different lines might diverge with substrates and media combinations. Our study highlights the importance of performing comparative culture analysis of different hESC and hiPSC lines.

Keywords: stem cell, pluripotency, benchmarking

Poster Abstracts

702 - B

DIFFERENTIAL EXPRESSION OF THE STRESS INDUCIBLE PROTEIN 1 IMPACTS EARLY DEVELOPMENT AND STEMNESS IN PLURIPOTENT STEM CELLS

Fernandes, Camila¹, Souza, Maria Clara¹, Soares, Samuel¹, de Araújo, João Pedro¹, Boccacino, Jacqueline¹, Iglesia, Rebeca¹, Coelho, Bárbara¹, Vilchez, David², Prado, Marco³, Lopes, Marilene¹

¹Department of Cell and Developmental Biology, University of São Paulo, Brazil, ²Cologne Excellence Cluster for Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Germany, ³Robarts Research Institute, The University of Western Ontario, London, ON, Canada

Intricated cellular mechanisms orchestrate early development, and despite significant research, our comprehension of its regulation remains limited. The stress inducible protein 1 (STIP1) plays a pivotal role in proteostasis by facilitating the assembly of the HSP70 and HSP90 chaperone complex. STIP1 knockout in mice is lethal, highlighting an unexplored function of this protein in mammalian embryogenesis. We analyzed single-cell RNA sequencing data from mouse embryos to unravel the molecular significance of STIP1 expression during development. We found that STIP1 expression peaks at the 8- and 16-cell stages, with strong co-expression with the core pluripotency transcription factors observed in the blastocyst phase. Seeking further to investigate the role of STIP1 in pluripotency maintenance, we derived mouse embryonic stem cell (mESC) lines from transgenic mice exhibiting differential STIP1 expression, including a knockdown (STIP1-HTKO) and an overexpression (STIP1-OE) model. mESCs bulk RNA-seq analysis demonstrated that the expression of STIP1 is intricately associated with pluripotency, DNA damage, and cell cycle molecular pathways. Notably, STIP1-HTKO correlated with decreased expression of essential pluripotency markers and impaired proliferation, accompanied by a heightened expression of apoptosis and DNA damage factors. Conversely, STIP1-OE led to elevated expression of pluripotency markers, enhanced proliferation, and reduced apoptosis and DNA damage. Furthermore, low levels of a truncated form of STIP1 recapitulated the results of STIP1-HTKO mESCs and showed disruption of the cell cycle in mESCs. Our findings also indicate that STIP1 is implicated in the differentiation potential of mESCs, as lower STIP1 levels impact embryoid body formation, and higher STIP1 levels are observed in active neural progenitors marked by increased mitosis compared to their inactive and committed counterparts. Remarkably, STIP1 knockdown in human induced

pluripotency cells (hiPSCs) recapitulates some of the results observed in mESCs with decreased levels of STIP1. Our study sheds light on the roles of pivotal yet underexplored players such as STIP1 and its interacting partners as promising regulators of the pluripotent phenotype and embryogenesis.

Funding Source: Research funded by Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP). Project numbers: C.F: 19/14741-9; 21/13114-0; M.C.S: 21/13070-3; S.S: 22/14445-3; J.P.A: 22/08106-1; M.L: 11/13906-2; 18/15557-4; 19/00341-9

Keywords: STIP1, pluripotency, development

703 - A

GENERATION OF HUMAN TRISOMIC IPSCS FROM DOWN SYNDROME PBMCS

Vautier, Mercedes¹, Clas, Giulia², Pertierra, Lucia¹, Marazita, Mariela¹, Isaja, Luciana¹, Rodriguez Varela, Soledad¹, Mucci, Sofia¹, Helou, Belén², Tapajóz, Fernanda², Magrath, Nahuel², Surace, Ezequiel², Romorini, Leonardo¹

