

Swiss Stem Cell Network

15th Annual Meeting 2020

Innovation in Clinical Bio-manufacturing

Organoids and other
specialized cell cultures
for clinical applications

Friday Dec 11, 2020


9:30 am - 5:30 pm

eMeeting by zoom

Confidentiality clause:
Limited to registered attendees.
No recordings of any kind.
No screen shots.



Gold/Silver:

 **biotechnet**
switzerland **CELLNTEC**
ADVANCED CELL SYSTEMS **Miltenyi Biotec** **Nikon** **Schweizerische Eidgenossenschaft**
Confédération suisse
Confederazione Svizzera
Confederaziun svizra
Innovations- / Schweizerische Agentur
für Innovationsförderung **sysmex**

Bronze:

CSL Behring

Supporter:

 **ZEISS**

Speakers

Organoids

Hayley Francies

Translational Cancer Genomics at
Wellcome Trust Sanger Institute Hinxton, UK

Matthias Lütolf

Laboratory of Stem Cell Bioengineering
EPFL Lausanne, Switzerland

Ivan Martin

Department of Biomedicine & Department of
Surgery, University Hospital Basel, Switzerland

Cell Therapies

Lukas Sommer

Institute of Anatomy
University of Zurich, Switzerland

Jérôme Guicheux

French National Institute for Health and Medical Research
INSERM U1229, Nantes Université, France

Michele de Luca

Centre for Regenerative Medicine «Stefano Ferrari»
University of Modena and Reggio Emilia, Italy

Meet the Experts

Hayley Francies

Translational Cancer Genomics at
Wellcome Trust Sanger Institute Hinxton

Marianna Kruithof-de Julio

DBMR, Medical Faculty and Urology, University of Bern

Scientific Advisory Board

BCPM

Mark Rubin (Lead)

Director of DBMR, Medical Faculty and Oncology,
University of Bern

Marianna Kruithof-de Julio

DBMR, Medical Faculty and Urology, University of Bern

Sven Rottenberg

DIP, Vetsuisse Faculty, University of Bern

SCRM

Eliane J. Müller (Lead)

DBMR, Medical Faculty and Molecular Dermatology,
University of Bern

Carsten Riether

DBMR, Medical Faculty and Oncology, University of Bern

Amiq Gazdhar

DBMR, Medical Faculty and Pneumology, University of Bern

Organizing Committee

BCPM

Timo Staub (Lead)

Bern Center for Precision Medicine (BCPM),
University of Bern

Stephan Christen

DBMR, Medical Faculty, University of Bern

Claudia Requeta

Bern Center for Precision Medicine (BCPM),
University of Bern

SCRM

Volker Enzmann (Lead)

