Session 10: Stem cells biochemistry

Lectures

L.10.1

Functional integration of reprogrammed human neurons and oligodendrocytes in the stroke-damaged brain network

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Stem cell therapy using human induced pluripotent stem (iPS) cell-derived neural precursors is a promising future therapy for stroke patients. Our data show that human skin-derived cortical progenitors can differentiate into cortical projection neurons and functionally integrate (forming afferent and efferent synaptic connections) not only into stroke-damaged rat cortical networks but also into organotypic cultures of the adult human cortex. The grafted cortical neurons respond to sensory stimulation in live animals and, importantly, affect spontaneous behavior when inhibited by optogenetic stimulation. Stroke leads to the loss of oligodendrocytes and axonal demyelination, contributing to functional impairment. Also, their axons should get myelinated for grafted neurons to become functional. Our recent data also show that human iPS cell-derived cells have the unique ability, in addition to differentiating into functional neurons also, to generate *bona fide* oligodendrocytes. The generated cells display human mature oligodendrocytes' structural, molecular, and functional characteristics. They can wrap both grafted human cell- and host-derived axons from cortical neurons in different set-ups after xenotransplantation into rat stroke-injured somatosensory cortex and the human adult cortical organotypic system. Our findings raise the possibility that stem cell transplantation might also restore injured neural circuitry in humans with stroke, which would have significant clinical implications.

L.10.2

Stem cell switch and angiogenesis

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Stem/progenitor cell transplantation is a potential novel therapeutic strategy to induce angiogenesis in ischemic tissue especially in chronic ischemic heart failure. Clinical cell therapy studies using stem cells in ischemic heart and peripheral vascular disease, however, did not report beneficial outcomes in contrast to animal studies. It has been presumed that in patients stem cell function is affected by underlying disease pathways modifying efficacy of cell therapy. We have indicated in RCT-trials that the response to stem cell therapy varies among patients associated to distinct patterns of RNA gene expression in peripheral blood. Angiogenesis response in heart tissue was found to be dependent on CD133+ hematopoietic stem cell proliferation. This suggests the role of genetic alterations, somatic mutations, and clonal hematopoiesis. Personalized stem cell-based therapy can be developed by analyzing individual stem cell reactivity to ischemic stimuli by application of whole-genome sequencing in peripheral blood. Unbiased analyses with state-of-the-art computational artificial intelligence methods, such as machine learning-based patient stratification, are suitable for predictions in clinical investigations and guide therapeutical interventions. The integration of these complex approaches into a unified analysis procedure for the identification of responders and non-responders before treatment is a new approach to unravel disease pathways and increase the efficacy of therapies.

L.10.3

Mitochondrial biogenesis and early brain development in the model of human brain organoids

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The study of mitochondrial biogenesis and its role in early brain development is crucial for understanding neurodevelopmental processes in health and disease. Here we used a human 3D brain organoid (BO) *in vitro* culture model to investigate the effects of low oxygen conditions (physioxia - 5% O_2) on mitochondrial morphology and connectivity during different stages of neuronal differentiation. Under normoxic oxygen conditions (21% O_2), as compared to physioxia, developmental stage-specific alterations in key parameters of mitochondrial morphology and connectivity were observed. Particularly at stage 44D-BO this changes were significant, indicating a critical period for mitochondrial development in BO. Additionally, this study provides the first insight into the modulatory role of physioxia on apoptosis, metabolism, and neural lineage specification. Physioxia influences neural cell fate commitment by modulating the glycolysis/oxidative phosphorylation switch. Furthermore, low oxygen conditions suppressed the expression of genes involved in apoptosis, suggesting a potential neuroprotective role of physioxia.
Our framework utilizing the brain organoids model provides a powerful tool for elucidating mitochondrial function and morphology at various stages of neuronal differentiation and contributes to our understanding of the dynamic relationship between mitochondrial biogenesis, oxygen levels, and neural development.

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Oral presentations

O.10.1

Extracellular vesicles-mediated communication between normal and cancer cells

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The therapeutic effects of stem cell therapies are partially based on extracellular vesicles (EVs) secretion. Stem cell-derived EVs induce effects comparable to parental cells as they share equivalent content. Therefore they may be considered a potential cell-free therapeutic approach. However, the biological impact of EVs is multidirectional and sometimes challenging to characterize. Thus, the present study aimed to evaluate the influence of EVs secreted by stem cells on the metabolism, proliferation, migration, and other characteristics of bladder cancer cells.
A panel of commercially available human cell lines was used in this study. Adipose-derived stem cells were cultured in a medium supplemented with an exosome-free serum to exclude the influence of exogenous EVs on the results. The isolated EVs were characterized before co-culture with cancer cells. Immunoblotting confirmed the expression of exosome-specific markers. This allowed us to assess the biological characteristics of tumor cells cultured in the presence of EVs.
We showed that both the viability and proliferation of cancer cells were reduced after culture with stem cell-derived EVs. Planned in vivo studies will make it possible to assess whether isolated vesicles can be a tissue engineering-based product that would regulate tissue renewal without activating cancer-related pathways.

