INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH

NEURAL STEM CELLS: CAPTURING COMPLEXITY AND PLASTICITY FROM THE CELL TO THE ORGANISM

An ISSCR International Symposium

3-4 APRIL 2025
ATHENS, GREECE

IN PARTNERSHIP WITH

STEM CELL REPORTS

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ABSTRACT BOOK

Dear Attendees,

We are delighted to welcome you to the 2025 ISSCR Athens International Symposium, Neural Stem Cells: Capturing Complexity and Plasticity from the Cell to the Organism! As we prepare for this exciting gathering of leading scientists, clinicians, and innovators from across the world, we are thrilled to host this unique event in the beautiful and historically rich city of Athens, Greece.

The symposium will focus on some of the most exciting advances in neural stem cell research. We will explore the intrinsic and extrinsic regulation of stem cells, epigenetics and metabolism, evolution and human-specific traits, neuron-glia interactions, and brain disease modeling. A central theme of our symposium is the enormous potential of neural stem cells in understanding development, aging, disease, and repair. While significant strides have been made, there remain many challenges in harnessing in vivo and in vitro models that can capture the full complexity of neural tissue and stem cell plasticity. We look forward to engaging with you in discussions around these cutting-edge topics.

We are also pleased to highlight that *Stem Cell Reports*, the official journal of this symposium, will feature a special issue on neural stem cells. This special issue is guest edited by two leading experts in the field who are part of our organizing committee for this event, Fiona Doetsch and Rebecca Matsas. Selected authors from this special issue will present their work at the symposium, providing an opportunity to explore their discoveries in depth.

Additionally, we would like to extend our great thanks to MaxWell Biosystems for their generous sponsorship. Their support is invaluable and plays a crucial role in making the 2025 ISSCR Athens International Symposium possible.

We are confident that this symposium will provide a unique platform to foster collaboration, inspire new ideas, and advance our collective understanding of stem cells, particularly neural stem cells. We are eager to interact with all of you and to the stimulating discussions that lie ahead.

Once again, thank you for being part of this exciting event, and we look forward to connecting with you in Athens!

Warm regards,

The 2025 ISSCR Athens International Symposium Organizing Committee
Fiona Doetsch
Rebecca Matsas
Marie Engelene J. Obien
Florentia Papastefanaki
Era Taoufik
Hynek Wichterle

maxwell BIOSYSTEMS

Dear Esteemed Colleagues,

On behalf of MaxWell Biosystems, we are delighted to welcome you to the 2025 International Society for Stem Cell Research (ISSCR) Athens International Symposium. We are honored to co-sponsor this distinguished event, held in partnership with *Stem Cell Reports*, focusing on the theme—**Neural Stem Cells: Capturing the Complexity andPlasticity from the Cell to the Organism.**

This symposium brings together leading scientists from around the globe to delve into the intricate dynamics of neural stem cells throughout development, aging, disease, and repair. The program encompasses a diverse array of topics, including intrinsic and extrinsic regulation of stem cells, epigenetics and metabolism, evolutionary perspectives, neuron-glia interactions, and brain disease modeling. Overall, this event fosters a rich exchange of ideas and insights in the field. We encourage all participants to engage fully in the sessions, discussions, and networking opportunities that this symposium offers.

At MaxWell Biosystems, we understand the value of stem cell 2D and 3D models to study the brain in health and in disease. Furthermore, we recognize the challenges researchers face, especially in characterizing the function of these models: whether in phenotyping, where detecting early, subtle activity changes is difficult, or in disease modeling, which requires insights at both population and single-cell levels. Across different applications utilizing neural stem cells, researchers need precise tools. The same applies to pharmacology and toxicology, where precise detection of response and reproducibility are essential.

MaxWell Biosystems is dedicated to address these challenges with next-generation high-density microelectrode arrays (HD-MEA) that facilitate precise characterization of neuronal activity and network dynamics. Our HD-MEA systems capture functional activity with unmatched precision, spatio-temporal resolution, and sensitivity. We believe that our innovative technologies can significantly contribute to the understanding and exploration of neural complexity and plasticity, aligning seamlessly with the symposium's objectives.

Since our founding in 2016, we have been at the forefront of electrophysiology, developing state-of-the-art products to equip the stem cell community with excellence in electrode technology. Building on the proven reliability of our systems and our commitment to leading innovation in electrophysiology, we are excited to further empower the stem cell and organoid research by launching **our next-generation MaxOne+ HD-MEA Chip** in this symposium. The new electrode design offers an optimized surface for cell attachment, viability and stability. Additionally, this new product improves the already exceptional signal quality of our original chips while enhancing the efficacy of electrical stimulation and ease of use. Visit our booth, posters and Innovation Showcase to discover how we can support and accelerate your research.

With the entire team of MaxWell Biosystems, we thank ISSCR for its outstanding organization and commitment to scientific excellence. Thank you for joining us in Athens. We look forward to a stimulating and wonderful symposium with all of you.

Best regards,

Marie Obien
Chief Commercial Officer

Urs Frey
Chief Executive Officer

ABOUT THE ISSCR

INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH

The International Society for Stem Cell Research
+1-224-592-5700
isscr.org

The International Society for Stem Cell Research (ISSCR) is a 501c(3) nonprofit organization with a mission to promote excellence in stem cell science and applications to human health. Our vision is a world where stem cell science is encouraged, ethics are prioritized, and discovery improves understanding and advances human health.

The ISSCR represents nearly 5,000 scientists, students, educators, ethicists, and business leaders from more than 80 countries. Each ISSCR member makes a personal commitment to uphold the ISSCR Guidelines for Stem Cell Research and Clinical Translation, an international benchmark for ethics, rigor, and transparency in all areas of practice.

Our work is made possible through generous support from our members and allied organizations towards strategic initiatives that support the mission:

- **Regulatory Affairs:** The ISSCR helps members navigate the regulatory landscape while assisting regulators by making scientifically informed recommendations for the development of stem cell therapies.
- **Policy:** The ISSCR advocates globally to support research funding, enforce ethical guidelines, and guard against unproven therapies.
- **Education:** The ISSCR provides resources and programs for the general public, educators, physicians, policy makers, and regulators. Aboutstemcells.org and ISSCR's patient handbook provide scientifically vetted resources for patients seeking unbiased and trusted information.
- **Standards and Guidelines:** The ISSCR sets international guidance for ethical and rigorous research, adopted by public and private organizations, regulatory bodies, funders, and publications. These references strengthen the pipeline of research and therapies, ultimately to benefit the patient.
- **International Conferences:** The ISSCR hosts a portfolio of international and digital meetings designed for knowledge sharing and collaboration to further the field. Discover upcoming programs, including the ISSCR 2025 Annual Meeting.
- **Publishing:** The ISSCR publishes *Stem Cell Reports*, an open access journal communicating basic discoveries in stem cell research alongside translational and clinical studies.

Our Board of Directors and Committees represent leaders across research, academia, and industry who are committed to advancing the Society's mission.

Learn more at isscr.org.

ABOUT *STEM CELL REPORTS*

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Stem Cell Reports

www.cell.com/stem-cell-reports/home

Stem Cell Reports is an open access forum communicating basic discoveries in stem cell research, in addition to translational and clinical studies. *Stem Cell Reports* focuses on manuscripts that report original research with conceptual or practical advances that are of broad interest to stem cell biologists and clinicians. *Stem Cell Reports* participates in Cell Press Multi-Journal Submission, allowing authors to simultaneously submit their papers for consideration by multiple journals at once.

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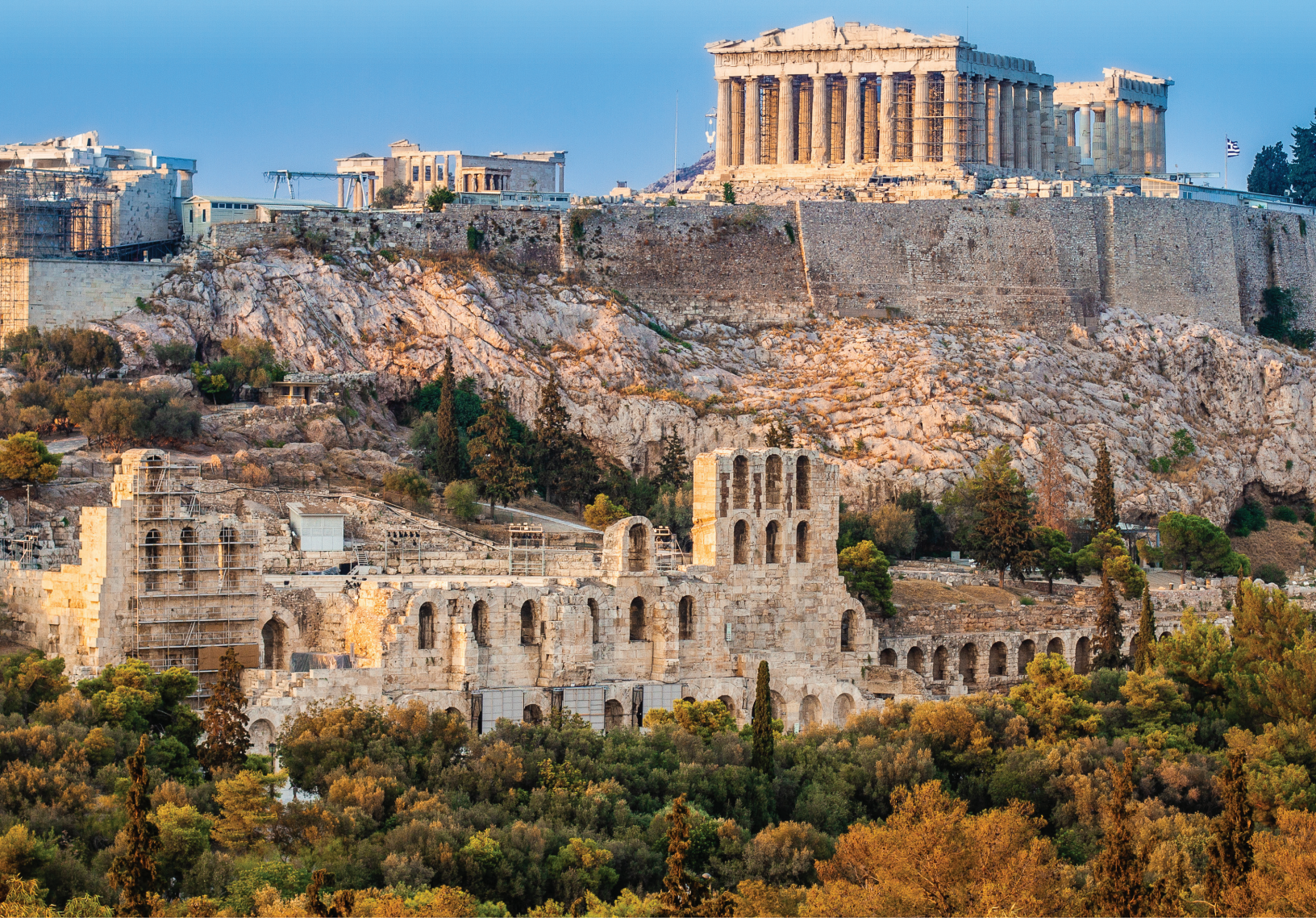

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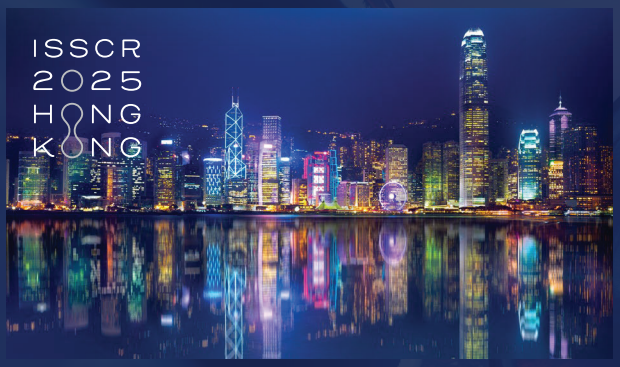
UPCOMING PROGRAMS



ISSCR 2025 ANNUAL MEETING

THE GLOBAL STEM CELL EVENT

11-14 JUNE 2025 | HONG KONG

Co-Sponsored By The University of Hong Kong, The Chinese University of Hong Kong, & The Hong Kong University of Science and Technology

SEATTLE INTERNATIONAL SYMPOSIUM

AI AND BIOLOGY: ACCELERATING DISCOVERY AND THERAPIES

9-10 OCTOBER 2025 | SEATTLE, USA

BOSTON INTERNATIONAL SYMPOSIUM

ACCELERATING PSC-DERIVED CELL THERAPIES: STARTING WITH THE END IN MIND

11-12 DECEMBER 2025 | BOSTON, USA

Learn more at ISSCR.org/upcoming-programs

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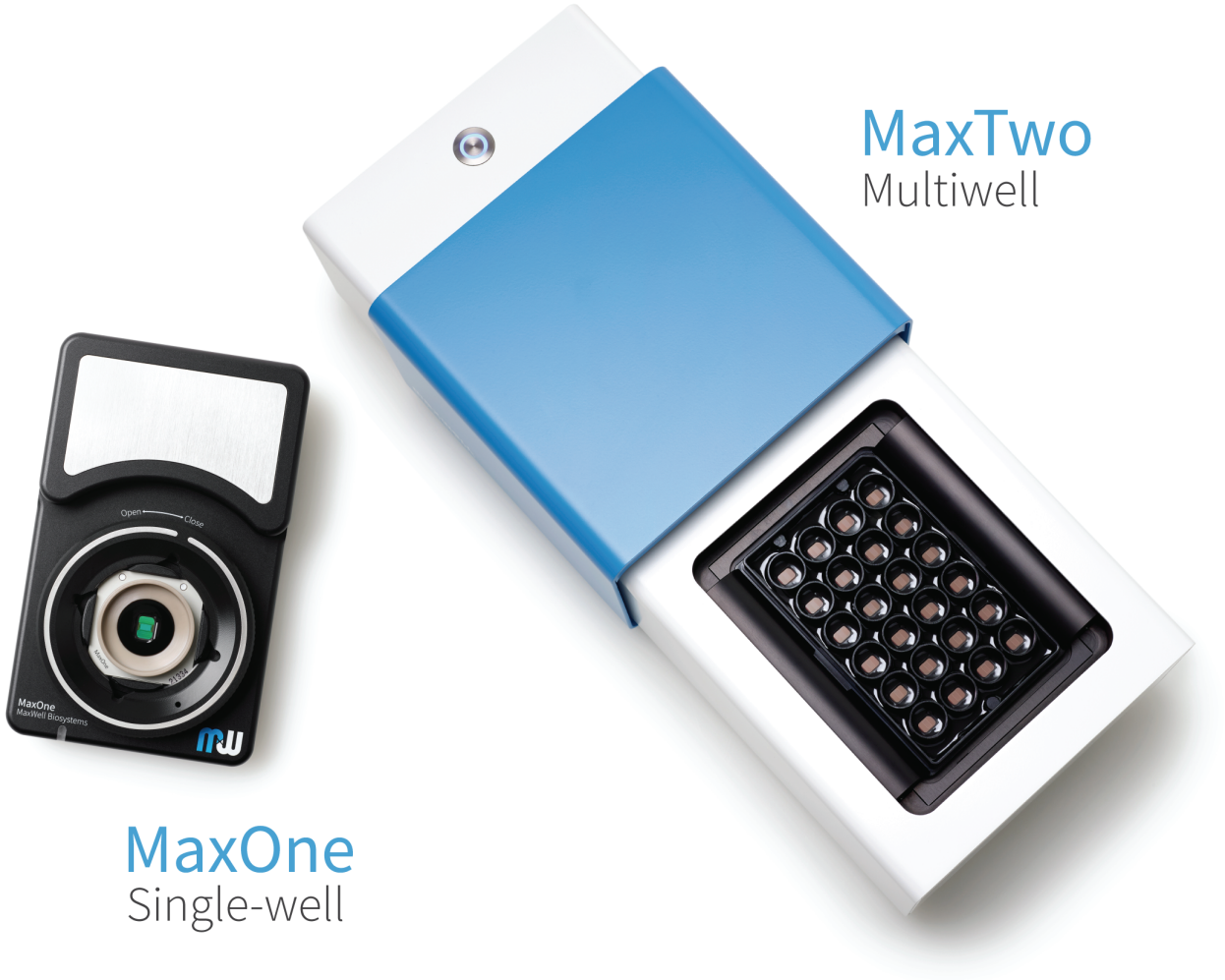

MaxWell Biosystems is a technology leader providing instrumentation and solutions to boost scientific research and development in neurosciences, stem cell and tissue engineering, ophthalmology, and other fields involving electrogenic cells. The company engineered advanced High-Density MicroElectrode Arrays (HD-MEAs) as the core of easy-to-use platforms, MaxOne (single-well) and MaxTwo (multi-well), that equip scientists to record electrical signals of neurons in in-vitro 2D and 3D models. MaxWell Biosystems' HD-MEA technology allows to capture neuronal activity across multiple scales, from sub-cellular to single cells, up to full networks in unprecedented detail. Ultimately, MaxWell Biosystems' platforms facilitate the understanding of neurological diseases, enhance the efficiency of cell-based assays for toxicity and safety pharmacology, and accelerate drug discovery.

What is your **Cell's** Story?

Capture the function of your neurons at
the network, cellular, and subcellular levels

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MEETING INFORMATION

ONSITE BADGE PICK UP

Pick up your name badge in the designated area during the posted hours below. Name badges are required for admission to all sessions, social events, meals/breaks, and the Exhibit & Poster area. Badges can be picked up during the following times:

Registration Desk Hours | Mezzanine Level at Hotel Titania

Thursday, 3 April	**8:30 AM – 7:00 PM**
Friday, 4 April	**8:00 AM – 4:00 PM**

ISSCR PROGRAM AGENDA

There will be no printed program book for the 2025 Athens International Symposium. You can access the online version of the program agenda here: Full Schedule

LIVESTREAMING

Livestream will not be available for this event. However, registrants can access the audio and slide recordings on-demand after the event by logging into the Member Library with their ISSCR credentials. An email will be sent approximately two weeks after the event to notify attendees that the on-demand content is ready for viewing.

ABSTRACT REVIEWERS

Laure Bally-Cuif, Benedikt Berninger, Sara Bizzotto, Nicolas Dray, Fiona Doetsch, Christina Kyrousi, Florentia Papastefanaki, Luca Peruzzotti-Jametti, Rebecca Matsas, Era Taoufik, Hynek Wichterle, Ping Wu

SMOKING

Smoking or the use of e-cigarettes is prohibited.

LOST AND FOUND

Please bring found items to the ISSCR registration desk during posted hours. If you lose an item, visit the registration desk during posted hours for assistance.

POSTER INFORMATION

Each poster will be presented during a 50-minute session in the Vergina-Olympia Halls at the Hotel Titania Athens. **Poster presenters must adhere to the scheduled date and time of their poster display and presentation.**

Poster presenters are responsible for removing their posters on Friday, 4 April between 1:30 PM – 1:45 PM. Any posters not removed at the end of this time will be discarded.

POSTER PRESENTATION SCHEDULE

Thursday, 3 April 2025

Poster Set-up:	**12:00 PM – 12:20 PM**
Poster Session 1:	**5:30 PM – 6:20 PM**
Poster Session 2:	**6:25 PM – 7:15 PM**

Friday, 4 April 2025

Poster Take-down:	**1:30 PM – 1:45 PM**

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Public Event

STEM CELL THERAPIES FOR NEURODEGENERATIVE DISEASES

Research advances- Clinical applications - Ethical issues

Wednesday
April 2
2025

18:00 -20:00

Central Hall of the Academy of Athens

28, Panepistimiou str, Athens, Greece

Speakers:

Lorenz Studer, Memorial Sloan Kettering Cancer Center, New York, USA & BlueRock Therapeutics

Achille Gravanis, Professor of Pharmacology, University of Crete

The discussion is coordinated by:
***Rebecca Matsas,** President of HSGTRM*
***Ioanna Soufleri,** Chief Editor of BHMAScience*

The event is held on the occasion of the Athens International Symposium on Neural Stem Cells, organized by the International Society for Stem Cell Research, Athens, April 3-4, 2025

The event is in English with simultaneous translation in Greek and is open to the public

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Cytosurge provides innovative solutions to shift boundaries in single-cell profiling and gene engineering based on the FluidFM® technology. The FluidFM OMNIUM platform with our biopsy solution, enables semi-automatic collection of cytoplasmic extractions (biopsies) from single cells in such a gentle manner that the cells survive. Biopsies represent a snapshot of a cell's transcriptional state at a specific point in time and can be used for downstream, temporal transcriptome readouts of single cells along their lifepath. In addition to extraction, the platform can also inject and pick cells providing a precision platform for single cell profiling. Next, the company offers CellEDIT, a gene editing service that utilizes intranuclear injection of CRISPR RNPs to avoid challenges associated with traditional cell transfection.

SPEAKER ABSTRACTS

All times are listed in Eastern European Summer Time (EEST)

THURSDAY, 3 APRIL 2025
10:20 AM – 12:05 PM
BRAIN DEVELOPMENT AND EVOLUTION

10:25 AM – 10:50 AM
OPENING KEYNOTE: TIMING MECHANISMS LINKING HUMAN BRAIN DEVELOPMENT, EVOLUTION, AND (DYS)FUNCTION

Pierre Vanderhaeghen, *VIB Center for Brain and Disease Research, Belgium*

The human cerebral cortex has undergone rapid expansion and complexification during recent hominid evolution, which is thought to be at the origin of some of the higher cognitive and social skills characteristic of our species. While the mechanisms of increase in human brain size have been studied for some time, those underlying the evolution of cortical circuits only start to be unravelled. These originate from selective divergence in gene regulatory networks, the emergence of human-specific genes, as well as species-specific cellular features such as mitochondria dynamics and metabolism. Newly discovered human-specific modifiers of cortical neuron development and function shed light on human evolution, and provide unexpected links to brain diseases to which our species is particularly sensitive.

10:50 AM – 11:15 AM
MOLECULAR SIGNATURE OF ASTROCYTE EVOLUTION IN PRIMATES

Aleksandra Pękowska, *Dioscuri Centre for Chromatin Biology and Epigenomics, Nencki Institute of Experimental Biology of the Polish Academy of Sciences, Poland*

Astrocytes contribute to the establishment and regulation of higher-level brain functions. Evolutionary changes in astrocyte activity during development and adulthood likely help establish the unique cognitive capacities of the human brain. However, the molecular signature of fetal astrocyte evolution is unsettled. We used human, chimpanzee, and

macaque induced pluripotent stem cell-derived astrocytes (iAstrocytes) to obtain fetal astrocytes. Human iAstrocytes are bigger and more complex than NHP iAstrocytes. We found new loci and cellular pathways related to the interspecies differences in astrocyte size and complexity. Strikingly, genes that feature lower expression in human than in NHP iAstrocytes frequently relate to neurological disorders, including intellectual disability, opening new questions on the relationship between evolution and the higher-level capacities of our brain. Evolution is largely fueled by changes in gene activity, which in turn arise as a corollary to genetic modification of distal DNA regulatory elements, including enhancers. Enhancers evolve fast. Yet, whether there are general and broadly applicable sequence changes that lead to functional activation of enhancers in evolution remains enigmatic. Through multilevel regulome analysis and machine learning, we reveal that functional activation of astrocytic enhancers coincides with a pervasive gain of binding sites of 'stripe' transcription factors, general transcriptional regulators with a previously unappreciated role in regulatory element evolution in humans. Altogether, we uncover genes and pathways linked to fetal astrocyte evolution and shed new light on a mechanism driving the acquisition of the regulatory potential of enhancers.

11:15 AM – 11:40 AM
DEVELOPMENT AND EVOLUTION OF THE HUMAN BRAIN

Nenad Sestan, *Yale University, USA*

The question of what makes human beings unique has fascinated humankind throughout modern history. Today, we view the brain as the core component of human identity, making an understanding of this organ crucial to explaining our species' characteristics. What distinguishes humans from other species is largely thought to reside in the unique features of brain development, especially in the wiring of the immensely complex neural circuits that underlie our cognitive and motor abilities. In my presentation, I will describe some of our recent efforts to better understand the molecular and cellular basis of how distinct properties of the human cerebral cortex emerge during development. This region, located on the outside of the mammalian brain, is responsible for processing our senses, executing motor functions, and facilitating higher-order cognitive abilities like language. The prefrontal cortex and hippocampus will be specifically highlighted, and the significance of diverse molecular and cellular processes in their evolution and development will be explored. The presentation will also examine how these complex developmental processes

have been modified in human evolution and may be vulnerable to compromise in neuropsychiatric conditions.

11:40 AM – 12:05 PM
REGULATION OF SPECIES-SPECIFIC DEVELOPMENTAL RATES BY REDOX METABOLISM

Margarete Diaz Cuadros, *Harvard Medical School, USA*
Gabriel Valdebenito, *Harvard Medical School, USA*
John Madden, *Harvard Medical School, USA*
Leslie Azurdia, *Harvard Medical School, USA*

Developmental processes during embryogenesis are conserved across mammals, yet the rate of development varies significantly between species. While embryogenesis in mice is completed in 15 days, human development spans 56 days. Understanding the molecular mechanisms underlying these differences is critical for improving the differentiation of human induced pluripotent stem cells (iPSCs) for disease modelling and regenerative therapies. We recently established an in vitro system that recapitulates the 2-3 fold difference in developmental rate between mouse and human embryos. This model relies on the differentiation of mouse and human pluripotent stem cells to presomitic mesoderm (PSM), a cell type that harbors a molecular oscillator known as the segmentation clock. This clock provides a high-resolution, quantitative proxy for developmental speed. Using this system, we previously found that the cytosolic NAD+/NADH ratio plays a key role in regulating developmental rates, with higher NAD+/NADH ratios associated with faster development. This redox balance is modulated by electron shuttling systems, notably the glycerol-3-phosphate (G3P) and malate-aspartate (MA) shuttles. We have found that the G3P shuttle is active in mouse PSM cells but not in human PSM cells due to species-specific expression patterns of G3P shuttle enzymes. Exogenously expressing G3P shuttle enzymes in human cells increases flux through this shuttle, raises the NAD+/NADH ratio, and accelerates the segmentation clock. By elucidating the molecular mechanisms governing developmental timing, this research provides critical insights into species-specific differences and enhances the utility of iPSCs for therapeutic applications.

1:50 PM – 3:20 PM
NEURAL STEM CELLS IN DEVELOPMENT

1:50 PM – 2:15 PM

3D HUMAN ORGANOIDS TO EXPLORE THE DEVELOPMENT OF THE CEREBRAL CORTEX AND ITS BARRIER

Simona Lodato, *Humanitas University, Italy*

Recent advances in stem cell-derived 3D organoid technology have paved the way for more detailed modeling of human brain development in vitro, providing insights into processes that have long remained elusive to neurodevelopmental researchers. The development of the mammalian cerebral cortex, including the human cortex, is shaped by a dynamic interplay of genetic, activity-dependent, and environmental factors, which drive its complexity from the earliest stages of formation. These factors influence neuronal diversity, synaptic connectivity, and the assembly of functional cortical networks, with their disruption often linked to the onset of neurodevelopmental disorders. Using well-established protocols to generate cortical organoids, we aim to model key aspects of human cortical development, particularly the emergence and modulation of spontaneous neuronal activity. In addition, we explore how alterations in early neuronal activity impact the formation of complex neural circuits. This approach underscores the critical role of early activity patterns and provides essential insights into modeling infantile epilepsy, where such patterns are often disrupted. By integrating single-cell omics with 3D calcium imaging, we adopt a holistic strategy to understand how cortical circuits are formed and maintained in different models of epilepsy, and what is the impact of genetic and environmental factors to the onset of the seizures. Given the dynamic environment surrounding the developing cortex, including the production of cerebrospinal fluid (CSF), we are also focused on defining the role of the choroid plexus (ChP) and other brain barriers in creating an optimal environment for cortical development and maturation. These barriers regulate molecular signals that govern neural differentiation, spontaneous activity, and network integration. To further investigate this, we developed a novel choroid plexus organoid model (ChP Orgs), which faithfully replicates the histological, functional, and ultrastructural characteristics of native human ChP tissue. This model demonstrates dynamic CSF secretion that closely mimics the ion composition found in vivo and responds actively to external stimuli. Through omics analysis, we longitudinally profiled the cellular landscape of ChP Orgs, revealing a diverse array of cell types, including neural, endothelial, and immune cells, reflecting the complexity of ChP tissue and its functional barrier activity. Our 3D ChP model offers crucial insights into cortical development and homeostasis by accurately recapitulating the complexity and function of native barrier tissue.

