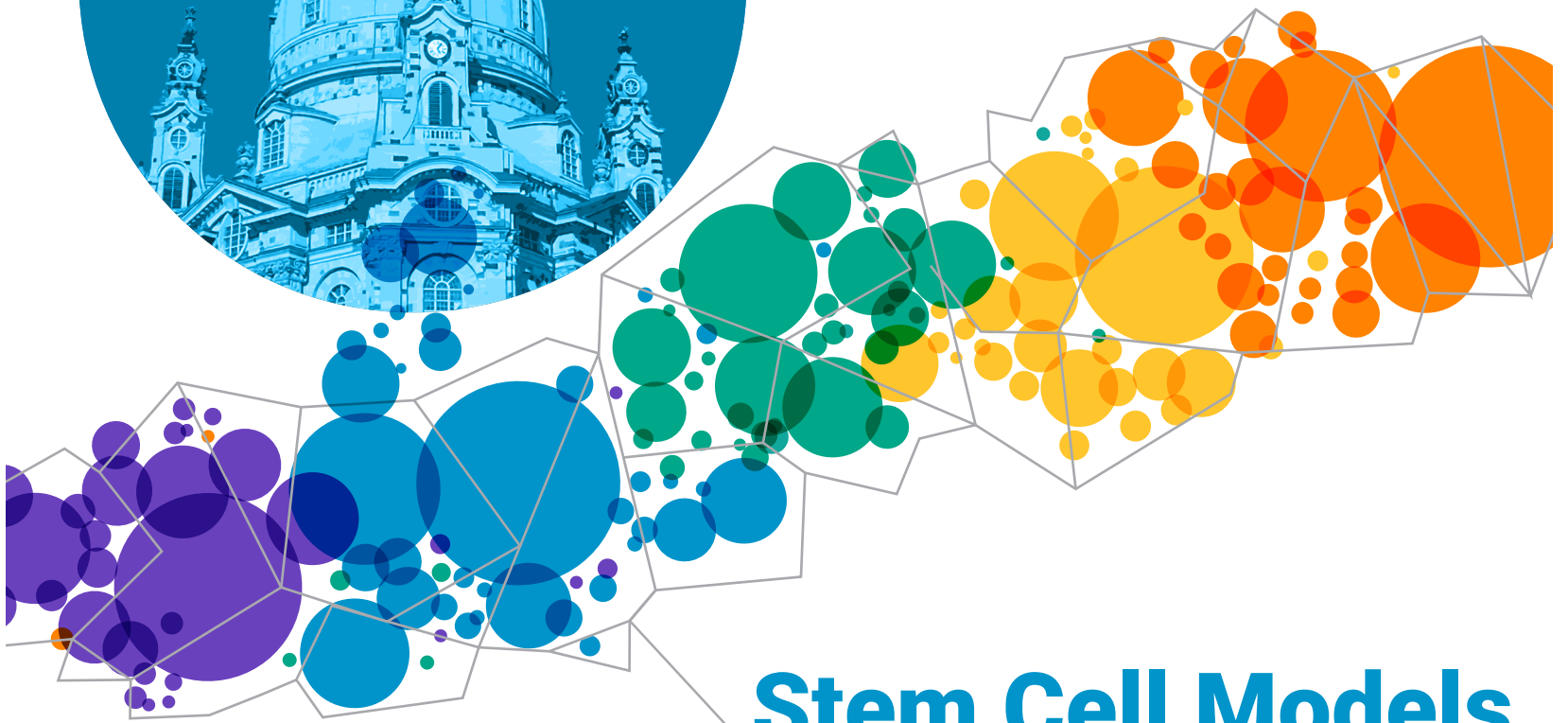


INTERNATIONAL **2016** SYMPOSIA



DRESDEN GERMANY **2016**

1-3 FEBRUARY



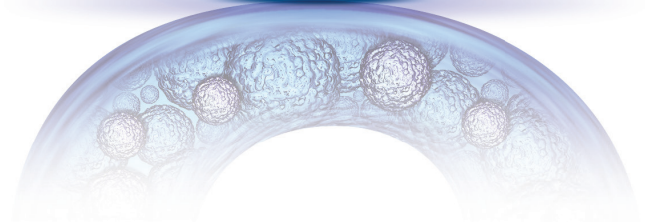
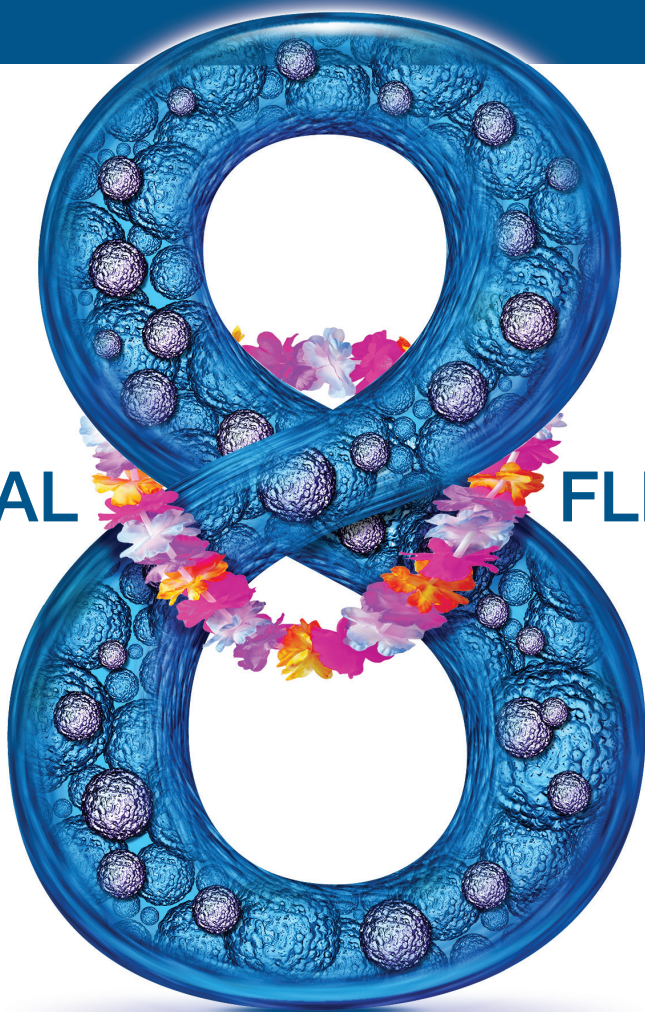
Stem Cell Models Of Neural Regeneration And Disease

PROGRAM GUIDE



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DRESDEN GERMANY

Stem Cell Models of Neural Regeneration and Disease



GENERAL INFORMATION

VENUE

The *Stem Cell Models of Neural Regeneration and Disease* International Symposium is located in the Center for Regenerative Therapies Dresden (CRTD) Research Center and Cluster of Excellence at the TU Dresden. All program sessions will be in The Auditorium, Ground Floor. The Tuesday, 2 February Break-out Sessions will be located in Auditorium Left and Right on the Ground Floor, Seminar Room 1 on the Second Floor and Seminar Room 3 on the Third Floor.

Registration and the Auditorium are located on the Ground Floor.

The Exhibition Hall is located on the Ground Floor. Refreshment breaks will be located on the Ground, First and Second Floors. Lunch will be served on the Ground Floor and First Floor. Poster presentations will take place on the First Floor and Second Floor.

Note: Food and drink are not permitted inside the Auditorium.

REGISTRATION AND BADGE PICKUP

Pick up your attendee name badge in the Ground Level, CRTD Research Center, during posted hours at the registration desk. Bring your Meeting Confirmation receipt to the registration area. Name badges are required for admission to all sessions, poster presentations, social events and the Exhibition Hall. Since the meeting badge serves as proof of participation, all attendees, speakers and exhibitors are required to wear their badges at all times during the International Symposium as well as the Networking Social Evening and all other special events. Access to events may be refused if the meeting badge is not displayed.

REGISTRATION DESK & BADGE PICKUP HOURS

MONDAY, 1 FEBRUARY

10:00 – 19:00

TUESDAY, 2 FEBRUARY

9:00 – 17:30

WEDNESDAY, 3 FEBRUARY

9:00 – 17:15

An administration charge of \$20 USD will be imposed on any replacement badge. A drop box for badge recycling will be provided on the Ground Floor.

INTERNET ACCESS

Complimentary access to the internet is available within the CRTD Research Center during the International Symposium. Individual log-in details will be given to attendees when they pick up their name badge.

As a courtesy to speakers, please be sure to silence any mobile phones and devices and refrain from using the internet during sessions. Please note that the bandwidth of this connection might be limiting.

HELP DESK

Please visit the registration counter located on the Ground Floor, CRTD Research Center with any questions.



GENERAL INFORMATION

SPECIAL EVENTS

REFRESHMENT BREAKS

Enjoy refreshment breaks on the Ground, First and Second Floors, CRTD Research Center. Complimentary coffee and tea will be served during the following days and times:

MONDAY, 1 FEBRUARY

15:30 – 16:00

TUESDAY, 2 FEBRUARY

10:15 – 10:45 & 15:45 – 16:30

WEDNESDAY, 3 FEBRUARY

10:30 – 11:00 & 15:45 – 16:15

LUNCH HOURS AND POSTER PRESENTATIONS

Refresh midday with a complimentary lunch served on the Ground Floor and the First Floor, CRTD Research Center. Lunch will take place during scheduled poster presentations and be served during the following days and times

TUESDAY, 2 FEBRUARY

12:15 – 14:30

WEDNESDAY, 3 FEBRUARY

12:15 – 14:30

RECEPTION

SUPPORTED BY THERMO FISHER SCIENTIFIC

The Reception will take place on **MONDAY, 1 FEBRUARY** from 17:00 – 19:00 on the Ground Floor, CRTD Research Center. Complimentary beer and wine as well as a light buffet will be provided.

NETWORKING SOCIAL EVENING AT BALLHAUS WATZKE

SUPPORTED BY GERMAN STEM CELL NETWORK

The Networking Social Evening at Ballhaus Watzke will take place on **TUESDAY, 2 FEBRUARY** from 19:00 – 22:00. Participation for this networking event requires pre-registration. Onsite registration for this event is not available. Attendees pre-registered for the Networking Social Evening at Ballhaus Watzke will have transportation provided.

DEPARTURE FROM CRTD RESEARCH CENTER:

18:00 (after the Breakout Sessions)

ARRIVAL AT BALLHAUS WATZKE:

19:00

RETURN DEPARTURE FROM BALLHAUS WATZKE:

22:00 (attendees will be dropped off at Hotel Steigenberger)

NOTE: Pre-registered attendees who board the bus at CRTD Research Center at 18:00 sharp will be given a city tour en route to the Ballhaus Watzke.

BALLHAUS WATZKE

Kötzschenbroder Str. 1, 01139 Dresden, Germany
+49 351 8529

RECORDING POLICY

Still photography, video and/or audio taping of the sessions, presentations and posters at the International Symposium is strictly prohibited. Intent to communicate or disseminate results or discussion presented at the meeting is prohibited until the start of each individual presentation.

DRESDEN GERMANY

Stem Cell Models of Neural Regeneration and Disease

POSTERS & EXHIBITS



POSTER HALL

POSTER SET-UP

The posters will be accessible for the duration of the International Symposium. Please place posters in their designated location on the First Floor and Second Floor, CRTD Research Center on **MONDAY, 1 FEBRUARY** from 12:00 – 17:00. Poster adhesive tape will be provided.

POSTER TAKEDOWN

Poster presenters are responsible for their own posters. Presenters will need to take down their posters on **WEDNESDAY, 3 FEBRUARY** from 14:30 – 16:15. Posters that have not been removed by **WEDNESDAY, 3 FEBRUARY** at 16:15 will be discarded by the organizer.

POSTER PRESENTATIONS

Poster presenters are required to be present at their posters during scheduled poster presentations. All poster presentations are scheduled during lunch breaks.

ODD-NUMBERED POSTERS TUESDAY, 2 FEBRUARY

12:15 – 14:30

EVEN-NUMBERED POSTERS WEDNESDAY, 3 FEBRUARY

12:15 – 14:30

EXHIBITION HALL

The Exhibition Hall features 17 leading suppliers and vendors. Walk through the hall on the Ground Floor, CRTD Research Center and support the Exhibitors who help make this Symposium possible.

The Exhibition Hall will be open throughout the International Symposium. Feel free to walk through the stands and learn about products and services with all exhibitors onsite.

EXHIBITION HALL HOURS

MONDAY, 1 FEBRUARY

10:00 – 19:00

TUESDAY, 2 FEBRUARY

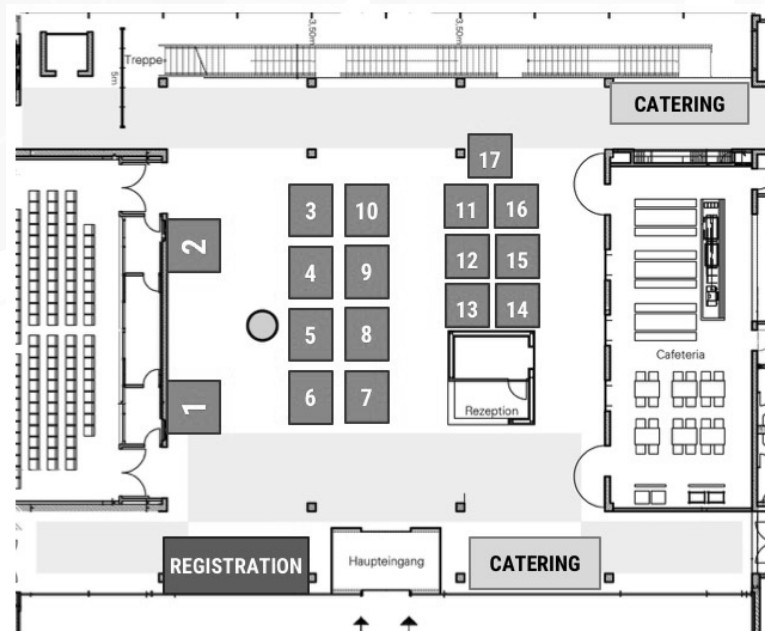
9:00 – 17:30

WEDNESDAY, 3 FEBRUARY

9:00 – 17:15

GROUND FLOOR

- 1 Thermo Fisher Scientific
- 2 German Stem Cell Network
- 3 STEMCELL Technologies
- 4 Irvine Scientific
- 5 LGC
- 6 ChemoMetec A/S
- 7 Wako Chemicals GmbH
- 8 Olympus Europa SE & Co. KG
- 9 Takara Clontech
- 10 PeproTech GmbH
- 11 PromoCell
- 12 BIOTREND Chemikalien GmbH
- 13 Miltenyi Biotec
- 14 Axol Biosciences LTD
- 15 BioLamina
- 16 Union Biometrica
- 17 City of Dresden, Office of Economic Development



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SYMPOSIUM ORGANIZING COMMITTEE

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University of Bonn Medical Center and LIFE & BRAIN,
Germany

Andrew Elefanty

Murdoch Children's Research Institute, Australia

Gordon Keller

McEwen Centre for Regenerative Medicine Ontario
Cancer Institute, Canada

Lorenz Studer

Sloan-Kettering Institute for Cancer Research, USA

Elly Tanaka

Center for Regenerative Therapies (CRTD), Technische
Universität Dresden, Germany

ABSTRACT REVIEWERS

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Max Planck Institute of Psychiatry, Germany

Mirella Dottori

University of Melbourne, Australia

Frank Edenhofer

University of Würzburg, Germany

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Universität Dresden, Germany

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University of Bonn Medical Center, Germany

Jonathan Niclis

The Florey Institute of Neuroscience and Mental Health,
Australia

Steven Petratos

Monash University, Australia

Laura Stappert

University of Bonn Medical Center, Germany

Jared Sternecker

Center for Regenerative Therapies (CRTD),
Technische Universität Dresden, Germany

Elly Tanaka

Center for Regenerative Therapies (CRTD),
Technische Universität Dresden, Germany

DRESDEN GERMANY

Stem Cell Models of Neural Regeneration and Disease

MONDAY, 1 FEBRUARY

MONDAY, 1 FEBRUARY

13:00 - 13:15

Welcome Remarks

Dr. Robert Franke, *City of Dresden, Office of Economic Development*

Nancy Witty, *ISSCR CEO*

13:15 - 14:00

Overview

Oliver Brüstle, *University of Bonn Medical Center and LIFE & BRAIN GmbH, Germany*

NEW FRONTIERS IN NEURO-BIOMEDICAL STEM CELL RESEARCH

14:00 - 15:30

Session 1: 3D Organoids

Chair: **Frederick Livesey**, *The Gurdon Institute - University of Cambridge, UK*

14:00 - 14:30

Doo Yeon Kim, *Massachusetts General Hospital, USA*

RECAPITULATING ALZHEIMER'S DISEASE PATHOLOGY IN A 3D HUMAN NEURAL CELL CULTURE MODEL

14:30 - 14:45

Gray Camp, *Max Planck Institute for Evolutionary Anthropology, Germany*

DISSECTING CEREBRAL ORGANOID AND FETAL NEOCORTEX USING SINGLE-CELL RNA-SEQ

14:45 - 15:00

Stefan Weiss, *Helmholtz Center Munich, Germany*

HIGH-CONTENT IMAGING OF STEM CELL-DERIVED NEURONS ALLOWS THE IDENTIFICATION OF A NEW NEURONAL LINEAGE SPECIFIER, GLYPICAN 4

15:00 - 15:30

Jürgen Knoblich, *IMBA, Austria*

MODELING HUMAN BRAIN DEVELOPMENT AND DISEASE IN 3D CULTURE

15:30 - 16:00

Break

16:00 - 17:00

Session 1: Organoids (continued)

16:00 - 16:15

Luis Zurkirchen, *University of Zurich, Switzerland*

YIN YANG 1 REGULATES CEREBRAL CORTEX DEVELOPMENT IN A DEVELOPMENTAL STAGE-SPECIFIC MANNER

PROGRAM SCHEDULE

16:15 – 16:45

Motosugu Eiraku, *RIKEN, Japan*

GENERATION OF COMPLEX TISSUE STRUCTURE IN STEM CELL CULTURE

16:45 – 17:00

Silvia Cappello, *Max Planck Institute of Psychiatry, Germany*

MODELING PERIVENTRICULAR HETEROTOPIA IN HUMAN CEREBRAL ORGANIDS WITH MUTATIONS IN FAT4 AND DCHS1

17:00 - 19:00

Reception

SUPPORTED BY THERMO FISHER SCIENTIFIC

TUESDAY, 2 FEBRUARY

8:00 - 8:30

Company Presentation *Light breakfast provided by Thermo Fisher Scientific prior to the presentation at 7:30 outside of the Auditorium*

David Kuninger, *Thermo Fisher Scientific*

PHYSIOLOGICALLY RELEVANT NEURAL MODELS FOR DISCOVERY AND SCREENING: STEM CELL DERIVED SYSTEMS FROM THERMO FISHER SCIENTIFIC

9:00 - 10:15

Session 2: Modeling Disorders

Chair: **Oliver Brüstle**, *University of Bonn Medical Center and Life & Brain GmbH, Germany*

9:00 - 9:30

Frederick Livesey, *The Gurdon Institute - University of Cambridge, UK*

INSIGHTS INTO ALZHEIMER'S DISEASE PATHOGENESIS FROM HUMAN STEM CELL SYSTEMS

9:30 - 9:45

Tongguang Wang, *National Institute of Neurological Disorders and Stroke, USA*

IN VITRO MODELING OF NEUROINFLAMMATION USING NEURAL STEM CELLS DERIVED FROM CD34 CELLS FROM PATIENTS WITH UNDIAGNOSED RARE DISEASES

9:45- 10:15

Guo-Li Ming, *Johns Hopkins University, USA*

MODELING NEUROLOGICAL DISEASES USING PATIENT IPSCS

10:15 - 10:45

Break

TUESDAY, 2 FEBRUARY

10:45 - 12:00

Session 2: Modeling Disorders (continued)

10:45 – 11:15

MONIKA EHRHART-BORNSTEIN MEMORIAL LECTURE

Ana Martin-Villalba, DKFZ German Cancer Research Center, Germany

STEM CELL HETEROGENEITY IN HOMEOSTASIS AND DISEASE

11:15 - 11:30

David Parfitt, UCL Institute of Ophthalmology, UK

PATIENT-DERIVED IPSC THREE-DIMENSIONAL OPTIC CUPS TO TEST DISEASE MECHANISMS AND RNA THERAPY FOR INHERITED BLINDNESS

11:30 -12:00

Lawrence S.B. Goldstein, University of California San Diego, USA

PROBING THE SECRETS OF ALZHEIMER'S DISEASE WITH PLURIPOTENT STEM CELL TECHNOLOGY

12:15 – 14:30

Lunch and Poster Session I (Odd numbered posters presented)

13:45-14:15

Company Presentation

Uwe Speck, BD Biosciences

HIGH THROUGHPUT SINGLE CELL SORTING FOR GENOMICS

14:30-15:45

Session 3: Modeling Regeneration

Chair: *Elly Tanaka, Center for Regenerative Therapies, Technische Universität Dresden, Germany*

14:30- 15:00

Frank Bradke, German Center for Neurodegenerative Diseases, Germany

TRANSCRIPTOME ANALYSIS IDENTIFIES THE CALCIUM CHANNEL SUBUNIT ALPHA2DELTA2 AS A KEY REGULATOR OF AXON REGENERATION

15:00 – 15:15

Jovica Ninkovic, Helmholtz Zentrum München, Germany

CELLULAR AND MOLECULAR CHANGES IN ADULT NEURAL STEM CELLS ALLOWING SUCCESSFUL REGENERATION IN THE ADULT ZEBRAFISH BRAIN

15:15 - 15:45

Jonas Frisen, Karolinska Institute, Sweden

CELLULAR PLASTICITY IN THE INTACT AND INJURED CENTRAL NERVOUS SYSTEM

PROGRAM SCHEDULE

15:45 - 16:30

Break

16:30 - 17:30

Break-out Sessions

3D CULTURES AND ORGANOIDS SUPPORTED BY GERMAN STEM CELL NETWORK

Leaders

Julia Ladewig, *University of Bonn, Germany*

Michael Karl, *Center for Regenerative Therapies at Technische Universität Dresden, Germany*

Jürgen Knoblich, *IMBA, Austria*

Motosugu Eiraku, *RIKEN, Japan*

MODELING NEURODEGENERATION

Leaders

Jared Sternecker, *Center for Regenerative Therapies at Technische Universität Dresden, Germany*

Michael Peitz, *University of Bonn, Germany*

Guo-Li Ming, *Johns Hopkins University, USA*

Lawrence S.B. Goldstein, *University of California San Diego, USA*

DIRECT CELL FATE CONVERSION AND ENDOGENOUS REGENERATION

Leaders

Volker Busskamp, *Center for Regenerative Therapies at Technische Universität Dresden, Germany*

Marisa Karow, *Ludwig-Maximilians-Universität München, Germany*

Jonas Frisén, *Karolinska Institute, Sweden*

CLINICAL TRANSLATION

Leaders

Marius Ader, *Center for Regenerative Therapies at Technische Universität Dresden, Germany*

Lorenz Studer, *Sloan-Kettering Institute for Cancer Research, USA*

Peter Coffey, *University College of London, UK*

Sally Temple, *Neural Stem Cell Institute, USA*

19:00 - 22:00

BALLHAUS WATZKE NETWORKING SOCIAL EVENT (PRE-REGISTRATION REQUIRED)

SUPPORTED BY GERMAN STEM CELL NETWORK

DRESDEN GERMANY

Stem Cell Models of Neural Regeneration and Disease



WEDNESDAY, 3 FEBRUARY

WEDNESDAY, 3 FEBRUARY

9:00 - 10:30

Session 3: Modeling Regeneration (continued)

9:00 - 9:30

Robin Franklin, *University of Cambridge, UK*

REGENERATING CNS MYELIN-FROM MECHANISMS TO MEDICINES

9:30 - 10:00

Steven Goldman, *University of Copenhagen, Denmark*

HUMAN GLIAL PROGENITOR CELL-BASED TREATMENT AND MODELING OF NEUROLOGICAL DISEASE

10:00 - 10:30

Malin Parmar, *Lund University, Sweden*

TOWARDS A STEM CELL BASED THERAPY FOR PARKINSON'S DISEASE

10:30 - 11:00

Break

11:00 - 12:00

Summary from Break-out Sessions

12:15 - 14:30

Lunch and Poster Session II (*Even numbered posters presented*)

14:30-15:45

Session 4: Road to the Clinic

Chair: **Steven Goldman**, *University of Copenhagen, Denmark*

14:30- 15:00

Lorenz Studer, *Sloan-Kettering Institute for Cancer Research, USA*

HUMAN PLURIPOTENT-DERIVED NEURONS FOR ADDRESSING DISORDERS OF THE GUT, MUSCLE, AND BRAIN

15:00 - 15:15

Paulina Ordonez, *University of California San Diego, USA*

IMPLICATIONS OF DISRUPTED AUTOPHAGY ON CHOLESTEROL TRAFFICKING, NEURONAL SURVIVAL AND STRATEGIES FOR DRUG DEVELOPMENT IN NPC1

15:15 - 15:45

Sally Temple, *Neural Stem Cell Institute, USA*

STEM CELLS IN THE ADULT HUMAN RETINAL PIGMENT EPITHELIUM AND THEIR THERAPEUTIC POTENTIAL

PROGRAM SCHEDULE

15:45 - 16:15

Break

16:15 - 17:00

Session 4: Road to the Clinic (continued)

16:15 - 16:30

Andrea Lampp, *University of Copenhagen, Denmark*

**OPCS DERIVED FROM HUNTINGTON DISEASE-DERIVED HESCS EXHIBIT
A SOX10 AND MYRF DISRUPTION-ASSOCIATED SUPPRESSION OF
OLIGODENDROCYTE DEVELOPMENT AND MYELINOGENESIS**

16:30 - 17:00

Peter Coffey, *University College of London, UK*

STEMMING VISIONLOSS USING HUMAN EMBRYONIC STEM CELLS

17:00 - 17:15

Closing Overview:

Elly Tanaka, *Center for Regenerative Therapies, Technische Universität Dresden, Germany*

DRESDEN GERMANY

Stem Cell Models of Neural Regeneration and Disease



SUPPORTERS & EXHIBITORS

ISSCR AND CRTD WOULD LIKE TO THANK THE FOLLOWING SUPPORTERS AND EXHIBITORS

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Thermo Fisher Scientific will be presenting *Physiologically Relevant Neural Models for Discovery and Screening: Stem Cell Derived Systems* on Tuesday at 8:00. A light breakfast will be available, courtesy of Thermo Fisher Scientific prior to the presentation at 7:30 outside of the Auditorium.

SILVER SUPPORT


GERMAN STEM CELL NETWORK (GSCN)

c/o Max Delbrück Center
Robert-Rössle-Str. 10
13125 Berlin
Germany

Phone: +49 30-9406-2488/-2487

www.gscn.org

The central task of the German Stem Cell Network (GSCN) is to pool the expertise in stem cell research in Germany and develop synergies between basic research, regenerative medicine and pharmacology. The network promotes and supports innovative research activities on a national and international level. In addition, targeted information is offered to encourage the public discourse on stem cell research.


GSCN
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DRESDEN GERMANY

Stem Cell Models of Neural Regeneration and Disease

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Medical Faculty Carl Gustav Carus
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01307 Dresden
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Phone: +49 351-458-5602

www.sfb655.de

The scientific vision of the SFB 655 is to understand the basic biology of somatic stem and progenitor cells in the context of tissue formation and, eventually, clinical application. Towards this goal, the SFB 655, funded by the DFG since 2005, has brought together the traditionally separate disciplines of cell biology, developmental biology, molecular bioengineering and medicine.



DRESDEN INTERNATIONAL GRADUATE SCHOOL FOR BIOMEDICINE & BIOENGINEERING (DIGS-BB)

DIGS-BB PhD Program Office
Technische Universität Dresden
DFG-Center for Regenerative Therapies Dresden
Cluster of Excellence
Fetscherstr. 105
01307 Dresden
Germany

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tu-dresden.de/exzellenz/graduierenschule/digsbb

The DIGS-BB was awarded to the TUD in the Excellence Initiative in 2006. Since then, the DIGS-BB offers excellent training and research opportunities for outstanding and enthusiastic graduates who wish to work towards a PhD degree in 4 multidisciplinary and interconnected fields: Regenerative Medicine, Biophysics and Bioengineering, Cell and Developmental Biology and Computational biology. Top-notch research, a structured curriculum and a highly international environment are hallmarks of the DIGS-BB.

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DRESDEN GERMANY

Stem Cell Models of Neural Regeneration and Disease

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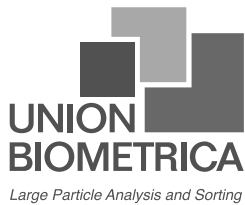
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- Thomas Eschenhagen
- Peter Zandstra
- Peter Coffey



SPEAKER ABSTRACTS

MONDAY, 1 FEBRUARY
13:00 - 14:00

MEETING OVERVIEW

NEW FRONTIERS IN NEURO-BIOMEDICAL STEM CELL RESEARCH

BRÜSTLE, OLIVER

University of Bonn Medical Center and LIFE & BRAIN GmbH, Bonn, Germany

This meeting will cover latest developments in stem cell research and its applications in biomedicine - a field, which is advancing at an ever-faster rate. This is particularly true for iPS cell-based disease modeling, where standardized in vitro differentiation protocols and efficient gene editing methods enable the establishment of cell models reliably reporting even subtle phenotypic changes with high fidelity. These robust and efficient tools have encouraged extension of iPS cell-based modeling from monogenic to complex diseases such as mental disorders. At the same time, traditional 2D systems are complemented by 3D matrix models, organoids and mouse-human neural chimeras, which provide experimental access to neurogenesis and patterning, cell-cell-interactions and disease-related extracellular deposits in a tissue context. In addition to classic reprogramming, direct transcription factor-based cell fate conversion is increasingly recognized as an attractive approach for both disease modeling and in situ transdifferentiation. At the translational end, transplants of ES cell-derived dopamine neurons and retinal pigment epithelial (RPE) cells derived from ES or endogenous RPE cells are emerging as promising clinical modalities for the treatment of Parkinson's disease and macular degeneration, respectively. Increasing insight into mechanisms recruiting resident progenitors into CNS lesions may eventually inform novel drug-based approaches for the promotion of endogenous repair.

MONDAY, 1 FEBRUARY
14:00 - 17:00

SESSION I: 3D ORGANOID

RECAPITULATING ALZHEIMER'S DISEASE PATHOLOGY IN A 3D HUMAN NEURAL CELL CULTURE MODEL

D'Avanzo, Carla, Choi, Se Hoon, Tanzi, Rudolph E., KIM, DOO YEON

Genetics and Aging Research Unit, MassGeneral Institute for Neurodegenerative, Massachusetts General Hospital, Charlestown, MA, USA

The "amyloid β hypothesis" of Alzheimer's disease (AD) has been the reigning hypothesis over the last two decades. According to this hypothesis, excessive accumulation of toxic β -amyloid peptides ($A\beta$) triggers a vicious pathogenic cascade including synaptic deficits, altered neuronal activity, hyperphosphorylation of tau/neurofibrillary tangles and finally, neuronal death. However, this hypothesis has not been fully validated in animal models, and several major unresolved issues remain. We recently developed a human neural cell culture model of AD based on a three-dimensional (3D) cell culture system. We showed that this unique cellular model recapitulates key events of the AD pathogenic cascade, including $A\beta$ plaques and neurofibrillary tangles. In follow-up studies, we explore the functional deficits along with $A\beta$ /p-tau pathologies, using single-clonal human ReNcell VM neural progenitor cells, which overexpress human APP and PSEN1 with familial AD mutations (FAD-ReN). As expected, we observed robust accumulation of $A\beta$ /p-tau pathologies in FAD-ReN cells after 7 weeks of 3D differentiation. FAD-ReN cells with high levels of p-tau, showed varicose-like structures that appear as irregular small swellings distributed along neurites. FAD-ReN cells also display significant increases in neuronal death after 7-9 week differentiation, which are measured by increased LDH releases, decrease ATP levels and accumulation of active caspase-3. We also detected abnormal intracellular Ca^{2+} accumulation and hyperexcitable cells only in FAD-ReN. These data suggest that accumulation of $A\beta$ and/or p-tau lead to functional deficits and possibly neurodegeneration in our 3D FAD-ReN cultures. Finally, we will also show preliminary data regarding our efforts to apply our 3D FAD-ReN cell culture models for a high-throughput drug screening.

SPEAKER ABSTRACTS

DISSECTING CEREBRAL ORGANIDS AND FETAL NEOCORTEX USING SINGLE-CELL RNA-SEQ

CAMP, GRAY¹, Badsha, Farhath², Florio, Marta², Pääbo, Svante¹, Huttner, Wieland², Treutlein, Barbara¹

¹Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany, ²Max Planck Institute of Cell Biology and Genetics, Dresden, Germany

Cerebral organoids - three-dimensional cultures of human cerebral tissue derived from pluripotent stem cells - have emerged as models of human cortical development. However, the extent to which in vitro organoid systems recapitulate neural progenitor cell proliferation and neuronal differentiation programs observed in vivo remains unclear. Here we use single-cell RNA sequencing (scRNA-seq) to dissect and compare cell composition and progenitor-to-neuron lineage relationships in human cerebral organoids and fetal neocortex. Covariation network analysis using the fetal neocortex data reveals known and novel interactions among genes central to neural progenitor proliferation and neuronal differentiation. In the organoid, we detect diverse progenitors and differentiated cell types of neuronal and mesenchymal lineages, and identify cells that derived from regions resembling the fetal neocortex. We find that these organoid cortical cells use gene expression programs remarkably similar to those of the fetal tissue in order to organize into cerebral cortex-like regions. Our comparison of in vivo and in vitro cortical single cell transcriptomes illuminates the genetic features underlying human cortical development that can be studied in organoid cultures.

HIGH-CONTENT IMAGING OF STEM CELL-DERIVED NEURONS ALLOWS THE IDENTIFICATION OF A NEW NEURONAL LINEAGE SPECIFIER, GLYPICAN 4

WEISS, STEFAN¹, Schorpp, Kenji², Hadian, Kamyar¹, Desbordes, Sabrina¹

¹Institute of Developmental Genetics, Helmholtz Center Munich, Oberschleißheim, Germany, ²Institute of Toxicology, Helmholtz Center Munich, Oberschleißheim, Germany

Autologous transplantation of stem cell-derived neurons might be a powerful approach for the future treatment of neurodegenerative diseases. Current protocols of stem cell-based neuronal differentiation mimic neurodevelopment in a dish using either activation or inhibition of various developmental signaling pathways. Although sophisticated protocols lead to desired cell types, a main limitation is the low differentiation efficiency to specific neural subtypes. It is therefore desired to identify new members involved in neuronal subtype-specific differentiation to further increase differentiation efficiency. In order to identify neural lineage specifiers,

we developed a high-content imaging assay to quantify pluripotent stem cell-derived dopaminergic (TH+) and cortical (CTIP2+) neural subpopulations. We then targeted candidate genes by RNA interference and analyzed their effect on the differentiation efficiency to these subpopulations. Our highly sensitive assay allowed us to identify Glypican 4, a membrane-anchored heparan sulphate proteoglycan, as a major regulator of cortical lineage specification. Further analysis demonstrated that the decrease of cortical neurons in Glypican 4-knockdown cells leads to an increase of the dopaminergic neuron subpopulation. This result revealed that Glypican 4 is a neuronal subtype decision gene. Furthermore, we demonstrated that Glypican 4 regulates early mouse neurodevelopment with a specific alteration of the Six gene family expression. Finally, our *in vivo* data confirmed its role as a regulator of mammalian fore-brain development, through the regulation of the Hh/Wnt pathways. High resolution 3D imaging allowed us to study in detail the cyclopic phenotype of the Glypican 4 KO embryos and to demonstrate the reminiscence of this KO embryos phenotype to human holoprosencephaly. Taken together, our results strongly support a role of Glypican 4 in neuronal subtype specification and open doors for novel therapeutic applications in neurodegenerative diseases.

MODELING HUMAN BRAIN DEVELOPMENT AND DISEASE IN 3D CULTURE

Lancaster, Madeline, Renner, Magdalena, **KNOBLICH, JÜRGEN A.**

IMBA-Institute of Molecular Biotechnology of the Austrian Academy of Science, Vienna, Austria

The human brain is highly unique in size and complexity. While many of its characteristics have been successfully studied in model organisms, recent experiments have emphasized unique features that cannot easily be modeled in animals. We have therefore developed a 3D organoid culture system derived from human pluripotent stem cells that recapitulates many aspects of human brain development. These cerebral organoids are capable of generating several brain regions including a well-organized cerebral cortex. Furthermore, human cerebral organoids display stem cell properties and progenitor zone organization that show characteristics specific to humans. Finally, we use both RNAi and patient specific iPS cells to model microcephaly, a human neurodevelopmental disorder that has been difficult to recapitulate in mice. This approach reveals premature neuronal differentiation with loss of the microcephaly protein CDK5RAP2, a defect that could explain the disease phenotype. Our data demonstrate an in vitro approach that recapitulates development of even this most complex organ, which can be used to gain insights into disease mechanisms.

SPEAKER ABSTRACTS



YIN YANG 1 REGULATES CEREBRAL CORTEX DEVELOPMENT IN A DEVELOPMENTAL STAGE-SPECIFIC MANNER

ZURKIRCHEN, LUIS, Varum, Sandra, Giger, Sonja, Klug, Annika, Sommer, Lukas
Institute of Anatomy, University of Zurich, Zurich, Switzerland

The development of the mammalian dorsal cortex is driven by a complex network of signaling pathways and transcriptional regulators. Neural progenitor cell (NPC) proliferation, fate decisions and survival need to be tightly controlled to ensure proper cortex development. The transcriptional regulator Yin Yang 1 (Yy1) has been shown to have context-dependent effects on these processes during the development and homeostasis of many tissues. However, the role of Yy1 during early cortical development has not been addressed yet. To investigate whether Yy1 regulates cortex development, we specifically ablated Yy1 in the dorsal cortex by crossing *Emx1Cre* mice to mice carrying Yy1 floxed alleles. Interestingly, ablation of Yy1 before the onset of embryonic neurogenesis resulted in microencephaly. Embryos lacking Yy1 exhibited decreased cortical size and thickness due to the depletion of the NPC pool at early stages of corticogenesis. Loss of Yy1 led to impaired proliferation and transient G1/S cell cycle arrest at embryonic day E12.5. In addition, Yy1 ablation transiently increased p53-dependent cell death at E12.5. Despite its constitutive expression during corticogenesis in NPCs and immature neurons, our data showed that Yy1 is only necessary during a specific time window of early cortex development. Conditional ablation of Yy1 at the onset of neurogenesis did not impair cortical development. To investigate the underlying molecular mechanisms we performed genome-wide expression analysis comparing control versus Yy1 mutant cortices. Intriguingly, genes associated with cellular metabolism were enriched among the differentially expressed genes. In summary, our results reveal a novel, developmental stage-dependent role for the transcriptional regulator Yy1 during cortical development. Yy1 is necessary to maintain proliferation and survival of NPCs at early stages of corticogenesis, possibly by regulating various metabolic pathways.

GENERATION OF COMPLEX TISSUE STRUCTURE IN STEM CELL CULTURE

EIRAKU, MOTOTSUGU
Center for Developmental Biology, RIKEN, Kobe, Japan
In vitro generation of a functional organ with complex

structures is a major challenge of cell biology. In recent decade, there has been an increasing interest in 3D tissue formation from stem cells. For example, stem cell researchers have demonstrated that embryonic stem (ES) cells or induced pluripotent stem (iPS) cells could steer to differentiate into 3D tissues, such as brain, retina, inner ear, liver, kidney and stomach. These 3D stem cell culture technologies are expected to contribute to a future regenerative medicine and drug discovery. We previously demonstrated that mouse ES cells self-formed apico-basally polarized cortical tissues using an efficient 3D aggregation culture. We also reported about self-organized formation of optic cup (retinal primordia) and a stratified neural retina from mouse ES cells. Using two-photon live imaging analysis and measurement of mechanical properties in developing retinal tissue, we revealed that the optic-cup morphogenesis can spontaneously occur in a three-dimensional stem-cell culture even in the absence of external forces, and that retinal progenitors have a latent intrinsic order to generate the optic-cup structure. In addition, we have attempted to apply these mouse ES cell culture to human ES cells. Thus, we have mainly focused on the 3D tissue generations from mouse and human pluripotent stem cells and endeavored to understand the molecular and cellular mechanisms underlying self-organization phenomena in neural development. I will also talk about our recent studies and a future direction of an *in vitro* histogenesis.

