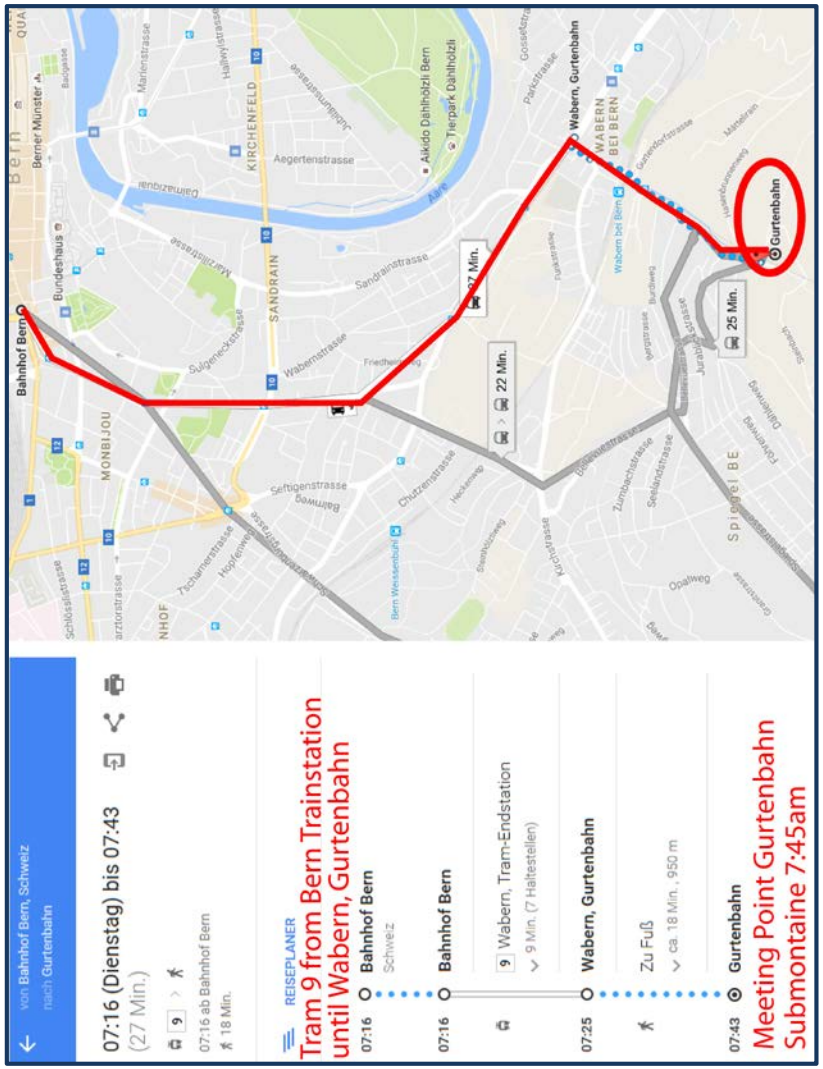
Map to get to the Retreat



In case you cannot make it in time or there is any other urgency please call 0779601230

4th SCRM PhD Students Retreat

Gurten, Bern
1st September 2017

Platform for
Stem Cell Research
in Regenerative
Medicine

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Graduate School
for Cellular and
Biomedical Scienc

UNIVERSITÄT BERN

Dear participants,

After three years of successful SCRM PhD Student Retreats, we are glad to welcome you once more top of the Gurten Mountain here in Bern.

We will start with a welcome coffee and a short address given by the organizing committee. The program then continues with sessions of PhD project presentations and two coffee breaks for networking and poster viewing.

We are extremely thankful to be receiving mentoring from Doctor Christine Weber and Professor Fredric Manfredsson, and look forward to their interesting Keynote lectures.
We are looking forward to meet you and we wish you a fruitful and pleasant time during the retreat.