Acknowledgements

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O.10.2

Modern technologies for stem cells and organoids imaging – an indispensable tool for biology understanding

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Stem cells and organoid biology is recently one of the most developing area of cellular sciences. Automated Imaging Cytometry and High Content Screening Microscopy offers standardized and quantitative tools for imaging, measuring and analyzing stem cells differentiation, 3D structure formulation and organoid behavior. Both technologies are very useful for basic research as well as for therapy or drug development. Automated Image Cytometry is dedicated for basic analysis of cells and spheroids giving opportunity to choose experimental conditions for High Content Screening samples imaging. Separately Imaging Cytometry and High Content Screening are powerful tools for advanced stem cells and organoids study. The lecture covers presentation of AIC and HCS technology and applications.

Posters

P.10.1

Osteogenic potential of human stromal fibroblast HS-27A cell line

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Human bone marrow, immortalized, fibroblastic stromal cell line HS-27A is defined as a cell line supporting the formation of the cobble area by isolated cells positive for CD34. We have investigated whether HS-27A cells display osteogenic potential and respond to osteogenic growth factors i.e. recombinant human BMP-2 (rhBMP-2), for potential use of these cells as a model to study osteogenesis in human bone marrow stromal cells (hBMSC). Cells were cultured in serum-containing or serum-depleted media and they were treated with different combinations of rhBMP-2, ascorbic acid (Asc), dexamethasone (Dex) and β-glycerophosphate (BGP), with and without ERK inhibitor (PD98059). Cells displayed the highest *MSX-2* mRNA levels after 7-day cultures with rhBMP-2 and PD98059 both in serum+ and serum- cultures, indicating the importance of decreased ERK activity for BMP osteogenic action. Osteocalcin (*OC*) mRNA levels were the highest upon BMP treatment in serum+ and bone sialoprotein (*BSP*) mRNA levels upon BMP-2 +PD98059 in serum- cultures. ALP activity was enhanced by BMP in both serum+ and serum- cultures and further enhanced by PD98059 in serum- cultures. Lipopolysaccharide (LPS) treatment together with Asc+Dex+BGP resulted in significantly increased ALP activity, suggesting the role of inflammatory cytokines in osteogenic response of HS-27A cells. Overall, we postulate HS-27A cell line as a good cellular model to study osteogenesis of hBMSC.

P.10.2

The role of angiomiotin-like 2 (AMOTL2) in human pluripotent stem cells and its effects on the Hippo signaling pathway and mitochondrial dynamics and functionality

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The Hippo signaling pathway plays a crucial role in regulating cell proliferation, development and homeostasis of various cell types. Using our single-cell RNA sequencing (scRNA-seq), we have identified angiomotin-like 2 (AMOTL2) as a gene with elevated expression in a newly identified subpopulation of delaminating endocrine progenitors biased to become pancreatic β-cells. Deletion of AMOTL2 gene in human pluripotent stem cells (hPSCs) changes cell and colony morphology, increases confluence and proliferation. High-resolution microscopy has revealed alterations in the organization and structure of F-actin cytoskeletal fibers and adhesion markers in AMOTL2 KO hPSCs. RNA-seq analysis has also revealed substantial alterations in the expression levels of markers regulating pluripotency, mitochondrial activity, and calcium ion homeostasis in AMOTL2 KO cells. Moreover, the influence of AMOTL2 on the activity of the Hippo pathway and its main effector, YAP1, was evaluated. This addressed the question of whether AMOTL2 mediates the transmission of mechanical signals through the cytoskeleton to YAP1. Spontaneous differentiation of AMOTL2 KO hPSCs suggests defective induction of endoderm *in vitro*. These findings indicate that AMOTL2 is a significant regulator of Hippo signaling pathway, and through the interaction with the cytoskeleton, may controls the function of hPSC homeostasis and development of human β-cells.

P.10.3

Unraveling the regulatory role of the splicing factor 3 complex in hematopoietic stem cell aging

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Aging is the primary risk factor for many human diseases. In blood, it manifests in myeloid lineage skewing, compromised immunity, & clonal hematopoiesis. That contributes to anemia, myeloproliferative disorders, & leukemia. Senile hematopoietic stem cells (HSCs) lose their ability to generate mature blood cells, indicating age-related stem cell exhaustion. We and others show that the loss of pre-mRNA splicing integrity appears to be detrimental for the upkeep of the homeostatic pathways in HSCs. Despite this, an in-depth analysis of alternative splicing (AS) patterns and spliceosome function upon HSC aging is lacking. We recently described that blocking c-Myc-driven regulation of SF3B1, results in the rewiring of aging-associated molecular circuitries. We posit that this molecular axis is involved in aging of HSCs.

To determine age-dependent splicing alterations in HSCs, we employ combinations of in vitro primary HSC cultures with transcriptomic analysis of AS patterns during physiological aging at single cell and population levels. Further, we delineate dynamics of individual SFs in aged vs. young individuals to chart landscape of alternative splicing upon HSC exhaustion. We find that certain SF3 complex components are more prone to the age-related interferon stress response.

Together, we postulate that expression of proteins from SF3 splicing nodes is 'tuned' to meet demands of HSC aging, which likely endows HSCs with specific splicing patterns critical for cellular responses in elderly.