MODELING PERIVENTRICULAR HETEROTOPIA IN HUMAN CEREBRAL ORGANIDS WITH MUTATIONS IN FAT4 AND DCHS1

Klaus, Johannes¹, Rusha, Ejona², Drukker, Micha², Robertson, Stephen³, Götz, Magdalena², **CAPPELLO, SILVIA**¹

¹Max Planck Institute of Psychiatry, Munich, Germany, ²Helmholtz Center Munich, Munich, Germany, ³University of Otago, Dunedin, New Zealand

Malformations of the human neocortex are present in about 1% of the general population and represent a major cause of developmental disabilities including severe epilepsy. To date, mouse lines carrying mutations of genes so far identified in human patients with cortical malformations only partially recapitulate the expected cortical phenotypes and therefore do not provide reliable models to entirely understand the molecular and cellular mechanisms responsible for these disorders. We have recently shown that mutations in the cadherin pair FAT4 or DCHS1 lead to periventricular heterotopia in humans, with neurons ectopically located at the ventricular surfaces of the brain. Acute downregulation of Fat4 or Dchs1 in the developing mouse cortex results in increase of proliferation and a concomitant decrease of differentiation of progenitors due to deregulation

SPEAKER ABSTRACTS

of the transcriptional regulator Yap. In the present study we model the neuronal heterotopia of human patients using induced pluripotent stem cells (iPSC) and cerebral organoids. Our results show that we can reproduce the cortical heterotopia in cerebral organoids, validating this model as excellent system to study these disorders. Furthermore, mutations in FAT4 or DCHS1 lead to changes in the cytoskeleton and in the number or localization of the centrosomes and cilia in neural stem cells and migrating neurons suggesting the cilium to be central in maintaining the polarity of NSCs and neurons. Taken together these results show that we can model human brain development and neuronal migration disorders using cerebral organoids and contribute to open new avenues in order to bridge the gap of knowledge between human brain malformations and existing mouse models.

TUESDAY, 2 FEBRUARY
8:00 - 8:30

COMPANY PRESENTATION

PHYSIOLOGICALLY RELEVANT NEURAL MODELS FOR DISCOVERY AND SCREENING: STEM CELL DERIVED SYSTEMS FROM THERMO FISHER SCIENTIFIC

DAVID KUNINGER,
Thermo Fisher Scientific

The presentation will cover newly developed products and ongoing research focused on derivation of specific neural cell types from human pluripotent stem cells. Systems to create bone fide, mid-brain floor derived Dopaminergic Neurons and ventral, forebrain GABAergic neurons will be described. Functional studies and the application of these cells in discovery and screening studies will be discussed. Additional tools for generation and study of more generalized neural populations will also be presented.

TUESDAY, 2 FEBRUARY
9:00 - 12:00

SESSION II: MODELING DISORDERS

INSIGHTS INTO ALZHEIMER'S DISEASE PATHOGENESIS FROM HUMAN STEM CELL SYSTEMS

LIVESEY, FREDERICK J.,
The Gurdon Institute, Cambridge, UK

The ability to differentiate human embryonic and induced pluripotent stem cells to human cortical neurons and neuronal networks permits the study of Alzheimer's disease mutations in the correct cellular and genetic contexts. We have used these systems as a platform to study the cell and molecular biology of Alzheimer's disease pathogenesis, starting from defined genetic changes associated with inherited forms of the disease. This seminar will report the intracellular and extracellular disease associated pathways that we have identified using these approaches, and their implications for the development of therapeutics.

IN VITRO MODELING OF NEUROINFLAMMATION USING NEURAL STEM CELLS DERIVED FROM CD34 CELLS FROM PATIENTS WITH UNDIAGNOSED RARE DISEASES

WANG, TONGGUANG, Medynets, Marie, Nath, Avindra
Translational Neuroscience Center, National Institute of Neurological Disorders and Stroke, Bethesda, MD, USA

Neurological complications are among the most frequently observed aspects in patients with mysterious conditions that have long eluded diagnosis. Although T cells involved neuroinflammation could be a mechanism underlying those neurological symptoms, it is difficult to confirm or study due to the difficulties in obtaining brain samples and the short of in vitro models. Recent advances in the field of cell transformation provide unique opportunities for in vitro human disorder modeling, such as using patient derived neurons for the study of neurological disorders. To delineate the roles of inflammatory cells and neuronal cells in mediating neuroinflammatory aspect of some of the undiagnosed diseases, we created mixed cultures consisting of autologous or heterologous inflammatory cells and neuronal cells derived from patient peripheral blood samples. We optimized conditions of PBMC separation and CD34 cells purification, and further generated induced neural stem cells (iNSC) by transfecting CD34 cells with Sendai virus vectors containing Yamanaka factors with the help of selective media. The neural stem cells-derived live neuronal cells were further labeled with fluorescent CM-Dil and cultured with PBMC or T cells labeled with CF-DA. By using this model, we found that autologous T cells-induced significant toxicity in neural cells derived from a patient with a rare atlastin-1 (ALT1) gene mutation compared to T cells from a normal donor. Further study also found

SPEAKER ABSTRACTS



that PBMCs from the patient also caused significant neurotoxicity in neural cells derived from CD34 cells from normal donor. These results indicated that inflammatory cells purified from the patient with ALT1 gene mutation were activated and neurotoxic. And the in vitro neuroinflammatory model is useful to delineate the mechanisms underlying the neurological symptoms of patients with undiagnosed diseases.

MODELING NEUROLOGICAL DISEASES USING PATIENT IPSCS

MING, GUO-LI

Institute for Cell Engineering, Johns Hopkins University, Baltimore, MD, USA

Schizophrenia and affective disorders are chronic and generally disabling brain disorders with a prominent genetic basis and with neurodevelopmental origin. A number of susceptibility genes have been identified, including DISC1, neuregulin, COMT, FEZ1. How dysfunction of these genes leads to aberrant neural development and contribute to the pathology of the disorder is largely unknown. DISC1 is by far the best-characterized risk genes for schizophrenia and other major mental disorders, and almost nothing is known about its function in human neural development. To understand how mutation of DISC1 gene in patients impacts the development of human neurons, we generated iPSC lines from multiple patients from one family with a DISC1 mutation and differentiated these iPSCs into forebrain neurons in high efficiency. We have identified critical roles of DISC1 in morphological developmental and synaptic development of human neurons derived from patient specific iPSCs.

MONIKA EHRHARDT-BORNSTEIN MEMORIAL LECTURE

STEM CELL HETEROGENEITY IN HOMEOSTASIS AND DISEASE

MARTIN-VILLALBA, ANA

German Cancer Research Center (DKFZ), Heidelberg, Germany

Adult mammalian neurogenesis is best characterized in the neurogenic niche of the subventricular zone of the lateral ventricles and the subgranular zone of the dentate gyrus. The process of neurogenesis includes activation of neural stem cell to generate neurogenic progenitors, and the subsequent progenitor's differentiation into distinct neuronal subtypes, and functional integration into the network. This can be influenced by signals present within the

microenvironment of the niche that are regulated by environmental factors such as physical exercise, mood disorders, maternity, and others. Several pathways are known to regulate multiple aspects of adult neurogenesis at the cellular and functional level. However, the study of heterogeneity in the response of neural stem cells remains to be addressed. In my talk I will discuss what we have learned about neural stem cell heterogeneity in the adult brain during homeostasis in the young and old brain and following injury.

PATIENT-DERIVED IPSC THREE-DIMENSIONAL OPTIC CUPS TO TEST DISEASE MECHANISMS AND RNA THERAPY FOR INHERITED BLINDNESS

PARFITT, DAVID¹, Lane, Amelia¹, Ramsden, Conor¹, Carr, Amanda¹, Munro, Peter¹, Jovanovic, Katarina¹, Schwarz, Nele¹, Kanuga, Naheed¹, Muthiah, Manickam¹, Hull, Sarah¹, Gallo, Jean-Marc², Da Cruz, Lyndon¹, Moore, Anthony³, Hardcastle, Alison¹, Coffey, Peter¹, Cheetham, Michael¹

¹UCL Institute of Ophthalmology, London, UK, ²Institute of Psychiatry, Kings College London, London, UK, ³Ophthalmology, UCSF School of Medicine, San Francisco, CA, USA

Leber congenital amaurosis (LCA) is a severe inherited retinal degeneration. The most common LCA mutation is a deep intronic change (c.2991+1665A>G) in *CEP290* that leads to aberrant splicing, inclusion of a cryptic exon and premature stop codon. CEP290 is known to be involved in ciliogenesis; patient cells with the deep intronic mutation have shorter and less frequent cilia. Currently, there are few therapeutic options available for LCA; however, antisense oligonucleotides (AONs) are a promising area of interest. Here, we have used induced pluripotent stem cells (iPSCs) reprogrammed from fibroblasts from a patient homozygous for the deep intronic mutation and used established protocols to differentiate them into retinal pigment epithelia (RPE) cells and three-dimensional organoid optic cups to probe disease mechanisms and test a potential AON therapy. Patient-derived iPSCs, RPE and 3D optic cups showed abnormal splicing and cilia defects, recapitulating the patient-derived fibroblast phenotype, but this did not appear to affect RPE or optic cup differentiation. An antisense-morpholino AON effectively blocked cryptic exon formation and restored the expression of full-length CEP290 in patient-derived fibroblasts, RPE and optic cups. This rescued cilia defects and reinstated cilia protein traffic, highlighting the potential of this approach for the treatment of LCA.

SPEAKER ABSTRACTS

PROBING THE SECRETS OF ALZHEIMER'S DISEASE WITH PLURIPOTENT STEM CELL TECHNOLOGY

GOLDSTEIN, LAWRENCE S.B

University of California San Diego, La Jolla, CA, USA

Our goal is to understand and then to develop effective therapies for Alzheimer's disease. To accomplish this goal we use neurons derived from induced pluripotent stem (iPS) cells carrying hereditary mutations causing early onset familial Alzheimer's disease (FAD). Specifically, rather than generating iPS lines from affected patients, we are using site directed mutagenesis to introduce defined FAD mutations in an isogenic background using iPS lines derived from the sequenced genome of J Craig Venter. Our analyses of these site directed mutants in the APP and PS1 genes thus far reveals that these mutations reduce the rate of trafficking of a key endosomal intermediate, which is required for a neuron specific transport event. This neuron-specific transport event is transcytosis from the somatodendritic compartment to the axonal compartment of the amyloid precursor protein and cholesterol containing lipoproteins needed for axonal growth and synaptic maintenance. We are also using iPS lines to dissect the function of loci identified in genome wide association studies using this iPS model.

TUESDAY, 2 FEBRUARY
13:45 - 14:15

COMPANY PRESENTATION

HIGH THROUGHPUT SINGLE CELL SORTING FOR GENOMICS

UWE SPECK,

BD Biosciences

The new BD FACSSeq™ cell sorter was developed to give researchers a fast and easy to use platform for single cell genomics and next generation sequencing (NGS). This affordable new machine has a dramatically simplified workflow: Technical innovation allow an almost fully automated setup and truly walk-away operation. Single cell may be deposited into 96-well PCR plates in less than 30 seconds with yield of > 95%. In combination with the BD Precise™ assay we offer a streamlined workflow for the first targeted RNA-seq platform capable of absolute and direct molecular counting of transcripts.

TUESDAY, 2 FEBRUARY
14:30 - 15:45

SESSION III: MODELING REGENERATION

TRANSCRIPTOME ANALYSIS IDENTIFIES THE CALCIUM CHANNEL SUBUNIT ALPHA2DELTA2 AS A KEY REGULATOR OF AXON REGENERATION

BRADKE, FRANK

German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany

Injuries to the adult central nervous system (CNS) often result in permanent disabilities because neurons lose the ability to regenerate their axon. Here, whole transcriptome sequencing and bioinformatics analysis followed by gain- and loss-of-function experiments identified *Cacna2d2*, the gene encoding the Alpha2delta2 subunit of voltage gated calcium channels, as a developmental switch that limits axon growth and regeneration. *Cacna2d2* gene deletion or silencing promoted axon growth in vitro. In vivo, Alpha2delta2 pharmacological blockade through Pregabalin (PGB) administration enhanced axon regeneration in adult mice after spinal cord injury. As PGB is already an established treatment for a wide range of neurological disorders, our findings suggest that this drug could be employed as a therapy to promote structural plasticity and regeneration following CNS trauma.

CELLULAR AND MOLECULAR CHANGES IN ADULT NEURAL STEM CELLS ALLOWING SUCCESSFUL REGENERATION IN THE ADULT ZEBRAFISH BRAIN

Barbosa, Joana, Sanchez-Gonzalez, Rosario, Di Giaimo, Rossella, Götz, Magdalena, **NINKOVIC, JOVICA**
Helmholtz Zentrum München, Neuherberg/ Munich, Germany

In the zebrafish brain, adult neural stem cells (aNSCs) exist not only in a more wide-spread manner as compared to mammals, but also react to injury by regenerating neurons. Indeed, after stab wound injury the cellular architecture is restored, including neurogenesis and recruitment of new neurons to the injury site. As aNSCs are positioned close to the brain surface, the behavior of aNSCs in the intact brain and during regeneration can be examined non-invasively by live *in vivo* imaging. We used repetitive imaging to follow single aNSCs in the intact and injured adult zebrafish telencephalon *in vivo* and found that neurons are generated by direct conversion of aNSCs into post-mitotic neurons, by

SPEAKER ABSTRACTS



asymmetric divisions or via intermediate progenitors amplifying the neuronal output. We also observed an imbalance of asymmetric and symmetric self-renewing divisions, depleting aNSCs over time. After brain injury, neuronal progenitors are recruited to the injury site and generate additional neurons. Our data suggest that several changes contribute to the response to injury: fewer stem cells remain quiescent compared to the intact brain and a pathway is added by which symmetric NSC division generates 2 intermediate progenitors. This new type of division observed only after injury gives rise to a larger neuronal progeny, but rapidly depletes the aNSC pool. The analysis of transcriptional changes in the aNSCs and their progeny revealed activation of the aryl-hydrocarbon receptor (AhR) signaling in a novel subset of aNSCs, characterized by the absence of PSA-NCAM expression in response to injury. Our data suggest that the activation of the AhR signaling is crucial for the change of the type of aNSC division and recruitment of aNSCs to the injury site, as observed also by live imaging. Comparing this pathway to mammalian brain injury in mice, we discovered that the activation of AhR signaling in reactive astrocytes after stab wound injury in the cerebral cortex enhances their de-differentiation and gain of the aNSC characteristics, as measured in the neurosphere assay. Our data imply conserved pathways in radial glia and reactive astrocyte reaction in zebrafish and mice and suggest that the increase in neurogenesis after injury comes at the expense of depletion of aNSCs, perhaps drawing on quiescent NSCs.

CELLULAR PLASTICITY IN THE INTACT AND INJURED CENTRAL NERVOUS SYSTEM

FRISÉN, JONAS

Department of Cell and Molecular Biology, Karolinska Institute, Stockholm, Sweden

The central nervous system was traditionally considered static, with little exchange of cells. Today we know that there are neural stem cells in both the brain and spinal cord. Most of these stem cells are quiescent and do not produce new cells under physiological conditions. Neurons are, however, continuously added throughout life in two discrete structures in the adult brain, the hippocampus and olfactory bulb, in most mammals. The generation of new neurons in the adult brain serves to maintain a pool of neurons with unique properties, present for a limited time after their birth, which enable specific types of neural processing. We have taken advantage of the massive increase in atmospheric ^{14}C by nuclear bomb testing during the cold war to birth date neurons, which has revealed a unique pattern of adult neurogenesis in humans. I will also describe how quiescent neural stem cells contribute to repair after central nervous system injuries.

WEDNESDAY, 3 FEBRUARY
9:00 - 12:00

SESSION III: MODELING REGENERATION

REGENERATING CNS MYELIN - FROM MECHANISMS TO MEDICINES

FRANKLIN, ROBIN

Wellcome Trust-MRC Cambridge Stem Cell Institute, University of Cambridge, Cambridge, UK

Remyelination, the process by which new myelin sheaths are restored to demyelinated axons, represents one of the most compelling examples of adult multipotent stem cells contributing to regeneration of the injured CNS. This process can occur with remarkable efficiency in multiple sclerosis (MS), and in experimental models, revealing an impressive ability of the adult CNS to repair itself. However, the inconsistency of remyelination in MS, and the loss of axonal integrity that results from its failure, makes enhancement of remyelination an important therapeutic objective. There is now compelling evidence that ageing is the major contributor to the declining efficiency of remyelination and that this is largely due to a failure of stem cell differentiation. This talk will review recent studies we have undertaken aimed at obtaining a detailed understanding of the mechanisms of regulating differentiation during remyelination and hence identifying novel therapeutic targets.

HUMAN GLIAL PROGENITOR CELL-BASED TREATMENT AND MODELING OF NEUROLOGICAL DISEASE

GOLDMAN, STEVEN A.

University of Copenhagen Faculty of Medicine, Copenhagen, Denmark

The most abundant precursor cells of the adult human brain are glial progenitor cells, which can give rise to both astrocytes and oligodendrocytes. As a result, diseases of glia may provide readily accessible targets for cell-based therapies. The myelin diseases, which involve the loss or dysfunction of oligodendrocytes, are among the most prevalent and disabling conditions in neurology, and may be particularly appropriate targets for progenitor cell-based therapy. This talk will focus on the potential utility of human glial and oligodendrocyte progenitor cell transplantation as a means of treating both congenital and acquired diseases of myelin. It will cover potential sources of both tissue and stem

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cell-derived glial progenitor cells, as well as the use of human hESC and iPSC-derived glial progenitors in myelin repair. The talk will also include a description of the glial chimeric mice that result from the neonatal implantation of human glial progenitors into the mouse brain. In these mice, the human glial progenitors out-compete their murine counterparts to eventually dominate the glial population of the recipient brains. Human glial chimerization has significant effects on neurophysiology and behavior, which suggest the importance of human-specific glial attributes to neural network function, and thus the potential for glial contributions to human-specific neurodegenerative and psychiatric disorders. By generating human glial chimeric mice using hiPSC-derived glial progenitors, we may now investigate the phenotype-selective role of glia in human brain disease, by producing patient-derived and disease-specific human glial chimeras. These mice provide us a new model system within which to study not only the myelin disorders, but the entire range of neurodegenerative and neuropsychiatric diseases in which glia may causally participate.

TOWARDS A STEM CELL BASED THERAPY FOR PARKINSON'S DISEASE

PARMAR, MALIN

Lund University, Lund, Sweden

Considerable progress has been made in generating fully functional and transplantable dopamine neurons from human embryonic stem cells (hESCs). Before these cells can be used for cell replacement therapy in Parkinson's disease (PD), it is important to verify their functional properties and efficacy in animal models. We have performed a comprehensive preclinical assessment of hESC-derived midbrain dopamine neurons in a rat model of PD. We show long-term survival and functionality using clinically relevant MRI and PET imaging techniques, and demonstrate efficacy in restoration of motor function with a potency comparable to that seen with human fetal dopamine neurons. Furthermore, we show that hESC-derived dopamine neurons can project sufficiently long distances for use in humans, integrate into the host brain circuitry, fully regenerate midbrain-to-forebrain projections, and innervate correct target structures. This provides strong preclinical support for translation of hESC-derived dopamine neurons using approaches similar to those established with fetal cells for treatment of Parkinson's disease. In preparation for the first clinical trial, we have established a fully GMP compliant differentiation protocol that results in differentiation of authentic and functional DA neurons after transplantation for a number of GMP hESC lines. Additionally, we are establishing a set of experimentally validated predictive markers correlating

to successful graft outcome by performing unbiased, retrospective RNA sequencing analysis of previously transplanted cells (30 different batches of hESC-derived mesDA progenitors, which have all been transplanted in the period of 2010-2015). By harmonising our predictive markers with other members of GForce-PD, we aim to jointly establish a gene expression analysis that will provide a direct comparison between the cell products at the different centres, which ultimately will provide a better ability to compare the outcome of such trials

WEDNESDAY, 3 FEBRUARY
14:30 - 17:00

SESSION IV: ROAD TO THE CLINIC

HUMAN PLURIPOTENT-DERIVED NEURONS FOR ADDRESSING DISORDERS OF THE GUT, MUSCLE, AND BRAIN

STUDER, LORENZ

Memorial Sloan-Kettering Institute for Cancer Research, New York, NY, USA

My presentation will discuss the derivation of specific CNS and PNS neurons in vitro from human embryonic and induced pluripotent stem cells. In particular, I will present new data on modeling and treating Hirschsprung's disease by recreating human enteric neuron lineages in vitro for disease modeling and for transplantation studies aimed at repopulating the postnatal colon in vivo. Another example of using human pluripotent stem cells for modeling neural disease include the establishment of an all-human functional assay to probe neuromuscular junction properties and to model related disorders. Finally, I will discuss our ongoing efforts to translate human pluripotent stem cell-based therapies for the treatment of Parkinson's disease, towards a first effort to harness the therapeutic potential of pluripotent stem cell-derived neurons in human patients.

IMPLICATIONS OF DISRUPTED AUTOPHAGY ON CHOLESTEROL TRAFFICKING, NEURONAL SURVIVAL AND STRATEGIES FOR DRUG DEVELOPMENT IN NPC1

ORDONEZ, PAULINA¹, Cabebe, Laura² Steele, John³, Goldstein, Lawrence³

¹Department of Pediatrics, University of California San Diego, La Jolla, CA, USA, ²Department of Biological Sciences, California State University San Marcos, San Diego, CA, USA, ³Cellular and Molecular Medicine,

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University of California San Diego, La Jolla, CA, USA

Niemann Pick type C1 (NPC1) is a pediatric dementia caused by mutations of the lysosomal cholesterol transporter NPC1. NPC1 has no cure or treatment, and affected patients die in their childhood or teenage years. Although rare, the disease imposes a burden on patients and families that is disproportionate to its relative infrequency, furthermore, NPC1 shares clinical and histologic features with Alzheimer's disease (AD), a more prevalent public health problem, suggesting common mechanisms of onset and progression. Successful development of effective therapeutic interventions for NPC1 and related neurodegenerative disorders will require a deeper understanding of mechanisms of disease initiation and progression. We used reprogramming technology to develop sets of NPC1 and control human induced pluripotent stem cell (hiPSC) lines, and we systematically generated patient-specific pure neuronal cultures using a standard differentiation protocol. We found that NPC1 neurons have disrupted mitochondrial turnover by autophagy that leads to mitochondrial depolarization and increased production of reactive oxygen species, all of which are likely to contribute to the neuronal failure observed in NPC1. Our data also raise the important and new possibility that NPC1 neurons initially survive cholesterol accumulation because they activate autophagy. We have evidence that in NPC1 mutant neurons, autophagy may function as a backup pathway that releases and distributes trapped cholesterol, albeit at lower efficiency, but sufficient to protect neuronal viability until birth and perhaps for a few additional years. Further mechanistic studies lead us to identify a potential new transporter that mediates autophagy-dependent cholesterol efflux from the late endosomal compartment. Our data highlight the central role that autophagy disruption plays in the selective neuronal failure observed in NPC1. Additionally, our approach establishes an hiPSC-based platform for the high-throughput screening of potential therapeutic compounds that can revert mitochondrial dysfunction while preserving bulk autophagy in NPC1 and related neurodegenerative diseases.

STEM CELLS IN THE ADULT HUMAN RETINAL PIGMENT EPITHELIUM AND THEIR THERAPEUTIC POTENTIAL

TEMPLE, SALLY

Neural Stem Cell Institute, Rensselaer, NY, USA

The retinal pigment epithelium (RPE) is a monolayer of central nervous system cells that provides essential support for the photoreceptors of the retina. The RPE degenerates in diseases such as age-related macular degeneration (AMD), leading to cell death in the macula, the center of the retina, and central vision loss. AMD is

the leading cause of vision impairment in the elderly, affecting approximately 1 in 5 people over age 70, and millions of patients globally. There are currently no disease-altering therapies for AMD, a significant unmet medical need. We recently discovered that the adult human RPE contains a subset of cells that are capable of extensive self-renewal and multi-lineage differentiation. These retinal pigment epithelial stem cells (RPESCs) offer several new therapeutic avenues for retinal repair and regeneration. Current studies include preclinical work to develop a transplantation therapy: RPESC are obtained from cadaver eyes (already donated to eye banks worldwide for corneal transplantation), and cultured into clinical grade cells that are being tested for their ability to rescue vision in animal models of retinal degeneration after sub-retinal transplantation. In addition, as RPESCs are present in the eyes of patients, we are determining ways to safely activate them to encourage endogenous repair. To this end, we are exploring molecules that control RPESC proliferative properties and factors regulating their plasticity.

OPCS DERIVED FROM HUNTINGTON DISEASE-DERIVED HESCS EXHIBIT A SOX10 AND MYRF DISRUPTION-ASSOCIATED SUPPRESSION OF OLIGODENDROCYTE DEVELOPMENT AND MYELINOGENESIS

LAMPP, ANDREA C.¹, Osipovitch, Mikhail¹, Benraiss, Abdellatif², Burm, Hayley², Chandler-Militello, Devin², Cornwell, Adam², Li, Xiaojie², Wang, Su², Windrem, Martha², Zou, Lisa², Goldman, Steve A.¹

¹University of Copenhagen, Copenhagen, Denmark, ²University of Rochester, Rochester, NY, USA

Huntington's Disease (HD) is characterized by striatal and cortical neuronal degeneration, but it is also associated with myelin loss, potentially reflecting dysfunction or failed replacement of myelin-producing oligodendrocytes. Nonetheless, the role of oligodendroglial pathology in HD has not been well explored. In preliminary experiments, we have generated oligodendrocyte progenitor cells (OPCs) from human embryonic stem cells, derived from either huntingtin (mHTT)-mutant embryos or controls, used fluorescence-activated cell sorting to isolate these cells, and performed RNA sequence analysis (RNAseq) on their extracted mRNAs to assess mHTT-dependent changes in gene expression in human oligodendrocyte progenitors. We found that a coherent set of key transcription factors associated with oligodendroglial differentiation from OPCs, and with myelin biosynthesis, were significantly, and often severely, down-regulated as a function of mHTT expression. Most notably, these included MYRF, Myelin-Regulatory Factor, a transcription factor that coordinately activates a number of genes

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necessary for myelin formation, including MBP, MAG, OMG, PLP1 and MOG, all of which were concurrently and significantly down-regulated. MYRF itself is driven by the early oligodendroglial regulators NKX2-2, OLIG2 and SOX10, all of which were sharply down-regulated in mHTT-expressing OPCs (fold change > 2.00: corrected $p < 0.01$). These data suggest that the hypomyelination and myelin loss of HD patients result from the mHTT-dependent suppression of NKX2.2, OLIG2 and SOX10, which results in MYRF inhibition and a consequent failure to transcribe critical mRNAs associated with myelinogenesis. Our data confirm a recent report of MYRF inactivation in PLP-150Q mice (Huang B. et al., *Neuron*, 2015), and extend that observation by confirming this process in human OPCs as well as mouse, and by identifying a host of upstream regulators of MYRF also suppressed by mHTT. Together, these data suggest that myelin failure in HD is a product of an mHTT-dependent block in oligodendrocytic differentiation by affected OPCs, followed by the serial failure of those oligodendrocytes that do develop to express sufficient MYRF to permit myelin biogenesis.

STEMMING VISION LOSS USING HUMAN EMBRYONIC STEM CELLS

COFFEY, PETER

*University College London Institute of Ophthalmology,
London, UK*

The London Project to Cure Blindness was launched by Prof Pete Coffey at the UCL Institute of Ophthalmology in June 2007, and aims to make the most of human embryonic stem cells to prevent blindness and restore sight in patients with Age-related Macular Degeneration (AMD). Our goal is to replace cells essential for "seeing" lost by disease at the back of the eye. We aim to repair and regenerate the aged diseased eye using human embryonic stem cells which have been transformed into the cells affected in AMD: the support cells for the photoreceptors (retinal pigment epithelium) and the photoreceptors. The cells will be surgically implanted into a clinical population of AMD patients.



POSTER ABSTRACTS

POSTER SESSION I

Note: Abstracts for Poster Session II (even-numbered posters) can be found on page 60

001

CELLULAR MODEL OF MERTK- ASSOCIATED RETINAL DYSTROPHY

Lukovic, Dunja¹, Artero Castro, Ana¹, **ERCEG, SLAVEN²**
¹*Stem Cell Therapies in Neurodegenerative Diseases, Research Center, Valencia, Spain,* ²*Stem Cell Therapies in Neurodegenerative Diseases, National Stem Cell Bank-Valencia Node, ISCIII, Research Center, Valencia, Spain*

Retinitis pigmentosa (RP) represents a genetically heterogeneous group of retinal dystrophies affecting mainly the rod photoreceptors and in some instances also the retinal pigment epithelium (RPE) cells of the retina. Clinical symptoms and disease progression leading to moderate to severe loss of vision are well established and despite significant progress in the identification of causative genes, the disease pathology remains unclear. Lack of this understanding has so far hindered development of effective therapies. Here we report successful generation of human induced pluripotent stem cells (iPSC) from skin fibroblasts of a patient harboring a novel Ser331Cysfs*5 mutation in the MERTK gene. The patient was diagnosed with an early onset and severe form of autosomal recessive RP (arRP). Upon differentiation of these iPSC towards RPE, patient-specific RPE cells exhibited defective phagocytosis, a characteristic phenotype of MERTK deficiency observed in human patients and animal models. Thus we have created a faithful cellular model of arRP incorporating the human genetic background which will allow us to investigate in detail the disease mechanism, explore screening of a variety of therapeutic compounds/reagents and design either combined cell and gene-based therapies or independent approaches.

003

NEW MODEL FOR NEURAL REGENERATION. 3-DIMENSIONAL DORSAL ROOT GANGLIA EXPLANTS FOR VISUALIZATION OF NEURAL CELL MIGRATION, AXON GUIDANCE AND BRANCHING

Semina, Ekaterina V., **RUBINA, KSENIYA A.**, Rysenkova, Karina D., Sysoeva, Veronika Yu, Tkachuk, Vsevolod V.
Faculty of Medicine, Lomonosov Moscow State University, Moscow, Russian Federation

The protocol describes isolation, culturing and immunofluorescent (IF) staining of axons in 3-dimensional ex vivo culture of dorsal root ganglion (DRG) explants in Matrigel (MG). Unlike other methods,

this approach can be used to evaluate axonal growth, navigation and neural cell migration in a 3D conditions. The method also allows to assess the impact of different agents on the growth and branching of neurites as well as cell migration - basic processes, reflecting regeneration of nerve tissue. To evaluate the rate of neurite elongation we used darkfield light microscopy; cell migration, axonal direction and branching were assessed using IF staining with confocal microscopy imaging. For experiments, mice were anesthetized and DRG were isolated under aseptic conditions using Olympus SZX16 microscope, placed in chambered coverglass (Lab-Tek), and covered with the drop of MG. Explants were cultured in sterile humidified incubator. Life imaging was performed using light microscopy (Zeiss Axiovert 2000) at low magnification for 2 weeks. Within 2 weeks neurite length and the number of migrating cells was evaluated using MetaMorph software (Meta Imaging Series). For staining samples were fixed, permeabilized and stained with 1st (NF200 for mature axon) and 2nd (AlexaFluor 594) antibodies, nuclei were counterstained by DAPI. Images were acquired by confocal laser scanning microscopy (TCS SP5, Leica) equipped with a Plan-Apo x10, 1.40 NA objective and 543-Argon laser. We tested the impact of exogenously added urokinase (uPA at 10 ng/ml) and the effect of antibody specifically blocking urokinase receptor (anti-uPAR) on the outgrowth and branching of axons, spontaneous cell migration (the rate and number of migrating cells) from DRG into MG. uPA administration significantly increased both, neurite outgrowth and cell migration. uPA stimulated axonal elongation (1.5 times longer compared to control, $p < 0.05$) and activated spontaneous cell migration (2 times more compared to control, $p < 0.05$). Blocking of uPA receptor resulted in increased axon branching and abnormal axonal growth trajectory.

005

PERTURBATION OF NUCLEOLAR ACTIVITY IN NEURAL PROGENITORS AND NEURONS DERIVED FROM ALZHEIMERS PATIENTS CARRYING PSEN1 MUTATIONS

Pires, Carlota¹, Turnbull, Hannah², Tubsuwan, Alisa³, Freude, Kristine¹, Nielsen, Jørgen⁴, Holst, Bjørn⁵, Hyttel, Poul¹, **HALL, VANESSA J.**¹

¹*University of Copenhagen, Fredriksberg C, Denmark,* ²*Plymouth University, Plymouth, UK,* ³*Mahidol University, Nakhon Pathom, Thailand,* ⁴*Rigshospitalet, Copenhagen, Denmark,* ⁵*Bioneer, Hørsholm, Denmark*

A large cohort of Alzheimer's disease (AD) patients carry a mutation in the presenilin 1 (*PSEN1*) gene and have a severe form of the disease. Mutations in *PSEN1* lead to a disruption in transmembrane protein cleavage and accumulation of toxic forms of Ab. Here we studied how defects in *PSEN1* might affect other key processes, such

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as RNA synthesis. We evaluated neural cells derived from patient-specific induced pluripotent stem cells (iPSCs) established from an individual carrying the *PSEN1* mutation, P150L. Two iPSC clones (PSEN1-iPSCs) were compared to healthy iPSC clones (CTRL-iPSCs). The iPSCs were differentiated into cortical neurons (CNs) using an established protocol for 72 days and were analyzed both at 24 days post differentiation (dpd) (i.e. neural progenitors (NPs) that expressed *SOX2*, *NES*, *VIM*) and at 72 dpd. The PSEN1-iPSC NPs expressed significantly higher levels of *NCL* at 24 dpd compared to the CTRL-iPSC NPs. In addition, the ultrastructure of 60 nucleoli from both the PSEN1-iPSC NPs and the CTRL-iPSC NPs revealed that the PSEN1-iPSC NPs had significantly larger nucleoli. Furthermore, all the inner nucleolar components, including the fibrillar centres, the dense fibrillar component, the granular component, and the nucleolus-associated chromatin areas were found to be significantly larger in the PSEN1-iPSC NPs. Expression of the rRNA genes *5.8S*, *28S* (forming part of the large subunit) and *18S* (forming part of the small subunit) were not significantly different to CTRL-iPSC NPs suggesting these genes do not contribute to the observed phenotype. In contrast, the PSEN1-iPSC CNs had significantly decreased *NCL* as well as decreased expression of *5.8S*, *28S* and *18S* ribosomal RNA compared to the CTRL-iPSC CNs, revealing that the RNA synthesis declined considerably from the NP stage to the CN stage and was significantly reduced compared to the CTRL-iPSC CNs. The discovery that *PSEN1* NPs have larger nucleoli and increased *NCL* is suggestive of increased RNA synthesis and therefore increased protein synthesis. Furthermore, significantly reduced *NCL* and decreased expression of both ribosomal RNA subunits in the CNs indicates a reduction in rRNA biogenesis. The dysregulation of *NCL* and RNA synthesis warrants further attention in AD in order to determine how this impacts on both cellular health and disease progression.

007

EXTRACELLULAR ADENOSINE MODULATES POST-NATAL NEUROGENESIS

CAVALIERE, FABIO, Benito, Monica, Matute, Carlos
Department of Neuroscience, University of Basque Country, Achucarro Basque Center for Neurosciences and Centro de Investigación Biomedica en Red en Enfermedades Neurodegenerativas (CIBERNED), Zamudio, Spain

Toxicity of extracellular purines is among the factors inhibiting adult neurogenesis during neurodegenerative diseases. After neurodegeneration extracellular ATP and adenosine are released at high concentrations and alter the homeostasis and survival of glia and neurons. In this study we examined the effects of adenosine in modulating the cellular fate of post-natal neural stem cells from the rat subventricular zone in a in vitro

model of purinergic cyto-toxicity and in vivo after MCAO ischemia. We observed that high concentrations of adenosine (100 micromolar) promote astrogliogenesis at the expense of neurogenesis. Although all adenosine receptors (A1, A2a, A2b and A3) are expressed in these cells, we found that only A1 is involved in the inhibition of neuronal differentiation, as demonstrated by qRT-PCR, Western blot and specific gene silencing. In turn, we found that the mechanisms by which adenosine inhibits neuronal differentiation and sustains astrogliogenesis involves the release of IL10 and further activation of the Bmp2/SMAD3 pathway. In vitro data were confirmed in in vivo experiments using intracerebroventricular infusion of the A1 agonist CPA that showed a drastic reduction of neurogenesis and a parallel increase of astrogliogenesis in the olfactory bulb of adult rats. In contrast, blocking the A1r with the specific antagonist DPCPX during focal ischemia in adult mice, we observed a rescue of neurogenesis in the infarcted area accompanied by a reduction of newly generated astrocytes. These data further supports the idea that purinergic signaling contributes to the regulation of adult neurogenesis, especially in pathological conditions when purines are present at high concentrations in the extracellular space. Supported by Gobierno Vasco, MINECO and CIBERNED.

009

GENERATION OF INTEGRATION-FREE INDUCED NEURAL STEM CELLS FROM MOUSE FIBROBLASTS

KWAK, TAE HWAN¹, Kim, Sung Min², Kim, Kee-pyo³, Lim, Kyung Tae¹, Lee, Seung Chan¹, Kim, Jonghun¹, Lee, Hoon Taek², Schöler, Hans³, Han, Dong Wook¹
¹*Department of Stem Cell Biology, School of Medicine, Konkuk University, Seoul, South Korea* ²*Department of Animal Biotechnology, Konkuk University, Seoul, South Korea* ³*Max Planck Institute for Molecular Biomedicine, Münster, Germany*

The viral vector-mediated overexpression of the defined transcription factors, Brn4/Pou3f4, Sox2, Klf4, and c-Myc (BSKM), could induce the direct conversion of somatic fibroblasts into induced neural stem cells (iNSCs). However, viral vectors may be randomly integrated into the host genome thereby increasing the risk for undesired genotoxicity, mutagenesis, and tumor formation. Here we describe the generation of integration-free iNSCs from mouse fibroblasts by non-viral episomal vectors containing BSKM. The episomal vector-derived iNSCs (e-iNSCs) closely resemble control NSCs and iNSCs generated by retrovirus (r-iNSCs) in morphology, gene expression profile, epigenetic status, and self-renewal capacity. The e-iNSCs are functionally mature, as they could differentiate into all the neuronal cell types both in vitro and in vivo. Our study provides a novel concept for generating functional iNSCs using a non-viral, non-integrating, plasmid-based system that could facilitate their biomedical applicability.

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011

A MOLECULAR BLUEPRINT OF NEUROGENESIS FROM HUMAN STEM CELLS

BUSSKAMP, VOLKER¹, Ng, Alex HM², Kutsche, Lisa K.¹, Lewis, Nathan E.³, Church, George M.²

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Advances in cellular reprogramming and stem cell differentiation now enable *ex vivo* studies of human neuronal differentiation. Still, it remains challenging to elucidate the underlying regulatory programs because differentiation protocols are laborious and often result in low and heterogeneous neuron yields. Here, we overexpressed two Neurogenin transcription factors in human induced pluripotent stem cells, and obtained neurons with bipolar morphology within four days, at greater than 90% purity. The high purity enabled mRNA and microRNA expression profiling over the entire time course of neurogenesis. We applied systems biology tools to reveal the genetic programs involved in the rapid transition from stem cell to neuron. The resulting cells exhibited transcriptional, morphological and functional signatures of differentiated neurons, with greatest transcriptional similarity to prenatal human brain samples as correlated with the BrainSpan atlas by the Allen Institute for Brain Science. Our analysis revealed a network of key transcription factors and microRNAs that promoted loss of pluripotency and rapid neurogenesis via progenitor states. Expression level manipulations of crucial transcription factors by sh- and siRNAs as well as Cas9/CRISPR knockouts were used to validate the gene regulatory networks. These perturbations affected homogeneity and phenotypic properties of the resulting neurons but failed to disrupt neurogenesis. Interestingly, manipulations of the microRNA profiles did not result in similar phenotypes. Combinatorial expression of additional transcription factors has also shown to result in additional neuronal cell types. Our data suggests that a systems-level view of the molecular differentiation blueprints may direct manipulation of human stem cells to rapidly engineer diverse neuronal types.