¹INEU, FLENI, Escobar, Argentina, ²INEU, FLENI, Belgrano, Argentina

Down Syndrome (DS) is the most common genetic form of intellectual disability. In recent years, there has been a significant increase in the life expectancy of individuals with DS, currently reaching the age of 60 or over. However, it has been observed that as from 40, these individuals experience higher risk of developing dementia, and almost all of them will finally exhibit histopathological characteristics of Alzheimer's disease (AD) in their brains. One possible mechanism that could explain this pathology of AD in individuals with DS is related to the APP gene, located in chromosome 21, which encodes the amyloid-beta precursor protein. The presence of an extra copy of APP would therefore increase the amount of A β and its propensity to aggregate, triggering a pathological cascade similar to that observed in AD. However, the exact mechanism of AD pathogenesis in individuals with DS is not yet fully understood. In order to study AD in the context of DS, differentiated cells derived from induced pluripotent stem cells (iPSCs) can be used as an experimental model. In this study, we present the generation of two iPSC lines derived from peripheral blood mononuclear cells (PBMCS) from individuals with Down Syndrome aged 50, along with the corresponding validation of both lines. To the best of our knowledge, very few iPSC lines are available from adults with DS. These lines represent a powerful model and tool to evaluate key molecular and cellular aspects of AD in the context of DS.

Keywords: Down Syndrome, Alzheimer's disease, induced pluripotent stem cells

Poster Abstracts

703 - B

HETEROCHROMATIN PROTEIN 1ALPHA DISTRIBUTION AND NANOG-BINDING DYNAMICS TO THE CHROMATIN ARE AFFECTED BY AKT1 IN A SUMOYLATION DEPENDENT MANNER

Francia, Marcos Gabriel¹, Oses, Camila¹, Roberti, Sabrina¹, Garcia, Mora¹, Cozza, Lucas¹, Diaz, Maria Candelaria¹, Rovner, Federico¹, Orsingher, Victor¹, Grecco, Hernan², Levi, Valeria¹, Guberman, Alejandra¹

¹IQUIBICEN (CONICET-UBA), Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina, ²IFIBA (CONICET-UBA), Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina

AKT is a serine-threonine kinase that regulates diverse processes in various cell types. In mouse embryonic stem cells (mESCs), it plays a key role in maintaining pluripotency, specially promoting the expression of the pluripotency transcription factor (TF) Nanog. AKT activation depends on its recruitment to the plasma membrane and subsequent phosphorylation, as well as other post-translational modifications (PTMs), including conjugation to the small ubiquitin-related modifier (SUMO), which altogether fine-tune its activity and target specificity. In this work, we explored the effects of SUMOylation on AKT1 distribution and function in mESCs. To this purpose, we generated mCherry-fused AKT1 variants with different SUMOylation capability and analyzed their distribution by confocal microscopy in mESCs. We found that this PTM impacts on the distribution and heterogeneity of AKT1 in the nucleus and cytoplasm. Specifically, both SUMOylation and the E17K mutation promoted AKT1 nuclear localization. These findings led us to hypothesize that this effect on AKT1 distribution could affect its interaction and affinity for its targets, ultimately impacting in its function. To explore this hypothesis, we studied the effect of AKT SUMOylatability on the distribution of Heterochromatin protein 1 α by quantitative confocal microscopy analysis, and on the dynamic interactions of NANOG with the chromatin, by Fluorescence Correlation Spectroscopy. We found that both SUMOylation and the E17K mutation impact on the overall chromatin landscape of mESCs and affect the chromatin-binding dynamics of NANOG. Remarkably, the oncogenic E17K AKT1 mutant produced the most prominent changes in these parameters, particularly increasing the binding of NANOG to its targets in a SUMOylation-dependent manner. These findings demonstrate that SUMOylation modulates AKT1 subcellular distribution, the chromatin landscape, and the TF dynamics, adding an extra layer of regulation to its function in mESCs.