Sincerely yours,

The organizing committee
Felix Baier
William Hariton
Magdalena Brunner

Program of the 4th Stem Cell PhD Student Retreat

7:45	7:55	Meeting point at the Gurtenbahn Bern (Submontane)
8:00	8:15	Welcome Coffee
8:15	8:20	Welcome by the Organizing Committee
		Morning Session Chair: Felix Baier
8:20	8:40	Gierin Thomi
8:40	9:00	Daniela Frauchiger
9:00	9:20	Rahel May
9:20	9:40	Pierre Balmer
9:40	10:00	Patricia Meyer
10:10	10:40	Coffee Break
10:40	11:00	William Hariton
11:00	11:20	Dzhuliya Dzhonova
11:30	12:30	Doctor Christine Weber, Associate Editor Nature Cell Biology (UK) **"Publishing in the Nature journals: the editor's perspective"**
12:30	14:00	Lunch Break
		Afternoon Session Chair: William Hariton
14:00	14:20	Stojiljkovic Ana
14:20	14:40	Felix Baier
14:40	15:00	Appiah Joddy
15:00	15:20	Anina Bauer
15:20	16:00	Coffee Break
16:10	17:10	Professor Fredric Manfredsson, Michigan State University (US) **"Gene Therapy Modalities of the 21st century"**
17:10	17:20	Conclusive Remarks by the Organizers and the SCRM Steering Committee
17:30		Apero Riché

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Anti-Inflammatory Potential of Extracellular Vesicles Derived from Wharton's Jelly Mesenchymal Stem/Stromal Cells on Microglia

Wharton's jelly mesenchymal stem/stromal cells (WJ-MSC) have the capacity to reduce neuroinflammation and induce tissue regeneration in perinatal brain damage despite of their low long-term survival in host tissue. The therapeutic function of WJ-MSC is mainly ascribed to their paracrine function including the shedding of cell-derived extracellular vesicles (EV). The aim of this study is to evaluate the anti-inflammatory potential of WJ-MSC-derived EV on microglia cells in vitro.

WJ-MSC-derived EV were isolated from cell culture supernatants using a protocol consisting of several steps of successive high-speed centrifugations and ultracentrifugations. The microglia cell line BV-2 was activated by 6 and 24 h of lipopolysaccharide (LPS) stimulation and used as an in vitro model for infection-associated perinatal brain damage. WJ-MSC-derived EV pre-stained with the fluorescent cell tracker dye CM-DiI were co-cultured with microglia cells before visualized with immunocytochemistry (ICC). After co-culture with WJ-MSC-derived EV, microglia cells were evaluated for their expression of activation markers and production of pro-inflammatory cytokines in response to LPS stimulation by real-time PCR and enzyme-linked immunosorbent assay (ELISA).

In co-culturing experiments, WJ-MSC-derived EV co-localize with microglia cells and suppress their activation-induced morphological changes in response to 6 h LPS stimulation. Furthermore, WJ-MSC-derived EV dampened the upregulation of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-6 and suppressed the production of inducible nitric oxide synthase (iNOS) in response to 6 h LPS stimulation. WJ-MSC-derived EV also suppressed the increase in TNF-α-secretion in response to 24 h LPS stimulation.

In conclusion, we demonstrate that WJ-MSC-derived EV are potent modulators of microglia activation. Hence not only WJ-MSC, but also WJ-MSC-derived EV are able to support tissue regeneration by reducing inflammation. As a result, WJ-MSC-derived EV might represent a novel cell-free approach to treat perinatal brain damage.

Financial support by Gottfried and Julia Bangerter-Rhyner Foundation.

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Nucleus pulposus progenitor cells – How to get them and their potential uses for intervertebral disc repair

Background A small fraction of precursor cells, positive for Angiopoietin-1 receptor Tie2, was recently identified and isolated within the nucleus pulposus (NP). These NP progenitor cells (NPPC) are of high interest for IVD regeneration [1]. So far, NPPC were isolated by fluorescence-activated cell sorting FACS, here we aim to test two other cell sorting protocols for bovine NPPC and their behaviour when injected back into a bovine degenerated IVD explant model.