2:15 PM – 2:40 PM

THE DIVERSITY OF NEUROGENESIS IN DEVELOPING BRAIN

Xiaoqun Wang, *Institute of Biophysics, Chinese Academy of Sciences, China*
Bo Zeng, *Institute of Biophysics, Chinese Academy of Sciences, China*
Mengdi Wang, *Institute of Biophysics, Chinese Academy of Sciences, China*
Zeyuabn Liu, *Institute of Biophysics, Chinese Academy of Sciences, China*
Suijian Zhong, *IDG/McGovern Institute for Brain Research, Beijing Normal University, China*
Xin Zhou, *IDG/McGovern Institute for Brain Research, Beijing Normal University, China*
Qian Wu, *IDG/McGovern Institute for Brain Research, Beijing Normal University, China*

The emergence of the three primary germ layers and the appearance of lineage-specific precursor cells orchestrating organogenesis represent fundamental milestones during early embryonic development. We analyzed the transcriptional profiles of over four hundred thousand cells from 14 human samples collected from postconceptional weeks (PCW) 3 to 12 to delineate the dynamic molecular and cellular landscape of early gastrulation and nervous system development. Using this unique dataset, we describe the diversification of cell types, the spatial patterning of the neural tube cells, and the signaling pathways involved in transforming epiblast cells into neuroepithelial cells and then into radial glia. We resolved 24 clusters of radial glial cells along the neural tube and outlined differentiation trajectories for the main classes of neurons. Lastly, we identified conserved and distinctive features across species by comparing early embryonic single-cell transcriptomic profiles between humans and mice. This comprehensive atlas sheds light on the molecular mechanisms underlying gastrulation and early brain development in humans.

Funding Source: New Corner stone foundation and NSFC.

2:40 PM – 2:55 PM

DECREASED PROGENITOR POOL SIZE TRIGGERS DRIVER-INDEPENDENT CLONAL EXPANSION DURING NEUROGENESIS

Giulia Di Muzio, *German Cancer Research Center (DKFZ), Germany*
Sarah Benedetto, *German Cancer Research Center (DKFZ), Germany*
Li-Chin Wang, *German Cancer Research Center (DKFZ), Germany*
Hsin-Jui Lu, *German Cancer Research Center (DKFZ), Germany*

Lea Weber, *German Cancer Research Center (DKFZ), Germany*
Yassin Harim, *German Cancer Research Center (DKFZ), Germany*
Jana Berlanda, *German Cancer Research Center (DKFZ), Germany*
Nina Claudino, *German Cancer Research Center (DKFZ), Germany*
Franciscus van der Hoeven, *German Cancer Research Center (DKFZ), Germany*
Brittney Armstrong, *German Cancer Research Center (DKFZ), Germany*
Thomas Höfer, *German Cancer Research Center (DKFZ), Germany*
Pei-Chi Wei, *German Cancer Research Center (DKFZ), Germany*

Clonal expansion is a key process in neurogenesis, driven by high-fitness progenitors that generate overrepresented neuronal populations sharing the same genomic variants. Driver mutations enhancing the proliferative capacity of progenitors can promote their positive selection. However, whole-genome sequencing of the human brain has revealed large neuronal clones lacking driver mutations, and therefore the mechanisms underlying driver-independent clonal expansion in neural stem and progenitor cells (NSPCs) remain unclear. To bridge this gap, we generated a novel mouse model to investigate whether reducing NSPC pool size induces driver-independent clonal competition akin to a bottleneck event. 30–50% of NSPCs were ablated using diphtheria toxin A at E12.5. Remarkably, the generation of the whole brain including all cell types was still completed by E17.5, indicating a compensatory shortening of the NSPC cell cycle. A dual-labeling thymidine analogue assay confirmed an accelerated cell cycle in intermediate progenitors (IPs) which, among others, generate cortical neurons. Variant allele frequency analysis of E17.5 forebrains revealed non-driver somatic variants, proving that NSPC depletion drives clonal expansion independent of known driver mutations. Yet, we found the oxidative damage signature SBS18, suggesting the early clonal expansion phase was characterized by high intracellular ROS levels. Single-cell RNA sequencing showed a reduction in radial glia cells (RGs) and neuroblasts, an increase in cortical neuron numbers, and a constancy in IP numbers, suggesting RGs underwent more consumptive divisions to sustain IP production. Moreover, both RGs and neuroblasts downregulated oxidative phosphorylation pathways, indicating delayed cell fate transitions in response to NSPC depletion. In conclusion, our findings demonstrate that a significant reduction of the NSPC pool promotes a clonal expansion independent of known driver mutations, suggesting an epigenomic adaptation of some NSPCs to become high-fitness progenitors. This driver-free expansion is incited by an increase in oxidative metabolism and is associated with changes in cell cycle speed and delayed cell fate transitions, potentially impacting postnatal brain development.

2:55 PM – 3:20 PM

SOMATIC MOSAICISM AND CELL LINEAGES IN HUMAN NEURODEVELOPMENT AND DISEASE

Sara Bizzotto, *Imagine Institute | Inserm, France*

How cell lineages shape the human central nervous system during normal and pathological neurodevelopment is a long-standing question. Somatic mosaicism has been providing a new way to look at cell lineages by interrogating human tissue directly. Indeed, somatic variants and especially single-nucleotide variants, occur frequently enough in progenitor cells to function as permanent markers of cell divisions and clones. Analysis of somatic variants in post-mortem human tissues reconstructed cell divisions of human embryonic development and revealed that early embryonic lineages give an unbalanced contribution to the human cortex. We estimated that 50 to 100 neuro-ectodermal progenitors give rise to the human forebrain, and showed that frontal lobe clones segregate from the posterior lobe during development. Somatic variants can have a deleterious impact on brain development when occurring in the wrong place at the wrong time such as variants activating the PI3K-mTOR pathway, which are a major cause of drug-resistant pediatric focal epilepsy, typically associated with focal cortical dysplasia type 2. Analyses of somatic mosaicism in pathological tissue identify the affected lineages and how they behave, providing insights into the pathophysiology of the disorder. Simultaneous single-nucleus somatic variant genotyping and gene expression analysis of post-surgical tissue showed that pathogenic variant-carrying cells have well-differentiated neuronal or glial identities, with enrichment of pathogenic variants in cells of the neuro-ectodermal lineage, pointing to neural progenitor cells as possible loci of somatic mutation in focal cortical dysplasia type 2. Within the lesion, variant-carrying neurons showed upregulation of PI3K-mTOR signaling and related pathways, while non-variant-carrying neurons showed downregulation of these pathways. Changes in microglial activation, cellular metabolism, synaptic homeostasis, and neuronal connectivity were also identified, all potentially contributing to epileptogenesis.

Funding Source: U01MH106883; R01NS032457; R01NS032457; U54HD090255; R01AG07092; R01CA269805; R01HG012573; UM1DA058230; Manton Center for Orphan Disease Research at BCH; Horizon2020 MSCA no. 101026484—CODICES.

3:50 PM – 5:20 PM
INTRINSIC REGULATION OF NEURAL STEM CELLS

3:50 PM – 4:15 PM
SINGLE CELL EPIGENOMIC RECONSTRUCTION OF EARLY HUMAN BRAIN ORGANOID DEVELOPMENT

Fides Zenk, *École Polytechnique Fédérale de Lausanne (EPFL), Switzerland*

A tightly controlled series of fate restrictions from pluripotent progenitors leads to the diversity of human cell types. These fate restrictions are governed by epigenetic mechanisms that control the activity of target genes and regulatory elements. However, to explore these pathways in human early brain development has been challenging. Here, we use single-cell profiling of histone modifications (H3K27ac, H3K27me3, and H3K4me3) in human central nervous system organoid cells across a developmental time course in order to reconstruct the epigenomic trajectories driving cell identity acquisition from human pluripotency. We analyze transitions from pluripotency to neuronal and glial terminal states as well as differentiation from progenitors to retinal and brain area specification through the neuroepithelium. We discover that decisions at each level can be predicted by switching between repressive and activating epigenetic modifications. We further establish a temporal census of regulatory elements and transcription factors and characterize them within the gene regulatory network human cerebral cell fate acquisition. Our single-cell genome-wide atlas of histone modification changes during the development of human brain organoids serves as a guide for further research into the cell fate choices made in human brain development in healthy physiology and in neurodevelopmental disorders.

Funding Source: EMBO Postdoctoral Fellowship, Swiss National Science Fond SNSF.

4:15 PM – 4:40 PM
MOLECULAR AND FUNCTIONAL HETEROGENEITY OF NEURAL STEM CELLS ACROSS LIFESPAN

Sebastian Jessberger, *University of Zürich, Switzerland*

Neural stem cells generate new neurons throughout life in distinct regions of the mammalian brain. This process, called adult neurogenesis, is critically involved in certain

forms of learning and memory. In addition, failing or altered neurogenesis has been associated with a number of neuro-psychiatric diseases such as major depression and cognitive aging. We aim to characterize the cellular and molecular mechanisms regulating neural stem cell activity and behavior on a single cell level across lifespan. We present new approaches to study the cellular principles underlying life-long neurogenesis using imaging-based tools and single cell molecular profiling. Further, we provide evidence for novel molecular mechanisms governing the neurogenic process in the mammalian brain. Thus, the data presented provide new insights into the cellular principles of life-long neurogenesis and identify novel mechanisms regulating the behavior of rodent and human neural stem cells.

4:40 PM – 4:55 PM
PROMYELOCYTIC LEUKEMIA PROTEIN (PML) IS CRUCIAL FOR NEURAL STEM CELL (NSC) DIFFERENTIATION, STRESS TOLERANCE AND MITOCHONDRIA INTEGRITY

Syrago Spanou, *Institute of Molecular Biology and Biotechnology (IMBB), Foundation for Research and Technology Hellas (FORTH), Greece*

The tumor suppressor PML has important roles in brain development; however, the molecular and cellular pathways regulated by PML in neuronal cells remain largely unknown. To address this issue, we analyzed gene expression changes caused by loss of PML in embryonic neural stem cells (E13.5). Our findings revealed that PML-deficient cells exhibited increased mTOR pathway activation and protein translation, as well as impaired autophagy and proteasome activity, resulting in increased formation of aggregates and stress-induced death. PML loss also disrupted mitochondrial integrity, affecting membrane potential, morphology and production of reactive oxygen species. We found that these abnormalities were caused by diminished PGC-1α expression and PPARγ signaling, both of which were rectified using a PPAR agonist. Together, our results indicate that PML is a critical regulator of neuronal survival and protection from stress. We propose that enhancing PML expression may offer therapeutic benefits in neurological disorders.

4:55 PM – 5:20 PM
ABSTRACT NOT AVAILABLE AT THE TIME OF PUBLISHING

Lorenz Studer, *Memorial Sloan Kettering Cancer Center and BlueRock Therapeutics, USA*

FRIDAY, 4 APRIL 2025
8:30 AM – 10:00 AM
ADULT NEURAL STEM CELLS AND AGING

8:55 AM – 9:20 AM
BLOOD FACTORS AS MEDIATORS OF BRAIN AGING AND REJUVENATION

Lida Katsimpardi, *Institute for Regenerative Medicine and Biotherapy, France*

Aging is associated with a decline in cerebrovascular function, which significantly contributes to reduced neurogenesis, impaired brain function, and cognitive decline. These changes also exacerbate the progression of neurodegenerative diseases such as Alzheimer's disease. We previously showed that factors present in young blood can reverse age-related cerebrovascular decline through heterochronic parabiosis in mice. In addition, young blood can enhance neurogenesis, promote vascular remodeling, and restore cerebral blood flow to youthful levels. Notably, we identified specific systemic factors such

as growth differentiation factor 11 (GDF11) as a key driver of these effects and demonstrated its ability to mimic the benefits of young blood by improving vascular integrity, promoting angiogenesis, and supporting neuronal health. Simultaneously, advances in organ-on-chip technology provide a complementary and powerful platform to accelerate the understanding and application of these rejuvenation strategies. With the integration of these models, the mechanisms underlying the actions of GDF11 and other therapeutic candidates can be studied in greater depth, enabling the refinement of targeted interventions and paving the way for personalized therapeutic strategies. The combination of blood factor therapies and organ-on-chip models represents a promising avenue for advancing our understanding of the aging brain and developing precision therapies.

9:20 AM – 9:35 AM

MOLECULAR TRAJECTORIES UNDERLYING QUIESCENCE RE-ENRY AMONG ADULT NEURAL STEM CELLS

Tanya Foley, *Institut Pasteur, , France*
Isabelle Foucher, *Institut Pasteur, France*
Jules Samaran, *Institut Pasteur, France*
Gaëlle Letort, *Institut Pasteur, France*
Nicolas Dray, *Institut Pasteur, France*
David Morizet, *Institut Pasteur, France*
Laura Cantini, *Institut Pasteur, France*
Christophe Zimmer, *Institut Pasteur, France*
Laure Bally-Cuif, *Institut Pasteur, France*

Adult vertebrate neurogenesis is continuous and sustained by the activity of neural stem cells (NSCs). Adult NSCs retain the capacity to proliferate and differentiate, generating neural progenitors (NPs) that further differentiate to neurons. Continued proliferation by NSCs leads to exhaustion of the NSC pool through differentiation. Therefore, to preserve NSCs over time, NSC divisions are followed by phases of quiescence (G0), a dynamic and reversible state of cell cycle arrest. Quiescent NSCs are transcriptionally heterogeneous and vary in quiescence depth, or resistance to activating cues. Using scRNA-seq, we previously identified a continuum of transcriptional substates among quiescent NSCs within the adult zebrafish telencephalon thought to reflect differences in quiescence depth. However, the molecular trajectories by which NSCs transition between these

substates remain unknown. To address this, BrdU-labelling of proliferating cells followed by varying chase times was used to estimate quiescence durations post-division. This was coupled to multiplexed single molecule RNA-fluorescence in situ hybridization (smRNA-FISH) that we adapted for use in the whole mount telencephalon, by which the expression of 12 genes that, when measured in combination, form a transcriptomic code that distinguishes between quiescence substates. With this approach, each substate identity was associated with a quiescence duration post-division, thereby revealing a temporal sequence of substates varying in quiescence depth through which cells transition and thus elucidating the molecular trajectories between quiescent substates upon quiescence re-entry. Our findings suggest that immediately post-division, NSCs returning to quiescence enter a transient state of deep quiescence in which they become highly resistant to activation. Resistance to activation resolves over time, however, as quiescent NSCs gradually transition to shallower quiescence substates, undergoing morphometric changes associated with increased likelihood for self-renewal upon division. This work not only describes previously unknown molecular trajectories between transcriptional substates among quiescent NSCs, but also highlights the importance of dynamic regulation of quiescence depth to balance neurogenesis with self-renewal over time.

9:35 AM – 10:00 AM
REGULATION AND DIVERSITY OF ADULT NEURAL STEM CELLS

Fiona Doetsch, *Biozentrum at the University of Basel, Switzerland*

Neural stem cells reside in specialized niches in the adult mammalian brain and contribute to brain plasticity throughout life. The adult ventricular-subventricular zone (V-SVZ) adjacent to the lateral ventricles is the largest germinal niche in the adult mouse brain. Stem cells in the V-SVZ give rise to different subtypes of olfactory bulb interneurons, as well as to some glia. Importantly, adult V-SVZ neural stem cells are a heterogeneous population, with distinct molecular identities and fates, depending on their spatial location in the niche. They constantly integrate intrinsic and extrinsic signals to either maintain their quiescent state or to become activated to divide and generate progeny. However, the functional significance of this heterogeneity has remained elusive. I will present our recent findings highlighting the key role of physiological states, as well as of long-range signals, in regulating regionally distinct pools of adult neural stem cells for on-demand adaptive brain plasticity.

10:30 AM – 11:50 AM
GLIA IN BRAIN DEVELOPMENT AND DISEASE

10:30 AM – 10:55 AM
IPS-CELL DERIVED MICROGLIA PROMOTE BRAIN ORGANOID MATURATION VIA CHOLESTEROL TRANSFER

Florent Ginhoux, *Singapore Immunology Network (SIgN), A*STAR, Singapore*

Microglia are specialized brain-resident macrophages that arise from primitive macrophages colonizing the embryonic brain. Microglia contribute to multiple aspects of brain development, but their precise roles in the early human brain remain poorly understood owing to limited access to relevant tissues. The generation of brain organoids from human induced pluripotent stem cells recapitulates some key features of human embryonic brain development. However, current approaches do not incorporate microglia or address their role in organoid maturation. Here we generated microglia-sufficient brain organoids by coculturing brain organoids with primitive-like macrophages generated from the same human induced pluripotent stem cells (iMac). In organoid cocultures, iMac differentiated into cells with microglia-like phenotypes and functions (iMicro) and modulated neuronal progenitor cell (NPC) differentiation, limiting NPC proliferation and promoting axonogenesis. Mechanistically, iMicro contained high levels of PLIN2+ lipid droplets that exported cholesterol and its esters, which were taken up by NPCs in the organoids. We also detected PLIN2+lipid droplet-loaded microglia in mouse and human embryonic brains. Overall, our approach substantially advances current human brain organoid approaches by incorporating microglial cells, as illustrated by the discovery of a key pathway of lipid-mediated crosstalk between microglia and NPCs that leads to improved neurogenesis.

10:55 AM – 11:10 AM
CREATING HUMAN MODELS FOR NEUROLOGICAL DISEASES: IPSC-DERIVED HUMAN MICROGLIA-LIKE CELLS AND CEREBRAL ORGANOIDS TO STUDY CSF1R-RELATED DISORDERS

Li Zeng, *National Neuroscience Institute, Singapore*

CSF1R-related disorder (CSF1R-RD) is a rare, inherited autosomal dominant, and progressive neurological disease. The clinical features of CSF1R-RD include cognitive decline, motor dysfunction, psychiatric symptoms, and white matter abnormalities. This disorder is caused by mutations in the CSF1R gene, which plays a crucial role in microglia and macrophages' regulation, development, survival, and function. Despite identifying many pathogenic mutations in CSF1R, the molecular mechanisms underlying CSF1R mutation-induced pathogenesis in CSF1R-RD remain elusive. Current studies have mainly utilized in vitro models or CSF1R knockout or overexpression mouse models to investigate CSF1R function in CSF1R-RD. To gain molecular insight into the CSF1R mutations in CSF1R-RD, we derived induced pluripotent stem cells (iPSCs) from a CSF1R-RD patient carrying the CSF1R-T567M mutation (the mutation occurs outside of the tyrosine kinase domain of the CSF1R gene) and its CRISPR/Cas9-corrected isogenic control. Using these iPSCs, we generated iPSC-derived human microglia-like cells (iMGL) and cerebral organoids (COs). We showed that CSF1R-MT-iMGL induced neuroinflammation, increased phagocytosis, and impaired migration. Transcriptomic RNA sequencing (RNA-seq) analysis and cytokine profiling revealed an upregulation of immune activity and downregulation of synaptic function. Furthermore, we found that CSF1R-MT promoted neural proliferation, inhibited neural differentiation, and delayed neural maturation. It caused neurodevelopmental defects in COs. Whole-cell patch-clamp recording showed an impaired synaptic function in CSF1R-MT-COs. Furthermore, CSF1R-MT-iMGL impaired synaptic protein expression when co-cultured with CSF1R-MT- COs. Together, this is the first study using CSF1R-MT patient iMGL and COs as models to study the pathogenesis of CSF1R-RD. It offers a robust platform for drug screening endeavors aimed at developing personalized medicine for CSF1R-RD.

Funding Source: SingHealth Duke-NUS Academic Medicine Research Grant (NO. AM/TP050/2021 (SRDUKAMR2150)).

11:10 AM – 11:25 AM

MULTIMODAL SINGLE CELL SPATIAL TRANSCRIPTOMICS TO MAP DORMANT-TO-ACTIVE NEUROGENIC NICHES

Roy Maimon, *University of California at San Diego, USA*

The potential to replace neurons lost to aging, injury, or neurodegeneration through induced adult neurogenesis offers groundbreaking therapeutic opportunities. Studying this

process has been challenging due to the rarity of neurogenesis events in the aging brain and limitations in existing tools. Here, we developed enhanced, multimodal single-cell spatial transcriptomics [Multimodal Multiplexed-Error-Robust-Fluorescence-In-Situ-Hybridization (MERFISH)] integrated with lineage-tracing tools to map endogenous neurogenesis in the two major neurogenic niches [Subventricular zone (SVZ) and Subgranular zone (SGZ)] across postnatal (P5), young adult (4- and 8-week-old), and aged (1-year-old) murine brains, profiling 2,085 genes across distinct cellular states. Our analysis demonstrates a transformation of the neurogenic niches from active to quiescent with age, characterized by altered neuronal stem cells positioning and persistent expression of cell identity regulators including the RNA-binding protein Polypyrimidine-Tract-Binding-Protein-1 (Ptbp1). In aged murine SVZ, we identify a quiescent neuronal stem cell-like population that, upon transient suppression of Ptbp1 via a single intracerebroventricular injection of a Ptbp1-targeting antisense oligonucleotide, reactivates and produces immature neurons via a canonical adult neurogenesis process. Cell proliferation (5-ethynyl-2'-deoxyuridine, EdU) labeling integrated with Multimodal MERFISH mapping, demonstrates that these neuronal stem cells re-enter the cell cycle, replicate DNA, and differentiate into Drd1 Medium Spiny Neurons. Genetic lineage tracing (Aldh1l1:Sun1:GFP) combined with Multimodal MERFISH further confirms the migration of these Ptbp1-suppressed glia-derived neurons to the striatum, where they integrate and acquire functional neuronal identities. Strikingly, we identify a parallel PTBP1-expressing neuronal stem cell-like glial population in dormant aged human and non-human primate SVZ, exhibiting conserved gene expression profiles. These findings position transient PTBP1 suppression as a transformative strategy to reactivate dormant neurogenic niches in the aged brain, paving the way for innovative therapeutic approaches to neurodegenerative diseases and age-related neuronal loss.

11:25 AM – 11:50 AM

ASTROCYTE DYSFUNCTION DRIVEN BY THE P.A53T-ALPHA-SYNUCLEIN MUTATION INDUCES LEWY-LIKE NEUROPATHOLOGY IN AN IPSC-DERIVED MODEL OF PARKINSON'S DISEASE

Rebecca Matsas, *Hellenic Pasteur Institute, Greece*
Christina Paschou, *Hellenic Pasteur Institute, Greece*
Olympia Apokotou, *Hellenic Pasteur Institute, Greece*
Anastasios Kollias, *Hellenic Pasteur Institute, Greece*
Martina Samiotaki, *BSRC Alexander Fleming, Greece*

Era Taoufik, *Hellenic Pasteur Institute, Greece*
Florentia Papastefanaki, *Hellenic Pasteur Institute, Greece*

Alpha-Synuclein (αSyn) plays a central role in Parkinson's disease (PD) and the p.A53T mutation causes an early-onset familial form of PD with severe manifestations. The pathological effects of p.A53T-αSyn have been extensively investigated in neurons, yet its consequences on astrocytes and the astrocytic contribution to PD pathology are understudied. To assess the effects of p.A53T-αSyn astrocytes on dopamine neurons, we differentiated induced pluripotent stem cells (iPSC) from PD patients carrying the mutation to ventral midbrain astrocytes, and characterized them through comprehensive molecular, biochemical, and functional analyses, along with proteome profiling. Astrocytes derived from healthy and gene-corrected iPSCs served as controls. Further, we established neuron-astrocyte co-cultures comprising iPSC-derived control and mutant cells at all possible combinations. Our analyses uncovered cell-intrinsic pathological phenotypes in p.A53T-αSyn astrocytes, including calcium dyshomeostasis, and accumulation of protein aggregates including those of phosphorylated αSyn. Proteomic and mechanistic studies demonstrated perturbed protein catabolic processes, involving proteasome and autophagy pathways, with associated lysosomal dysfunction and disturbance of mammalian target of rapamycin signaling. These impairments affected the endocytic clearance capacity of p.A53T-aSyn astrocytes compromising their ability to process exogenous αSyn cargo. Furthermore, p.A53T-αSyn dopamine neurons co-cultured with p.A53T-αSyn astrocytes displayed hallmark Lewy-like pathologies, similar to those identified in human post-mortem PD brains, and exhibited exacerbated neurodegeneration, in both morphological and functional terms. The aggravated neuropathology was alleviated by control astrocytes, suggesting a prominent neuroprotective effect. In contrast, p.A53T-αSyn astrocytes exerted a toxic influence on control neurons, inducing PD-relevant neuropathology. Our findings demonstrate a critical impact of the p.A53T-αSyn mutation in causally disrupting astrocytic protein quality control mechanisms, and corroborate astrocytes as important contributors to PD neuropathology. Additionally, our study presents a two-dimensional co-culture model that reliably recapitulates key aspects of PD pathology, offering a useful tool for mechanistic and drug discovery studies.

Funding Source: The work was supported by the Hellenic Foundation for Research and Innovation project 1019 and the TAA TAEDR-0535850 - Brain Precision Medicine Project.

1:45 PM – 3:15 PM

PROGRAMMING AND REPROGRAMMING THE BRAIN

1:45 PM – 2:10 PM

ENGINEERING INHIBITORY NEUROGENESIS IN VIVO VIA LINEAGE REPROGRAMING

Benedikt Berninger, *King's College London, UK*
Alexis Cooper, *King's College London, UK*
Sydney Leaman, *King's College London, UK*
Nicolas Marichal, *King's College London, UK*
Araceli Garcia Mora, *King's College London, UK*
Ana Beltrán Arranz, *King's College London, UK*
Filipe Ferreira, *King's College London, UK*
Adil Khan, *King's College London, UK*

Lineage reprogramming of resident glia into induced neurons emerges as an experimental strategy to remodel diseased brain circuits. A key challenge to the approach consists in the generation of neurons with neuronal subtype-specific features. Our previous work has highlighted the possibility of converting postnatal cortical astrocytes into induced neurons with hallmarks of parvalbumin-expressing, fast-spiking interneurons. This was achieved by forced expression of a phosphosite-deficient form of the proneural transcription factor achaete-scute complex like-1 (Ascl1SA6) together with the cell death regulator Bcl2. Here, I will discuss our recent efforts to uncover the transcriptional changes that underpin the conversion of early cortical astrocytes into interneuron-like cells. Using single cell transcriptomics, we found that while Ascl1SA6/Bcl2 co-expression drives expression of a battery of classical Ascl1 target genes, some aspects of cortical interneurogenesis remain incomplete. For instance, Ascl1SA6/Bcl2 failed to induce detectable levels of Dlx2 which is typically expressed in immature cortical interneurons. We are currently exploring the consequences of Dlx2 co-expression alongside Ascl1SA6. Another important question concerns the ability of induced neurons to integrate into cortical circuits. Here, I will present data from two-photon live microscopy that show that induced neurons can become recruited into active cortical circuits and respond to sensory stimuli.

Funding Source: Wellcome Trust (206410/Z/17/Z), European Research Council (grant agreement no. 101021560, IMAGINE), ERA-NET Neuron grant (Brain4Sight, 01EW2202).

2:10 PM – 2:35 PM

NEURONAL TIME-TURNER: REPROGRAMMING MOTOR NEURON VULNERABILITY TO ALS

Hynek Wichterle, *Columbia University, USA*

Our lab has shown that neuronal maturation is a protracted process that, even in mice, takes up to a month. During this period, there are dramatic changes in gene expression and chromatin accessibility. We have begun dissecting genetic programs that contribute to the maturation process, with the hope that we will be able to control the developmental age of spinal motor neurons. Our data suggest that transcriptional reprogramming of postnatal motor neurons with factors that control the specification of immature motor neuron identity is a promising method for increasing the resistance of nerve cells to mutations that cause late-onset degenerative diseases, such as ALS.

2:35 PM – 2:50 PM
DISSECTING STEM CELL HIERARCHIES IN HUMAN BRAIN DEVELOPMENT AND DISEASE

Daniel Dan Liu, *Stanford University, USA*

ABSTRACT NOT AVAILABLE AT THE TIME OF PUBLISHING

2:50 PM – 3:15 PM
DECONSTRUCTING NEURONAL DIVERSITY

Kristin K. Baldwin, *Columbia University, USA*

Within the nervous system, diverse types of neurons have been shown to arise through a highly choreographed temporal series of cell fate changes. However, the robustness of methods to generate neurons using direct reprogramming has shown that many aspects of neuronal identity and diversity can be produced without transit through all developmental intermediates. Interestingly, more recent developmental studies of brain evolution have uncovered alternative developmental pathways involved in brain development. Here we report results of a large unbiased screen for factors that can produce elements of neuronal identity and diversity. Analyses of these data allow us to develop mathematical models of cell fate stability. Our results also supply a roadmap for generating new neuronal subtypes for disease modeling and make inroads into more predictive methods for reprogramming.