Funding Source: This work was supported by Universidad de Buenos Aires (20020160100025BA to ASG) and Agencia Nacional de Promoción Científica y Tecnológica, Fondo para la Investigación Científica y Tecnológica (FONCYT) (PICT-2018-01516 to ASG).

Keywords: mouse embryonic stem cells, AKT SUMOylation, E17K mutant

704 - A

N6-METHYLADENOSINE AS A DETERMINANT OF R-LOOP METABOLISM AND GENOME STABILITY IN HUMAN PLURIPOTENT STEM CELLS

Ruzov, Alexey

Institute of Biotechnology, Federal Research Centre Fundamentals of Biotechnology, Moscow, Russia

R-loops are formed by an RNA/DNA hybrid and an unpaired single stranded DNA and involved in a number of important biological processes, ranging from transcriptional regulation to DNA repair in mammalian cells. Although R-loops were initially considered as a harmful by-product of transcription representing a source of genomic instability, a number of studies imply that these structures may also encode higher order regulatory information. We recently demonstrated that m6A, a modification involved in regulation of several steps of messenger RNA metabolism, is detectable on the majority of RNA/DNA hybrids in human pluripotent stem cells. We showed that the m6A reader promoting mRNA degradation, YTHDF2, interacts with R-loop-enriched loci and prevents accumulation of R-loops in dividing cells. Here we show that another m6A nuclear reader involved in mRNA processing and alternative splicing, heterogeneous nuclear ribonucleoprotein A2/B1 (HNRNPA2B1), cooperates with YTHDF2 in resolving RNA/DNA hybrids that accumulate in intronic regions in human pluripotent stem cells. According to our results, knockout of HNRNPA2B1 results in the accumulation of R-loops on a number of introns that are prone to the formation of RNA/DNA hybrids and on SINE/Alu repeats, which often act as splice acceptors in the human genome. Interestingly, in contrast to YTHDF2 knockout, the HNRNPA2B1 knockout does not lead to a significant increase in the number of double-stranded DNA breaks and does not substantially affect rates of cellular proliferation. Collectively, our results imply that m6A represents one of the key determinants of R-loop metabolism directly involved in regulation of mRNA processing and safeguarding genome integrity in human pluripotent stem cells.

Funding Source: Russian Science Foundation (RSF) Interdisciplinary Grant 22-65-00022

Keywords: pluripotent stem cells, R-loops, N6-methyladenosine

Poster Abstracts

704 - B

OBTENTION OF HUMAN BETA-PANCREATIC CELLS FROM INDUCED PLURIPOTENT STEM CELLS**Lizcano, Fernando**¹, Arroyave, Felipe²¹Center of Biomedical Investigation, Universidad de La Sabana, Chia, Colombia, ²Doctoral Program in Biosciences, Universidad de La Sabana, Chia, Colombia

Diabetes mellitus is a chronic, non-transmissible disease that causes high costs in health systems worldwide. Despite the multiple advances in the medical therapy of diabetes mellitus, the high prevalence of cardiovascular complications, mainly in developing countries, requires specific strategies to mitigate the disease. Pancreatic β -like cells (P β LC) differentiated from induced pluripotent stem cells (iPSCs) in vitro may be considered a promising approach in regenerative medicine as a cure for diabetes mellitus. Differentiation protocols attempt to mimic pancreatic embryonic development by activating and inhibiting molecular signaling pathways that control the differentiation of iPSCs into pancreatic β cells with the ability to produce insulin and regulate blood glucose. Here, we present different cofactors and small molecules that differentiate iPSCs into P β LC without detecting other endocrine hormones. A four-step sequential in vitro protocol differentiated pancreatic β cells from iPSC (20b). The differentiated cells expressed the characteristic molecular markers of a pancreatic β cell (pdx1, nkx6.1, ngn3, and insulin) observed by western blot and PCR and compared to a positive control of producing insulin cell line (Min6). In addition, the continuous use of molecules such as vitamin C and retinoic acid in the protocol's final stages promotes and enhances the expression of the β cell characteristic genes. These results allow us to consider the importance of vitamin C and retinoic acid on pancreatic development. Also, these results provide a new perspective on these cofactors' crucial role in maintaining and maturing differentiated cells in vitro.