Method Bovine NP cells were isolated from fresh 1-year-old NP tissue. Subsequently, one out of three selection methods for Tie2 was applied using the same primary polyclonal antibody against CD202b (Bioss inc.). 1) FACS (FACSaria III, BD Biosciences), 2) Magnetic-activated cell sorting (MACS) (Miltenyi Biotec, Inc.) and 3) size-based sorting using pluriBead® technology (pluriSelect, inc.). Colony forming unit (CFU) assay was carried out with Tie2+ and Tie2- cells respectively by suspending them in Methocult (Stem Cell Technologies) and culturing for 7 days. Labelled FACS sorted Tie2 cells were injected with PBS or fibrin hydrogel into papain degenerated discs and cultured for 7 days. Then, live/dead stain was performed.

Results NPPC could be sorted with all three methods. Although, cell yield differed among sorting methods (FACS: 5.033 ± 4.041, n=9; MACS: 2.075 ± 3.734, n=4; pluriSelect: 0.3376 ± 0.1568, n=2). An explanation for the better cell yield might be a more generous gating. When comparing CFU assay for FACS (n=6, p=0.015) and pluriSelect (n=2) sorted cells a higher colony count was observed for Tie2 positive than negative cells. Injected Tie2 cells could be located with cLSM and showed a higher CV when mixed with PBS. If in mixture with fibrin hydrogel, the CV dropped below 50%.

Conclusion Both MACS and pluriSelect offer a less complex and time consuming sorting method for NPPC. Despite the lower cell yield, they facilitate investigation of in vitro expansion conditions for this cell type and further studies regarding their regeneration potential.

Outlook We aim to investigate the parameters for in vitro expansion of NPPC and to perform further experiments in situ to study behavior and regeneration potential of NPPC.

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Stimulation of Primary Human Osteoblasts and Human Stromal Cells with the BMP Antagonists Gremlin and Noggin

Introduction: The standard treatment for intervertebral disc (IVD) degeneration is discectomy followed by spinal fusion. During spinal fusion the removed IVD is replaced by bone substitute or an autograft and the two adjacent vertebral bodies are fixed with a cage. Clinical observations showed, that partial removal of the IVD during discectomy can lead to a failure of bone formation during the spinal fusion of the affected section. One possible explanation for this phenomenon could be a secretion of BMP antagonists by IVD cells. The BMP pathway plays a crucial role in bone turnover by inducing osteogenesis. BMP antagonists like Gremlin (GREM) and Noggin (NOG) inhibit the osteogenesis by binding to bone morphogenic protein-4 (NOG and GREM) or bone morphogenic protein-2 (GREM) (1). We hypothesized that human primary osteoblasts (OB) or primary human mesenchymal stem cells (MSC) stimulated with NOG and GREM show an inhibition of the osteogenic phenotype.

Methods: OB were isolated from patients undergoing total knee replacement and MSC from spinal surgery patients. Cells were cultured in monolayer up to passage two to minimize dedifferentiation effects. All protocols involving human tissue were ethically approved. OB were seeded at a density of 10'000 cells/cm2 and MSC at a density of 5'200 cells/cm2. OB/MSC were stimulated with 10 or 100 ng/mL NOG and GREM respectively and were applied with osteogenic medium. The control groups were stimulated with osteogenic medium (positive control) and with control medium, α-MEM + 10% FCS (negative control). The respective medium for each experimental group was refreshed every second or third day. After 21 days of culturing in hypoxic conditions (2% O2), matrix mineralization of OB and MSC monolayers were measured by Alizarin red (ALZR) staining. ALZR was quantified by absorbance and normalized to cell activity (Resazurin assay).

Results: After 21 days, a significant increase of ALZR staining of mineralized matrix was observed in the OB culture in osteogenic medium (0.341 ± 0.040, mean ± SEM) compared to OB grown in control medium (0.056 ± 0.009) (M-W test: P = 0.0286). OB stimulated with 100 ng/mL of NOG and GREM showed a significant higher mineralization than the negative control (NOG 0.820 ± 0.336 (Friedman test: P = 0.0124) and GREM 0.659 ± 0.180, Friedman test: P = 0.0393). The same trend was observed in stimulated MSC.

Discussion: In this study we investigated the exogenous effect of BMP antagonists like NOG and GREM on human OB and MSC. After the monitoring and quantification of calcium deposit could we not confirm the hypothesis, that the exogenous stimulation of OB and MSC will lead to an inhibition of the osteogenic phenotype and so to a reduction of matrix mineralization. Conversely, the calcium deposition in both, OB and MSC monolayer, were stimulated with the addition of the BMP antagonists. These findings, question the role of GREM1 and NOG for bone inhibition pathway.

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Unravel cellular and molecular functions of SUV39H2 in skin homeostasis and establish the causative role of a SUV39H2 variant for a canine genodermatosis

Background: Genodermatoses are hereditary disorders affecting the skin. They characteristically exhibit altered epidermal homeostasis known to be driven by stem and/or progenitor cells. In this study, we investigate a canine Hereditary Nasal Parakeratosis (HNPK). HNPK is a monogenic autosomal recessive disorder that is associated with a missense variant in SUV39H2. SUV39H2 encodes a histone 3 lysine 9 methyltransferase, implicated in epigenetic processes, assumed to lead to gene silencing. The identified SUV39H2 variant results in an inactivation of the catalytic domain leading to a non-functional protein. So far the role of SUV39H2 in epidermal homeostasis is unknown while the contribution of epigenetic mechanisms to this process is only starting to being revealed.We hypothesize that SUV39H2 is involved in skin homeostasis through regulating the onset and progression of the orderly differentiation process of epidermal progenitor cells. Accordingly, the aim of my project is to investigate the cellular and molecular contribution of SUV39H2 to epidermal proliferation and differentiation and establish a causative role of the SUV39H2 variant in HNPK disorder.

Methods: RNAseq, RT-qPCR and western blot analyses are used to study the impact of SUV39H2 on keratinocyte differentiation. ChIPseq will be used to measure the degree of methylation of candidate gene promoters identified by RNAseq analysis and luciferase reporter gene assays will be applied to measure the activity of signaling pathways.

Results: Gene expression profiling on skin biopsies of three affected and three healthy control dogs using RNAseq showed several gene expression misregulations, especially in genes i) involved in the transition between proliferation and differentiation of keratinocytes, and ii) in those known to play a role in late keratinocyte differentiation. Pathway analyses on dog patients' biopsies confirmed a misregulation of the Notch and Wnt pathways known to be important for epidermal keratinocytes proliferation and differentiation, as well as for the formation of the cornified envelope.

Conclusion and outlooks: Preliminary observations from a time course study of isolated affected dog nose keratinocytes confirmed an aberrant expression pattern of key proliferation and differentiation markers, including effectors of Notch and Wnt signaling pathways. Together these results suggest that SUV39H2 plays a critical role in the balance between keratinocytes proliferation and differentiation, at least in the nose of these dogs. Causality of the SUV39H2 variant for HNPK is currently being established by siRNA experiments on healthy keratinocytes expected to recapitulate the aberrant proliferation and differentiation pattern. Finally, morphological and immunofluorescence studies will be performed using formalin fixed skin sections from HNPK and control dogs to confirm results obtained on cultured keratinocytes.

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Evaluation of neuronal progenitor cells grafting as regenerative therapy in an ex vivo model of ischemic brain damage consecutive to cardiac arrest

Background:
Sudden cardiac arrest (CA) is the most important cause of global brain ischemia. Due to a lack of effective therapies to treat the subsequent brain damage, most patients are left with incomplete neurological recovery. Several brain regions, including the hippocampus, are particularly affected by a transient hypoxic-ischemic insult. This "selective vulnerability" is observed in rodent models as well as in humans. The aim of this project is to develop an ex vivo surrogate for the in vivo rat model of cardiac arrest/resuscitation, using organotypic hippocampal cultures (OHCs) for the evaluation of regenerative therapeutic approaches using grafting of neuronal stem and progenitor cells (NPCs).

Methods:
Hippocampi from rats pups were cut into slices, cultivated for one week, and then subjected to oxygen-glucose deprivation (OGD) to reproduce in vitro the hypoxic-ischemic injury observed after CA. Duration of OGD was optimized to reproduce the extent and pattern of damage observed in vivo. Neuronal damage was quantified by Fluoro-Jade B (FJ) staining, specific for degenerating neurons. For grafting experiments, NPCs were isolated from hippocampi of newborn rats, expanded as neurospheres and grafted into injured cultures. Immunohistochemistry was used to characterize the cellular composition of neurospheres.

Results:
OHCs submitted to 33 minutes of OGD developed hippocampal damage to a similar extent as observed in the in vivo model. A significantly higher amount of FJ-positive cells was found after OGD in the hippocampal CA1 segment compared to the normoxic control. The cellular composition of the neurospheres, tested by IHC, showed the presence of numerous nestin-, doublecortin- and Ki67-positive cells, confirming the presence of NPCs. Differentiation of these cells towards neurons for one week yielded a significant proportion of βIII-tubulin-positive cells. After NPCs transplantations into OGD-injured OHCs, viable cells were found at different time points after grafting, confirming the overall feasibility of the procedure.

Conclusions and outlook:
We successfully reproduced damage to CA1 neurons of the hippocampus ex vivo after OGD, as observed in vivo after CA. Neurospheres contained proliferating neuronal progenitors with the potential to differentiate into mature neurons. The survival, migration and differentiation of grafted cells are currently assessed using NPCs isolated from Green Fluorescent Protein transgenic rats, therefore facilitating the identification of the grafted cells. This project represents a first step towards a cell-based regenerative therapy for ischemic brain damaged consecutive to CA.

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Desmosomal cadherin Dsg3 transadhesion balances quiescence versus activation in repair of the hair follicle stem cell niche

Hair follicle (HF) stem cells (SC) are set aside in a specialized niche called the bulge (BuSC) to support lifelong HF cycling and regeneration. How the HF bulges are repaired in case of injury is poorly understood. We used the skin disease pemphigus vulgaris (PV) with anti-desmoglein 3 antibodies (AK23) as a model to induce blisters in the mouse telogen (quiescent) HF stem cell niche and study molecular mechanisms of repair. This mouse model replicates the acantholysis seen in PV patients and repair in Dsg3-/- knockout mice.

Combining functional analyses with SC marker expression and associated pathways, we here focused on the specific signaling pathways modulating acantholytic (pathological) and repair processes. During the pathological phase, we observed that loss of Dsg3 mediated cell-cell adhesion (inducing bulge blisters) is preceded by activation of quiescent BuSC resulting in a loss of stemness, as seen by reduced colony forming efficiency and BuSC markers (such as CD34). PV pathology and spontaneous repair leading to full stemness recovery was found to occur through proliferative mechanisms different from anagen induction, involving the EGFR and Wnt (c-Myc, Stat3, Lef-1, β-catenin and plakoglobin) but not Shh signaling pathways.

The functional implication of these pathways in pathology and repair was confirmed via treatment with EGFR inhibitor Lapatinib, Wnt activator BIO and the Shh agonist SAG. We thereby uncovered entirely novel aspects of drug treatment to be taken into consideration for PV and other diseases involving SC, such as cancer or degenerative disorders; i) pharmacological drugs need to be carefully titrated as over-inhibition or over-activation of pathways required for equilibrated tissue homeostasis can worsen the condition, ii) preventing pathogenesis may abrogate repair mechanisms which is a likely scenario in PV patients with existing and newly initiated blisters; e.g. EGFR inhibitors prevented PV pathogenesis but hampered repair, while Wnt activators had a dual function by preventing pathogenesis and supporting repair.

Taken together, we here report on entirely novel aspects of combined mechanisms of pathogenesis and repair in the HF stem cell niche, holding great potential to serve as a basis for novel treatment approaches. Our results also highlight the suitability of the PV mouse model to delineate optimal drug concentrations for a beneficial outcome of skin disorders while avoiding detrimental side effects.