Department of Ophthalmology, University of Bern

Benjamin Gantenbein

Department of Orthopaedic Surgery and Traumatology,
University of Bern

Rene Aeberhard

DBMR, Medical Faculty, University of Bern

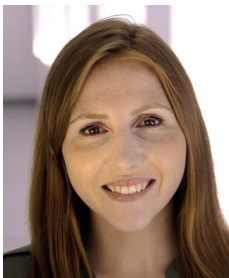
Program schedule

9:30 – 9:45	Welcome Prof Mark A. Rubin (BCPM) / Prof Eliane J. Müller (SCRM)
	Organoids – chair: Prof Mark A. Rubin
9:45	Trailer Sysmex Suisse AG
9:45 – 10:30	Keynote Hayley Francies <i>Translational Cancer Genomics at Wellcome Trust Sanger Institute, Hinxton, UK</i> Towards the next-generation of cancer cell models
10:30 – 10:45	Selected Speaker from Abstract #1 – Simon Leonard April-Monn , University of Bern
10:45 – 11:15	Matthias Lutolf <i>Laboratory of Stem Cell Bioengineering, EPFL Lausanne, Switzerland</i> Engineering scalable organoid-based assays for disease modelling
11:15 – 11:45	Coffee Break / 11.45 Trailer Nikon (Schweiz) GmbH
11:45 – 12:15	Ivan Martin <i>Department of Biomedicine and Department of Surgery, University Hospital Basel, Switzerland</i> Engineering 3D organotypic niches
12:15 – 12:30	Selected Speaker from Abstract #2 – Simon Blanchoud , University of Fribourg
12:30 – 14:00	Lunch break / Sponsor trailer loop
13:00 – 13:55	Meet the Experts <i>on Organoid culture during lunch e.g. Marianna Kruithof-de Julio / Hayley Francies</i>
	Cell therapies – chair: Prof Eliane J. Müller / Volker Enzmann
13:55	Trailer Miltenyi Biotec B.V. & Co. KG
14:00 – 14:30	Lukas Sommer <i>Institute of Anatomy, University of Zurich, Switzerland</i> Neural crest stemness in regeneration and cancer formation
14:30 – 14:45	Selected Speaker from Abstract #3 – Catherine Pfefferli , University of Fribourg
14:45 – 15:00	5 Selected Flash Presentations from Abstracts #4–8, 2 minutes – Laura Jahnke, Thomas Bise, Hendrik Oudhoff, Andreas Shaun Croft, Patricia Renz
15:00 – 15:30	Jérôme Guicheux <i>French National Institute for Health and Medical Research INSERM U1229, Regenerative Medicine and Skeleton Research Center, Nantes Université, France</i> Exploring stem cells for the regenerative medicine of intervertebral disc: a paradigm shift in spine surgery
15:30 – 15:45	Selected Speaker from Abstract #9 – Nenad Slavko Suknovic , University of Geneva
15:45 – 16:15	Coffee Break / 16.15 Trailer CELLnTEC Advanced Cell System AG
16:15 – 17:00	Keynote Michele De Luca <i>Centre for Regenerative Medicine «Stefano Ferrari», University of Modena and Reggio Emilia, Italy</i> Epithelial stem cells in cell and gene therapy
17:00 – 17:15	Poster Award / Closing Remarks Prof Volker Enzmann (SCRM / SSCN board) Prof Mark A. Rubin (BCPM) and Prof Eliane J. Müller (SCRM)

Keynote

Cancer, translational, functional genomics, drug screening, CRISPR

Towards the next-generation of cancer cell models



Hayley Francies

*Translational Cancer Genomics at Wellcome Sanger Institute,
Hinxton, UK*

Cell lines derived from patient tumours have contributed tremendously to our understanding of cancer biology and therapeutic drug response. However, cancer cell lines grown in 2D in vitro culture have several limitations including failing to represent the heterogeneity of cancer. The derivation of human epithelial organoid models grown in 3D culture could transform the preclinical cancer setting by better reflecting the biology of the tumour of origin, and by providing more predictive models of patient responses to therapies. The Human Cancer Models Initiative (HCMI), an international consortium, intends to derive the next-generation of cancer cell models. Across the UK, tumour specimens following biopsy or surgery are collected and sent for organoid derivation. Clinical history for each sample is acquired including age, disease stage and prior lines of therapy. Derived organoids are subjected to targeted gene sequencing to confirm tumour origin as well as whole genome and RNA sequencing, as well as drug and gene perturbation screens. All models and datasets are made available to the research community via ATCC and web-portals respectively. To date the Sanger Institute has generated and genetically characterised more than 150 cancer organoid models. Very good concordance is observed between the genomics of the tumour and matched organoid. Continual genomic analysis during extended culture indicates acquisition of further driver mutations is rare and consistent variant allele fractions of original driver mutations. A new web-portal, the Cell Model Passports has been developed as a mechanism to share clinical as well as genomic and phenotypic datasets for these models. Over the coming years the Sanger Institute hopes to contribute in the region of 600 genomically and phenotypically characterised cell models from a variety of cancer types to the HCMI. We hope these transformative datasets will contribute to the identification of new drug targets and biomarkers of drug response in order to improve patient care.

Main speaker

Organoids, stem cells

Engineering scalable organoid-based assays for disease modelling



Matthias P. Lutolf

Laboratory of Stem Cell Bioengineering, Institute of Bioengineering, School of Life Sciences and School of Engineering, École Polytechnique Fédérale de Lausanne (EPFL), Switzerland

Organoids form through poorly understood morphogenetic processes in which initially homogeneous ensembles of stem cells spontaneously self-organize in suspension or within permissive three-dimensional extracellular matrices. Yet, the absence of virtually any predefined patterning influences such as morphogen gradients or mechanical cues results in an extensive heterogeneity. Moreover, the current mismatch in shape, size and lifespan between native organs and their in vitro counterparts hinders their even wider applicability. In this talk I will discuss some of our ongoing efforts in developing next-generation organoids that are assembled by guiding cell-intrinsic self-patterning through engineered stem cell microenvironments. The improved reproducibility, scalability and function of these engineered organoids opens up new prospects for drug discovery and regenerative medicine.