013

COMPREHENSIVE HUMAN TRANSCRIPTION FACTOR SCREEN CONVERTS HIPSCS INTO THE THREE GERM LAYERS

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¹Harvard Medical School, Boston, MA, USA, ²Center for Regenerative Therapies Dresden, Dresden, Germany

Engineered organoids display remarkable similarities to native organs, but do not yet fully recapitulate cell

type composition and spatial organization. For cerebral organoids, endothelial cells and glial lineages are missing, and certain brain regions are randomly localized or absent. Therefore, to complement the differentiation cues that guide organoid self-organization, there is a need for targeted genetic control to specify cell type composition and localization within organoids. Our goal is to generate a catalogue of differentiated cell types from human induced pluripotent stem cells (hiPSCs) to be co-cultured within organoids. To achieve this, we aimed to identify transcription factors (TFs) that differentiate hiPSCs into a broad spectrum of cell types. First, we have assembled a complete human TF overexpression library (TFome) containing 1626 isoforms that represent 1522 unique TFs. To screen the TFome for TFs that promote differentiation, we established a multiplexed screening platform and identified 142 high-scoring candidates. These include well-characterized differentiation factors such as MyoD1, Ascl1, and NeuroG1, as well as many TFs not known to induce differentiation in hiPSCs. Using systems-level analyses, we found that these TFs are enriched for organ and tissue development and are implicated in the development of diverse lineages. To map which TFs differentiate hiPSCs into particular cell types, we adapted the multiplex platform to screen for the three germ layers, as well as neuronal, glial and endothelial lineages, which are often missing or mislocalized in cerebral organoids. Furthermore, we are constructing a catalogue of inducible single TF hiPSC lines for validation and extensive characterization. In summary, our TFome overexpression resource and multiplex screening platform allow us to build towards a cell types "parts list", which may be used to precisely supplement, control and improve engineered organoids.

015

ELECTROPHYSIOLOGY IN IPSC DISEASE MODELING: MICROELECTRODE ARRAY (MEA) AND AUTOMATED PATCH-CLAMP APPROACHES

DRAGICEVIC, ELENA¹, Becker, Nadine¹, Millard, Daniel², Clements, Isaac², Nicolini, Anthony², Stoelzle-Feix, Sonja¹, George, Michael¹, Fertig, Niels¹

¹Nanion Technologies, München, Germany, ²Axion BioSystems, Inc., Atlanta, GA, USA

Dissecting electrophysiological characteristics and activity patterns of induced pluripotent stem cell (iPSC) derived neurons, recently became a priority in disease modeling research. Here, we describe different electrophysiological methods, used to achieve this goal. Multi-well microelectrode array (MEA) systems (Axion Biosystems) provide simultaneous measurements of extracellular electrophysiological activity of excitable cells over long periods of time. Each electrode is capable of capturing extracellular action potentials

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of excitable cells in ultra-high resolution (millisecond events with microvolt amplitudes), while multiple recording sites within each well allow population network activity measurements. MEAs offer unbiased, label free, non-invasive recordings of natural cell functions, in a regulated physiological environment. Multiwell optogenetic stimulation further excels MEA-based disease modeling and drug discovery. These features enabled the usage of MEAs in development and characterization of *in vitro* disease models - Parkinson's disease, ALS, epilepsy, fragile X, and autism - with the ultimate goal of identifying treatments. The high throughput and accuracy that Axion's MEA systems provide will significantly accelerate progress toward such treatments. Automated, high-throughput planar patch clamp systems (Nanion Technologies), complement the need for predictive neurotoxicity screening *in-vitro* assays. Specialized protocols for reduced cell usage, increased throughput and integration into robotic environments improve cost efficiency, precision and are speeding up the HTS process of drug development and safety screening as a whole. Our special cell handling protocols enable usage of both control and patient derived, iPSC derived neurons on planar automated patch clamp systems. Our chip-based approaches, allow parallel patch clamp recordings without compromising neither data quality nor sophistication regarding technical features. In summary, we present the data obtained from neuronal disease modelling experiments, by using both MEA and automated patch clamp approaches. These platforms together provide unmatched possibilities to study specific neurological disorders by developing phenotypic disease-in-a-dish cellular models.

017

HUMAN MOTOR NEURONS DERIVED FROM FUS MUTANT IPS CELLS AS A MODEL SYSTEM TO IDENTIFY PATHWAYS ALTERED IN AMYOTROPHIC LATERAL SCLEROSIS.

DE SANTIS, RICCARDO¹, Lenzi, Jessica¹, Santini, Laura¹, de Turris, Valeria², Bozzoni, Irene¹, Rosa, Alessandro¹
¹University of Rome La Sapienza, Rome, Italy, ²Istituto Italiano di Tecnologia/CNLS@Sapienza, Rome, Italy

Induced Pluripotent Stem Cells (iPSCs) provide an opportunity to model neurodegenerative diseases *in vitro*. Amyotrophic Lateral Sclerosis (ALS) is a fatal condition caused by motor neurons (MNs) loss and iPSC-derived MNs allow to study the behavior of ALS-linked mutant proteins in the appropriate cellular and genetic background. Several iPSC lines carrying ALS mutations in the FUS gene have been recently derived in our lab. Our iPSCs collection includes lines derived by reprogramming from patients (FUS-R514S and FUS-R521C) or raised by TALEN-directed mutagenesis (FUS-P525L), all of which express physiological levels of mutated proteins.

We show that aberrant cytoplasmic localization of mutated FUS was recapitulated in iPSC-derived MNs. Moreover, different kinds of stress caused aberrant recruitment of mutated FUS into stress granules. These cellular phenotypes have been proposed as the initial steps in ALS FUS, validating our system as a proper platform for disease modeling. In order to dissect the molecular pathways underlying mutant MN degeneration, we aimed to implement our model system by stably integrating in iPSCs a reporter construct that would allow to specifically isolate MNs in differentiated neural populations. We made an effort to compare multiple reporter systems, raised in our lab or obtained by others, based on different technologies. These include: integration of the reporter by a piaggyBac transposon (or a Zinc-Finger Nuclease) and CRISPR/Cas9-mediated modification of an endogenous MN-specific locus. This comparative analysis will be instrumental to fully exploit the potential of iPSC-based systems for the modeling of ALS and other MN diseases.

019

CYTOPLASMIC CALCIUM LEVELS AND AUTOPHAGY INHIBITION CONSPIRE IN THE AGGREGATION OF ATAXIN-3 IN MACHADO-JOSEPH DISEASE-SPECIFIC NEURONS

JUNGVERDORBEN, JOHANNES¹, Breuer, Peter², Koch, Philipp¹, Wüllner, Ullrich², Peitz, Michael¹, Brüstle, Oliver¹
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Machado-Joseph disease (MJD) or spinocerebellar ataxia type 3 is the most frequent form of inherited spinocerebellar ataxia worldwide. Expansion of a polyQ repeat increases the aggregation propensity of ataxin-3, leading to the formation of ataxin-3-positive inclusions, a hallmark of MJD. Our previous studies employing patient-specific MJD neurons showed that excitation by glutamate or NMDA and subsequent Ca²⁺ entry via voltage-gated calcium channels activates calpain-mediated cleavage of ataxin-3, which initiates the generation of SDS-insoluble ataxin-3 microaggregates (Koch et al., Nature 480:543-46, 2011). Here we set out to explore whether and to what extent intracellular calcium release contributes to this disease initiating step. We investigated the early expressed purinergic receptor family for the induction of cytosolic calcium increase and found that ATP-stimulation of P2Y receptors triggered induction of microaggregates as early as 6 days after initiation of neuronal differentiation. Microscopically visible neuronal intranuclear inclusions (NIIs) positive for ataxin-3 and ubiquitin could be detected selectively in ATP-stimulated MJD cultures. To dissect the role of intracellular calcium release we also explored the potential contribution of ubiquitously expressed ryanodine receptors as well as sarco/endoplasmic



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reticulum calcium-ATPase (SERCA) to aggregation of ataxin 3. Both activation of ryanodine receptors and SERCA inhibition resulted in ataxin-3 NLLs after only 8 days of differentiation with SERCA inhibition yielding >80% MJD neurons carrying NLLs. However, these paradigms still showed high variability of NLL induction between independent experiments. We reasoned that this variability might be due to fluctuations in proteasomal degradation and autophagy. Indeed, calibration of media conditions towards inhibition of autophagic flux resulted in robust and disease specific NLL formation in MJD neurons. Furthermore, autophagy inhibition in mature neurons induced microaggregates whereas its induction prevented their formation upon glutamate stimulation. Our data point to a complex pathological interplay where dysregulation of both, intracellular calcium homeostasis and autophagy conspire to initiate the process of protein aggregation in MJD neurons.

021

BEHAVIORAL CHANGES AND NEUROGENESIS DYSFUNCTION IN A MODEL OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD), the most common form of dementia of the elderly, affects more than 35 million people worldwide, and is clinically characterized by cognitive deficits and memory loss. During the past decade, much of the focus in AD pathogenesis has turned to soluble oligomers of the A β peptide (A β O). These neurotoxins are known to accumulate in AD brains and have been considered as the actual pathogenic culprit of AD. A single intracerebroventricular injection of A β O (10 pmol) induced rapid and prolonged impairment in object recognition, object location and fear memory in mice (24 hours and 8 days after injection). As depression is one of the most common psychiatric symptoms in AD, we also compared vehicle-injected controls with A β O-injected mice in tasks designed to evaluate depressive-like behavior in rodents. A β O-injected mice exhibited a significant increase in immobility time in the Forced Swim Test (FST) and tail suspension test (TST), results indicative of depressive-like behavior. These animals also showed anhedonia-like behavior, evaluated using the Sucrose Preference Test (SPT). Although hippocampal neurogenesis has been evaluated in many AD models, its relevance for the pathology of the disease is still controversial. Whether or not

neurogenesis is modulated by AD progression, it could have an important compensatory role in memory loss and depressive behavior. We found that the number of DCX+/Ki67+ cells in the hippocampal dentate gyrus of A β O-injected mice was significantly reduced after 8 days, but not 24 hours post-injection. The current findings suggest that A β O impair the generation of new neurons in the hippocampus, and support a dual role of A β O in memory impairment and depressive-like behavior in this mouse model of AD. Understanding how adult neurogenesis is involved in AD may help us connect the behavioral and cognitive sides of the disease, and find effective targets for therapy.

023

TAKE FIVE TO FORM NEURAL ROSETTES, A NEURAL DEVELOPMENTAL MODELING SYSTEM

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Morphogenesis is an important process during animal development, by which animals shape their body plane, organise tissues, and form organs. Our brains originate from a sheet of epithelium (a neural plate), which wraps up to form a tube (a neural tube). During morphogenesis in the neural tube, cells arrange in a rosette shape (called neural rosettes). Studies showed that neural rosettes recapitulate neurogenic events proceeding in the ventricular region of the embryonic cortex, which can be used as a neural development modelling system. How neural rosettes form remains to be elucidated. Our study found that the neural rosette formation consists of five steps, cell intercalation, cell constriction, cell polarisation, cell elongation, and lumen formation. We employed molecular and biochemical approaches to decipher several elements, β -catenin, ZO-1, PARD3, actin and tubulin, in each step of the rosette formation.

025

MINIPIG SPINAL CORD CONTUSION MODELS TO ASSESS THE EFFICACY AND SAFETY OF CELL-REPLACEMENT-BASED THERAPIES

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In recent years several minipig models of acute or chron-

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ic spinal cord injury (SCI) were developed. In our previous experiments, we have developed and characterized minipig spinal cord contusion models targeting cervical, thoracic and lumbar levels of injury and have demonstrated the presence of similar spinal pathological changes as seen in human patients. These SCI contusion models have been successfully used to test the engraftment of porcine (allogeneic graft) or human (xenograft) fetal tissue - or iPSC-derived neural precursors (566RSC, PE8SC. Ubc-EGFP) once grafted into injured spinal cord during acute or chronic stage after spinal injury. The effect of short-lasting immunosuppression on induction of allograft-versus-host long term immune tolerance was also studied. Collectively, the grafting data show: i) consistent xenograft survival in continuously immunosuppressed pigs if cells are grafted into naive or mechanically-injured spinal cord at 1,3, 7 days or at 2 or 5 months post-injury, ii) long-term engraftment and synapse formation of porcine fetal tissue-derived or iPSC-derived NPCs once grafted into injured spinal cord of transiently (1 month) immunosuppressed pigs. These data show that the use of the pig acute or chronic spinal injury model coupled with allogeneic or xenogeneic cell grafting represents an important preclinical model to validate the potency and safety of cell-replacement-based therapies. In addition, this model permits effective utilization and potential modification of clinical immunosuppression protocols to optimize long-term engraftment of allogeneic neural precursor grafts.

027

HUMAN EMBRYONIC STEM CELL-BASED NEURAL DEVELOPMENTAL MODELING REVEALS THE VERSATILE ROLES OF FGF2 SIGNALING

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The invention of human embryonic stem cell (hESC) and induced pluripotent stem cell (hiPSC) technologies have shifted the research approach from the adaptation of conventional animal models to the human pluripotent stem cell (hPSC)-based modeling, which offers potential for studying human conditions using human systems. However, despite hPSCs holding great potential, it is imperative to validate how faithful hPSC-based neural developmental modeling is in recapitulating the developmental process *in vivo*. This study undertook to substantiate the validity of hPSC-based neural developmental modeling by comparing it with many facets of neural developmental events occurring in mice. We found that the hPSC-based system mimicked the process from the neural plate to the neural tube, then to the neocortex, during which the system captured characteristics of brain development. We also showed that the system reserved the similar

neural stem cell (NSC) niche residing in the ventricular region of the cortex. Moreover, FGF2 signaling exhibited a different repertoire of regulatory activities depending upon neural stage progression.

029

IN VIVO REPROGRAMMING OF REACTIVE ASTROCYTES TO OLIGODENDROCYTE PROGENITOR CELLS USING TRANSCRIPTION FACTORS AND MICRO-RNA IN THE ADULT BRAIN

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Reactive astrocytes are the main cellular component of astrogliosis as the main landmark of neurodegenerative disorders including multiple sclerosis. Therefore *in vivo* reprogramming of reactive astrocytes to functional oligodendrocyte progenitor cells (OPCs) exerts a dual beneficial effect by converting the repair inhibitor cells into repairing cells. Using viral vectors which express transcription factors Oct4, Sox2, Sox10, Olig2 and miR-302/367 we have tried to transdifferentiate the reactive astrocytes into OPCs. Both primary adult mouse astrocytes and human astrocyte line were used as starting cells. Following transduction, tracking of transduced cells showed that astrocytes changed their cellular fate and expressed both OPCs and myelinating phenotypes over the post-transduction period. We could not detect pluripotency markers and teratoma following viral transduction which implies direct conversion of astrocytes to OPCs. Our data suggest that application of transcription factors or miRNA can increase the capacity of brain for myelin repair and promise for novel therapeutic approaches.

031

OVEREXPRESSION OF SLP-2 RESCUES MITOCHONDRIAL PHENOTYPES IN IPSC-DERIVED NEURONS FROM PATIENTS WITH PARKIN-LINKED PARKINSON'S DISEASE

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In many Parkinson's disease patients, a modest decrease of the activity of the mitochondrial respiratory chain



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complex I in the *substantia nigra* has been reported, although the mechanism for this deficit is unknown. The deficiency of Parkin, a gene causing a familial form of early-onset parkinsonism, results in a specific reduction of complex I activity in various cell and animal models. Here we demonstrate for the first time that Parkin interacts with the mitochondrial protein Stomatin-like protein 2 (SLP-2), which is involved in the assembly of respiratory chain proteins. SH-SY5Y cells with reduced expression of Parkin or SLP-2 and induced pluripotent stem cell-derived dopaminergic neurons from patients with *Parkin* mutations showed deficiencies in mitochondrial respiration, complex I activity, ATP production, and mitochondrial morphology. Overexpression of SLP-2 corrected the altered mitochondrial phenotypes caused by loss of Parkin, highlighting a functional relationship between these two proteins. This finding places further emphasis on the relevance of Parkin for the maintenance of mitochondrial function in neurons and provides a novel target for therapeutic strategies for treatment of Parkinson's disease.

033

EFFECTS OF INDUCED PLURIPOTENT STEM CELL LONG TERM PASSAGING ON PLURIPOTENCY, NEURONAL DIFFERENTIATION AND STRESS RESPONSE

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Induced pluripotent stem cells (iPSCs) have been proposed as a homogeneous source of cells which can reproducibly be differentiated into various neuronal phenotypes. Therefore they might constitute ideal tools for pharmaceutical research and for addressing the mechanisms underlying the function of specific neurons. In order to test reproducibility of iPSC cultures and derived neurons differentiated into a midbrain dopaminergic phenotype, we are monitoring the effects of long time cultivation of iPSCs on pluripotency, neuronal differentiation and rotenone sensitivity by immunocytochemistry for marker proteins, functional assays and next generation sequencing (NGS). We are comparing three iPSC clones originating from one fibroblast donor, which have additionally been divided into two daughter cell lines each. Neuronal progenitor cell lines (NPCs) were generated from these six lines and cultured in parallel. The iPSC lines and the NPC lines were cultivated over 30 passages and samples were taken on a regular basis. Furthermore, the NPCs were differentiated into dopaminergic neurons at an early,

middle and late time point of the study. Differentiated neurons were treated with rotenone at different concentrations to induce oxidative stress by inhibiting mitochondrial respiration. The six iPSC lines expressed typical pluripotency markers over 30 passages and maintained their proliferation rates. Similarly, the six NPC lines stably expressed neuronal precursor markers over the course of the study and could be differentiated into dopaminergic neurons with comparable percentages of TH positive neurons and morphology. However, rotenone sensitivity varied between individual experiments. The gene expression profile is currently under evaluation. First analyses indicate that the gene expression profiles of iPSCs change over the 30 passages. Variation of gene expression between daughter clones of the three iPSC lines is smaller than between the tree lines. Unexpectedly, the number of genes differentially expressed between passages follows a regular pattern which seems to depend on medium changes. NGS of NPCs and differentiated neurons is ongoing. We expect that these data will give insight into the stability of iPSC and NPC cultures over time and guide us to create a stable cell culture model in future.

035

INTRATHECAL TRANSPLANTATION OF HUMAN ASTROCYTES ATTENUATES CLINICAL SYMPTOMS IN SOD1^{G93A} ALS RAT MODEL

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ALS is a Motor Neuron (MN) disease that is characterized by the loss of MNs in the central and peripheral nervous systems. As motor neurons die, patients progressively lose their ability to control voluntary movements, become paralyzed and eventually die from respiratory/deglutition related failure. To date, Riluzole, which extends lifespan by approximately three months, is the only drug treatment for ALS. Despite the selective MN cell death in ALS, there is growing evidence that astrocytes play a crucial role in ALS disease progression. Astrocytes from both ALS patients and ALS animal models were found to be malfunctioning in protecting the MNs. Therefore, finding a novel and available source for functional human astrocytes offers a new approach to protecting MNs in ALS. Here we describe a new cellular therapy that is based on the derivation of healthy and enriched population (>90% GFAP+ cells) of astrocytes from human embryonic stem cells (AstroRx). In vitro, these astrocytes exhibit both gene and specific markers' expression similar to primary human astrocytes. They also demonstrate functional properties of "healthy" astrocytes', including uptake of glutamate from the medium, secretion of neurotrophic

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factors such as BDNF, GDNF, IGF-I and VEGF, and protection from oxidative stress. In-vivo, intrathecal injections of AstroRx into the cerebrospinal fluid (CSF) of transgenic SOD1^{G93A} rats, prolonged survival of transgenic SOD1^{G93A} rats compared to sham-injected rats. In addition, rats that were injected with AstroRx significantly maintained their body weight over time. We also observed a significant delay in disease onset. A set of motor performance tests also demonstrated the efficacy of AstroRx over sham-injected rats. AstroRx significantly delayed the development of clinical symptoms as measured by neurological scoring, longer duration on a rotating "rotarod" and increased forelimb muscle strength as measured by a grip strength test. These pre-clinical studies demonstrated the possible promising therapeutic effects of AstroRx in the treatment of ALS. AstroRx can be expanded ex-vivo in large quantities under GMP conditions and has the potential to be kept frozen as an "off the shelf" product. AstroRx is currently being developed towards FDA IND submission and initiation of clinical trials.

037

SYNAPTIC PROTEIN TURNOVER AT HUMAN SYNAPSES

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Many proteins are continuously involved in organizing efficient synaptic transmission and recycling of synaptic vesicles. Yet, these synaptic contacts are often situated far from the soma, signifying unique challenges for these proteins to remain functionally stable to prevent strong reliance on new protein transport from the nucleus. We hypothesize that failing protein quality control at synapses and proteopathic stress contribute to early synaptic defects in neurodegeneration. To study the protein homeostasis in human neurons we are genetically manipulating and differentiating human ES cells into glutamatergic neurons. We are growing cells with specific mutations in autaptic micro-island cultures, allowing us to use elaborate imaging of specific fluorescent marker tools that reveal specific types of autophagy at synapses (micro- macro and chaperone mediated autophagy) and vesicle/endosome trafficking routes. Furthermore, by using viral transductions with 'timer fusions' constructs - which slowly convert from blue to red fluorescence - fused with synaptic proteins, we provide a read out of how fast proteins are turned over at synapses and what the effects of defective autophagic pathways are on this. Finally, we will be able to conduct detailed electrophysiological studies to assess the role of protein homeostasis alterations in the control of synaptic output. Our work aims to reveal the role of synaptic protein homeostasis on synapse function in healthy synapses but also in the context of neurodegenerative disease conditions.

039

NOTCH3 TARGETS INVOLVED IN NEURAL STEM CELL QUIESCENCE AND STEMNESS

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Constitutive adult neurogenesis in the mammalian brain remains a spatially and quantitatively limited phenomenon confined to few subregions of the telencephalon. Unlike in rodents, the zebrafish adult brain displays widespread neurogenesis. The pallial ventricular zone accommodates (at least) two populations of progenitor cells. Cells of the first population express glial markers and can both self-renew and generate neurons, including at the single cell level, identifying them as genuine neural stem cells (NSCs). These NSCs, which are radial glia (RG), are predominantly quiescent, even though 10% of them express proliferation markers at any time. The second population of progenitors proliferates but does not express glial markers. Recently, our laboratory demonstrated the prominent role of Notch3 signaling in gating the initial activation step of NSC recruitment. More recently, we also observed a direct or indirect loss of stemness in RG cells following Notch3 abrogation, and we set out to identify potential Notch3 targets involved in maintaining RG quiescence or stemness by comparing the transcriptional profiles of RG in wild-type and *notch3* homozygous mutant backgrounds. Pallial NSCs were isolated by FACS based on their expression of a *gfap:gfap* transgene in 7dpf *notch3*^{-/-} mutant larvae versus wildtype siblings, and RNAseq was conducted followed by GO and GSEA analyses. Using a similar combination of FACS and RNA-seq with adult wild-type zebrafish expressing dRFP in RG and eGFP in proliferating progenitors, we also compared the transcriptome of quiescent versus activated RG. The intersection of both analyses delineates the potential Notch3 targets involved in the regulation of RG states. One of the candidate genes transcriptionally associated with the stemness state encodes *Hey1*. We confirmed the selective expression of *hey1* in pallial NSCs, and characterized its function by electroporating fluorescein-tagged morpholinos into the pallial ventricular zone in vivo in adult fish. We showed that the abrogation of *Hey1* function results in the generation of neurons at the expense of the activated NSC state. These results functionally implicate *Hey1* in the maintenance of a progenitor state in activated RGs of the adult pallial germinal zone.



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041

EFFICIENT HUMAN PLURIPOTENT STEM CELL BIOPROCESS DEVELOPMENT: UPSCALING OF EXPANSION AND DIFFERENTIATION POTENTIAL

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Cell-based therapies have the potential to contribute to overall health care, whereby living cells can be introduced as medical therapies. To obtain a sufficient cell number for biomedical applications and therapeutic purposes, human pluripotent stem cells (hPSCs) are the ideal cell source because of their self-renewal ability and pluripotency. The dynamic suspension culture technique is one of the promising methods. However, current technologies for hPSC large-scale bioprocessing have not fulfilled the demands of clinical use, as they are associated with low cost-efficiency, lack of quality control, or do not meet Good Manufacturing Practices (GMP) standards. In this study, both a spinner flask (125 mL, Corning) and a bioreactor (Mobius 3L, Merck Millipore) were used for hPSC expansion. In both of these stirred suspension culture vessels, cultivations were started using a human induced pluripotent stem cell (hiPSC) single cell suspension without any microcarriers or additional matrices. Our data demonstrates the maintenance of pluripotency in hiPSC dynamic suspension culture in these vessels. Moreover, the process is easily scalable, in our case from 50 to 1000 mL, and generates hiPSC aggregates which maintain both homogeneity (diameter 300-400 μm , at day 7) and a high percentage of pluripotency-marker positive cells (>97.8% TRA-1-60, at day 7). As a parameter of bioreactor productivity, the growth rates were evaluated by fold increase per day (FIPD) relative to the initial number of inoculated cells. Recent references concerning hiPSC bioprocessing similar to our protocol describe FIPD of 1.5 (6 fold in 4 days) or FIPD of 0.9 (6.3 fold in 7 days). In contrast, we were able to achieve an improved FIPD of 3.00 (21 fold in 7 days) in a 100 mL spinner flask and a FIPD of 1.27 (8.9 fold in 7 days) in a 1 L bioreactor. According to our protocol, we successfully generated more than 10^9 cells (1.6 billion in 7 days) in the bioreactor. This amount of hiPSCs is sufficient for cell transplantations, tissue patches, and high-throughput drug screening. Currently, our procedure is being adapted to other cell lines such as neural progenitor cells (NPCs) to get robust and cost-efficient scale-up and scale-out strategies for neural repair.

043

DEVELOPMENT AND VALIDATION OF A HIGH THROUGHPUT SCREENING ASSAY TO IDENTIFY STIMULATORS OF PHAGOCYTOSIS IN THE RETINAL PIGMENT EPITHELIUM

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The RPE (retinal pigment epithelium) is a single monolayer of pigmented epithelial cells that is essential for the survival of overlying retinal photoreceptors and preservation of vision. Among other roles, the RPE is responsible for the phagocytosis and degradation of POS (photoreceptor outer segments), shed daily by the photoreceptors. Unfortunately, the efficiency of phagocytosis, and thus, degradation of POS decreases with age. This leads to the accumulation of toxic by-products; retinal degeneration and photoreceptor loss, which together with other factors contribute to the development of retinal dystrophies, such as; retinitis pigmentosa (RP), and age-related macular degeneration (AMD), the prime cause of vision impairment and blindness in industrialized societies. Previously, we have shown that the three-dimensional (3D) culture of stem cells in matrigel combined with neural induction results in cysts with defined neuroepithelial identity within 5 days. These neuroepithelial cysts can be further differentiated quantitatively into retinal pigment epithelial (RPE) cells within 30 days. More recently, the availability of unlimited source of RPE tissue that, unlike other available RPE cell lines, resemble primary RPE phenotypically and functionally, allowed us to develop a high through put screening (HTS) assay to screen for stimulators of POS phagocytosis. Our preliminary results show that our assay is robust and reproducible and could be used to identify stimulators of phagocytosis. Additionally, we have done a pilot screen of 1600 FDA approved library of small molecules, and we identified not only stimulators but also inhibitors of POS phagocytosis. In the future, we would like to confirm these hits and validate them in secondary assays and animal models of retinal degeneration.

045

NON-MODIFIED MRNAS FOR THE DERIVATION OF CLINICALLY RELEVANT IPS CELL LINES FROM HUMAN BLOOD-DERIVED EPCS AND ADULT HUMAN DERMAL FIBROBLASTS

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Many groups have demonstrated that the repeated transfection of fibroblasts with a cocktail of reprogramming associated mRNAs results in the derivation of integration-free human induced pluripotent stem (iPS) cells. While many advancements have been made to refine this process on fibroblasts (incorporation of ECMs, miRNAs, and more efficient transfection reagents), to date no group has been able to demonstrate efficient, RNA-based reprogramming of a blood derived cell type. This is due to the inability to efficiently and repeatedly deliver mRNA to blood derived cell types without inducing cytotoxicity. Peripheral blood provides easy access to adult human cell types for reprogramming purposes. Notably, endothelial progenitor cells (EPCs) are a rare, circulating population of cells that can be clonally isolated from both fresh and frozen human peripheral blood and cord blood samples. The EPCs adherent nature and high proliferative capacity while maintaining their cell identity makes them highly desirable for repeated transfection with RNA when compared to hematopoietic suspension cell types commonly isolated from peripheral blood which typically have limited expansion potential and exhibit maturation in culture. Lastly, the ability to generate clinical grade iPS cells from EPCs using RNA reprogramming technologies presents a unique therapeutic opportunity to treat myeloproliferative disorders in which the disease-causing somatic mutations are restricted to cells in the hematopoietic lineage. Here we present an RNA-based reprogramming method that utilizes a novel cocktail of synthetic, non-modified reprogramming and immune evasion mRNAs and reprogramming enhancing miRNAs for the generation of clinical grade iPS cell lines from human blood-derived EPCs. This novel, reprogramming protocol results in the highly efficient generation of stable, fully pluripotent iPS cell lines from both blood-derived EPCs (0.4%) and HUVECs (3%) as well as human adult dermal fibroblasts (3%), all within 11 days.

047

DERIVATION AND STABILIZATION OF NOVEL EARLY HUMAN NEURAL PROGENITOR CELLS FROM PRIMARY TISSUE CAPABLE OF DIFFERENTIATION INTO CENTRAL AND PERIPHERAL LINEAGES

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In recent times, major progress in cellular reprogramming for modeling neurological and neuropsychiatric diseases

has been achieved. The generation of iPSCs and their differentiation into neural progenitor cells (NPCs) and the direct conversion of somatic cells into NPCs emerged into a promising strategy to obtain patient-specific cells. Nevertheless, it remains unclear if those NPCs represent the physiological state. Primary NPCs from fetal brain tissue might serve as a bona-fide-model and standard population for comparative studies. Moreover it could elucidate neural developmental mechanisms. Earlier studies demonstrate the derivation of rosette-forming or radial-glia-like cells from primary tissue. We hypothesized that the modulation of crucial developmental signaling pathways such as TGF β and Shh is instrumental for the stabilization of early progenitors. Thus we assessed the potential of small molecules for the stable cultivation of early NPCs from primary tissue. Indeed, we identified conditions allowing robust formation of neuroepithelial colonies displaying a homogeneous morphology and high proliferation rate. Moreover they could be isolated and monoclally expanded for more than 40 passages. Characterization by immunofluorescent stainings and quantitative PCR show a neural stem cell profile including SOX1, PAX6, Nestin and SOX2. FACS- analysis reveals a characteristic expression of the early neural stem cell marker Prominin-1 (CD133). Differentiation analysis indicates a strong neurogenic potential with a high percentage of TUJ1-positive neurons among which GABAergic, glutamatergic, dopaminergic and serotonergic subtypes can be found. Interestingly, rare neurons stain positive for the peripheral nervous system marker Peripherin. By applying directed differentiation protocols we increased the percentage of dopaminergic neurons along with several subtypes of peripheral neurons and GFAP-positive cells representing the glial lineage. In summary, we here present a study showing the derivation of a novel fetal-derived early NPC population by defined media conditions. These cells could serve as a comparative cell population for conversion studies and provide a cell source for biomedical applications such as cell replacement therapy approaches and tissue engineering.

049

INTRANASAL DELIVERY OF HUCB-MSC : A NONINVASIVE STEM CELL THERAPY TO TARGET NIEMANN PICK TYPE C1

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Niemann pick type C1 (NPC) disease is a neurological disorder in which cholesterol and gangliosides accumulate in late endosomes/ lysosomes, followed by rapid death of purkinje neurons. Our previous data have demonstrated that human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) have the multifunctional abilities to ameliorate NPC symptoms



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of NPC transgenic mice model. Given that Safety and efficacy of cell-based therapies for neurodegenerative diseases depends on the mode of cell administration, direct transplantation of cells to the CNS can be invasive and precluded to apply in the clinic. Therefore, the present study examined the therapeutic efficacy of intranasal delivered hUCB-MSCs using NPC mice. After nasal delivery, GFP expressing hUCB-MSCs were detected in the olfactory bulb, corpus callosum, hippocampus and cerebellum. Motor function analysis evaluated by Rota-rod test also showed that hUCB-MSCs led to behavioral improvement of NPC mice. The transplantation of hUCB-MSCs increased purkinje cell survival as well as reduced cholesterol accumulation in the neurons. In addition, compromised autophagy in the cerebellum of NPC mice was quarreled by reducing the increased level of LC3-II. Taken together, intranasal administrated hUCB-MSCs provides highly encouraging alternative to the traumatic surgical transplantation and a promising potential therapeutic strategy for NPC patients.

051

CYTOPROTECTIVE ROLE OF P53 IN ADULT HIPPOCAMPAL NEURAL STEM CELLS

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Programmed cell death (PCD) of neural stem cells (NSCs) plays a critical role in the development and function of the central nerve system. Previously, we have reported that adult hippocampal neural stem (HCN) cells undergo autophagic cell death (ACD) following insulin withdrawal despite their intact apoptotic capabilities. Here, we demonstrate that p53 is involved in regulation of HCN cell survival. The expression level of p53 decreases in hippocampus during brain development, but basal p53 expression level remains relatively high in HCN cells. Interestingly, p53 is degraded by autophagy following insulin withdrawal. This is the first report on the autophagic degradation of wild type p53. p53 is transcriptionally inactive and localized mostly in the cytosol in HCN cells in vitro and in vivo. Overexpression of p53 mutant targeted to the cytosol significantly reduced autophagy and cell death rates following insulin withdrawal. In addition, p53 also protected HCN cells from apoptotic inducers. In summary, high expression level and cytosolic location of p53 and the protective role of p53 against cell death suggest the interesting transcription-independent, pro-survival role of p53 in HCN cells. Understanding the role of p53 in response to cell death signals can help to understand the cell death mechanism of HCN cells with implications for regenerative therapies using endogenous stem cells.

053

COMPARATIVE ANALYSES OF NETWORK ACTIVITIES IN NEURONAL POPULATIONS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

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Neurons derived from human pluripotent stem cells (hPSC) are a valuable resource for establishing in vitro models of neurological function and dysfunction. An imperative step for modelling the nervous system in vitro is to measure and characterize neuronal network activities. Here, we use microelectrode arrays (MEAs) to measure network activity of hPSC-derived neurons, thereby characterizing their ability to form functional neuronal networks. These measurements were conducted over the time course of cells in culture and also in neurons derived using different methodologies for hPSC induction to derive different cell types. Our data show differences in firing activities between various hPSC-derived neuronal populations, including cortical glutamatergic and GABAergic neurons. These studies are key for modelling neural circuitry systems using hPSC-derived neurons.

055

NEURODEVELOPMENTAL DEFECTS IN SPG11 RELATED MOTOR NEURON DISEASE USING A HUMAN IPSC DERIVED MODEL

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Mutations in the Spastic Paraplegia Gene 11 (*SPG11*), encoding spatacsin, cause the most frequent complicated form of autosomal recessive (AR) hereditary spastic paraplegia (HSP) and juvenile onset amyotrophic lateral sclerosis (ALS5). When *SPG11* is mutated, patients frequently present with spastic paraparesis, a thin corpus callosum, and cognitive impairment. We previously delineated a neurodegenerative phenotype in neurons of these patients. Our aim was to recapitulate the early developmental phenotypes of *SPG11* and outline the cellular and molecular mechanisms in the patients-specific induced pluripotent stem cells (iPSCs) derived cortical neural progenitors (NPCs). We generated and characterized induced pluripotent stem cells (iPSCs) derived NPCs and neurons from three *SPG11* patients and two age-matched controls (CTRLs). Gene expression profiling of *SPG11*-NPCs

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revealed widespread transcriptional alterations in neurodevelopmental pathways. These include changes in cell cycle, neurogenesis, callosal developmental guidance cues, in addition to autophagic deficits. More importantly, the GSK3 β signaling pathway was found to be dysregulated in SPG11-NPCs.

Impaired proliferation of SPG11-NPCs resulted in a significant diminution in the number of neurons. The decrease in mitotically active SPG11-NPCs and neurogenesis anomalies were rescued by GSK3 modulation. This iPSC-derived NPC model provides the first evidence for an early neurodevelopmental phenotype in SPG11, with GSK3 β as a potential novel target to reverse the disease phenotype.

057

SCALABLE EXPANSION OF HUMAN EMBRYONIC STEM CELL-DERIVED NEURAL PROGENITORS IN MICROCARRIER-FREE 3D SUSPENSION

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We developed a method for suspension culturing and massive expansion of human embryonic stem cells (ESCs) in spinner flasks and bioreactors under microcarrier-free 3D suspension conditions. While cultured as clusters in bioreactors, cell numbers increased by 1,700 fold during 18 days of culture, reaching a concentration of over 40 million cells per ml and >95% viability rate. The same cells maintained features of pluripotent stem cells (PSCs), including karyotype stability, developmental and differentiation potential and expression of key markers. To validate the similarity of cells cultured in suspension to PSCs characteristics, we profiled the transcriptome of I3 (TE03) using RNA-sequencing technology. As expected, we found a clear pluripotency signature characteristics of human ESC lines. RT-PCR analysis of 88 stemness-related and differentiation markers further showed a high concordance between suspension (3D, as clusters) and adherent (2D) systems ($r = 0.85$). However, in contrast to adherent cells, 3D cultured cells express significantly higher levels of key markers of the naïve pluripotent state (FGF4 and REX1) and very low levels of FGF5, an established marker of primed stem cells. Remarkably, under 3D conditions, we found a substantial decrease (17-fold) in XIST mRNA, suggesting a shift in X-inactivation status towards naïve pluripotency. We derived neural progenitor-like cells in 2D and 3D suspension culture conditions. Both methods allowed

massive expansion of the neural progenitor-like cells in spinner flasks and bioreactors with an increase of 8-fold in cell proliferation every 7 days. We derived two types of progenitor-like cells, one that highly expressed the neural precursor marker PS-NCAM and a second that highly expressed the neuroepithelial markers PAX-6 and Nestin. Notably, we demonstrated the ability of the cells to further differentiate into neurons and glial cells. Taken together, our novel technology for suspension culturing of PSCs, enables directed differentiation and mass production of neural progenitors needed for industrial and clinical applications.

059

DECCELLULARIZED DERMAL FIBROBLASTS AS EXTRACELLULAR MATRICES FOR EFFICIENT PRIMARY EXPANSION OF HUMAN DENTAL PULP STEM CELLS

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Extracellular matrices (ECMs) play an important role in stem cell niche for proliferation, differentiation, and maintaining characters. Decellularization techniques have been developed for the regeneration of target tissues and organs, and some studies showed that decellularized matrix (DM) can support mesenchymal stem cell expansion and replace feeder cells for embryonic stem cell culture. To get a sufficient amount of human dental pulp stem cells (hDPSCs) for in vivo transplantation, it takes several weeks to months under serum-free condition, and such a long-term cultivation give rise to safety concerns for clinical application. In this study, we aimed to delineate the potential of DM derived from cultured human dermal fibroblasts (hDFs) for the primary expansion of hDPSCs under serum-free condition. To compare the effect of fibrillar structure within DM, PBS-washed DM (W-DM) following decellularization of hDFs was also prepared. hDPSCs were divided into four groups and cultured; gelatin (Gel), poly D-lysine/laminin (PL), DM, and W-DM. Cell numbers of hDPSCs in DW was the highest, and those in W-DM were the second at 2 weeks among four culture conditions. The fibroblasts originated from hDPSCs was not found under all four culture conditions. We concluded that the period for the primary expansion of hDPSCs was dramatically shortened when the cells were cultured on DM and W-DM derived from hDFs compared to those on Gel or PL, and decellularization techniques might be helpful to obtain sufficient hDPSCs for in vivo transplantation.