Funding Source: Universidad de La Sabana**Keywords:** iPSC, human pancreatic beta cells, differentiation

705 - A

QUANTIFICATION OF MAJOR HISTOCOMPATIBILITY COMPLEX I IN UNDIFFERENTIATED INDUCED PLURIPOTENT STEM CELLS AND IPSC DIFFERENTIATED MICROGLIA**Wood, Connor**, Mothé, Bianca*Biological Sciences, California State University, San Diego, CA, USA*

Induced pluripotent stem cells (iPSCs) provide an infinite source of human cells that can be used for therapies and to help understand processes of the immune system. Microglia, a major factor in the immune response in the central nervous system (CNS), play an important role in brain infections and inflammation. Recent evidence has pointed to major histocompatibility complex (MHC) class I antigen display that is triggered by microglia activation in neuronal diseases with inflammation in the CNS. There is little known about the quantity of MHC that is present in microglia, more specifically, the presence of MHC as microglia develops. To better understand this, we will develop a model for microglia differentiation. Starting with iPSCs, MHC expression will be quantified with Cytex Northern Lights Flow Cytometer and MHC class I abundance will be quantified using RT-qPCR. Stem Cell Technologies Hematopoietic Kit will then provide the necessary mediums to differentiate the iPSCs into hematopoietic progenitor cells for MHC quantification. Finally, Stem Cell Technologies Microglia Differentiation Kit will be used to further differentiate the hematopoietic progenitor cells into mature microglia for MHC quantification. We expect to see varying expression of MHC class I as the stem cells differentiate, with more MHC expression at the microglia stage than that of the other two. The expectation of increased MHC expression as cells differentiate can provide context for future experimentation on the effect that varying quantities of MHC can have in inflammatory responses in cells.

Keywords: induced pluripotent stem cells, microglia, major histocompatibility complex class 1

Poster Abstracts

705 - B

STIP1'S ROLE IN PLURIPOTENCY: INSIGHTS FROM MOUSE EMBRYONIC STEM CELLS OVEREXPRESSION

Soares, Samuel Ribeiro, Fernandes, Camila, Souza, Maria Clara, Araújo, João Pedro, Lopes, Marilene

Department of Cell and Developmental Biology, Instituto de Ciências Biomédicas ICB-USP, São Paulo, Brazil

Mouse embryonic stem cells (mESCs) have been a major research focus in recent decades for their unique ability to give rise to all three embryonic germ layers, thus being termed pluripotent. This capability is regulated via different mechanisms, among them a network of transcription factors like OCT4, SOX2 and NANOG, who are in turn orchestrated by several cell signaling pathways. In this context of early development, recent studies have pointed out the importance of the stress inducible protein 1 (Stip1), a highly conserved cochaperone capable of binding with heat-shock proteins 70/90kDa, and whose deletion in mouse embryos is fatal through yet unknown mechanisms. In tumor models, Stip1 has been shown to have direct involvement with the Wnt canonical signaling, a pathway heavily involved with early embryo development and the maintenance of pluripotency in mESCs. In this study, we use mESC STIP1OE cell line, overexpressing Stip1, to investigate the role this protein plays in the maintenance of pluripotency and whether it is related to the canonical Wnt signaling pathway. In the first phase of this study, we characterized our overexpression model in terms of pluripotency and differentiation markers, as well as canonical Wnt signaling expression profile using Western blotting and RT-qPCR. We also evaluated phenotypical aspects including cell death and proliferation markers via immunofluorescence to better comprehend changes brought upon Stip1 modulation. Our early results in STIP1OE show not only changes in core pluripotency marker expression at transcript and protein levels, but also indicate increased expression of a subset of naïve markers targeted by canonical Wnt signaling, as well as increased proliferation and decreased cell death. Taken together, our preliminary results suggest the involvement of Stip1 in the maintenance of pluripotency through the canonical Wnt signaling pathway, and may help to further underpin the mechanisms by which the lethal deletion phenotype is presented in early development.