Main speaker

Bone marrow model, 3D tumor model, stromal niche

Engineering 3D organotypic niches



Ivan Martin

Department of Biomedicine and Department of Surgery, University Hospital Basel, Switzerland

Culture models based on human cells and capturing features of tissue physiology and pathology are key to advance fundamental understanding of development, to learn how to regulate homeostatic processes, and to test new therapies in degenerative or malignancy settings. This lecture will provide an overview of past and ongoing work of the group on the generation and use of 3D human organotypic bioreactor-based models. Following establishment of mesenchymal cell-based stromal tissues and embedding of vascular structures using perfusion flow, the miniaturization into microfluidic models will be introduced to increase throughput, include mechanical loading features and identify compounds regulating lineage commitment and rescuing from catabolic processes.

Generated tissues will also be presented as stromal niches to sustain hematopoietic stem cell growth and differentiation, thus recapitulating in vitro the structure and function of bone marrow organs. Introduction in the system of malignant hematopoietic cells (e.g., from patients with myeloproliferative neoplasm or acute myeloid leukemia) will exemplify the possibility to model bone marrow pathologies in personalized settings.

Finally, use of cancer cell lines and human primary tumor specimens will illustrate that the developed culture platform allows to engineer tumor models and to generate patterns of response to drugs or immunotherapy strategies, which cannot be mimicked by 2D cultures or by more simple spheroids. Ongoing studies are targeting the connection of bone/bone marrow with tumor models to investigate metastatic processes of prostate and breast tumor cells.

Main speaker

Neural crest stem cells, skin repair, skin tumors

Neural crest stemness in regeneration and cancer formation



Lukas Sommer

Institute of Anatomy, University of Zurich, Switzerland

During embryonic development of vertebrates, neural crest stem cells (NCSCs) give rise to a variety of cell types including peripheral neurons and glia as well as melanocytes. Our lab has shown that cells with NCSC properties cannot only be isolated from embryos, but also emerge in adult tissue, for instance upon wounding of the skin, initiation of melanoma, or establishment of melanoma metastases. NCSC-like cells are crucial for both wound repair and tumorigenesis by contributing cells to new tissue as well as by interacting with other cell types, including fibroblasts and immune cells. In our research, we aim to identify the regulatory networks regulating a “NCSC state” in development, regeneration and tumorigenesis. Moreover, we investigate how NCSC-like cells modulate their cellular environment. Knowledge of the similarities and differences in the mechanisms controlling neural crest stemness in development and disease might point to novel treatment strategies.

Main speaker

Stem cells, intervertebral disc, low back pain, biomaterials

Exploring stem cells for the regenerative medicine of intervertebral disc: a paradigm shift in spine surgery



Jerome Guicheux

INSERM UMRS 1229-RMeS, Regenerative medicine and skeleton research centre, Nantes University School of Dental Medicine, Nantes University Hospital, Nantes, France

Degenerative disc disease (DDD) is one of the major causes of low back pain (LBP). Currently, LBP is primarily managed by pharmacological treatments and if unsuccessful by surgical procedures (spine fusion or arthroplasty) that are reserved for severe debilitating LBP. To clinically address LBP earlier in the degenerative cascade of IVD, biology-inspired regenerative strategies could offer less invasive and etiological alternatives to spinal reconstructive surgery. Considering their tissue regenerative abilities, anti-inflammatory and immunomodulatory properties and their promising clinical outcomes in knee OA, the intradiscal injection of mesenchymal stem/stromal cells (MSC) have been contemplated with a growing interest.

In this context, we will first share our view of the mesenchymal stromal cells (MSC)-based therapeutic approaches that have been preclinically developed and, for some of them, clinically transferred in patients with DDD-associated LBP. Then, we will comment on the recent biomaterial-assisted MSC therapies that recently entered the preclinical and clinical scene and discuss whether injectable biomaterials that could be used as cell carriers and percutaneously transplanted into degenerated IVDs may be a relevant therapeutic strategy. We will also discuss whether induced pluripotent stem cells (iPS) could be a relevant cell source for the production of regenerative IVD cells and whether iPS-derived disc cells may warrant clinical use.

Finally, we will present some recent perspectives (3D Bioprinting, extracellular vesicles, endogenous regeneration, annulus repair...) and whether these concepts may pave the way of future therapeutic developments for DDD-associated LBP.