3:45 PM – 5:20 PM
MODELING BRAIN DEVELOPMENT AND DISEASE

3:45 PM – 4:10 PM
HUMAN IPSC-DERIVED GLIAL CELL MODELS FOR INVESTIGATING MULTIPLE SCLEROSIS AND NEURODEGENERATIVE DISEASES

Valentina Fossati, *New York Stem Cell Foundation, USA*

Human induced pluripotent stem cell (iPSC)-derived models of glial cells are emerging as critical complementary tools to animal models for studying neurological diseases unique to humans. Over the years, we have developed robust protocols for generating oligodendrocytes, astrocytes, and microglia to investigate their roles in neurodegenerative diseases. These reductionist systems offer the advantage of isolating cellular mechanisms without the complexity of the in vivo milieu. In the context of multiple sclerosis (MS), our iPSC-derided system has enabled the study of glial cells independently from peripheral immune cell infiltration, which drives neuroinflammation during disease progression. Using single-cell transcriptomic analysis of glial cultures derived from MS patients and healthy controls, we identified proinflammatory subpopulations within MS oligodendrocytes and astrocytes, along with increased oligodendrocytes vulnerability in primary progressive MS, even in absence of inflammation and peripheral immune cells. These findings emphasize the cellular heterogeneity of glial cells in MS and suggest that targeting glial-driven inflammatory pathways may lead to novel, more personalized therapeutic interventions.

Funding Source: DOD award number W81xWH-15-1-0448; The New York State Stem Cell Science (NYSTEM), award number C32586GG.

4:10 PM – 4:35 PM
DECODING BRAIN DEVELOPMENT AND NEURODEVELOPMENTAL, NEUROLOGICAL AND PSYCHIATRIC DISORDERS

Christina Kyrousi, *National and Kapodistrian University of Athens, Greece*

Brain development is a highly orchestrated process that ensures proper brain formation and function. Thus, dysregulation of genetic, molecular or cellular mechanisms essential for neurodevelopment may lead to perturbation of its structure causing a range of defects like Neurodevelopmental, Neurological and Psychiatric Disorders. In recent years, research aims at deciphering the etiology of such disorders, focusing on key neurodevelopmental mechanisms. Amongst those, both extracellular signaling and intrinsic properties regulating cell function and lineage progression of neural progenitors have been highlighted, however, the exact mechanisms mediating their intercommunication are still elusive. Primary cilia (PC), sensory organelles that act as mediators of various signaling pathways which also regulate intrinsic properties of cells, affect proper corticogenesis and have been recently implicated in neurodevelopmental and psychiatric disorders. PC in mice is known to regulate neural progenitor cells' division and neuronal migration, however, no concrete knowledge exists regarding human brain development. Interestingly, mutations in centrosomes- or ciliary-associated genes have been identified in subclasses of Neurodevelopmental Disorders like microcephaly, periventricular heterotopia and polymicrogyria, suggesting that centrosome-cilia-axis is essential for proper corticogenesis in humans. We aim to investigate the role of PC during cortical development and scrutinize the PC-mediated mechanisms that lead to brain-related diseases. To dissect the role of PC in human corticogenesis, we focus on PC-related genes with species-specific expression differences. We then provoke ectopic manipulation of PC candidate genes in the developing mouse cortex and human brain organoids and we generate mutant brain organoids to decipher their function.

4:35 PM – 4:50 PM

MULTIVARIABLE MACHINE LEARNING REVEALS COMMON FIRING PATTERNS IN IPSC MODELS OF KCNQ2 DEVELOPMENTAL EPILEPTIC ENCEPHALOPATHY

Evangelos Kiskinis, *Northwestern University, Feinberg School of Medicine, USA*
Dina Simkin, *Northwestern University, USA*
Adil Wafa, *Northwestern University, USA*
Alfred George, *Northwestern University, USA*

Heterozygous loss-of-function mutations in KCNQ2 cause developmental and epileptic encephalopathy (DEE), a devastating disorder characterized by neonatal seizures and a diverse spectrum of cognitive and developmental deficits with no effective therapeutics. Here, we used induced pluripotent stem cell (iPSC) models to investigate the

pathophysiological mechanisms of multiple KCNQ2-DEE patient mutations. We find a dyshomeostatic, transcriptional and functional enhancement of Ca2+-activated SK channels across patient lines, that is associated with faster action potential repolarization and larger post-burst after-hyperpolarizations. As a result, KCNQ2-DEE patient neurons exhibit an irregular burst-suppression-like firing pattern and long-term synaptic deficits. Application of multivariable machine learning algorithms on rich multi-electrode array (MEA) recording datasets revealed patient-specific, as well as common firing features across multiple KCNQ2-DEE lines. Critically, using our machine learning model we show that early and chronic treatment with the Kv7 activator retigabine, restores the temporal pattern of neuronal activity and the ability of patient neurons to respond to electrical stimulation. Our work highlights SK channel upregulation as a pathophysiological mechanism in KCNQ2-DEE, provides an MEA-based machine learning platform that can be used to decipher phenotypic diversity amongst patients, as well as identify effective drug treatment modalities.

Funding Source: New York Stem Cell Foundation, NIH/NINDS.

4:50 PM – 5:15 PM
KEYNOTE: CEREBRAL ORGANOIDS: GROWING HUMAN BRAIN TISSUE FROM STEM CELLS TO STUDY DEVELOPMENT AND DISEASE

Jürgen Knoblich, *Institute of Molecular Biotechnology, Austrian Academy of Sciences, Austria*

The human brain is unique in its size and complexity. We have developed cerebral organoids, 3D cell cultures derived from patient stem cells that recapitulate the development of the human brain. Using this technology, we have identified developmental processes unique to humans, studied the mechanistic basis for brain diseases and reconstituted human neural network activity (Lancaster et al., Nature 2013; Esk et al., Science 2020; Eichmüller et al., Science 2022; Li et al., Nature 2023). Our efforts to recapitulate human specific processes of brain development and to replicate disease pathology on the circuit level will be presented. Specifically, I will cover our recent efforts to use electrophysiology and barcoded connectome analysis with single-cell resolution in order to determine and understand how neural network activity and network architecture change in patients that develop epilepsy.

POSTER ABSTRACTS

All times are listed in Eastern European Summer Time (EEST)

THURSDAY, 3 APRIL 2025
POSTER SESSION 1: 5:30 PM – 6:20 PM

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ENHANCING OLFACTORY ENSHEATHING CELL VIABILITY AND FUNCTIONALITY THROUGH 3-DIMENSIONAL CULTURE COMBINED WITH S100β: IMPLICATIONS FOR SPINAL CORD INJURY REPAIR

Yan Han, *Griffith Institute for Drug Discovery, Australia*
Mo Chen, *Griffith University, Australia*
Mariyam Murtaza, *Griffith University, Australia*
James St. John, *Griffith University, Australia*

Spinal cord injuries (SCI) are challenging to treat due to the nervous system's limited capacity for regeneration. Olfactory ensheathing cells (OECs), with neuro-regenerative properties, offer promise for SCI repair through transplantation. However, the therapeutic potential of OECs is limited by high post-transplantation cell death, highlighting the need for strategies to enhance their survival and viability. This study aims to enhance the survival of OECs post-transplantation by employing three-dimensional cell culture (3D) over traditional two-dimensional culture (2D), combined with S100β treatment, to boost the therapeutic potential of OECs. Six concentrations of S100β were applied to treat OECs in both 2D and 3D cultures, revealing changes in cell proliferation, migration, morphology, and spheroid formation. Bulk RNA-seq analysis of two human OECs samples cultured under 2D conditions identified significant differential gene expression between S100β-treated and untreated groups, highlighting the potential influence of cellular architecture on gene regulatory mechanisms. Notably, the comparison between S100β-treated and untreated OECs revealed a distinct transcriptomic profile. Key upregulated genes in S100β-treated cells were associated with pathways governing cell survival, extracellular matrix remodeling, and adhesion proteins, suggesting that S100β exerts a modulatory effect on OECs plasticity and enhances their capacity to interact with the extracellular environment. This study highlights the potential of 3D culture combined with S100β treatment in enhancing OEC function, which could open new avenues for SCI therapy. By improving

OEC viability, migration, and intercellular adhesion in a 3D environment, these strategies offer a promising approach to boost the therapeutic efficacy of OECs in SCI repair.

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THE ADULT NEURAL STEM CELL VASCULAR NICHE

Dimitrios Dimitrakopoulos, *University of Basel, Switzerland*
Fiona Doetsch, *University of Basel, Switzerland*

In the adult mammalian brain, neural stem cells (NSCs) reside within specialized niches that continuously generate new neurons and some glial cells. The ventricular-subventricular zone (V-SVZ), the largest germinal zone in the adult mouse brain, is a thin layer of cells adjacent to the lateral ventricles and comprises two distinct regions: the lateral and septal walls. NSCs in the lateral wall are actively dividing and highly neurogenic, whereas those in the septal wall are more quiescent and gliogenic. A key component of the V-SVZ niche is the vasculature, which possesses unique structural and signaling properties critical for regulating NSC behavior, including quiescence, activation and proliferation. The lateral V-SVZ contains an extensive planar vascular plexus with which dividing NSCs and transit-amplifying cells are tightly associated, often contacting blood vessels at sites that lack astrocyte endfeet and pericyte coverage. Endothelial cells and pericytes in the V-SVZ provide contact-mediated and secreted signals that influence NSC behavior. Despite the recognized importance of the vasculature, its heterogeneity within and between the lateral and septal walls has been underexplored. Using confocal microscopy and light-sheet imaging, we show differences in the vascular architecture between these regions. Lateral wall blood vessels are predominantly planar and parallel to the ventricle, whereas those in the septal wall are more tortuous. Blood vessels sometimes connect the septal and lateral walls at rostral V-SVZ levels, appearing as “bridges” which are covered by actively cycling cells. In the septal wall, NSCs exhibit distinct morphologies and distributions, and are closely associated with the vasculature. Proliferating cells in this wall are also associated with the vasculature, frequently extending processes to contact blood vessels. Interestingly, the vascular plexus within the V-SVZ itself exhibits regional characteristics, with areas of higher proliferation associated with distinct vascular plexus features as compared to those with lower proliferative activity. Thus, different features of the vasculature may shape NSC behavior. Ongoing research aims to unravel the molecular mechanisms underlying vascular-NSC interactions and their impact on adult neurogenesis.

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QUANTIFICATION OF EMERGENT NEURON NETWORK MORPHOLOGY IN VITRO

Cassandra Hoffmann, *University of Melbourne, Australia*
Simon Maksour, *University of Wollongong, Australia*
Mirella Dottori, *University of Wollongong, Australia*
Ellie Cho, Biological Optical Microscopy Platform, *University of Melbourne, Australia*
Andrew Zalesky, *University of Melbourne, Australia*
Maria A. Di Biase, *University of Melbourne, Australia*

Throughout brain development, neural stem cells self-organise into refined networks, establishing the infrastructure for efficient neuronal communication. Here, we established an in vitro platform to study the formation and perturbation of these networks using a live-cell imaging protocol and bioimage analysis pipeline. Cortical neurons, derived from human embryonic stem cells via Neurogenin-2 and green fluorescent protein (GFP) viral transduction, were imaged over 33 days. To minimise phototoxic stress under repeated fluorescence imaging, we optimised culture conditions by testing three factors: extracellular matrix (human- or murine-derived laminin), culture media (Neurobasal or Brainphys Imaging media), and seeding density (1×105 or 2×105 cells per cm2). Brainphys Imaging medium was observed to support cell survival to a greater extent than Neurobasal medium with either laminin type, as quantified by PrestoBlue viability assay ($p = 0.0052$) and neurite outgrowth analysis ($p < 0.0001$). However, a combination of Neurobasal medium and human laminin detrimentally impacted neurite outgrowth ($p < 0.0001$). Using our optimised protocol, we captured timeseries microscopy images and built a computational pipeline to evaluate multiscale network features. At global resolution, we introduced two measures of population-wide somata clustering and neurite fasciculation. At local resolution, we developed a tool that generates spatially-embedded models of neuron networks, representing cell bodies as points (network nodes) and neurite connections as links between these points (network edges). Application of this pipeline to pharmacologically-induced disease models of schizophrenia (treated with MK-801) and epilepsy (treated with kainic acid) revealed distinct topological profiles. Graph theoretical analysis demonstrated significantly reduced clustering coefficients ($p = 0.0398$) and small-world indices ($p = 0.0268$) in schizophrenia relative to epilepsy models. These analytical tools reveal, for the first time, disrupted anatomical connectivity across neuronal networks,

extending beyond standard neurite outgrowth measures to capture precise motifs in neuron organisation.

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THE CHROMATIN REMODELLER CHD7 ACTS AS A CHROMATIN HUB COORDINATING DIFFERENTIATION OF MULTIPLE CELL LINEAGES DURING MOUSE HIPPOCAMPAL DEVELOPMENT

Yassin Harim, *German Cancer Research Center (DKFZ), Germany*
Chunxuan Shao, *German Cancer Research Center (DKFZ), Germany*
Heike Alter, *German Cancer Research Center (DKFZ), Germany*
Changwen Wang, *German Cancer Research Center (DKFZ), Germany*
Giulia Di Muzio, *German Cancer Research Center (DKFZ), Germany*
Li-Chin Wang, *German Cancer Research Center (DKFZ), Germany*
Yue Zhuo, *German Cancer Research Center (DKFZ), Germany*
Gözde Bekki, *German Cancer Research Center (DKFZ), Germany*
Asya Sayin, *German Cancer Research Center (DKFZ), Germany*
Nadja Stöffler, *German Cancer Research Center (DKFZ), Germany*
Katharina Hartmann, *German Cancer Research Center (DKFZ), Germany*
Anna Neuerburg, *German Cancer Research Center (DKFZ), Germany*
Pei-Chi Wei, *German Cancer Research Center (DKFZ), Germany*
Weijun Feng, *Children's Hospital of Fudan University, China*
Hai-Kun Liu, *Children's Hospital of Fudan University, China*

Chromatin remodelling is a crucial process in development, controlling gene expression during organogenesis and cell differentiation. Mutations of chromatin remodellers are thus often implicated in neurodevelopmental disorders (NDDs) such as CHARGE syndrome, which results from a heterozygous mutation of the chromatin remodeller CHD7. Some CHARGE symptoms such as learning and memory deficits suggest a link to the forebrain, specifically to the hippocampus. However, the molecular role of CHD7 there remains unknown. To address this point, we used a dorsal forebrain-specific Chd7 mutant mouse model, which also targets the hippocampus, to investigate forebrain development in absence of Chd7. Upon Chd7 ablation, the adult hippocampus displays a patterning defect, manifesting in a 41% reduction in size and partial morphological disorganisation. Subsequent integrative analysis of scRNA- and scATAC-seq as well as histological data of the developing mouse brain suggests that Chd7 is essential for migration and maturation of

multiple cell lineages during hippocampal development. Immunostainings and pseudotime analysis highlighted impaired neural progenitor migration and differentiation, with progenitor cells accumulating and partially failing to establish a mature identity in the dentate gyrus granule neuron and hippocampal pyramidal neuron lineages. Furthermore, Cajal-Retzius neurons, a transient cell population that controls cell migration and orchestrates the spatial organisation of cortex and hippocampus, are reduced in their numbers and localise aberrantly in Chd7 mutant animals. This phenotype could functionally compromise their control of neural progenitor migration. Altogether, we propose that Chd7 has a key role in activating and fine-tuning gene expression programs including maintenance of neural progenitor stemness, fate determination and cell differentiation in a lineage-specific manner. Our data support a novel concept where Chd7 not only acts in a specific developmental process, but rather is a central chromatin coordinator of multiple lineages during organogenesis. Unravelling the essential molecular networks of Chd7 will further point towards new potential approaches for CHARGE syndrome treatment and improve our understanding of epigenetically caused NDDs.

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DYSREGULATION OF mTOR SIGNALING IS A CONVERGING MECHANISM IN LISSENCEPHALY

Angeliki Louvi, *Yale School of Medicine, USA*
Ce Zhang, *Yale School of Medicine, USA*
Dan Liang, *Yale School of Medicine, USA*
Murat Gunel, *Yale School of Medicine, USA*
Kaya Bilguvar, *Yale School of Medicine, USA*

Lissencephaly (‘smooth brain’) spectrum disorders comprise a group of rare, genetically heterogeneous congenital brain malformations commonly associated with epilepsy and intellectual disability. However, the molecular mechanisms underlying disease pathogenesis remain unknown. We characterized two types of cerebral organoid derived from individuals with genetically distinct lissencephalies with a recessive mutation in p53-induced death domain protein 1 (PIDD1) or a heterozygous chromosome 17p13.3 microdeletion leading to Miller–Dieker lissencephaly syndrome (MDLS). We integrated cellular, single-cell transcriptomic and proteomic analyses to study disrupted processes that lead to lissencephaly. PIDD1-mutant organoids and MDLS organoids recapitulated the thickened cortex typical of human lissencephaly and demonstrated dysregulation of

protein translation and metabolism underlain by hypoactivity of the mTOR pathway. A brain-selective activator of mTOR complex 1 prevented and reversed cellular and molecular defects in the lissencephaly organoids. Our findings show that a converging and clinically relevant molecular mechanism contributes to two genetically distinct lissencephaly spectrum disorders.

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ALZHEIMER'S DISEASE RISK FACTOR BIN1: LINKING MICROGLIAL DEFICIENCY TO NEUROINFLAMMATION AND REGULATION OF ADULT HIPPOCAMPAL NEUROGENESIS

Irini Thanou, *Hellenic Pasteur Institute, Greece*
Maria Margariti, *Neural Stem Cells and Neuro-imaging Group, Greece*
Eirini Portokali, *Hellenic Pasteur Institute, Neural Stem Cells and Neuro-imaging Group, Greece*
Elsa Papadimitriou, *Hellenic Institute Pasteur, Neural Stem Cells and Neuro-imaging Group, Greece*
Evangelia Xingi, *Hellenic Institute Pasteur, Greece*
Maritsa Margaroni, *Hellenic Pasteur Institute, Immunology of Infection Laboratory, Greece*
Marcos Costa, *Institut Pasteur de Lille, Univ. Lille, Inserm, Greece*
Dimitra Thomaidou, *Hellenic Pasteur Institute, Neural Stem Cells and Neuro-imaging Group, Greece*

Bridging Integrator 1 (BIN1) is the second major genetic risk factor for Late-Onset Alzheimer's Disease (LOAD) identified through GWAS. While BIN1's role in neuronal functions is well-documented, its role in microglia remains underexplored. Given the central role of microglia in neuroinflammation and Adult Hippocampal Neurogenesis (AHN)—both key aspects of Alzheimer's pathology—studying BIN1 in this context may shed light on disease progression. To address this, we used a conditional double transgenic mouse model with microglia-specific BIN1 knockout to investigate their role in hippocampal function. Consistent with snRNA-Seq data from our group on cortical samples, real-time qPCR experiments demonstrate upregulation of type I interferon pathway genes in LPS-treated hippocampi lacking microglial BIN1. This upregulation occurs alongside with a notable decrease in inflammatory chemokines and complement genes, indicating a complex interplay between interferon signaling and inflammatory

responses. Moreover, morphometric analysis of microglia revealed a hyper-ramified phenotype indicative of an intermediate microglial activation state. Furthermore, our analysis revealed that neuroinflammation in microglial BIN1cKO mice increased the percentage of proliferating microglia, although there was no overall microglial population expansion. Additionally, hippocampal microglia in BIN1cKO mice exhibited an elevated expression of the phagocytic marker CD68 under homeostatic conditions, which was further increased after LPS treatment. Interestingly, loss of microglial BIN1 under homeostatic conditions led to an increase in DCX+ neuroblasts in the Subgranular Zone, suggesting that BIN1 participates in regulating AHN. Our findings indicate that the loss of BIN1 in hippocampal microglia under inflammatory conditions promotes the expansion of proliferative and IFN-I-responsive, reactive microglial subpopulations. Additionally, microglial BIN1 deficiency independently modulates both microglial phagocytic capacity and neurogenesis, with the molecular mechanisms underlying these effects being still under investigation.

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HARNESSING CRISPR-READY IOGLUTAMATERGIC NEURONS AND IOMICROGLIA AS FUNCTIONAL GENOMICS TOOLS FOR DRUG DISCOVERY

Rebecca Northeast, *bit.bio, UK*
Lisa Grabner, *bit.bio, Austria*
Magdelana Gamperl, *bit.bio, Austria*
Barnabas Koenye, *bit.bio, Austria*
Callum Talbot-Cooper, *bit.bio, Austria*
Huriye Ceylan, *bit.bio, Austria*
Kemal Arat, *bit.bio, Austria*
Nora Kapai, *bit.bio, Austria*
Benj Klapholz, *bit.bio, Austria*
Libby Spencer, *bit.bio, UK*
Germano Belli-Valletta, *bit.bio, UK*
Karl Firth, *bit.bio, UK*
Ann Byrne, *bit.bio, UK*

Manos Metzakopian, *bit.bio, UK*
Farah Patell-Socha, *bit.bio, UK*
Tillmann Buerckstuemmer, *bit.bio, Austria*
Sejla Salic-Hainzl, *bit.bio, Austria*
Mark Kotter, *bit.bio, UK*

The complexity of neurodegenerative diseases demands innovative approaches in drug discovery, particularly models that closely reflect human physiology. Traditional methods, including animal models and immortalised cell lines, often fail to replicate the nuances of human cellular behaviour. Induced pluripotent stem cells (iPSCs) offer an improved solution but come with differentiation challenges, including variability and scalability issues. These challenges are addressed with bit.bio's opti-ox™ technology, enabling rapid and deterministic programming of cell types at scale. The emergence of CRISPR-Cas9 technology has revolutionised functional genomics, offering unprecedented insights into the genetic underpinnings of diseases. In this context, bit.bio's CRISPR-Ready ioCells represent a cutting-edge functional genomics tool for CRISPR-based screening in drug discovery. These cells, including CRISPR-Ready ioGlutamatergic Neurons and CRISPR-Ready ioMicroglia, are engineered to express Cas9 and facilitate high-throughput CRISPR screening, enabling consistent, scalable and precise gene editing and functional analysis. Our data demonstrates the power of single-cell CRISPR (scCRISPR) screening workflows in these iPSC-derived cell types. Proof-of-concept screens targeting neurodegenerative disease-associated genes in CRISPR-Ready ioGlutamatergic Neurons revealed critical insights into the genetic drivers of disease. Single-cell transcriptomic analyses further identified key genes whose knockout produced significant transcriptomic changes, highlighting potential therapeutic targets. Notably, in CRISPR-Ready ioMicroglia, CRISPR knockout screens elucidated genes influencing the response to LPS stimulation, providing deeper understanding of microglial activation in neuroinflammation. By combining the precision of CRISPR technology with the robustness of opti-ox technology, we present a novel approach to drug discovery. These functional genomics tools enable detailed exploration of disease-relevant genetic networks, accelerating the identification and validation of new therapeutic targets for neurodegenerative diseases.

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EXOSOMES ISOLATED FROM INDUCED PLURIPOTENT STEM CELLS RESCUE CELLULAR PHENOTYPES AND BEHAVIORAL DEFICITS IN SHANK3 IPSC-DERIVED NEURONS AND MICE MODEL

Ashwani Choudhary, *University of Haifa, Israel*
Idan Rosh, *University of Haifa, Israel*
Yara Hussein, *University of Haifa, Israel*
Shai Netser, *University of Haifa, Israel*
Aviram Shemen, *University of Haifa, Israel*
Tagreed Suliman, *University of Haifa, Israel*
Wote Amelo Rike, *University of Haifa, Israel*
Lilach Simchi, *University of Haifa, Israel*
Boris Shklyar, *University of Haifa, Israel*
Assaf Zinger, *Israel Institute of Technology, Israel*
Daniel Offen, *Tel Aviv University, Israel*
Shlomo Wagner, *University of Haifa, Israel*
Shani Stern, *University of Haifa, Israel*

Exosomes are small extracellular vesicles secreted by various eukaryotic cells including neurons and carry heterogeneous cargoes of RNA, protein and other biomolecules. Shank3, a scaffolding protein, is critical for synaptic structure and function, particularly for the formation and maintenance of dendritic spines. Shank3 mutations are strongly implicated in autism spectrum disorder (ASD) and related neuropsychiatric conditions such as Phelan-McDermid Syndrome (PMS). Previous work has recognized early hyperexcitability in cortical neurons derived from ASD patients with various gene mutations as a common endophenotype. Here, we examined the effects of exosomes extracted from several cell types on the neurophysiological properties of human cortical neurons derived from Shank3 mutant induced pluripotent stem cells (iPSCs) as well as Shank3B-/- transgenic mice. First, we sought to understand the implications of exosome-based intercellular communication on the neurophysiology of Shank3 mutant and control neurons by switching their respective secreted exosomes in vitro. We found that while control neuron-derived exosomes do not change the neurophysiology of Shank3 neurons, the Shank3 neuron-derived exosomes transfer their early hyperexcitability and other ASD-related phenotypes to control neurons. Next, we explored the therapeutic potential of mesenchymal stem cells (MSC) and iPSC-derived exosomes from healthy donors in Shank3 mutant cortical neurons. We demonstrate that both MSC and iPSC-derived exosomes rescue the early hyperexcitability and accelerated maturation of Shank3 mutant neurons. Proteomic analysis of exosomes derived from Shank3 mutant and control neurons, as well as from exosomes derived from MSCs and iPSCs, revealed distinct protein cargoes that likely alter the neurophysiological properties of the recipient mutant neurons.

To extend our findings and the therapeutic potential of iPSC-derived exosomes, we evaluated their effects by performing intranasal administration of iPSC-derived exosomes in Shank3B-/- transgenic mice. Notably, treatment with iPSC-derived exosomes significantly ameliorated ASD-related behavioral deficits in vivo, indicating their potential as a therapeutic intervention for ASD. Our results thus emphasize the potential to alter disease course through the introduction of novel intercellular communication via exosomes derived from stem cells.

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AGING ASSESSMENT OF NEURAL CREST STEM CELL OF DENTAL ORIGIN

Zornitsa Mihaylova, *Medical University, Bulgaria*
Evgeniy Aleksiev, *Medical University, Bulgaria*
Nikolay Ishkitiev, *Medical University, Bulgaria*

The cultivation of stem cells of neural crest origin from dental pulp (DPSC), and periodontal ligament (PDLs) has unveiled their remarkable potential for proliferation and differentiation. These human DPSC, and PDLs have become recognized as reliable sources of cells with significant promise for a wide array of regenerative therapies. Yet, a substantial knowledge gap surrounds the influence of extended cultivation on the characteristics of these stem cells. Thus, the principal objective of this study is to address this gap by examining the effects of prolonged cultivation on DPSC, and PDLs, with a specific focus on identifying potential signs of cellular senescence. Healthy third molars extracted from patients was utilized to procure DPSC tissue, along with PDLs, from which stem cells were isolated and cultured under standard conditions for an extended in vitro period. The cultivation of DPSC, and PDLs spanned nearly four months, encompassing passages ranging from the initial (1st-3rd) to the intermediate (10th-12th) and concluding with the late passages (18th-20th). The harvested cells from these passages underwent a comprehensive assessment to identify any indicators of cellular aging, including evaluations of cell proliferation and apoptosis. In addition, the study delved into the examination of telomerase and beta-galactosidase enzymatic activity, which are well-established markers associated with cell senescence. Finally, an MTT test was conducted, and all cells were subjected to an assessment of HLA expression. We have effectively

isolated and expanded DPSCs, and PDLs in an in vitro setting. Our findings reveal that there is no statistically significant reduction in the proliferative capacity of stem cells during both early and late passages ($p>0.05$). We did observe a slightly elevated number of apoptotic cells in the late-passage stem cells. Nonetheless, the enzymatic activity of telomerase and beta-galactosidase remained relatively consistent in both early and late passages, with no statistically significant differences detected across our stem cell lineages. Furthermore, there were no notable variations in the expression of HLA. In conclusion, our study highlights the remarkable potential of neural crest stem cells derived from the dental pulp (DPSC), and periodontal ligament (PDL) in the context of regenerative medicine. These cells have proven to be reliable sources for various regenerative therapies. These findings affirm the resilience of DPSC, and PDL cells even after extended cultivation, positioning them as promising candidates for future regenerative therapies and tissue engineering applications. This study provides a foundation for further research and clinical exploration of these dental-derived stem cells in the field of regenerative medicine.

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SEARCHING FOR THE ORIGIN OF ADULT NEURAL STEM CELLS DURING HIPPOCAMPAL DEVELOPMENT

Aixa V. Morales, *Instituto Cajal (CSIC), Spain*
Cristina Medina-Menéndez, *Instituto Cajal (CSIC), Spain*
Lingling Li, *Instituto Cajal (CSIC), Spain*
Paula Tirado-Melendro, *Instituto Cajal (CSIC), Spain*
Pilar Rodríguez-Martín, *Instituto Cajal (CSIC), Spain*
Elena Melgarejo-de la Peña, *Instituto Cajal (CSIC), Spain*

Neural stem cells (NSCs) in the dentate gyrus (DG) enter quiescence during early postnatal development, before the adult hippocampal neurogenic niche is fully established. However, the mechanisms controlling NSC first quiescence entry and the correct level of quiescence are largely unknown. Using conditional mutant mouse during embryonic or postnatal stages, we have determined that transcription factor Sox5 is required to restrict first entry in quiescence. Moreover, we have found a critical window during the second postnatal week when NSCs build up a shallow or primed quiescent state. Loss of Sox5

leads to an excess of primed NSCs prone to activate, leading to a neurogenic burst in the adult DG and precocious depletion of the NSC pool. Mechanistically, Sox5 prevent an excess of BMP canonical signaling activation, a pathway that we have now determined is associated to NSC primed state. In conclusion, our results demonstrate that Sox5 is required to control the correct balance between primed and deep quiescence during the first postnatal weeks of DG development, a balance which is essential for establishing long-lasting adult neurogenesis.