Funding Source: This research was mainly funded by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

Keywords: stip1, pluripotency, Wnt signaling

706 - A

STRUCTURAL AND FUNCTIONAL INVESTIGATION OF A COCULTURE MODEL OF THE NEURO-CARDIAC JUNCTION USING HUMAN IPSC-DERIVED CARDIOMYOCYTES AND RAT SYMPATHETIC NEURONS

Mohammadi, Neda, Sanchez-Alonso, Jose, Leonov, Vladislav, Seyedmousavi, Sama, Diakonov, Ivan, Harding, Sian, Gorelik, Julia

National Heart and Lung Institute, Imperial College London, UK

The sympathetic nervous system densely innervates all cardiac chambers, yet this neuro-cardiac relationship has scarcely been investigated using a stem cell-based model at a single cell level. This study aims to investigate the effects that sympathetic neurons have on human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) in vitro, and whether these effects induce any degree of maturity in these conventionally immature cells. IMR90 stem cells were differentiated into hiPSC-CM, and sympathetic neurons were isolated from superior cervical ganglia of neonatal rat pups and cocultured together. Structural changes of hiPSC-CM were analysed by SICM and confocal imaging of key target proteins. Ca²⁺ and contractile activity was measured by CytoCypher, which combines ratiometric Ca²⁺ measurements with pixel correlation as a novel method to study hiPSC-CM. FRET was used to measure production of second messenger molecule cAMP, in hiPSC-CM. We found that hiPSC-CM become significantly more elongated in the presence of neurons. β -adrenergic response of hiPSC-CM is also significantly larger in coculture upon stimulation with nicotine, as shown by increased cAMP production. Contraction peak height is increased in paced hiPSC-CM in coculture, with shorter kinetic parameters, indicating that innervation makes them contract more and faster. Similarly, ratiometric Ca²⁺ transient amplitude is significantly larger in coculture. Interestingly, spontaneous contraction studies using β -adrenergic receptor agonist, isoproterenol, show that hiPSC-CM have a similar beat rate per minute at baseline between conditions, but the response is significantly lower in coculture than monoculture. Sympathetic neurons can modulate hiPSC-CM in vitro, reducing their circularity and causing a more mature functional response due to larger baseline contraction peaks and Ca²⁺ transient amplitudes and larger cAMP production in coculture. This may result from hiPSC-CM adjustments towards a more ventricular phenotype, possibly caused by spontaneous norepinephrine release from neurons. Sympathetic neurons can be used as a reliable tool to study the structure and function of hiPSC-CM and contribute to engineered heart tissues or models of sympathoadrenergic disease in vitro.

Keywords: IPSC, cardiomyocyte, neuron

Poster Abstracts

706 - B**THE DYNAMICAL ORGANIZATION OF THE CORE PLURIPOTENCY TRANSCRIPTION FACTORS RESPONDS TO DIFFERENTIATION CUES IN EARLY-S PHASE**

Oses Oliveto, Camila Maite, Francia, Marcos, Veneri, Paula, Vazquez Echegaray, Camila, Guberman, Alejandra, Levi, Valeria

IQUIBICEN - FCEyN, UBA, Buenos Aires, Argentina

Pluripotent stem cells (PSCs) are a promise for regenerative medicine and disease modeling due to their capabilities to self-renew indefinitely and differentiate into all somatic cell types. In the last years, the cell cycle has come under the spotlight for its role in pluripotency maintenance and cell fate decisions. Relevantly, the cell cycle of PSCs presents unique properties since it is faster than that of somatic cells and has a very short G1 phase. Moreover, DNA replication in PSCs is a major challenge for pluripotency preservation since this process involves massive changes in the chromatin architecture and the reorganization of many transcription-related molecules. Pluripotency is controlled by a large regulatory network directed by the core transcription factors (TFs) OCT4, SOX2 and NANOG which induce genes that promote pluripotency and repress those involved in differentiation. It is now widely known that the dynamical distribution of the TFs defines their interactions with chromatin targets and modulates gene expression. Particularly, pluripotency TFs distribute between the nucleoplasm and condensates in mouse embryonic stem cells (ESCs), and their dynamics and distribution change during early differentiation stages preceding their downregulation. In this context, we hypothesized that the S phase could be a window of opportunity to execute changes that impact on the chromatin landscape and the dynamical distribution of the TFs. We explored the dynamical organization of the pluripotency TFs in living ESCs expressing OCT4, SOX2 or NANOG fused to fluorescent proteins through quantitative fluorescence imaging and identified cells in different stages of the S phase through the distribution of PCNA-RFP. We found that differentiation cues in G1 trigger the dissolution of OCT4 and SOX2 foci in the subsequent S phase and favor the interactions of these TFs with the chromatin; contrary, NANOG detaches from chromatin. Our results provide new insights into the regulation of the core pluripotency TFs during DNA replication of ESCs and highlight their different roles at early differentiation stages.

Keywords: transcription factors, fluorescence correlation spectroscopy, cell cycle

Author Index

PRESENTER NAME	PAGE NUMBER	PRESENTER NAME	PAGE NUMBER	PRESENTER NAME	PAGE NUMBER
Ahmed, Ahmed Khalid		Costa Ribeiro, Paula Do		Griesi Oliveira, Karina	87
Mohamed Albashir	38	Amaral	51	Guerrero, Erika N.	41
Alcoforado Diniz, Juliana.....	60	da Costa, Vitor Rodrigues.....	46	Guimaraes, Marilia Zaluar	85
Almeida, Valéria	72	da Silva Chaves, Juliana		Gutierrez, Patricia Noemi	77
Alvarez, Carla Barragan.....	65	Cristina	67	H. Real, Fernando	46
Alves, Rodrigo Nunes.....	83	da Silva Goncalves, Carlos E....	23	Harvey, Kieran.....	21
Apostolou, Eftychia	20	da Silva Souza, Maria Clara		Hayashi, Mirian	56
Araña, Matheus C.	89	Da Silva	66	Horianski, Romina Soledad	68
Azevedo, Evellyn M.	48	de Sousa Vieira, Afonso.....	75	Hsieh, Jenny.....	80
Barcelos, Stefhani M.	45	de Castro, Mateus V.	49	Hutschalik, Thomas.....	30
Barros, Barbara Avelar		de Matos, Bruno M.	40	Izsvak, Zsuzsanna.....	22
Ferreira.....	92	Didamson, Onyisi Christiana....	82	Jaén Alvarado, Yanelys	
Bello, Claudia	79	Doetsch, Fiona	23	Lourdes	65
Benvenisty, Nissim	19	Dos Santos, Marcelo Garrido ...	38	Jarriault, Sophie.....	23
Berti, Joselina.....	59	Egli, Dieter	22	Josino, Raphaella.....	37
Beyerstedt, Stephany.....	36, 47	Felismino, Daniel Minatti	55	Julio, Alison	72
Bim, Larissa V.	61	Fernandes, Camila.....	93	Kadowaki Komatsu, Sabrina	
Butenas, Alexandre B.	70	Fernández, Edelmira A.....	74	Kaori	63
Carlone, Robert Leo.....	44	Ferreira, Nikolas Dresch	64	Kim, Hyunjin	86
Carreira, Ana Claudia.....	85, 89	Fontecilla Escobar, Javiera	43	Knock, Erin.....	25
Carvalho, Antonio Carlos C.....	33	Frade, Bianca Braga.....	78	Kovacs, Gergo	92
Chao, George	88	Francia, Marcos Gabriel.....	94	Lanuza, Guillermo.....	22
Chiaradia, Ilaria	58	Franco, Marcella L.	44	Leite, Nayara	33
Clark, Amander	19	Frontini Lopez, Yesica		Lizcano, Fernando	95
Costa, Isabela de Souza.....	36	Romina Romina	47	Machado, Caio Bezerra.....	59
Correia Croda Catharino,		Ganguly, Mohuli Roy	56	Mallick, Sumit.....	53
Luana.....	50	Garcez, Emanuella Melgaco....	43	Manzini, Bruna M.....	91