Keynote

YAP/TAZ, JEB, Gene Therapy

Epithelial stem cell in cell and gene therapy



Michele De Luca

*Centre for Regenerative Medicine «Stefano Ferrari», University of
Modena and Reggio Emilia, Italy*

Purpose: LAMB3-dependent generalized Junctional Epidermolysis Bullosa (JEB) was targeted by transplantation of epidermal cultures originated from transgenic epidermal stem cells. We report life-saving regeneration of the entire epidermis on a seven-year-old JEB child suffering from a devastating form of JEB.

Methods: The regenerated transgenic epidermis remained stable throughout the entire follow-up period and did not form blisters, even upon shear force. The proviral integration pattern was maintained in vivo and epidermal renewal did not cause any clonal selection. Clonal tracing showed that the human epidermis is sustained by a limited number of long-lived stem cells, detected as holoclones, that can extensively self-renew and produce short-lived progenitors that replenish terminally differentiated keratinocytes.

Results: In studying the different behaviour of JEB and COL7A1-dependent generalized Dystrophic EB (RDEB) cultures we discovered a pivotal role of YAP in sustaining human epidermal stem cells, which explains the progressive stem cell loss observed in JEB. Epidermal stem cell depletion of primary JEB keratinocytes is due to perturbation of the YAP/TAZ pathway. YAP/TAZ expression is significantly decreased in JEB keratinocytes, which do not contain nuclear YAP but only phosphorylated, inactive YAP. The JEB phenotype is recapitulated by Laminin 5 ablation and consequent YAP/TAZ down-regulation in normal cells. Restoration of adhesion properties by Laminin 5-gene therapy rescues normal nuclear levels of YAP/TAZ and clonogenic potential. Enforced YAP recapitulates Laminin 5-gene therapy in JEB cells, thus uncoupling adhesion from proliferation in epidermal stem cells.

Conclusion: This work has important clinical implication for an efficient ex vivo gene therapy of JEB.

Abstract #1

Organoids, Precision Medicine, Tumor, 3D culture, Pancreatic Tumor, Neuroendocrine, Drug screening

Patient-derived islet-like tumoroids – towards more personalized medicine in PanNETs

Simon Leonhard April-Monn^{1,2}, Renaud Maire¹, Marco Schiavo Lena³, Mafalda Trippel¹, Francesca Muffatti⁴, Valentina Andreasi⁴, Claudio Doglioni^{3,5}, Corina Kim-Fuchs⁶, Beat Gloor⁶, Stefano Partelli^{4,5}, Massimo Falconi^{4,5}, Aurel Perren¹, Ilaria Marinoni¹

¹*Institute of Pathology, Switzerland;*

²*Graduate School for Cellular and Biomedical Sciences University of Bern, Bern, Switzerland;*

³*Unit of Pathology, San Raffaele Scientific Institute, Milan, Italy;*

⁴*Pancreatic Surgery Unit, Pancreas Translational & Clinical Research Center, San Raffaele Scientific Institute, Milan, Italy;*

⁵*Università "Vita e Salute", Milan, Italy;*

⁶*Inselspital, University of Bern, Switzerland;
simon.april@pathology.unibe.ch*

Purpose: Current preclinical models of pancreatic neuroendocrine tumors (PanNETs) do not accurately reflect the patient situation. With this project we aimed at establishing a 3-dimensional (3-D) patient-derived PanNET model that adequately reflects individual tumor characteristics and that allows exploration of personalized in vitro pharmacotyping.

Methods: Tumor cells from 33 cryopreserved PanNET patients were isolated, molecularly- and histomorphologically characterized and matched with original tumor mirror blocks. Established 3-D cultures were kept in a PanNET specific medium based on patient transcriptional expression signatures. Live-cell imaging and time course drug sensitivity were assessed in single-well resolution relying on a non-lytic viability metabolic surrogate. Short- and long-term drug sensitivity profiles were determined using novel per-division based parametrized sensitivity metric approach.

Results: Our PanNET screening platform allows multi-center sample collection. 3-D culture and pharmacotyping pipeline demonstrate a high success rate of 85%. Live cell imaging revealed formation of structures similar to non-neoplastic human pancreatic islets. These patient-derived Islet-like tumoroids (PDIT) retained the histomorphology of original tumors and phenocopied patient proliferation indices. PDIT displayed noticeable inter-patient susceptibilities to clinically approved therapies. Of note, parametrized in vitro drug sensitivity matched with clinical response. Hierarchical cluster analysis revealed three pharmacotypes potentially reflecting patient responses.