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**Intra-graft Tacrolimus-loaded hydrogel –
a local alternative to systemic immunosuppression in composite allografts?**

Background
The life-long need for immunosuppression is a major drawback to a broader application of vascular composite allotransplantation (VCA). The opportunity to treat the exposed VCA grafts locally could reduce the side effects related to systemic immunosuppression. Here we demonstrate that periodic injections of a hydrogel loaded with Tacrolimus subcutaneously into the graft induce indefinite VCA survival. Further, we compare the hydrogel therapy to conventional systemic immunosuppression in terms of toxicity and tolerance markers.

Methods/Materials
Brown Norway-to-Lewis hind-limb transplantations were performed. Rats were randomly divided in two groups (n=6 per group): Controls (systemic treatment with Tacrolimus, 1 mg/kg daily) and experimental group (Intra-graft injection of 1 mL hydrogel loaded with 7 mg Tacrolimus, repeated every 70 days). Graft survival was monitored for 280 days or until grade III rejection. Tacrolimus (LC-MS/MS), toxicity markers, hematopoietic chimerism and Tregs (Flow Cytometry) in blood and skin biopsies were examined.

Results
All grafts survived until the endpoint of 280 days post transplantation, except for one from the experimental group, who underwent rejection and was sacrificed after 149 days. Mixed hematopoietic chimerism was detectable but declining until the endpoint, with experimental animals having higher levels compared to controls. Tregs were comparable in the two groups. Systemically treated animals had significantly higher Creatinine and Blood Urea Nitrogen levels, while hydrogel treated animals were comparable to naive rats.

Conclusions
Repeated injections of hydrogel loaded with Tacrolimus promote indefinite graft survival in a rat hind limb transplantation model with an up to 3 times lower total dose of Tacrolimus. They furnished higher levels of mixed hematopoietic chimerism in comparison with controls and reduced nephrotoxicity. The results suggest that a hydrogel-based drug delivery system may be a feasible approach for immunosuppression in VCA.

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Bmi1-hTERT-PiggyBac™ Transposon System is a promising tool to rescue senescent canine adipose-derived mesenchymal stem cells in vitro

Background:
Cellular senescence going along with a loss of proliferative capacity is a major drawback in view of future therapeutic applications of canine adipose-derived mesenchymal stem cells (cAD-MSCs) in the field of regenerative medicine. Therefore, we are interested in investigating mechanisms underlying cellular senescence and finding strategies to prevent or at least delay senescence in vitro. For this purpose we are investigating the immortalization of cAD-MSCs by using a PiggyBac™ transposon system containing the human polycomb complex protein Bmi1 (Bmi1) and the telomerase reverse transcriptase (hTERT) gene. Compared to lentivirus-based immortalization constructs, the PiggyBac™ system has the great advantage of being a non-viral genome editing system with the unique feature of reversibility by footprint-free removal of the edited sequence at any time, thus restoring the properties of the primary cells.

Methods:
Isolation of cAD-MSCs was achieved by enzymatic digestion and selection based on plastic adherence. Gene and protein expression levels of Bmi1 were investigated by RT-qPCR and immunohistochemistry. Bmi1 positive cells were quantified by high-content analysis. For the immortalization transposon construct we cloned the human Bmi1cDNA in a PiggyBac™ transposon system already containing the hTERT coding sequence.

Results:
RT-qPCR and immunohistochemical data show a decreased expression of Bmi1 during the course of cell passaging. Preliminary data show that the PiggyBac system can be used to efficiently transfect cAD-MSCs, with best results obtained using an electroporation method (Neon Transfection System, Invitrogen).

Conclusion:
cAD-MSCs are showing senescence in vitro, but the involved key pathways remain to be elucidated. The non-viral PiggyBac™ Transposon System containing hTERT and Bmi1 is a promising tool for the immortalization of cAD-MSCs, enhancing the suitability of cAD-MSCs for cellular therapies.

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Gallbladder Derived Stem Cells: A New Chance for Cell Therapy in the Liver

Background:
Liver disease related mortality has a growing incidence worldwide. Cell therapy has been proposed as an alternative to overcome the major drawbacks of the current treatment options. We recently reported the Gallbladder as a potent source of adult hepatic stem cells, which can be expanded in the form of organoids. As pre-requisite for successful engraftment and function in the recipient livers, we now explore the in vitro differentiation potential of these cells towards the hepatocyte lineage.

Methods:
Adult stem cells are isolated from C57BL/6J mouse gallbladders and expanded in Matrigel. Expansion is based on growth factors including Wnt activation and TGF-beta inhibition. Differentiation conditions include epigenetic modulation, Notch inhibition, synthetic Hepatocyte growth factor or Oncostatin M.

Results:
Modification of the organoid culture medium with differentiation promoting factors induced hexagonal cell shapes, multi- nucleation, decreased proliferation, upregulation of hepatocyte lineage markers (HNF4a, Cyp3a11, AFP, Pparg) and downregulation of stem cell markers (CD44, Sox9).

Conclusions:
Our current culture protocol allows a differentiation of the organoids towards a hepatoblast-like state. Ongoing refinements of the differentiation media formulation may allow the formation of committed hepatocyte progenitors in the future. The differentiated cells may then be applied in a mouse model of liver failure.

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Characterization of anti-A2ML1 Autoantibodies in Paraneoplastic Pemphigus

Paraneoplastic pemphigus (PNP) is an autoimmune blistering disease associated with lymphoproliferative neoplasms. Its pathogenesis is incomplete making targeted therapies challenging. About 60% of PNP patients produce autoantibodies against alpha 2 macroglobulin-like 1 (A2ML1), an extracellular broad-range protease inhibitor mainly expressed in the granular layer of epidermal keratinocytes. However the function of A2ML1 in the epidermis and the role of anti-A2ML1 autoantibodies in PNP disease are yet to be defined.

It has been shown that anti-A2ML1 autoantibodies increase cell-cell dissociation in keratinocyte cultures therefore we will generate monoclonal antibodies (mAbs) against A2ML1 by immortalizing B cells from the sera of PNP patients. With the generated mAbs, we will be able to confirm the loss in intercellular adhesion by dispase dissociation assay. To detect the major protease targets of A2ML1, we will co-immunoprecipitate from normal skin extract the A2ML1-protease complexes and carry out mass spectrometry analysis. Interestingly, anti-A2ML1 pAbs increase the activity of plasmin, a serine protease involved in basement membrane regulation.

If we confirm these findings, we will determine the source of the increase by determining the plasminogen:plasmin ratio by western blot. Otherwise we will quantify the activity of the other two known plasmin activators urokinase-type plasminogen activator (uPa) and kallikreins (KLKs) using fluorescent activity assays. Lastly, to understand the funct ion of A2ML1 in the granular layer, we will grow skin equivalents and knock down its expression by siRNA.

Currently we are amplifying A2ML1 from differentiated keratinocytes and improving our transfection efficiency in HEK cells for the B cell immortalization screen.From these, we will then have all the tools to determine the function of A2ML1 in the skin and the role of its autoantibodies in PNP disease.

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Genetic analysis of genodermatoses:
A de novo variant in the ASPRV1 gene in a dog with ichthyosis

Background: The skin is the largest organ of the body and serves as a barrier between the organism and the environment. In humans, hundreds of genetic disorders affecting the skin or its appendages are described, many of them assumed to be monogenic. However, the causative gene or the gene function remains unclear for many of them. The skin undergoes a constant process of self-renewing and keratinocytes migrate from the basal layer of the epidermis to the uppermost layer, the stratum corneum, as they differentiate. A defect in the differentiation of keratinocytes can lead to cornification disorders such as ichthyosis (scaling disease).

Methods: We studied a German Shepherd dog with a novel form of ichthyosis. The whole genome of the affected dog was sequenced and compared to the canine reference genome and 288 genomes from non-affected dogs of various different breeds. Sanger sequencing was used for confirmation of the candidate variant. Immunofluorescence staining on skin sections of the affected dog and a control dog was used to investigate the impact of the variant on filaggrin expression.