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BIOENGINEERED 3D CORTICAL CONSTRUCTS FOR ENHANCED BRAIN REPAIR FOLLOWING TRAUMATIC BRAIN INJURY

Luana Campos Soares, *University of Oxford, UK*
Elisa Marozzi Cruz, *University of Oxford, UK*
Mona Barkat, *University of Oxford, UK*
Gretchen Greene, *University of Oxford, UK*
Marlene Lawston, *University of Oxford, UK*
Mingyu Li, *University of Oxford, UK*
Mirriam Domocos, *University of Oxford, UK*
Yufan Xu, *University of Oxford, UK*
Gabriel Moser, *University of Oxford, UK*
Pawel Swietach, *University of Oxford, UK*
Mootaz Salman, *University of Oxford, UK*
Linna Zhou, *University of Oxford, UK*
Zoltán Molnár, *University of Oxford, UK*
Francis Szele, *University of Oxford, UK*

Traumatic brain injuries (TBI) have the highest incidence of all common neurological disorders and pose a major public health burden due to the lack of effective treatments and long-term complications including disability. Recent advances in regenerative medicine and bioengineering have offered fresh hope to help facilitate recovery and improvement of the prognosis. However, achieving neuronal survival, maturation, and integration into the host tissue are among the key challenges. This study investigates the therapeutic potential of bioengineered 3D constructs designed to mimic the structure and cellular heterogeneity of the cerebral cortex. We used human neural progenitor cells (hNPCs), murine astrocytes and microfluidic-based technology to generate implantable 3D

constructs. Layer-specific hNPCs were isolated and shown to generate both upper and lower glutamatergic cortical neurons. Our bespoke microfluidic device enabled the production of laminated 3D constructs with distinct upper and lower compartments. Functional neuronal networks were confirmed in vitro through the analysis of fluorescence traces of spontaneous calcium oscillations obtained with calcium imaging. Our in vitro results also show that astrocytes are essential for neuronal recovery through increasing their viability and density while enhancing their maturation. Following implantation into the somatosensory cortex of immunocompromised mice, physiological astrocytic phenotypes were increased in astrocyte-enriched constructs. This was evidenced by increased astrocyte endfeet coupling with blood vessels and greater polarization of aquaporin-4 when compared with neurons-only constructs. Moreover, inclusion of astrocytes reduced lesion size and promoted tissue healing following TBI. In conclusion, our research highlights the importance of microfluidic technologies and astrocyte neuronal co-culture systems in developing viable 3D cortical constructs that can be used for therapeutic purposes for TBI and neurological diseases. These findings represent a significant step towards improving the efficacy of neural implants, paving the way for future clinical applications in brain repair.

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DEVELOPING HUMAN CORTICAL NEURONS TO MODEL DEVELOPMENTAL AND EPILEPTIC ENCEPHALOPATHY CAUSED BY VARIANTS IN THE STRETCH-ACTIVATED TMEM63B CHANNEL

Cristiana Pelorosso, *Meyer Children's Hospital IRCCS, Italy*
Rodolfo Tonin, *Meyer Children's Hospital IRCCS, Italy*
Amelia Morrone, *Meyer Children's Hospital IRCCS, Italy*
Valerio Conti, *Meyer Children's Hospital IRCCS, Italy*
Simona Balestrini, *Meyer Children's Hospital IRCCS, Italy*
Renzo Guerrini, *Meyer Children's Hospital IRCCS, Italy*

TMEM63A, TMEM63B, and TMEM63C constitute the TMEM63 gene family in humans and were initially identified as the closest homologs of the OSCAs (reduced hyperosmolality-induced [Ca2+]i increase) ion channels in plants. OSCA proteins sense osmotic stress-induced mechanical forces across the plasma membrane and activate a signaling pathway

responsible for regulating water transpiration and root growth. In mammalian cells, members of the TMEM63 family mediate stretch-activated cation currents in response to osmotic and mechanical stimuli affecting membrane tension, a process critical for cell volume regulation and viability. Variants in TMEM63A and TMEM63C have been recently associated with human neurodevelopmental disorders. We described a cohort of patients with severe developmental and epileptic encephalopathy (DEE) and progressive neurodegenerative brain changes carrying heterozygous variants in TMEM63B. Functional studies in transfected Neuro2A cells demonstrated inward leak cation currents across the mutated channel even in isotonic conditions and impaired Ca2+ transients generated under hypo-osmotic stimulation. To deepen our understanding of the pathophysiological mechanisms underlying the TMEM63B-associated DEE, we aimed to develop an in vitro model of patients' neural cells (neurons, oligodendrocyte precursor cells - OPC - and oligodendrocytes). By studying neurons, we will characterize the pathophysiological mechanisms underlying epilepsy. Studying OPCs and oligodendrocytes will clarify whether and what defects in one or both cell types are involved in progressive neurodegenerative brain changes (i.e. abnormal myelination) observed in our patients. We reprogrammed fibroblasts of two patients carrying TMEM63B variants into induced pluripotent stem cells (iPSC) and differentiated iPSC into neuronal progenitor cells (NPC) and mature cortical neurons. We will present unpublished data from recent and ongoing experiments aimed at characterizing cells by immunocytochemistry, electron microscopy, and electrophysiological recordings.

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PHARMACEUTICAL REJUVENATION OF AGED NEURAL STEM CELLS TO ENHANCE COGNITION IN AGE-RELATED AND ALZHEIMER'S DISEASE-ASSOCIATED COGNITIVE DECLINE

Wynnie Nguyen, *University of Southern California (USC), USA*
Jonathan Levi, *University of Southern California (USC), USA*
Mohammad Shariq, *University of Southern California (USC), USA*
Lei Peng, *Stanford University, USA*
Michael Bonaguidi, *University of Southern California (USC), USA*

Adult neural stem cells (NSCs) generate new neurons that modify existing neural circuits to support learning and memory. This process, known as adult neurogenesis, relies on NSCs transitioning from a quiescent to an activated state to enter the proliferative phase, which,

unfortunately, is compromised at early chronological age. To better target this premature aging of NSCs, our lab compared single-cell transcriptomic trajectories of NSCs in young and older mice as they shift from quiescence to activation. Our analyses revealed a gene signature that is robustly activated as young NSCs proliferate but not in aged NSCs. Using in silico screening and subsequent in vivo validation, we identified SS as a leading candidate compound that successfully reactivates this transition signature, rejuvenating NSCs in middle-aged rodents and improving cognitive performance. Building on these findings, we extended our validation to Alzheimer's disease (AD), where NSC exhaustion and neurogenesis dysregulation is exacerbated, contributing to more severe cognitive deficits. In both 5xFAD and 3xTg-AD mouse models, we observed significantly reduced NSC numbers, proliferation, and neurogenesis compared to age-matched wild-type controls. Remarkably, SS treatment via intracerebroventricular infusion for just six days significantly enhanced NSC proliferation in 5xFAD mice at both 6 and 8 months of age, which corresponds to early and advanced stages of cognitive impairment in this model. These results highlight that NSC dysfunction is accelerated in AD and demonstrate that SS effectively rejuvenates NSCs by targeting aging-related mechanisms, offering a promising therapeutic approach for AD-related cognitive decline.

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DECIPHERING THE ROLE OF P75 NEUROTROPHIN RECEPTOR IN ADULT NEUROGENESIS IN ALZHEIMER'S DISEASE: INSIGHTS FROM MOUSE AND HUMAN NEURAL STEM CELLS

Ioannis Charalampopoulos, *Medical School, University of Crete & Institute of Molecular Biology & Biotechnology (IMBB), Foundation for Research and Technology Hellas (FORTH), Greece*
Maria Anna Papadopoulou, *Medical School, University of Crete & Institute of Molecular Biology & Biotechnology (IMBB), Foundation for Research and Technology Hellas (FORTH), Greece*
Konstantina Chanoumidou, *Medical School, University of Crete, Herakl Medical School, University of Crete & Institute of Molecular Biology & Biotechnology (IMBB), Foundation for Research and Technology Hellas (FORTH), Greece*

The pan-neurotrophin p75 receptor (p75NTR), a member of the TNF death receptors superfamily, presents a pleiotropic expression in neural tissue and multifaced regulatory functions, ranging from neuronal differentiation to cell death. The receptor's altered expression profile combined with its controversial signaling, acting as pro-apoptotic and/or pro-survival mediator, makes p75NTR an appealing target in neurotherapeutics. A plethora of experimental results, have implicated p75NTR in Alzheimer's Disease (AD) pathology, while its contribution to adult hippocampal neurogenesis, which drops sharply in AD, remains poorly understood. Contradictory evidence in mouse model studies suggests either a pro- or anti-neurogenic effect of p75NTR, while its function in human neural stem cells (hNSCs) is still unknown. Based on our expertise in p75NTR signaling in neural cells, we investigated receptor's effects in rodent and human neurogenesis, under physiological and AD conditions. In order to study the adult hippocampal neurogenesis in mice, we performed BrdU injections for detection of cell proliferation, immunocytochemical analyses of key neurogenic markers using p75KO, 5xFAD and 5xFAD/p75KO mice of different ages, as well as RNAseq analysis for differential gene expression. Finally, we have generated NSCs from human iPSCs and examined the activity of p75NTR signaling in cell survival upon the addition of toxic Aβ-42 oligomers. The present study demonstrates the role of p75NTR in neurogenesis, both in mouse and human cells, emphasizing on its role in AD neurogenic deficits. Pharmacological targeting of p75NTR signaling in NSCs could enable the enhancement of the endogenous regenerative processes, thus contributing to the armamentarium against AD.

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DIFFERENT STRATEGIES TO GENERATE NEURONAL DIVERSITY IN DISTINCT NEUROGENIC REGIONS OF THE DROSOPHILA OPTIC LOBE

Rana Eldanaf, *New York University Abu Dhabi, United Arab Emirates*
Katarina Kapuralin, *University of Rijeka, Croatia*
Raghuvanshi Rajesh, *New York University, USA*
Filipe Pinto-Teixeira, *University of Toulouse, France*
Claude Desplan, *New York University, USA*

The complexity of the brain is largely due to the presence of a vast pool of neurons that perform different functions and control various behaviors. The Drosophila visual system has emerged as a model for understanding how this diversity is generated during development. In particular, the optic lobes contain ~270 well-defined neuronal types, which are produced from neural stem cells (neuroblasts-NB) emerging from two main neuroepithelial domains: the outer proliferation center (OPC) and the inner proliferation center (IPC). The optic lobe is made up of four neuropils that mediate different aspects of visual processing: lamina, medulla, lobula and lobula plate. The OPC can be divided into 8 spatial domains and gives rise to the majority of neurons in the medulla. The main IPC gives rise to neurons that project to the lobula plate or feedback neurons in the medulla. Its ventral tip (ventral surface IPC, vs-IPC) marked by Wg expression gives rise to poorly characterized neurons. The patterning mechanisms of the OPC and main IPC have been investigated in detail. They involve temporal and spatial factors patterning mechanisms to achieve neuronal diversity. However, little is known about how the vs-IPC is patterned, including the mechanisms of NB division and the neurons they produce. For example, do temporal transcription factors play a role in the neurogenesis of vs-IPC neurons? If so, are they similar to the cascades found in the OPC? Additionally, do vs-IPC neurons innervate different optic lobe regions or are they confined to a single domain? To investigate this, we used single cell RNA sequencing of FACSed neurons from a fly line in which GFP is mostly expressed in the vs-IPC at larval stages. We profiled the NBs and the identity of neurons generated from this domain and investigated the transcription factors that they express. Here, we show that the vs-IPC can be divided into at least three domains marked by the neuronal expression of Rx, Acj6 and Toy. However, NBs appear to be short-lived and to not progress through a long series of temporal factors. Among the neurons produced, we find different visual projections neurons (also known as Lobula columnar neurons -LCN). These LCNs seem to originate from at least 2 separate vs-IPC domains, the first one giving rise to LC4/ LC12/LC17, while another one giving rise to LC20. Interestingly, we found that other LCNs originate from the OPC (dorsal and ventral regions) and the central brain. Our findings add to the growing evidence of the convergence of LCNs from different neurogenic regions, and highlight the complexity of neural stem cell division and mechanisms.

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MECHANISTIC INSIGHTS INTO THE MIR-124/ISX9 INSTRUCTED DIRECT REPROGRAMMING OF MOUSE CORTICAL ASTROCYTES TO INDUCED NEURONS

Elsa Papadimitriou, *Hellenic Pasteur Institute, Greece*

Alexandra Frazeskou, *Hellenic Pasteur Institute, Greece*
Lucas daCC Iohan, *Federal University of Rio Grande do Norte, Brazil*
Marcos R. Costa, *Univ. Lille, Inserm, CHU Lille, Institut Pasteur de Lille, France*
Dimitra Thomaidou, *Hellenic Pasteur Institute, Greece*

Direct neurogenic reprogramming of astrocytes remains, despite challenges, a promising in vivo therapeutic approach aiming at alleviating neuronal loss, which is a hallmark of neurodegeneration and brain injury. To this end, conversion of astrocytes to induced neurons (iNs) has been well studied in vitro and in vivo using combinations of neurogenic transcription factors (TFs), miRNAs and chemical compounds. We have previously shown that miR-124 is an efficient driver of the astrocytic fate switch towards an immature neuronal fate in vitro and that addition of the neurogenic compound ISX9 leads to iNs' differentiation and functional maturation. We have also shown that miR-124 is potent in guiding direct conversion of reactive astrocytes to immature iNs in vivo following cortical trauma, while ISX9 administration confers a survival benefit to newly generated iNs. Along these lines, we aimed to dissect the molecular mechanisms through which miR-124 instructs the astrocytic fate switch and along with ISX9 establishes the neuronal fate. For this, we used our RNA-seq data performed in miR-124-iNs and miR-124+ISX9-iNs at day 7 of reprogramming to identify novel miR-124 direct targets with pivotal role in reprogramming. By comparing our RNA-seq data with publicly available AGO-CLIP data from mouse cortex we identified many miR-124 direct targets, among which the RNA binding protein (RBP) Zfp36l1, implicated in mRNA decay. Further analysis revealed that Zfp36l1 targets for degradation a set of neurogenic genes, among which neuronal-specific RBPs, being de-repressed by miR-124. In parallel we were interested in identifying the core TFs that instruct the reprogramming process and for this using our RNA-seq data we constructed the TF activity network established by miR-124 and further expanded by ISX9. Among the top TFs that exhibited high activity in both networks was the DNA demethylase, Tet1. Silencing of Tet1 using specific siRNAs greatly reduced reprogramming efficiency and iNs' differentiation, pointing to a central role of Tet1 in the transcriptional changes imposed by miR-124 and ISX9 during reprogramming. Concluding, in this work we have unraveled transcriptional and post-transcriptional mechanisms that instruct the direct neurogenic reprogramming of mouse cortical astrocytes by miR-124 and ISX9.

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REPROGRAMMING ADULT PORCINE ASTROCYTES INTO NEURONS IN VITRO

Eva Kamenna, *Charles University, Czech Republic*
Jiri Klima, *Institute of Animal Physiology and Genetics, Czech Republic*
Ivona Valekova, *Institute of Animal Physiology and Genetics, Czech Republic*
Jana Juhasova, *Institute of Animal Physiology and Genetics, Czech Republic*
Stefan Juhas, *Institute of Animal Physiology and Genetics, Czech Republic*
Zdenka Ellederova, *Institute of Animal Physiology and Genetics, Czech Republic*

Direct reprogramming of somatic cells into neurons holds promise for cell replacement therapies and disease modelling. While extensive research has focused on human and murine cells, only one study has reported reprogramming of porcine fibroblasts using miRNAs and factors Ascl1, and Brn2. Here, we explore the direct reprogramming of primary porcine astrocytes from adult animals, focusing on the roles of PTBP1 downregulation and combinations of pro-neural factors. Contrary to the hypothesis that PTBP1 downregulation alone can induce astrocyte-to-neuron conversion, our preliminary data show it is insufficient in adult porcine astrocytes. However, expression of Ascl1, Brn2, and Ngn2 together with PTBP1 downregulation altered astrocyte transcriptomes, inducing the expression of neuronal markers such as Tuj1, MAP2, and Syp. We further test driver factors -Ascl1, Brn2, Ngn2, Myt1L, and Dlx alongside different REST-complex modulators via siRNA/shRNA targeting PTBP1, REST, and miR-9/miR-124. Our findings could contribute to understanding PTBP1's role in reprogramming and establishing a protocol for generating neurons from porcine astrocytes. These reprogrammed neurons could model Huntington's disease in our transgenic minipig model, bypassing the challenge of isolating medium spiny neurons (MSNs) from the porcine brain. Additionally, the possibility of transplanting reprogrammed cells into the porcine brain offers a platform to study graft survival and integration in a large mammalian brain.

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THE ROLE OF FULL-LENGTH LAMININS IN ENHANCING IN VITRO CELLULAR MICROENVIRONMENTS

Zhijie Xiao, *BioLamina, Sweden*

Laminins, a family of 16 distinct extracellular matrix (ECM) proteins, play an essential role in tissue formation, maintenance, and homeostasis due to their spatially and temporally specific expression. These proteins are integral to the structure of basement membranes (BM), with intact laminins being essential for healthy tissue function. Mutations in laminin-encoding genes can lead to laminin diseases, a diverse group of disorders affecting organs such as muscles, kidneys, nerves, skin, and eyes, as exemplified by Pierson syndrome and Epidermolysis bullosa. In vivo, laminins interact with integrin and non-integrin receptors, such as dystroglycan and syndecan, and bind essential growth factors (GF) with high affinity, modulating GF release kinetics. As large trimeric proteins (~800 kDa), intact laminins provide structural integrity and bioactivity critical for BM functionality. In contrast, fragmented laminins lack these properties, are not naturally produced in healthy tissues, and fail to support normal tissue homeostasis. Our study highlights the superior bioactivity of intact laminin-521 in supporting human pluripotent stem cell (PSC) culture. Compared to fragmented laminin products, full-length laminin-521 significantly enhances PSC survival, proliferation, and migration, enabling single-cell seeding without requiring ROCK inhibitors. Cells cultured on full-length laminin-521 demonstrate exceptional migratory capacity, achieving complete migration closure whereas fragmented variants reach a maximum of 50% closure. These findings highlight the importance of full-length laminin-521 in recreating natural cellular microenvironments, which is crucial for advancing PSC culture techniques, improving differentiation protocols, and refining disease modeling and gene-editing strategies.

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NEXT-GENERATION ELECTROPHYSIOLOGY FOR FUNCTIONAL CHARACTERIZATION OF HUMAN NEURAL ORGANOIDS AND ASSEMBLOIDS

Laura D'Ignazio, *MaxWell Biosystems AG, Switzerland*
Elvira Guella, *MaxWell Biosystems, Switzerland*
Simon Sennhauser, *MaxWell Biosystems, Switzerland*
Zhuoliang Li, *MaxWell Biosystems, Switzerland*
Anastasiia Tourbier, *MaxWell Biosystems, Switzerland*

Silvia Oldani, *MaxWell Biosystems, Switzerland*
Praveena Manogaran, *MaxWell Biosystems, Switzerland*
Marie E. Obien, *MaxWell Biosystems, Switzerland*

Three-dimensional neural models derived from human-induced pluripotent stem cells (hiPSCs), including organoids and assembloids, have emerged as indispensable systems for recapitulating fundamental aspects of human brain development. These models have proven critical for studying neurological disorders like Alzheimer's and Parkinson's disease. To fully understand the intricate dynamics of the neural networks within these self-organizing in-vitro cellular models, there is a need for real-time and label-free electrical activity measurement. High-density microelectrode arrays (HD-MEAs) provide a non-invasive approach to high-content electrical imaging by allowing for real-time electrophysiological recordings from a variety of electrogenic materials, such as neural organoids, assembloids, retinal or brain tissue explants. We utilized the MaxOne and MaxTwo HD-MEA platforms, each equipped with 26.400 electrodes per well, to record extracellular action potentials from various 3D neural models at multiple scales, ranging from network-level activity to single-cell and subcellular analyses. We demonstrated the flexible electrode selection for recording neural activity and how it improves the collected data's statistical power and reproducibility. Key parameters like firing rate, spike amplitude, and network burst profile were extrapolated. We used the AxonTracking Assay to trace action potential propagation along axonal branches, enabling a detailed examination of axon morphology and function, including conduction velocity, latency, axonal length, and branching patterns. This breakthrough assay allows for high-resolution investigation of disease models targeting axon initial segments, axonal branching, development, and conduction. The HD-MEA platforms' capability for targeted electrode selection improves data consistency and enables more comprehensive statistical insights. Furthermore, automated data visualization and metric extraction make these systems a robust and user-friendly choice for in-vitro disease modeling and drug testing in both acute and longitudinal studies.

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NEURAL STEM CELL QUIESCENCE UNCOUPLES THE TRANSCRIPTOME FROM THE PROTEOME

Alice Rossi, *The Francis Crick Institute, UK*
Antoine Coum, *King's College London, UK*
Stephanie Strohbuecker, *The Francis Crick Institute, UK*
Manon Madelenat, *King's College London, UK*
Fursham Hamid, *King's College London, UK*
Francois Guillemot, *The Francis Crick Institute, UK*
Rita Sousa-Nunes, *King's College London, UK*

Neural stem cells (NSCs) can exist in either a proliferative or quiescent state, which consists of reversible cell cycle arrest accompanied by low biosynthetic activity. Quiescence preserves stem cells from replicative exhaustion and protects them from various environmental insults, thus ensuring tissue homeostasis during development and into adulthood. Though only few NSCs persist post-natally, these become increasingly quiescent as a function of age, and by adulthood most of them are in this state. Due to the lack of specific markers, quiescence is difficult to study, leaving many unanswered questions surrounding it. Importantly, when acquired by cancer stem cells, quiescence makes them refractory to therapy, highlighting the clinical imperative of understanding this state. We found that nucleocytoplasmic partitioning of polyadenylated RNAs was altered in quiescence in both Drosophila and mammalian NSCs. In adult mouse NSCs, around 2000 transcripts changed their subcellular bias significantly, the majority of which became more nuclear in quiescence. Crucially, this results in the uncoupling of the transcriptome from the proteome as a result of quiescence induction. We showed that polyadenylated RNAs accumulate in nuclear speckles in quiescent NSCs, likely because nuclear-biased transcripts are preferentially enriched in purine-rich repetitive sequences bound by SR proteins. A similar mechanism, termed interstasis, has been recently independently described by our collaborators, who showed its function to be that of regulating the abundance of disordered proteins, thus preventing their condensation in potentially harmful aggregates. We propose that altered nucleocytoplasmic portioning of RNAs is a novel and underappreciated mechanism to inhibit protein translation in quiescent cells, whilst likely priming them for quick reactivation, and potentially contributing to their proteostasis.

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NUCLEAR HORMONE RECEPTOR NR2E1 IS THE KEY REGULATOR OF SELF-RENEWAL AND DIFFERENTIATION OF HUMAN NSC

Dong Eun Seo, *University of Georgia, USA*
Xingzhi Sun, *Yale University, USA*
Ke Xu, *Yale University, USA*
Brett Phelan, *Yale University, USA*
Sumner Magruder, *Yale University, USA*
Alberto Taddia, *Center for Molecular Medicine, USA*
Alexander Davis, *University of Georgia, USA*
Sawsan Mahmoud, *University of Georgia, USA*
Robert J. Woods, *University of Georgia, USA*
Adam Barb, *University of Georgia, USA*
Serge van De Pavert, *Aix-Marseille University, France*
Smita Krishnaswamy, *Yale University, USA*
Fabio R. Santori, *Center for Molecular Medicine, USA*
Natalia B. Ivanova, *University of Georgia, USA*

During brain development, neural stem cells (NSCs) are responsible for generating the proper numbers of neurons and glial cells. Defects in NSC self-renewal and differentiation have been linked to neurodevelopmental disorders which exert tremendous burden on both the patients and the society. The latest transcriptomics studies generated cellular and molecular profiles of NSCs in the developing human brain. However, functional interrogation of NSC gene networks has not yet been conducted on a significant scale. NR2E1 is a NSC-enriched transcription factor that regulates NSC maintenance in mice. In humans, mutations in the regulatory region of NR2E1 were found in patients with schizophrenia and cortical defects. To define NR2E1 functions in human brain development, we generated NR2E1-deficient (NR2E1-KO) human embryonic stem cells (hESCs) and interrogated their differentiation in several NSC models. Neurospheres derived from NR2E1-KO cells had smaller sizes and numbers and showed downregulation of cell cycle genes when compared to WT neurospheres. Reduction in size and cell numbers were also observed in cortical organoids generated from NR2E1-KO hESCs. Sc-RNA seq analyses throughout organoid development revealed that the number of proliferating NSCs was reduced in NR2E1-KO organoids, which became more pronounced as organoids matured. Moreover, NR2E1-KO organoids exhibited altered ratios of neuronal subtypes and a premature switch from neurogenesis to gliogenesis. ChIP-seq profiling in NSCs revealed critical cell cycle and NSC regulators as direct targets of NR2E1. Proximity

labeling studies identified several chromatin remodeling complexes as interaction partners of NR2E1 thus providing insights into NR2E1 target gene regulation. NR2E1 is a metabolite-regulated transcription factor. Through metabolite screening and biochemical studies, we identified metabolites of the phosphatidyl inositol pathway (PIP) as candidate ligands for NR2E1. Our work suggests that NR2E1 is a key component of the NCS regulatory network that links PIP signaling to the regulation of cell cycle entry, neurogenesis and gliogenesis during human cortical development. This work will advance the development of therapies for neurological diseases.

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PROXIMAL AND DISTAL ENHANCER CLUSTERS INDEPENDENTLY CONTRIBUTE TO SOX2 EXPRESSION AND NEURAL IDENTITY MAINTENANCE IN MOUSE NEURAL STEM AND PROGENITOR CELLS

Mariia Cherednychenko, *University of Toronto, Canada*
Jennifer A. Mitchell, *University of Toronto, Canada*
Ian C. Tobias, *University of Guelph, Canada*
Sakthi D. Moorthy, *CCRM, Canada*

Transcription factor SOX2 is essential for early nervous system development and adult neural stem cell maintenance in mammals. Previous data shows that Sox2 expression is maintained in a time- and tissue-type-specific manner by different enhancer sequences. Recently, a proximal enhancer cluster Sox2 regulatory regions 2-18 (SRR2-18) was identified, located 2-18 kb downstream from the Sox2 gene. These enhancers act coordinately as a cis-regulator to control Sox2 allelic dosage during neural differentiation and maintain a more anterior regional identity of mouse neural stem and progenitor cells (mNSPCs). Another putative Sox2 enhancer region, active in mNSPCs, termed the Distal Neural Enhancer (DNE) is located over 400 kb downstream of Sox2 and was also shown to play a significant role in Sox2 expression in mNSPCs. RNA-sequencing analysis of mNSPCs with a homozygous deletion of SRR2-18 or DNE displayed both Sox2 and global transcriptome disruption. ΔDNE-/- clones demonstrated an 80% loss of Sox2 expression whereas ΔSRR2-18-/- shows only a 30% loss of Sox2 expression compared to wild-type mNSPCs. Globally, while the two enhancer deletions share 219 downregulated and 799 upregulated genes compared to the wild-type control, the ΔDNE-/- clones had 1,263 additional downregulated and 1,138 upregulated genes compared to the ΔSRR2-18-/-, likely due to the lower Sox2 levels in ΔDNE-/- cells. Gene ontology analysis of the common

differentially expressed genes revealed an enrichment of genes involved in anterior/posterior pattern specification, regionalization, and neural tube formation, displaying a significant activation of the Hoxb6-9 and Cdx2 genes. ΔDNE-/- clones also expressed neuromesodermal progenitor markers, in contrast to ΔSRR2-18-/- clones which did not. Together, these results show that whereas DNE and SRR2-18 both support Sox2 expression and NSPC regional identity, they differ in their transcription-driving capacity, which affects the degree to which they support cell identity. By studying Sox2 enhancers and their interplay, we can better understand the molecular mechanisms behind CNS formation and diseases that arise due to neurodevelopment disruptions.

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INVESTIGATING HIPPOCAMPAL NEURAL STEM CELL DYNAMICS IN THE HUMAN DEVELOPING BRAIN

Piero Rigo, *The Francis Crick Institute, UK*
Katherine Long, *King's College London, UK*
Francois Guillemot, *The Francis Crick Institute, UK*

Neural stem cells (NSCs) are the source of all neurons and glia in the mammalian brain, but in mice, they persist beyond birth in few brain areas, including the hippocampus. Hippocampal NSCs support neurogenesis, which reaches its highest rates around birth in rodents and declines to low levels during the first few postnatal months, as most NSCs enter quiescence, allowing for the lifelong maintenance of NSCs and neurogenesis. Conversely, there is no general agreement on whether NSCs and neurogenesis persist in the adult human hippocampus. However, human hippocampal neurogenesis rates peak around gestational week (GW) 14, raising the possibility that NSCs enter quiescence before birth. Our preliminary analysis using immunofluorescence and single cell transcriptomics suggests that a large fraction of hippocampal NSCs persists in a non-proliferative state after the GW14 peak, suggesting, by analogy with mouse hippocampal neurogenesis, the formation of a reservoir of quiescent NSCs that could support long-term postnatal neurogenesis. Moreover, when reanalysing published adult human hippocampus scRNA-Seq datasets, we found a cell cluster with a transcriptomic profile similar to that of embryonic human hippocampal NSCs. Altogether, our preliminary results are compatible

with a model whereby human hippocampal NSCs enter quiescence around mid-gestation and are maintained in adults.

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ASTROCYTE-GENERATED TRANSIENT LGE CLASS INTERNEURONS FUNCTIONALLY INTEGRATE INTO THE LESIONED STRIATUM

Giulia Nato, *University of Turin, Italy*
Marco Fogli, *University of Turin, Italy*
Nicolas Marichal, *King's College, UK*
Valentina Cerrato, *University of Turin, Italy*
Valentina Proserpio, *University of Turin, Italy*
Ilaria Ghia, *University of Turin, Italy*
Giovanni Zanotto, *University of Turin, Italy*
Matteo Bergami, *University of Cologne, Germany*
Salvatore Oliviero, *University of Turin, Italy*
Paolo Peretto, *University of Turin, Italy*
Benedikt Berninger, *King's College London, UK*
Annalisa Buffo, *University of Turin, Italy*
Federico Luzzati, *University of Turin, Italy*

In the adult brain, subsets of astrocytes generate new neurons that integrate into pre-existing circuits in two specialized niches. Outside of these niches, new neurons have been observed only in specific conditions, mostly in the striatum. In this region, neurogenesis occurs spontaneously in adult rabbits and around weaning in guinea pigs, while in mice and rats it is induced by both progressive and acute striatal degeneration. However, the fate and the functional integration of the lesion-induced neurons remain unclear. To address this issue, here we performed an in-depth molecular, morphological, and functional characterization of immature neurons induced by excitotoxic lesion in mice. We previously showed that in this model, striatal astrocytes undergo spontaneous neurogenic activation, leading to the local generation of a huge number of neuroblasts for at least six months post-lesion. Like other models of physiological and pathological striatal neurogenesis, Quinolinic acid (QA)-induced neuroblasts do not express typical markers of striatal neurons and have a transient lifespan. Single cell-RNA sequencing analyses indicated that these cells belong to the lateral ganglionic eminence (LGE)-derived GABAergic interneurons class and are distinct from striatal projection neurons (LGE-derived) or

striatal interneurons (MGE-derived). Through the analysis of public datasets, we demonstrate that cells of this class transiently populate the brain during development, and that adult cortical and striatal astrocytes retain the potential to generate them. Despite their transient life, morphometric analysis of individually reconstructed neuroblasts reveals that these cells often attain complex morphologies with long dendrites and variable amounts of dendritic spines. Similarly to other LGE-derived interneurons, they are axonless. Electrophysiological recordings in acute brain slices showed that many of these locally-generated cells acquired membrane properties typical of immature neurons, displaying transient inward currents and generating single action potentials in response to depolarizing current steps. A few cells were further able to generate action potentials repetitively. Interestingly, many of the recorded neuroblasts received spontaneous excitatory synaptic inputs while only the cells with more mature features also received inhibitory postsynaptic currents. Rabies virus-based monosynaptic tracing confirmed the presence of excitatory inputs to these neuroblasts originating from various cortical and thalamic areas, particularly those projecting to the medial striatum. Thus, astrocyte-generated neurons in the striatum do not replace degenerated cells but still transiently integrate into pre-existing circuitry, thereby potentially contributing to post-lesion network plasticity supporting functional recovery after damage.

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PANCREATIC CANCER-ASSOCIATED DEPRESSION (PCAD) IS LINKED TO ADULT NEUROGENESIS IMPAIRMENT

Dimitrios Troumpoukis, *Biomedical Research Foundation of Academy of Athens, Greece*
Adriana Papadimitropoulou, *Biomedical Research Foundation of Academy of Athens, Greece*
Paraskevi Kogionou, *Biomedical Research Foundation of Academy of Athens, Greece*
Chrysanthi Charalampous, *Biomedical Research Foundation of Academy of Athens, Greece*
Effrosyni Koronaiou, *Biomedical Research Foundation of Academy of Athens, Greece*
Alexia Polissidis, *Biomedical Research Foundation of Academy of Athens, Greece*
Nicolas Nicolaides, *National Kapodistrian University of Athens, Greece*
Yassemi Koutmani, *Biomedical Research Foundation of Academy of Athens, Greece*

Ioannis Serafimidis, *Biomedical Research Foundation of Academy of Athens, Greece*

Pancreatic Cancer (PC) is a very aggressive type of cancer, associated with a high incidence of major depression (PCAD) manifesting before formal diagnosis. Our study aims to shed light on the mechanism underpinning this correlation, by investigating how PC affects the brain, leading to depression. For this purpose, we behaviorally tested a PC mouse model, generated by orthotopically injecting human pancreatic Panc-1 cells in immunocompromised NOD-SCID mice. Our results demonstrated that these mice exhibit a depressive-like phenotype as compared to sham-operated control mice. Brain serotonin levels in the injected mice were significantly altered as shown by HPLC analysis, and this alteration was also evident in a genetic mouse model of PC (Pdx1Cre-AKrasG12D) that mimics human PC development by constitutively expressing the KrasG12D mutation specifically in the pancreas. Interestingly, both PC mouse models exhibit impaired hippocampal neurogenesis, as demonstrated by the reduced number of DCX+ cells found in the dentate gyrus of the hippocampus, compared to controls. Moreover, the pool of hippocampal neural stem cells (NSCs) was also reduced in both mouse models at a later stage of PC, as revealed by the number of GFAP+/radial glia-like NSCs suggesting an impact of PC on hippocampal neurogenesis. To assess the involvement of systemic factors on adult neurogenesis during PC progression, we isolated and cultured wild-type hippocampal NSCs in the presence of serum collected from the above PC mouse models. Exposure of NSCs to both sera caused a reduction of their proliferative activity as revealed by BrdU incorporation assay while negatively affected their survival capacity as assessed by TUNEL assay. A Luminex-based multiplex panel was used to quantify the levels of 20 major cytokines in serum samples from PC mice, and our results demonstrated significantly increased levels of specific pro-inflammatory factors, largely distinct in the two PC models used. More specifically, the levels of IL-6, MCP-1 and G-CSF were higher in NOD-SCID mice with PC, while the levels of TNFa, IL-1b, IL-1a and G-CSF were higher in Pdx1Cre-KrasG12D mice. Our findings suggest an adult neurogenesis-linked mechanism for PCAD and identifies specific pro-inflammatory factors as key components of this process.

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EXPRESSION AND FUNCTIONAL PROFILING OF DIFFERENT LINES OF HUMAN STEM CELL-DERIVED MICROGLIA

Kovacs Gergo, *Technical University of Denmark, Denmark*

Yee Ying Lim, *Technical University of Denmark, Denmark*
Marie V. Lukassen, *Technical University of Denmark, Denmark*
Kedar N. Natarajan, *India*
Lisa Riedmayr, *Technical University of Denmark, Denmark*

The proper functioning of the human brain highly depends on the neuroimmune system, with microglia serving as its primary resident immune cells. These cells are essential for maintaining homeostasis, supporting central nervous system development, and have been strongly linked to neurodegenerative disorders, including Alzheimer's Disease and Parkinson's Disease. As the study of human microglia remains challenging due to their limited availability and their non-propagative nature, human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) are valuable tools for generating human microglia models. In this study, we assess the differentiation efficiency and dynamics of multiple hESC and hiPSC lines into microglia through a macrophage intermediate by tracking morphological changes, analyzing gene expression via qPCR and FACS, and using fluorescence video microscopy to evaluate microglial functional activity. Additionally, we perform bioinformatic analysis of proteomics data across various differentiation stages to explore expression dynamics during microglial development and assess heterogeneity across the different cell lines. Our findings offer valuable insights into the molecular mechanisms governing human microglia differentiation. We observed minor variations in differentiation efficiency, and gene and protein expression across different hESC and hiPSC lines as well as more pronounced differences regarding differentiation speed and dynamics. These line-to-line variations, along with dynamic transcriptional changes during differentiation, provide important insights into the robustness of current differentiation protocols and their capacity to be applied across diverse cell lines. Furthermore, the high sensitivity of the developed multifaceted assays may help reveal key differences in the differentiation process associated with genetic backgrounds, which could be critical for identifying pathways linked to neurodegenerative diseases and for discovering potential therapeutic targets.

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MICROGLIAL TREM2 SIGNALING IN HIPSC-DERIVED MODELS OF aSYNUCLEIN-MEDIATED NEURODEGENERATION AND NEUROINFLAMMATION

Foteini Vasilopoulou, *Hellenic Pasteur Institute, Greece, and UCL Queen Square Institute of Neurology, University College London, UK*
Jennifer M. Pocock, *University College London, UK*
Era Taoufik, *Hellenic Pasteur Institute, Greece*

Parkinson's disease (PD) is considered to be caused by a combination of genetic and environmental factors that may synergistically propagate neuronal death in the diseased central nervous system (CNS). Besides the well-established αSynuclein (αSyn)-induced neurodegeneration that characterizes PD, the disease pathophysiology has been linked to aberrant microglia-regulated neuroinflammation, and there is evidence to suggest that inflammatory triggers including viral infections increase the risk of developing PD. Microglia, the immune cells of the brain, are crucial for antiviral defence and the microglial triggering receptor expressed on myeloid cells 2 (TREM2) has been shown to regulate virus replication and pathogenesis, accelerating neurodegenerative processes or mediating neuroprotection. However, whether genetic or virus-induced microglia activation and subsequent TREM2 activity alterations predispose to post-infection PD or other neurological disorders is underexplored. To address this, we generated human iPSC-derived microglia and neurons that harbour G209A, a point αSyn gene mutation that results in the expression and accumulation of pathological A53T (p.A53T), TREM2-/- and healthy cells. We then assessed how infection by human cytomegalovirus, a neurotropic virus associated with late-appearing CNS disorders, affects TREM2 activity and TREM2-dependent functions in healthy and PD patient-derived microglia and neuron-microglia co-cultures. We found that pathological aSYN expression affects microglia responsiveness to viral infection, TREM2 signalling and downstream functions including phagocytosis. We also investigated how PD microglia impact neuronal function and connectivity throughTREM2-dependent and/ or TREM2-independent mechanisms under basal or after viral infection conditions. Our data provide insights into how TREM2 regulates microglia functions and neuronal health upon viral infection in healthy and diseased CNS, extending findings to mechanisms that link infectivity with late-appearing neurodegenerative diseases.

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INVESTIGATING p.A53T ALPHA-SYNUCLEIN-INDUCED ASTROCYTIC SENESCENCE IN A PARKINSON'S DISEASE INDUCED PLURIPOTENT STEM CELL MODEL

Christina Paschou, *Hellenic Pasteur Institute, Greece*
Constantinos Sideris, *Hellenic Pasteur Institute, Greece*
Sofia Dede, *Hellenic Pasteur Institute, Greece*
Olympia Apokotou, *Hellenic Pasteur Institute, Greece*
Anastasios Kollias, *Hellenic Pasteur Institute, Greece*
Era Taoufik, *Hellenic Pasteur Institute, Greece*
Rebecca Matsas, *Hellenic Pasteur Institute, Greece*
Florentia Papastefanaki, *Hellenic Pasteur Institute, Greece*

Cellular senescence is characterized by irreversible cell cycle arrest, resistance to cell death, and a pro-inflammatory phenotype known as the senescence-associated secretory phenotype (SASP). While a normal feature of aging, senescence can also be triggered by environmental and cellular stressors. Increasing evidence suggests that senescent astrocytes contribute to neurodegenerative disorders, including Parkinson's Disease (PD). PD, the second most common neurodegenerative disorder, is characterized by α-synuclein (αSyn) aggregation and progressive loss of dopaminergic neurons in the substantia nigra pars compacta, leading to motor and non-motor symptoms. Previous studies provide evidence of senescence in both post-mortem PD brains and experimental PD models. A major genetic risk factor for early-onset and severe PD is the p.A53T mutation in αSyn (SNCAG209A). Our recent work with p.A53T-αSyn patient-derived induced pluripotent stem cells (iPSC) demonstrated that PD astrocytes exhibit pathological protein aggregation, disturbed autophagy, and deleterious effects on dopaminergic neuron health. This study investigates the link between the p.A53T mutation in astrocytes and cellular senescence, aiming to elucidate astrocytic contributions to PD and assess the potential of senotherapeutics. To this end, we compared senescence traits in PD and healthy iPSC-derived ventral midbrain astrocytes. Our results show that PD astrocytes exhibit elevated p21 mRNA levels, enlarged cell and nuclear sizes, increased lysosome content, and enhanced autofluorescence likely due to accumulation of lipofuscins, a fluorescent mixture of partially digested proteins and lipids, within lysosomes, a hallmark of senescence. These findings indicate that the p.A53T-αSyn mutation drives astrocytic senescence, potentially contributing to early PD neuropathology. Ongoing experiments aim to validate the senescent phenotype of mutant astrocytes and investigate SASP factors as potential paracrine mediators of neuronal dysfunction. Ultimately, our goal is to explore

senomorphic molecules for their ability to reverse or delay PD progression by targeting astrocytic senescence.

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FROM PHENOTYPING TO DRUG DISCOVERY OF NEDAMSS

Sara Del Rey Mateos, *Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC), Germany*
Louise Bomholtz, *Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC), Germany*
Daniel Bauersachs, *Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC), Germany*
Agnieszka Rybak-Wolf, *Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC), Germany*
Niels R. Weisbach, *Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC), Germany*
Ralf Kühn, *Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC), Germany*
Pawel Lisowski, *Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC), Germany*
Jakob J. Metzger, *Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC), Germany*

Rare diseases affect over 350 million individuals globally, yet effective treatments exist for only 5% of them. Neurodevelopmental Disorder with Regression, Abnormal Movements, Loss of Speech, and Seizures (NEDAMSS) is a rare neurodevelopmental and neurodegenerative disorder characterized by early-onset neuronal protein aggregation and/or mislocalization. NEDAMSS is caused by mutations in the Interferon Regulatory Factor 2 Binding-Like (IRF2BPL) gene, which encodes a poorly understood transcriptional regulator. To address the limited understanding of its function and role in neural disorders, we generated isogenic controls for patient- and control-derived induced pluripotent stem cell (iPSC) lines carrying IRF2BPL mutations and differentiated them into 3D forebrain

organoids. Initial single-cell transcriptomic analysis of organoids generated from mutant and corrected cell lines revealed significant differences in cellular composition and developmental pathways. Mutant organoids exhibited reduced abundance of astrocytes, glial cells, and mature neurons, along with increased neural progenitors and immature neurons. Dysregulated pathways included those involved in neurodevelopment, synaptic connectivity, extracellular matrix organization, and metabolic regulation, highlighting critical molecular and cellular defects associated with IRF2BPL mutations. To build on these findings, further analyses will be conducted on organoids generated from different IRF2BPL mutations and corresponding isogenic controls. These datasets will inform the design of high-content drug screening to identify compounds that can reverse the disease phenotypes. This work not only aims to identify potential therapies for NEDAMSS but also to establish a general pipeline for drug discovery in rare neurodevelopmental disorders, addressing critical unmet needs in rare disease therapeutics.

Funding Source: We acknowledge support from the IRF2BPL Support Group Europe, Eva Luise und Horst Köhler Stiftung, and iDream for a Cure.

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PARACRINE EFFECTS OF ASTROCYTES ON NEURONS IN AN IPSC-DERIVED PARKINSON'S DISEASE MODEL WITH THE p.A53T-ALPHA-SYNUCLEIN MUTATION

Olympia Apokotou, *Hellenic Pasteur Institute, Greece*
Eirini Dima, *Hellenic Pasteur Institute, Greece*
Christina Paschou, *Hellenic Pasteur Institute, Greece*
Sofia Dede, *Hellenic Pasteur Institute, Greece*
Anastasios Kollias, *Hellenic Pasteur Institute, Greece*
Era Taoufik, *Hellenic Pasteur Institute, Greece*
Rebecca Matsas, *Hellenic Pasteur Institute, Greece*
Florentia Papastefanaki, *Hellenic Pasteur Institute, Greece*

Parkinson's Disease (PD) is characterized by the progressive loss of midbrain dopaminergic neurons and the presence of α-synuclein (αSyn) inclusions, known as Lewy bodies and Lewy neurites. Approximately 10% of cases are linked to mutations in specific genes, such as the p.A53T-αSyn mutation. Despite intensive research on neuronal dysfunction, the role of astrocytes in PD remains underexplored. Previous work in our lab has shown that A53T PD patient induced pluripotent stem cell (iPSC)-derived astrocytes exhibit intrinsic

proteostasis defects and contribute to non-cell-autonomous neurodegeneration in direct astrocyte-neuron co-culture systems. Healthy astrocytes, by contrast, mitigate neuronal pathology by efficiently clearing αSyn aggregates. Additionally, astrocytes have been proposed to modulate neuronal autophagy and fitness in a paracrine manner. However, it remains unresolved whether astrocytes regulate selective autophagy, particularly aggrephagy, in neurons and by which mechanisms this may impact neuronal pathophysiology. To address these questions, we treated iPSC-derived dopaminergic neurons with conditioned medium from iPSC-derived ventral midbrain astrocytes. Our results revealed that treatment with healthy astrocyte-conditioned medium (H-ACM) reduced the protein and αSyn aggregate load in A53T dopamine neurons and enhanced their viability. Conversely, exposure to PD astrocyte conditioned medium (PD-ACM) significantly decreased the viability of healthy dopaminergic neurons, suggesting a neurotoxic paracrine effect of A53T astrocytes. Ongoing experiments aim to further elucidate the effects of H- or PD-ACM on neuronal function using rabies virus-based retrograde monosynaptic tracing, electrophysiological recordings in a multi-electrode array system, and Ca2+ imaging. In parallel, we are investigating the paracrine effects of healthy and PD astrocytes on both global and selective neuronal autophagy. In summary, our findings underscore the detrimental role of A53T astrocytes in PD pathology, under both contact- and non-contact conditions and highlight the neuroprotective potential of H-ACM. These results suggest that yet unidentified astrocytic paracrine mediators may represent promising therapeutic targets for PD.

Funding Source: Empeirikeion Foundation; HFRI Project 1019-DiseasePhenoTarget; GSRI project TAEDR-0535850 - Brain Precision [European Union (NextGenerationEU); National Recovery and Resilience Plan Greece 2.0].

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GUT MICROBIOME MANIPULATION REJUVENATES THE AGED MOUSE BRAIN

Caterina Gasperini, *Harvard University, USA*
Kris M. Holton, *Harvard University, USA*
Francesco Limone, *Harvard University, USA*
Mahitha Juttu, *Harvard University, USA*
Cristina C. DeMeo, *Harvard University, USA*
Richard M. Giadone, *Harvard University, USA*
Kaylee M. Wells, *Harvard University, USA*

Lee L. Rubin, *Harvard University, USA*

Brain aging is accompanied by cellular and structural changes, including reduced neurogenesis, impaired vasculature, and increased inflammation, all of which contribute to cognitive decline and increased susceptibility to neurodegenerative disease. Emerging evidence suggests that age-related shifts in gut microbiome composition negatively impact brain health through poorly understood mechanisms. Given the influence of the gut-brain axis on neurological function, understanding how microbial changes contribute to brain aging is crucial for developing novel therapeutic strategies. To address this, we investigated the effects of microbiome depletion on brain aging by administering antibiotics to aged mice. We hypothesized that attenuating age-related microbial dysbiosis would mitigate cellular and molecular hallmarks of brain aging, thereby restoring neural function. Using single-nucleus RNA sequencing and immunohistochemistry, we discovered that microbiome depletion led to broad transcriptional and structural rejuvenation across diverse brain cell types. Specifically, we observed increased neurogenesis, enhanced myelination, improved vascular integrity, and a significant reduction in neuroinflammation. Strikingly, microbiome depleted mice showed a potential improvement in hippocampal-dependent memory, providing functional evidence that supports the molecular and cellular changes observed. To further explore the molecular mechanisms underlying these effects, we performed proteomic analyses on blood plasma and brain lysates, identifying candidate factors that may mediate the rejuvenating influence of microbiome depletion. Our findings reveal that targeting the gut microbiome can profoundly influence the aging brain, suggesting a novel and promising approach for mitigating neurodegeneration and cognitive decline. By uncovering a link between microbial composition and brain health, our study highlights the potential of gut microbiome as a therapeutic target for preserving brain function and resilience in aging populations.

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REGIONALIZED INNERVATION AND NEUROTRANSMITTER REGULATION OF THE V-SVZ ADULT NEURAL STEM CELL NICHE

Eleni Makarouni, *University of Basel, Switzerland*

Alex Paul, *Rockerfeller University, USA*
Fiona Doetsch, Biozentrum, *University of Basel, Switzerland*

Neural stem cells (NSCs) within the adult ventricular-subventricular zone (V-SVZ) represent a highly diverse population, exhibiting heterogeneity in morphology, proliferative behavior and regional identity. NSC proliferation and activation can be modulated by neurotransmitters released both locally and by neurons from distant brain areas. Here, we performed an in-vitro screen using FACs purified quiescent NSCs (qNSCs), to identify signals regulating activation. Interestingly, neurotransmitters emerged as the main class of qNSC-activating signals. We found that local and long-range neurons regionally innervate the V-SVZ, suggesting that they may regulate spatially distinct pools of NSCs. Spatial transcriptomics data also revealed regionalized expression of some neurotransmitter and G-protein-coupled receptors along the rostro-caudal and dorsal-ventral axes of the V-SVZ. In vivo experiments, using cholinergic agonists resulted in increased stem cell proliferation in specific SVZ domains during homeostatic and regenerative conditions. Future work will investigate whether neurons in distinct brain regions affect the dynamics of region-specific NSC domains, their progeny, and behavior under various physiological settings.

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GENE REGULATION NETWORKS IN NEURAL DEVELOPMENT AND CANCER PROGRESSION: IDENTIFICATION OF NEW THERAPEUTIC TARGETS

Panagiotis Politis, *Biomedical Research Foundation of the Academy of Athens, Greece*
Dimitrios Gkikas, *Biomedical Research Foundation of the Academy of Athens, Greece*
Athanasios Stergiopoulos, *Biomedical Research Foundation of the Academy of Athens, Greece*
Maximilianos Elkouris, *Biomedical Research Foundation of the Academy of Athens, Greece*
Valeria Kaltezioti, *Biomedical Research Foundation of the Academy of Athens, Greece*
Daphne Antoniou, *Biomedical Research Foundation of the Academy of Athens, Greece*
Popianna Tsiortou, *Biomedical Research Foundation of the Academy of Athens, Greece*

In the developing central nervous system (CNS), the generation of cell diversity depends on precisely coordinated proliferation and differentiation decisions in neural stem cells (NSCs). These decisions are orchestrated by gene regulatory networks, and dysregulation of these networks is linked to the onset and progression of nervous system cancers. Recent evidence also highlights the importance of long non-coding RNAs (lncRNAs), a newly

discovered class of regulatory RNAs, as integral components of such networks. Elucidating the roles of these molecules in organogenesis could transform our fundamental understanding of developmental and stem cell biology. Here, we present evidence that Prox1, a homeobox transcription factor; Ariel, a lncRNA transcribed in an antiparallel manner from the Prox1 gene locus; and NR5A2, an orphan nuclear receptor, form a cross-regulatory network with essential functions in NSCs during CNS development. While the role of Prox1 as a key regulator of NSC differentiation is well established, we now demonstrate that NR5A2 and Ariel also critically influence neural development. Specifically, NR5A2 promotes cell cycle exit and neurogenesis by inducing Prox1 expression, whereas Ariel suppresses neurogenesis and favors astrogenesis in part by repressing Prox1 and NR5A2. Moreover, our findings reveal that this network is implicated in the tumorigenesis of glioblastomas and neuroblastomas. We are currently evaluating small-molecule agonists of NR5A2 as potential therapeutic agents using patient-derived cell lines and orthotopic xenograft mouse models. Altogether, these results advance our understanding of how gene regulatory networks drive NSC differentiation and identify novel targets for the treatment of CNS malignancies.

Funding Source: HFRI Call “Basic research Financing (Horizontal support of all Sciences)” under the National Recovery and Resilience Plan“Greece 2.0”funded by the European Union–NextGenerationEU(Project Number 16238).

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EFFECTS OF THE NR2E1 MUTATION ON CELLULAR AND MOLECULAR DETERMINANTS OF NEUROGENESIS

Kevin Lei, *Baylor College of Medicine/Texas Children's Hospital, USA*
Gerarda Cappuccio, *Baylor College of Medicine/Texas Children’s Hospital, USA*
Mirjana Maletic-Savatic, *Baylor College of Medicine/Texas Children’s Hospital, USA*

Nuclear receptor subfamily 2, group E, member 1 (NR2E1) is a transcription factor and master regulator of neurogenesis that governs neural stem cell (NSC) division and ensures the neuronal differentiation of progeny. NR2E1 typically associates with co-repressors to suppress downstream target genes. However, upon binding to its endogenous ligand, oleic acid, NR2E1 recruits co-activators and promotes genes responsible for NSC proliferation and neurogenesis. While in mice, loss of Nr2e1 results in hippocampal hypoplasia, impaired learning and memory, and aggressive behaviors, the functional significance of

NR2E1 and the pathological implications of its mutations in humans remain largely unknown. Our search of gnomAD, a consortium that analyzes human genetic variants, identified no individuals with NR2E1 loss-of-function variants, underscoring its critical role in human brain development. Surprisingly, using databases such as DECIPHER, which connects clinicians and researchers, we identified several patients with unique single nucleotide variants causing missense mutations in the NR2E1 coding sequence. These patients exhibited undiagnosed neurological disorders characterized by mild-to-moderate intellectual disability and behavioral issues, including aggressiveness. To examine whether these mutations cause aberrant activity of the NR2E1, we performed several biophysical and functional assays. Luciferase assays revealed that some of these mutations significantly impaired NR2E1's transcriptional activity, suggesting that the mutations disrupt NR2E1's function rather than its expression. To establish causality, we focused on a specific mutation, Ile385Thr, located within the NR2E1 ligand-binding domain. We hypothesized that this mutation compromises NR2E1 function, impairing neurogenesis, learning and memory, and mood regulation. Biophysical assays confirmed that Ile385Thr significantly reduces NR2E1's binding to its co-repressors, thereby affecting its transcriptional activity. To explore its physiological effects, we generated a Nr2e1 Ile385Thr knock-in mouse model. Mutant mice exhibited increased expression of GFAP, an NR2E1 target gene and astrocytic marker, along with a slight reduction in NSC proliferation. While young adult mutant mice displayed no overt phenotypes, older (8-month-old) homozygous mutants exhibited a fearless or less anxious behavioral phenotype, consistent with findings from previously published Nr2e1 knockout mouse models. Our ongoing research seeks to determine whether this mutation negatively impacts hippocampal neurogenesis, cognition, and mood, thereby recapitulating the patient phenotype. This study will advance our understanding of NR2E1's role in human pathology and guide the development of targeted therapies to restore NR2E1 function and promote hippocampal neurogenesis.

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SEX CHROMOSOME OVERDOSAGE ALTERS EPIGENOMIC AND TRANSCRIPTOMIC LANDSCAPES OF NEURAL STEM CELLS AND IMPAIRS THEIR NEUROGENIC POTENTIAL

Antonio Adamo, *King Abdullah University of Science and Technology (KAUST), Saudi Arabia*
Veronica Astro, *King Abdullah University of Science and Technology (KAUST), Saudi Arabia*
Kelly J. Cardona-Londoño, *King Abdullah University of Science and Technology (KAUST), Saudi Arabia*

Lorena V Cortes-Medina, *King Abdullah University of Science and Technology (KAUST), Saudi Arabia*
Rwan Alghamdi, *King Abdullah University of Science and Technology (KAUST), Saudi Arabia*
Jair Dilme' Capo', Sequentia Biotech, Spain
Santiago Radio, *Sequentia Biotech, Spain*

Sex chromosome overdosage in Klinefelter Syndrome (KS; 47,XXY), Jacobs Syndrome (JS; 47,XYY), and high-grade sex chromosome aneuploidies (SCAs; 48,XXXY and 49,XXXXY) results in a spectrum of clinical manifestations, including intellectual disabilities and delays in motor, speech, and language development. The severity of neurological symptoms correlates with the number of supernumerary sex chromosomes; however, the in vitro modeling of these diseases remains largely unexplored. In this study, we employed an induced pluripotent stem cell (iPSC)-based approach to elucidate the effects of sex chromosome aneuploidies on early neurodevelopment. Differentiation of iPSCs from individuals with KS, JS, high-grade SCAs, and 46,XY controls into neural stem cells (NSCs) and neurons revealed X chromosome dosage-sensitive impairments in NSC differentiation and survival. Neurons derived from high-grade SCAs exhibited reduced neurite branching, diminished number of MAP2-positive dendrites, and failed to establish functional networks. Integrated methylation and transcriptomic profiling of NSCs demonstrated widespread epigenomic and transcriptomic alterations in X aneuploidies, characterized by X-linked hypermethylation proportional to X chromosome dosage. In contrast, Y chromosome aneuploidy was associated with subtle epigenomic changes. We quantified gene expression changes across X and Y chromosome complements using linear regression modeling. Our analysis uncovered modular transcriptomic alterations specific to X or Y chromosomes, with ~30% of these changes shared between KS and JS. Notably, a subset of X-linked genes escaping X-inactivation, located in the pseudoautosomal (PAR) regions of X and Y chromosomes, defined both X and Y transcriptomic signatures and may contribute to the overlapping neurodevelopmental deficits observed in SCAs. To further dissect the molecular mechanisms, we developed 46,XY NSCs overexpressing individual candidate PAR genes, identifying their roles in transcriptomic dysregulation associated with aneuploidies. Additionally, we created an open-access, web-based platform to facilitate exploration of the epigenetic and transcriptomic landscapes of SCAs, offering a valuable resource for investigating the genetic basis of X and Y overdosage during early neurodevelopment.

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POSTER SESSION 2: 6:25 PM – 7:15 PM

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INHIBITING THE DIFFERENTIATION OF RADIAL GLIA NEURAL STEM CELLS TOWARDS CONTINUING NEUROGENESIS BEYOND ITS NORMAL END

Anthi C. Krontira, *Helmholtz Center Munich, Germany*
Magdalena Götz, *Helmholtz Center Munich, Germany*

In most brain regions in mammals, neurogenesis ends around birth, transitioning to gliogenesis. However, a few regions retain adult neural stem cells and continue neurogenesis. As these regions differ among species, this is a developmentally regulated trait. One such brain region, where neurogenesis ends at birth, is the murine cerebral cortex, uniquely responsible for producing glutamatergic projection neurons. Thus, glutamatergic projection neurons lost during injury or disease are not replaced by endogenous means. Here, we aim at prolonging cortical neurogenesis postnatally. To do so we target the differentiation of radial glia cells (RGCs), the neural stem cells (NSCs) of the developing brain, to ependymal cells, the last step in their differentiation to gliogenesis. We use in vivo CRISPR knock-outs of the main drivers of the ependymal lineage, Gmnc, McIdas, Prdm16 and Trp73, via in utero electroporations. Excitingly, this shows that we can maintain cells with characteristics of NSCs in the cortex at postnatal day 4 (P4), without affecting developmental neurogenesis. These cells express PAX6 and BLBP, two markers of cortical, neurogenic NSCs. While there is a reduction in ependymal cells, this does not result in hydrocephalus due to the focused editions in a subset of the cells of the ventricular zone. Molecular analysis of the persisting NSCs will be presented as well as how to expand their neurogenic capacity if needed, using an orthogonal approach of simultaneous CRISPR-knockouts and CRISPR-activations in the same cells in vivo. Maintaining a neurogenic NSC population in the cerebral cortex could be a valuable avenue for neuronal replacement therapy for the frequent injuries in this region.

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AN INNOVATIVE APPROACH FOR CONDUCTING 3D ELECTROPHYSIOLOGICAL RECORDINGS WITHIN INTACT ORGANOIDS

Bastian Roth, *Multi Channel Systems, Germany*
Tom Stumpp, *NMI Natural and Medical Sciences Institute at the University of Tübingen, Germany*
Sara Mirsadeghi, *Department of Neuroscience, Developmental and Regenerative Biology, The University of Texas at San Antonio, USA*
Michael Mierzejewski, *NMI Natural and Medical Sciences Institute at the University of Tübingen, Germany*
Angelika Stumpf, *NMI Natural and Medical Sciences Institute at the University of Tübingen, Germany*
Haein Chang, *NMI Natural and Medical Sciences Institute at the University of Tübingen, Germany*
Udo Kraushaar, *NMI Natural and Medical Sciences Institute at the University of Tübingen, Germany*
Ali Hosseini, *Neuroscience Area, International School of Advanced Studies, Italy*
Michele Giugliano, *Neuroscience Area, International School of Advanced Studies, Italy*
Jenny Hsieh, *Department of Neuroscience, Developmental and Regenerative Biology, The University of Texas at San Antonio, USA*
Peter D. Jones, *NMI Natural and Medical Sciences Institute at the University of Tübingen, Germany*

Organoid technology is a pivotal tool for exploring human physiology and diseases. Despite its potential, current readout capabilities constrain organoid electrophysiological research. Classical microelectrode arrays (MEA) fall short in capturing data from intact organoids, which may flatten in the 2D-MEA surface, jeopardizing physiological responses and data validity. To overcome this, we pioneered a mesh MEA, reducing morphological deformations, fostering 3D growth, and facilitating electrical activity recording within intact organoids over an extended period. Electrophysiological recordings of human brain organoids were performed in an MEA-2100 headstage from MultiChannel Systems, accommodating classical MEA and mesh MEA chips. Extracellular neural activity, sampled at 25 kHz and filtered at 400 Hz for spike detection, accurately reflected action potential events on the membrane. Neuronal migration around the mesh was monitored using light microscopy. The mesh MEA integrates 60 titanium nitride electrodes (30 µm diameter) at the nodes of a 2D polymer mesh with a pitch of 200 µm and filament width of ~20 µm and thickness of ~10 µm. The mesh scaffold is suspended 2 mm from the bottom of the well. From preliminary measurements, spike time analysis revealed heightened activity after seven days on the mesh MEA (mean firing rate 34 Hz) compared to acute recordings on a classical MEA (5 Hz). Microscopy images illustrated neuronal migration, dendritic growth,

and axon development around the mesh structure and electrodes. These findings suggest that the mesh MEA holds great promise for comprehensive, long-term organoid electrophysiological studies, providing deeper insights into human functions and disorders.

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NEUROTOXICITY PLATFORM DEVELOPMENT OF HUMAN IPSC-DERIVED NEURONAL CULTURES

Anouk Spruit, *Leiden University Medical Centre/Dutch Center for RNA Therapeutics, Netherlands*
M. C. Lauffer, *Dutch Center for RNA Therapeutics/Leiden University Medical Center (LUMC), Netherlands*
W. M.C. van Roon-mom, *Dutch Center for RNA Therapeutics/ Leiden University Medical Center (LUMC), Netherlands*
A. M. Aartsma-Rus, *Dutch Center for RNA Therapeutics/Leiden University Medical Center (LUMC), Netherlands*

Predicting drug-induced neurotoxicity in humans from pre-clinical studies is challenging. However, screening for neurotoxicity is essential in early drug development. Standardized, reproducible, and high-throughput testing platforms are critical for improving translatability, particularly for time-sensitive applications like N=1 treatments. The Dutch Center for RNA Therapeutics (DCRT) is a consortium that develops individualized RNA therapies for patients with nano-rare (N=1+) disorders of the brain and eye. To promptly validate potential lead candidate compounds, we are developing a human induced pluripotent stem cell (hiPSC)-derived platform to determine in vitro neurotoxicity. Here, we present our platform that makes use of lentiviral TET-on expression systems to generate both hiPSC-derived neurons and astrocytes. Overexpression of NGN2-ASCL1 generates iNeurons, while overexpression of NFIB-SOX9 generates iAstrocytes. This system allows us to generate a controlled co-culture of both iNeurons and iAstrocytes. With quick turnaround times in generating these co-cultures, we will be able to screen for drug-induced neurotoxicity for a variety of compounds, starting with antisense oligonucleotides for the DCRT. To this date, we have successfully converted multiple control iPSC lines into iNeurons. Preliminary data illustrates that we are able to generate iNeurons by cell type specific morphology and expression of characteristic markers. The establishment of iAstrocytes is ongoing. After successful differentiation of iAstrocytes and iNeurons, the

correct ratio of these cell types needs to be established. Neurotoxicity assays that we will use on this platform will include neural network activity, neurite outgrowth and cellular viability. The platform can later be advanced with the additional CNS-cell types like microglia and oligodendrocytes.

Funding Source: Leiden University Medical Center (LUMC), Human Genetics (HG) department.

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GENERATION OF HUMAN CENTRAL NORADRENERGIC NEURONS IN BRAINSTEM ORGANOIDS

Rosario Sanchez-Pernaute, *Biobizkaia Health Research Institute, Spain*
Silvia Iniguez, *Biobizkaia Health Research Institute, Spain*
Marina Ozalla, *Biobizkaia Health Research Institute, Spain*
Soledad Romero, *Biobizkaia Health Research Institute/ University of the Basque Country, Spain*

The loss of central noradrenergic (NA) neurons occurs early in several neurodegenerative diseases such as Parkinson disease (PD), Alzheimer disease and multiple system atrophy, and it is related to cognitive, autonomic and sleep symptoms. The majority of NA neurons in the human brain are located in the Locus Coeruleus (LC), a small nucleus located in the brainstem, that originates dorsally in the first rhombomer and migrates to occupy the lateral part of the 4th ventricle floor. These neurons are born very early during development and innervate broad areas of the forebrain and spinal cord, regulating stress response, arousal, attention and sleep. Notwithstanding, there is a lack of human cellular models and only recently NA neurons have been generated from human iPSC (Tao et al 2024), partly because in contrast to mouse cells, BMPs inhibit the NA fate in human hindbrain progenitors while Activin A promotes it. We have adapted a protocol for 3D models implementing a sequential inductive protocol to generate hindbrain organoids combining small molecules to inhibit SMAD, Wnt1 and BMPs followed by graded exposure to Activin A to induce the expression of PHOX2B, a master regulator for noradrenergic fate, in organoids from different iPSC lines. These cells express other markers of NA neurons like DBH (dopamine beta hydroxylase) and ADRA1A (adrenergic receptor 1A) and show the expected developmental downregulation in the expression of PHOX2B and PAX7. We have also tested the effect of different extracellular matrices in the efficiency of organoid formation

and induction of LC neurons finding no benefit of Matrigel embedding. We are now examining the maturation profile of these neurons and evaluating differences in organoids derived from genetic PD cell lines and their isogenic controls to investigate early events in the pathogenesis of the disease.

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MODELING GLIOMA INTRATUMORAL HETEROGENEITY WITH PRIMARY HUMAN NEURAL STEM AND PROGENITOR CELLS

Daniel Gao, *Stanford Institute for Stem Cell Biology and Regenerative Medicine, USA*
Daniel Liu, *Stanford Institute for Stem Cell Biology and Regenerative Medicine, USA*
Anna Eastman, *Stanford Institute for Stem Cell Biology and Regenerative Medicine, USA*
Nicole Womack, *Stanford Institute for Stem Cell Biology and Regenerative Medicine, USA*
Benjamin Ohene-Gambill, *Stanford Institute for Stem Cell Biology and Regenerative Medicine, USA*

Gliomas are primary tumors of the central nervous system that are notorious for their significant intratumoral heterogeneity, which is believed to drive therapy resistance. The cells making up these tumors have been observed to mimic a neural stem cell hierarchy reminiscent of normal brain development. However, how intratumoral heterogeneity is shaped by cell-of-origin and different driver mutations is still not fully understood. Here, we develop a model of glioma initiation using neural stem and progenitor cells (NSPCs) purified from midgestational human brain tissue. We previously described a method to prospectively isolate and culture tripotent neural stem cells (NSCs), bipotent glial progenitor cells (GPCs), and unipotent oligodendrocyte precursor cells (OPCs). We transduced these isogenic lines with dominant-negative TP53R175H and NF1 knockdown, a commonly used genetic model of GBM in mice. These reprogrammed lines robustly engrafted when transplanted into the brains of immunodeficient mice, and showed aggressive invasive properties and expansion over time. We find that compared to NSC- and GPC-derived lines, OPC-derived tumors harbor a higher proportion of differentiated oligodendrocyte-like cells, reminiscent of low grade oligodendrogliomas. We furthermore generate NSC lines with TP53R175H plus either CDK4, EGFR, or PDGFRA overexpression, and find that while CDK4 expectedly drives a neuron-like subtype, EGFR and PDGFRA actually drive a GPC-like subtype. Our platform is highly adaptable and allows for modular

and systematic interrogation of how cell-of-origin and specific driver mutations shape the tumor landscape.

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TATTON-BROWN-RAHMAN-SYNDROME ASSOCIATED MUTATIONS IN DNMT3A DE-REPRESS CORTICAL INTERNEURON MATURATION CAUSING NEURONAL NETWORK DYSFUNCTION

Gareth D. Chapman, *Washington University in St. Louis, USA*
Julianna Determan, *Washington University in St. Louis, USA*
John R. Edwards, *Washington University in St. Louis, USA*
James E. Huettner, *Washington University in St. Louis, USA*
Haley D. Jetter, *Washington University in St. Louis, USA*
Sydney Crump, *Washington University in St. Louis, USA*
Timothy Ley, *Washington University in St. Louis, USA*
Harrison Gabel, *Washington University in St. Louis, USA*
Kristen Kroll, *Washington University in St. Louis, USA*

Mutations in several related chromatin modifying proteins cause both brain overgrowth and intellectual disability, presenting a unique opportunity to understand the mechanisms underpinning the etiology of these phenotypes. A characteristic example is Tatton-Brown-Rahman-Syndrome (TBRS), which results from mutation of the DNA methyltransferase DNMT3A, and involves overgrowth of somatic tissues including the brain and intellectual disability (ID). Despite evidence that DNMT3A plays critical roles in human development and the significance of DNMT3A mutations to human disease, requirements for DNMT3A activity during human cortical development and their disruption to cause TBRS remain undefined. Using human pluripotent stem cell models of TBRS, we examined how TBRS-associated DNMT3A mutations alter GABAergic and glutamatergic cortical neuron development. We identified loss of repressive DNA methylation across TBRS models specific to both neuronal cell types and developmental time points. Ultimately, we define GABAergic cortical interneuron development as particularly sensitive to TBRS-associated mutation, which causes persistent up-regulation of genes associated with neuronal maturation, beginning during neuronal precursor cell specification. Ultimately, this epigenetic dysregulation causes the early onset of GABAergic neuron maturation resulting in neuronal hyperactivity and precocious neuronal network formation. By contrast, during glutamatergic neuron development, TBRS-associated DNMT3A mutation had more muted

consequences, in part due to compensatory increases in repressive histone H3 lysine 27 tri-methylation. This caused TBRS-associated transcriptomic changes consistent with a failure to repress early neurodevelopmental gene expression programs but had little effect on glutamatergic neuron maturation or function. Together, this work provides insight into requirements for DNMT3A in human cortical development, defines how pathogenic mutations perturb neurodevelopment to cause TBRS, and identifies GABAergic neurons as a key driver of neuronal network dysfunction associated with TBRS related ID.

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UNRAVELING STRESS RESPONSE PATHWAYS IN HUMAN ASTROCYTES: A MULTI-MODEL APPROACH

Tim Schäfer, *Karolinska Institutet, Sweden*
Aikaterini Zilaki, *Karolinska Institutet, Sweden*
Dominic Kaul, *University of Wollongong, Australia*
Anthi C. Krontira, *Helmholtz Zentrum München, Germany*
Christina Kyrousi, *National and Kapodistrian University of Athens, Greece*
Darina Czamara, *Max Planck Institute of Psychiatry, Germany*
Silvia Capello, *LMU Munich, Germany*
Elisabeth Binder, *Max Planck Institute of Psychiatry, Germany*
Cristiana Cruceanu, *Karolinska Institutet, Sweden*

Astrocytes are the most abundant non-neuronal cell type in the human brain and play a key role in stress response. Despite robust evidence from in vitro and in vivo rodent models demonstrating a link between stress-induced astrocyte dysfunction and psychiatric phenotypes, such evidence in human systems remains limited. This is partly due to challenges in isolating developing human astrocytes, and the quantity of astrocyte subtypes present in humans. Subsequently, the derivation methods can vary greatly. The aim of this project is to utilize a selection of in vitro cell culture systems to identify robust pathway responses to test stress paradigms in astrocytes that are consistently replicated across multiple in vitro systems. In the first model, human-induced pluripotent stem cells (hiPSCs) were used to generate unguided neural organoids. Following 8-15 months in culture, the organoids were dissociated and selected to form a 2D astrocyte culture. In a second model, hiPSC-derived neural progenitors were transduced with a lentiviral vector system to overexpress SOX9 and NFIB, to induce astrocytes. A third model system employed primary human fetal-derived astrocyte cells, while a fourth model used U87

glioblastoma cells. All four cell culture systems were exposed to acute and chronic stress paradigms, using physiologically relevant concentrations of cortisol and dexamethasone (a synthetic cortisol analogue). Gene expression changes were assessed using bulk-RNA sequencing and qPCR analyses. The results showed robust gene expression changes in pathways involved in stress response, such as FKBP5 and TSC22D3, and also identified some new genes of interest, all of which are relevant for neuropsychiatric disorders, including SEMA3A, GLUL, BDNF, and PER1. By utilizing a comprehensive array of stem cell-, primary cell-, and cancer cell-derived human astrocyte culture models, we demonstrated robust gene expression changes following stress response activation via cortisol and dexamethasone. These findings lay a foundation for using human-derived stem cell systems to investigate complex cellular responses to psychological stress indicators. This approach aims to enhance our understanding of molecular pathways involved in the development of psychiatric disorders from the earliest stages of brain development.

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A VERSATILE TOOLBOX OF HUMAN IPSC-DERIVED MICROGLIA FOR DISEASE MODELLING, CRISPR SCREENS, AND MULTICELLULAR IN VITRO MODELS FOR NEURODEGENERATION DRUG DISCOVERY

Rebecca Northeast, *bit bio, UK*
Camilla Fairbairn, *bit.bio, UK*
Phillipa Barton, *bit.bio, UK*
Euan Yates, *bit.bio, UK*
Alessandro Abram, *bit.bio, UK*
Richard Hickman, *bit.bio, UK*
Hannah Garnett, *bit.bio, UK*
Lukasz Kowalksi, *bit.bio, UK*
Stefan Milde, *bit.bio, UK*
Magdalena Gamperl, *bit.bio, UK*
Barnabás Könye, *bit.bio, Austria*
Sejla Salic-Hainzl, *bit.bio, Austria*
Samir-Pierre Barbaria, *bit.bio, Austria*
Tilmannn Buerckstuemmer, *bit.bio, Austria*
Farah Patell-Socha, *bit.bio, UK*
Karl Firth, *bit.bio, UK*
Will Bernard, *bit.bio, UK*

Manos Metzakopian, *bit.bio, UK*
Mark Kotter, *bit.bio, UK*

Microglia, the resident macrophages of the brain, play critical roles in neural function by regulating neurogenesis, synaptic remodelling, and serving as first responders to infection. They are also highly implicated in the pathology of neurodegenerative diseases, including Alzheimer's disease (AD). To advance drug discovery efforts in complex diseases such as AD, scientists need a diverse toolkit for advanced research applications to model disease, generate gene knockouts and track cells in co-culture. Using opti-ox™, a deterministic cell programming technology, we have successfully generated human-induced pluripotent stem cell (iPSC)-derived microglia from both male and female genetic backgrounds in a consistent and scalable manner. These derived microglia express key markers, including CD45, P2RY12, CD11b, CD14, IBA1, and TREM2. Functionally, both male- and female-derived microglia exhibit robust phagocytic activity and secrete pro-inflammatory cytokines, however, background-specific responses are observed. To provide new models for investigating mechanisms involved in neurodegeneration, we engineered ioMicroglia in the male genetic background with specific AD-relevant mutations. These include point mutations in TREM2 (R47H) and APOE (C112R), the latter of which exhibits a phagocytic phenotype associated with AD. Developing CRISPR-compatible iPSC-derived cells has traditionally been a lengthy and complex process. To address this, we developed CRISPR-Ready ioMicroglia, which constitutively express Cas9, enabling high-throughput CRISPR screening and significantly reducing workflow duration from months to days. Proof-of-concept experiments validate the functionality of this system, demonstrating efficient single-gene knockouts and arrayed CRISPR screens. To support the development of complex multicellular neurobiology models, we created GFP ioMicroglia, which constitutively express GFP throughout the cytosol for live-cell imaging, antibody-free cell sorting, and cell tracking in co-cultures. These microglia were successfully co-cultured with ioGlutamatergic Neurons and evaluated using live-cell imaging assays. In summary, opti-ox-mediated deterministic programming enables the generation of iPSC-derived microglia from diverse genetic backgrounds and serves as a versatile platform for disease modelling and the development of advanced co-culture systems.

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LAB LIFE, SEASONS AND CHROMOSOME FUSIONS RESTRICT NON-CELL-AUTONOMOUSLY PROLIFERATION AND NEUROGENESIS, BUT NOT OLIGODENDROGENESIS, IN MICE AND VOLES

Athanasia Rapti, *National and Kapodistrian University of Athens, Greece*
Theodosia Androutsopoulou, *University of Patras, Greece*
Evangelia Andreopoulou, *University of Patras, Greece*
Maria Mellou, *University of Patras, Greece*
Georgios Leventakos, *University of Patras, Greece*
Maria Anesti, *University of Patras, Greece*
Konstantina Mastori, *University of Patras, Greece*
Myrto Chatzopoulou, *University of Patras, Greece*
Paraskevi Smyrli, *University of Patras, Greece*
Georgios P. Mitsainas, *University of Patras, Greece*
Ilias Kazanis, *University of Westminister, UK*

Environmental and behavioral factors have been shown, in experimental settings, to affect neurogenesis in the mouse brain. We found that the density of proliferating neural stem/progenitor cells (NSPCs) and of neuroblasts was significantly lower in the Subependymal Zone stem cell niche of lab mice when compared with mice and pine voles captured in the wild, with seasonal variation observed only in voles. Moreover, levels of proliferation and neurogenesis were found to decrease in proportion to the decrease in the numbers of chromosomes (from the typical 2n = 40 down to 2n = 26) caused by Robertsonian fusions. In contrast, oligodendroglial progenitors and microglial cells were unaffected by wildlife, seasons and chromosomal fusions. When NSPCs were grown in cultures no differences were detected, suggesting that environmental and genetic effects are mediated by non-cell-autonomous mechanisms. These “real-world” data provide a platform for the identification of systemic factors and genetic loci that control postnatal brain neurogenesis.

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IMPACT OF PROLONGED CULTIVATION ON THE BIOLOGICAL PROPERTIES OF STEM CELLS FROM APICAL PAPILLA (SCAP)

Marina Miteva, *Medical University Sofia, Bulgaria*
Kameliya V. Kercheva, *Medical University Sofia, Bulgaria*
Silvia Kalenderova-Valkova, *Medical University Sofia, Bulgaria*
Maria V. Praskova, *Medical University Sofia, Bulgaria*

Stem cells from the apical papilla (SCAP) have demonstrated considerable potential for proliferation and differentiation when cultivated under appropriate conditions. As a promising cell source for various regenerative therapies, human SCAP has attracted significant attention. However, the effects of aging on the properties of these stem cells are still not well understood. This study aims to explore the impact of long-term cultivation on SCAP and identify potential markers of cell senescence. Healthy patients' routinely extracted third molars were utilized to obtain SCAP tissue, from which stem cells were isolated and cultured under standard conditions (DMEM supplemented with 10% fetal bovine serum) for an extended period in vitro. The SCAPs were cultivated for nearly four months, spanning from the first to the twentieth passage. To identify any signs of aging, cells from early (1st-3rd), medium (10th-12th), and late (18th-20th) passages were harvested and evaluated for cell proliferation and apoptosis. Additionally, telomerase and beta-galactosidase enzymatic activity, known to be associated with cell senescence, were thoroughly investigated. Finally, an MTT test was administered. We successfully isolated and expanded SCAPs in vitro. Our results show no statistically significant decrease in the proliferative capacity of stem cells between early and late passages ($p>0.05$). While we noted a slight increase in the number of apoptotic cells in late passage stem cells, the enzymatic activity of telomerase and beta-galactosidase remained relatively consistent across both early and late passages, with no significant differences observed in our stem cell lines. Consideration of changes during long-term cultivation is essential when using stem cells for research or therapeutic purposes. However, our findings show no statistically significant differences in the proliferative characteristics or enzymatic activity of SCAPs between early and late passages.

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INVESTIGATING THE ROLE OF A PROXIMAL SOX2 ENHANCER IN BRAIN DEVELOPMENT AND PSYCHIATRIC DISORDERS

Natalia Gajewska, *University of Toronto, Canada*
Jennifer A. Mitchell, *University of Toronto, Canada*
Iva Zovkic, *University of Toronto Mississauga, Canada*

SOX2, a transcription factor required for neural stem cell self-renewal is implicated in anophthalmia and brain malformations in human developmental disorders. Furthermore, the 1 megabase gene desert surrounding the SOX2 gene contains non-coding polymorphisms linked by GWAS to schizophrenia, depressive behaviours, and cognitive impairment. As 93% of disorder-linked genetic variants are found in the non-coding regions of the DNA, housing enhancers, Sox2 provides an interesting case study. Mouse models revealed tissue-specific enhancers are active during brain development and an enhancer cluster downstream of Sox2 is required for anterior neural stem cell identity in culture. Sox2 Regulatory Region 2 (SRR2) is a known enhancer within this cluster active in embryonic and neural stem cells as well as the telencephalon of the developing murine central nervous system. Previous work in the lab shows deletion of SRR2 in embryonically derived neural stem cells causes a significant decrease of Sox2 expression. This led us to investigate the role of this enhancer in a mouse deletion model, with a focus on adult behavior phenotypes. Constitutive deletion of SRR2 did not result in overt changes in the mouse brain, generating adult fertile mice. We investigated to what extent the loss of SRR2 affects mouse behavior with a focus on behavioural assays that investigate phenotypes representative of the disease links identified in human GWAS studies. Mice with constitutive deletion of SRR2 displayed no significant difference compared to wild-type littermates in memory, anxiety, or hyperactivity. Mice with a homozygous SRR2 deletion show a 33% decrease in social interaction compared to heterozygous and wild-type mice ($p<0.05$). Mice with loss of SRR2 on one or both alleles displayed an impairment in attentional filtering evidenced by a loss of latent inhibition ($p<0.05$), a phenotype commonly observed in schizophrenia patients. These findings are the first mechanistic link between Sox2 transcriptional regulation and schizophrenia, however, when Sox2 expression was assessed in brain regions implicated in these behaviours at 12 weeks (medial prefrontal cortex, amygdala, hippocampus, and cerebellum) there was no significant difference in the levels of expression. These findings suggest that disruption of Sox2 regulation in the embryonic or early postnatal period can alter brain development affecting adult behaviour. This is in line with the current understanding of the onset of schizophrenia as a neurodevelopment disorder, with root causes beginning in abnormal brain development and symptoms manifesting later in life. This SRR2 enhancer deleted mouse model will allow us to further investigate the mechanisms through which Sox2 and neural stem cells are linked to neurological disease, specifically schizophrenia.

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INVESTIGATING THE ROLE OF VASCULAR CELLS ON NEURAL STEM CELL FUNCTION DURING NEONATAL DEVELOPMENT

Marissa A. Lithopoulos, *University of British Columbia, Canada*
Jasmine Yang, *University of British Columbia, Canada*
Dina Karamboulas, *The Hospital for Sick Children, Canada*
David R. Kaplan, *The Hospital for Sick Children, Canada*
Freda D. Miller, *University of British Columbia, Canada*

The neural stem cell (NSC) vascular niche is known to provide important cues that regulate NSC maintenance, proliferation, and differentiation. However, the mechanisms by which vascular endothelial cells (ECs) interact with NSCs during the neonatal period remain largely unknown, as previous studies have focused on embryonic or adult signalling, where EC secreted cues largely increased proliferation and differentiation of NSCs. We hypothesize that during neonatal development, ECs of the subventricular zone (SVZ) stem cell niche prevent hyperproliferation and differentiation of NSCs, and that the signals from ECs change over the lifespan to instead drive NSC lineage progression and proliferation. Our objective is to investigate the role of ECs on neonatal NSC behaviour and to compare EC signalling cues onto NSCs across the lifespan. Utilizing single-cell RNA sequencing, we examined SVZ niche transcriptional changes and conducted ligand-receptor modelling to predict whether EC signalling cues differ with age. We utilized single-cell spatial transcriptomics to validate ligand-receptor interactions in neonatal forebrain mouse tissue. We also assessed the effect of conditioned media from ECs on cortical precursor proliferation and fate. SVZ ECs from postnatal day (P)7 mice showed an altered gene expression profile compared to ECs from adult mice, with over 5,000 differentially expressed genes. Gene ontology analysis revealed an upregulation of pathways involved in inflammation and oxidative phosphorylation in adult ECs compared to those from the neonate. Ligand-receptor modelling showed a distinct set of potential interactions between ECs and neural progenitors during the neonatal period compared to the adult, with 1.5 times more ligands identified during the neonatal period. Initial data showed that conditioned media from ECs from the neonatal SVZ restricted cortical precursor proliferation, gliogenesis, and neurogenesis in vitro. Our data suggest that during neonatal development, ECs provide cues that restrict precursor proliferation and differentiation, which may help maintain the NSC pool. Future work will determine when EC signalling towards NSCs switches from restrictive to permissive and validate EC-expressed ligands that regulate these processes.

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THE FUNCTION OF PHF3 IN NEURONAL DIFFERENTIATION AND NEURONAL DISORDERS

Magdalena Engl, *Max Perutz Labs, Austria*
Filip Horvat, *Max Perutz Labs, Austria*
Lisa-Marie Appel, *Max Perutz Labs, Austria*
Vedran Franke, *Max Delbrück Center, Germany*
Melania Bruno, *The National Institutes of Health, USA*
Dea Slade, *Max Perutz Labs, Austria*

During neural development, neuronal stem cells differentiate into functional neurons that make up the human brain. Transcription regulators ensure the timely expression of genes required for neuronal differentiation. Perturbed transcription can cause neurological and neurodevelopmental disorders as well as lead to the development of cancer. It is therefore crucial to identify and characterize transcriptional regulators involved in these processes to understand disease origin and develop new treatment strategies. We identified PHD finger protein 3 (PHF3) as a key player in the regulation of neuronal gene expression and neuronal differentiation. PHF3 directly binds RNA polymerase II and regulates transcription and mRNA stability. The molecular mechanism of how PHF3 chooses its targets and regulates neuronal differentiation remains unresolved. Our preliminary data indicate that loss of PHF3 in human induced pluripotent stem cells (hiPSCs) due to gene knock-out (KO) or acute degradation impairs differentiation into functional neurons. Single-cell RNA-sequencing (scRNA-seq) revealed accelerated neuronal differentiation in PHF3 KO cells with a higher proportion of neurons and a lower proportion of radial glial and intermediate progenitor cells. This is coupled with deregulation of transcription factors known to be essential for neurogenesis. As a result, neuronal functionality is compromised in PHF3 KO, which show reduced neurite length and impaired spontaneous excitatory input measured by patch-clamp recordings. Insights gained from this project will provide a comprehensive understanding of PHF3 function in neuronal differentiation, paving the way for new therapeutic approaches tackling neurodevelopmental diseases.

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GEMININ AND GEMC1 REGULATE ADULT NEURAL STEM CELL DYNAMICS AND NEUROGENESIS IN THE SUBVENTRICULAR ZONE

Anna Chantzara, *University of Patras School of Medicine, Greece*
Maria-Eleni Lalioti, *University of Patras School of Medicine, Greece*
Christina Kyrousi, *University of Patras School of Medicine, Greece*
Konstantina Kaplani, *University of Patras School of Medicine, Greece*
Georgia Lokka, *University of Patras School of Medicine, Greece*
Zoi Lygerou, *University of Patras School of Medicine, Greece*
Stavros Taraviras, *University of Patras School of Medicine, Greece*

The subventricular zone (SVZ) is a neurogenic niche in the adult mammalian brain, essential for neural plasticity and regeneration, located along the lateral ventricles. Neural stem cells (NSCs) and multiciliated ependymal cells (ECs) are key cellular components of the SVZ, driving respectively adult neurogenesis and structural and trophic support of the niche. The understanding of the mechanisms contributing to the homeostatic mechanisms maintaining the SVZ are poorly understood. In our present work we provide evidence that Geminin family members, Geminin and GemC1, coordinate the balance between NSC and EC populations. Our findings demonstrate that in the absence of Geminin, EC production is increased at the expense of NSCs, prompting NSCs to increase self-renewal while reducing differentiation. This shift is accompanied by enhanced cycle re-entry and S-phase activity in NSC progenitors. Furthermore, Geminin-KO SVZ colonies exhibit a composition skewed towards neuronal lineage. Finally, transcriptomics analysis reveals that Geminin regulates key pathways involved in NSC activation and neurogenesis. Collectively, these results highlight Geminin's role in maintaining the balance between NSC maintenance and differentiation within the SVZ niche. In addition, in the absence of GemC1, cells adopt an NSC phenotype at the expense of EC differentiation. This shift is accompanied by an increase in NSC numbers, proliferation, and enhanced neurogenesis in the postnatal SVZ. Finally, GemC1-KO cells exhibit altered chromatin organization at several loci, further supporting a NSC identity. In conclusion, our findings highlight that Geminin and GemC1 play a crucial role in establishing the SVZ and balancing ECs and NSCs. The antagonistic interplay between Geminin, promoting NSC self-renewal, and GemC1, driving EC commitment, underscores the interconnected fate decisions of these lineages, offering insights into NSC dynamics and brain regeneration.

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EFFECT OF DIFFERENT SUPPLEMENT VARIANTS ON CELL CULTURE APPLICATIONS: A COMPARATIVE ANALYSIS

Sara Perez Munoz, *Thermo Fisher Scientific, USA*
Richard Josephson, *Thermo Fisher Scientific, USA*
Adam Ibrahimi, *Thermo Fisher Scientific, USA*
Pradip Shahi Thakuri, *Thermo Fisher Scientific, USA*
David Kuninger, *Thermo Fisher Scientific, USA*

B27, a proprietary supplement widely used in diverse cell culture applications such as neurons, human and mouse pluripotent stem cells (PSCs), and their differentiation, has prompted the development of alternative formulations by various vendors. While these formulations cannot be referred to as B27 or replicate its exact composition, they aim to provide similar functionalities. In this study, we investigated the effects of these alternative supplements on multiple cell culture applications, including mouse and rat neurons, human neural stem cell (NSC) proliferation, and human PSC differentiation. Through a comparative analysis, we examined the impact of these alternative formulations on cell culture outcomes. Analytical analyses were conducted to elucidate the underlying differences between B27 and these alternative supplements. Our findings offer valuable insights into the potential variations in cell culture outcomes resulting from the use of these alternative formulations, without explicitly focusing on the vendors behind them. This study contributes to the understanding of the effects of alternative supplement variants on cell culture applications, aiding in the optimization of cell culture protocols and promoting reproducibility in experimental studies.

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COMPLEXITY AND PLASTICITY OF CEREBRAL BRAIN ORGANOIDS DERIVED FROM IPSC-DIFFERENTIATED FOREBRAIN NEURAL PROGENITOR CELLS

Jing Fan, *Hopstem Biotechnology, USA*
Anxin Wang, *Hopstem Engineering, China*

Yanshuang Xiao, *Hopstem Engineering, China*
Xinni Zhu, *Hopstem Engineering, China*
Zhenwen Cai, *Hopstem Engineering, China*
Binjie Zhao, *Hopstem Engineering, China*
Tan Zhou, *Hopstem Engineering, China*
Yinggang Yan, *Hopstem Engineering, China*
Fang Ren, *Hopstem Engineering, China*

Human iPSC-derived forebrain neural progenitor cells (fNPCs) and differentiated cerebral neural cells or organoids hold great promises for disease modeling, drug screening, as well as therapeutic products targeting the largest portion of neurological disorders. Understanding the fate of fNPCs and the function of the complex progeny neural cell population is essential for all these applications. In this study, we utilized human induced pluripotent stem cells (hiPSCs) differentiated cerebral organoids as a model system to investigate the fate and differentiation process of fNPCs with different methods, including immunofluorescence staining, single cell RNA-sequencing (scRNA-seq), as well as single nucleus RNA-sequencing (snRNA-seq). Readout from both methods showed that these cerebral organoids contain a diverse range of cell types similar to those in human forebrain at late development stage, including 6 cortical layer neurons with interneurons, astrocytes and oligodendrocyte progenitor cells. The function and plasticity of these fNPCs formed cerebral organoids were assessed by calcium imaging and multi-electrode array (MEA) recordings. Synchronized spontaneous activities of these cerebral organoids can be observed starting at 20 days of, while responses to NMDAR agonist and antagonist can be recorded at 40 days, and brain wave-like periodic and rhythmic activity can be seen around 60 days of the fNPCs in vitro culture. In conclusion, the in vitro differentiated cerebral organoids are complex and functional models of the in vivo neural development of fNPCs.

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ASCORBIC ACID AMELIORATES MOLECULAR AND DEVELOPMENTAL DEFECTS IN HUMAN INDUCED PLURIPOTENT STEM CELL AND CEREBRAL ORGANOID MODELS OF FRAGILE X SYNDROME

Keith Gunapala, *University of Basel, Switzerland*
Aseel Gadban, *The Hebrew University of Jerusalem, Israel*
Faiza Noreen, *University of Basel, Switzerland*
Primo Schär, *University of Basel, Switzerland*

Nissim Benvenisty, *The Hebrew University of Jerusalem, Israel*
Verdon Taylor, *University of Basel, Switzerland*

Fragile X Syndrome (FX) is the most common form of inherited cognitive impairment and falls under the broader category of Autism Spectrum Disorders (ASD). FX is caused by a CGG trinucleotide repeat expansion in the non-coding region of the X-linked Fragile X Messenger Ribonucleoprotein 1 (FMR1) gene, leading to its hypermethylation and epigenetic silencing. Animal models of FX rely on the deletion of the Fmr1 gene which fail to replicate the epigenetic silencing mechanism of the FMR1 gene seen in human patients. Human stem cells carrying FX repeat expansions have provided a better understanding of the basis of the epigenetic silencing of FMR1. Previous studies have found that 5-Azacytidine (5Azac) can reverse this methylation; however, 5Azac can be toxic and may limit its therapeutic potential. Here, we show that the dietary factor Ascorbic Acid (AsA) can reduce DNA methylation in the FMR1 locus and lead to an increase in FMR1 gene expression in FX iPSCs and cerebral organoids. In addition, AsA treatment rescued neuronal gene expression and the morphological defects observed in FX iPSC-derived cerebral organoids. Hence, we demonstrate that the dietary co-factor AsA can partially revert molecular and morphological defects seen in human FX models in vitro. Our findings have implications for the development of novel therapies for FX in the future.

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STUDYING THE DIRECT AND GLIAL-CELL MEDIATED EFFECTS OF HYPERGLYCEMIA ON HUMAN DOPAMINERGIC NEURONS

Konstantina Chanoumidou, *IMBB-FORTH, Greece*
Chrystalla Konstantinou, *University of Crete, Greece*
Ioanna Zota, *University of Crete, Greece*
Marianna Papadopoulou, *University of Crete, Greece*
Alexandros Tsimpolis, *University of Crete, Greece*
Maria Tziortziou, *University of Luxembourg, Luxembourg*
Ioannis Charalampopoulos, *University of Crete, Greece*

Elevated blood glucose levels, commonly referred to as hyperglycemia, is a defining characteristic of Diabetes Mellitus (DM), a metabolic disorder with a growing prevalence worldwide. DM is a high-risk factor for neurodegeneration and the development of Parkinson's Disease. Animal models and patients with DM present with striatal dopaminergic dysfunction and have increased risk for parkinsonian symptoms (Iara Pérez-Taboada et al.,2018, Iara Pérez-Taboada et al.,2020). However, the mechanistic interlink between DM and PD is still unclear. p75 neurotrophin receptor (p75NTR) belongs to the TNF-receptor superfamily and signals neuronal apoptosis in different settings. p75NTR is upregulated in the plasma of diabetic patients and has been previously implicated in diabetic retinopathy (Mysona et al., 2013). Though, its role in diabetic encephalopathy is unknown. To date, most studies address glucose neurotoxicity in animal diabetic models. Here, we aim to study the neurological manifestations of high glucose in human dopaminergic neurons with emphasis on the role of p75NTR. We generated iPSC-derived dopaminergic neurons and found that cell treatment with high glucose (HG, 100mM) for 48h led to DNA damage, activation of the JNK signaling and significant cell death. Additionally, glucose overload makes neurons more susceptible to the cytotoxic effect of 6-OHDA. The expression of p75NTR is up-regulated in HG-treated neurons while inhibition of p75NTR activity rescues neuronal cell death highlighting p75NTR as a mediator of glucose neurotoxicity. Additionally, we demonstrate that BNN27, a synthetic neurosteroid that can penetrate the Blood Brain Barrier, can protect neurons from glucotoxicity via the TRKA and p75NTR receptors. Finally, we studied the role of glial cells on neurodegeneration in hyperglycemia. We generated iPSC-derived astrocytes and microglia and treated them with HG (100mM) for 48h. Strikingly, HG induced the release of neurotoxic factors in both cell types with a detrimental effect on dopaminergic neurons as showcased by the increased neuronal cell death after treatment with the astrocyte and microglia conditioned media. In summary, our results provide insights into the mechanism of glucose neurotoxicity in dopaminergic neurons, suggest for the first time the involvement of p75NTR in diabetic encephalopathy and highlight the critical role of glial cells on neurodegeneration in DM.

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THE BIG NICHE: STRIATAL AND SVZ ASTROCYTES SHARE A WIDESPREAD NEUROGENIC POTENTIAL AND COMMITMENT TOWARDS LGE-CLASS INTERNEURONS

Marco Fogli, *University of Turin, Italy*
Giulia Nato, *Neuroscience Institute Cavalieri Ottolenghi, Italy*
Philip Greulich, *University of Southampton, UK*
Jacopo Pinto, *University of Turin, Italy*
Marta Ribodino, *University of Turin, Italy*
Gregorio Valsania, *University of Turin, Italy*
Valentina Proserpio, *Candiolo Cancer Institute, FPO-IRCCS, Italy*
Salvatore Oliviero, *University of Turin, Italy*
Benedikt Berninger, *King's College London and Johannes Gutenberg University, UK*
Paolo Peretto, *University of Turin, Italy*
Annalisa Buffo, *University of Turin, Italy*
Federico Luzzati, *University of Turin, Italy*

In the postnatal and adult brain, descendants of various cortical and subcortical embryonic progenitor domains differentiate into astrocytes, which continue to generate neurons in a specialized periventricular niche: the subventricular zone (SVZ). The cell fate potential of these astrocytes converges towards the generation of lateral ganglionic eminence (LGE)-class interneurons, which migrate to the olfactory bulb (OB) and differentiate into distinct OB interneuron subtypes. Under specific conditions, new neurons can also be generated outside these niches in the adult brain parenchyma, particularly in the striatum. In this region, neurogenesis occurs both under physiological conditions (such as in pre-pubertal guinea pigs, adult rabbits, and possibly humans) and in pathological contexts, (in mouse models of progressive or acute neuronal degeneration). Lineage tracing studies have demonstrated that parenchymal astrocytes can generate neuroblasts in response to stroke or excitotoxic lesions. However, the prevalence, distribution, and behavior of these latent neural stem cells (NSCs) remain poorly understood. To address these questions, we reconstructed the spatiotemporal pattern of striatal astrocyte neurogenic activation following excitotoxic lesions in mice. Similar to canonical niches, steady-state neurogenesis in the striatum is driven by their continuous stochastic activation. Activated astrocytes rapidly return to quiescence, while their progeny undergo transient expansion, characterized by an accelerated propensity for differentiation. Interestingly, the activation rate of striatal astrocytes is comparable to that of SVZ astrocytes, suggesting a similar prevalence of NSC potential between these two populations. Furthermore, single-cell RNA sequencing (scRNAseq) reveals that, like SVZ NSCs, the immature neurons generated by striatal astrocytes also belong to the LGE-class interneuron lineage. The primary distinction between striatal parenchymal astrocytes and

those in canonical niches may lie in the availability of neurogenic activation stimuli, which, in the parenchyma, occur only under specific conditions.

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SOX21 SUPPRESSES GBM GROWTH BY REPRESSING AP-1 REGULATED TARGET GENES

Maria Bergsland, *Karolinska Institutet, Sweden*
Juan Yuan, *Karolinska Institute, Sweden*
Eltjona Rrapaj, *Karolinska Institute, Sweden*
Jonas Muhr, *Karolinska Institute, Sweden*

Treatment resistant glioblastoma stem cells (GSCs) are responsible for glioblastoma (GBM) growth and recurrence. This study investigates the potential of the transcription factor SOX21 to inhibit GSC properties and thereby counteract GBM progression. The findings reveal that increasing SOX21 levels in primary GSCs induces an anti-tumorigenic gene expression profile. This is supported by a significant correlation between high SOX21 expression and improved survival rates of GBM patients. Furthermore, induced SOX21 expression in pre-established GBM reduces tumor growth and significantly extends the survival of orthotopically transplanted mice. Mechanistically, SOX21 acts as a tumor suppressor by binding to and epigenetically repressing AP-1 targeted chromatin, thereby downregulating adjacent genes. Consistently, the anti-tumorigenic activities of SOX21 are replicated by small molecules that inhibit AP-1 activity, whereas overexpression of an AP-1 transcription factor reverses these effects. Collectively, these findings underscore the potent role of SOX21 in restricting GBM progression through the repression of AP-1 stimulated tumor-promoting gene expression, highlighting SOX21 as a promising therapeutic target.

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A MULTI-OMICS APPROACH TO STUDY THE EPIGENETIC MECHANISMS THAT CONTROL THE MAINTENANCE AND ACTIVATION OF ADULT HIPPOCAMPAL NEURAL STEM CELLS

Sara Ahmed, *The Francis Crick Institute, UK*
François Guillemot, *The Francis Crick Institute, UK*

Neural stem cells (NSCs) in the postnatal hippocampus persist in a state of reversible growth arrest or quiescence. Adult neurogenesis involves activation of quiescent NSCs which generate dentate granule neurons before differentiating. With age, a growing fraction of active NSCs return to quiescence instead of differentiating. On the other hand, NSCs that do not activate move progressively into deeper quiescence with age. Previous work in our lab has shown that the increasing capacity of active NSCs to return to quiescence instead of differentiating during adulthood is due to the progressive reduction of ASCL1 protein levels (Lachlan, H. et al. 2021 Cell Stem Cell). Several studies have provided evidence for the role of ASCL1 as a pioneer transcription factor associating with condensed chromatin to facilitate the binding of additional transcription factors and modulating chromatin accessibility (Park, N. I. et al. 2017 Cell Stem Cell, Raposo, A. A. S. F. et al. 2015 Cell reports, Soufi, A. et. al. 2015 Cell, Wapinski, O. L. et al. 2013 Cell). Here we are characterising the transcriptome, chromatin accessibility and Ascl1 binding profile of adult hippocampal NSCs to shed more light on how ASCL1 controls hippocampal NSCs states in an age-dependent manner. Considering earlier results, in the present project we will explore the possible role of ASCL1 in promoting the epigenetic changes required for the transition of hippocampal NSCs from quiescence to activation by acting as a pioneer transcription factor and opening chromatin at NSCs activity-related loci.

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IMPORTANCE OF HIGH-DENSITY MICROELECTRODE ARRAYS FOR RECORDING MULTI-SCALE EXTRACELLULAR POTENTIAL AND LABEL-FREE CHARACTERIZATION OF NETWORK DYNAMICS IN IPSC-DERIVED NEURONS

Francesco Modena, *MaxWell Biosystems AG, Switzerland*
Zhuoliang Li, *MaxWell Biosystems AG, Switzerland*
Elvira Guella, *MaxWell Biosystems AG, Switzerland*
Anastasiia Tourbier, *MaxWell Biosystems AG, Switzerland*
Laura D'Ignazio, *MaxWell Biosystems AG, Switzerland*
Silvia Oldani, *MaxWell Biosystems AG, Switzerland*
Praveena Manogaran, *MaxWell Biosystems AG, Switzerland*
Marie E. Obien, *MaxWell Biosystems, Switzerland*

Advances in microelectrode array (MEA) technology for in-vitro electrophysiological recordings have made it possible to study neuronal networks across multiple scales, from subcellular properties to network-level dynamics. These devices are essential for exploring the phenotypes of neurological disorders and accelerating drug discovery, offering unique insights into the behaviour of neuronal networks. Key factors such as electrode density, spacing, and size significantly impact signal quality, noise, and sensitivity. To exhaustively characterize neuronal networks, MEAs must combine single-cell and subcellular resolution with high-throughput capabilities, maintaining sensitivity to small extracellular action potentials to capture the full range of network activity. In this study, the MaxOne and MaxTwo high-density (HD) MEA systems (MaxWell Biosystems, Switzerland) were utilized to record activity from induced pluripotent stem cell-derived neurons. These systems, with 26,400 electrodes per well, demonstrated the benefits of increased statistical power in longitudinal data collection. HD-MEA recordings were compared to simulated low-density recordings, where adjacent electrodes on HD-MEAs were clustered to mimic larger, lower-density electrodes. Additionally, the AxonTracking Assay, an automated tool for analysing individual axonal arbours from multiple neurons simultaneously, was used to evaluate axonal structures and network functionality in the recorded cultures. Results showed that higher electrode density and smaller electrode size enhanced sensitivity, allowing for the detection of smaller spikes and capturing the complete spectrum of network dynamics. The high-resolution analysis of network activity, combined with subcellular insights from the AxonTracking Assay, offers a robust platform for drug screening and disease modelling.

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FROZEN NEURAL PROGENITORS, NEURON PRECURSORS, AND ASTROCYTES DERIVED FROM THE HIGHLY CHARACTERIZED REFERENCE SCTi003-A LINE

Erin Knock, *STEMCELL Technologies Inc., Canada*
Alym Moosa, *STEMCELL Technologies Inc., Canada*
Kenzie Wrightson, *STEMCELL Technologies Inc., Canada*
Benjamin Streeter, *Axion Biosystems, USA*
Andrew Gaffney, *STEMCELL Technologies Inc., Canada*
Sharon A. Louis, *STEMCELL Technologies Inc., Canada*
Allen C. Eaves, *STEMCELL Technologies Inc., Canada*

Cryopreserved cells can serve as a flexible and reproducible starting point for downstream differentiation. We developed highly pure cryopreserved neural progenitor cells (NPCs), neuron precursors, and astrocytes derived from the highly-characterized human induced pluripotent stem cell (hiPSC) line, SCTi003-A. SCTi003-A hiPSCs were seeded in complete STEMdiff™ Neural Induction Medium + SMADi and cultured for 21 days to generate NPCs. Day 21 NPCs were then cryopreserved in STEMdiff™ Neural Progenitor Freezing Medium at a density of 1.3 x 106 cells/mL. After thawing, NPCs were differentiated to forebrain neuron precursors and astrocytes using STEMdiff™ Forebrain Neuron Differentiation Kit and STEMdiff™ Astrocyte Serum-Free Differentiation and Maturation Kits, respectively. Forebrain neuron precursors were cryopreserved on day 7 at a density of 1.5 x 106 cells/mL and day 54 astrocytes were cryopreserved at 1.5 x 106 cells/mL. The majority of thawed SCTi003-A-derived NPCs expressed PAX6 (96.4 ± 3.4%) and SOX1 (91.5 ± 2.4%), and exhibited little spontaneous neuronal differentiation (3.5 ± 1.3% βIIITUB+; mean ± SEM; n = 4). NPCs maintained in STEMdiff™ Neural Progenitor Medium and expanded for 5 passages displayed a 2.8 ± 0.5-fold increase (mean ± SEM; n = 4) in cell number/passage while retaining NPC phenotype (97.8 ± 1.3% PAX6+SOX1+). Forebrain precursor cells thawed into STEMdiff™ Forebrain Maturation medium and cultured for a further 14 days were 95.2% positive for neuronal marker βIIITUB with only 3.4% off target differentiation to glial cells as marked by S100β (n = 2). Microelectrode array analysis of the neural activity using the Maestro Pro™ MEA System showed an increasing mean firing rate reaching over 1.5 Hz with over 300 firing bursts and a synchronicity index over 0.06 by day 42 (n = 1). Astrocytes thawed into STEMdiff™ Astrocyte Serum-Free Maturation Medium and cultured for a further 7 days were positive for astrocyte markers S100β (84.3 ± 4.7%) and GFAP (79.0 ± 4.4%) by immunochemistry with low levels of immature neuron marker doublecortin (DCX; 3.3 ± 1.5%; n = 3). In summary, we have generated highly pure, hiPSC-derived NPCs, forebrain neuron precursors, and astrocytes to provide a standardized and reproducible tool box for performing disease modeling studies and large-scale neural research

Funding Source: STEMCELL Technologies is a private, for profit, biotechnology company.

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HUMAN STEM CELLS BUILT WITH HIGH PLASTICITY REGENERATE DAMAGED TISSUES

Min Hu, *APstem Therapeutics, Inc., USA*

The high plasticity of transplanted stem cells to integrate into local tissue and further differentiate into tissue-constituting cells reflects the therapeutic efficacy of the cells. In the study, we describe a novel group of human adult high-plastic stem cells (hAPSC, CD52+ /CD90+) derived from cell-cell communication with adult blood cells (CD52+/CD90-) and human umbilical cord mesenchymal stromal cells (UC-MSC, (CD52-/CD90+). Genomic analysis shows that hAPSCs have 4,511 genes' differential expression compared to blood CD52+/CD90$^-$cells and 1,131 genes to UC-MSCs. hAPSCs express representative markers from three germ layers although having different pluripotent marker panels: Sox2$^-$/Nanog$^-$/Lin28A$^-$/Oct4weak. When mixed with hydrogel or simply as cell suspension to be either topically or systematically applied to damaged skin, small intestine tissues of FVB mice or damaged brain of Shiverer mice, AHPSCs showed capabilities to homing, reside, and differentiate into local constituent cells to repair damage or regenerate tissues. The high plasticity and non-tumorigenic properties make hAPSCs excellent seed cells for stem cell-based therapy.

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METHODS AND THERAPEUTIC APPROACHES FOR SETD5 SYNDROME

Georgia Chaldaiopoulou, *Sanford Consortium for Regenerative Medicine, USA*
Isa R. Fernandes, *Sanford Consortium for Regenarative Medicine, USA*
Janayna de Souza, *Sanford Consortium for Regenerative Medicine, USA*
Xiwei Shan, *Sanford Consortium for Regenerative Medicine, USA*
Miguel Tenheiro, *Sanford Consortium for Regenerative Medicine, USA*
Sandra Sanchez, *Sanford Consortium for Regenerative Medicine, USA*
Angels Almenar-Queralt, *Sanford Consortium for Regenerative Medicine, USA*
Muotri R. Alysson, *Sanford Consortium for Regenerative Medicine, USA*

Autism spectrum disorder (ASD) affects approximately 1 in 36 individuals, imposing lifelong challenges on patients. Yet still, effective treatments for ASD remain elusive. Rare mutations in the SETD5 (SET Domain Containing 5) gene, a chromatin regulator, have been firmly linked to ASD with high confidence. To date, reports studying the molecular role of SETD5 are limited and how exactly SETD5 haploinsufficiency leads to ASD remains unclear. Setd5-haploinsufficient mice have successfully demonstrated ASD-like behaviours, yet

they fail to accurately replicate human brain development and pathologies. To overcome the constraint of human brain tissue inaccessibility, we have generated the first (to our knowledge) human induced pluripotent stem cell (hiPSC)-derived model related to ASD-SETD5. Our preliminary data revealed a reduced size of disease brain organoids, suggesting SETD5-dependent disturbances in NPC proliferation and differentiation. Data obtained from 2D cultures indicated disease-related neuronal abnormalities, as well as astrocyte reactivity characterized by increased IL-6 secretion, among others. The increased astrocytic IL-6 was shown to result from abnormal activation of the JAK/STAT (Signal Transducer and Activator of Transcription) signal transduction pathway in the reactive disease astrocytes, and it was sufficient to induce ASD-related alterations in control neurons. Significantly, SETD5 astrocytes pre-treated with CYT387 (Momelotinib), an effective disruptor of the JAK/STAT pathway, rescued the impaired neuronal phenotypes of SETD5 neurons, demonstrating non-cell autonomous and reversible deficiencies in SETD5 mutant neurons. Future experiments will elucidate the effects of SETD5 haploinsufficiency in cell and non-cell autonomous mechanisms, will uncover neurons-astrocytic interactions, and guide the development of effective therapeutic tools.

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ABSENCE OF THE PERIPHERAL IMMUNE SYSTEM HIGHLY AFFECTS HIPPOCAMPAL NEUROGENESIS AT EARLY POSTNATAL STAGES

Zoe Kotsikou, *Biomedical Research Foundation of the Academy of Athens, Greece*
Dimitrios Troumpoukis, *Biomedical Research Foundation of the Academy of Athens, Greece*
Ioannis Serafimidis, *Biomedical Research Foundation of the Academy of Athens, Greece*
Yassemi Koutmani, *Biomedical Research Foundation of the Academy of Athens, Greece*

Although the brain, unlike other organs, is separated from the peripheral immune machinery, it is now considered to be an organ that actively intercommunicates with the peripheral immune system in order to obtain homeostatic support for its proper function. Immune cells of both innate and adaptive immune system can modulate the characteristics of neural stem cells (NSCs) and their progeny. The hippocampal dentate gyrus (DG) is a brain region that hosts a population of NSCs that remain across development and continue to generate new neurons throughout life. Although there is emerging evidence that immune system deficiency or malfunction leads to impaired adult hippocampal neurogenesis, the underlying mechanisms remain elusive. Our study aims to

unravel whether this impairment is the result of a developmental deficit or an altered neurogenic niche in the adult hippocampus. To this end, we used as a model of study the immunodeficient NOD-SCID mice with defects in both innate and adaptive immunity. More specifically, we conducted a comparative study between NOD-SCID and control C57BL/6J mice at different developmental stages by analyzing the expression of specific markers of the neurogenic lineage combined with morphometrical criteria. Our results showed that immunodeficient mice at postnatal day 21 (P21), P30, and in adult life (P60) have significantly reduced numbers of GFAP+/radial glia-like NSCs and reduced DCX+ neuronal precursors in the DG, when compared to controls. We further analysed the local brain immune system that consists mainly of microglial cells by immunofluorescence against the marker Iba-1. Preliminary results showed that in early stages the number of microglial cells in NOD-SCID mice did not differ significantly from the control. Overall, these results suggest that the lack of cellular and biochemical components of the immune system is associated with impaired adult hippocampal neurogenesis, possibly by affecting the population of neural stem cells. These findings expand our knowledge of the fundamental role of the immune system in the homeostasis of the nervous system and contribute to our understanding of the complex regulation of neurogenesis.

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THE ROLE OF PLACENTAL CRH IN HUMAN BRAIN DEVELOPMENT

Yassemi Koutmani, *Biomedical Research Foundation of the Academy of Athens, Greece*
George Ellinas, *Center of Experimental Surgery, Clinical and Translational Research, Biomedical Research Foundation of the Academy of Athens, Greece*
Iliana Theodorou, *Center of Experimental Surgery, Clinical and Translational Research, Biomedical Research Foundation of the Academy of Athens, Greece*
Dimitris Valakos, *Center of Basic Research, Biomedical Research Foundation of the Academy of Athens, Greece*
Florentia Papastefanaki, *Laboratory of Cellular and Molecular Neurobiology, Hellenic Pasteur Institute, Greece*
Georgia Kouroupi, *Laboratory of Cellular and Molecular Neurobiology, Hellenic Pasteur Institute, Greece*
Katia K.P. Karalis, *Regeneron Pharmaceuticals, Inc., USA*
Panagiotis K. Politis, *Center of Basic Research, Biomedical Research Foundation of the Academy of Athens, Greece*

Rebecca Matsas, *Laboratory of Cellular and Molecular Neurobiology, Hellenic Pasteur Institute, Greece*
Ioannis Serafimidis, *Center of Basic Research, Biomedical Research Foundation of the Academy of Athens, Greece*
Yassemi Koutmani, *Center of Experimental Surgery, Clinical and Translational Research, Biomedical Research Foundation of the Academy of Athens, Greece*

Corticotropin-Releasing Hormone (CRH) was first identified as a neurohormone secreted by the hypothalamus in response to stressful stimuli. However, large amounts of CRH are also secreted by the placenta of anthropoid primates during pregnancy. Although the role of hypothalamic CRH has been extensively studied, there is a remarkable lack of evidence regarding the role of placental CRH, while the biological significance of its unique expression pattern in anthropoid primates remains elusive. In order to investigate the effects of placental CRH on human brain development and to overcome the limitations raised in experimenting with human tissue, we have generated human 3D-neural spheroids and human cerebral cortical organoids from human embryonic stem cells (hESCs). Exposure of neural spheroids or/and cortical organoids to CRH results in significant differences in their size and cellular composition. In addition, immunohistological analyses using cortical layer-specific antibodies, revealed differences in the cytoarchitecture of the organoids exposed to CRH as compared to control. Pharmacological disruption of CRH signaling using the specific CRH receptor 1 antagonist, NBI, reverses the effects of CRH. In addition, RNA sequencing analysis of the CRH and NBI exposed organoids revealed altered expression of genes related to neurodevelopmental processes such as HOXB9 and FOXG1, depicting CRH as an essential modulator of human brain development. Our findings suggest that this in vitro approach provides a unique tool for our understanding of the mechanisms underlying the role of stress hormones in human brain development.

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COMPREHENSIVE ANALYSIS OF DIFFERENTIATION CAPACITY OF EARLY NEUROEPITHELIAL STEM/PROGENITOR CELL LINES DERIVED FROM HUMAN EMBRYONIC BRAIN TISSUE

Angeliki Spathopoulou, *University of Innsbruck, Austria*
Katharina Günther, *University of Innsbruck, Austria*
Marcel Tisch, *University of Innsbruck, Austria*
Marc-Christian Thier, *HI-STEM, German Cancer Research Center, Germany*

Amelie Schurer, *University of Innsbruck, Austria*
Simone Liebscher, *Eberhard Karls University Tübingen, Germany*
Katja Schenke-Layland, *Eberhard Karls University Tübingen, Germany*
Andreas Trumpp, *HI-STEM, German Cancer Research Center, Germany*
Frank Edenhofer, *University of Innsbruck, Austria*

Cell lines derived from early embryonic stages have significantly contributed to our understanding of mammalian development. However, the available human neural stem/progenitor cell culture models typically represent relatively late stages of neurodevelopment, post-neural tube closure, such as rosette-like or radial glial cells, or fail to maintain long-term clonal self-renewal. We successfully isolated and established a tissue-derived human neural stem/progenitor cell population mirroring human neuroepithelium at five weeks of in vivo development. These embryonic neural stem/progenitor cells (eNSPCs) exhibit a distinct naïve, non-polarized, pre-rosette phenotype, demonstrate virtually unlimited self-renewal, and possess the potential to differentiate into diverse lineages of both the central and peripheral nervous systems, including dopaminergic neurons, V2a progenitors, interneurons, and neural crest cells. Additionally, single-cell RNA sequencing revealed that eNSPCs represent a homogeneous population of multipotent stem cells, rather than a mixture of committed neural progenitors, with distinctive expression of early neurodevelopmental markers. Our findings establish eNSPCs as the earliest stabilized human neural stem/progenitor cell model to date, characterized by very broad plasticity, making them an invaluable tool for studying human neurodevelopment and exploring regenerative medicine applications.

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NEURONAL DIFFERENTIATION OF INDUCED PLURIPOTENT STEM CELLS OF A CORNELIA DE LANGE SYNDROME PATIENT

Marika Foglia, *Mario Negri Institute for Pharmacological Research, Italy*
Mineko Terao, *Mario Negri Institute for Pharmacological Research, Italy*
Luca Guarrera, *Mario Negri Institute for Pharmacological Research, Italy*

The Cornelia de Lange Syndrome (CdLS) is a rare genetic disease, which is characterized by a cohesinopathy. Mutations of the NIPBL gene are observed in 65% of the CdLS patients and are associated with a severe phenotype. The mutations cause a distinctive craniofacial appearance and impair the correct growth and development of different organs such as

heart, brain and gut. A novel iPSC (induced Pluripotent Stem Cells) line was reprogrammed from the leukocytes of a CdLS patient carrying a missense mutation of the NIPBL gene at nucleotide 5483 G>A that causes a single amino acid change at Arg1828Gln. iPSC cell lines derived from parents were used as control. The iPSC lines were differentiated along the hepatocyte-like cells. From our published results obtained from undifferentiated and differentiated iPSCs, we observed a deficit in the differentiation along the hepatocyte-lineage in our CdLS patient due to a transcriptional dysregulation resulting from a cohesin dependent alteration of chromatin accessibility. In fact, some of the transcriptionally silenced loci, such as the DPP6 gene on chromosome 7q36.2 and the ZNF gene cluster on chromosome 19p12, are located in closed-chromatin regions. Interestingly, most of the selectively down-regulated genes code for transcription factors and proteins regulating neural differentiation. It is also known that the disease causes intellectual disability, microcephaly, epilepsy and autism. Therefore, we planned to differentiate CdLS patient-derived iPSCs in cortical neurons and motor neurons to investigate the effects of the mutation on distinct differentiation processes and the possibility to clarify the molecular mechanism at the basis of neuronal differentiation. Currently, differentiated cortical neurons at D14 and motor neurons at D28, showed a lower expression of neuronal markers (NGN2, βIII tubulin) compared with the control cell line as assessed by immunofluorescence analyses. This result demonstrated a deficit in the differentiation along the two neuronal cell lineages. In addition to DPP6 and ZNF gene cluster, we hypothesized that some differentially regulated genes will be newly identified as potential pharmacological targets that could be modulated for the treatment of CdLS patients.

Funding Source: The financial support from Dr. Gino Del Bon covered expenses for the experimental works. The study was also financially supported by the Consiglio Nazionale delle Ricerche (CNR).

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EPENDYMAL CELL REPROGRAMMING A PROOF OF PRINCIPLE STUDY FOR A THERAPEUTIC INTERVENTION FOR HYDROCEPHALUS

Stavros Taraviras, *University of Patras School of Medicine, Greece*
Konstantina Kaplani, *University of Patras, Medical School, Greece*
Maria Eleni Lalioti, *University of Patras, Medical School, Greece*
Styliani Vassalou, *School of Medicine, University of Patras, Greece*
Georgia Lokka, *School of Medicine, University of Patras, Greece*

Evangelia Parlapani, *School of Medicine, University of Patras, Greece*
Giorgos Kritikos, *Greece*
Zoi Lygerou, *School of Medicine, University of Patras, Greece*
Stavros Taraviras, *School of Medicine, University of Patras, Greece*

Hydrocephalus is a frequently occurring neurological disorder, affecting approximately 1 in 1000 births, defined by abnormal accumulation of cerebrospinal fluid in the brain's ventricles. A major factor in the pathophysiology of hydrocephalus is the dysfunction of multiciliated brain ependymal cells. These cells are essential for regulating cerebrospinal fluid (CSF) flow and composition while also sustaining neural stem cells within the ventricular/ subventricular neurogenic niche. Moreover, disturbances in the niche's structural organization play a significant and lasting role in hydrocephalus progression. The primary treatment for hydrocephalus typically involves CSF's neurosurgical diversion, a procedure associated with high rates of morbidity and failure. This underscores the urgent need to develop innovative therapeutic strategies for hydrocephalus. We have shown that ectopic expression of GemC1 and McIdas reprograms cortical astrocytes and programs mouse embryonic stem cells into ependyma. McIdas is sufficient to induce functional activity in reprogrammed astrocytes. Additionally, we demonstrated that McIdas expression supports ependymal cell regeneration in two distinct postnatal hydrocephalus mouse models—one involving intracranial hemorrhage and the other a genetic form of hydrocephalus—while also improving the structural integrity of the neurogenic niche. Our study demonstrates that GemC1 and McIdas induce direct cellular reprogramming towards ependyma. In addition, we provide evidence supporting the idea that restoration of ependymal cells in hydrocephalus, could offer a future therapeutic intervention for hydrocephalus.

Funding Source: Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "2nd Call for H.F.R.I. Research Projects to support Faculty Members & Researchers" (2735), the Hydrocephalus Association USA.

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DERIVATION OF A CLONAL AND STABLE ISTHMIC-LIKE PROGENITOR WITH HIGH CAPACITY FOR MID-HINDBRAIN BOUNDARY NEURON PRODUCTION IN VITRO AND IN VIVO

Ceren Thier, *University Hospital Heidelberg, Germany*

Sascha Conic, *HI-STEM, Germany*
Sarah Lang, *HI-STEM, Germany*
Nils Wörner, *DKFZ, Germany*
Hannah Monyer, *University Hospital Heidelberg, Germany*
Andreas Trumpp, *HI-STEM, DKFZ, Germany*
Marc C. Thier, *HI-STEM, DKFZ, Germany*

The isthmic organizer, a critical signaling hub at the midbrain-hindbrain boundary, orchestrates the development of neighboring brain regions during early vertebrate embryogenesis. In this study, we report the generation of a stable, clonal human neuroepithelial progenitor population with characteristics of the isthmic region, termed isthmic region-like neural progenitor cells (istNPCs). These cells demonstrate robust self-renewal, clonal growth, and stable culture while expressing key markers of the isthmic organizer such as EN1, FGF8, GBX2, PAX2. Through a short induction protocol, istNPCs are derived from directly reprogrammed induced Neural Plate Border Stem Cells (iNBSCs). Furthermore, using our istNPC culture media, we successfully isolated and cultured the isthmic organizer region from E8 mouse embryos, validating the compatibility of our culture conditions with in vivo counterparts. Notably, the inherent plasticity of istNPCs enables positional specification along the anterior-posterior axis of the isthmic region under tailored media conditions. Evidenced by OTX2 or GBX2 expression at the anterior or posterior border, respectively, allows the derivation of the full regional identity of the isthmus. Consistent with this regional identity, istNPCs efficiently differentiate into mid-hindbrain neuronal lineages, including dopaminergic and serotonergic neurons, both in vitro and in vivo. Functional validation using mass spectrometry confirmed the release of dopamine, serotonin, and their metabolites, underscoring the physiological relevance of these neurons. This direct conversion of human somatic cells into stable, clonal neural progenitors representing the isthmic region offers a powerful platform for investigating early human neural development, specifically the deveopment of the mid-hindbrain regions. Critically, due to the high degree of overlap between the istNPCs and the embryonic source of the dopaminergic neuron progenitors during development, this new cell population represents a significant shortcut towards a rapid and efficient production of dopaminergic neurons for potential applications in cell replacement therapies for neurodegenerative diseases.

DEFINING THE ROLE OF NDD-ASSOCIATED mTOR MUTATIONS ON CELL COMPETITION AND DIFFERENTIATION AMONG NPCS

Samrat Thapa, *Stanford University, USA*
Anna E. Eastman, *Stanford University, USA*
Suyash Raj, *Stanford University, USA*
Nicole Womack, *Stanford University, USA*
Claudia K. Petritsch, *Stanford University, USA*
Irving L. Weissman, *Stanford University, USA*

During neurogenesis, a high frequency of somatic mutations resulting from single nucleotide variations (SNVs) is linked to future neurodevelopmental disorders (NDDs), such as focal cortical dysplasia (FCD) and hemimegalencephaly (HME). Many of these somatic mutations lead to the overactivation of the mammalian target of rapamycin (mTOR) pathway. To define the role of these NDD-associated mutations in cell competition and differentiation, we genetically engineered a somatic point mutation mTOR (p.L2427P) in iPSC-derived neural progenitor cells (NPCs). Using competitive transplantation xenograft assays, we mixed control NPCs with mutant NPCs at a 1:1 ratio and transplanted them into the murine subventricular zone (SVZ). A two-color fluorescent reporter strategy, using mClover for control NPCs and mScarlet for mutant NPCs, enabled us to distinguish these populations. At the one-month endpoint, a flow cytometry readout revealed that the mutant Scarlet population consistently outcompeted the Clover control population. To discern possible mechanisms underlying mutant competition, we measured levels of surface-expressed CD47, a "don't eat me" signal previously discovered by our lab. We found that the mutant NPCs upregulate CD47 relative to the control, indicating immune evasion as a possible mechanism of stem cell competition. Single-cell RNA sequencing analysis is underway to identify potential differentiation biases and other abnormalities in mutant NPC progeny. Following these in vivo results, we have begun testing combinatorial therapeutic strategies in vitro with rapamycin to decrease mTOR activity and anti-CD47 antibodies to reduce immune evasion by mutants.

Funding Source: The Cancer Grand Challenges Partnership funded by Cancer Research UK (CGCATF-2021/100005), National Cancer Institute (OT2CA278686), and the Mark Foundation for Cancer Research.

IN VITRO ASSAYS TO STUDY NEUROPROTECTION AND AXON REGENERATION IN IPSC-DERIVED NGN2-INDUCED NEURONS

Maheswara Reddy Emani, *Genentech Inc., USA*

Drug discovery in neurodegenerative diseases is one of the most challenging therapeutic areas due to the lack of suitable models. Developing physiologically relevant in vitro neuroprotection and axon regeneration models is crucial for elucidating the mechanisms underlying neurodegeneration and designing effective therapeutic interventions. Neuroprotection focuses on maintaining the health of surviving neurons after injury, while regeneration seeks to repair or replace damaged neurons and synapses. Relevant in vitro experimental models of neuronal damage are necessary to evaluate any treatment's effectiveness. The discovery of induced pluripotent stem cells (iPSCs) has revolutionized the modeling of human diseases, significantly boosting the confidence of in vitro neurological disease models. We have developed in vitro assays using some common insults to model and study neuroprotection/axon regeneration, including chemical toxicity and mechanical damage in Neurogenin-2 engineered iPSC-derived human neurons (iNeurons). Integrating automated imaging and artificial intelligence (AI) analysis methods to measure axon degeneration/regeneration showed robust neuroprotection and regeneration in vitro, with little hands-on time. These models could help us accelerate therapeutic candidate validation and selection for neurodegenerative diseases.

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PML REGULATES PROTEOSTASIS AND MITOCHONDRIA INTEGRITY IN NEURAL STEM CELLS AND MODULATES MICROGLIAL FUNCTIONS UPON NEUROINFLAMMATION

Syrago Spanou, *University of Crete/ IMBB-FORTH, Greece*
Takis Makatounakis, *IMBB-FORTH, Greece*
Ioanna Pandi, *IMBB-FORTH, Greece*
Martina Samiotaki, *BSRC Alexander Fleming, Greece*
Christoforos Nikolaou, *BSRC Alexander Fleming, Greece*
Panayiota Poirazi, *IMBB-FORTH, Greece*
Joseph Papamatheakis, *University of Crete/ IMBB-FORTH, Greece*
Androniki Kretsovali, *IMBB-FORTH, Greece*

The Promyelocytic Leukemia Protein (PML) originally characterized as a tumor suppressor, has important roles in brain development; however, the molecular and cellular pathways regulated by PML in neuronal cells remain largely unknown. To address this issue, we analyzed gene expression changes caused by PML loss in embryonic neural stem cells (eNSC). Our findings revealed that PML-deficient cells exhibited impaired autophagy and proteasome activity, resulting in increased formation of aggregates and stress-induced death. PML loss also disrupted mitochondrial integrity, affecting membrane potential, reactive oxygen species and mitochondria morphology. We attributed these abnormalities to diminished PGC-1α expression and PPARγ signaling, both of which were functionally restored using a PPAR agonist. Having seen that PML deficient eNSC share commonalities with cells undergoing aging and/or neurodegeneration we investigated the role of PML in neuroinflammation using in vivo models. Intracerebroventricular injections of oAβ1-42 in WT and Pml-/- mice show in the absence of PML, a failure in microglia recruitment and activation leading to increased neurotoxicity in the hippocampus, as examined by immunohistochemistry, protein and gene expression analysis. Additionally, in two-month-old 5xFAD (familial Alzheimer's disease) mice, PML nuclear expression is reduced in cortical neurons. At later stages of pathology, PML is expressed in activated microglia in a diffuse nuclear and cytoplasmic pattern in both the prefrontal cortex and the hippocampus. The above indicate a role for PML in amyloidogenic responses of microglia. To further study this, we currently generate Pml-/- mice bred to the 5xFAD transgenic background to examine how PML affects the disease course at the molecular and cognitive level. Together, our findings demonstrate that PML regulates neuronal survival and is essential for neuroinflammatory responses.

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INTERROGATING ALS SIGNATURE IN EARLY NEURAL PROGENITOR CELLS: A DIFFERENT PERSPECTIVE

Edvige Vulcano, *University of Milano-Bicocca, Department of Biotechnology and Biosciences, Italy*
Elisa Perciballi, *Fondazione IRCCS Casa Sollievo della Sofferenza, Institute for Stem-Cell Biology, Regenerative Medicine and Innovative Therapies (ISBReMIT), Italy*
Ivan Lombardi, Department of Biotechnology and Biosciences, University of Milano-Bicocca, Italy
Rose Mary Carletti, *Fondazione IRCCS Casa Sollievo della Sofferenza, Italy*

Bovio Federica, Department of Biotechnology and Biosciences, University of Milano-Bicocca, Italy
Matilde Forcella, *Department of Biotechnology and Biosciences, University of Milano-Bicocca, Italy*
Jessica Rosati, *Fondazione IRCCS Casa Sollievo della Sofferenza, Italy*
Gelati Maurizio, *Fondazione IRCCS Casa Sollievo della Sofferenza, Institute for Stem-Cell Biology, Regenerative Medicine and Innovative Therapies (ISBReMIT), Italy*
Daniela Profico, *Fondazione IRCCS Casa Sollievo della Sofferenza, Institute for Stem-Cell Biology, Regenerative Medicine and Innovative Therapies (ISBReMIT), Italy*
Fusi Paola, *Department of Biotechnology and Biosciences, University of Milano-Bicocca, Italy*
Angelo Luigi Vescovi, *Link Campus University, Italy*
Daniela Ferrai, *Department of Biotechnology and Biosciences, University of Milano-Bicocca, Italy*

Amyotrophic lateral sclerosis (ALS) is a relentlessly fatal neurodegenerative disease characterized by a selective degeneration of upper motor neurons in motor cortex and lower motor neurons in brainstem and spinal cord, leading to rapid paralysis and death. To date, while the molecular basis of the earliest pathogenesis is still unknown, few findings highlight a compromised adult neurogenesis in ALS. Nonetheless, an increasing amount of evidence shows the pivotal role of abnormalities in energy metabolism and mitochondrial dysfunction in ALS, thus suggesting a strong association between mitochondria metabolism disorder and neurodegeneration. In this study, starting from skin biopsies, we generated a collection of induce pluripotent stem cell (iPSCs) lines from a TARDBP family carrying p.G376D mutation, spanning diverse clinical stages of the disease – asymptomatic, early symptomatic, and late symptomatic – to obtain human induced Neural Stem Cells (hiNSCs) and human induced Neural Progenitor Cells (hiNPCs). The preliminary results showed that hiNSCs carrying p.G376D mutation exhibit impairments in the growth rate, mitochondrial functionality, and ATP production compared to healthy controls. This evidence suggests that possible disease perturbations may occur temporally earlier starting from hiNSCs, potentially increasing the vulnerability of mature cells to subsequent degeneration. By investigating the initial dysfunctions, this study aims to provide a platform to unravel critical gaps in understanding the disease progression, paving the way for innovative and targeted treatments.

MODELING FETAL ALCOHOL SYNDROME USING MOUSE NEURAL STEM CELLS

Raja Kittappa, *National Institutes of Health (NIH), USA*
David Goldman, *NIAAA-NIH, USA*

In the USA, about 14% of pregnant women report alcohol use, and about 5% of these women engage in binge drinking. Fetal Alcohol Syndrome (FAS) is a condition in a child that results from ethanol exposure during the mother's pregnancy. Drinking during pregnancy can cause the child to have disabilities related to fetal brain development and to behavior and learning throughout the individual's lifetime. Here, we show that the development and differentiation of embryonic neural progenitors in vitro is profoundly affected by treatment with ethanol. Specifically, sonic hedgehog signaling is attenuated by ethanol altering cell fate and the developmental trajectory of treated neural stem cells. By this mechanism, ethanol causes the holoprosencephaly and microcephaly observed in fetal alcohol syndrome. The approach outlined here may prove to be generally useful in studying the effects of teratogens on brain development.

Funding Source: National Institute on Alcohol Abuse and Alcoholism, USA.

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ADVANCING NEURAL CELL FUNCTIONALITY: LAMININS AS KEY ECM COMPONENTS IN CELLULAR MODELS AND TRANSLATIONAL RESEARCH

Zhijie Xiao, *BioLamina, Sweden*

Cells within tissues interact with an extracellular matrix (ECM), a protein-rich framework essential for cellular adhesion, proliferation, differentiation, and maturation. Laminins, critical ECM components, comprise 16 isoforms tailored to specific tissues, playing crucial roles in tissue-specific cellular behaviors. Mimicking the natural cell microenvironment is supreme for developing advanced differentiation protocols, reliable disease models, and effective gene-editing platforms. Additionally, the use of defined, xeno-free, and application-specific matrices supports innovative cell therapy approaches. In neural systems, laminins are required for both development and function. Full-length laminin matrix, Biolaminin®, supports the long-term culture of specialized neural cells and enhances the purity of transplantable progenitors. Laminin-based protocols have demonstrated significant improvements: a 43-fold yield increase of hESC-derived

dopaminergic progenitors using laminin-111 compared to conventional methods (Kirkeby et al., 2016), and successful CRISPR/Cas9-mediated gene editing and cortical neuron differentiation (Dias et al., 2022). Clinically relevant disease models have been developed for dopaminergic neurons (Kirkeby et al., 2016; Kirkeby et al., 2023; Park et al.,2024) and astrocytes (Lundin et al., 2018; Izrael et al., 2018; Park et al., 2024) using Biolaminin 111 and 521. Moreover, tissue-specific laminin matrices can be augmented with 3D recombinant platforms, such as Biosilk, to create advanced organoids. Laminin augmentation has further improved brain microenvironment modeling, as evidenced by the cooperative effects of laminin-111 and -521 in enhancing neural differentiation and reducing neuroinflammation in human brain organoids (Bae et al., 2024). This approach enables the generation of long-term functional midbrain and cerebral organoids (Fiorenzano et al., 2021; Sozzi et al., 2022). In conclusion, the integration of laminins into cell culture systems exemplifies their transformative impact on translational research and regenerative medicine. By replicating natural cellular interactions, laminins facilitate the development of refined in vitro models and therapies, setting a foundation for advancements in neural cell functionality and treatment efficacy.

INNOVATION SHOWCASES

All times are listed in Eastern European Summer Time (EEST)

THURSDAY 3 APRIL 2025
1:00 PM – 1:15 PM
THE LATEST ADVANCEMENT TO IMPROVE EFFICIENCY AND VIABILITY FROM GENE-EDITING PSCS TO NEURAL DIFFERENTIATION

Presented by Thermo Fisher Scientific
Sara Pérez Muñoz, *Thermo Fisher Scientific, Spain*

Advancements in cell culture media development and transfection methods have led to improved cell culture and gene editing efficiency of most cell types. However, for sensitive cell types like PSCs, poor cell survival, recovery and efficient differentiation to neural cells as well as the later maintenance of neural cultures remain a significant challenge. Here we present the latest advancements to improve gene editing efficiency, cell survival and neural cell differentiation. The Neon™ NxT Electroporation System will be presented, highlighting its flexibility and ease of use while high transfection efficiency and cell viability are achieved. In the context of cell survival, we will introduce the versatile CultureCEPT™ Supplement and its excellent performance compared to existing recovery reagents. As the last step of the PSC culture and differentiation workflow, we will discuss the B-27 Plus Supplement and its advantages in maturation and long-term culture of neurons.

THURSDAY 3 APRIL 2025
1:20 PM – 1:35 PM
CYTOPLASMIC LIVE-CELL BIOPSIES FOR THE TEMPORAL PROFILING OF SINGLE-CELLS

Presented by CYTOSURGE
Tamás Gerecsei, *Cytosurge, Switzerland*

Single-cell transcriptomics (scRNA-seq) has transformed biological research, but it stayed an end-point measurement, as the cells are destroyed during sequencing. We present a streamlined workflow utilizing the FluidFM OMNIUM platform to perform live-cell cytoplasmic biopsies and RNA sequencing on Panc-1 cells, enabling the repeated sampling of the same cell over time without compromising its viability. This non-destructive approach allows for temporal transcriptomic profiling, making it possible to

track dynamic gene expression changes within individual cells. By preserving cell integrity, researchers can study long-term cellular processes such as differentiation, adaptation, and response to stimuli, unlocking new possibilities for high-resolution, time-resolved single-cell analysis in biomedical research. Additionally, technology offers a powerful tool for studying neural development, enabling repeated transcriptomic profiling of neurons and their progenitors to uncover dynamic gene expression changes during differentiation, maturation, and response to stimuli.

FRIDAY 4 APRIL 2025
1:00 PM – 1:30 PM
REVOLUTIONIZING THE FUNCTIONAL CHARACTERIZATION OF NEURAL STEM CELLS: STRATEGIES TO UNLOCK THE FULL POTENTIAL OF HIGH-DENSITY MICROELECTRODE ARRAYS

Presented by MaxWell Biosystems
Laura D'Ignazio, *MaxWell Biosystems, Switzerland*
Silvia Ronchi, *MaxWell Biosystems, Switzerland*
Yoshiho Ikeuchi, *Institute of Industrial Science, The University of Tokyo, Japan*

Human-induced pluripotent stem cell (iPSC)-derived 2D and 3D cellular models are invaluable tools for disease modeling and drug development. Recently, these models have enabled the reconstruction of neural circuits outside the body, offering deeper insights into human brain function. However, capturing the full network dynamics of these complex cellular models while also detecting the smallest neural signals remains a major challenge. To address this, high-density microelectrode arrays (HD-MEAs) provide a powerful, non-invasive approach for functionally characterizing electrogenic neural cells. Platforms such as MaxOne and MaxTwo HD-MEAs ensure the detection of even the smallest spikes while offering direct access to both cellular and subcellular activity with unparalleled precision.

In this session, together with our guest Speaker, we will showcase innovative solutions designed to enhance electrophysiological recordings from various biological samples, including networks of multiple neural organoids. We will introduce advanced strategies that improve cell culture surfaces, optimize signal quality, and enhance electrical stimulation capabilities. These advancements pave the way for more precise and scalable research, accelerating discoveries in neuroscience and therapeutics.