Conclusion: We present a 3-D human primary PanNET screening platform optimized for fast and efficient in vitro pharmacotyping holding potential clinical utility and promoting more personalized treatment in PanNETs.

Abstract #2

marine, aquatic, invertebrate, european, networking

Marine/aquatic invertebrate stem cells (MARISTEM): from basic research to innovative applications

L Ballarin¹, B Rinkevich², I Domart-Coulon³, A Varela Coelho⁴,
P Oliveri⁵, A Burzynski⁶, K Bartscherer⁷, E Berezikov⁸, U Berzins⁹,
S Blanchoud¹⁰, N Blom¹¹, S Cambier¹², M Cammarata¹,
R Cardoso Neves¹¹, J Delroisse¹³, D Drobne¹⁴, J Encinas¹⁵, U Frank¹⁶,
B Galliot¹⁰, A Genevieve³, B Hobmayer¹⁷, A Karahan¹⁸, S Krstic¹⁹,
D Krysko¹³, H Löhelaid²⁰, D Lyons²¹, P Martinez¹⁵, S Mladjenovic²²,
N Odintsova²³, E Oruc¹⁸, L Peric²¹, O Petukhova²³, S Piraino¹,
A Pirkovi¹⁹, S Rakers⁷, A Ramšak¹⁴, F Rentzsch²⁴, A Rosner²,
P Schembri Wismayer²⁵, T Silva⁴, I Somorjai⁵, K Stensvåg²⁴,
S Suleiman²⁵, S Vasileiadis²⁶, Z Ziaka²⁶

¹Italy; ²Israel; ³France; ⁴Portugal; ⁵United Kingdom; ⁶Poland; ⁷Germany; ⁸Netherlands;
⁹Latvia; ¹⁰Switzerland; ¹¹Denmark; ¹²Luxembourg; ¹³Belgium; ¹⁴Slovenia; ¹⁵Spain; ¹⁶Ireland;
¹⁷Austria; ¹⁸Turkey; ¹⁹Serbia; ²⁰Estonia; ²¹Croatia; ²²Bosnia and Herzegovina; ²³Russia;
²⁴Norway; ²⁵Malta; ²⁶Greece; simon.blanchoud@unifr.ch

The “stem cells” discipline represents one of the most dynamic areas in biology and biomedicine. The vast majority of research on stem cells is currently being conducted in vertebrate models, which usages are being increasingly restricted and regulated. However, stem cell research has already greatly benefited from discoveries in evolutionary more distant animals. Yet, the study of these organisms has not been pursued vigorously. In this context, marine and aquatic invertebrate stem cell (MISC) biology is of prime research and medical interest.

Marine and aquatic invertebrates as a whole show the largest biodiversity and the widest phylogenetic radiation on Earth, from morphologically simple organisms (e.g., sponges, cnidarians), to the more complex mollusks, crustaceans, echinoderms and protochordates. Likewise, they illustrate a kaleidoscope of MISC-types that participate in the production of numerous bioactive-molecules as well as in aging and regeneration phenomena, including whole-body regeneration. Consequently, their study is of significant interest for human welfare. Up to now, the European MISC-community is highly fragmented and very scarce ties were established with biomedical industries to harness this potential. This European COST Action aims at fostering the study of MISCs by strengthening the European MISC community, and integrating the MISC field with biomedical disciplines for innovative applications. Our action organizes topical workshops, training schools and short term scientific mission grants on MISCs.

26 European or associated countries are already participating in the COST Action MARISTEM, the main network for MISC research. Join the emergence of marine and aquatic invertebrate models, join our action: <http://www.maristem.eu>!

Abstract #3

Zebrafish, heart, regeneration, neoplastic growth

HRas-induced cardiac neoplasia shares common features with heart regeneration in zebrafish

Catherine Pfefferli, Marylène Bonvin, Steve Robatel, Désirée Koenig, Anna Jazwinska

University of Fribourg, Switzerland; catherine.pfefferli@unifr.ch

Purpose: The mammalian heart is devoid of regeneration-competent cells and cardiac tumors are extremely rare. The zebrafish heart can efficiently regenerate after injury through cardiomyocyte dedifferentiation and proliferation. Whether the high cellular plasticity of zebrafish cardiac cells might cause a susceptibility to neoplasia remains unknown.