Results: Comparing the genome sequence of the affected dog to 288 control dogs revealed a private heterozygous variant in the ASPRV1 gene encoding “aspartic peptidase, retroviral-like 1”, also known as skin aspartic protease (SASPase). The identified candidate variant was a missense variant (c.1052T>C) affecting a conserved residue close to an autoprocessing site (p.L351P). Since the variant was not present in the non-affected parents, it was most likely due to a de novo mutation event in the germline of one of the parents or during the early embryonal development of the case. The retroviral-like protease encoded by ASPRV1 is known to be involved in profilaggrin-to-filaggrin processing. By immunofluorescence staining, we showed that the filaggrin expression pattern was altered in the affected dog.
Conclusion: Our findings provide evidence that the identified de novo variant is causative for the ichthyosis in the affected dog and that ASPRV1 plays an essential role in skin barrier formation. ASPRV1 is thus a novel candidate gene for unexplained human forms of ichthyoses.

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Involvement of Müller glia cells in retinal degeneration/regeneration in zebrafish and mouse

The aim of the project is to identify and compare the role of Müller glia cells (MCs), a radial-glial-like neural stem cells, during retinal degeneration/regeneration in two different animal models: one with high regeneration capacity (zebrafish) and the other with low regeneration capacity (mouse). Even if it might be difficult to compare MC role between species as mouse and fish inhabit different environment, understanding the mechanisms by which zebrafish can regenerate a damaged retina may suggest strategies for stimulating retinal regeneration in mammals and, in particular, in humans. Furthermore, modulation of endogenous repair mechanism will minimize adverse effects including rejection or tumor formation seen after transplantation of retinal or stem cells. Therefore, activation, proliferation and differentiation of MCs and the pathways involved in these processes will be investigated.

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The mammalian retina lacks regenerative capacities. However, a cell population expressing the adult stem cell marker Lgr5 was recently found. Might this be a quiescent stem cell population of the adult retina?

Recently it has been shown that the retina harbors a cell population which expresses the Leucine-rich repeat containing G-protein receptor 5 (Lgr5). This marker was identified as an adult stem cells marker in the intestine and was found to be expressed in several other adult SC populations of epithelial tissues. In the retina Lgr5 is expressed in glycinergic amacrine interneurons. Even though Lgr5-postive amacrine cells demonstrate properties of differentiated interneurons they also contribute to the generation of new retinal cells in adult animals. Therefore, we would like to investigate if Lgr5-postive cells can be cultured and expanded in vitro and whether they can differentiate into different retinal cell types.

For the experiments Lgr5EGFP-Ires-CreERT2 knock-in mice were used. In a first step whole retinae from P1 and P5 animals were cultured as spheres in low adherence culture plates. In the neonatal mouse retina Lgr5 can be detected from P4. In accordance with this finding no GFP-positive cells were initially found in P1 cultures. Yet after 8 days in culture GFP expression could be detected. Immunohistochemistry performed on 3rd generation spheres showed that not all Lgr5-positive cells expressed Syntaxin, a marker of mature amacrine cells. Furthermore, Lgr5-positive cells from P5 retinae were sorted by FACS and cultured as spheres. However, the cells did not proliferate under the chosen culture conditions.

These preliminary results demonstrate that Lgr5-positive cells can be cultured in vitro, albeit no proliferation was seen. Therefore, in a next step different culture conditions will be assessed. Furthermore, Syntaxin-negative Lgr5-cells will be further investigated to analyze whether these cells correspond to a progenitor-like cell type.

Keynote Speakers

Dr. Christine Weber

Prof. Fredric Manfredsson

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Guests

Prof. Ben Gantenbein

Prof. Volker Enzmann

Dr. Stefano Di Santo

Prof. Hans Ruedi Widmer

Emeritus Prof. André Haeberli

Prof. Eliane Müller

Dr. Amiq Gazdhar

Special acknowledgement

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and

Emeritus Prof. André Haeberli

We want to thank you for your support, which made this event possible for the fourth time already.

1st Retreat, Munchewiler Castle, 06.08.2014

2nd Retreat, Gurten Mountain, 04.09.2015

Previous Retreats

3rd Retreat, Gurten Mountain, 06.09.2015