5th SCRM PhD Student Retreat

Paul Klee Center
31st August 2018

Graduate School for Cellular and Biomedical Sciences

Platform for Stem Cell Research in Regenerative Medicine

UNIVERSITÄT BERN

Universitätsklinik für Thoraxchirurgie

Program

8:00	8:15	Welcome Coffee
8:15	8:20	Welcome by the Organizing Committee

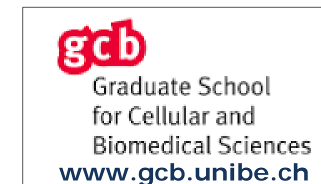
Morning Session Chair: Rahel May

8:20	8:40	Pierre Balmer
8:40	9:00	Nathalie Vielle
9:00	9:20	Haibin Deng
9:20	9:40	Magdalena Brunner
9:40	10:00	Felix Baier
10:10	10:40	Coffee Break
10:40	11:00	Emina Dzafo
11:00	11:20	Silvia Erni
11:30	12:30	Professor Joerg Huelsken, EPFL Lausanne: "Targeting Cancer Stem Cells"
12:30	14:00	Lunch Break

Afternoon Session Chair: Magdalena Brunner

14:00	14:20	Lijuan Ma
14:20	14:40	Rahel May
14:40	15:00	Yanyun Gao
15:00	15:20	Xingshuo Zhang
15:20	16:00	Coffee Break
16:10	17:10	Doctor Susan Kirkland, Novartis Pharma: " Stem cell fate regulators for regenerative medicine indications"
17:10	17:20	Conclusive Remarks by the Organizers and the SCRM Steering Committee
17:30		*Apero Riche*

This event was made possible with the generous support of:



Emeritus Prof. André Haeberli

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

1st Retreat, Münchenwiler Castle, 9th August, 2014

2nd Retreat, Gurten Mountain, 4th September 2015

3rd Retreat, Gurten Mountain, 6th September 2016

4th Retreat, Gurten Mountain, 1st September 2017

Dear participants,

Welcome to the 5th SCRM PhD students retreat!

We are happy to continue this successful history of seminars with you, which started in 2014, initiated by our colleague Dr. Luca Tamò.

The day starts with a welcome coffee and a short address given by the organizing committee. The program will continue with sessions of PhD project presentations and two coffee breaks for networking and discussions.

Then we are again looking forward to two interesting keynote lectures, which will be given by our this year's mentors **Prof. Joerg Huelsken** from the EPFL Lausanne, and **Dr. Susan Kirkland from Novartis Pharma**. We are very grateful to them for being our mentors for a day.

The retreat will be finalized by an address given by the SCRM steering committee, followed by the *Apero Riche*.

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
Magdalena Brunner
William Hariton
Rahel May

Pierre Balmer
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The methyltransferase SUV39H2 plays a key role in epidermal homeostasis

Background: In this study we addressed the underlying cause of canine Hereditary Nasal Parakeratosis (HNPK). This disorder is associated with a monogenic autosomal recessive missense variant in SUV39H2, reported to inactivate the catalytic domain. SUV39H2 encodes a histone 3 lysine 9 methyltransferase implicated in epigenetic processes and gene silencing. However, SUV39H2 has so far not been implicated in epidermal processes while epigenetic phenomena just start to emerge.
We hypothesized that SUV39H2 is involved in skin homeostasis by 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 function of SUV39H2 in epidermal proliferation and differentiation and establish the causality of the SUV39H2 variant in the HNPK disorder.

Methods: RNAseq on biopsies of three affected and three healthy control dogs, RT-qPCR and siRNA time course studies on cultured HNPK keratinocytes and immunofluorescence validation on tissue and cells, were used for functional analyses and to confirm causality. ChIP will be used to associate deregulated gene expression profiles to aberrant promoter methylation.