Methods: We establish a strategy to conditionally overexpress the oncogene HRASG12V in the zebrafish myocardium.

Results: The temporal HRASG12V overexpression in the mature myocardium resulted in heart overgrowth. The malformed myocardium displays common characteristics with the regenerative myocardium, such as reactivation of cardiac embryonic programs, incomplete differentiation, enhanced cell proliferation, metabolic changes, intramyocardial matrix remodeling and leucocyte recruitment. We found that HRAS-dependent cardiac tumorigenesis was mediated by mTOR signaling, as visualized by phosphorylation of its target ribosomal protein S6. Inhibition of TOR by rapamycin counteracted hyperproliferation, partially rescuing the phenotype. A similar involvement of TOR signaling in regulation of cell proliferation was also evident during heart regeneration.

Conclusion: We show that the juvenile and the adult zebrafish hearts are susceptible to neoplastic transformation, and the underlying mechanisms share similar molecular signatures involved in regeneration. Our comparative study of zebrafish cardiomyocytes challenged to either regenerative or neoplastic growth may provide insights about the permissive and restrictive factors regulating organ renewal and homeostasis.

Abstract #4

Gliosis, Retina, Regeneration, Collagen, Zebrafish

Involvement of fibrillary collagens in the regeneration process after laser-induced retinal degeneration in the zebrafish

Laura Jahnke^{1,2,3}, Federica Conedera⁴, Volker Enzmann^{1,2}

¹Department of Ophthalmology, University Hospital of Bern, University of Bern, Bern, Switzerland;

²Department of BioMedical Research, University of Bern, Bern, Switzerland;

³Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland;

⁴Department of Dermatology, Massachusetts General Hospital, Boston, USA;
laura.jahnke@extern.insel.ch

Purpose: After injury, various collagens and the extracellular matrix build temporarily a scaffold to stabilize the surrounding tissue and thus support the wound healing. We want to understand the role of fiber-forming collagens during glial scar formation after damage in the vertebrate retina and their role in the ensuing repair.

Methods: Focal retinal damage was induced by a 532 nm diode laser and spots were visualized via optical coherence tomography (OCT). To investigate the influence of collagens during regeneration, the ZF were treated 1 h post laser injury (PI) with the fibrinolysis inhibitor tranexamic acid (TXA; concentration 20 mg/L) given once a day via the tank water for up to 7 days. Six Sensory retinas with 10 laser spots were prepared for qRT-PCR analysis to quantify the gene expression of different collagens. Furthermore, eyes bearing 4 laser spots were enucleated on days 3 and 7 PI for immunohistochemical staining.

Results: The retinal samples showed the expression of all tested collagen types. Thereby, time course of collagen expression after laser injury showed significant differences between the known fiber-forming collagens at day 3 and day 7 PI. The most attention was given to the collagens 5A3B and 1A2, which have the potential to form short time scaffolds for the repair mechanism of the proliferative phase during wound healing. Collagen 5 was detected by immunohistochemistry on day 7 PI.

Conclusions: Based on the results we can exclude the involvement of certain collagen subtypes in the glial scar development during regeneration. On the other hand, the overexpressed collagens present a possibility for modulation of the glial scar formation that hinders endogenous retina regeneration in mammals.

Abstract #5

careg, Retina, Regeneration, Müller Glia

Careg element as a new marker to study müller-glia mediated retina regeneration in adult zebrafish

Thomas Bise, Catherine Pfefferli, Anna Jazwinska

University of Fribourg, Switzerland; thomas.bise@unifr.ch

Purpose: Zebrafish can regenerate organs such as the heart, fins and retina throughout their entire life. A previous study from our lab has demonstrated that regenerating cells of the fin and the heart upregulate a transgenic reporter called *careg*. In this study, we investigate the activation of this reporter during retina regeneration in adult zebrafish.

Methods: To investigate this topic, we induce retinal injury by N-Methyl-N-Nitrosourea (MNU) treatment that depletes rod photoreceptors in adult zebrafish. scRNAseq was then performed on regenerating retinas at different stages to ensure a transcriptomic overview of the main regenerating phases.

Results: In this study, we identified that the *careg* reporter is also activated during retina regeneration. We found that after MNU-induced damage, both *careg:eGFP* and *careg:dmKO2* are expressed in Müller glia and this expression persists until completion of regeneration. A cell-lineage tracing analysis showed that replaced photoreceptors indeed derived from *careg* expressing cells. A TGFb pathway inhibition with SB431542 induced a decreased *careg* expression in regenerating cells.