Author Index

PRESENTER NAME	PAGE NUMBER	PRESENTER NAME	PAGE NUMBER	PRESENTER NAME	PAGE NUMBER
Marques, Juliana Moreira	73	Paes de Camargo, Bruno T.	58	Serrano, Grecia Vanessa.....	42
Marchetto, Maria Carolina.....	26	Pagotto, Romina	76	Shablii, Volodymyr.....	54
Marconi, Pedro Batista.....	69	Paladini, Carlos Antonio	52	Shlomi, Shlomo	64
Markin, Alexander	82	Palma, Verónica	29	Shu, Jian	26
Martinez-Alarcon, Omar.....	61	Parmar, Malin.....	19	Silva, Karina Ribeiro Da	42
Martins Oliveira, Natássia.....	48	Paskevicius, Gabriela de Oliveira.....	49	Smolinska, Agnieszka	39
Martins, Vilma Regina	86	Pereira, Lygia da Veiga	20	Soares, Samuel Ribeiro.....	96
Mazzoni, Esteban O.....	24	Peterson, Quinn	34, 53, 63	Solis, Mairim Alexandra	32, 80
Medeiros, Mariana	51	Pinheiro, Luisa Machado	81	Supakul, Sopak	69
Medina-Arellano, Maria de Jesús	31	Pitossi, Fernando	31, 67	Tavormina, Penny	84
Meléndez, César D	27	Polanco, Angel	61	Teles E. Silva, Andre L.	30
Mendez-Otero, Rosalia.....	23	Polo, Jose.....	27	Terra, Sara Carolina Vieira.....	41
Mohammadi, Neda	96	Porcionatto, Marimelia A.	29	Trabish, Sophie	55
Monteiro Da Rocha, Andre	71	Prišlin, Marina	76	Vautier, Mercedes	93
Morais, Gabriel S.....	40	Quintero, Carolina	57	Velasco, Ivan	24
More Milan, Thais	90	Rao, Mahendra S.....	33	Viegas, Juliane O.....	21
Munsie, Megan.....	32	Rehen, Stevens K.....	25	Wang, Xi.....	24
Munteanu, Raluca S.	68	Rivron, Nicolas	28	Wernig, Marius	24
Muotri, Alysson R.....	28	Rizal, Rizal.....	66	Woltjen, Knut.....	25
Nava, Jessica.....	73	Rosa, Viviane.....	54	Wood, Connor	95
Nicolicht-Amorim, Priscila	91	Roy, Debashish.....	90	Wu, Jun	28
Nolasco, Patricia	84	Ruginsk, Bruna Estelita.....	75	Zaboroski Silva, Isabelle L.	70
Nunes, Catarina de Jesus.....	79	Ruzov, Alexey	94	Zuliani, Carolina Coli	39
Oropeza, Anderson	60	Salazar, Alejandro	83	Zych Nadolny, Jaiesa	88
Osés Oliveto, Camila Maite	97	Salles, Geisa Rodrigues	81		
Pacheco, Lara	62	Santillan Cigales, Juan Jair	71		



Your Access to a World of Stem Cell Discoveries

JOIN OUR GLOBAL COMMUNITY

The ISSCR supports members with access to breakthrough science, career resources, global advocacy, and collaboration through global events. There is strength in numbers, and our numbers are growing.

LEARN MORE AT

<https://www.isscr.org/membership>