Results: Gene expression profiling on skin biopsies and cultured keratinocytes showed significant mis-expression of genes involved in the transition between proliferation and differentiation as well as in terminal differentiation. Pathway analyses on HNPK dog biopsies confirmed impaired cell cycle exit paralleled by deregulated Wnt and Notch pathway activities, known to be key in epidermal homeostasis from onset of differentiation to the formation of the cornified envelope. The knock-down of SUV39H2 in non-affected canine keratinocytes further confirmed that loss of SUV39H2 function is sufficient to mimic the HNPK phenotype.

Conclusion and outlook: Our results underscore that the missense SUV39H2 variant perturbs epidermal homeostasis by interfering with Wnt and Notch signaling in HNPK dogs. Together these results suggest that SUV39H2 plays a critical role in the balance between keratinocyte proliferation and differentiation, at least in the canine nasal planum.

- Prof. Joerg Huelsken, EPFL Lausanne
- Dr. Susan Kirkland, Novartis Pharma

Additional Participants

- Irene Häfliger, PhD student (Genetics)
- Anna Letko, PhD student (Genetics)

Guests

- Dr. William Hariton
- Prof. Eliane Müller
- Prof. Volker Enzmann
- Prof. em. Thomas Krause
- Prof. Daniel Surbek
- Prof. Benjamin Gantenbein
- Dr. Amiq Gazdhar
- Rene Aeberhard
- Dr. Stefan Bärtschi, CELLnTEC
- Dr. Thomas Schnibbe, CELLnTEC

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Investigating the neurotropism of flaviviruses using human brain organoids

A spectrum of clinical syndromes complicates flavivirus infections in humans, ranging from mild fever and arthralgia to severe encephalitic manifestations. Flaviviruses share a similar genomic organization and replication strategy, and yet cause a range of distinct clinical diseases in humans. Once in the central nervous system (CNS), flaviviruses induce neuronal damage and cell death by different mechanisms this leading to the clinical manifestations of disease. Antiviral signaling is critical for controlling virus infection but the impact of interferon (IFN) responses in neurons remains unclear. Neurons are capable of detecting the presence of pathogen-associated molecular patterns and viral infection, and subsequently mounting an effective immune response against several neurotropic viruses. In line with IFN production, many studies demonstrate neuronal upregulation of key antiviral effector molecules and other IFN-stimulated genes (ISGs) in response to neurotropic RNA viral infection.

We aim at analyzing neurotropic flavivirus invasion of the CNS and its relevance in virus-associated neuropathogenesis using an advanced 3D culture system based on human cerebral organoids generated from human pluripotent stem cells. This novel in vivo-like system to will allow us to investigate flavivirus replication, cellular tropism and the mechanism of potential virus-induced neuronal injury during flavivirus infection.

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Background: The therapy for intervertebral disc (IVD) degeneration is limited by the regenerative potential which even undergo reduction with aging. The strategy of regeneration medicine might be a promising approach. Comparing with spinal fusion or artificial disc replacement, stem cell therapy by injection is preferably as less invasive. However, the appropriate cell source of Stem cell therapy strategies is not clear. Endothelial marker Angiopoietin-1 receptor (= Tie2) has been demonstrated as specific surface markers of NNPC (Tekari et al. 2016, Sakai et al. 2012). However, the expression of Tie2 was lost during monolayer culture (Tekari et al. 2016). Here, we propose to maintain Tie2 expression during expansion.

Methods: I cultured the NP cells from same donor and passage them in media add 25uM PPAR delta which has been shown could increase the Tie2+ cells in hematopoietic cells with a DMSO control. Than the death cells will detect by DAPI and Tie2 receptor will detect by Antibody from Bioss under flow cytometry.

Results: The cells with PPAR treated shows twice times Tie+ cells in total population than the control group. The cells in PPAR treated group also shows less death cells.

Conclusion: PPAR delta could increase the progenitor NP in Intervertebral disc

Outlook: Upregulation of Progenitor NP cells percentage in total population

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Novel insights into the pathways regulating the canine hair cycle and their deregulation in alopecia X

Alopecia X is a hair cycle arrest disorder in Pomeranians. An intact hair coat is maintained by lifelong cycling of the hair follicles through periodic stages of growth (anagen), regression (catagen), and relative quiescence (telogen). In alopecia X, histologically, hair follicles in their quiescent phase predominate, whereas anagen follicles are sparse. The induction of anagen relies on the activation of hair follicle stem cells and their subsequent proliferation and differentiation. This stem cell activation is mediated by Wnt and Shh signaling.

We performed transcriptome profiling of skin biopsies to analyze altered molecular pathways in alopecia X. Biopsies from five affected and four non-affected Pomeranians were investigated using the Illumina HiSeq3000 with 2x150 bp paired-end sequencing cycles. We used DESeq2 v.1.6.3 to assess differential expression between alopecia X and controls. Transcripts were considered to be differentially expressed with a FDR of < 0.01. The differentially expressed genes were mapped to biological networks using the database GeneGo MetaCore™ and its pathway analysis software. In addition the Panther Classification System was used for the enrichment analysis.

Transcriptome profiling showed, that hair cycle regulation is similar in mice and dogs: We specifically analyzed genes of the Wnt, Shh, Bmp, Fgf and Tgf-β signaling pathways which are also known to play a role in HF biology in mice. We identified a total of 47 differentially expressed genes involved in one of these pathways. Additionally, we observed a significant downregulation of the stem cell and progenitor markers SOX9, LHX2, LGR5, TCF7L1 and GLI1 in Pomeranians with alopecia X, whereas NFATc1, a quiescence marker, was upregulated. Furthermore, the Shh and Wnt pathways were significantly downregulated.
To summarize our results from the trancriptome analysis we can state that the hair cycle regulation is similar in mice and dogs. Furthermore, our results showed a downregulation of Shh and Wnt pathways suggesting an impaired anagen induction and deregulation of the hair cycle in dogs with alopecia X. The downregulation of stem cell and progenitor markers indicates a reduced stem cell function of hair follicles.

In our ongoing project we would like to discover whether there is a reduced stem cell capacity in hair follicles of dogs with alopecia X or an induced quiescence of hair follicle stem cells caused by factors within the micro- and /or macroenvironment of the hair follicles. For this purpose we established epidermal organoids from microdissected hair follicle keratinocytes. We are going to compare the growth rate and morphology of organoids, assess the colony forming efficiency of keratinocytes derived from organoids and using RNA-seq to identify differentially expressed genes of organoids from dogs with alopecia X in comparison to organoids from control dogs.

Rahel May
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Comparison of gene expression of discs from Diffuse Idiopathic Skeletal Hyperostosis (DISH) and trauma/degenerative patient

Background: Diffuse idiopathic skeletal hyperostosis (DISH) is a common disease, affecting mostly mid-aged and elderly patients. Abnormalities like formation of osteophytes, following an ossification anterior to the vertebral bodies and intervertebral discs (IVD) are frequently observed in DISH patients. However, the nucleus pulposus remains unaffected from this disease. We have recently shown, that the IVD cells are expressing BMP antagonists, which could lead to inhibition in bone formation for spinal fusion. The aim of this study was to compare the transcriptome of discs of the BMP-pathway from DISH patients with traumatic or degenerative discs.

Methods: Fresh DISH-IVDs and trauma or degenerated IVDs were obtained from patients undergoing spinal surgery (approved by the Ethics Committee of the Canton of Bern, CH). IVD cells were released from their native extracellular matrix by digestion of the tissue with pronase (1.9 mg/mL Roche, Basel, Switzerland) for one hour and subsequent mild overnight collagenase II digestion (129 U/mL, Worthington, London, United Kingdom). Cells were then filter-strained (100 µm) and cultured up to passage 2 and then lysed in TRI reagent for total RNA extraction. RNA integrity and quantity was monitored using Experion™ RNA electrophoresis prior qPCR (Bio-Rad). PrimePCR™ (TGFβ BMP Signaling Pathway Plus H96, 90 genes, Bio-Rad, US) was run on CFX96 machine (Bio-Rad). Gene expressions of three DISH patients were tested against three control patients.

Results: In all DISH-IVDs an up-regulation of Interleukin 6 (IL-6) was detected (mean ± SEM of all five comparisons) 122.32 ± 110.67. Furthermore, were Early Growth Response 2 (EGR2) and Insulin-like growth factor 1 (IGF-1) up-regulated in two of the DISH-IVD donors (26.62 ± 17.11-fold and 27.20 ± 27.17-fold, respectively). Whereas, the two Growth and Differentiation Factors 5 and 6 (GDF5 and 6) were down-regulated.

Conclusion: Most interestingly, the disc cells of DISH patients showed a considerable change in IGF-1 and IL-6. These two factors lead to a proliferation of osteoblasts and to bone deposition. The increased production of these two growth factors was also hypothesised before. IGF-6 was already determined as a serum marker for rheumatic diseases, such as DISH. GDF5 and GDF6 showed a down-regulation in DISH-IVDs. However, the exact role of these two members of the TGFβ pathway in DISH disease is yet unknown.

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Cisplatin resistant lung cancer cells can be identified by increased mitochondrial mass and are sensitive to pemetrexed treatment

Background: Lung cancer is the most common cause of cancer-related deaths in developed nations. More than 80% of lung tumors are non-small-cell lung cancers (NSCLC). Cisplatin plus pemetrexed combination therapy is considered the standard treatment for patients with advanced non-squamous NSCLC. However, advanced NSCLC has a low 5-year survival rate of approximately 30%. This is mainly due to the development of therapy resistance. We previously showed that the NSCLC cell line A549 harbors different subpopulations including a mesenchymal-like subpopulation featuring increased chemo- and radiotherapy resistance. Very recently, therapy resistance in hematological and solid tumors has been associated with increased mitochondrial activity. Thus, the aim of this study was to investigate the role of the mitochondrial activity in NSCLC chemotherapy resistance.
Methods: Mito Tracker Deep Red (M22462, Invitrogen) was used to stain the mitochondrial mass of the mesenchymal-like population from NSCLC cell line A549. Real Time PCR(RT-PCR) was used to quantify the mitochondrial DNA copy number. Basal respiration of cells was measured by high-resolution respirometry (OROBOROS). FACS (Fluorescence-Activated Cell Sorting) was used to sort two subpopulations Mito-High (10%) and Mito-Low (10%). The cisplatin and pemetrexed response of these two populations was tested by colony formation assay.
Results: Mito-high (10%) and Mito-Low (10%) subpopulations isolated based on mitochondrial mass feature significant differences in mitochondrial DNA content and basal respiratory capacity. The Mito-High subpopulation showed 1.4-fold increase in mitochondrial DNA copy number, relative to the Mito-Low cell population. Besides, Mito-High cell subpopulation showed 1.6-fold enhancement in the basal respiration rate, compared with Mito-Low cell population. The cells with high mitochondrial mass increased proliferation activity. In detail, the cell number of the Mito-High subpopulation was 1.7-fold higher than Mito-Low subpopulation after 7-day growth. The Mito-High subpopulation was more resistant to cisplatin than the Mito-Low cell subpopulation, as showed by colony formation assay with different cisplatin concentrations. Interestingly, the Mito-High cell population was sensitive to pemetrexed, relative to the Mito-Low cell subpopulation.
Conclusions: This study revealed that the level of mitochondrial mass is positively associated with cisplatin resistance in the mesenchymal-like A549 subpopulation. In contrast, high levels of mitochondrial mass confer pemetrexed sensitivity. Thus, cisplatin resistant cells characterized by high levels of mitochondrial mass are susceptible to pemetrexed treatment, which could explain the increased efficiency of the combination therapy in the clinical setting.

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Targeting mitochondria in lung cancer tumor initiating cells

Background: Lung cancer is the most common cause of cancer-related mortality worldwide. It was postulated that tumor initiation and propagation are mediated by so called cancer stem cells (CSCs). In various solid tumors, it has been shown that CSCs are highly dependent on oxidative phosphorylation as indicated by a high mitochondrial activity whereas bulk tumor cells are dependent on glycolysis.

Our group currently revealed that the NSCLC cell line A549 contains phenotypically distinct subpopulations. A549 holoclone cells were characterized by an epithelial and stem-like phenotype, paraclone cells featured a mesenchymal phenotype whereas meroclone cells featured an intermediate phenotype.

In summary, the aim of this study is to dissect the contribution of the two parameters in tumor initiation for A549 and primary lung cancer cells.

Methods & Research Plan
Aim 1: Prospectively isolate and characterize in vitro the sphere formation capacity of distinct A549 subpopulations.
Aim 2A: Prospectively determine the tumor initiation capacity of the distinct A549 subpopulations by extreme limited dilution assay.
Aim 2B: Prospectively determine the tumor initiation capacity of the distinct subpopulations from primary lung cancer samples by extreme limited dilution assay.
Aim 3: Identify pathways differentially dysregulated in A549 subpopulations with high versus low tumor initiating capacity
Aim 4: Investigate how the inhibition of enzymes specifically dysregulated in TICs affects in vitro cancer cell growth and tumorigenicity.

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Delaying the Senescence of Human Bone Marrow Derived Mesenchymal Stromal Cells in vitro

Background: The regenerative potential of mesenchymal stromal cells (MSC) is being investigated in many clinical trials for various health issues, from bone fractures to autoimmune diseases.1 One of the major problems involved in such cell therapy is the necessity to rely on fetal bovine serum (FBS) for cell expansion which proved to have major disadvantages. Additionally, MSC undergo senescence during expansion in vitro, which impairs their therapeutic potential.2 Recently, it was shown that oral treatment with nicotinamide riboside (NR) rejuvenated hematopoietic stem cells in mice.3 However, it is currently unknown whether NR alleviates the senescence of MSC in vitro.

Methods: Bone marrow aspirates were obtained from the vertebrae of donors undergoing spinal surgery with their written consent. MSC were expanded either in α-MEM+10% FBS+2.5ng/ml bFGF-2 (α-MEM/FBS), or two CnT-Prime MSC Media: Xeno Free (CnT-XF, containing 2% human serum), and Chemically Defined (CnT-CD) (CELLnTEC, Bern Switzerland). In the first part of the project, NR was tested in α-MEM/FBS in concentrations of 10, 100 and 1'000 µM. For each group, we measured population doubling level (PDL), differentiation potential, Resazurin salt cell activity, senescence-associated β-galactosidase assay (SA-β-gal) and NAD/NADH ratio. In the second part, the growth kinetics between the three media were assessed by using PDL, population doubling time (PDT), and IncuCyte S3® Analysis System. Immunophenotyping was performed by flow cytometry. The cytotoxicity of NR was tested in CnT-CD at 28 concentrations ranging from 10 to 0.01 µM.

Results: There was 80.17 ± 54.34% (mean ± SD) higher cell activity at passaging in 10 µM NR relative to the basal medium in two donors, while one donor exhibited no difference. The responsive donors reached also a higher PDL compared to the control. The NAD/NADH ratio tended to be higher in all experimental groups at all measurements. Cells treated with 10 µM NR were 1.48 ± 1.40% positively stained upon SA-β-gal staining in contrast to 18.17 ± 18.18% in the basal medium (mean ± SD, N=1). In CnT-XF the cells had a PDT of 114.01 ± 123.54h and PDL of 13.61 ± 1.45 compared to 340.70 ± 338.37h and a PDL of 8.17 ± 1.59 in α-MEM/FBS (mean ± SD, N=1). The growth kinetics of CnT-CD could not be measured due to weak plastic adherence of the cells. Flow cytometry revealed that cells in all three media were CD73+, CD90+, CD14-, CD34- and CD45- (N=3 for α-MEM/FBS and CnT-XF, N=1 for CnT-CD). Interestingly, only cells expanded in α-MEM/FBS were also CD105+. All experimental groups underwent osteogenic and adipogenic differentiation, except cells grown in CnT-CD, which showed no osteogenic potential. NR was cytotoxic to cells in CnT-CD at concentrations ≥1 µM (p=0.0004, N=4).

Conclusion and Outlook: 10 µM NR improved the growth kinetics of α-MEM/FBS. The mechanism of this has yet to be determined by analysing gene expression of mitochondria-relevant genes and relative telomere shortening. In CnT-XF cells reached a higher PDL and had a lower PDT which is an advantage for cell therapies. NR might improve the growth kinetics of CnT-CD which should be further investigated at concentrations from 0.9 to 0.01 µM.

1. Squillaro, T., Peluso, G. & Galderisi, U. Clinical Trials with Mesenchymal Stem Cells: An Update. Cell Transplant. 25, 1–53 (2015).
2. Sepúlveda, J. C. et al. Cell senescence abrogates the therapeutic potential of human mesenchymal stem cells in the lethal endotoxemia model. Stem Cells 32, 1865–77 (2014).
3. Zhang, H. et al. NAD+ repletion improves mitochondrial and stem cell function and enhances life span in mice. Science (80-.). 352, 1436–1443 (2016).

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Validation of a New Generation Gamma-Secretase Inhibitor to Induce Sensory Hair Cell Regeneration in Vitro and in Vivo after Pneumococcal Infection

Background: Over 5% of the world's population suffer from hearing loss. Sensorineural hearing loss is also the most common long-term deficit after pneumococcal meningitis (PM), occurring in up to 30% of surviving patients. Treatment options for inner ear pathologies are limited and novel pharmaceutical treatments are highly needed.

Methods: Inhibition of Notch signaling in vitro and in vivo induces supporting cell transdifferentiation to hair cells and restores partially hearing thresholds in noise-deafened mice. Here, we tested the oto-regenerative potential of a novel gamma secretase inhibitor (GSI) in experimental bacterial meningitis-induced hearing loss, and the role of Notch signaling in this process.
Organotypic organ of Corti explants from young Wistar rats (d2-4) were incubated 4 days with/without GSI and analyzed histologically or for gene expression by PCR. For in vivo assessment, infant rats were infected intracisternally with Streptococcus pneumoniae. After antibiotic treatment and recovery from PM, hearing thresholds were measured by Auditory Brainstem Response. GSI was locally delivered to the round window membrane. Animals were assessed for hearing function 2 weeks later and sacrificed for histological analysis of the hair cells.

Results: In vitro, the number of hair cells and supporting cells in the sensory epithelium did not differ significantly between the groups (n=2-4). However, preliminary data of GSI treated versus untreated explants showed an increased expression level of Atoh1 (1.42 fold) and Jag2 (2.45 fold) whereas Hes1, Hes5 and Notch1 were downregulated, confirming the expected effect of GSI on Notch signaling.
In vivo, we successfully established the local route of delivery, without negative effects of the surgery on hearing thresholds. PM induced a mild hearing loss in the tested animals (n=10). Hearing thresholds for broad band clicks did not differ after GSI treatment, however for specific sound frequency, we observed improvement, namely at 16 kHz (-6.25dB±8.34) and 32 kHz (-9.28dB±14.56). Hair cell counts showed a slight increase in GSI treated ears, however without statistical significance.

Conclusion: In vitro and in vivo models have been established to study the regenerative properties of Notch inhibition by GSI. Further experiments are needed to confirm beneficial effect of GSI on auditory function.

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The comprehensive analysis of epithelial-to-mesenchymal transition (EMT) in endometriosis with, and without, recurrence

Introduction: Endometriosis corresponds to the growth of endometrial epithelial (EEC) and stromal (ESC) cells outside the uterus (ectopic lesions). Although surgery and medication can alleviate pain, relief may only be temporary for many women, the recurrence of endometriosis after treatment is very high [1]. To date, there are no reports that Epithelial-to-mesenchymal (EMT) might be related to recurrence of endometriosis

Aims: To explore whether EMT plays any role in the pathogenesis of endometriosis recurrence. And to validate the dysregulation of EMT in targeted tissues.

Methods:
Set up the group 1 as recurrence endometriosis, the group 2 as endometriosis without recurrence, and the group 3 as the control with endometriosis-free women.

Isolate the cells as a function of the presence of epithelial and/or mesenchymal markers positive.

Apply nanostring analysis to identify the difference of EMT related mRNAs between groups. The method will be applied to the detection of mRNA.

Analyse the data on pathways included in nCounter® Pancancer Progression Panel (nanostring).

Reference:
1. Cea Soriano, L., et al. Eur J Contracept Reprod Health Care, 2017. 22(5): p. 334-343.

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Importance of Tight Junction Regulation for Liver and Disease

Background: Hepatic tight junction proteins fulfil key roles in creating tissue compactness and restriction of bile flow from the blood circulation. Dysregulation of tight junctions is often associated to liver pathologies including cholestatic diseases, abnormal hepatocyte proliferation and others. Yet, concise literature about expression of individual hepatic tight junction genes and their contribution to liver disease and repair is not available. In this study, we give an overview on the expression of tight junction associated genes in mouse and human liver tissue. We identified a highly transcribed tight junction gene, claudin-3, and elucidate its function in two different liver disease models, compensatory regeneration and severe cholestasis. To find regulators of hepatic tight junctions, we studied liver regeneration in mice deficient for an important liver cytokine, IL-22.

Methods: Tight junction gene expression was evaluated by RNAseq and RT-PCR data and within GEO databases. CLDN3-/- mice were used to study the functional consequences of claudin-3 loss for liver regeneration (70% partial hepatectomy model) or cholestasis (common bile duct ligation for 48 hours). Immunohistochemistry of proliferation markers (KI67, pHH3), qRT-PCR of cell cycle regulators (FoxM1, Birc5, Ccnb1 and p21), western blotting (WB) for ductular reaction markers (CK7), cytokine expression array and total bile acid quantification were used. IL-22-/- mice were subjected to 70% partial hepatectomy and expression of tight junctions was quantified by qRT-PCR and WB. Isolated human hepatocytes were stimulated with recombinant IL-22 protein to study the effect on Cldn3 expression.

Results: By RNA analysis, we determined that murine and human liver express a distinct set of tight junctions which are localized to the various liver cell types, such as hepatocytes, immune cells, cholangiocytes and sinusoidal endothelial cells. Claudin-3 was among the highly expressed genes. Upon induction of liver regeneration, there was a significant down regulation of Cldn3 mRNA and protein expression at 6 hours followed by a restoration by 24 hours. By immunofluorescent microscopy, CLDN3 was localized to cholangiocytes in bile ducts and to bile canaliculi of hepatocytes, mostly in the peri-central region. In regenerating livers of CLDN3-/- mice, hepatocyte proliferation and cell-cycle gene expression (FoxM1, Birc5, Ccnb1) was impaired. Common bile duct ligation (BDL) in CLDN3+/+ and CLDN3-/- mice showed that CLDN3-/- mice surprisingly had less signs of severe cholestasis, shown by fewer biliary infarcts and less hepatic bile acids. Junction leakiness was implied by the increased serum bile acid levels in CLDN3-/- mice. The cytokine IL-22 regulates tight junction expression, since IL-22-/- mice showed lower expression of Cldn1, Cldn3, Cldn12, Tjp1, Ocln and Jam-A after partial hepatectomy. Treatment with recombinant IL-22 protein induced Cldn3 transcription in human primary hepatocytes.

Conclusion: Human and mice express distinct sets of hepatic tight junctions, with claudin-3 being among the highly expressed genes. CLDN3 knock out impairs hepatocyte proliferation after partial hepatectomy, indicating that it contributes to optimal liver regeneration by a mechanism that remains to be defined. In a model of obstructive cholestasis, CLDN3-/- mice had lower hepatic bile acids, but higher serum bile acid levels than in CLDN3+/+ mice, showing a leak of bile acids into the blood circulation. Hence, CLDN3 sustains the canalicular paracellular barrier against bile acids. Finally, IL-22 acts as a regulator of liver tight junctions, since IL-22 deletion impairs expression of CLDN3 during regeneration, while its addition to primary human hepatocyte cultures induces CLDN3 expression.