Conclusion: Cell-lineage tracing coupled with scRNAseq analysis demonstrates that *careg* transgenic element is a new marker of activated Müller glia. Moreover, our study supports the model that common genetic programs, such as the TGFb pathway, are involved in the activation of regeneration-competent cells in various zebrafish organs.

Abstract #6

Preconditioning, serum analysis, proteomics

Preconditioning effects of serum injection in zebrafish adult hearts

Hendrik Oudhoff, Thomas Bise, Anna Jazwinska

University of Fribourg, Switzerland; hendrik.oudhoff@unifr.ch

Purpose: Zebrafish cannot only fully regenerate their heart after injury, but display a fascinating preconditioning ability, for example through thoracotomy. Immediate effectors of preconditioning are believed to be present in the blood circulation of the animal. In order to further elucidate the proteins and pathways involved a proteomic analysis of the conditioned serum is to be performed. Ideal timepoints of blood collection can be determined by injection of conditioned serum into uninjured zebrafish.

Methods: Cryoinjury after Chablais et al. (2012). Blood collection following heart cryoinjury according to Zang et al. (2005), centrifugation and collection of serum. Conditioned serum injection in Tg(cmlc2:DsRed2-Nuc) adult fish.

Results: Significant myocardial cell proliferation is observable in Tg(cmlc2:DsRed2-Nuc) hearts after injection of 6 hours post cryoinjury (hpci) serum, compared to control hearts. Very early as well as advanced timepoints of serum collection fail to convey the same effect. Interestingly a preconditioning response is noticeable again with serum collected 4 days after cryoinjury. In the pilot proteomic analysis of zebrafish serum of 6 and 96 hpci, protein intensity profiles show distinctive patterns compared to control hearts.

Conclusion: Factors responsible for precondition can be transferred to an unconditioned fish for similar effect. These effectors could possibly be a systemic reaction to any injury. With the proteomic data new coherences of precondition and its protein expressions as well as involved pathways in the serum might be elucidated.

Abstract #7

cryopreservation, trilineage differentiation, culture media, stem cell, biological therapies

Trilineage potency of human nucleus pulposus cells before and after cryopreservation

Andreas Shaun Croft

University of Bern, Switzerland; andreas.croft@dbmr.unibe.ch

Purpose: In the past, progenitor cells have been identified within the nucleus pulposus (NP) of human intervertebral discs (IVD). However, little is known about the effect of expansion and cryopreservation on here called “heterogenic” human NP cells (hNPCs) and their stemness. Therefore, we aimed i) to expand hNPCs and to investigate their differentiation potential before and after cryopreservation and ii) to find an optimal approach for cryopreservation.

Methods: HNPs were obtained from six patients (4 trauma and 2 degenerated) undergoing spinal surgery. Cells were then differentiated into osteogenic, adipogenic or chondrogenic lineages for 21 days or were cryo-preserved for one week at -150°C in five different cryo-media to compare their effect on the cell's viability (CV) and multipotency. CV was determined by flow cytometry using propidium iodide. The differentiation potential was assessed using qPCR and histology.

Results: HNPs from trauma patients showed the presence of lipid droplets and upregulated adiponectin (up to 2300-fold). Furthermore, cells cultured in chondrogenic medium expressed up to 750-fold more collagen type 2. Remarkably, osteogenic differentiation was less pronounced and was subjected to large donor variability. After cryopreservation, the hNPCs' CV was comparable for all five tested cryo-media (~82% CV) and no significant changes in the cells' stemness were observed. However, hNPCs from degenerated discs could only undergo chondrogenesis.

Conclusion: HNPs from trauma IVDs, which were presumably non-degenerated, may differentiate into a chondrogenic lineage, and also partially into an adipo- and osteogenic lineage. Furthermore, all five tested cryopreservation media seem to perform the same in terms of CV and maintaining hNPCs' stemness.