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HUMAN EMBRYONIC STEM CELL-DERIVED NEURAL STEM CELLS: A POWERFUL MODEL TO INVESTIGATE THE REGENERATIVE CAPACITY OF THE BRAIN AFTER AN INSULT

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Regeneration of the nervous system requires either repair or replacement of nerve cells that have been damaged by injury or disease. The maintenance of the stem cell pool is therefore of particular importance. Yet, any accumulation of damage in stem cells may affect their function as well as the behavior of their progeny. To investigate damage repair potential and neurogenesis of neural stem cells (NSC), and functionality of their progeny (neurons) after treatment with potential health hazards such as ionizing radiation (e.g. X-rays), we differentiated human embryonic stem cells (hESC, H9) into NSC using an established differentiation protocol. Differentiation of NSC progeny was initiated by the formation of three dimensional neurospheres (NS). The functionality of NS was characterized using non-invasive microelectrode arrays (MEA). Already one day after plating, electrical signals were recorded as random single spikes. After two days, the cells started to express burst-like spiking activity representing the mature signaling activity of the network. The NS were viable over a period of several weeks on the MEA chips enabling long-term experiments. NSC were characterized by analyzing neural progenitor marker expression, such as PAX6, SOX2 and Nestin in terms of gene and protein expression. After exposure to X-rays, we observed a dose-dependent cell cycle block in G2/M phase for NSCs, in a time-frame of 8-16 hours post irradiation. A dose-dependent effect was also observed in terms of apoptosis (TUNEL assay) and survival. Data on the impact of X-rays on activation of DNA damage repair pathways, gene expression during neural differentiation as well as functionality of NS will be presented. This work was supported by the BMBF, Bonn, Germany (02NUK025 and 02NUK034C).

063

THE EUROPEAN BANK FOR INDUCED PLURIPOTENT STEM CELLS (EBISC): ENABLING UTILISATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS FOR DRUG DISCOVERY APPLICATIONS

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In Drug Discovery more predictive preclinical cellular disease models are required to assess and rank the efficacy of compounds in a cost-effective manner. Induced pluripotent stem cell derived models are becoming an integral part of drug discovery, disease stratification and basic research. The consistent derivation and banking of iPSC from healthy donors or patients with disease causing genome mutations is an important step to fully utilise the potential of this technology. The EBISC consortium consists of twenty seven partners, distributed across nine separate European nations and includes seven universities, seven pharmaceutical companies, five biotechnology companies, four government run agencies and a single charity. A central pillar of the EBISC vision is to support applications in disease modelling and patient stratification based on cellular phenotyping. The goal is to establish in Europe a centralized, not-for-profit iPSC line banking, testing and distribution activity, providing all qualified users with access to scalable, cost-efficient and customized cell line products, enriched data-sets and associated services. Based on this provision, the centralised facility is projected to become self-sustaining by 2019. This poster will present the process of large scale banking and use of imaging tools to characterise iPSC lines at the population and single cell level which will ultimately guide the quality control and release of lines to the general scientific community.

065

MODELING ALZHEIMER'S DISEASE WITH IPSC-DERIVED NEURONS

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Alzheimer's disease (AD) is the most common neurodegenerative disorder. Extensive research on Alzheimer's disease has broadened our understanding of the disease but failed to generate an effective therapeutic approach to prevent onset or progression. Animal models of AD often use overexpression of familial APP mutations, which may not closely reflect the situation in patients. Therefore, dermal fibroblast from AD patients and non-demented controls were reprogrammed to induced pluripotent stem cells (iPSCs) by episomal expression of the Yamanaka factors. Stem cell colonies were selected by morphology and multiple clones were tested for expression of pluripotency factors, stable karyotype and vector integration. Differentiation to induced neuronal (iN)

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cells was achieved by overexpression of Neurogenin2. After three weeks of differentiation iN cells were tested for expression of neuronal markers by western blot and immunofluorescence. Neurogenin2 lead to an efficient and robust conversion of iPSCs to iN cells, which express the major players of AD pathology, APP and tau. We aim to elucidate pathomechanisms of AD in human neurons by the use of iPSCs, derived from AD patients.

067

MODELING FUS-ALS HALLMARK NEUROPATHOLOGY USING PATIENT-SPECIFIC IPSCS & IPSC-DERIVED CORTICAL NEURONS

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Amyotrophic lateral sclerosis (ALS) is an adult onset disorder in which about 5% of familial cases are caused by autosomal-dominant mutations within the *FUS* (fused in sarcoma) gene. *FUS* is a ubiquitously expressed nuclear protein which, in *FUS*-ALS, is mislocalized to the cytoplasm and forms aggregates. A cytoplasmic translocation of *FUS* was suggested as a key event in *FUS*-ALS pathology, while *FUS* aggregation is thought to be caused by a second hit. Here we present the first human *in vitro* model of *FUS*-ALS that allows the (patho-)physiological investigation of *FUS* distribution in patient-derived cortical neurons carrying endogenous mutation. We could show that observed pathological phenotypes can be related to clinically early (R495QfsX527) vs. late (R521C) disease onset. We found that the amount of cytoplasmic *FUS* as well as cellular vulnerability depends on the severity of the underlying mutation. Cytoplasmic *FUS* inclusions formed spontaneously in mutated iPSC-derived cortical neurons depending both on the severity of *FUS* mutation and neural aging but independent of a second hit. Finally, neurodegeneration was not specific to layer V cortical neurons, which are mainly affected in ALS. Pathological phenotypes were also seen in layer II/III cortical neurons typically affected in Frontotemporal lobar degeneration (FTLD), even though *FUS* mutations do not cause FTLD. Our study thereby highlights the value and usefulness of patient-derived cell models in *FUS*-ALS and the importance to study pathophysiology in cell types specifically affected in disease.

069

MODELING DRAVET SYNDROME IN IPS CELL-DERIVED NEURONS

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Dravet syndrome (DS) is a debilitating, congenital form of childhood epilepsy. Most patients carry mutations in the *SCN1A* gene leading to insufficient functional Nav1.1 protein, a major voltage-gated sodium channel in mature inhibitory interneurons. Typically, myoclonic seizures arise between 3-18 months of age, coinciding with Nav1.1 expression in developing GABAergic interneurons. To model DS *in vitro*, patient fibroblasts harboring monoallelic loss-of-function mutations in *SCN1A* were reprogrammed to induced pluripotent stem (iPS) cells. Long-term self-renewing neuroepithelial stem (It-NES) cells were derived as a stable intermediary population that efficiently differentiates into GABAergic neurons with anterior hindbrain identity (Koch et al., 2009). DS-specific neurons differentiated for 6 weeks exhibit voltage-dependent inward and outward currents, action potential generation and spontaneous synaptic activity. RT-qPCR analysis of patient-specific neurons revealed a 50% reduction in *SCN1A* wild-type mRNA, mutant mRNA making up half the total amount. Analysis on protein level confirmed a 50% reduction while, expectedly, no truncated protein was detected. We then set out to increase neuronal Nav1.1 levels via an endogenous regulator (Kim et al., 2007). To this end, the intracellular domain of Nav channel subunit $\beta 2$ was fused to GFP via a self-cleaving 2A peptide and incorporated into a lentiviral doxycycline-inducible construct. Successfully transduced DS It-NES cells were enriched by FACS. Construct cleavage was confirmed by Western blot analysis, and transgenic $\beta 2$ -ICD was found in cell nuclei by 3D microscopy. $\beta 2$ -ICD transgenic cells retained an It-NES identity remained able to generate GABAergic neurons. Transgene induction in 6-week-old neurons increased intracellular Nav1.1 protein levels 4-8-fold. Furthermore, biotinylation studies demonstrated a similar increase in Nav1.1 on the cell surface, implying a putative functional impact of the additional channels. These results indicate a regulatory activity of $\beta 2$ -ICD on sodium channel homeostasis in authentic human neurons, which might be exploited to counteract Nav1.1 deficiency in DS-specific neurons.



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TARGETED NEURONAL CIRCUIT ASSEMBLY OF HUMAN STEM CELL DERIVED NEURONS

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Every aspect of brain function is based on precisely wired neuronal circuits. However, studying human neuronal circuits in health and disease remains quite challenging to date. Induced pluripotent stem (iPS) cells can be differentiated to neurons forming functional circuits *in vitro*. However, they tend to interconnect randomly. In order to generate a suitable human model system, we assemble iPS cell-derived neurons into functional circuits in a targeted fashion. First, we trigger rapid neurogenesis in human iPS cells via the inducible expression of certain neurogenic transcription factors yielding highly homogeneous neuronal populations. Next, we print bioactive coating substrates on non-permissive surfaces to generate circuit matrices that guide the growth of stem cell-derived neurons. As soon as a network is reliably and reproducibly constructed, we will characterize it on the morphological and functional level. Adding simple circuits together can create more complex neuronal systems that will help to study basic circuit formation and function. For biomedical applications, diseased circuits can be mimicked.

073

TNF ALPHA SIGNALLING IN NSC: POTENTIAL ROLE IN SEZ REGENERATION.

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Neurogenesis persists in specific niches of the adult mammalian brain and is supported by long-lived stem cells present in these locations. The subependymal zone (SEZ) of the adult murine brain is a very active neurogenic niche in which a relatively quiescent population of radial glia/astrocyte-like GFAP⁺ neural stem cells (NSC) continually produce new neurons and oligodendrocytes, via a population of rapidly-dividing transit-amplifying progenitor cells. Although some intrinsic determinants are known to regulate stem cell division, the observation that stem cells can respond to excessive cellular demand in pathological situations or after traumatic injury suggests that signals present in their microenvironment or niche contribute to the regulation. Tumor necrosis factor alpha (TNF α), a

pro-inflammatory cytokine, is a multifunctional protein with a broad range of activities in different systems. Increasing evidence indicates that immune cells and immunological mediators modulate neurogenesis. In this context, effects of pro-inflammatory cytokines that are produced under non-physiological conditions, such as irradiation, inflammation, status epilepticus or stroke, on neurogenesis have been described. However, their effects appeared sometimes contradictory, suggesting potentially distinct effects depending on the cell or receptor type involved. In the present work, we have evaluated potential roles of TNF α and its receptors in SEZ remodeling/regeneration analyzing direct effects of this cytokine on proliferation/self-renewal of NSC in culture and assessing its relevance in different *in vivo* scenarios where SEZ homeostasis is compromised as acute inflammation or chemotherapy. We have also analyzed the role of the two TNF α receptors, both *in vivo* and *in vitro* neurosphere cultures using specific TNFR1 and TNFR2 agonist and TNFR *knock-out* mice. We found that TNF α modulates proliferation, self-renewal and the balance of symmetrical/asymmetrical divisions of neural stem cells in culture and that each receptor mediates a different biological response. Furthermore, both TNF α and its receptors, among other tested inflammatory and neurogenic genes, appear up-regulated after SEZ homeostasis disruption indicating a hypothetical role in NSC activation and SEZ remodeling/regeneration.

075

ANALYSIS OF OF LRRK2 MEDIATED PARKINSON'S DISEASE PATHOGENESIS USING FUNCTIONAL PHOSPHO PROTEOMICS FROM HUMAN IPSC DERIVED NEURONS

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Parkinson's disease (PD) affects over a million Europeans and is expected to significantly increase. No effective treatments are available to prevent or delay disease pathogenesis, and better treatments are urgently needed. The mutation G2019S in the gene *LRRK2* is the most common genetic cause of PD currently known, and is thought to cause PD by increasing LRRK2 kinase activity. Consistent with this finding, inhibition of LRRK2 kinase activity protects against *LRRK2* G2019S induced pathogenesis. However, the exact targets of LRRK2 kinase activity in human neurons remain unknown. To better understand the mechanism of mutant *LRRK2*-induced PD, our group recently generated isogenic induced pluripotent stem cells (iPSCs) from patients

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with *LRRK2* G2019S, as well as gene-corrected wild type control iPSCs thereof. We demonstrated that iPSC-derived midbrain dopaminergic neurons (mDANs) recapitulated key aspects of PD pathogenesis including increased degeneration and increased alpha-Synuclein protein compared to isogenic controls. Inhibition of LRRK2 kinase activity with various LRRK2 kinase inhibitors protected against PD pathogenesis, suggesting that LRRK2 actively phosphorylates a disease-relevant target. To identify LRRK2 kinase targets in iPSC-derived mDANs, we used mass-spectrometry based phospho-proteomics. Interestingly, we found specific serine residues in TAU as being phosphorylated in a LRRK2-dependent manner. This is particularly interesting because TAU mutations cause Parkinsonism, and TAU pathology has been reported in some PD patients with mutant *LRRK2*. Moreover, we demonstrated previously that TAU expression is higher in human iPSC-derived neurons with *LRRK2* G2019S, compared to isogenic controls. Currently, we are using CRISPR/Cas9-mediated gene targeting to mutate these phospho-sites to further assess their contribution to mutant LRRK2-mediated PD. These results suggest a novel and direct connection between two key mediators of PD pathology: TAU and LRRK2.

077

ORGANELLE TRAFFICKING IS SEVERELY IMPAIRED IN HUMAN NEURONS DERIVED FROM CHOREA ACANTHOCYTOSIS PATIENTS

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Chorea Acanthocytosis (ChAc) is an autosomal recessive inherited disease caused by loss- of-function mutation in the *VPS13A* gene which encodes CHOREIN protein. The yeast homolog of *VPS13A* functions in vesicle and mitochondrial trafficking. These processes are aberrantly regulated in Chorea Huntington (HD) which is phenotypically very similar to ChAc, and suggests that they play an important role in ChAc pathogenesis. Therefore, this study focuses on vesicle and mitochondria trafficking. Live imaging of neurons differentiated from induced pluripotent stem cells (iPSCs) revealed a decreased lysosomal count in neurites and reduced motility of mitochondria. Mitochondria were abnormally shortened, reminiscent of vesicles. Characterization of patient mitochondria

by respirometry showed diminished function. Feasibly, the disease progression of HD and ChAc share a common underlying mechanism. Furthermore differentiated neurons show an increased susceptibility to proteotoxic stress and unfolded protein response. Currently we are investigating genes identified as hallmarks of trafficking impairment in HD, cytoskeleton dynamics and applying selective treatment to further characterize the observed phenotypes.

079

MODELLING NEURODEGENERATIVE DISEASES ON ISOGENIC GENE CORRECTED IPS CELLS

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Induced pluripotent stem (iPS) cell technology allows studying diseases in a relevant cell type and therefore has the potential to capture pathomechanistic effects that may not be present in conventional *in vitro* model systems. iPS cells derived from patients with a pathogenic familial mutation can be used to investigate dysfunction of such mutations in a patient-specific context without artificial overexpression. Gene-editing using TALEN or CRISPR technology is a powerful tool that enables the generation of isogenic controls differing for example in only a single DNA base. As such, the use of isogenic lines allow investigating the dysfunction of a pathogenic familial mutation very precisely on an identical genomic background. Here, we report the generation of iPS cells from patients with a familial form of Alzheimer's disease (AD), frontotemporal dementia (FTD) and spinocerebellar ataxia type 2 (SCA2). All patients harbored either a single base mutation (AD and FTD) or an elongated CAG repeat (SCA2) causing the respective diseases. iPS cells were generated using a plasmid-based, non-integrative system. Through TALEN or CRISPR mediated gene-editing, we obtained both gene-corrected isogenic controls as well as healthy iPS cell lines with inserted hetero- or homozygous mutations. Upon differentiation to particular neuronal subtypes, we are investigating diverse disease-related phenotypes by comparing them to their isogenic gene-corrected controls. Interestingly, phenotypes are often not detectable when patient iPS cell-derived neurons are compared to neurons from age and gender matched healthy controls, potentially due to too high genetic variability. Our work therefore aims to highlight the power of iPS cell-derived neurons in combination with gene-editing techniques to uncover specific disease-related phenotypes in the culture dish.



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LONG-TERM EXPANSION OF DOPAMINERGIC PROGENITORS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS (iPSCs) AND DIFFERENTIATION INTO MATURE AND FUNCTIONAL DOPAMINERGIC NEURONS IN VITRO AND IN VIVO

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Mesencephalic dopaminergic neurons (mDA) are the main source of dopamine in the brain. The degeneration of mDA cells and the consequent destruction of the nigrostriatal network cause Parkinson's disease (PD). Despite numerous advances in our understanding having been made in the last few years, the molecular mechanisms leading to PD are not fully understood. Functional DA neurons derived from human induced pluripotent stem cells (iPSCs) are an important system to improve our knowledge of mDA development and differentiation, and potentially to identify new molecular mechanisms that underlie the disease. Dopaminergic neuron differentiation from human pluripotent stem cells has been described previously. Important morphogens for midbrain development, especially FGF8 and SHH, promote the appearance of LMX1A⁺ and FOXA2⁺ progenitors on day 11 *in vitro*. In order to expand these ventral mid/hindbrain progenitors as a potential source of enriched mDA neurons, we have developed and refined a new protocol. We describe a method for generating large numbers of mDA progenitors that can be maintained and passaged for more than one month *in vitro* as a homogeneous population while retaining their marker expression and differentiation potential. Most importantly, the mDA progenitor expansion results in a 5-fold increase in the percentage of TH⁺ cells. At later stage of differentiation, expanded mDA progenitors give rise to higher number of TH⁺ neurons that co-express the typical midbrain markers including NURR1, DAT, AADC, GIRK2 and VMAT2. Furthermore, we confirmed the generation of mature and functional human iPSC-derived mDA neurons *in vitro* by dopamine release and electrophysiology properties. Transplantation of expanded mDA progenitors in the mesencephalon of mouse embryos shows a successful cell survival and cell integration in the host. Human iPSC-derived expanded mDA progenitors give rise to TH⁺ neurons *in vivo*, they exhibit neuronal morphology and express the typical dopaminergic markers such as FOXA2 and NURR1. In summary, our results describe a novel method for long-term expansion of large number of dopaminergic

progenitors that retain their differentiation potential to generate mature and functional mDA neurons *in vitro* and *in vivo*.

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ASTROGLIAL CELLS PRODUCE A STEM CELL NICHE-LIKE EXTRACELLULAR MATRIX AFTER CORTICAL LASER LESIONS IN THE MOUSE

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The mammalian central nervous system (CNS) shows a limited regeneration capacity after lesion. In contrast, other species like amphibians regenerate nervous tissue successfully. This indicates the existence of limiting factors that inhibit proper recovery of patients. In this regard, the extracellular matrix (ECM) is of interest, as it contains signaling molecules that regulate survival, proliferation, migration, differentiation of cells as well as neurite outgrowth. Therefore, the postlesional ECM composition and cells that produce this matrix are candidates for the development of new treatment strategies. We use laser lesions of the mouse visual cortex as a model for regeneration and plasticity because of its convenient accessibility and the extensive knowledge about plasticity on the cellular and molecular level in this region. Plasticity induced by laser lesions has been well described in the cat and rat cortex, whereas the mouse system allows to use knockout animals. Immunohistochemical analysis revealed reactive gliosis with changes in the ECM composition that were associated with astroglia: The stem cell-related DSD-1 carbohydrate epitope was detected on cells near the lesion. Tenascin-C, a glycoprotein that regulates differentiation and axon growth and that is expressed during development and after injury, was also up-regulated. After three and 14 days, astroglial cells expressing one or more of the markers nestin, glial fibrillary acidic protein (GFAP), vimentin, or S100 β were not distributed equally. Nestin expression was restricted to a small area adjacent to the lesion, GFAP showed a broader up-regulation, whereas vimentin was found in an intermediate pattern. With the DSD-1 epitope and tenascin-C, we identified ECM components of astroglial origin that resemble a stem cell niche-like environment for weeks. In fact, stem/progenitor-like cells have been described in the injured CNS and the marker expression indicates the existence of astroglial subpopulations that might be multipotent, at least *in vitro*. Multipotent, neurosphere-forming cells have been shown in the rat. This suggests that astrocytes adopt, depending on the distance to the lesion and hence in presence of a specific ECM composition, stem cell-like features and represent a potential intrinsic source of new neurons.

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DIRECTLY REPROGRAMMED OLIGODENDROCYTE PRECURSOR CELLS ENHANCE FUNCTIONAL RECOVERY FOLLOWING SPINAL CORD INJURY IN RATS

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Spinal cord injury (SCI) results in neuronal degeneration and demyelination due to the secondary injury and oligodendrocyte apoptosis at the lesion epicenter. Demyelination leads to prolonged latency and slowed conduction velocity after SCI and finally affect to inhibit functional restoration. Direct reprogramming of somatic cells into oligodendrocyte precursor cells (iOPCs) may enhance remyelination following SCI without the risk of tumor formation and immune reactions. In this study, we had transplanted iOPCs into contused spinal cord of rats for the first time. Eight weeks after transplantation, transplanted iOPCs had survived and migrated adjacent to the injured spinal cord, and successfully differentiated into NG2⁺, APC-CC1⁺, O4⁺, and MBP⁺ cells which indicates mature oligodendrocytes. Transcranial magnetic motor-evoked potentials (MEPs) also revealed that conduction velocity and onset latency were improved, and the bladder and locomotor dysfunctions were decreased than control at 8 weeks after transplantation. Therefore, we conclude that iOPCs are helpful for the functional following SCI in rats and might be a good *source* for clinical translation of stem cell therapy to SCI patients.

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SURVEY TO SPINAL CORD INJURY PATIENTS: AWARENESS FOR STEM CELL THERAPY IN SOUTH KOREA

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This study is analysis of survey to patients with spinal cord injury (SCI) for investigation of their awareness on clinical trials of stem cell therapy (SCT). Although, SCT have been tried to SCI and continued related researches in many countries including South Korea last decade, SCT has not yet been approved in the clinical parts. Here, we performed the survey to SCT- received and SCT-non received SCI patients about SCT via individuals in South Korea and analyzed the responses of the questionnaire. The questionnaire consisted with information of respondents, participation motivation, willingness to participate, expectations, complications, opinion about personal cost, necessity of modified rehabilitation,

perception of problems, and improvement measures for SCT on SCI patients. We categorized the subjects to fundamental distinction based on the questionnaire, such as age, gender, education, damage level and status of SCI, duration of SCI, and complications. Expectation of slight-to-definite functional improvement following SCT was higher on SCT-received patients (91.3%) than on SCT-non received patients (58.4%), and there was no difference between genders. Source of information about SCT was higher on healthcare provider (36%) or word of mouth (44%) than on television or internet (20%) in SCT-received patients, in contrast, was greatest on television or internet (73%) in SCT-non received patients. Both SCT-received and -non received patients have apprehension about side effects following SCT and the reason for not participating to SCT was highest on complications (54%) in SCT-non received patients. All the patient groups concurred with the necessity of modified active rehabilitation program considered to SCT. In summary, the highest concern about SCT was side effects concurrently in both SCT-received and -non received patients and SCT-received patients group has higher expectation to SCT.

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NEURONAL MACROAUTOPHAGY MODULATION AS A THERAPEUTIC STRATEGY FOR NEURODEGENERATIVE DISEASES

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Macroautophagy is a key degradation pathway that controls protein homeostasis by eliminating misfolded proteins, protein aggregates and damaged organelles. Macroautophagy begins with formation of double-membrane-bounded autophagosomes that engulf cargo and target it to lysosomes for degradation. Defects in the macroautophagy process may theoretically occur at a variety of steps, from the initial formation of a pre-autophagosome limiting membrane, to the ultimate fusion of mature autophagosomes with the lysosomal compartment. Defective macroautophagy has been associated with a variety of neurodegenerative disorders, including AD, PD and FTD. In addition, stimulation of macroautophagy has been shown to reduce the levels of toxic protein aggregates in these proteinopathies. Blood brain barrier penetrating compounds enhancing neuronal autophagy flux are thus expected to alleviate macrophagy defects in brains of patients suffering from a neurological condition. Importantly recent studies indicated that autophagy might be differentially regulated in proliferating cells and post-mitotic neurons. We are establishing biochemical assays that could be used for quantification of autophagy flux markers LCII and p62 in neurons obtained by directed differentiation of human



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induced pluripotent stem cells. Our aim is to use these assays to discover small molecule that can enhance the autophagic pathway for the benefit of patients with neurodegenerative disorders.

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GLYCOCONJUGATES ARE DIFFERENTLY EXPRESSED ON DISTINCT TYPES OF NEURAL STEM CELLS DERIVED FROM HIPSCS

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The differentiation capacity of human induced pluripotent stem cells (hiPSCs) enables to study early human development. The progression of hiPSCs is characterized by different morphological stages with the expression of various marker molecules. The utilization of surface antibodies as biomarkers is a useful tool to identify specific glycan motifs at distinct stages of differentiation from hiPSCs to human neural stem cells (hNSCs). We used the monoclonal antibodies (mAbs) 487^{LeX}, 5750^{LeX}, and 473HD to analyze the expression pattern during neural differentiation, starting with hiPSCs. Thereby the LeX antibodies detect structural variants of the LewisX glycan (LeX) and the mAb 473HD detects the DSD-1 chondroitinsulfate glycosaminoglycan (GAG) epitope. We reveal that 487^{LeX}, 5750^{LeX}, and 473HD-related glycans are differently expressed at the pluripotent stage. Furthermore, we show that these surface antibodies can be used to detect cells of the three germ layers. After neuralization of human embryoid bodies (hEBs) into human neural aggregates (hNAs), a subpopulation of cells can be detected by these antibodies. At human rosette-stage NSCs (hR-NSCs) we demonstrate that LeX- and DSD-1-related epitopes are differently expressed and show antibody-specific expression patterns. In addition these mAbs can be used to distinguish hR-NSCs from human small rosette-stage NSCs (hSR-NSCs). The characterization of human NSCs cultivated in FGF-2 and EGF (hNSCs^{FGF-2/EGF}) as a later stage reveals that both LeX antibodies and the 473HD antibody label only a subpopulation of these cells. Taken together, this study reveals that the 487^{LeX}, 5750^{LeX}, and 473HD-related glycan expression is spatially and temporally regulated during neural differentiation. These mAbs can be used as biomarkers for specific stages during early human development.

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DEVELOPMENTAL ENDOTHELIAL LOCUS-1 IS A FACTOR OF HEMATOPOIETIC STEM CELL NICHE.

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HSC reside in a specific micro-anatomical region in the bone marrow (BM), termed as hematopoietic stem/progenitor cell (HSPC) niche. Endothelial cells are a main player in HSPC niche, having critical role in the maintenance of HSPC. Developmental endothelial locus-1 (Del-1) has been previously identified as a ligand for the integrins LFA-1 and $\alpha\beta 3$. Herein, we study the role of Del-1 in the regulation of hematopoiesis. We identified sinusoidal endothelial cells as the main cell population that expresses Del-1 in the BM. Del-1 deficiency induced increased quiescence of HSPCs in mice under homeostasis, as shown by cell cycle analysis. Reduced proliferation potential in situations that mimic stress hematopoiesis, including transplantation, LPS and G-CSF administration, was further observed, resulting in an impaired HSPC expansion and production of myeloid progenitors and mature cells, proposing a role for Del-1 in myeloid lineage development. Endothelial overexpression of Del-1 resulted in the expansion of HSPC pool. Downregulation of the cyclin D1 was correlated with the decreased proliferation potential of HSPC in Del-1 deficient mice. Del-1 was further shown to promote cyclin D1 expression through direct interactions with the $\alpha\beta 3$ integrin expressed in HSPCs. In conclusion, we identified Del-1 as a novel niche factor in the regulation of HSC, promoting HSPC proliferation and myeloid lineage development under stress hematopoiesis.

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EXPANSION OF OLIGODENDROCYTE PROGENITOR CELLS FOR REGENERATION

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Oligodendrocyte progenitor cells (OPC) represent the most abundant and homogeneously distributed cycling cell population of the central nervous system (CNS). These cells give rise to mature myelinating oligodendrocytes that are proposed to play neuroprotective effects during injury or disease. The length of OPC cell cycle is remarkably long, in the range

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of weeks, and is longer in the cortex than in the corpus callosum, which is primarily due to a long G1 phase. Our group has shown that G1 can be shortened by overexpression of the cyclin D1/cdk4 (4D) complex and that this leads to the expansion of neural stem and progenitor cells and increased neurogenesis. Here, we developed a triple transgenic mouse line to conditionally and transiently overexpress 4D by Tet-On system specifically in cells of the oligodendrocyte lineage. Overexpression of 4D for three weeks resulted in a 3-fold increase in the proliferation of OPC in the cortex and the corpus callosum as compared to controls. Following this time, 4D overexpression was terminated and OPC fate assessed for the subsequent weeks. This showed that OPC proliferation returned to physiological levels upon turning off 4D, and current analyses are characterizing mature oligodendrocytes that originated from manipulated OPC. Excitingly, overexpression of 4D yielded an increased number of young mature oligodendrocytes in the corpus callosum, a differentiation-promoting environment, whereas the same could not be observed in the cortex. Characterization of survival of manipulated OPC, their differentiation and myelinating-abilities will be presented. Re-myelination is fundamental to promote the functional recovery of the CNS after injury or disease, for which we expect our expansion of OPC to be beneficial. Towards this we are currently establishing a model of photothrombotic stroke.

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THE ROLE OF NFκB IN NEURAL SPECIFICATION OF HUMAN EMBRYONIC STEM (HES) CELLS

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Nuclear factor kappa B (NFκB) is a family of transcription factors most notable its role in inflammation and tumorigenesis. However, in more recent years a number of studies have demonstrated that NFκB plays an integral part in neurogenesis and ageing. In terms of neural specification, activation of the canonical NFκB signalling pathway has been shown to be necessary for early neural stem cell (NSC) differentiation and inhibition of NFκB activity *in vitro* blocks neural maturation. The aim of this project is to clarify the role of NFκB throughout neural specification of hES cells using a reproducible targeted differentiation protocol. We have utilised a lentiviral reporter system to assess temporal NFκB activity during normal neural differentiation. We have found that as neural stem cells are serially passaged, they upregulate canonical NFκB signalling. Transcriptomic microarray analysis at key stages of

neural differentiation indicates that many NFκB targets, involved in a number of processes such as metabolism and cell cycle regulation are significantly increased in older neural progenitor cells.

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PHENOTYPIC PROFILING OF COMPARTMENTALIZED IPSC-DERIVED NEURONS WITH LIVE IMAGING TOOLS FOR MOLECULAR DISSECTION AND THERAPEUTIC INVESTIGATION OF NEURODEGENERATION

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The use of Induced pluripotent stem cells (iPSC) have pioneered modelling of human diseases. Since valid models of neurodegenerative diseases are often still lacking, iPSCs offer great opportunities especially for investigating the pathogenesis of neurodegeneration. Our group utilizes iPSC-derived neurons from patients of hereditary Amyotrophic Lateral Sclerosis (ALS), Chorea Acanthocytosis (ChAc), Neuronal Ceroid Lipofuscinosis (NCL), Parkinson and others. Our overall aim is to understand how perturbed axonal trafficking and metabolism of mitochondria and other organelles contributes to neurodegeneration, as the lengthy architecture of these outgrowths renders them particularly vulnerable for compromised delivery of energy, mRNA and other cargo to distal outposts. Using Xona Microfluidic Channel (MFC) cultures, we perform fast multi-channel live imaging on compartmentalized neurons with standardized readout windows in distal versus proximal axon parts with defined directionality (retrograde vs anterograde). Our tracking analysis has revealed distinct phenotypes across our library of iPSC clones, depending on affected gene and mutation. For example, one type often found for ALS mutations is perturbed motility and membrane potential of mitochondria in distal axons whereas in the proximal part trafficking functions normal ('gradient phenotypes'). Conversely, another distinct phenotype features abnormal elongation of mitochondria along with a moderate decrease of membrane potential on both the proximal and distal site ('global phenotypes'). Beyond a purely descriptive cataloguing, our phenotypic profiling comprises a standardized chemical and genetic interrogation protocol with bioactive compounds of known targets, thereby revealing the underlying pathways of distinct axonopathies. Of particular interest are compounds that either mimic or



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rescue a phenotype. Based on the paradigm of Modular Cell Biology, the response of a newly interrogated disease model allows its assignment to a phenotypic class, thereby pointing to the underlying mechanism. Collectively, we have established a powerful, analytic imaging platform of compartmentalized neurons for comprehensive modeling of neurodegeneration, molecular dissection and identifying novel therapeutic targets.

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THE ROLE OF CELL FATE CONVERSION IN VIVO DURING ADULT ZEBRAFISH TELENCEPHALON REGENERATION

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Zebrafish can regenerate several organs and tissues upon injury including the central nervous system (CNS). In contrast, traumatic brain injury (TBI) to the adult mammalian CNS leads to life-long loss-of-function without neural regeneration. The cellular and molecular mechanisms that enable or prevent adult CNS regeneration have not been completely elucidated but a comprehensive understanding of cellular contribution towards newly generated neurons plays a pivotal role in better understanding CNS regeneration. To study these mechanisms in adult zebrafish telencephalon, a stab lesion assay was developed in our laboratory, and cellular reactions to TBI have been analyzed. Several cell types have been shown to proliferate upon TBI. Furthermore a subtype of ventricular radial glial stem cells, a known neural progenitor, proliferates and generates neuroblasts that migrate to the lesion site and express mature neuronal as well as synaptic markers. However, the contribution of other reactively proliferating cells and their fate towards neural lineage was not addressed so far. In vivo lineage conversion is currently discussed as a possible therapeutic strategy for neurodegenerative conditions in mammals, using misexpression of lineage converting transcription factors. Here, we used hematopoietic stem cell transplantation and Cre/loxP lineage tracing to determine whether proliferating non-neuronal progenitor cell lineages show any plasticity and transdifferentiate to neuronal-marker expressing cells in vivo after TBI. So far, we find no new neurons of donor descent. Microglia / macrophages, oligodendrocyte precursors, myelin expressing mature oligodendrocytes, endothelial cells and pericytes do not convert to a neuronal lineage following TBI. Moreover, nitroreductase based radial glial cell ablation impaired reactive neurogenesis and regeneration. Our results indicate that radial glial cells are the key source of newly generated neurons during regeneration and in vivo transdifferentiation is a rare event or even absent in the regeneration-capable adult zebrafish telencephalon, in spite of the challenges of TBI.

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DERIVATION OF RETINAL ORGANIDS FROM HEALTHY AND JNCL PATIENTS' IPSCS

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Neuronal Ceroid Lipofuscinosis (NCL) are a group of rare progressive neurodegenerative lysosomal storage disorders characterized by the accumulation of autofluorescent lipofuscin in many tissues, including neurons. The most common form is the juvenile (JNCL or Batten disease), caused by a mutated version of *CLN3* gene. JNCL leads to retina degeneration, followed by CNS neurodegeneration and premature death. Pathomechanisms are mostly unknown, therapies are missing and animal models do not completely recapitulate the disease. In fact, whereas in JNCL patients retina is affected first, retinal defects are not severe and appear only later in JNCL mouse models. Therefore, human cell based-models might enable new insight into pathomechanisms and therapy development. hiPSC have previously been generated from fibroblasts of two JNCL patients, one healthy donor carrier for the common mutation and one healthy donor not carrier for the mutation. Our aim is to generate retinal progenitor cells and retinal neurons from JNCL and healthy donors-derived hiPSC in culture using a 3D retinal organoid approach. Our results show that both JNCL patients and healthy donors-derived hiPSC could be differentiated towards eyefield with high efficiency ($\approx 79\% \pm 13$, $n=116$, $N=3$). Further, we found that both lines have the capacity to develop into neural retina and into RPE. In the retinal organoids generated from both hiPSC-lines, we detected retinal ganglion cells, amacrine, cone and rod photoreceptors, born in the same order as in mammals in vivo. We conclude that JNCL hiPSCs can be used to obtain JNCL patients'-specific neural retina and pigmented epithelium. Because of its early retinal disease onset, progressive neurodegeneration and potential involvement of different retinal cell types, we hypothesize that iPSCs-derived retinal organoids will be a great tool to model JNCL disease, decipher the identity and timing of JNCL pathology in retinal neurons, investigate pathomechanisms of JNCL disease and possibly enable developing and validation of therapeutic approaches. Further human retina JNCL disease model would represent a proof of principle for other retinal

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diseases with lipofuscin accumulation, such as Best disease and age-related macular degeneration (AMD).

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ASSESSING THE CONTRIBUTION OF TAU PHOSPHORYLATION TO MUTANT LRRK2-INDUCED PARKINSON'S DISEASE USING GENE EDITED IPS CELLS

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LRRK2 G2019S is the most common genetic mutation known to cause Parkinson's disease (PD). **G2019S** increases LRRK2 kinase activity, and inhibition of **LRRK2** G2019S kinase activity ameliorates neurodegeneration. Previously, we used phospho-proteomics to identify potential LRRK2 kinase targets in human iPSC-derived neurons, and identified specific serine residues, in TAU as being phosphorylated in a LRRK2-dependent manner. In order to validate this data, we treated mature midbrain dopaminergic neurons (mDAN) for 24h with a specific LRRK2 kinase inhibitor. Western blot analysis indicated that TAU phosphorylation in mDANs is significantly decreased by LRRK2 kinase inhibition. To assess the contribution of TAU phosphorylation to mutant **LRRK2**-mediated PD, we abolished one phosphorylation site within the **MAPT** locus, which encodes TAU, using CRISPR/Cas9-mediated homology-directed repair (HDR). For this purpose, we tested two different strategies: In one approach we used a single-stranded DNA oligonucleotide (ssODN) as a repair template whereas the other approach is based on a specifically designed repair vector harboring an additional zeocin resistance cassette to increase gene editing efficiency. Gene targeted clones are currently being differentiated into mDANs via an expandable neural progenitor in order to facilitate phenotyping of the isogenic iPSC lines with wild type and mutant TAU. We were therefore able to generate a valuable new cell line to investigate LRRK2-mediated PD.

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DIRECT REPROGRAMMING OF REACTIVE ASTROCYTES TO DIFFERENTIATED NEURONS IN VITRO AND IN VIVO FOLLOWING FORCED EXPRESSION OF NEUROGENIC MOLECULES AND MIRNAS

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Recent studies demonstrate that astroglia from non-neurogenic brain regions can be reprogrammed into

functional neurons through forced expression of neurogenic factors. Following brain injury, astroglia surrounding the injury area acquire or reactivate stem cell potential as part of reactive gliosis, therefore highlighting a cell source outside the stem cell niche being directed towards a neurogenic fate. For the above reasons, in our studies we have explored the role of astrocytes as the cell source to restore connectivity following brain injury. To this end, we have tested the regenerative potential of neurogenic genes Cend1 and Neurog2 and miRNA miR-124 in directing the reprogramming of activated astrocytes, both in vitro and in vivo following traumatic brain injury, towards a neuronal phenotype. Forced expression of Cend1, Neurog2, miR-124 or their combinations in vitro resulted in acquisition of induced neuronal cells expressing β III tubulin and the subtype-specific markers GABA, glutamate or tyrosine hydroxylase. Surprisingly, a subpopulation of Cend1/Neurog2-double transduced astrocytes formed highly proliferative spheres exhibiting neural stem cell-like properties and multipotentiality, while, knock-down of endogenous Cend1 mRNA demonstrated that it is a key downstream mediator of Neurog2-induced neuronal reprogramming of astrocytes. Importantly our vivo data also suggest that 3- weeks following injury and subsequent viral transduction, a significant percentage of Cend1, Neurog2, or miR-124-transduced cells in the astrogliosis area surrounding the lesion site not only exhibit neuronal morphology, but also express the early neuronal marker Doublecortin, indicating that transduced cells have acquired an immature neuronal phenotype. Further phenotypic analysis and intravital imaging studies are in progress to follow in real time activated astrocytes dynamics and in vivo trans-differentiation to neurons following neurogenic factors forced expression. We expect that utilizing endogenous reactive astrocytes as a neuron-producing cell source and studying the spatiotemporal sequence of events occurring in the neurovascular niche following brain injury, will contribute towards a better planning of strategies to treat neurodegeneration.

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DEFINED DIFFERENTIATION TOWARDS LOW GFAP EXPRESSING HUMAN ASTROCYTES FOR DISEASE MODELING

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Pioneer studies over the last decade have enlightened the importance of astrocyte heterogeneity in the human brain. Moreover, vast differences between rodents and human astrocytes (for eg. increase in cell volume, number of processes) make human astrocytes preferable to investigate their basic physiology and role in neuro-



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degenerative disorders. However, *in vivo* studies from human samples are restricted due to ethical concerns and limited availability. Thus, *in vitro* approach becomes handier, especially with the potential of human induced pluripotent stem cells (hiPSCs) to generate distinct cell types. Although serum is widely used for primary cultures and astrocytic differentiation, it has been shown to induce non physiological changes, rendering these astrocyte preparations inconsistent and unsuitable for comparison to *in vivo* condition. In the last couple of years, several studies have reported differentiation of astrocyte from hiPSCs using serum, CNTF or BMP and LIF in a time span of about 6 months. However, all of these studies focus on producing high-GFAP expressing astrocytes, thereby leading to under appreciation of astrocyte heterogeneity. In this study, using a small molecule based approach; we have developed a defined protocol to generate low-GFAP expressing, functionally active astrocytes from hiPSCs, following the conventional STAT1/3 pathway. Astrocyte identity was confirmed by the expression of different markers like ALDH1L1, Acsbg1, EAAT1 etc. Furthermore, maturation of differentiated cells was confirmed by a significant abrogation of the proliferation marker- Ki67. At the functional level, these cells lack spontaneous or K⁺ evoked calcium oscillations; however, they exhibit calcium transients upon stimulation with L-glutamate or ATP. Similar to *in vivo* astrocytes, they display the potential to uptake glutamate from the extracellular solution. Also, these low-GFAP astrocytes up regulate GFAP and other reactivity markers when exposed to acidic condition or inflammatory agents like TNF- α and LPS. With these functional characteristics and short differentiation time, small molecule derived low GFAP expressing astrocytes provide a platform to explore the role of astrocyte subtypes in brain physiology as well as pathology.

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TARGETING DYSFUNCTIONAL MEMORY SYSTEMS WITH ADULT SKIN DERIVED NEURAL PRECURSORS

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Repopulation of brain circuits by neural stem cells is a promising therapeutic strategy under investigation in animal models of neurodegeneration. Using our novel adherent culture system we are able to isolate a population of neural precursor cells from small samples of adult skin. Here, we assess the *in vitro* and *in vivo* features of these cells and their therapeutic potential following intra-hippocampal transplantation. These skin derived neural precursors are highly homogenous in culture, rate-limited, and differentiate almost exclusively into neurons without expression of glial molecular markers. Cell transplantation into the hippocampus is safe and leads to widespread neuronal engraftment and synaptogenesis. Donor cells also become electrophysiologically active, establish synaptic connections and integrate into host brain neuronal circuitry. Moreover, in aged rats, selective hippocampal-dependent place recognition memory deficits are reversed, restoring memory function back to levels equivalent with young rats. This unique population of neural precursor-type cells surmounts many of the major limitations preventing the clinical translation of other stem cell based therapies, namely a propensity for glial differentiation and the use of genetic modifications. Adult skin derived neural precursors are easily accessible, readily available, patient specific, and show a significant therapeutic effect following transplantation in aged rats. Consequently, they may have potential for use in a cell-based therapy to regenerate or replace neurons lost from neurodegenerative disorders.

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FULLY AUTOMATED EXPANSION AND NON-INVASIVE QC OF HUMAN IPSCS ON THE STEMCELLFACTORY

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The biomedical prospects of human induced pluripotent stem cells (hiPSCs) have created an urgent need for standardized and automated processes for reprogramming and expansion of hiPSC lines from large patient cohorts. This need can be met by the StemCellFactory (www.stemcellfactory.de), a large system integration that automates all required cell culture steps, ranging from adult human dermal fibroblast (HF) expansion via feeder-free, Sendai virus-based reprogramming to clonal selection and enzyme-free expansion of the obtained hiPSC clones and lines. The implementation of on-line measurement technologies is key for the establishment of a fully automated production process on the StemCellFactory.

Here we report the implementation and biological validation of non-invasive measurement technologies for QC of hiPSC cultures and for scheduling of fully automated

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splitting processes on the StemCellFactory. For on-line assessment of metabolic activity and detection of potential bacterial contamination we implemented a plate reader and established instruments setting that enabled us to detect a shift of pH and/or turbidity. We show that absorption can be used as a parameter for automated QC of hiPSC cultures. Furthermore, an automated microscopy platform was implemented to schedule and perform fully automated long-term cultivation of hiPSCs. To that end we developed a confluence detection assay that enables dynamic feedback by computing confluence-based splitting ratios. For parallel and fully automated hiPSC generation and cultivation, well-based and plate-based automated splitting protocols were developed, which enable clonal expansion of individual hiPSC clones in 24-well plates as well as scaled production of hiPSCs in 6-well plates. Validation by immunocytochemical and in vitro differentiation assays confirmed that automatically expanded hiPSCs remain pluripotent for at least 10 passages. Our data show that dynamic feedback via generation and analysis of in-process data largely facilitates automation of highly dynamic cell culture processes.

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TRANSPLANTATION OF MOUSE ESC-DERIVED PHOTORECEPTORS INTO THE MOUSE RETINA

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Vision is dependent on light sensitive cells in the retina - photoreceptors - and their degeneration is the leading cause of blindness in the developed world. To date, there is not cure available and several therapeutic approaches are being developed including gene therapy and neuroprotection but most treatments rely on the presence of endogenous photoreceptors. Thus, photoreceptor replacement presents a promising alternative to restore visual function of degenerated retinas. Most photoreceptor replacement studies used primary cells transplanted into slow retinal degeneration mouse models, which does not recapitulate an eventual clinical scenario. Additionally, the use of primary human donor cells would raise several ethical, logistic and supply concerns that would be avoided with the use of a renewable and expandable donor cell source such as embryonic- or induced pluripotent stem cells. Hence, we took advantage of Sasai's protocol for the in vitro generation of 3-dimensional optic cups to successfully

differentiate mouse embryonic stem cells (mESC) along the photoreceptor lineage. mESC-derived photoreceptors were characterized and enriched using magnetic activated cell sorting (MACS), reaching a purity of 85%. Enriched photoreceptors were subsequently transplanted into the subretinal space of different mouse models and analyzed by immunohistochemistry three weeks after transplantation. Engrafted mESC-derived photoreceptors survived in the subretinal space up to three weeks and expressed mature photoreceptor and synaptic markers. In conclusion, we demonstrate the possibility of mESC-derived photoreceptor enrichment and transplantation into the subretinal space of the mouse retina. These results show, as proof-of-principle, the potential of mESC-derived photoreceptors transplantation for future cell replacement therapies for retinal diseases such as age-related macular degeneration and retinitis pigmentosa.

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JOINING THE DOTS: A MULTI-FACETED PHENOTYPE INCLUDING THE SELECTIVE VULNERABILITY OF THE SNCA (A30P) PATIENT-DERIVED MIDBRAIN DOPAMINERGIC NEURONS TO ER STRESS

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Parkinson's disease (PD) is the most common movement disorder characterised by the selective degeneration of A9 dopaminergic neurons in the Substantia nigra pars compacta of the midbrain and the presence of intracytoplasmic inclusions: Lewy Bodies, in those neurons that remain. PD is typically characterised by its 4 cardinal motor symptoms: bradykinesia, resting tremor, gait difficulty and postural instability. However PD has many non-motor symptoms such as depression that can occur up decades before diagnosis. PD currently affects over 1.2 million people across Europe with close to 1200 confirmed PD cases in Luxembourg. The annual cost of PD is estimated to be 13.9 billion euros while the number of people with PD is set to double by 2030 with an increasing ageing population. Following the discovery that an A53T point mutation in **SNCA** encoding the α -synuclein protein leads to an autosomal dominant form of inheritance in a familial case of PD, further point mutations, duplications and triplications of **SNCA** have been identified to predispose an individual towards PD, with several Genome Wide Association Study (GWAS) implicating the variability at the **SNCA** locus as a major risk factor in idiopathic PD. Patient-derived fibroblasts were donated with informed consent from the index patient with an A30P point mutation in **SNCA** and reprogrammed into induced pluripotent stem



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cells (iPSCs). These iPSCs were then differentiated using small molecules into neuronal precursor cells (smNPCs) to generate a renewable and homogeneous population that can be further differentiated into midbrain dopaminergic neurons (mDANs). Comparing the *SNCA* (A30P) mDANs to an age- and gender-matched non-PD controls, we were able to functionally characterise these mDANs, observing synchronous firing and also the detection of dopamine using gas chromatography mass spectrometry (GCMS). Furthermore phenotypic differences were also found within the mitochondria, the ER and also on the metabolomic level. It would be the subsequent aim of this research to further explore the mechanism behind these phenotype(s) and attenuate these differences using small molecules in order to attempt to rescue and prevent the selective vulnerability of these mDANs which contributes to the pathogenesis of PD.

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GENERATION OF HIPSC-DERIVED MYOGENIC CELLS TO STUDY MUSCLE DEVELOPMENT AND AXON-MUSCLE CONTACTS IN AUTISM SPECTRUM DISORDERS

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Phelan-McDermid syndrome (PMS), also known as 22q13.3 deletion syndrome, is a genetic disorder resulting from a heterozygous de novo deletion of the distal long arm of chromosome 22. The majority of neurological defects is thought to result from a haploinsufficiency of the gene *SHANK3*, which is deleted in almost all PMS patients. *SHANK3* belongs to the *SHANK* proteins, a scaffolding protein family of the postsynaptic density at excitatory synapses but also in motor endplates of muscle cells. PMS is classified as an Autism Spectrum Disorder, as the core symptoms of PMS include global developmental delay, absent or severely delayed speech, intellectual impairment, an increased risk for autism or autistic-like behavior and neonatal hypotonia. Hypotonia, usually the first presenting symptom in affected children, manifests in a general muscle weakness and has great influence on the patient's development. Therefore, the investigation of myogenesis and the neuromuscular junction (NMJ), the site of connection between a lower motor neuron and a skeletal muscle fiber, of PMS patients is of great interest. Human induced pluripotent stem cells (hiPSCs) offer a great tool for the generation of patient-derived cells carrying a specific mutation, including motor neurons and skeletal muscle cells. In the present study, hiPSC-derived myogenic cells were generated using different protocols. Firstly, myogenic cells were generated by PAX7 induction and secondly using a non-

genetic approach by adding myogenic factors. Mature myogenic cells revealing cell fusion, multi-nucleation and cross striation were used for the analysis of developmental expression of myogenic markers as well as morphological features in patient and control cells lines. In addition, when myogenic cells were co-cultured with motor neurons NMJs were formed, revealing a motor unit formation between hiPSC-derived cells from the same patient. A reliable generation of hiPSC-derived muscle cells provides new insights into the properties of human muscle cells and is a prerequisite for mimicking the formation of the neuromuscular junction *in vitro*, which is of great importance for patients suffering from hypotonia, for example PMS patients.

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GENERATION OF HUMAN IPSC-DERIVED ASTROCYTES TO INVESTIGATE ASTROCYTE BIOLOGY IN NEURODEGENERATIVE DISEASES

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For more than 100 years the neuron was accepted as the most important cell type for cognitive function in the brain. Astrocytes were discussed to have only supportive functions in the central nervous system "filling the gap" between neurons. However, there is growing evidence that the contribution of astrocytes is far more complex and critical for brain function in physiology and pathology. Astrocytes are important regulators of brain homeostasis metabolism and synaptic activity thus play a critical role to achieve normal brain function. Astrocyte dysfunction is also indicated to be critical in neurodegenerative disorders like Alzheimer's Disease (AD). A better understanding of astrocyte biology and their interplay with neurons will help to develop disease modifying treatments. In this study, we differentiated mature human astrocytes from control and AD-patient derived iPSCs to study their role in disease in greater detail. To uncover the potential role of human astrocytes in synaptic maturation hiPSC-derived astrocytes were used in co-culture experiments with hiPSC-derived neurons. In addition the extracellular matrix (ECM) of these cells was used as a substrate for hiPSC-derived neurons in the same context. Synaptic marker expression and excitotoxicity were measured to assess neuronal maturity. Both, direct co-culture as well as astrocyte-derived ECM lead to increased neuronal survival. Furthermore, astrocyte ECM had a proliferative effect on neuronal precursor cells. In a next step we successfully differentiated human astrocytes from AD-patient derived hiPSCs. IF staining and qPCR revealed the expression of astrocyte markers (e.g. GFAP, S100 β). Using a next generation sequencing approach we aim to identify

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novel disease relevant genes in hiPSC-derived astrocytes from AD patients. These cells will also be used to build *in vitro* models for AD dissecting their role in the disease process, ultimately identifying patient-relevant astrocyte pathology. This project was supported by the IMI project "STEMBANCC" (Grant number 115439-2). Disclosures: All authors are employees of AbbVie. The design, study conduct, and financial support for this research were provided by AbbVie. AbbVie participated in the interpretation of data, review, and approval of the publication.

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ROLE OF ASTROCYTES IN FORMATION OF FUNCTIONAL NEURONAL NETWORKS IN VITRO

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Astrocytes are the most numerous cell type in the central nervous system involved in normal development as well as dysfunctions of neuronal networks. Besides providing metabolic support for neurons, recent studies show astrocytes having also more active roles in the brain function. They can control synaptogenesis and have even been suggested to participate in synaptic transmission and plasticity, although still controversial. On the other hand, astrocytes are increasingly being recognized as contributors to several nervous system dysfunctions including neurodevelopmental diseases (Down's, Fragile X and Rett syndrome), psychiatric diseases (schizophrenia) and neurodegenerative diseases (epilepsy, Alzheimer's disease). Advances in the stem cell field have enabled differentiation of human pluripotent stem cells (hPSCs) into neurons and astrocytes. hPSC-derived *in vitro* cultures may provide additional platforms for studying astrocyte role in neuronal networks with the possibility to expand to disease modeling of astrocyte contributory diseases. Here, we differentiated human pluripotent stem cells into 1) neuron enriched and 2) astrocyte enriched cultures. Properties of these cultures were compared with immunocytochemistry, microelectrode array measurements (MEA) and calcium imaging. The results show that amount of astrocytes does affect the functional development of neuronal networks. These results suggest that astrocytes should be taken into account when developing *in vitro* disease models as they are part of normal neuronal network development.

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MODELING ALZHEIMER'S DISEASE WITH IPSCS WHAT IS DOWNSTREAM OF ABETA?

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Alzheimer's disease (AD) is a neurodegenerative disorder, characterized by β -amyloid deposits, neuronal death and dementia. The disease is prevalently sporadic, but the hereditary cases provided a great insight into its etiology. In the familial forms, mutations typically involve genes encoding amyloid precursor protein (APP) or presenilin, which both result in abnormal production of the amyloidogenic peptide A β 42 over A β 40. The advent of iPSCs allows for the modeling and investigation of different aspects of the disease using patient-derived cells. Nonetheless, researchers face major challenges in obtaining fast, reproducible and robust differentiation and, especially, disease relevant phenotypes. This is particularly true for an inherently heterogeneous tissue like the brain and complex disorders such as AD. Therefore, we first established a robust and scalable differentiation protocol into neural precursor cells and neurons. We then proceeded to use iPSCs derived from four familial AD patients to investigate disease mechanism. For this we derived neurons from these iPSC lines and measured the production of amyloid peptides (A β 42 and A β 40); in all patient-derived cultures the ratio of A β 42 over A β 40 was significantly increased compared to those obtained from healthy controls. These changes were reflected in the expression of known disease relevant genes, such as MAPK1, PRKCB and SERPINA3. We went on to analyze other pathological hallmarks of AD, such as hyperphosphorylated MapT. While the relevance of β -amyloid production in the context of neuronal death needs to be elucidated further, our model provides a robust platform to investigate the cause of the disease and to test therapeutically relevant compounds *in vitro*.

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YAP1 PROMOTES EXPANSION OF NEUROGENIC PROGENITORS IN THE DEVELOPING MOUSE NEOCORTEX

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The neocortex is the site of higher cognitive functions and evolutionarily represents the youngest part of the mammalian brain. To understand the development and evolution of mammalian neocortex, it is important to



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identify the cues that determine brain size. Developing neocortex contains two main types of progenitors, that are apical progenitors (APs) the nuclei of which reside in the ventricular zone (VZ), and basal progenitors (BPs) the nuclei of which are located in the subventricular zone (SVZ). Evolutionary expansion of neocortex is tightly linked with the increased generation of BPs and formation of the outer SVZ (oSVZ). Our lab previously performed transcriptome analyses of distinct germinal zones and progenitor types in the developing mouse and human neocortex. These analyses revealed that YAP1 mRNA is differentially expressed between mouse and human BP pools of the SVZ. Level of YAP1 mRNA in the mouse SVZ is low, whereas in human SVZ, YAP1 mRNA is highly expressed. In this study, we aimed to explore the role of the Hippo signaling pathway in the neocortical expansion during evolution. Staining for YAP1 in mouse, ferret, and human developing neocortical wall revealed that YAP1 expression is confined within the VZ in mouse, whereas in ferret and human, YAP1 protein is expressed in VZ and SVZ. Our hypothesis is that high activity of YAP1 in the oSVZ maintains proliferative capacities of BPs in the SVZ of gyrencephalic species, which contributes to the expansion of the neocortex. To test this hypothesis, we conditionally overexpressed constitutively active YAP1 in the developing mouse neocortex, specifically in the neurogenic progenitor lineage. We show that upon YAP1 overexpression, a portion of the overexpressing cells has a delayed migration towards the cortical plate. We also show that YAP1 overexpressing cells continue to proliferate. Together, these data suggest that YAP1 is sufficient to alter the fate of neurogenic progenitors from differentiation to proliferation.

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EFFECTS OF METABOTROPIC GLUTAMATE RECEPTOR 5 ANTAGONIST ON THE DIFFERENTIATION OF HUMAN AND MOUSE NEURAL PROGENITORS LACKING FRAGILE X MENTAL RETARDATION PROTEIN

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Fragile X syndrome (FXS) is the most common cause of inherited intellectual disability and a well characterized form of autism spectrum disorder. A triplet repeat expansion in the *FMR1* gene leads to transcriptional silencing and the absence of FMR1 protein (FMRP) in FXS. FMRP is a RNA binding protein essential for maturation and function of synapses and neuronal networks. The absence of FMRP results in alterations of neurogenesis which underlie impaired learning in FXS. Interference of metabotropic glutamate receptor 5 (mGluR5) signaling prevents the main features in the phenotype of *Fmr1* knockout (KO) mice, a mouse

model for FXS. We have compared the effects of mGluR5 antagonist, 2-methyl-6-(phenylethynyl)-pyridine (MPEP) on the differentiation of FMRP-deficient human and mouse neural progenitors. We reprogrammed somatic cells of males diagnosed with FXS and healthy controls to induced pluripotent stem (iPS) cell lines, which were characterized by pluripotency markers and expression of *FMR1*. We compared functional responses in differentiating mouse and iPS-derived human FXS neural progenitors to those of controls by intracellular calcium recordings after exposure to specific ligands of glutamate receptors. We found that intracellular calcium responses to type 1 mGluR agonist, (S)-3,5 dihydroxyphenylglycine (DHPG) were augmented in both human and mouse FXS progenitors when compared to controls. The increased responsiveness was not associated with expression changes of mGluR5. Treatment with the mGluR 5 antagonist, 2-methyl-6-(phenylethynyl)-pyridine (MPEP) normalized an increase in the differentiation of a subset of cells responsive to activation of both metabotropic and ionotropic glutamate receptors in mouse but not in human FXS neural progenitors. MPEP increased neurite length of new born neurons and reduced morphological defects of both glial and neuronal cells in FXS neurospheres. In the developing brain of *Fmr1* KO mice, MPEP prevented the accumulation basal progenitors in the subventricular zone. Our results show differential effects of MPEP on the differentiation of human and mouse FXS progenitors which could contribute to different outcome of treatment with mGluR5 antagonists in human and mouse.

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MODELING MITOCHONDRIAL RECESSIVE ATAXIA SYNDROME (MIRAS) WITH PATIENT IPSC

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Mitochondrial dysfunction has shown to be an important cause of spinocerebellar ataxias (SCAs), often combined with sensory neuropathy and epilepsy. Mitochondrial recessive ataxia syndrome (MIRAS) is caused by recessive nuclear gene mutations W748S + E1143G in mtDNA maintenance protein, DNA polymerase gamma (POLG). In Finland, the carrier frequency is 1:89 (n=6118, SiSu project). MIRAS patients manifest complex nervous system dysfunction including ataxia combined with dysarthria, sensory neuropathy, oculomotor defects, tremor, and psychiatric symptoms. The age of onset is typically during young adult age, but can be juvenile or middle-age. Interestingly, although all Finnish patients carry the same pathogenic allele, the juvenile early-onset form of MIRAS manifests primarily with difficult-to-

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treat epilepsy whereas late-onset patients do not suffer from epilepsy. MIRAS patients exhibit tissue-specific mtDNA instability and respiratory chain deficiency in brain and liver and sometimes also a subtle phenotype in the muscle. Why such a wide variety of symptoms between patient groups exist, is unknown. We engineered iPSC lines from 4 early-onset epileptic and 3 late-onset not-epileptic MIRAS patients as well as control lines. The iPSC lines were differentiated towards neuronal phenotype and the molecular biological properties of these cells were studied. The electrophysiological properties of neuronal networks from these cells was measured at basal level and with pharmacological modulation utilizing multielectrode array (MEA) platform. All iPSC lines differentiated similarly. We were able to demonstrate physiological changes in the patient iPSC-derived neurons, related to epileptic activity, as early-onset iPSC-derived neuronal networks showed repeatedly abnormal elevated signaling when compared to the late-onset or control networks. The early-onset neuronal networks also showed a lowered activity threshold with concomitant pharmacological testing. The detailed analysis of the neuronal cell phenotype and mitochondrial trafficking is currently ongoing. Here we describe this novel disease model and present preliminary mechanistic insights of MIRAS pathogenesis.

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MODELING EPILEPTIC LIKE EVENTS IN VITRO

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Induced human pluripotent stem cells and human embryonic stem cells have revolutionized the field of disease modeling in vitro. Use of these human pluripotent stem cells have given us tools to model both genetic and acquired diseases in a dish. Modeling neurological diseases in vitro has already taken huge steps forward during the last few years. At the same time, neurological diseases are one of the most challenging groups of diseases to model in a dish. The human brain is the most complex organ in our body. In addition, detailed mechanisms behind neurological diseases are often unknown and diseases have varying phenotypes. Thus, one big question is how can we examine the disease in a dish? Epileptic seizures are symptoms of epilepsy. Epilepsy itself can be the primary disease or develop as a secondary disease. Epileptic seizures are abnormal functions of neuronal networks detectable with electroencephalography (EEG) monitoring. In vitro, neuronal network functions

are monitored with microelectrode arrays (MEA). Our aim was to develop a model platform where we could be able to detect epileptic like events in vitro from neuronal networks derived from patients suffering from epilepsy. Further, the aim was to characterize the detected epileptic like events in detail. Neuronal networks were created from 7 patients and 3 controls. 4/7 patients had been diagnosed with severe epilepsy of a metabolic cause. Neuronal networks were cultured in MEAs for 4 to 6 wks with repeated recordings. In addition to naïve recordings, pharmacological testing pattern was optimized for detecting hyperexcitable nature of these cultures. Abnormal functional behaviors were detected in the networks derived from patients diagnosed with epilepsy. In the preliminary analysis, the abnormal activity was detected as an increased total neuronal network activity and as alterations in signal shapes. These changes were detectable in repeated experiments. These results suggest that we have been able to detect epileptic like events in vitro, thus being able to create link with the in vivo and in vitro disease. The more detailed analysis of the detected epileptic like events are currently ongoing.

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DRUG SCREENING IN ZEBRAFISH IDENTIFIES ANTIOXIDANTS WITH A DUAL ROLE IN BETA-CELL PROTECTION AND REGENERATION

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Chronic oxidative stress and islet inflammation in type 2 diabetes are two contributing factors to β -cell failure and apoptosis occurring during the disease progression. Thus, the identification of compounds that stimulate β -cell proliferation while protecting against oxidative stress and inflammation is a key factor for the improvement of the current diabetes treatments. For this purpose, we performed an *in vivo* chemical genetic screen of anti-oxidant compounds to identify the ones that promote β -cell proliferation using zebrafish. We identified four anti-oxidant compounds that stimulate β -cell proliferation. The "hit" compounds include Rosmaniric acid, AA-861, Ascorbyl Palmitate and Butylated Hydroxyanisole (BHA). Notably, in addition to their antioxidant properties, these small molecules play a role in the regulation of arachidonic acid metabolism. Additionally, we investigated if these compounds can activate the transcription factor Nrf2, which has been shown to play an essential role in β -cell protection



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against oxidative stress and inflammation. For this purpose, we generated a β -cell specific reporter of the transcription factor Nrf2a *Tg(ins: Nrf2a-GFP)*, allowing for monitoring the nuclear levels of Nrf2a. Using this transgenic zebrafish line, we showed that a brief treatment of zebrafish larvae with BHA increased the levels of nuclear Nrf2a in β -cells. Consistent with this role in promoting Nrf2-activation, our preliminary results show that BHA enhances mouse islet cells survival after exposure to inflammatory cytokines. In conclusion, we identified four novel antioxidants that can stimulate β -cell proliferation, one of which seems to induce the activation of Nrf2 and protect islet cells against inflammatory cytokines.

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UNDERSTANDING THE SOUL OF LIMB REGENERATION - TRACING THE CONNECTIVE TISSUE LINEAGE

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Axolotl limb regeneration is a fascinating phenomenon, which leads to restoration of fully functional and patterned limb after amputation. Formation of undifferentiated cell mass at the amputation plane known as blastema is the hallmark of limb regeneration. Connective tissue, which is the major constituent of blastema is a highly heterogeneous tissue in the mature limb and can be grossly subdivided into 5 subtypes 1) Dermis 2) Fascia 3) Tendons 4) Perichondrium and 5) Chondrocytes. Generations of developmental biologists have debated regeneration capability of each of these subtypes and it is unclear if there is a unique progenitor cell within each of these population or all these subtypes participates in a diverse manner. Here in first of its kind study, we have created different connective tissue driven inducible cre lines and with the help of loxP reporter line, we have attempted to define role of each of these connective tissue subtypes during limb regeneration. Our results suggest that chondrocytes have no ability to participate in limb regeneration whereas tenocytes are lineage restricted. Interestingly, dermis and fascia possess multi-potent progenitors that can differentiate into one another and also possess ability to differentiate into tenocytes and chondrocytes. Further, our study using *Prrx1* driver shows that *Prrx1*^{+ve} connective tissue cells of dermis and fascia contributes at least three fold more as compared to *Prrx1*^{-ve} cells and identifies *Prrx1* as a progenitor marker of the connective tissue in the limb.

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GENERATION OF 3D CORTICAL SPHERES FROM INDUCED PLURIPOTENT STEM CELLS TO ESTABLISH A CELLULAR MODEL FOR NEURODEVELOPMENTAL DISEASES

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Three-dimensional cortical-like structures are a promising tool to study neurodevelopmental disorders. The differentiation of induced pluripotent stem cells (iPS cells) towards 3D-cortical spheres seems to follow a similar pattern than in vivo cortical development with emerging cortical layers during the process generating a complete cortical structure in the dish. The cellular network within the organoids most probably supports the maturation of the generated neurons, yielding a functional neuronal model in a dish. Here, we show 3D cortical spheroid differentiation starting from iPS cells. The organoids were cultured in floating culture and analyzed at different time points during the differentiation and maturation process. Hematoxylin/Eosin staining together with Immunocytochemistry revealed rosette structures within the spheroids, reminiscent of ventricular structures during in vivo development. Cortical layering, however, has not been observed, yet. The cells within the organoids show measurable calcium transients as well as network activity which can be picked up in multi-electrode array (MEA) recordings. Further characterization of our cellular model includes neurotransmitter release studies to consolidate our functional data.

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ELASTIC MODULUS CHANGES IN MOUSE BRAINS WITHOUT MYELIN

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To date, it is well recognized that the mechanical characteristics of a cell's environment can have a profound influence on its biological properties. It has been shown, that neurons and glia cells are sensitive to mechanical input during development, in disease and regenerative states. Axonal myelination is an important factor in aging and disease. Demyelinated axons are impaired in their function and degenerate over time. Oligodendrocytes, the cells responsible for myelination of axons, are sensitive to mechanical properties of their environment. By using atomic force microscopy,

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we investigated these properties in a mouse model of complete myelin absence to shed further light onto the mechanics of axonal myelination.

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MECHANISM UNDERLYING INDUCTION OF THE FLOOR PLATE IN THE NEUROEPITHELIAL CYSTS IN VITRO

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The mechanism underlying formation of the floor plate (FP), ventral signaling center in the neural tube, remains under debate for many years. One of the main hypotheses implies induction of the FP by the SHH secreted from the notochord. Recently established protocol allows production of patterned neuroepithelial organoids (cysts) with neural tube properties from mouse embryonic stem cells in vitro. This opened up an opportunity to study neural tube formation and patterning in a simplest 3D environment and demonstrated that induction of the localized FP occurs in such cysts spontaneously upon global application of retinoic acid (RA). This observation questions the role of notochord and Shh in the onset of the FP and extends the role of RA in neurodevelopment. In this study we aim to understand the mechanism of FP formation in the neuro-epithelial cysts in vitro. We identified transcription factor FoxA2 as one of the earliest response genes. We show that in response to RA treatment cells in the cyst upregulate FoxA2 and gradually shape future floor plate prior to Shh induction. To elucidate mechanism underlying such a remarkable self-organization phenomenon in the neuroepithelial cysts we want to analyze molecular factors interacting with FoxA2 to restrict its expression to a single locus. To find regulatory pathways involved in this process we employ deep sequencing of total RNA obtained from cysts at different time points with and without RA application. Understanding of mechanisms controlling neural tube development may help to find therapeutical approaches to different diseases caused by neural tube defects.

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MINIPIG BRAIN ISCHEMIC AND TRAUMATIC INJURY MODEL TO ASSESS THE SAFETY OF GRAFTED HUMAN AND PORCINE NEURAL PRECURSORS

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The incidence of stroke varies from 63.0 to 239.3 per 100,000 in the European population with higher incidence in men. A comparable incidence of trauma brain injuries (TBI) (about 235 per 100,000) was also derived from European reports. For successful clinical translation of cell-replacement based therapies to modulate brain ischemic or traumatic injury there is a need to develop a reproducible brain ischemia and traumatic model in large animals. In a previous study we have developed and characterized a minipig unilateral brain ischemia model and provided a correlative assessment between the qualitative/quantitative neurological deficit and corresponding neurodegenerative changes at specific brain regions at different time points after post-ischemic reflow. Recently, using a Fogarty embolectomy catheter introduced directly into the brain striatum, we have developed a reproducible TBI in minipigs. A consistent transient neurological deficit accompanied by well-defined regional neurodegenerative changes was also detected. Both of these models were/are successfully used for preclinical testing of human spinal stem cells (HSSC; Neuralstem Inc.) once grafted into areas of previous ischemic or traumatic injury in continuously immunosuppressed minipigs. Post mortem immunofluorescence analysis at 3-6 weeks after cell grafting showed well incorporated cell grafts in all animals. Phenotypic characterization of grafted cells showed a preferential neuronal differentiation and extensive axonal sprouting into host tissue. Minimal or no secondary inflammatory effect was seen in cell-grafted regions.

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DEVELOPING OF SPATIAL-TEMPORAL-CONTROLLED GENE KNOCKOUT METHOD USING CRISPR SYSTEM IN AXOLOTL TO STUDY SPINAL CORD REGENERATION

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Salamanders can fully regenerate their lost organs, e.g. limb and spinal cord, upon natural injury or amputation, making them a very powerful model for studying tissue regeneration. Understanding the underlying molecular mechanisms of salamander tissue regeneration may also shed light on human regenerative therapies. We previously established an efficient gene knockout method using the CRISPR technique in the salamander *Ambystoma mexicanum* (axolotl) via injection the mixture of Cas9 mRNA and gRNAs into the fertilized



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eggs, and showed that knockout of Sox2 resulting in the failure of spinal cord regeneration. Although this constitutive gene knockout approach was successfully used to dissect Sox2 gene function during spinal cord regeneration, the potential application of this method is limited due to the lack of temporal and spatial specificity. To overcome this roadblock, we demonstrated the establishment of a temporally and spatially controlled gene knockout method by *in vivo* injecting CRISPR-gRNA complexes into the spinal cord lumen of the axolotl, followed by electroporation. This method leads to comprehensive knockout of Gfp and Sox2 target gene expression. Sox2 knockout in the axolotl spinal cord by CRISPR electroporation gave rise to similar regeneration defects as the phenotype achieved by the zygote-injection protocol. We further showed that simultaneous delivery of CRISPR-gRNA complexes directed against Sox2 and GFP yields efficient knockout of both genes in GFP-reporter animals. Finally we successfully applied this method to other tissues such as skin and limb mesenchyme for GFP knockout. This efficient delivery method opens up the possibility for rapid *in vivo* genetic screens during axolotl regeneration and can in principle be applied to other vertebrate tissue systems.

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A CUTTING EDGE COMBINATORIAL DESIGNED AGONISTIC STEM CELL PROLIFERATION INDUCER BASED ON A FGF2-OVEREXPRESSED TRANSCRIPTOMIC EFFECT WITH REGENERATION COMPETENCE IN CORD BLOOD TETANUS TOXOID TLR-FREE SIGNALING ASSOCIATED CELL CULTURE SYSTEM.

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Adult human fibroblasts grown in low oxygen and with FGF2 supplementation have the capacity to tip the healing outcome of skeletal muscle injury - by favoring regeneration response *in vivo* over scar formation. It has been previously been reported that the transcriptomes of control adult human dermal fibroblasts and induced regeneration-competent (iRC) fibroblasts to identify transcriptional changes may be related to their regeneration competence. HSPCs express TLRs and are activated by TLR2 and TLR4 agonists *in vitro* through MyD88 signaling. Lastly, presence of various growth factors in culture media can have a significant effect on a cell transcriptional activity and consequently its phenotype. FGF2 is a mesenchyme-derived growth factor that displays mitogenic, migratory, and morphogenic functions and is also known to play role in angiogenesis,

organ development, organ regeneration, and wound healing. Contrary to its predominantly mitogenic effects on differentiated cell types, FGF2 is absolutely required for maintenance of expression of stemness-related genes. With respect to wound healing, FGF2 has been studied as a potential therapeutic anti-scarring agent. It has been previously investigated the effects of the aforementioned important cell culture conditions, FGF2 and culture surface, on adult human fibroblasts. In this study, we introduced an infectious tetanus toxoid multi-algorithmic simulation model, *tetanus toxoid* induced by cecal ligation and puncture (CLP), to examine the regulation of *in vitro* HSPC expansion and a newly introduced binding energy funnel 'steepness score' for the evaluation of the inducing of IFN- β (TRIF)/Toll-IL1 protein-peptide-multi-ligand complexes binding affinity simulations predicted high binding affinity for native protein-peptide-hyper-ligand complexes benchmark and low affinity for low-energy decoy complexes for the *in silico* generation of HSP activators by the TLR3 agonist polyinosinic-polycytidylic acid mimetic hyper agonist chemostructures through IFN-I signaling (5, 6) simulating a vaccinia virus infection like quantum parameteromechanic environment causing a MyD88-dependent HSPC expansion effect.

004

TRANSPLANTATION OF NEURAL STEM CELLS CULTURED IN ALGINATE SCAFFOLD FOR SPINAL INJURY

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Spinal cord injury is one of the leading causes of disability which has no treatment. Spinal cord injury also could cause sensory impairment. These days stem cells therapy has been used for different central nervous system and it could make some neurologic improvement for spinal cord injury. Neural stem cell is one type of stem cells that are able to differentiate to three neural lineages. Some previous studies showed that culturing neural stem cells in 3D scaffolds like alginate could enhance neural differentiation. We have designed this study to investigate the effects of alginate encapsulated neural stem cells transplantation on spinal cord injury. The neural stem cells were isolated from rat embryo 14 days. The isolated neural stem cells were cultured in NSA media containing basic fibroblast growth factor and endothelial growth factor. The cells were characterized by differentiating to three neural lineages and they were cultured in alginate scaffold. After 7 days the cells were encapsulated and transplanted to rat model of spinal cord injury. The neurological functions were assessed for 6 weeks after transplantation and also the histology study and activity of Caspase 3 were evaluated. Our data showed that culturing in 3D scaffold and transplantation

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of neural stem cells could improve neurological outcome for rat model of spinal cord injury. The inflammation and lesion size and also the activity of Caspase 3 was less in encapsulated neural stem cells transplantation. Transplantation of neural stem cells which were cultured in alginate scaffold could make better clinical and histological outcome for spinal cord injury model in rats.

006

STUDIES ON NEURAL CELLS DERIVED EXOSOMAL MICRORNAS

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Exosome, a new kind of major paracrine secretor, is released into the extracellular environment by the outward budding of various types of cells including neural cells and stem cells. Exosome is revealed as an important mediator and carrier of cell-to-cell communication as it always surrounded by a phospholipid bilayer and containing proteins, mRNAs, and microRNAs, which may function as regulators in many biological and pathological processes as well as neural degeneration and regeneration. In our study, we focus on a group of exosomal microRNAs (miR-X) which were predicted as regulators for A β processing proteins such as DYRK, APP, PSEN1 and BACE1. In cell models, we found that cellular miR-X expression levels are correlated with Abeta levels in vitro. These microRNAs directly regulated Abeta generation in neural cells, and the exosomal microRNAs in supernatant were also decreased in Abeta-treated cells. For in vivo analysis, we also found that serum exosomal miR-X are decreased in APP/PS1 mice comparing to wildtype mice, and the expression levels of serum exosomal microRNAs were correlated with brain Abeta levels in APP/PS1 mice. Furthermore, in 60 Alzheimer's disease (AD) patients, we also found that serum exosomal microRNAs were significantly decreased in AD patients comparing to the peoples in control group. Interestingly, we also found that serum exosomal miR-X levels were decreased in aging mice and patients, which indicated that these exosomal microRNAs may be related to neural aging. We therefore suggested that exosomal miR-X may be potential biomarkers for Alzheimer's diseases as well as some other neural degeneration diseases. And these findings also lead to further implications using exosomal miR-X as new therapy strategies for neural regeneration investigations.

008

ANALYSIS OF THE FOXG1 KNOCKOUT PHENOTYPE IN MURINE iPSC USING SFEBQ DIFFERENTIATION

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Foxg1 is a transcription factor critical for the development of the mammalian telencephalon. It is responsible for the proliferation of dorsal telencephalon progenitors and the specification of the ventral telencephalon. A homozygous knockout of Foxg1 in mice leads to a severely reduced size of the dorsal telencephalon and complete loss of the ventral telencephalic structures. In this study, the effects of a homozygous Foxg1 mutation were studied *in vitro* after differentiation of mouse iPSC to telencephalon progenitor cells, using serum free embryoid body culture with quick reaggregation (SFEBq) technology. The phenotype will be verified using *in vivo* chimera formation. Mouse iPSC were differentiated using the SFEBq method and collected after 10-25 days to analyze mRNA expression of several telencephalic markers and Foxg1 protein expression. Quantitative real-time PCR revealed that Foxg1 and telencephalic markers are strongly upregulated after 15 days of differentiation and Foxg1 protein is reproducibly detectable with Western Blot latest after 20 days of differentiation. The Foxg1 knockout was established using the CRISPR/Cas9 system in murine iPSC. After two rounds of limited dilution and clonal expansion, four different lines with mutations in Foxg1 that lead to a premature stop codon with loss of the DNA binding domain were identified. One line homozygous for an 80 bp deletion (Foxg1^{-80bp/-80bp}) was used to validate the functionality of the Foxg1 knockout after differentiation. In Foxg1^{-80bp/-80bp} cells, ventral telencephalic markers such as Nkx2.1 and Gsx2 were less upregulated compared to wildtype miPSC after 25 days. Moreover, they expressed higher levels of the marker for post-mitotic neurons Tuj1 after 20 days of differentiation. These results are in accordance with previously described Foxg1^{-/-} effects *in vivo*, where ventral telencephalic areas were not specified and dorsal telencephalic neurons differentiated prematurely at the expand of the progenitor pool. Next, the additional Foxg1^{-/-} miPSC lines will be differentiated and analyzed at the mRNA and protein level. Then, the *in vivo* contribution of Foxg1^{-/-} miPSC to chimeras, especially to telencephalic progeny, will be investigated.

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010

CDK4/CYCLIN D1 OVEREXPRESSION IN THE SUBVENTRICULAR ZONE EXPANDS NEURAL STEM CELLS AND INCREASES OLFACTORY BULB NEUROGENESIS**BRAGADO ALONSO, SARA**¹, Reinert, Janine², Massalini, Simone¹, Marichal, Nicolas³, Kuner, Thomas², Berninger, Benedikt³, Calegari, Federico¹¹Center for Regenerative Therapies Dresden, Dresden, Germany, ²Institute for Anatomy and Cell Biology, University of Heidelberg, Heidelberg, Germany, ³Institute of Physiological Chemistry, University Medical Center, Johannes Gutenberg University Mainz, Mainz, Germany

The subventricular zone is the main source of neural stem cells (NSCs) in the mammalian brain and, although the physiological role of adult neurogenesis remains controversial, current strategies aim to manipulate this process as a promising approach towards therapy. Despite several efforts, controlling the proliferation versus differentiation of NSCs remains a challenge. Our group has shown that the expansion of endogenous NSCs can be controlled during embryonic development and adulthood by shortening the G1 phase of their cell cycle upon overexpression of Cdk4/cyclin D1 (4D) (Artegiani et al., 2011; Nonaka-Kinoshita et al., 2013). We generated a transgenic mouse line that allows the temporal and reversible control of 4D overexpression. We found that switching on 4D increases the population of NSCs while switching it off afterwards allows their physiological differentiation. As a consequence of the transient expansion of NSCs, the final neuronal output is increased. The 4D-derived neurons survive for more than two months and seem to integrate in the local circuit as assessed by the morphology of their synapses and marker expression. Finally, electrophysiology and olfactometry analyses are being performed to assess the nature of the additional neurons and their effect on behavior.

012

STAGE-SPECIFIC KRAB ZNF GENE EXPRESSION SIGNATURES DURING NEURAL DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS**LORENZ, PETER**¹, Peitz, Michael², Koczan, Dirk¹, Ziems, Bjoern³, Steinbeck, Felix¹, Li, Shengdi⁴, Li, Yixue⁴, Bruestle, Oliver², **THIESEN, HANS-JUERGEN**¹¹Institute of Immunology, University Medicine Rostock, Rostock, Germany, ²Institute of Reconstructive Neurobiology, University Bonn, Bonn, Germany, ³Steinbeis-Transferzentrum Proteom-Analyse, Rostock, Germany, ⁴Shanghai Center for Bioinformation Technology, Shanghai, China

C2H2 Krüppel-type zinc finger (ZNF) genes encode an evolutionarily conserved domain that was initially described as a heptad repeat of leucines in KOX1/ZNF10, and later designated **K**rüppel-associated **B**ox (KRAB).

The KRAB domain represents one of the strongest repressors found in mammalian cells and it has been successfully utilized as a genetic tool to modulate transcriptional gene regulation by fusion to tetracycline repressor or to inactivated CRISPR-Cas9 endonuclease. Although the approximately 400 human KRAB-ZNFs are assumed to have regulatory functions in many developmental processes, little is known on their role in lineage specification. Here, we set out to investigate KRAB ZNF gene expression signatures during neural differentiation of human pluripotent stem cells (hPSCs). To this end, we employed TaqMan arrays to quantitatively analyze RNA expression of 367 different KRAB ZNF genes in human induced pluripotent stem cells (hiPSCs), human embryonic stem cells (hESCs) and long-term self-renewing neuroepithelial stem cells (It-NES cells). Data on KRAB ZNF expression in hiPSCs were further compared to profiles obtained from parental human fibroblasts. Principal component analysis (PCA) revealed specific expression clusters for hPSCs, It-NES cells and fibroblasts. Differential expression analysis identified one subset of KRAB ZNF genes to be highly upregulated in It-NES cells compared to fibroblasts and hiPSCs, whereas another subset was highly expressed in hPSCs compared to It-NES cells and fibroblasts. In summary, KRAB ZNF genes show distinct expression signatures during neural differentiation that discriminate specific stages and may thus be used to select candidate genes for functional studies on the role of this class of genes in human neural development.

014

HUMAN INDUCED PLURIPOTENT STEM CELL-BASED APPROACHES TO MODEL AND TREAT CEREBELLAR DEGENERATION IN ATAXIA TELANGIECTASIA**FRANCO, SONIA**¹, Bhatt, Niraj¹, Ghosh, Rajib¹, Roy, Sanchita¹, Gao, Yongxing², Armanios, Mary³, Cheng, Linzhao²¹Radiation Oncology, Johns Hopkins University, Baltimore, MD, USA, ²Medicine, Johns Hopkins University, Baltimore, MD, USA, ³Oncology, Johns Hopkins University, Baltimore, MD, USA

Ataxia Telangiectasia (A-T) is an autosomal recessive syndrome caused by mutations in ATM, a large serine-threonine kinase encoded by the ATM locus at 11q22. Among its pleiotropic clinical manifestations, ataxia develops early in childhood and typically results in severe disability in the second decade of life. Consistently, radiology of affected patients reveals progressive cerebellar atrophy and pathology of postmortem A-T cerebellums shows loss of Purkinje neurons. A-T has no cure and most patients die in early adulthood due to severe recurrent infections or cancer. The goal of our work is to develop stem cell-based approaches to: 1) understand the requirement for ATM in the maintenance of cerebellar neurons; and 2) develop cell-based regenerative therapies for the disease. Towards these

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goals, we have recently generated and characterized induced pluripotent stem (iPS) cells from an A-T patient. Previous studies had reprogrammed A-T fibroblasts using feeder layers and integrating vectors and found a marked decrease in reprogramming efficiency. In contrast, we find that A-T peripheral blood (PB) is a robust source of A-T iPSCs. Moreover, our experiments were conducted in xeno-free, feeder-free conditions and using episomal vectors, in preparation for clinical implementation in the future. Importantly, we find that A-T iPS cells maintain pluripotency with passage, have a normal karyotype and reset telomere length similar to ATM-proficient iPS cells. Based on these findings, we are currently developing protocols to 1) differentiate A-T iPS cells to cerebellar neural stem cell progenitors and mature cerebellar neurons, in order to understand the mechanisms leading to cerebellar atrophy in A-T; and 2) upon gene defect correction via CRISPR/Cas9 technology, generate autologous cerebellar grafts and *in vitro* and *in vivo* models to test their function.

016

MIR-302/367 CLUSTER AND VALPORATE ENHANCES MYELIN REPAIR IN CUPRIZONE INDUCED DEMYELINATION

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The enhancement of repair potential in the context of degenerative brain diseases has been a priority for research activities during recent years. Considering recent advancement in the field of direct transdifferentiation, conversion of astrocytes as a prominent component of glial scars to the repairing cells seems interesting. Recently, we reported miR-302/367-mediated *in vivo* conversion of astrocytes into neuroblasts and neurons. Here, using miR-302/367 and valproate we showed the possibility of conversion of astrocytes to OPCs and myelinating cells in cuprizone induced model of demyelination. This approach enhanced remyelination as it was detected by luxol fast blue staining and immunostaining against MBP and PLP. Evaluation of memory impairment following cuprizone and consequent treatments showed functional recovery of lesions. Tracing of transduced cells using GFP, revealed their contribution in generation of new myelinating cells. These findings suggest that *in vivo* administration of miR-302/367 cluster and VPA may increase the potential of brain for myelin repair by

targeting of astrocytes. This finding may open a new avenue for treating demyelination diseases such as multiple sclerosis.

018

GENERATION OF HYPOTHALAMIC NEURONS FROM PRADER WILLI SYNDROME iPSCS

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Prader Willi Syndrome (PWS) is a genomic imprinting disorder that affects 1 in 15,000 patients. Approximately 70% of patients with PWS inherit the disorder through deletions on the paternally inherited chromosome 15, specifically the 15q11-q13 region. Most other cases of PWS result from uniparental disomy of the maternal chromosome 15. In the earliest stage of PWS, infants display hypotonia and failure to thrive. As development proceeds, PWS is characterized by obesity due to hyperphagia as well as other behavioral disorders, dysregulated sleep and infertility. Taken together, these symptoms indicate improper regulation of the endocrine system and, more specifically, the function of the hypothalamus, a ventral diencephalon structure that serves to connect the endocrine system to the central nervous system. Within the hypothalamus are located several nuclei, including the medially located arcuate nucleus (ARC). The ARC contains neurons that regulate sleep, growth, fertility, and hunger, through expression of neuropeptides and hormones, like oxytocin, growth hormone-releasing hormone (GHRH), and gonadotropin-releasing hormone. In order to investigate the potential for misregulation or impaired development of the hypothalamus in PWS patients, we have generated several induced pluripotent stem cell (iPSC) lines from patients with PWS, both those with deletions in the 15q11-q13 region and those with uniparental disomy. Using protocols for organoid and spheroid three-dimensional methods of neural differentiation, in addition to standard monolayer differentiation, we have modified existing protocols to in order to efficiently generate hypothalamic neurons from these iPSC lines. Following the generation and maturation of hypothalamic neurons from PWS and control lines, we aim to investigate the potential for PWS iPSC-derived neurons to adopt hypothalamic cell fates, comparable to the ability of control iPSC lines, through gene expression analysis and assays for neuropeptide expression. We are also differentiating PWS iPSC lines in which the silenced maternal PWS genes have been re-activated, through disruption of the epigenetic machinery, and are generating hypothalamic neurons from the "rescued" cells in an effort to develop potential therapeutic applications.



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020

AMYLOID-BETA42-INDUCED NEURODEGENERATION ENHANCES STEM CELL PLASTICITY AND NEUROGENESIS IN ADULT ZEBRAFISH BRAIN

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Humans cannot regenerate after neurodegeneration due to difficulties in recruiting endogenous neural stem/progenitor cells (NSPCs) towards neurogenesis in vivo. Yet, zebrafish effectively regenerates its brain after injuries due to the neuroregenerative outcome of NSPCs. Therefore, zebrafish – a relatively unexplored model in neurodegeneration field – can help elucidate the way vertebrate brains regenerate, and the underlying molecular programs. To generate an adult zebrafish model of neurodegeneration, we synthesized Amyloid- β 42 (A β 42) – a hallmark of the neuropathology of Alzheimer's disease – using a specific surface chemistry. We coupled human A β 42 to a cell-penetrating peptide for intracellular delivery, and performed microinjection into adult zebrafish brains. A β 42 deposition was prominent in neurons, leading to phenotypes reminiscent of human pathophysiology: apoptosis, inflammation, synaptic degeneration, and learning deficits. Interestingly, zebrafish responded to A β 42-mediated neurodegeneration by inducing proliferation of NSPCs and neurogenesis in a microglia-dependent manner indicating the presence of neurodegeneration-induced NSPC plasticity in adult zebrafish brain. We also profiled the transcriptome of her4.1-positive neural progenitor cells. This novel A β 42-mediated neurodegeneration model and transcriptome analysis in the adult zebrafish could help us understand the molecular basis of how a regenerative response can be elicited, and could progress to neuronal repair in human brains.

022

ABERRANT DNA METHYLATION IN HUMAN NEURAL STEM CELLS UNDER HIGH GLUCOSE CONDITION

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Maternal hyperglycemia in pre-gestational period potentially altered expression of several signaling molecules and transcription factors involved in developing central nervous system (CNS) resulting in neural tube defects (NTDs) in the embryo. The DNA methyltransferase (DNMT) families, including of maintenance DNMT1 and *de novo* DNMT3a and DNMT3b have been highly expressed and play important functions in DNA methylation during neurogenesis. The disturbance of those DNMT activities potentially emanates the inhibitory effects on proliferation, migration and differentiation of neural stem cells (NSCs). In this study, we aimed to determine whether high glucose exerts the negative effects of high glucose on neurogenesis through the aberrant of DNA methylation. Human NSCs were cultured in neuronal medium (17.5 mM of glucose for the control group) added with 30 and 60 mM of glucose for 72 hours. Mannitol was used as osmolality control. By MTT assay, the viability of NSCs was gradually decreased in a glucose-concentration manner. KI-67⁺ proliferative cells of 60 mM glucose-treated NSCs were markedly fewer than in the untreated group. Meanwhile, the percentages of PI-positive cells from high glucose-treated groups were significantly higher than the control culture. However, there was no significant difference in percentage of PI-positive cells among the different glucose concentrations. The level of DNMT3b gene expression in both 30 mM and 60 mM of glucose-treated NSCs markedly lower than the untreated groups. While DNMT1 and DNMT3a level of NSCs were significantly decreased at 60 mM glucose addition. Particularly, the results of global DNA methylation showed a remarkable decrease in high glucose-treated NSCs compared with control. Taken together, our finding endowed the possibility of epigenetic alteration underlying the negative influences of high glucose condition on human neurogenesis. Nevertheless, the role of DNA methylation-regulated NSCs under high glucose condition is still needed to be emphasized.

024

A COCKTAIL OF SMALL MOLECULES DRIVES A DIRECT CONVERSION OF HUMAN FIBROBLASTS INTO NEURAL STEM CELLS

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A next generation of patient-specific cell therapies for neural degenerative disorders, such as Alzheimer's and Parkinson's diseases, are based on direct conversion technologies to generate target neural cell types. As recently reported in many studies, human fibroblasts can be directly converted into functional neuronal cells, bypassing an induced pluripotent stem cell (iPSC) stage and also removing exogenous transgenes. However, sources of such direct conversions have been restricted

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and the converted neurons have a limited capacity to proliferate. Here, we report a direct conversion of human fibroblasts into expandable, induced neural stem cells by four chemicals (ciNSCs), without passage of a transient pluripotent stage. These ciNSCs resembled hiPSC- and human fibroblast-induced NSCs, with neurosphere formation and NSC-specific gene and protein expressions. After a further maturation, ciNSCs can be differentiated into three main neural lineages, namely neurons, astrocytes and oligodendrocytes *in vitro/in vivo*. Additionally, they can be terminally differentiated into specific neuronal subtypes, such as dopaminergic neurons, under defined culture conditions. Thus, our data demonstrate that a specific chemical cocktail can drive the neural lineage-specific direct conversion of human somatic cells into progenitors with self-renewal and multipotency. This chemical-only approach for iNSCs can, therefore, be suggested as an alternative technique to produce transgene-free and large-scale sources for patient-specific cell-based therapies.

026

THE REGULATION OF OLIGODENDROGLIA IN DEVELOPMENT AND REGENERATION IN ZEBRAFISH SPINAL CORD

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Successful regeneration of spinal cord lesions in humans is a medical challenge that still requires a lot of research. Unlike mammals, zebrafish are able to regenerate CNS tissue after spinal cord damage, which makes it an ideal model for research into successful neural regeneration. For functional regeneration axonal regrowth as well as remyelination of axons is important for effective signal transduction. Remyelination relies upon two factors: recruitment of oligodendrocyte precursor cells (OPCs) and generation of axon wrapping myelin sheaths by oligodendrocytes. The aim of my work is to identify novel factors that influence OPC recruitment and generation of new myelin sheaths after injury.

028

'CUREMOTORNEURON': NOVEL IPSC APPROACH TO ADVANCE ALS RESEARCH

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ALS is a neurodegenerative disorder that affects both

lower motor neurons in brainstem and spinal cord, and the upper motor neurons in the motor cortex. Loss of motor neurons with both familial and sporadic ALS is relentlessly progressive, and most patients die within three to five years after symptom onset. The high unmet medical need is compounded by clinical heterogeneity, lack of robustly predictive *in vitro/in vivo* disease models and limited understanding of the molecular mechanisms of disease pathogenesis. 'Cure MotorNeuron', our strategic partnership with the Harvard Stem Cell Institute, aims to use motor neurons derived from a panel of well characterized human induced pluripotent stem cell lines both from familial and sporadic ALS patients as basic models of disease. We have adapted and standardized motor neuron differentiation and phenotypic screening in 384-well format and are progressing towards further disease modeling and systematic screening for new mechanisms, targets and compounds that have therapeutic value for this life-threatening disease.

030

BIOHYBRID HYDROGELS ENABLE DEFINED 3D CULTURES OF HUMAN CORTICAL NEURONS

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Extracellular matrix composition (ECM) and architecture is an integral parameter for stem cell activity and tissue patterning. However, due to the complex interplay between multiple ECM derived signals and their pleiotropic effects, *in vivo* assays pose a challenge in identifying the roles of exogenous cues on tissue patterning. *In vivo*-like three-dimensional (3D) cell culture platforms can therefore serve as advantageous assays to dissect the roles of matrix properties on stem cell activity and differentiation. To emulate the three-dimensional neuronal networks we used *in situ*-forming, matrix metalloproteinase (MMP)-responsive starPEG (polyethylene glycol) -heparin [PEG-HEP] hydrogels¹ as scaffolds to encapsulate embryonic human cortical cells (HCCs) from 21st gestation week. We grow the cells within the hydrogels for 21 days and we analyze cell morphology and network in real time using cell tracking dyes. Apart from live monitoring, we perform immunofluorescent stainings at different time points. Our 3D scaffolds enable cells to be embedded in 3D with high viability. Matrix metalloproteinase (MMP)-cleavable crosslinkers, enabling cell-responsive remodeling of the hydrogels, were found to be essential for neural stem cell proliferation and neuronal network formation as determined by immunocytochemical stainings for general neuronal markers and specific cortical



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neuronal markers. Additionally, when amyloid-beta42 was incorporated into the matrix, hampered network formation and elicited cell death were found, suggesting that amyloid-toxicity and other ECM-related disorders might be mimicked with functionalized starPEG-heparin hydrogels. We believe that our new 3D culture platform provides an excellent tool to study the plasticity of human neural stem cells and disease states in real time. In extension, the 3D cultures can be further decorated with various ECM associated signals (e.g. growth factors and adhesion ligands) and be used to perform screens for drugs that may affect neuronal plasticity and network formation.

032

A MURINE "MINI EAR" ORGANOID MODEL FOR PHENOTYPIC HIGH-CONTENT SCREENING IN HEARING RESEARCH

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In vitro models to study ototoxicity, otoprotection or otoregeneration have utilized inner ear cell lines, zebra fish, and to a certain extent whole organ culture. Since cell lines and non-mammalian models for ototoxicity may behave distinct from primary cells in response to drug treatment, we developed an in vitro standardized assay for ototoxic drug screening derived from murine organ of Corti progenitor cells, representing "Mini Ears". We isolated cells from the postnatal day 0 organ of Corti of NMRI mice that are known to give rise to stem cell derived otospheres (Oshima et al., 2007, J Assoc Res Otolaryngol). From these spheres, differentiated epithelial islands - "Mini Ears" - populated with hair- and supporting cell-like cells can be obtained. Primary cells were cultured in a proliferative environment for 5 days in vitro (DIV). With the removal of the growth factors, plating on 96-well plate format, and pursued culture for 14 DIV in adherent conditions, the spheres differentiate into prosensory cell patches. The "Mini ears" were fixed, immunohistochemically stained, and automatically analyzed using an ImageXpress Micro XLS High-Content Screening microscope (Molecular Devices). Varying culture conditions and testing markers for hair and supporting cell-like cells, we found reproducible conditions for the generation of "Mini-Ears". A fully automated data acquisition algorithm that identifies, images, and analyses "Mini Ears" with no user intervention for up to 5 different markers and z-stacks was established. Using the inducible reporter mouse line Fgfr3-iCreERT2, proliferation could be monitored selectively in specific sub types of supporting cells. The

assay now provides a screening platform to conduct "Mini Ear" based high-content screening for ototoxicity, otoprotection, and otoregeneration.

034

ESTABLISHMENT OF HUMAN OLIGODENDROCYTE PROGENITOR CELL-BASED ASSAYS FOR DRUG SCREENING: EFFECT OF BMP INHIBITORS ON HUMAN OLIGODENDROCYTE DIFFERENTIATION AND MYELINATION

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Mechanistic studies of human oligodendrocyte differentiation and myelination have been hindered by the lack of human specific oligodendrocytes culture system in-vitro. We have developed a robust *in vitro* protocol to derive oligodendrocytes from human pluripotent stem cells (hPSC). In this protocol hPSCs are differentiated toward glial restricted cells (hGRC), expanded and kept frozen as hGRC cell banks. Upon thawing, these cells efficiently differentiate into mature functional oligodendrocytes. Here we describe a new versatile high-throughput and high content screening (HTS and HCS) cell-based assays to screen for new compounds that affect human oligodendrocytes precursor cell (hOPC) proliferation, differentiation and myelination. The HCS analysis results in quantification of multiple parameters, including oligodendrocyte number, morphology and capacity to myelinate axons. To demonstrate the capabilities of this system to detect an effect of a compound on oligodendrocyte differentiation, we tested escalating doses of Noggin (a bone morphogenetic protein family inhibitor) on our hOPC culture. We found that Noggin, significantly increased the number of OLIG2 and O4 positive cells, number of processes per cell, total processes length and branching in a dose-dependent manner. The 50% effective dose (EC₅₀) of Noggin was found to be 1.57 nM. The assay's robustness was confirmed by a profound Z' factor. Based on these results we screened an array of small molecules with a known BMP inhibition activity. We found that LDN-193189 (inhibitor of Alk2, 3, and 6) promoted oligodendrocyte differentiation and myelination (IC₅₀ 30nM). To evaluate the myelination capacity of hOPC using our screening system, we co-cultured hOPC and neurons from rodent dorsal root ganglia (DRGs). Myelination of axons was quantified by measuring area overlap between myelin basic protein (MBP) and neurofilament positive fibers. To demonstrate the feasibility of this co-culture system to screen for myelin promoting agents we tested the possible effect of LDN-193189 to enhance myelination.

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We found that LDN-193189 also stimulated myelination of neuronal axons. These new hOPC cell-based screening assays create new opportunities to discover compounds with a therapeutic potential to treat demyelinating diseases.

036

ENGINEERING HIGH AND LOW COMPLEX 3D NEURAL TISSUE FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Three dimensional cell cultures have proven to mimic natural tissues and organs more closely than cultures grown in two dimensions. Next to authentic cell-to-cell interactions the 3D culture system allows improved cell adhesion to the extracellular matrix and increases cell survival. For neural cultures growth in the third dimension opens the possibility for the formation of highly complex multi-layer structures and enhances neuronal differentiation and maturation. In this study fibroblasts and mononuclear blood cells were isolated from individuals and reprogrammed into iPSCs using non-integrating techniques. Human iPSC were induced to become multipotent neural progenitor cells which can be further differentiated into various neuronal and glial cell types. Using an air-liquid interface based, scaffold-free culture system, high complex and low complex 3D engineered neural tissue (ENT) were generated. After 6 to 8 weeks of differentiation the 3D ENTs were characterized by immunocytochemical and electron microscopic methods as well as by electrophysiology (multi-electrode array, MEA) and calcium imaging techniques. Immunocytochemistry analyses confirmed the different complexity states of the two ENT types. Both types revealed the presence of several neuronal and glial markers such as beta-III Tubulin, MAP2, NF200kD, GFAP, OSP and also various synaptic proteins were detectable. MEA recordings and calcium imaging revealed that ENT neurons exhibited spontaneous firing activity indicating the presence of functional synapses. The majority of the cells in the low complex ENTs differentiated into a homogenous layer of neurons and glia cells. In contrast, high complex ENTs were composed of a rather heterogeneous cell population ranging from undifferentiated neuronal precursors to mature neurons, astrocytes and oligodendrocytes. While low complex ENTs, due to their homogeneity, are suited for quantitative and functional downstream assays,

high complex ENTs are the better choice when highly differentiated neurons and glial cells are required for qualitative assays. In conclusion, these experiments demonstrate that 3D ENTs offer a flexible assay system for drug development and disease modelling of various neurological and psychiatric disorders. This work was supported by grants from EU FP7 and H2020 projects (STEMMAD, PIAPP-GA-2012-324451; EpiHealth, HEALTH-2012-F2-278418; EpiHealthNet, PITN-GA-2012-317146; D-BOARD, FP7-HEALTH-2012-INNOVATION-1-305815 and EuToxRisk H2020- PHC-2015-681002).

038

MODELLING AMYOTROPHIC LATERAL SCLEROSIS USING GENE TARGETED ISOGENIC IPSC REPORTER LINES

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder that selectively affects upper and lower motor neurons. Approximately 5 to 10 % of cases are familial and result from inherited genetic mutations. Of the known mutations involved in ALS pathogenesis, those affecting the *Fused in Sarcoma (FUS)* gene have recently gained attention. FUS is a ubiquitously expressed protein with transcriptional activation activity and is involved in RNA biogenesis and processing, as well as in DNA damage response. Moreover, FUS shuttles between nucleus and cytoplasm for the purpose of mRNA export, in addition to being recruited to form granules under conditions of stress. Hence, mutations affecting the *FUS* gene may interfere with a wide range of cellular functions, causing severe phenotypes in terminally differentiated cells such as motor neurons (MNs). The majority of studies investigating the role of FUS in ALS pathogenesis have used cellular models that fail to recapitulate the complex biology of MNs. To overcome this limitation, induced pluripotent stem cells (iPSCs) were selected for the present study due to their unique ability to undergo immortal self-renewal and differentiate into specialized cell types, including affected MNs. To facilitate the investigation of FUS behavior, the CRISPR/Cas9 technology was used to generate an iPSC line expressing the eGFP reporter sequence fused to one allele of the endogenous *FUS* gene. Additionally, the P525L-causing mutation, associated with early-onset ALS, was knocked-in to generate a suitable isogenic line. MNs were then derived using a differentiation protocol developed in our lab. These reporters uniquely enable the use of state-of-the-art technologies, including live cell imaging of axonal transport dynamics and stress granule formation,

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high-throughput compound screening, protein-protein interaction studies, as well as seeding studies to examine the potential prion-like behavior of the protein. In conclusion, the herein described isogenic iPSC reporter lines represent a powerful tool to elucidate mutant FUS-dependent molecular mechanisms involved in one form of familial ALS pathogenesis.

040

NEURAL STEM CELLS EXPANSION AND ITS IMPACT ON HIPPOCAMPAL FUNCTION THROUGHOUT LIFE

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Previous experiments in our lab showed that a transient cdk4 and cyclinD1 (4D) overexpression controls the balance between proliferative and neurogenic divisions of adult hippocampal neural stem cells (NSC). We developed a system in which HIV-1 based multicistronic viral particles are stereotaxically delivered into the adult hippocampus triggering 4D overexpression and NSC expansion. The particular design of these viral constructs permits 4D recombination in the infected NSC upon tamoxifen administration in Nestin-CreERT2 mice. After 4D removal, the previously expanded NSC differentiate and give rise to new immature neurons that are indistinguishable from physiologically generated neurons, thus increasing neurogenesis by two fold. Adult neurogenesis is known to decrease during life but it is still debated whether this is due to a depletion of NSC or, alternatively, a loss of their proliferative potential. Either way, we thought that 4D could be an ideal system to compensate for the age-dependent decrease in neurogenesis. To address this, we first induced 4D in senescent (over 16 month old) mice. In parallel, second, we induced 4D in young mice and let them reach senescence. Either manipulation induced a substantial increase in neurogenesis and, remarkably, the effect of the latter was an order of magnitude greater. Our data show that 4D induces the expansion of NSC without causing their long-term depletion. Moreover, our data suggest that NSC depletion during life has a bigger impact on age-related decrease in neurogenesis than a loss of proliferative activity. In parallel, behavioural analyses are being performed to assess the impact of increased neurogenesis on cognitive function. Our data in young mice suggest that 4D increase in neurogenesis improves flexible learning in the water maze, which paves the way for future experiments trying to restore cognitive function during senescence. Altogether, these experiments could help us to better understand the role of adult neurogenesis and investigate the use of NSC for therapy.

042

RETINAL STEM CELL TRANSPLANTATION ONTO THE INNER RETINA OF THE LARGE MAMMALIAN EYE REQUIRES CONSIDERATION OF THE ANATOMICAL DIFFERENCES BETWEEN SMALL RODENTS AND LARGE MAMMALS.

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Human Müller glia with stem cell characteristics (hMSC) have been shown to improve retinal function following differentiation into retinal ganglion cells (RGCs) *in vitro* and transplantation into a rat model of retinal ganglion cell depletion. However, the translational potential of these cells may depend upon successful engraftment and improvement of retinal function in experimental models with anatomical and functional features resembling those of the human eye. We have investigated the effect of allogeneic transplantation of feline Müller glia differentiated into RGCs following retinal ablation of RGCs by NMDA in this species. Unlike previous observations in the rat, xeno-transplantation of RGCs derived from hMSC into the feline vitreous resulted in the formation of cellular aggregates and elicited a severe inflammatory response without improving visual function. In contrast, allogeneic transplantation of RGCs derived from feline Müller glia into the vitrectomized eye improved the scotopic threshold response (STR) of the electroretinogram (ERG). Despite causing functional improvement, cells did not attach onto the retina and formed aggregates on residual vitreous, suggesting that vitreous may constitute a barrier for cell attachment onto the retina. This was confirmed by observations that cellular scaffolds of compressed collagen and enriched preparations of feline Müller-derived RGC facilitated cell attachment. Although cells did not migrate into the RGC layer or the optic nerve, they significantly improved the STR and the photopic negative response of the ERG, indicative of increased RGC function. The results indicate that the transplanted cells may have had a neuroprotective ability that promoted partial recovery of RGC function, and more important, they suggest that methods used for transplantation of cells onto the inner retina of small rodents may need to be refined if similar approaches are to be implemented in the human eye.

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044

ESTABLISHING CHRONIC NEURODEGENERATION MODELS IN ZEBRAFISH BRAIN

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Human brain is prone to neurodegenerative disorders where neurons chronically die. However, we cannot regenerate the lost neurons in part due to inefficient stem cell activation and regenerative neurogenesis. In contrast, adult zebrafish has a profound ability to regenerate lost neurons after various modes of tissue loss. So far the regenerative ability of adult zebrafish brain has not been well studied in a chronic neurodegeneration situation. We aim to model chronic neurodegeneration in zebrafish brain by expressing variants of human proteins that are associated with pathology of neurodegeneration in humans. In this study, we generated a conditional zebrafish line expressing P301L-mutant version of human Tau protein, which has previously been associated with neurodegenerative diseases such as Frontotemporal Lobar Degeneration and Alzheimer's Disease. We analyzed the effects of chronic Tau expression on phosphorylation and tangle formation, immune response, cell death and stem cell proliferation in adult zebrafish brain. We believe that this humanized zebrafish Tau model can be used to further test and model tauopathies in the regenerative system of zebrafish, and findings in this model can potentially be used to design new therapeutic strategies in humans.

046

GENERATION OF CLINICALLY RELEVANT IPS CELL LINES FROM HUMAN PERIPHERAL AND CORD BLOOD DERIVED ENDOTHELIAL PROGENITOR CELLS USING SELF-REPLICATIVE RNA

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In 2010, it was first published that repeated transfection of fibroblasts with a cocktail of reprogramming mRNAs resulted in the generation of stable, integration-free human iPS cells. While many advancements have been made to refine this process on fibroblasts, to date no group has been able to apply this same technology to reprogram a blood derived cell type. Peripheral blood provides easy access to adult human cell types for reprogramming purposes. Notably, blood-outgrowth endothelial progenitor cells (EPCs) can be clonally isolated from both fresh and frozen human peripheral blood and cord blood. The EPCs adherent nature and high prolifer-

ative capacity while maintaining their cell identity makes them highly desirable for transfection with RNA. In 2013, published results demonstrated the reprogramming of human neonatal fibroblasts into iPS cells using self-replicative RNA (srRNA), with as few as one transfection. Subsequently, we have extended the application of srRNA for cellular reprogramming to peripheral and cord blood derived EPCs as well as adult fibroblasts. The generation of RNA-EPC-iPS cell lines required optimization of mRNA delivery, culture media composition and transitions, as well as incorporation of reprogramming associated microRNAs. These improvements resulted in a simple and robust two transfection, no-split protocol on extracellular matrix without the need for conditioned medium. These integration-free EPC-derived iPS cell line exhibit unique genetic stability, making them an exceptional choice for applications requiring clinical grade cells. Lastly, those clinical grade EPC-iPS cells generated using this novel srRNA reprogramming technology present a therapeutic opportunity to treat myeloproliferative disorders in which the disease-causing somatic mutations are restricted to cells in the hematopoietic lineage. Here we present data demonstrating the unique combined application of microRNA and srRNA for the cellular reprogramming of human EPC lines derived from human blood as well as adult fibroblasts into stable, pluripotent and clinically relevant iPS cells.

048

ROLE OF CATHEPSIN S AND CX3CL1-CX3CR1 AXIS IN THE REGULATION OF ABNORMAL MICROGLIAL ACTIVATION IN NIEMANN-PICK DISEASE TYPE C1 MICE

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Niemann-Pick disease type C1 (NPC1) is a fatal metabolic disorder caused by a mutation in the NPC1 gene, which leads to the disruption of the lipid trafficking system and lipid sequestration within the lysosomal and late endosomal compartments. In our previous study, we have demonstrated that excessive microgliosis contribute to the progress of olfactory deficit in NPC1 mice via increasing neuronal apoptosis and impeding the maturation of newborn neurons. Although usage of microglial inhibition seems to produce therapeutic outcomes as proved in prior data, understanding how microglia react to NPC1 dysfunction is still important because microglia also provides beneficial effects during the neurodegenerative process. Therefore, we aimed to extend our knowledge via investigating the underlying mechanisms of microglial dysregulation and its detrimental impact in NPC1 status. Herein, we elucidate the novel role of Cx3cl1-Cx3cr1 signaling in olfactory dysfunction in NPC1 mice. First, we showed that expression level of microglial Cx3cr1 is increased accompanied with



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enhanced secretion of neuronal chemokine Cx3cl1 into CSF in NPC1 mice compared to WT mice, implying that up-regulated Cx3cl1-Cx3cr1 interaction might be responsible for astrocyte-independent microglial activation in NPC1. Interestingly, blocking of this interaction using Cx3cl1 neutralization by nasal infusion of Cx3cl1 specific antibody successfully led to transformation of microglia from amoeboid form into ramified, resting form as well as reduced the total number of microglia. Notably, excessive microgliosis is attenuated by blocking Cathepsin S activity followed by recovery of olfaction, suggesting that cathepsin S is responsible, in part, for this phenomenon. In addition, further in vitro data revealed that cholesterol accumulation induced by NPC1 mutation or U18666A treatment enhances Cathepsin S expression via abnormal P38 MAPK activation in microglia. These observations elucidate a causal relationship between cathepsin S-mediated Cx3cl1-Cx3cr1 signaling and microglia dependent degenerative process in neurological disorders with altered cholesterol metabolism.

050

BRAINPHYS™ NEURONAL MEDIUM: A NEW MEDIUM OPTIMIZED TO SUPPORT THE NEUROPHYSIOLOGICAL ACTIVITY OF PRIMARY AND HUMAN PLURIPOTENT STEM CELL-DERIVED NEURONS

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Neuronal cultures derived from human pluripotent stem cells (hPSCs) and primary tissues are useful models for studying neurological development and disease. The relevance of these studies is increased when neurons are cultured in a medium that closely resembles the physiological milieu of the central nervous system. Traditional media, such as DMEM/F-12 and Neurobasal™, were designed to promote cell survival; however, they acutely inhibit normal electrophysiological functions, including action potential generation and synaptic communication. Consequently, these culture media must be replaced with artificial cerebrospinal fluid (ACSF) immediately prior to electrophysiological evaluation, thereby complicating the procedure. BrainPhys™ is a novel neurophysiological medium, designed to circumvent these issues (Bardy et al., 2015). Based on the published formulation, we developed BrainPhys™ Neuronal Medium (NM) and demonstrated that it supports both maturation of primary neurons and the differentiation and maturation of hPSC-derived neurons. Neural progenitor cells derived from hPSCs were cultured in BrainPhys™ NM, supplemented with growth factors. After 21 - 30 days, the cells displayed neuronal morphology and appropriate expression of MAP2, class III β -tubulin, and Synapsin 1, and exhibited normal electrophysiological profiles. We also developed a two-

step protocol for primary neuronal culture that resulted in improved survival and neurophysiological function. E18 rat cortices were dissociated and plated in a traditional neuronal culture medium (NeuroCult™ Neuronal Basal Medium or Neurobasal™ Medium, supplemented with NeuroCult™ SM1). After 5 days, half of the cultures were transitioned to BrainPhys™ NM, and half were maintained in the traditional medium throughout the culture period (control). After 21 days, the number of neurons was approximately 2-fold greater in cultures matured in BrainPhys™ NM, compared to the control. Furthermore, these neurons expressed MAP2 and Synapsin 1, and showed increased frequency and amplitude of spontaneous AMPA and GABA_A receptor currents, relative to the control. Together, these data demonstrate that BrainPhys™ NM supports the growth and maturation of hPSC-derived and primary neurons under physiologically relevant conditions.

052

DIRECT CONVERSION OF PATIENT SPECIFIC HUMAN NEURAL STEM CELLS FROM NIEMANN PICK DISEASE TYPE C PATIENT DERIVED FIBROBLASTS

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Niemann-Pick disease type C is a neurodegenerative and lysosomal lipid storage disorder, characterized by abnormal accumulation of unesterified cholesterol and glycolipids, which is caused by mutations in NPC1 or NPC2 genes. Here, we report the generation of human induced neural stem cells from NPC patient-derived fibroblasts (NPC-iNSCs) using only two reprogramming factors without going through the pluripotent state. NPC-iNSC lines were stably expandable and showed trilineage neural differentiation potential. However, NPC-iNSCs displayed cholesterol accumulation, defective self-renewal, and neuronal differentiation, suggesting that NPC-iNSC lines retain main features of NPC disease. We found that valproic acid (VPA), a histone deacetylase (HDAC) inhibitor, significantly ameliorated the cholesterol accumulation and restored cholesterol homeostasis. Furthermore, VPA corrected the impaired self-renewal and neuronal differentiation of NPC-iNSC lines. Taken together, these findings suggest that NPC-iNSCs could provide a powerful platform for pathological study or drug screening in a patient specific manner and that this direct conversion technology may extend to other human neurodegenerative diseases.

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054

CHARACTERIZATION OF LRRK2 MUTANT IPS-DERIVED DOPAMINERGIC NEURONS AND ASTROCYTES

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Parkinson's disease (PD) is the second most common neurodegenerative disease that affects approximately 1-2% population over 60. The pathology is characterized mainly by the progressive degeneration of dopaminergic neurons in the *substantia nigra* and by intracellular inclusions known as Lewy bodies. Despite extensive research, there is no cure for this devastating disorder. As most cases of PD are sporadic, the exact cause of disease pathogenesis is unknown, and multiple events, such as genetic mutations and exposure to environmental toxins, are likely to cause PD. Mutations in LRRK2 gene are the most common genetic cause of PD. The pathology of patients with LRRK2 G2019S resembles that of patients with sporadic PD and may thus be a model for at least some of these cases. We have generated iPSCs from patients with LRRK2 G2019S mutation and further differentiated them into dopaminergic (DA) neurons and astrocytes. DA neuron-enriched cultures (40% of DA neurons) of LRRK2-mutant lines manifested increased α -synuclein levels while tyrosine hydroxylase and dopamine transporter levels were decreased when compared to corresponding cultures derived from normal control patients. In addition, phosphorylation of extracellular signal-regulated kinase $\frac{1}{2}$ (ERK) was increased in the cultures of the mutant lines. In LRRK2-mutant astrocytes (> 90% GFAP+), the level of cytoplasmic α -synuclein was higher in comparison to control astrocytes. Moreover, the levels of MAO-B were increased in LRRK2-mutant astrocytes, a finding which is in agreement with previous reports suggesting MAO-B to act as an inducer of PD phenotype. On the other hand, the expression level of glial cell line-derived neurotrophic factor, essential for the survival of dopaminergic neurons, was increased and also transcription factor NURR1 was upregulated in LRRK2-mutant astrocytes.

In conclusion, DA neurons derived from patients carrying LRRK2 G2019S mutation were positive for many characteristics of PD pathology, whereas astrocytes derived from these same patients exerted both neuroprotective, possibly adaptive responses and neurodegenerative alterations. Additional studies addressing possible alterations in neuron-astrocytes interaction in LRRK2-mutant cell lines might help understand the mechanisms of cell-to-cell spreading of the disease.

056

CONTROL OF SPINAL CORD DEVELOPMENT AND REGENERATION IN ZEBRAFISH

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Coordinated development of brainstem and spinal target neurons is pivotal for the emergence of precisely functioning locomotor system.

Signals that match the development of these far apart regions of the CNS may be redeployed during spinal cord regeneration. Here we show that descending dopaminergic projections from the brain promote motor neuron generation at the expense of V2 interneurons in the developing zebrafish spinal cord by activating the D4a receptor, which acts on the hedgehog pathway. Inhibiting this essential signal during early neurogenesis leads to a long-lasting reduction of motor neuron numbers and impaired motor responses of free-swimming larvae. Importantly, during successful spinal cord regeneration in adult zebrafish, endogenous dopamine promotes generation of spinal motor neurons and dopamine agonist augment this process. Hence, we describe a novel supraspinal control mechanism for the development and regeneration of specific spinal cell types that uses dopamine as a signal.

058

AN APPROPRIATE INTRACELLULAR CONTENT OF THYROID HORMONE IS REQUIRED TO OVERCOME THE BLOCK OF OPCs DIFFERENTIATION INDUCED BY INFLAMMATION

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The active form (T3) of thyroid hormones (TH) induces cell cycle exit and terminal differentiation of oligodendrocyte precursor cells (OPCs) into myelinating oligodendrocytes (OL). A block of this process is a pre-eminent cause of remyelination failure in demyelinating diseases. In the CNS, deiodinase D2 is responsible for the activation of T4 into T3, while D3 is the inactivating enzyme of T4 and T3, to rT3 and T2 respectively. D2 and D3 activities are altered by inflammatory cytokines in several tissues including CNS, during acute and chronic diseases. We investigated if a dysregulation of T3 intracellular content is involved in the inflammation-induced OPCs differentiation block. By using the deiodinases inhibitor iopanoic acid (IOP), we first proved that our OPCs system (OPC -NG2-IR- derived from the subventricular zone of adult rats) needs T3 for an



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efficient maturation/differentiation process. In fact, we found that the block of T3 production by IOP significantly decreases the % of mature/myelinating oligodendrocytes (CNPase-IR: $P < 0.0001$; MBP-IR $P = 0.0124$). Therefore, we exposed oligospheres to an inflammatory cytokine cocktail (TGF- β 1, TNF- α , IL-1 β , IL-6, IL-17 and IFN- γ ; 20ng/ml), then inducing differentiation (12 days) by T3 and cytokines withdrawal. The exposure to cytokines results in an increase in NG2-positive cells ($P = 0.0015$) and a drastic decrease of CNPase- ($P = 0.0002$) and MBP-IR cells ($P = 0.0030$). In this conditions, we observed a significant increase in the % of D3-IR cells ($P = 0.0001$). Accordingly, we used IOP to block D3 and restore T3 intracellular content in the presence of cytokines. This treatment restored the maturation of OL from OPC. At 12 days in culture, we obtained the following % of cells along the maturation lineage of OPC. In the presence of T3: NG2 17.1%, CNPase 40%, MBP 22.3%; in the presence of T3 + cytokines (vs T3): NG2 46.9% $P < 0.0001$; CNPase 4.9% $P < 0.0001$; MBP 0.7% $P < 0.0001$; in the presence of T3 + cytokines + IOP (vs T3 + cytokines): NG2 20.7% $P < 0.0001$, CNPase 22.3% $P = 0.0008$, MBP 13.1% $P < 0.0001$). We then concluded that in our oligosphere assay, inflammatory cytokines induce an increase of the D3 enzyme, that inactivates T3 in OPC. This mechanism could take part in the cytokines-induced OPC differentiation impairment in inflammatory-demyelinating diseases.

060

SCAFFOLDS FOR CELL THERAPIES IN NEURAL REPAIR: STANDARDIZATION OF SAFETY AND EFFICACY IN VITRO TESTING FOCUSING ON APPROPRIATE CELLULAR SYSTEMS, HIGH-CONTENT ANALYSIS AND REGULATORY CONCERNS.

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The potential applications of tissue engineering products for regenerative medicine in acute and chronic diseases of the nervous system are quite extensive. The process of creating "a smart scaffold" involves not only materials science and fabrication, but also calls for extensive biological testing. The third-generation of biomimetic materials is actually designed to stimulate specific cellular responses at the molecular level. Therefore, in vitro testing must be optimized to explore the impact of novel materials on cellular and molecular responses involved in each pathogenic process. In particular, appropriate cell systems and technologies

must be standardized also taking regulatory issues into consideration. In this study the biological and molecular effects of different scaffolds on three neural systems were compared, i.e. the neural cell line SH-SY5Y, primary cortical neurons and neural stem cells. The effect of poly(L-lactic acid) (PLLA) scaffolds having different surface geometry (conventional 2D flat surface, random or aligned fibres as semi3D structure) and chemical functionalization (laminin or extracellular matrix extract) were compared. The end-points were defined for efficacy (i.e. neural differentiation and neurite elongation) and for safety (i.e. cell death/survival) using statistically sound approaches supported by high-content analysis. It is demonstrated that (i) the characterization of biological properties of biomaterials is profoundly influenced by the test system used; (ii) the definition of the in vitro safety profile of biomaterials for neural repair is also influenced by the test system; (iii) cell-based high-content screening may well be successfully used to characterize both the efficacy and safety of novel biomaterials, thus speeding up and improving the soundness of this critical step in material science having a medical application. Lastly, an example of a "Safety Study Report" performed in compliance with Good Laboratory Practice is presented. In conclusion, an appropriate consideration of experimental variables could truly improve the pipeline of "third-generation biomaterials" development to support cell-based regenerative medicine of the nervous tissue.

062

HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED MIXED EXCITATORY INHIBITORY NEURONAL NETWORK CULTURES

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Cortical activity is determined by the complex interplay between excitation and inhibition. Inhibitory interneurons provide functional identity to local networks and give rise to synchronized oscillations. To maintain the integrity of a neuronal network during early and late development, different molecular and physiological mechanisms are involved. Disruption of this balance between excitatory and inhibitory activity is thought to underlie different neurological disorders. Patient induced pluripotent stem

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cell (iPSC) - derived neuronal cultures give the potential to model disease phenotypes *in vitro* with the ultimate goal to study underlying disease mechanisms and to screen for therapeutic targets. Despite existing studies using iPSC technology to generate neuronal networks, current cultures often lack the presence of both inhibitory and excitatory neuronal populations. Here we present an *in vitro* differentiation protocol which generates a mixture of functionally mature inhibitory and excitatory iPSC-derived neurons. We show that the low-density sandwich culture mode offers the opportunity to study neuronal changes in physiology and gene expression.

064

TRANSPLANTED EMBRYONIC NEURONS INTEGRATE INTO ADULT NEOCORTICAL CIRCUITS

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Transplantation studies in animal models of neurological disease or injury have shown host-graft synapse formation and extension of efferents from the grafted cells to proper anatomical targets through the adult brain. However, it remains largely unexplored whether new afferent synapses are adequate and thus able to convey genuine input information. To tackle this question, we induced cell death of upper layer callosal projection neurons in the primary visual cortex (V1) of adult mice and investigated whether young neurons transplanted at the lesion may integrate properly or aberrantly into the pre-existing circuitry, a critical question for functional reconstruction. We demonstrate here that most of transplanted cells display the appropriate upper layer neuron identity and morphology, acquiring synaptic structures over time. Using the monosynaptic rabies virus-based tracing approach we dissected the whole brain host-to-graft circuits. Local neuronal populations within the visual cortex connect massively with transplanted neurons, but also other sensory and associative areas including the somatosensory cortex, motor cortex, auditory cortex, retrosplenial cortex and the posterior parietal association cortex. Moreover, synaptic input from subcortical distant areas including the thalamic lateral geniculate nucleus, which relays information from the retina and the contralateral V1 was found. A comparative analysis with the endogenous developmentally-generated connectivity shows that the newly formed circuits strikingly resemble the normal afferents of V1 neurons. In addition, using two-photon calcium imaging we demonstrate that transplanted

neurons become visually responsive and show orientation and/or direction selectivity. Altogether, our results indicate that new neurons may properly integrate and function in an injured neocortex.

066

ANALYZING OLIGODENDROCYTES AND REMYELINATION AFTER INJURY OF THE ADULT ZEBRAFISH SPINAL CORD

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A major component of the loss of axonal function after spinal cord injuries in mammals is the death of oligodendrocytes and consequent loss of myelin sheaths that are essential for proper axonal signal transduction. Therefore, we decided to study the mechanisms of de- and consequent remyelination using the regeneration permissive adult zebrafish spinal cord as a model. Using a spinal cord transection lesion paradigm and a transgenic mbp:GFP reporter line we find that mature oligodendrocytes and myelin sheaths are lost around the site of injury within the first week after lesion. However, during the course of axonal regeneration from 2 to 6 weeks after lesion we find that the normal myelination pattern is largely reestablished. Hence, the adult zebrafish spinal cord transection model is ideally suited to study the cellular and molecular mechanisms of remyelination. A better understanding of these processes in zebrafish could help to develop novel therapeutic strategies for the diseased or injured human spinal cord.

068

DISEASE MODELING OF FTD WITH PATIENT IPSC-DERIVED NEURONS AND THEIR ISOGENIC CONTROLS

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Frontotemporal dementia (FTD) is a clinically, pathologically and genetically heterogeneous disease. One unique subtype of FTD was previously described in a large Danish family, linked to chromosome 3 (FTD3), resulting in a C-terminal truncated version of the charged multivesicular body protein 2B (CHMP2B) which is a component of the endosomal ESCRT-III complex. This dominant gain of function mutation affects the functionality of the endo-lysosomal system, which is important for protein degradation and cell surface receptor recycling. Here, we present a disease model based on human induced pluripotent stem cells (hiPSCs) from FTD3 patients and their isogenic controls generated via the CRISPR/Cas9 method. We derived forebrain region-specific neurons from our patient and isogenic hiPSC lines expressing NESTIN, PAX6 and OTX2 proteins. After subsequent maturation, we obtained glutamatergic cortical neurons expressing MAP2AB, TUJ1, TAU, VGLUT1, TBR1 and CTIP2 proteins. Neuronal functionality was validated by application of fluorescent probes and image analysis showing increased intracellular calcium levels in response to the neurotransmitters glutamate/glycine, GABA and acetylcholine as well as membrane depolarization by application of extracellular potassium. Patient-derived neurons displayed enlarged endosomes, as earlier described in patient brain samples and fibroblasts thereby validating our hiPSC-derived neurons as a useful in vitro tool to study FTD3 and in particular endo-lysosomal dysfunction. Moreover we identified a unique and novel phenotype in the patient's iPSC-derived neurons characterized by mitochondria with poorly developed cristae. Accordingly, these neurons also had decreased basal respiration and a reduced spare respiratory capacity. All of the observed phenotypes were reversed after targeted gene corrections in the isogenic controls. Taken together, we present here for the first time a human FTD3 in vitro model based on hiPSC-derived neurons including their isogenic controls, which show enlarged endo-lysosomal complexes and mitochondria abnormalities and dysfunction. *Supported by the EU FP7 Programme (PIAPP-GA-2012-324451), Danish Innovation Fund (4108-00008B) and Copenhagen Consortium for Designer Organisms, University of Copenhagen.*

070

MODELING INTERNEURONAL DIFFERENTIATION PROCESSES USING INDUCED PLURIPOTENT STEM CELLS DERIVED FROM ALZHEIMER'S DISEASE PATIENTS BEARING MUTATIONS WITHIN GENES ASSOCIATED WITH INFLAMMATORY PROCESSES

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Recently, a *TREM2* missense mutation, R47H, and a functional copy number variation (CNV) in *CR1* have been associated with Alzheimer's Disease (AD) risk. *TREM2* is defined as an innate immune receptor expressed on myeloid cells in the brain, while the complement receptor type 1 (*CR1*) is found on microglia and neurons, but little is known about the influence these mutations have on neuronal development in the central nervous system of Alzheimer-disease patients. Here we used patient-specific induced pluripotent stem cells (iPS) and analysed the distinct progression steps of cortical inhibitory GABAergic interneurons (GABA-IN) by comparing the neural-derived networks of the *TREM2* mutation and the CNV *CR1* to healthy control lines. First, mimicking by embryoid bodies, neural rosettes and neurospheres, the migration steps of the medial ganglionic eminence (MGE)-born progenitor cells were analysed by ventral forebrain markers such as *NKX2.1*, *FOXG1*, *LHX6* and *DLX*. The maturation steps to functional interneurons were classified by subtype-specific expression of neuropeptide Somatostatin and calcium-binding protein markers Calbindin2 and Parvalbumin. Together with GABAergic and neuronal marker, e.g. GABA, *GAD1/2*, Tau and pre-/post-synaptic proteins, the developing progression from immature to inhibitory interneuron was monitored. To sum up, we used cell morphology, transcriptome profiling as well as subtype-specific protein marker expression to track the diverse maturation steps from (a) the undifferentiated pluripotent state, (b) the MGE-like progenitor cell and (c) the GABAergic interneuronal cell in relation to the *TREM2* mutation and CNV in *CR1*. In conclusion, our study has shown the ability to differentiate patient-specific cortical inhibitory GABA-IN, the neuronal cell type that is associated with dementia, thus demonstrating the advantages of studying various maturation steps within the context of innate immunity. Data obtained in this preliminary study will help to understand the basic pathological mechanism underlying the onset of neurodegenerative diseases and could provide potential therapeutic targets for interneuronal-driven disorders such as AD, epilepsy and autism.

072

INJECTABLE POROUS SCAFFOLD PROMOTES NEUROGENESIS FOR STROKE REPAIR

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Stroke is the leading cause of adult disability in the US. Although tissue regeneration is limited after stroke, neurogenesis, or recruitment and infiltration of endogenous Neural Progenitor Cells (NPCs) to the damaged site has been described as the critical phenomenon in post-stroke tissue regeneration and functional recovery. Advances in biomaterials have developed hydrogels that support cell infiltration and tissue regeneration when injected in the wound area. However, to date, all gels injected in the brain have failed at promoting neurogenesis, likely due to the high inflammatory response and the astrocytic scar formation after stroke. Microporous gels have shown to promote a significant reduction in inflammation and scar formation; therefore, we hypothesize the injection of a microporous Hyaluronic Acid (HA) gel in the stroke cavity should reduce microglial and astrocyte activation, thereby allowing recruitment and infiltration of endogenous NPCs in the damaged site. However, until now, the creation of pores required the gel to be pre-cast before transplantation, making it not injectable. Using microfluidics, we engineered the first injectable hydrogel made of spherical HA beads that anneals *in situ* and demonstrates interconnected porosity. The gel stiffness was engineered to directly match that of the brain tissue. Furthermore, due to the random packing of these HA beads a void space is generated between the beads that is 10-15% of the scaffold volume, allowing for easier cell infiltration. The size of each individual pore can be modulated by changing the size of the HA beads. Mice were subjected to an ischemic stroke and injected 5 days later with porous HA directly into the stroke cavity, and sacrificed 10 days post injection. Sections were stained and analyzed for neurogenesis (Dcx), microglial response (Iba-1) and astrocytic scar (GFAP). We found that the Iba-1 % area in the infarcted core and the GFAP scar thickness were significantly reduced in the porous HA condition compared to the non-porous HA. Moreover, the infiltration of Dcx cells in the gel was significantly greater in the porous condition. Overall, this experiment demonstrates that the injection of a porous gel reduces the inflammatory and astrocytic response after stroke and promotes the recruitment and infiltration of NPCs in the damaged site.

074

DYNAMIC REMODELLING OF NEURAL CELLULAR AND EXTRACELLULAR SIGNATURES DEPICTED IN 3D IN VITRO DIFFERENTIATION OF HUMAN iPSC-DERIVED NSC

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Brain microenvironment plays an important role in

neurological development, function and degeneration. Extracellular matrix (ECM) components modulate neural cell survival, migration, proliferation and neuronal function. We hypothesized that three-dimensional (3D) differentiation of human neural stem cells can sustain microenvironment remodelling and consequently recapitulate key cell-ECM interactions. Aggregation and 3D neural differentiation of human neural stem cells derived from induced pluripotent stem cells (hiPSC-NSC) was performed in perfusion stirred-tank bioreactors. Neural differentiation was successfully induced with recapitulation of neurogenesis developmental pathways events. Complex tissue-like 3D structures containing neuronal, astroglial and oligodendroglial cells were generated by 1 month of culture. Major neuronal subtypes were detected, including dopaminergic, glutamatergic and GABAergic lineages. These neurons spontaneously elicited calcium transients and performed stimuli-induced synaptic vesicle exocytosis. Differentiated glial cells presented typical astrocytic functions, such as extracellular glutamate clearance and glutamine synthesis. Concomitantly, a significant enrichment in neural adhesion molecules involved in neuronal migration and differentiation (e.g. L1CAM and NCAM) was observed; typical neural ECM proteoglycans, such as neurocan and versican were also identified. In summary, we demonstrated that 3D neural differentiation of hiPSC-NSC enabled *in vitro* recapitulation of neural cellular and extracellular developmental features. Moreover, the developed culture platform, combined with the use of patient-derived iPSC could be applied to the development and interrogation of human 3D neurological disease models. Support from Brainvectors (FP7-286071) funded by the EU; SFRH/BD/78308/2011 and SFRH/BD/52473/2014 PhD fellowships funded by FCT, Portugal.

076

DEVELOPMENT OF PARKINSONS DISEASE CELL MODEL BASED ON DISEASED IPS CELLS - DERIVED MATURE NEURONS

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Parkinson's disease (PD) is the second most common neurodegenerative disorder (after Alzheimer's disease). Parkinson's disease is a gradually progressive degenerative neurologic disorder. Mutations in some



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genes have been identified as a cause of familial PD. Autosomal dominant mutations in the leucine-rich repeat kinase 2 (LRRK2; PARK8 gene) are the most common cause of familial and sporadic PD identified to date. Mutations in the E3-ubiquitin ligase parkin (PARK2 gene) cause an autosomal recessive early onset PD. Although some progress on LRRK2 and parkin involvement in the disease progression has emerged during last years, the absence of adequate model complicates investigation of human diseases and development of novel therapies. Recent advances in cell reprogramming technologies facilitate the development of cellular models that allows precise investigation of disease mechanisms. Using skin biopsies from PD patients with PARK8 gene mutation (G2019S) and with PARK2 gene compound heterozygous mutations we reprogrammed dermal fibroblasts using lentiviral constructs carrying Yamanaka's factors (Oct4, Sox2, Klf4, c-Myc). We developed an effective protocol of iPSC differentiation into tyrosine hydroxylase - positive dopaminergic neurons. This protocol allows cryopreserving of neuronal progenitors at two stages of differentiation: early neuronal progenitors and specialized dopaminergic progenitors. Efficiency of differentiation was determined by flow cytometry. Expression of synaptophysin and electrophysiological activity indicates mature state of these neurons. A high percentage of tyrosine hydroxylase - positive cells in resulted population (up to 80%) allows performing phosphoproteomic and transcriptomic assays. We developed mitochondrial potential assay based on flow cytometry approach for analyzing the state of mitochondria in cells carrying mutation in parkin compared with non-mutated cells.

078

DIRECT CONVERSION OF HUMAN BLOOD DERIVED CELLS INTO STABLY EXPANDABLE TRIPOTENT INDUCED NEURAL PROGENITORS (INPCs)

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Reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) represents an attractive approach to generate donor cells for disease modelling, drug discovery and neuro-restorative approaches. However, generation and subsequent neural differentiation of human iPSCs is time-consuming, and the extended cultivation periods may result in the acquisition of mutations. Here, we present a robust approach for the direct conversion of human cord blood and adult human peripheral blood-derived cells into homogenous and transgene-free induced neural progenitor cells (iNPCs). After transduction of the donor cells with non-integrating Sendai viruses expressing Sox2 and c-MYC, neuroepithelium-like iNPC colonies emerge within a

week in chemically defined conditions. Single colony-derived iNPC lines can be stably maintained for up to 25 passages and express early neuroectodermal markers such as PAX6, SOX2, NESTIN, ZO1 and PLZF as well as neural crest markers AP2 α and HNK1. iNPCs respond to instructive patterning and differentiation cues promoting, e.g., specification of neuronal and glial subtypes including spinal motoneurons, mesencephalic dopamine-like neurons and astrocytes. iNPC-derived neurons show mature electrophysiological properties including the spontaneous postsynaptic currents indicating functional synapse formation. The direct conversion of human blood-derived cells into transgene-free iNPCs may enable the establishment of alternative patient-specific cellular resources for disease modelling, regenerative medicine and other biomedical applications.

080

CENTROSOME POSITIONING DURING DEVELOPMENT IS INSTRUCTIVE FOR THE SEEDING OF ADULT NEURAL STEM CELLS

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The developmental mechanisms regulating the generation of the appropriate number of adult (a) neural stem cells (NSCs) are largely unknown. Here we show that the positioning of centrosomes during M-phase in embryonic radial glial cells (RGC) is crucial to determine the number of aNSCs in the adult subependymal zone (SEZ). Manipulating *mlnsc* or *LGN* each leads to a reorientation of the division plane, which in turn results in a change in the progenitor pool composition. Live imaging demonstrates that the frequency of self-renewing divisions producing apical RGCs is reduced upon acute overexpression of *mlnsc* or a dominant negative form of *LGN*. These alterations in the embryo ultimately lead to a dramatic reduction of aNSCs in the adult SEZ with an accordingly reduced neuronal output. However, *mlnsc* overexpression in aNSCs did not affect their behavior suggesting a specific role for centrosome positioning in embryonic RGCs in order to determine the initial number of aNSCs.

082

WITNESSING PERICYTE TO NEURON CONVERSION WITHIN 3D CEREBRAL ORGANIDS

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Work from our lab has shown that brain pericytes derived from the adult human cerebral cortex can be successfully reprogrammed into induced neurons (iNs) *in vitro* via overexpression of the transcription factors Sox2 and Ascl1, thus identifying brain pericytes as a new target cell population for *in vivo* reprogramming (Karow et al., 2012). One of the major challenges for human tissue organ repair consists in devising appropriate experimental paradigms for testing the functionality of reprogrammed human cells in the context of human tissue. Here we established an experimental platform to assay lineage conversion of human pericytes within 3D human cerebral organoids using a recently developed protocol (Lancaster et al., 2013). As previously reported, different developmental stages of brain development can be discerned within these cerebral organoids including the ventricular/subventricular-like germinative zones as well as different stages of neurogenesis. Cultures of human brain pericytes were transduced *in vitro* with a polycistronic retrovirus encoding Sox2 and Ascl1 together with a reporter gene and FAC-sorted cells were subsequently transplanted into cerebral organoids. With this approach we aimed at assessing whether i) Sox2-Ascl1 transduced pericytes can still undergo lineage conversion within the context of human developing nervous tissue; ii) whether exposure of transplanted cells to the milieu of the organoids results in an improvement of conversion efficiency and/or affects neuronal subtype selection of the reprogrammed cells; and iii) whether cells undergoing reprogramming are preferentially recruited to specific regions within the organoids. Indeed we find that pericytic cells transduced with Sox2-Ascl1 not only survive within cerebral organoids but successfully undergo lineage reprogramming into iNs as indicated by acquisition of the neuronal marker bIII-tubulin. Using continuous 2-photon live imaging we could observe transplanted pericytes undergoing reprogramming within human cerebral organoids. Using this semi-*in-vivo* approach we will utilize cerebral organoids as experimental brain incubators to better understand and refine the direct conversion of pericytes into iNs and evaluate the potential application of pericyte-based cellular regeneration approaches.

084

MARKED DIFFERENCES IN HYPERMETHYLATION BETWEEN C9/ALS-FTD HESC LINES AND PATIENT-DERIVED IPS CELLS

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We established two human embryonic stem cell lines (SZ-ALS1, SZ-ALS3) with the GGGGCC expansion with nearly 300 repeats (C9 mutation). The embryos, which were obtained through preimplantation genetic diagnosis (PGD), were donated by a couple in which the mother was an expansion carrier (30 yrs-old). Our newly established C9/ALS-FTD HESC lines display key features of pluripotent cells; namely unrestricted growth in culture, expression of undifferentiated cell specific markers and potential to differentiate into a wide range of cell types by forming teratomas. In addition, we have generated iPS cells (more than 15 different clones) from a skin biopsy of the yet asymptomatic mother, and compared methylation levels upstream to the GGGGCC repeats in the iPS clones with that of HESC, blood and fibroblast cell counterparts. Interestingly, we find that the iPS cells are unusual in their hypermethylation levels. While the HESC, blood and fibroblasts are completely unmethylated, methylation is at its maximum in the iPS cells (50% in all examined clones representing levels of 100% on the mutant allele), indicating that direct cell reprogramming incorrectly hypermethylates this region as a consequence of the mutation. To further substantiate these findings and examine whether methylation is elicited with age or disease symptoms, we also generated iPS cells from a 65 year old C9/ALS patient in whom disease commenced 2 years ago. Here again, we observed dramatic gain of aberrant methylation (50% in all clones) as a consequence of cell reprogramming. Hypermethylation was not associated with age or manifestation of the disease. Taken together, our findings suggest that hypermethylation in C9/ALS-FTD is normally gained later during development, and that direct cell reprogramming may inappropriately enhance abnormal methylation in C9orf72.

086

PREPARATION AND IN VIVO TRANSPLANTATION OF DENTAL PULP DERIVED NEURAL STEM LIKE CELLS UNDER XENO-FREE CONDITION

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Even though neural stem cells (NSCs) could provide an invaluable resource for neuroregenerative medicine, embryo derived-, induced pluripotent stem cell derived-, or direct reprogrammed-NSCs have many hurdles for clinical application, such as ethical, immunological and safety (e.g animal components used to stem cell culture) concerns. Neural crest cells and ecto-mesodermal originated dental pulp stem cells are good candidate of autologous cell source, based on highly proliferative activity and differentiation potential to neural cell types, however



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animal serum have been used unwillingly for obtaining sufficient cells on primary culture. In this study, we aimed to set up the method to select and expand neural stem-like cells (NSLCs) from human dental pulp without animal components and serum in media from the initial step for advantages on safe clinical application and this study is the first trial for selective expansion of NSLCs from human dental pulp. We verified the NSLCs' ability of paracrine effects via secreting growth factors and cytokines, which were important to regulate environmental niche for survival and differentiation of stem cells. Then NSLCs were transplanted into rat brain and injured spinal cord and survived well within rat central nervous systems. In conclusion, NSLCs can be selectively expanded from human dental pulp using NSC media at initial step and these findings will be useful for clinical application of stem cells as an autologous source on neuroregenerative medicine

088

RETINAL ORGANOID TRISECTION FACILITATES EFFICIENT RETINOGENESIS FROM PLURIPOTENT STEM CELLS

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Recently, protocols for the differentiation of 3D retinal organoids from embryonic stem cells (ESCs) have been developed, which either recapitulate complete eyecup morphogenesis or maximize photoreceptor genesis. Here, we optimized the generation of large 3D stratified retina organoids from mouse ESCs, so that it does not require optic-vesicle or eyecup formation. Similar to previous reports we observed efficient eyefield induction (82 ± 12 SD % of aggregates, N=7), but a less efficient optic vesicle evagination limits the yield of retina organoids (35 ± 10 SD % of aggregates form Rax+ evaginations). We demonstrate that aggregate trisection at the eyefield neuroepithelium stage circumvents this limitation and facilitates an efficient and synchronized development of organoids with big, stratified retinal tissue, reminiscent of early postnatal retina *in vivo* (87 ± 3 SD % of aggregates, N=4). A given starting aggregate results on average in 1.8 ± 0.6 organoids (N=12) each with 1.4 ± 0.4 SD mm retinal circumferential length. Temporal gene expression comparative analysis of individual organoids (5 timepoints, 60 organoids, 22 genes) and cell birthdating experiments indicate efficient, reproducible, and temporally regulated retinogenesis in this organoid system. Further, inhibition of Notch signaling by DAPT treatment at early or late

stages of the differentiation protocol enables the generation of organoids enriched with cone or rod photoreceptors, respectively, demonstrating the power of our improved organoid system for future research work in stem cell biology and regenerative medicine.

090

DEVELOPMENT OF A HIGH THROUGHPUT-COMPATIBLE ASSAY TO QUANTIFY TAU AGGREGATION USING ADENOVIRALLY TRANSDUCED HUMAN IPSC-DERIVED CORTICAL NEURONS

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Tau aggregation is the pathological hallmark that best correlates with the progression of Alzheimer's Disease (AD). The presence of neurofibrillary tangles (NFTs), formed of hyperphosphorylated tau, leads to neuronal dysfunction and loss, and is directly associated with the cognitive decline observed in AD patients. Next to targeting β -amyloid pathologies the blocking tau phosphorylation, aggregation and/or spreading as alternative therapeutic entry points to treat AD has recently gained increased interest. Identification of novel therapies requires disease-relevant and scalable assays capable of reproducing key features of the pathology in an *in vitro* setting. Current tau aggregation models lack physiological relevance or are incompatible with high throughput screening (HTS) formats. To overcome these limitations we used induced pluripotent stem cells (iPSC) as a virtually unlimited source of human cortical neurons to develop a robust and scalable tau aggregation model. We optimized cell culture conditions to 384-well plate format using matrigel, and complemented the assay with AlphaLISA technology for the accurate quantification of tau aggregates in a high throughput-compatible format. The resulting assay is reproducible across users and works with different commercially available iPSC-lines, providing a reliable tool for better understanding tau pathophysiology and for the identification of novel treatments against AD.

092

TRANSCRIPTOME ANALYSIS OF STEM CELL-DERIVED NEURONS FOR MODELING ALZHEIMER'S DISEASE

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Recent efforts to develop *in vitro* models for Alzheimer's

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Disease (AD) have involved the differentiation of neurons or neural progenitors from pluripotent stem cells, such as induced pluripotent stem cells and embryonic stem cells. The early loss of basal forebrain cholinergic neurons (BFCNs) is a consistent feature of AD and the inclusion of BFCNs in *in vitro* models may help elucidate the pathogenesis of AD. To characterize stem cell-derived neural lineages that can be used in engineering an *in vitro* model of AD, we performed RNA sequencing of BFCNs at various stages of differentiation. We observed significant expression level changes in $\sim 10^5$ genes as pluripotent stem cells are differentiated to BFCNs. At least 20% of the changes in gene expression occurred after forebrain patterning. Consistent with the differentiation process, genes associated with pluripotency were downregulated while neuronal genes were upregulated. The BFCN transcriptome can be used as a basis for comparison in future studies, allowing us to determine how the transcriptome differs in BFCNs that are associated with an Alzheimer's Disease state.

094

BIOENGINEERING HYDROGELS FOR BRAIN REPAIR AFTER STROKE

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Research in my laboratory over the past nine years has focused on the generation of hydrogel biomaterials to support the formation of a reparative niche within diseased or injured sites that can block or prevent inhibitory signals from dominating the repair process, while providing pro-repair signals that can guide new tissue formation. The goal of our approach is to use engineered materials to "unlock" the regenerative capacity of damaged or diseased tissue to promote repair. The premise of our approach is that all tissues in the body have the capacity to repair through local stem or progenitor cells, but that due to unfavorable environmental conditions during the normal healing process they are not able to do so. Our general strategy has been to combine our biomaterials engineering with designing materials that promote the formation of a space filling vascular plexus that could serve as part of a reparative niche directly at the wound site. The idea is that this vascular plexus would lay the groundwork for the recruitment of endogenous stem cells located in the local tissue surrounding the damaged area and generate an environment that would foster repair rather than scarring. In this talk we focus on our efforts to bioengineer hydrogels for brain repair after stroke. In particular our efforts to engineer injectable hydrogel materials that gel *in situ* and present multivalent aggregates of vascular endothelial growth factor (VEGF), fibronectin fragment proteins, and the appropriate me-

chanical properties will be presented.

096

NON CELL AUTONOMOUS EVENTS AND DISEASE TRANSMISSION IN ALS PATHOLOGY: TDP43 AND ITS DEADLY COLLEAGUES

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Amyotrophic lateral sclerosis (ALS) is one of the most progressive and lethal variant of motor neuron diseases (MND). Efficient therapeutic approaches are limited by the lack of sufficient cell models of investigation that allow mutation specific analysis. Various different genes were found to cause ALS, including SOD1, TAR DNA binding protein 43 (TDP43) and Fused in Sarcoma (FUS). How these genes cause ALS pathology is not yet precisely identified. Next to studies of gain- and loss of function hypotheses a few hints of non-cell autonomous mechanisms as well as disease transmission processes are of interesting impact, however those are barely studied in the more recently identified ALS causing genes. Here, we generated stem cell-based models of ALS using murine embryonic stem cell- and human iPSC-derived neuro-ectodermal derivatives expressing human wild-type and mutant TDP43, FUS or SOD1 to investigate non-cell autonomous events. In the murine ES cell model the co-cultivation of healthy motor neurons on TDP43 or FUS transgenic muscle cells resulted in the degeneration of motor neurons. In the human model system iPSC-derived neural precursor cells were differentiated into motor neurons to analyze their behavior after disease transmission obtained by the treatment of healthy motor neurons with pathogenic supernatants and cell homogenates of the disease-associated ones. Thereby we detect gene mutation specific effects. Cell homogenates of TDP43 mutated neurons or either both supernatant and cell homogenate of FUS and SOD1 mutated neurons led to the disruption of motor-neuronal filaments, morphology and the formation of intra-nuclear and cytoplasmic FUS and TDP43 protein inclusions in healthy control motor neurons. Similarly, protein inclusions and golgi disruption were observed in motor neurons of healthy controls when treated with cerebrospinal fluid (CSF) of sporadic ALS patients. Together we can strengthen the evidence for non-cell autonomous events as inducement for motor neuron disease in ALS-specific SOD1, FUS and TDP43 murine and human cell models with characteristics of a transmissible disease.



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IMMATURE RESPONSES TO GABA IN HUMAN FRAGILE X NEURONSTelias, Michael¹, **MAYSHAR, YOAV**², Segal, Menahem³, Ben-Yosef, Dalit²¹University of California Berkley, Berkley, CA, USA, ²Tel Aviv Sourasky Medical Center, Tel Aviv, Israel, ³Weizmann Institute of Science, Rehovot, Israel

Fragile X Syndrome (FXS) is the most common form of inherited cognitive disability. However, functional deficiencies in FX neurons have been described so far almost exclusively in animal models. In a recent study we found several functional deficits in FX neurons differentiated in-vitro from human embryonic stem cells (hESCs), including their inability to fire repetitive action potentials, and their lack of synaptic activity. Here, we investigated the responses of such neurons to pulse application of the neurotransmitter GABA. We found two distinct types of responses to GABA and sensitivity to the GABA-A receptor antagonist bicuculline; type 1 (mature) characterized by non-desensitized responses to GABA as well as a high sensitivity to bicuculline, and Type 2 (immature) which are desensitized to GABA and insensitive to bicuculline. Type 1 responses were age-dependent in WT neurons. In contrast, FX neurons expressed primarily type 2 phenotype in an age-independent manner. Expression analysis of GABA-A receptor subunits demonstrated that this bias in human FX neurons could be the consequence of a significant alteration in the expression of the GABA-A receptor subunits $\alpha 2$ and $\beta 2$. Our results show that an immature response to GABA is more frequent and more persistent in human FX neurons, and the inactivation of FMR1 during neurogenesis in FXS probably alters also the composition of the GABA-A receptor in FX neurons, affecting their synaptic function.

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IN VITRO MODELING OF PARKINSON'S DISEASE AND SEIZURE EFFECTS IN HUMAN IPSC-DERIVED NEURONS GROWN ON MICRO ELECTRODE ARRAYS (MEAS) FOR FUNCTIONAL PHENOTYPIC SCREENING APPROACHES.**BADER, BENJAMIN M.**, Pielka, Anna-Maria, Ehnert, Corina, Juegelt, Konstantin, Gramowski-Voss, Alexandra, Schröder, Olaf H.-U.

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Primary cultures are widely used for phenotypic testing of drug and test compounds. Therefore, the use of human induced pluripotent stem cell-derived (hiPSC) neurons is relevant to evaluate whether these models can be applied to human cells with the goal to increase predictability, sensitivity and specificity of test systems. Our goal was to evaluate toxin-induced cell-based models for Parkinson's disease and seizure using hiPSC neurons

and to compare them with primary neurons. We cultured hiPSC neurons and primary embryonic mouse neurons on micro electrode arrays and recorded the spontaneous network activity during in vitro development using micro electrode arrays. For the Parkinson model we treated the cultures containing more than 50% dopaminergic neurons with MPP⁺ in order to induce a strong functional but a weak morphological stress. Then we tested growth factors such as GDNF to prevent the functional impairment. For seizure induction we used a selection of seizure-inducing compounds in acute treatment paradigms. Using multi-parametric data analysis of the functional phenotypic activity patterns, we compared the effects on primary networks with those on hiPSC-neurons. We demonstrate that the activity of both primary and hiPSC neurons is affected by MPP⁺ which can be prevented by treatment with compounds. Seizure-inducing compounds affect hiPSC neuron activity, partly more potently than in primary neurons. In conclusion, despite our limited understanding of the maturation status and correlation to the in vivo developmental stage, hiPSC-derived neurons can be used for functional in vitro screening of compounds and exhibit comparable response patterns compared to known primary mouse neurons. This study was supported by CrackIT/NC3R UK.

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DEFINING THE MOLECULAR UNDERPINNINGS OF ASCL1-SOX2-MEDIATED LINEAGE CONVERSION OF ADULT HUMAN PERICYTES INTO NEURONSKarow, Marisa¹, Fournier, David², Garding, Angela², Schichor, Christian³, Tiwari, Vijay K.⁴, **BERNINGER, BENEDIKT**³¹Physiological Genomics, Institute of Physiology, Ludwig-Maximilians University, Mainz, Germany, ²Institute of Molecular Biology Mainz, Mainz, Germany, ³Physiological Chemistry, University Medical Center Johannes Gutenberg University of Mainz, Mainz, Germany, ⁴Physiological Chemistry, Institute of Molecular Biology Mainz, Mainz, Germany

Lineage reprogramming of brain-resident cells into induced neurons (iNs) in vivo is emerging as a promising cell-based strategy for the treatment of neurodegenerative diseases. Previous work has led to the identification of a new potential cell target for lineage conversion within the adult human cerebral cortex: brain-derived pericytes (Karow et al., 2012). In fact cells expressing a large panel of pericyte markers could be converted into functional iNs by combined expression of the transcription factors (TFs) *Ascl1* and *Sox2*. Importantly, neither of these factors was sufficient to induce fate switch on its own, strongly arguing for a synergistic mode of action. This mutual requirement is even more remarkable as *Ascl1* is recognized as a pioneer TF capable of binding and trans-activating target genes in closed chromatin (Wapinski et al., 2013; Raposo et al., 2015). To unravel the role of *Sox2* and its synergism with *Ascl1*, we performed

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RNA-Seq on sorted pericytes undergoing Ascl1- and Sox2-induced conversion. Brain pericytes were obtained from 3 individuals and transduced with retroviruses encoding Ascl1, Sox2 or both factors as experimental group and reporter-only for control. Given the protracted reprogramming of adult pericytes, cells were sorted at day 2 and 7 to determine early gene expression changes. Very little alterations in gene expression were observed after Ascl1- or Sox2-only expression, consistent with the failure of reprogramming by either factor alone. By contrast, combined expression significantly induced partially overlapping sets of genes at day 2 and 7. In accordance with the acquisition of neuronal properties, up-regulated genes included many TFs and chromatin remodelers known to regulate neurogenesis during development. Taken together, Ascl1 and Sox2-mediated iN reprogramming represents a unique experimental system to study the transcriptomic and epigenetic remodeling underlying the direct lineage conversion of reprogramming-resilient cells.

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SINGLE CELL TRANSCRIPTOMICS REVEAL ALTERNATIVE FATES DURING DIRECT REPROGRAMMING FROM FIBROBLASTS TO NEURONS

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Direct lineage reprogramming represents a remarkable conversion of cellular and transcriptome states. However, the intermediates through which individual cells progress are largely undefined. Here we used single cell RNA-seq at multiple time points to reconstruct the direct reprogramming path from mouse embryonic fibroblasts (MEFs) to induced neuronal (iN) cells mediated by the transcription factor Ascl1. Compared to reprogramming towards pluripotency, the process is fairly synchronous suggesting a deterministic mechanism, yet we still observed marked heterogeneity at each time point. When ordered by transcriptome similarity rather than time we find that cells transition through graded states at each step along the reprogramming path. Ascl1 overexpression results in a well-defined initialization causing cells to exit the cell cycle and re-focus gene expression through distinct neural transcription factors. We find that alternative non-neuronal fates can emerge during reprogramming through mechanisms attributed to Ascl1 silencing. These data provide a high-resolution approach for understanding transcriptome states during lineage differentiation.

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FUNCTIONAL PROFILING OF HUMAN NEURAL STEM CELLS

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Regenerative medicine aims to regenerate or replace tissues or organs, which cannot function properly due to damage, aging, etc.. Characteristics of stem cells, self-renewal and differentiation, render stem cell research one of the main foci of regenerative medicine. Beyond most of the stem cell research done on model organisms, no previous study applied a systematic comparison on human stem cells (hSC), due to great genetic diversity between individuals. Hence we perform a comparative study by genome-wide RNAi screens on human hematopoietic stem cell (HSC), iPSC, and neural stem cell (NSC). To be able to have cells with the same genetic background, HSC derived from a healthy donor, are reprogrammed to iPSC, which are differentiated to NSC. After obtaining these three hSC, RNAi screens on each cell type are performed by pooled epigenetic shRNA library. By analyzing and characterizing genes special for NSC self-renewal and differentiation, we aim to contribute to deciphering NSC biology and improve gene therapies for potential neural disorders.

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AN HOURGLASS MODEL OF PANCREAS GROWTH: MULTI-COLOR IMAGING REVEALS REPLICATION AND AGE-DEPENDENT POSITIONAL HETEROGENEITY AMONG BETA-CELLS IN JUVENILE ZEBRAFISH

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Organ growth is a highly dynamic process requiring precise co-ordination among cells of the tissue, surrounding organs and the environment. Growth of the β -cells mass within the zebrafish pancreas occurs by replication of existing cells and differentiation of ductal cells. It is not known what the relative contribution of the two processes to organ growth is and the contribution from individual cells to the final structure. To understand cellular contribution, we have adapted the multi-color lineage tracing strategy (Brainbow) to zebrafish β -cells. Using this approach, we were able to track multiple individual cell lineages within the same animal. Pulse chase during growth revealed non-uniform contribution from single cells; thereby suggesting unequal expansion of pre-existing cells. The labelling experiment also helped us track the localization of siblings within the final tissue.



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To our surprise, we saw two interesting phenomena:
1. Daughter cells did not form a cluster, and could be separated by large distances; 2. The labelled cell population was not uniformly distributed throughout the islet, but clustered in the posterior end. The anterior half of the islet develops from β -cells neogenesis of a precursor population; some of which are Notch-responsive. Overall, the β -cell mass in zebrafish encloses two levels of heterogeneity: first, the unequal contribution from individual cells, and the polarized addition of de-novo β -cells. The study sheds light into the patterning of β -cell mass; lessons from which could be utilized for tissue engineering and organ fabrication.

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REGENERATION OF THE ADULT ZEBRAFISH BRAIN: THE ROLE OF LINEAGE CONVERSION

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Severe traumatic brain injury (TBI) of the adult mammalian central nervous system (CNS) leads to life-long loss-of-function, and neuronal regeneration does not occur. In contrast, adult zebrafish have a remarkable ability to regenerate adult brain, retina and spinal cord. Neurogenesis in adult rodents is limited to only two sub-regions of the telencephalon, but in adult zebrafish occurs along the entire length of the neural axis, suggesting a mechanistic link to its regeneration ability. The cellular and molecular mechanisms that enable or prevent adult CNS regeneration are little known. To study these mechanisms in adult zebrafish, we developed TBI lesion assays, and analyzed cellular reactions to TBI. We find that adult zebrafish can efficiently regenerate brain lesions and lack permanent glial scarring. Several cell types proliferate as a consequence of the TBI. Using conditional Cre-loxP-based genetic lineage tracing, we asked which stem/progenitor cell types react to injury, proliferate and which contribute to neuronal replacement. We previously described that a subtype of ventricular radial glial stem cells proliferates and generates neuroblasts that migrate to the lesion site. The newly generated neurons survive for at least 3 months, are decorated with synaptic contacts and express mature neuronal markers. Here, we used hematopoietic stem cell transplantation and Cre/loxP lineage tracing to determine if proliferating non-neuronal stem cell lineages convert to generating neuronal-marker expressing cells after TBI, as was reported after HSC transplantation in mammals. So far, we find no new neurons of donor descent. Leukocytes, oligodendrocyte progenitors, mature myelin, endothelial cells and pericytes appear not to convert to a neuronal lineage following TBI. In vivo lineage conversion is currently discussed as a possible therapeutic strategy

for neurodegenerative conditions in mammals, using misexpression of lineage converting transcription factors. Our results indicate that lineage conversion is rare in regeneration-capable adult zebrafish brain, in spite of the challenges of a traumatic brain lesion.

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THE USE OF MULTIPOTENT MESENCHYMAL CELLS IN THE TREATMENT OF AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder leading to the death of upper and lower motoneurons (MN). To date there is no cure. Cell therapy may present a new possibility to cure ALS by providing neurotrophic support to host MN and/or replace dysfunctional glial cells. In the current study, 500,000 human bone marrow mesenchymal stromal cells (hMSCs) (Bioinova s.r.o.) suspended in 50 μ l of growth media were delivered intrathecally into symptomatic SOD1 G93A transgenic rats (n=11). Sham-treated animals (n=9) were injected with growth media, after the first symptoms of ALS appeared. Overall survival in the hMSC-treated group was prolonged by 13.6 days compared to the sham-treated group. The cell-treated rats showed significantly better motility and grip strength when compared to vehicle-injected animals. The difference in motor activity started to appear shortly after the cells were delivered, whereas there were no changes in the dynamics of the body weight loss between the groups. Electrophysiological evaluation of transgenic SOD1 rats showed a progressive loss of motor units in the gastrocnemius muscle, with no major changes in nerve conduction velocity. Quantitative analyses of wisteria floribunda (WFA) fluorescence intensity measured in the ventral horns of the cervical and lumbar levels of the spinal cord, revealed greater numbers of perineuronal nets in the hMSC-treated animals compared to the sham-treated group. We are currently performing a clinical study: "Autologous multipotent mesenchymal stem cells from 3rd passage, A Prospective, Non-randomized, Open Label Study to Assess the Safety and the Efficacy of Autologous Multipotent Mesenchymal Stem Cells in the Treatment of Amyotrophic Lateral Sclerosis" (ALS ID: AMSC-ALS-001, Eudra CT nr. 2011-000362-35) sponsored by Bioinova s.r.o. (Czech Republic). 26 patients are enrolled in our study (phase I/IIa), and have undergone transplantation of MSCs via lumbar puncture. No complications have been observed. Several patients reported temporary improvements e.g.

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in speech and swallowing. We are currently preparing phase II of the clinical trial. Supported by: GAČR 14-10504P, 15-06958S, P304/12/G069

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GENERATION, CHARACTERIZATION AND GENE PROFILING OF HUMAN iPSCS ORIGINATED FROM FAMILIAL MENIERES DISEASE

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Meniere's disease (MD) is an inner ear disorder characterized by sensorineural hearing loss, episodic vertigo and tinnitus. We have identified for the first time two pathogenic variants in the *FAM136* and *DTNA* genes in a family with autosomal dominant inheritance using whole exome sequencing. To investigate the functional role of these genes in familial MD, we have generated iPSCs from peripheral blood mononuclear cells (PBMCs) of a MD patient and a healthy control using non-integrative Sendai viruses containing Sox2, Oct3/4, c-Myc, and Klf4. The efficiency of iPSC generation was 0.01% and 0.03% for patient and control respectively. Two clones, MD-iPSCs (W7) and control-iPSCs (A2), were established and characterized. Both cell lines express the endogenous pluripotency markers, OCT4, SOX2, KLF4, and NANOG, at the mRNA levels, and they also express OCT4, SSEA4, TRA-1-60, and Tra-1-81 proteins as assessed by flow cytometry analysis. They have been shown normal karyotyping, W7 (46, XX) and A2 (46, XY) grown on irradiated human foreskin fibroblasts (iHFF) feeder cells, after several passages by mechanical transfer. Both cell lines demonstrated pluripotency by differentiating into three germ layers *in vitro* and teratoma formations *in vivo*. Of notice, both cell lines showed different proliferation rates, W7 cells have a doubling time of 20 hr, while A2 cells divide every 26 hr. Furthermore, pathogenic variants in both *FAM136* and *DTNA* genes in W7 were verified by Sanger sequencing. These results demonstrate that we successfully established two iPSC cell lines from a MD patient and a healthy control. These iPSC cell lines can be contributed to understand the phenotypic differences and molecular pathways involved with MD as the human cellular MD models in the future studies.

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DEVELOPMENT OF A HUMAN PLURIPOTENT STEM CELL DERIVED MODEL OF HUMAN NEURITOGENESIS AND ITS APPLICATION TO STUDY THE MECHANISM OF NEURITE INHIBITION IN DISORDERS OF THE CENTRAL NERVOUS SYSTEM.

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Inhibition of neurite outgrowth is common to many pathologies of the central nervous system (CNS) and forms an obstacle in their treatment. Trauma to the CNS, such as spinal cord injury (SCI), results in the activation of astrocytes and release of inhibitory molecules forming a glial scar. Similarly, peptide deposition associated with neurodegenerative diseases, such as the β -amyloid peptide in Alzheimer's disease (AD), has been reported to inhibit neurite outgrowth via a similar mechanism. Although neurite outgrowth is reduced in both AD and SCI, the detailed molecular mechanism remains relatively unknown. We have developed a novel *in vitro* human pluripotent stem cell based model of neuritogenesis, to support investigation of mechanisms that underpin neurite inhibition. Pluripotent stem cells were cultured in suspension (1.5×10^6 cells per Petri dish), 24 hours prior to retinoid treatment. Aggregates were maintained with retinoids for 21 days before being transferred to ECM coated surfaces. Aggregates were fixed with 4% PFA following 10 days culture with mitotic inhibitors and prepared for immunocytochemistry. The differentiation of stem cell aggregates was optimised and photostable synthetic retinoids (EC23, AH61) were found to induce neurite outgrowth to a greater extent than the natural all-*trans* retinoic acid. Treatment with $0.01 \mu\text{M}$ EC23 produced significantly more neurites than any other condition. The model was adapted into a 3D culture system using an ECM coated scaffold producing significant neurite outgrowth. Application of inhibitory molecules, such as chondroitin sulphate proteoglycans (CSPGs) found within the glial scar, inhibited neurite outgrowth in a dose dependent manner. Similarly, both addition of β -amyloid to culture media and coating of growth substrates inhibited neurite outgrowth. The data demonstrate that synthetic retinoids efficiently induce neural differentiation of human pluripotent stem cells, resulting in robust and reproducible neurite outgrowth. The application of CSPGs and β -amyloid resulted in dose dependent neurite inhibition. The system can now be utilised to investigate the use of small molecules to overcome CSPG and β -amyloid mediated neurite inhibition, to elucidate the molecular mechanisms underpinning neurite inhibition within CNS pathologies.



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NORMAL OSTEOGENESIS OF COSTELLO SYNDROME-INDUCED PLURIPOTENT STEM CELLS VIA NEURAL CREST LINEAGE

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The generation of human induced pluripotent stem cells (iPSCs) via cellular reprogramming of somatic cells from individuals with genetic disorders can provide a great opportunity to understand pathophysiology of the genetic disorders *in vitro*. Costello syndrome (CS) is one of RASopathies that are a group of genetic disorders having mutations on RAS/MAPK pathway. Constitutive activation of HRAS resulting from *HRAS* mutation causes CS. CS patients have severe musculoskeletal abnormalities such as short stature, stooped standing posture, hip dysplasia, kyphoscoliosis and osteoporosis. To elucidate molecular mechanism of musculoskeletal defects in CS, CS-iPSCs were generated from patient fibroblasts by ectopic expression of OCT4, SOX2, C-MYC and KLF4. Mesenchymal stem cells (MSCs) were differentiated from CS-iPSCs by two separate methods. MSCs could be developed through neural crest (NC) and paraxial mesoderm (PM) lineages, respectively. NC-MSCs expressed MSC-surface markers such as CD73, CD90 and CD105 and normally differentiated into osteoblasts. However, PM-MSCs did not express CD105 and failed further differentiation into osteoblasts. The results indicate that aberrant osteogenesis in paraxial mesoderm lineage may be responsible for musculoskeletal defects in CS patients.

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AMYOTROPHIC LATERAL SCLEROSIS (ALS) SPECIFIC PATHOLOGY IN HIPSCS AND SPINAL MOTONEURONS EXPRESSING MILD TO VERY AGGRESSIVE ALS-FUS MUTANTS

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Amyotrophic lateral sclerosis (ALS) is characterized by the degeneration of upper and lower motoneurons (MN). In 5% of familial ALS (fALS) cases mutations

in FUS (Fused in Sarcoma) have been identified as a genetic cause of the disease. FUS is a RNA binding protein involved in mRNA splicing, translation and mRNA transport. Mainly it is located in nuclei but also presents a dendritic localization and is detectable in synaptic spines in neurons. Most of the ALS related FUS mutations are clustered in the nuclear localization signal. Due to this the protein exhibits an abnormal redistribution between nucleus and cytoplasm. In brain and in spinal cord of affected patients, FUS is found in cytoplasmic protein inclusions. FUS is also implicated in DNA damage response by showing a direct interaction with Histone deacetylase 1. FALS related mutations interfere with this interaction and therefore this might lead to increased DNA damage in neurons. Since it is known that the overexpression of WT FUS has toxic effects to neurons, hiPSC-derived MN may represent a suitable system to model ALS associated neuropathology. Therefore, the pathophysiological phenotypes associated with ALS related FUS mutations were investigated in hiPSCs from different fALS-FUS patients harboring a benign late onset missense mutation (R521C) and/or two juvenile onset mutations: the malign R495QfsX527 and most severe Asp502Thrfs*27. Cells were analyzed under physiological and stress conditions as well as after induced DNA damage. Furthermore, the intracellular distribution of FUS in differentiated spinal MN was also characterized. We found that the vulnerability to DNA damage in hiPSC was dependent on the severity of the mutation. Similarly, mislocalization of FUS protein in MN correlated the severity of the underlying mutation and could lead to a complete absence of FUS in the nuclei and an aberrant distribution of FUS granules along the neurites. These findings correlated to the clinical age of onset of the patients from which the cell were derived. Thus, ALS-FUS hiPSC are suitable to model ALS pathology and can be valuable tools to study specific pathophysiology in diseased cells.

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DEVELOPMENT OF CO-CULTURE MODEL FOR NEURON-OLIGODENDROCYTE INTERACTIONS

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In vitro co-culture models represent an important research tool in addition to animal models. Limitations of animal models are linked to species differences and furthermore, animal studies are very laborious, time-consuming and expensive. Thus, other model systems are also needed. Microfabrication and

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microfluidic technology enables the generation of multi-chamber *in vitro* cell culture platforms that allow the compartmentalized culture of different cell types and guidance of cell growth. In addition, the environment of segregated cell regions can be manipulated. Microfluidic-based co-culture models for neuron-oligodendrocyte interactions exist but lack important features. For example, myelination event is still challenging to detect in these models. The aim of this study is to develop an *in vitro* co-culture model which contains controlled culture conditions for neuron-oligodendrocyte co-culture. Microfluidic co-culture platform is manufactured from polydimethylsiloxane (PDMS). The platform contains chambers for neurons and oligodendrocytes and microchannels to guide and restrict the growth of cells. The platform is designed to enable multiple measurement methods including electrophysiological approaches. Human pluripotent stem cell (hPSC)-derived neurons and oligodendrocytes are used as cell components in the platform. Cell culture and differentiation are performed according to the routine methods developed in our laboratory. Co-culture platform can be successfully and repeatedly generated from PDMS. hPSC-derived neurons and oligodendrocytes are viable in the platform and can be successfully cultured in the platform for a long period. Microchannels of the platform were shown to restrict and guide the growth of both cell types and biochemical measurements can be performed in the system. According to our studies, the developed model is suitable for the co-culture of hPSC-derived neurons and oligodendrocytes and has potential for various applications related to neuron and oligodendrocyte research.

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SOX9 AS A NOVEL PLAYER IN NEOCORTICAL DEVELOPMENT AND EVOLUTION

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The neocortex is the outermost part of the cerebral cortex and it has expanded greatly during the course of evolution. This expansion is due to the increase in the proliferation capacity of embryonic neural progenitors, especially the basal progenitors. It is hypothesized that this increase is caused by the ability of human basal progenitors to autonomously produce extracellular matrix (ECM) components and create their own niche. Based on previous interspecies comparative transcriptomics studies, we have found that transcription factor Sox9 is a putative candidate that regulates ECM expression. In this study we analyzed Sox9 expression in mouse, ferret and human developing neocortices, and found that Sox9 is expressed in progenitors with high self-renewal potential. In ferret and human basal progenitors, Sox9 is

expressed by proliferating progenitors, not expressed in Tbr2 expressing intermediate progenitors. Furthermore, in the ferret neocortex ~20% of the Sox9 expressing basal progenitors are gliogenic. Next, we ectopically overexpressed Sox9 specifically in mouse basal progenitors, which do not express Sox9 endogenously and are limited in self-renewal capacity. Overexpression of Sox9 in basal progenitors generated: i) more proliferating basal progenitors, ii) less Tbr2 expressing intermediate basal progenitors, iii) ectopic expression of gliogenic marker Olig2. These findings suggest that Sox9 expression results in a higher number of self-renewing progenitors, concomitant with a heterogeneity in mouse basal progenitors, more similar to the composition of neural progenitors seen during human neocortical development. In addition, we show that Sox9 exerts its effects in a dose dependent manner, where high doses of Sox9 induce Olig2 expression and repress Tbr2 mRNA levels. Convergence of Sox9 expression and ECM production thus appears to be crucial for basal progenitor proliferation and fate choice, and therefore for the evolutionary expansion of the neocortex. Our studies now focus on elucidating the role of Sox9 in ECM production, and in progenitor cell fate choice.

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AGING-DEPENDENT DECLINE IN BETA-CELL PROLIFERATION ASSOCIATES WITH IMPROVED GLUCOSE TOLERANCE IN ZEBRAFISH.

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Type 2 diabetes is caused in part by compromised function and regenerative capacity of the pancreatic β -cells associated with aging. Previous studies have suggested that pancreatic β -cell replication and function in humans declines with age. Elucidating the basis for this deterioration may reveal strategies for reversing the age-related dysfunction of β -cells, a long-sought goal for diabetes therapy. Previously, we had reported that nutrients stimulate a robust increase in β -cell proliferation in juvenile zebrafish. This increase in proliferation may happen in order to compensate for the increased insulin demand. This phenomenon is also observed in obese individuals that exhibit an increase in pancreatic β -cell mass. Taking advantage of this knowledge, we investigated the proliferative and functional capacities of β -cells in young and aged adult zebrafish. Notably, we observed a decline in β -cell proliferation in response to nutrients with increased age of the animal. Strikingly, we find improved glucose tolerance in aged animals compared to the younger adults, suggesting improved β -cell function. We are now performing transcriptome profiling of β -cell from



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juvenile, young adult and aged animals in order to identify differences in gene expression signatures between these stages. Identifying the signalling mechanisms underlying active β -cell proliferation in young zebrafish along with improved function in aged animals might provide a therapeutic tool towards reversing the effects of age associated β -cell dysfunction in humans.

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NEUROMUSCULAR JUNCTION FORMATION USING HIPSC CO-CULTURE SYSTEM. RELEVANCE TO AMYOTROPHIC LATERAL SCLEROSIS

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In amyotrophic lateral sclerosis (ALS) a neuropathological hallmark is the degeneration of upper and lower motoneurons (MNs), this leads to muscular atrophy and respiratory failure. However, in ALS there is not only MN pathology but also the skeletal muscle is affected more in particular at the neuromuscular junction (NMJ). Myoblasts can be cultured from muscle tissue *in vitro*. If muscle tissue is unavailable, myogenic cells can be generated from human induced pluripotent stem cells (hiPSCs) and could be co-cultured with MNs to form axon-muscle contacts at the NMJ to model neuromuscular pathologies. Thereby, hiPSC derived myogenic cells were generated by overexpressing PAX7 and expanded in myogenic conditions. Serum deprived hiPSC-derived myoblast-like cells fused and formed multinucleated striated myotubes that expressed a set of key markers for muscle differentiation. In addition, these hiPSC derived-myotubes contracted upon electrical stimulation, generated action potentials and responded to acetylcholine. Finally, MNs and myotubes generated from the same hiPSCs cell lines were co-cultured. In one hand, we could observe the early aggregation of acetylcholine receptors in muscle cells of immature co-cultures. At later stages, we identified and characterised mature NMJs in mature cultures from healthy donors. On the other hand, in MN-myotube co-cultures generated from hiPSC from a patient harbouring a malign R495QfsX527 or a most severe Asp502Thrfs 27 Fused in Sarcoma (FUS)-ALS related mutation NMJ did not fully mature, suggesting that FUS-ALS mutations may impede proper NMJ formation. In summary, we describe here the successful generation of a functional cellular system consisting of two distinct communicating cells types in healthy and in ALS related mutations. This *in vitro* co-culture system could therefore contribute to research on diseases in which the MN and the NMJ are predominantly affected, such as in ALS.

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MOLECULAR AND CELLULAR MECHANISMS UNDERLYING THE ROLE OF INSM1 IN THE GENERATION OF BASAL PROGENITOR CELLS

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During the development of the mammalian neocortex, neurons arise from neurogenic divisions of neural progenitor cells (NPs). Two main classes of neural progenitors have been identified: apical progenitors (APs) that undergo mitosis at the ventricular surface of the ventricular zone (VZ), and basal progenitors (BPs) that undergo mitosis at an abventricular location, typically in the subventricular zone (SVZ). One of the most striking differences between the human and mouse developing brain is the increase in the pool size, diversity and proliferative capacity of the BPs and the consequent increase in the size of the SVZ. Therefore, a crucial question in developmental neuroscience is to identify the molecular mechanism(s) responsible for the generation of BPs. Hence, we studied *Insm1*, a zinc-finger transcription factor involved in the generation of BPs. We set out to identify its downstream targets and mechanisms, both at the molecular and at the cellular level. We show that *Insm1* is highly expressed in newborn BPs that are leaving the VZ, both in the mouse and human developing cortex. Moreover, *Insm1* seems to be part of the cascade of subsequently expressed BP genes. The forced expression of *Insm1* in the mouse developing neocortex causes delamination of the targeted cells and, as a consequence, drastically affects their distribution through the neocortical wall. Moreover, *Insm1* overexpression changes the identity of the targeted NPs within the VZ, inducing BP fate, without affecting neuronal differentiation. Finally, we used transcriptome analysis of *Insm1*-overexpressing cells to identify *Insm1* downstream target genes. *Insm1* overexpression resulted in differential expression of genes involved in cell and neuronal morphogenesis and, also, ECM-associated genes. Moreover, *Insm1*-overexpression affected the expression of genes important for the retention of the apical contact. This work thus contributes to the dissection of the molecular mechanisms that leads to the production of BPs, by characterizing the role of *Insm1* in this process and identifying different genes that might be important for the retention of the apical endfoot.

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CHEMICAL REGULATORS OF BETA CELL PROLIFERATIVE HETEROGENEITY IN ZEBRAFISH

POSTER ABSTRACTS

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Organ growth during development is supported by cell proliferation and differentiation. Though this is well appreciated, the contribution of individual cells towards final organ size and shape is not well understood. We wish to study the heterogeneity among individual β -cells to distinguish the proliferative population from the quiescent one; and find small molecule inducers of quiescent cells - a model better resembling adult human β -cells - in hope that such drugs pave the way for better treatment of Diabetes mellitus. For this purpose we developed two novel techniques: 1. Multi-color fate mapping of multiple individual β -cells within the same tissue; 2. A novel bioluminescence based reporter for high-throughput screening. This assay expresses Firefly luciferase specifically in proliferating β -cells; and is capable of screening proliferation at stages where the pancreas is inaccessible. We adapted the Brainbow system for multi-color lineage analysis, which allowed us to survey the clonal development of neighbouring cells within the tissue. We observed unequal contribution of individual β -cells to islet growth; thereby suggesting presence of quiescent cells within the tissue. Next, we carried out a chemical screen using the luminescent transgenic line to identify enhancers of β -cell proliferation. We are combing fate mapping of individual β -cells with drug treatment to evaluate the effect of chemical enhancers on the heterogeneity among the β -cells. We aim to identify drugs that enhance replication of quiescent β -cells. The mechanism behind such drugs could be investigated in future for potential therapeutic application in humans as a treatment of Diabetes mellitus.

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IPHEMAP: AN ATLAS OF PHENOTYPE TO GENOTYPE RELATIONSHIPS OF HUMAN INDUCED PLURIPOTENT STEM CELL MODELS OF NEUROLOGICAL DISEASES.

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Our goal was to perform a phenogenetic correlation of iPSCs models of neurological disease. Reprogramming adult human somatic cells to model neurological diseases is poised to provide unprecedented insights in complex neurological disorders; however, the wealth of information and the multiple phenotypes generated from these studies has become increasingly difficult to follow and interpret. A system biology metanalysis was performed from iPSCs experiments

from 77 studies. We used Circos plot and ideogram to correlate neuronal phenotypes with mutations from neurodevelopmental and neurodegenerative disease. We used cytoscape to build a map of all phenotypes and genes. We performed network analysis of gene expression from diseased derived cells and controls to generate molecular phenotypes. We catalogued 517 phenotypes from 55 different mutations in 25 adult and pediatric neurological disorders. We established a novel taxonomy to group all the neuronal phenotypes from iPSCs experiments into nine distinct clusters mapped to the human genome. In addition, these phenotypic clusters mapped to distinct derived cells, we found that phenotypes clustered to neural cells depending of gene mutations. We characterized the resulting relationships between genes and phenotype into a phenogenetic map, revealing differential grouping of phenotypes. We found that these relationships follow a scale-free power law relationship, revealing novel correlations between gene and phenotypes. We performed pair-wise statistical comparison of functional annotations derived from well-established gene ontologies and our new phenotype ontology validating novel correlations. We designed a web platform, to make our data accessible to scientists studying the modeling of neurological diseases with pluripotent stem cells derived from patients. Our findings provide new insights into *in-the dish* model of neurological diseases and our database provide for the first time a field synopsis of iPSCs phenotypes.

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RETINAL TRANSPLANTATION - DO DONOR AND RECIPIENT PHOTORECEPTORS "FUSE"?

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Photoreceptor replacement by cell transplantation has been proposed as a therapeutic approach to treat retinopathies. Pre-clinical studies provided evidence for successful transplantation of photoreceptors into mouse models of retinal degeneration. Here donor photoreceptors were suggested to integrate into the host outer nuclear layer (ONL) developing a mature photoreceptor morphology with the potential to repair visual responses. A potential "fusion" of donor and host cells, as it was observed in other transplantation paradigms e.g. in bone marrow transplants was ruled out as reporter labeled cells contained a single nucleus and some donor cell processes showed no co-labeling when transplanted in fluorescent labeled hosts. By using techniques to separate cytoplasm and nuclear labeling we reinvested the potential of "fusion" events following photoreceptor transplantation. Therefore donor photoreceptors were isolated from male photoreceptor-



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specific GFP reporter mice (Nrl-GFP) and transplanted into female recipients. Analysis of cells located at the site of injection, the sub-retinal space (SRS), revealed that virtually all GFP+ cells contained a Y-chromosome identifying them as donor photoreceptors. Interestingly, of all GFP+ photoreceptors located within the host ONL, less than 3% showed co-staining for the Y-chromosome, suggesting that cytoplasm content but not the nucleus of donor cells is located within the host ONL. To further investigate a potential exchange of plasma contents between donor and host photoreceptors donor cells were isolated from a floxed reporter mouse line (Ai9) and transplanted into cre-recombinase expressing hosts (B2-Cre^{+/+}; a rod photoreceptor-specific cre-driver line). Reporter expression was detected in both cells in the SRS and photoreceptors within the ONL while no reporter expression was detected upon transplantation of Ai9 donor cells into wild-type recipients. These results suggest that cre-recombinase produced by the host could enter donor cells leading to expression of the reporter. Therefore, we hypothesise that donor photoreceptor precursors transplanted into the adult mouse retina can “fuse” with host photoreceptors but without translocation of the nucleus. Further studies will be needed to elucidate the mechanism of this observation.

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ASYMMETRIC CONTRIBUTION OF PROGENITOR CELLS TO REGENERATING BETA CELL MASS IN ZEBRAFISH

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Perfect organ regeneration not only involves replacement of lost cells, but also replication of the original pattern. Though it is well known that ablated β -cells in zebrafish are capable of recovering via self-duplication and differentiation from ductal lineage progenitors; we still lack information about the nature of regenerated islet pattern and the factors (internal and environmental) contributing to the process. To study regeneration pattern, I have developed a new tool for efficient and near-complete targeted ablation of zebrafish β -cells. Post-ablation, we expected a homogenous recovery of the β -cell mass. Surprisingly, the regenerated islet did not have the characteristic spherical shape, but seemed to be devoid of β -cells in the posterior region. This suggested an asymmetric addition of β -cells to the islet. Utilizing the β -cell developmental timing assay, we observed β -cell neogenesis to be localized to the anterior of the primary islet. β -cell differentiation is thought to occur from Notch-responsive ductal cells. We found this population to be responsible for β -cell addition - however such process was restricted to the anterior region. Interestingly, not

just β -cells, but differentiation of all pancreatic endocrine lineages was found to be localized at the anterior of the pancreas. We are currently investigating the mechanisms driving β -cell birth in the anterior of the pancreas, and the factors attracting these cells to the primary islet. The work sets a stage for further dissection of the processes involved in pancreas regeneration. Extending these findings to adult human population might help induce β -cell mass increase for treatment of diabetes.

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MRNA ANALYSIS ON IPSC DERIVED PHOTORECEPTOR CELLS OF STARGARDT PATIENTS REVEALS THAT THE C.5461-10T>C VARIANT RESULTS IN TRUNCATIONS OF THE PREDICTED ABCA4 PROTEIN

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Mutations in the gene encoding the ATP-binding cassette transporter type A4 (*ABCA4*) are the most frequent cause of inherited retinal dystrophies (IRDs). The *ABCA4* gene is associated with retinal disease in almost all persons with recessive Stargardt disease (STGD1), about 25% of cases with recessive cone-rod dystrophy (CRD), and 5% of persons with panretinal dystrophy. STGD1 cases primarily show defects in the center of the retina, harbouring most of the cone cells responsible for high acuity daylight and colour vision. Mutation in the *ABCA4* gene give rise to recessive diseases, and affected persons should carry mutations in both copies of the *ABCA4* gene. About 25% of persons with STGD1 carry only one or no *ABCA4* mutation; one plausible explanation for the ‘missing mutations’ is that they are located outside the protein-coding parts of the gene. The second most frequent *ABCA4* variant, c.5461-10T>C, on the basis of prediction programs has a very small effect on splicing efficiency. In our classical STGD1 patients, it is very often found *trans* to a mild variant (c.2588G>C), suggesting that it is a severe *ABCA4* variant. We investigated the effect of the c.5461-10T>C variant on splicing by analyzing mRNA from photoreceptor progenitor cells (PPCs) derived from persons with maculopathies who carry this variant in a homozygous or heterozygous state. In addition, we performed *in vitro* minigene splicing studies to show that this variant results in a truncation of the predicted *ABCA4* protein and show that it resides on an ancestral ‘founder’ haplotype.

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CHARACTERIZATION AND SEPARATION OF ALGINATE PARTICLES LOADED WITH CELLS FROM EMPTY PARTICLES AS A PREREQUISITE FOR XENOGRAFT EXPERIMENTS.

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Fulfilling the promise of regenerative medicine requires overcoming several hurdles related to the specific approach that one adopts. For example, allografts and xenografts must contend with the immune response of the recipient to the donor cells. This is often accomplished by suppressing the recipient's immune response and encapsulating the donor cells within a hydrogel, such as alginate, that functions as a physical barrier to the recipient's immune system. The donor cells can continue to function therapeutically, releasing and responding to small diffusible biomolecules within the host. Technologies exist to encapsulate donor cells and tissues within hydrogel particles but most approaches create a near random distribution of particles that range from having no cells within them to ones heavily loaded with cells and cell clusters. There are several steps in this process that can create variation. The characteristics of the encapsulated sample can vary from one batch to the next. The sizes of the particles might vary. The distribution in the numbers of cells or cell clusters within the particle might vary by batch. It is likely that the size of the loaded alginate particle will have an effect on the therapeutic benefit it can provide. Therefore, a beneficial quality control step would be to assess the characteristics of the sizes and cell density level of the generated particles. Furthermore, testing the effectiveness of encapsulated particles for their therapeutic benefit will require control of particle size and number and cell density characteristics of the particles donated to the recipient. These quality control steps can be accomplished using the COPAS large particle flow cytometer. We show that cells encapsulated in alginate can be analyzed and sorted accurately providing both the metrics and the method to repeatedly generate nearly identical samples of encapsulated cells for research, discovery, and in the future, for therapeutic use.

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IMPLICATIONS OF DISRUPTED AUTOPHAGY ON CHOLESTEROL TRAFFICKING, NEURONAL SURVIVAL AND STRATEGIES FOR DRUG DEVELOPMENT IN NPC1

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Niemann Pick type C1 (NPC1) is a pediatric dementia caused by mutations of the lysosomal cholesterol transporter NPC1. NPC1 has no cure or treatment, and affected patients die in their childhood or teenage years. Although rare, the disease imposes a burden on patients and families that is disproportionate to its relative infrequency, furthermore, NPC1 shares clinical and histologic features with Alzheimer's disease (AD), a more prevalent public health problem, suggesting common mechanisms of onset and progression. Successful development of effective therapeutic interventions for NPC1 and related neurodegenerative disorders will require a deeper understanding of mechanisms of disease initiation and progression. We used reprogramming technology to develop sets of NPC1 and control human induced pluripotent stem cell (hiPSC) lines, and we systematically generated patient-specific pure neuronal cultures using a standard differentiation protocol. We found that NPC1 neurons have disrupted mitochondrial turnover by autophagy that leads to mitochondrial depolarization and increased production of reactive oxygen species, all of which are likely to contribute to the neuronal failure observed in NPC1. Our data also raise the important and new possibility that NPC1 neurons initially survive cholesterol accumulation because they activate autophagy. We have evidence that in NPC1 mutant neurons, autophagy may function as a backup pathway that releases and distributes trapped cholesterol, albeit at lower efficiency, but sufficient to protect neuronal viability until birth and perhaps for a few additional years. Further mechanistic studies lead us to identify a potential new transporter that mediates autophagy-dependent cholesterol efflux from the late endosomal compartment. Our data highlight the central role that autophagy disruption plays in the selective neuronal failure observed in NPC1. Additionally, our approach establishes an hiPSC-based platform for the high-throughput screening of potential therapeutic compounds that can revert mitochondrial dysfunction while preserving bulk autophagy in NPC1 and related neurodegenerative diseases.



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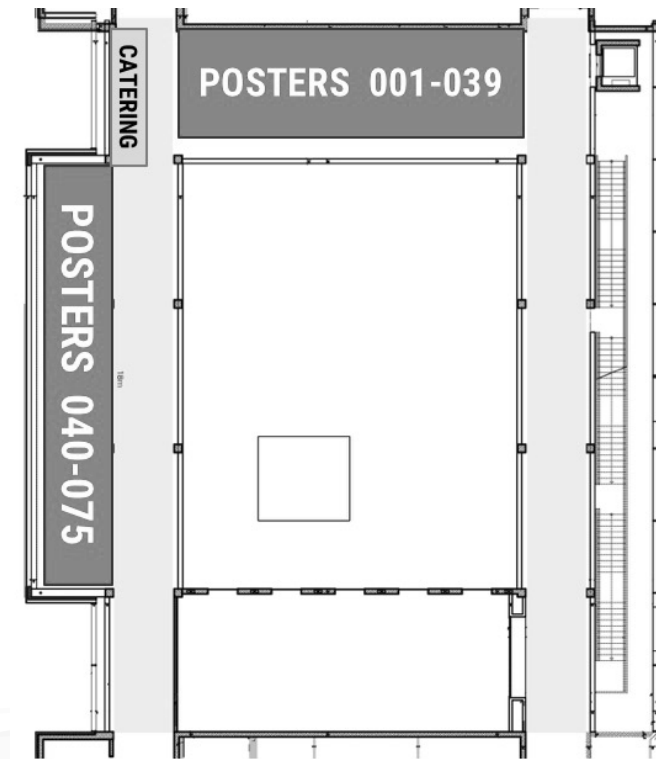
CELLULAR AND MOLECULAR EFFECTS OF LYSOPHOSPHATIDIC ACID ON CULTURED HUMAN NEURAL PROGENITOR CELLS

MEDELNIK, JAN, Tanaka, Elly M.

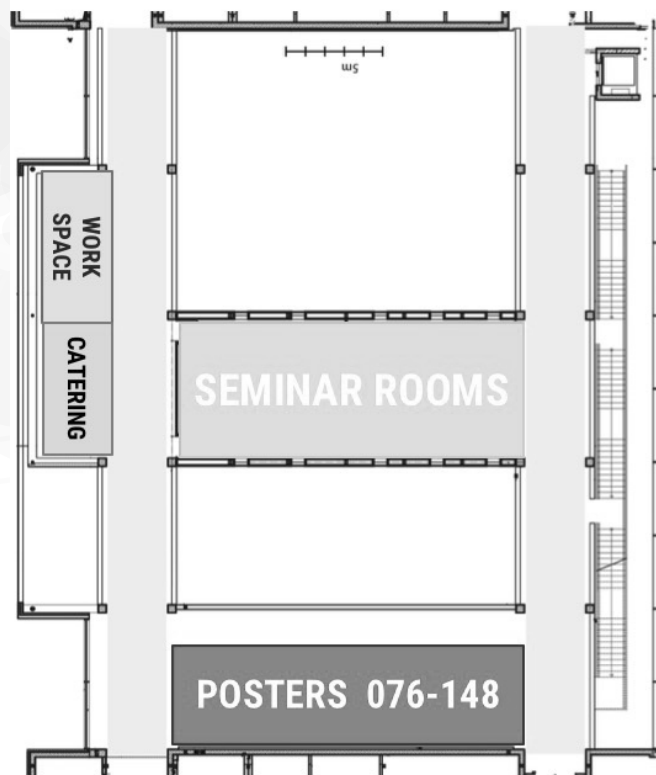
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In the lab we have established a protocol to culture axolotl spinal cord-derived neuroepithelial cells in a cell culture dish. The cells spontaneously self-organize into neural rosettes. Rosettes are two-dimensional tube-like structures of polarized epithelial cells which radially organize and form a lumen at their apical site. The polarized and pseudostratified-structure of a rosette highly resembles the morphology of a developing neural tube. In the lab we found that adding serum or lysophosphatidic acid (LPA), a major serum component, to axolotl neural stem cells (NSCs) induces the formation of very large rosette-like structures. In this project we aim to find out if serum and LPA also promote large rosette formation in cultured human NSCs. Here we present that serum and LPA also increase rosette size of cultured human NSCs and that this effect is independent of cell proliferation. We show that LPA dynamically regulates the amount of epithelialized area and NSC-apical domain size in a concentration-dependent fashion. Furthermore we show involvement of the Rho-signaling pathway in the rosette formation process and present downstream genes that are upregulated by LPA and possibly involved in NSC apical domain increase.

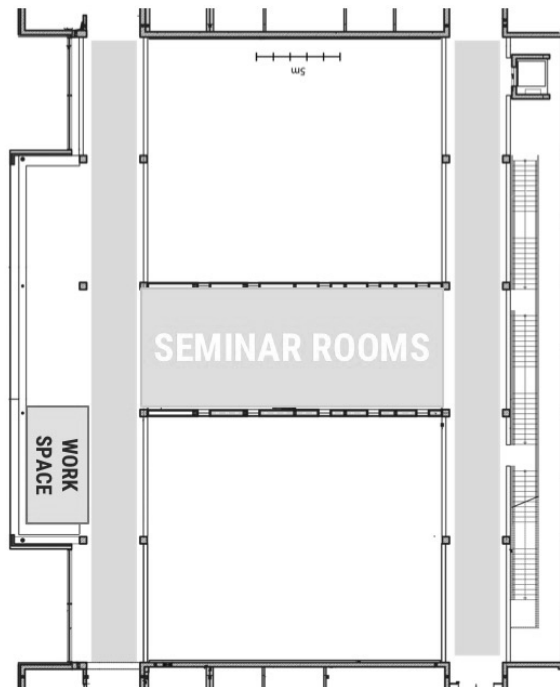
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