Abstract #8

White matter injury, astrocyte, inflammatory reactive astrocytes, immunopanning, preterm birth

Neuroprotection in preterm birth by modification of astrocyte polarization

Patricia Renz^{1,2}, Vera Tscherrig¹, Valérie Haesler¹, Shane Liddelow³,
Daniel Surbek¹, Andreina Schoeberlein¹, Amanda Brosius Lutz¹

¹Department of Obstetrics and Gynecology, Inselspital, Bern University Hospital and
Department for BioMedical Research (DBMR), University of Bern, Switzerland;

²Graduate School for Cellular and Biomedical Sciences (GCB), University of Bern,
Switzerland;

³Neuroscience Institute, Department of Neuroscience and Physiology, New York
University (NYU), New York City, New York, USA; patricia.renz@dbmr.unibe.ch

White matter injury (WMI) is the most common form of brain injury in preterm infants. It is characterized by reactive microgliosis and astrocytosis, defective oligodendrocyte maturation, and in severe cases, neuronal death. Recent studies in the mature brain show the formation of diverse reactive astrocyte subtypes after injury, some favoring brain repair and other «inflammatory» reactive astrocytes contributing to neurodegeneration. The specific nature of astrocyte reactivity after WMI remains obscure. Here we report the results of experiments aimed to investigate inflammatory astrocyte formation in WMI.

WMI was induced in rats at postnatal day 2 (P2) using a combination of hypoxic-ischemic and inflammatory insults. In situ hybridization (ISH) with probes for inflammatory astrocyte-specific mRNA transcripts was performed on injured and healthy brains at multiple post-injury timepoints. To confirm WMI, immunohistochemistry (IHC) was performed on injured and healthy brains at P11. We used immunopanning to purify astrocytes from injured and healthy brains. mRNA isolated from these cells was used for microfluidic qRT-PCR analysis using known markers of reactive astrocyte subtypes. ISH demonstrate a significant increase of inflammatory astrocytes in subcortical white matter tracts in our rodent models, while IHC showed the severity of the WMI. An astrocyte immunopanning protocol optimized for our disease model yields acutely purified viable primary astrocytes.

We demonstrate the formation of inflammatory reactive astrocytes in rodent models of WMI. This result is an important step towards understanding astrocyte polarization in WMI and opens the door to experiments investigating whether preventing the formation of this astrocyte subtype ameliorates WMI disease outcomes.

Abstract #9

stem cells, paracrine signaling, injury-induced ROS signals, mitochondrial superoxide, injury-induced cell death

ROS signaling and injury-induced cell death drive head regeneration in hydra

Nenad Slavko Suknovic, Silke Reiter, Brigitte Galliot

Department of Genetic and Evolution, Institute of Genomics and Genetics in Geneva (iGE3), Faculty of Sciences, University of Geneva, Switzerland; nenad.suknovic@unige.ch

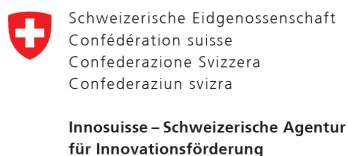
Purpose: To understand how injury-induced signaling triggers regeneration, we use Hydra, a bilayered animal made up of epithelial and interstitial stem cells (ESCs, ISCs). After mid-gastric bisection, head regeneration (HR) relies on an asymmetric activation of MAPK/ERK kinase followed by massive cell death among interstitial-derived cells, while MAPK/ERK activity and cell death remain low in the foot-regenerating (FR) half. Our aim was to understand how asymmetric injury-induced signaling arises.

Methods & Result: We focused on ROS signals and identified with MitoSOX an immediate symmetrical production of mitochondrial superoxide (mtO_2^-) in ESCs on each side of the cut. By contrast, we measured higher levels of hydrogen peroxide (H_2O_2) in HR- than in FR-halves. Upon mtO_2^- scavenging by the antioxidant Tiron, H_2O_2 production is decreased, cell death no longer observed in HR halves while wound healing and HR are delayed. To enhance mtO_2^- levels, we knocked down by RNAi the superoxide dismutase *sod1*, and noted the ectopic production of mtO_2^- post-injury together with an enhanced wound healing. Animals treated with the NOX inhibitor DPI show a partial blockade of H_2O_2 production after bisection, a delay in wound healing and head regeneration, pointing to additional non-mitochondrial sources of injury-induced ROS.

Conclusion: We propose a scenario where the injury-induced symmetrical production of mtO_2^- triggers wound healing independently of the regenerative process. In HR tips, the low levels of H_2O_2 generated by mtO_2^- triggers apoptosis of the surrounding interstitial cells. Dying cells amplify H_2O_2 production and paracrine signaling between cell-death resistant ESCs and cell-death-sensitive ISCs, a process essential for launching head regeneration.

Sponsors und Supporter

Gold/Silver



Bronze:

CSL Behring

Supporter:

