9th SCRM PhD Students Retreat

Gurten Park
2 September 2022

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Bern Stem Cell Research
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Gurten Park, Bern
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08:30	08:35	Funicular from Wabern to Gurten
08:45	09:00	Welcome Coffee
09:00	09:10	Welcome by Organizing Committee
Morning Session Chair: Elisa Rodrigues Sousa		
09:10	09:30	Siavash Rahimi
09:30	09:50	Ainhoa Asensio Aldave
09:50	10:10	Andreas Croft
10:10	10:30	Juening Kang
10:30	11:00	**Coffee Break**
11:00	11:20	Vera Tscherrig
11:20	11:40	Francesco Bonollo
11:40	12:00	Katherine Crump
12:00	13:00	**Mentor Talk: Prof. Adrian Zürcher**
13:00	14:00	**Lunch Break**
Afternoon Session Chair: Ainhoa Asensio Aldave		
14:00	14:20	Yuebing Li
14:20	14:40	Franziska Strunz
14:40	15:00	Jingyi Zhang
15:00	15:30	**Coffee Break**
15:30	15:50	Ines Monteiro
15:50	16:10	Slavko Corluka
16:10	16:30	Chaonan Jin
16:50	18:00	**Mentor Talk: Prof. Alexandre Reymond**
18:00	18:10	Conclusive Remarks and Thanks from the SCRM Steering Committee
18:15		**Apéro**

This event was made possible with the generous support of:

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We want to thank you for your support, which made this event possible

6th Retreat, Gurten Park, 30th August 2019

7th Retreat, Gurten Park, 4th September 2020

8th Retreat, Gurten Park, 3th September 2021

Dear participants,

Welcome to the 9th SCRM PhD Students Retreat!

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

The day starts with a coffee and a short welcome from the organizing committee. The program will continue with a morning and an afternoon session of PhD project presentations and two coffee breaks for networking and discussions.

We are also excited to attend two interesting keynote lectures, which will be given by this year's mentors **Prof. Adrian Zürcher**, the Head of Research Europe and Head of Plasma Protein Research at CSL Behring and **Prof. Alexandre Reymond**, Director of the Center for Integrative Genomics from University of Lausanne. We are very grateful to them for being our mentors today.

The retreat will be concluded with brief remarks and thanks by the SCRM Steering Committee, followed by the Apéro.

We are looking forward to meeting you and we wish you a fruitful and pleasant time during the retreat.

Sincerely yours,
The organizing committee

Elisa Rodrigues Sousa
Ainhoa Asensio Aldave
Siavash Rahimi
Vedat Burak Ozan

Protective measures

In order to prevent any risk of SARS-Cov2 infections spreading, we will keep official safety measures in place during the whole day.

We thank you all for your understanding and compliance with the rules.

Mentors

- Prof. Alexandre Reymond, Director of the Center for Integrative Genomics from University of Lausanne.
- Prof. Adrian Zürcher, Head of Research Europe and Head of Plasma Protein Research at CSL Behring.

Guests

The SCRM Steering Committee:

- Prof. Eliane J Müller
- Prof. Marianna Kruithof-de Julio
- Prof. Deborah M. Keogh-Stroka
- Prof. Volker Enzmann
- PD Dr. Amiq Gadzhar
- Prof. Benjamin Gantenbein
- Prof. em. Thomas Krause
- Prof. Carsten Riether
- Prof. Daniel Surbek

Principal Investigators of the PhD students

- Rene Aeberhard

Siavash Rahimi

A Multi-omics approach to Pemphigus vulgaris

Purpose:

The autoimmune bullous disease pemphigus vulgaris (PV) is a devastating skin disease where the sole uncoupling of desmosomal cadherin desmoglein 3 (Dsg3) cell-cell adhesion is sufficient for pathogenesis. Published and unpublished data from our laboratory converge to predict that the major autoimmune target Dsg3 is a significant signaling node of transcellular communication surveying biophysical, biochemical, epigenetic and genetic signaling responses.

Methods:

In this study, we use a multi-omics approach (total RNA-sequencing, proteomics, phosphoproteomics, and Dsg3 Co-IP proteomics) on 2D cell culture of human primary epidermal keratinocytes treated with experimental antibodies, AK23 (anti-Dsg3) or PX43 (anti-Dsg1/3), to elucidate the Dsg3 signalling network using systems biology. For RNA-seq, three biological replicates were sampled at 5, 10 and 24 hours post antibody treatment. Although RNA-seq data for AK23 vs mIgG and PX43 vs hIgG treated groups revealed only a few statistically significant differentially regulated genes (26 and 62, respectively), biostatistical analyses identified as top candidates (adjusted p-value ≤0.05) pathways with known function in PV.

Results:

In both treatment groups, in hallmark gene sets MYC, E2F, and TNF-α were enriched. Furthermore, reactome pathways included modulation of replication, cell cycle, WNT, Rho GTPases and apoptosis, but also new pathways pertaining to epigenetic regulation and pluripotency. In addition to abovementioned clusters, the PX43 group also showed significant enrichment of NOTCH, Hedgehog, mTORC1, and p53 signaling pathways, as well as keratinization. Due to the limited number of differentially regulated genes in our analysis, we are currently generating more robust data by increasing the biological replicates.

Ainhoa Asensio Aldave

Mapping origins of replication and DNA damage in aged and diseased livers

Purpose:

Origins of replication (ORIs) are specific chromosomal locations where the replication machinery is loaded during the S-phase of the cell cycle. The correct initiation and coordination of ORIs during replication is key to ensure the correct completion of the process, and therefore the transmission of DNA information to daughter cells. Our group has successfully established a novel method to map ORI firing profiles and replication fork progression *in vivo* at a genome level in mouse regenerating livers post-partial hepatectomy. With this technique, altered low-synchronized ORI patterns were identified in aged mice, which were already known to have a high liver inflammation background and a low proliferative potential. We are interested in assessing if similar ORI pattern alterations can be found in other liver backgrounds with chronical inflammation, and chose specifically to look into the non-alcoholic fatty liver disease (NAFLD). This model has a high clinical relevance since NAFLD is one of the most common forms of chronic liver disease in western countries, linked to obesity and diabetes. Nearly 25% of NAFLD patients progress to NASH, a fibrotic non-reversible and more severe stage of the disease; ultimately leading to cirrhosis or even hepatocellular carcinoma (HCC).

Methods:

We worked with BL/6 WT mice and the MC4R line (lacking the melanocortin-4-receptor and therefore the satiation sensation). Steatosis levels and hepatocyte proliferation were assessed through oil red and Ki67 histology stainings, respectively. Liver replication was induced through partial hepatectomy surgery. Senescence was assessed by measuring the gene expression of different SASP (Senescence-Associated Secretory Phenotype) with qRT-PCR.

Results:

MC4R heterozygotes fed with HFD for 4-6 weeks before harvesting were stablished as the mouse model we will use to mimic human NAFLD: homogeneity among samples, a significant weight increase compared to the WT (~2/3 increase) and steatosis development (>5% liver fat accumulation). Ki67 immunohistochemistry stainings confirmed significantly lower hepatocyte proliferation in the MC4R heterozygotes after induced liver replication. Nevertheless, these mice did not show an average differential expression of SASP factors, concluding that we do not have increased hepatocyte senescence in these mice.

Conclusions:

Altogether, these experiments have allowed us to characterize the MC4R heterozygote mice and stablish a model to mimic human NAFLD disease. Future experiments include isolating these mice's hepatocytes for ORI mapping (currently on-going) and establishment of next generation error-corrected sequencing protocols to assess mutagenesis.

Andreas Croft

Annulus fibrosus repair using methacrylated gellan gum in combination with novel silk in a dynamically loaded bovine organ culture model

Purpose:

Annulus fibrosus (AF) rupture within the intervertebral disc (IVD) can lead to herniation and is a key contributor to low back pain. A potential solution could be the implementation of a methacrylated gellan gum (MA-GG) hydrogel. MA-GG is a polysaccharide produced by the bacterium Sphingomonas paucimobilis and is considered as a promising biomaterial for IVD tissue engineering applications. Therefore, we aimed to create an AF repair approach using 2% MA-GG as a filler material and sealing it with an embroidered silk yarn fabric, which mimics the AF's morphology.

Methods:

For this ex vivo organ culture model, coccygeal IVDs were isolated from fresh bovine tails. Next, the AF was injured using a biopsy puncher (⌀ 2 mm). The damaged site was either left untreated or filled with 2% MA-GG and then sealed with an embroidered silk yarn fabric sutured directly onto the IVD. Then, the IVDs were cultured for 14 days under different loading profiles, i.e. i) no load, ii) static load at 0.2 MPa, and iii) dynamic load at 0.2 ± 0.1 MPa and a superimposed torsion of 2°.

Results:

After 14 days of culture, no IVD herniation occurred, and the silk ribbon successfully managed to seal the repaired site regardless of the loading profile. Moreover, repaired IVDs showed a better disc height recovery than damaged samples under the dynamic loading profile. However, the metabolic activity and the amount of extracellular matrix within the IVDs remained mostly unchanged.

Conclusion:

The presented approach using MA-GG and silk fibroin to repair an AF injury was a success as no displacement of the biomaterials and no IVD herniation was observed. Furthermore, the cytocompatible properties of MA-GG and silk were confirmed as the tissue's metabolic activity remained stable.

Juening Kang

Identifying drug sensitivity of multifocal prostate cancer towards personalized screens and treatment decision

Purpose:

Prostate cancer (PCa) is a highly heterogeneous disease with multiple and distinct cancer foci within the same prostate gland. We hypothesize that heterogeneous lesions, in terms of genomic aberrations and tumour staging, may be linked to different functional properties e.g. differential drug response sensitivity. Here we investigate which histopathological and molecular properties are associated with ability to maintain PCa cells as ex vivo organoid cultures and specific drug responses.

Methods:

To simultaneously assess the histopathology, genetic profile and organoid drug sensitivity, we employed mirror biopsies for FFPE and fresh tissue preparations. Four cores of prostate tissue from radical prostatectomies (N=13) were used. Patient-derived organoids (PDOs) were characterized for morphology, number, and diameter. Targeted genomic sequencing was performed on PDOs and their parental FFPE tissues. Drug response was evaluated by ATP-based viability assay after treatment with standard-of-care (AR inhibitors), and approved compounds for other malignancies (DNA synthesis, receptor tyrosine kinase (RTK) and mTOR inhibitors).

Results:

Heterogeneous cores with different Gleason score range 6 to 9 per prostate were found in 76% of cases (N=10/13). Regarding formation efficiency, 78% of tumor-containing cores (N=22/28) and 66% of benign cores (N=16/24) showed organoid formation. Solid, cystic, and mixed morphologies were overall observed; tumor-containing cores formed higher number of mixed structures and solid structures of larger diameter ($p<0.05$, $p<0.01$, vs normal cores, respectively). PCa somatic mutations were found in 19 cores with 11 cores sharing mutations with the deriving PDOs. Different cores from the same prostate showed different drug response, while RTK inhibitors, Crizotinib and Ponatinib showed efficacy in the majority of cases ($p<0.01$ over vehicle, N=29/32 and N=30/32, respectively).

Conclusion:

The majority of primary PCa cases showed genetic and histopathological multifocality, representing a highly heterogeneous cohort. PDOs recapitulated the genetic signature of the parental tissue and formed solid, adenocarcinoma-like morphology. Drug responses of PDOs did not follow patient-specific patterns but rather revealed high intra-patient heterogeneity. Overall low response to AR and mTOR inhibitors and strong response to RTK inhibitors was observed, possibly providing new options for PCa treatment.

Vera Tscherrig

Shedding light on the neuro-regenerative potential of small extracellular vesicles (sEV) from Wharton's jelly mesenchymal stromal cells

Purpose:

One of the most common neurological complications of preterm birth is perinatal white matter injury. It is a global health problem and leads to long-lasting neurological and neuro-behavior disabilities. Small extracellular vesicles derived from mesenchymal stromal cells (MSC-sEV) show very promising results as a therapeutic approach for neural injury. The cargo of MSC-sEV includes small non-coding RNAs such as microRNAs (miRNAs), which are predicted to target mRNAs encoding for proteins that are involved in premature birth-related white matter injury (WMI). Thus, we hypothesize that the observed beneficial effects of MSC-sEV can largely be attributed to the miRNAs released by sEV upon uptake by their target cells.

Methods:

We isolated MSC from the connective tissue of human umbilical cords, the so-called Wharton's jelly. The cells were stained for typical MSC-markers by immunohistochemistry. We purified small EV from the conditioned cell medium by serial ultracentrifugation, followed by size exclusion chromatography (SEC). With a NanoVue Plus™ we checked each SEC fraction for their protein and RNA content. The fractions with the highest protein content were characterised by proteomics, Western blot, ImageStream and ZetaView analyses. Quantitative PCR was used to measure the miRNA content of the sEV. Pathway enrichment of the miRNAs was analysed following Next Generation Sequencing (NGS). A miRNA luciferase reporter assay was established in order to evaluate the regulatory activity of sEV miRNA.

Results:

The SEC fractions 5-7 were positive for the sEV markers CD81, CD63 and CD9, and the MSC markers CD73, CD90 and CD105. Moreover, they contained high amounts of miRNAs, such as miR-21-5p, miR-22-5p, miR-27b-3p, and members of the let-7 family. TP53 was identified as a target of 11 of the 32 most abundant MSC-sEV miRNA by NGS. Therefore, the 3'UTR sequence of TP53 was cloned into the pMirTarget 3'UTR assay vector downstream of a firefly luciferase sequence and co-transfected with MSC-sEV into the luciferase reporter cell line HEK293T. MSC-sEV significantly reduced the luciferase signal, strongly indicating an inhibitory effect of MSC-sEV miRNAs.

Conclusions:

Our data identify miRNAs as a functional unit in the therapeutic effect of MSC-sEV in preclinical WMI models. The regulatory potential of MSC-sEV miRNAs in disease-related gene expression is currently being further analysed using agomir / antagomir assays in an in vitro model of WMI.

Francesco Bonollo

Elucidating the stromal contribution to androgen deprivation therapy resistance in prostate cancer

Purpose:

Several studies have shown that Cancer-associated Fibroblasts (CAFs), the most abundant component of Prostate Cancer (PCa) stroma, promote resistance to androgen deprivation therapies (ADTs) and metastasis. The aim of this project is to investigate the role of CAFs in promoting the development of advanced and hormone-resistant PCa.

Methods:

To study tumor cell-CAF interactions, we use Patient-derived Xenograft (PDX) models representative of different PCa stages: early-stage PNPCa (androgen-dependent soft tissue metastasis), and advanced stage models BM18 (androgen-dependent bone metastasis), and LAPC9 (androgen-independent bone metastasis). Tumor epithelial and stromal cells (human and mouse origin respectively) were separated through magnetic-associated cell sorting (Mouse Cell Depletion) to obtain PDX-derived tumor organoids and fibroblasts. PDX-derived fibroblasts were characterized by analyzing the expression of prostate CAF markers at the RNA and protein level (RT-qPCR, Western Blot, and Immunofluorescence). Organoids and fibroblasts were then recombined to generate in vitro transwell 3D co-cultures, in which organoids are grown in conical nanowells (Sphericalplate 5D, Kugelmeiers) in the bottom compartment, while fibroblasts were cultured in transwell inserts in the upper compartment. Organoid viability was measured after 9 days of co-culture via CellTiter-Glo 3D Cell Viability Assay (Promega).

Results:

PDX-derived fibroblasts express typical CAF markers as α-smooth muscle actin, Tenascin C, and Fibroblast-specific protein 1, as well as Androgen Receptor (AR) at both RNA and protein level. Moreover, AR protein expression is increased after 24h of treatment with Dihydrotestosterone (DHT) in both PNPCa and LAPC9 fibroblasts (obtained from androgen dependent and independent setting, respectively), and this effect is abrogated upon co-treatment with the AR inhibitor Enzalutamide. Transwell co-culture of PNPCa tumor organoids with both PNPCa and LAPC9-derived fibroblasts determines a higher organoid viability compared to PNPCa organoid mono-culture.
Conclusions:

Conclusions:

Our data suggest that PDX-derived fibroblasts, even though derived from the host stroma (mouse), represent a reliable tool to study the role of CAFs in advanced PCa progression, given the expression of typical CAF markers and AR protein expression modulation following DHT treatment. Transwell co-culture experiments suggest that PDX-derived CAFs are able to support tumor organoid viability, possibly through the establishment of a paracrine communication between tumor cells and fibroblasts.
As future perspective, we will apply tumor-CAF 3D co-cultures to study how CAFs promote PCa cell viability in low-DHT co-cultures or following ADTs (e.g. Enzalutamide treatment). In addition, we aim at characterizing the gene expression profile of PDX-derived fibroblasts and organoids in mono-culture vs co-culture settings through RNA-seq.

Katherine Crump

In vitro and *In Silico* Investigation of Dynamic Compression on Cartilage Endplate Cells in Agarose

Purpose:
Intervertebral disc (IVD) degeneration is one of the main causes of low back pain. Nevertheless, the initiating risk factors of IVD degeneration are poorly understood[1]. The cartilage endplate (CEP) covers the top and bottom of the IVD and acts to transmit compressive loads and transport water, nutrients, and waste in and out of the disc[2]. However, with aging and degeneration the CEP experiences structural changes and altered permeability[1]. Although most research focuses on the nucleus pulposus and annulus fibrosis, early CEP degeneration is believed to be a cause of nucleus dehydration and play a key role in IVD degeneration[3]. Therefore, a better understanding of the mechanobiology within the CEP is necessary to develop early therapies that target the CEP.
Methods:
In vitro: Bovine tail CEP cells were expanded until passage three. Afterwards, a 1:1 mixture of CEP cells and agarose was pipetted into silicon molds to create 2% agarose and $1x10^7$ cells/ml carriers of 6 mm in diameter and 3 mm in thickness and then cultured for 2 days for phenotype recovery. Cell-agarose carriers were placed in custom-made chambers, stimulated with 10 ng/ml TGF-β1 throughout the entirety of the experiment and dynamically compressed up to 7% strain for one hour at 1.5 Hz every day for up to 14 days. Those that were not dynamically loaded were only experiencing the constant weight of the chamber lid exerting ~5.1 Pa per carrier. Carriers were collected on Days 0, 7, and 14 for downstream analysis of cell viability and relative gene expression. *In silico:* A 2D axisymmetric porohyperelastic, compressible, Neo-Hookean finite element model (FEM) of a cell-agarose carrier was developed in Abaqus using literature-derived material properties. The model was loaded with the same dynamic compression as the *in vitro* experiment. A newly developed mechanotransduction network model, based on a previously developed biochemical regulation model,[4] was used to predict protein activation levels by initial perturbations of nodes that represent mechanoreceptors sensitive to dynamic compression (α5β1, αvβ3), physioosmotic pressure (TRPV4), tensile strain (αvβ5), and chondrogenic media (TGF-β). Predicted protein activation was normalized by the baseline conditions.
Results:
After seven and 14 days of culture, the cell-agarose carriers in all conditions demonstrated increased expression of the anabolic genes aggrecan (*ACAN*) and collagen II (*COL II*), alongside decreased expression of catabolic gene matrix metalloproteinase 3 (*MMP3*). Cell viability decreased between 14-28% after 14 days for all conditions, except for the dynamically loaded chondrogenic carriers which increased 6.6% (Fig. 1d). Cell viability was generally higher at the edge of the carrier in comparison to the center. FEM and network model results were validated using the experimental results. Reaction forces from the FEM (0.07N) match force data collected during loading (0.06N). The FEM showed that hydrostatic pressure varies from the center to edge of the carrier. General trends of increased/decreased gene expression and protein activation match between experimental and network model results.
Conclusions:
A novel framework coupling 3D cell culture with *in silico* methods is presented, in which the FEM provides details about loads experienced at each point within the carrier, while the network model uses these mechanical cues and environmental perturbations to predict the protein expression. Increased expression of anabolic genes *ACAN* and *COL II* convey that the agarose carriers provided an anabolic environment for CEP cells under static and dynamic compression. However, chondrogenic media and type of compression did not appear to affect gene expression. Additional biological replicates being done to validate the *in vitro* results. Interestingly, the FEM demonstrated that pressure is not uniform within the carrier. Comparison is limited because the network model predicts protein activation while the experiment measures gene expression. Future work will validate the mechanoreceptors activated during loading using immunohistochemistry and measure protein expression using multiplex assays.

Yuebing Li

Rho-kinase Involvement in an Animal Model of Subretinal Fibrosis

Purpose:

Subretinal fibrosis can evolve in the course of neovascular age-related macular degeneration (AMD). Until now, there is no successful treatment nor established animal model for subretinal fibrosis. Intravitreal anti-VEGF treatment can reduce the choroidal neovascularization (CNV), but not the subretinal fibrosis. Rho-associated protein kinases (ROCK) have multiple functions, including the regulation of smooth muscle cell contraction, cell migration, maintenance of cell viability and morphology, in part by regulating stress fibers and focal adhesions. ROCK has also been implicated in fibrosis formation. Rho-kinase inhibitors are compounds that target rho-kinase and inhibit the ROCK pathway. We aim to investigate the impact of the ROCK inhibitor fasudil on subretinal fibrosis after CNV in AMD.

Methods:

To induce CNV-related fibrosis. We used a 532-nm laser inserted in a slit-lamp delivery system. After development of fibrosis on day 35, the mice(C57BL/6J, 8-9w, mix gender) were treated intraperitoneally with fasudil for two weeks. The volume of fibrosis was quantified with optical coherence tomography (OCT) measurements every week. Additionally, we performed autofluorescence and fluorescence angiography at every time point to document fibrotic changes over time. Choroidal flat mounts were used for immunohistochemistry (Collagen 1, Isolectin B) in order to visualize development in vascularization and extracellular matrix. Western blot were performanced to evaluate the protein level of fibrotic related markers.

Results:

The leakage in the fluorescence angiography decreased after treatment and the subretinal fibrosis in the OCT images increased over the investigated time. Correspondingly, from day 21 to day 49 after laser injury, the expression of Collagen 1 increased, whereas isolectin B decreased. After treatment, we can see the volume of leakage decreased. The westernblot indicate there are 2 peaks appeared during the whole 49 days after laser.The pan-ROCK inhibitor, Fasudil, substantially reduced subretinal fibrosis in vivo.

Conclusions:

The current results indicate that the pan-ROCK inhibitor fasudil may has a therapeutic potential for the treatment of subretinal fibrosis in neovascular age-related macular degeneration.

Franziska Strunz

Repair of a critical size defect in estrogen-deficient mice treated with bisphosphonates

Purpose:

To prevent loss of bone mass and deterioration of microarchitecture in osteoporosis, bisphosphonates (BP) are the therapy of choice. Treatment with BP, however, due to their effects on bone metabolism, may impair the healing of fractures and large bone defects in these patients. Currently, critical size defects are filled with calcium phosphate-based bone grafts, often combined with the osteoinductive growth factor Bone Morphogenetic Protein-2 (BMP2). Within the present study, the potential of L51P, an engineered BMP2 variant binding and blunting BMP antagonists, to increase the biological efficacy of BMP2 was investigated in estrogen deficient mice, an established model for post-menopausal osteoporosis. In addition, the interference of BP therapy with biomaterial turnover was tested by implanting β-tri-calcium phosphate (βTCP) ceramics in a long bone critical size defect.

Methods:

Animals were either ovariectomized (*OVX*) or *sham* operated, 8 weeks later BP treatment was started. At this time point loss of bone mass was observed for the estrogen-deficient *OVX* animals. 5 weeks later, a critical size defect was generated in the left femur. βTCP cylinders loaded with 0.25 µg or 2.5 µg BMP2, 2.5 µg L51P, and 0.25 µg BMP2 + 2.5µg L51P and empty controls were fitted into the defects. The implantation site was rigidly fixed, using a titanium osteosynthesis system. Femora were collected 6 and 12 weeks post-op.

Results:

Micro-computed tomography (MicroCT) analysis 12 weeks post-op revealed preservation of the implant material in the control, 2.5 µg L51P and 0.25 µg BMP2 groups with low remodelling of the defect site in both *sham* and *OVX* mice with or without BP therapy. In contrast, 0.25 µg BMP2 + 2.5 µg L51P and 2.5 µg BMP2 cylinders showed higher turnover of the βTCP with stronger bone integration. After 12 weeks, no significant differences in levels of transcripts encoding bone and cartilage markers were detected by PCR among the experimental groups. Relative expression levels of transcripts encoding collagen I were 150 times higher than those for collagen II and around 5000 times higher than those for collagen X. The different implants did not influence the gene expression pattern of collagens significantly.

Conclusions:

The MicroCT results demonstrated the potential of L51P to increase the osteogenic efficiency of BMP2 in the healing of ceramic filled critical size bone defects. The relative expression profile of the different collagens suggests intramembranous bone formation in the rigidly fixed defects.

Jingyi Zhang

Inducing ferroptosis to overcome primary resistance to KRAS-G12C inhibitor therapy in lung cancer

Background

Lung cancer is the most common cancer with an aggressive clinical course and high mortality rates. Lung adenocarcinomas (LUAD) are the most common histological subtype, 25% of which are driven by an activating mutation in KRAS. KRAS proteins belong to GTPases family anchored to the plasma membrane and play an essential role in cellular signaling for proliferation, differentiation, and survival. KRAS (G12C) variants are most commonly found in LUAD. Development of effective therapeutics to inhibit KRAS-driven oncogenesis has eluded the field and KRAS has for long thought to be 'undruggable', until a breakthrough in targeting KRAS oncoprotein KRAS directly through a novel approach to covalently target the reactive $KRAS^{G12C}$. However, despite the encouraging clinical results, about half of the patients with $KRAS^{G12C}$-mutant LUAD in clinical trials did not experience significant tumor shrinkage with specific $KRAS^{G12C}$ inhibitors. These findings strongly suggest the existence of intrinsic resistance that leads to the heterogeneous response to $KRAS^{G12C}$ inhibition and the urgent need new strategies to overcome intrinsic resistance. Ferroptosis is a form of regulated cell death characterized by the iron-dependent accumulation of lipid hydroperoxides to lethal levels. It plays an important regulatory role in the occurrence and development of tumorigenesis. Previous studies from our laboratory indicate that ferroptosis evasion may contribute intrinsic resistance and limit the efficacy of $KRAS^{G12C}$ inhibitor therapy.

Aims:

1: To validate the synergistic effects of $KRAS^{G12C}$ inhibitors and ferroptosis inducers in $KRAS^{G12C}$-mutant lung cancer cells in vitro & in vivo.
2: To investigate the molecular mechanisms of the combination treatment.

Methods:
1. In vitro effects of G12C inhibition combined with ferroptosis induction on KRAS-mutant NSCLC.
2. In vivo effects of G12C inhibition combined with ferroptosis induction on KRAS-mutant NSCLC.
3. The molecular mechanisms by which ferroptosis resistance limits the susceptibility of KRASG12C-mutant NSCLC to G12C inhibitors.

Significance

There is a challenging thesis that intrinsic resistance limits the efficacy of $KRAS^{G12C}$ inhibitor therapy. In view of this situation, my project concentrates on new strategy to overcome this resistance. It is a promising treatment to optimize G12C inhibition therapy combined with ferroptosis inducers in NSCLC.

Ines Monteiro

ILC2s' direct effect upon cancer stem cells

Purpose:

It is the aim of this study to characterize the direct interaction between type 2 innate lymphoid cells (ILC2s) and cancer cells, particularly cancer stem cells (CSCs), in the settings of colorectal cancer (CRC) and acute myeloid leukemia (AML).

Methods:

Induction of AML: an AML mouse model was generated by the injection of retrovirally transduced granulocyte-macrophage progenitors (GMPs) with the mixed lineage leukemia (MLL)-AF9 oncogene coupled to a green fluorescent protein (GFP) into non-irradiated recipients. Mouse model of ILC2 depletion: RORαfl/fl IL7rCre mice were used. Overnight culture of leukemic stem cells (LSC) and ILC2s and colony forming assay (CFA): fluorescence activated cell sorting (FACS)-purified LSCs with/without ILC2s were co-cultured overnight (1000:1000). Methylcellulose colony assays were performed for 7 days for each round of plating. ILC2s' cytokine production assay: 5000 FACS-purified ILC2s were cultured 24h in IMDM with 10% of FCS and IL-2 0.05 U/ul, IL-7 50 ng/ml, with/without IL-33 10 ng/ml. The supernatant was analyzed using a multiplex immunoassay (Meso Scale Discovery). Isolation of ILC2s from colon: as described by Moro et al. 2015, with some modifications. Mouse model of CRC: CRC was induced with azoxymethane (intraperitoneal injection of 10ug/gr, at two time points) and dextran sodium sulfate (2% in water for 5 days, for three cycles) (AOM/DSS). CRC organoids: CRC organoids were derived from the AOM/DSS model and from the ApcMin/+,Vil-Cre, Krasf/f (ACK) model. Organoids were established by isolating colonic crypts and propagated in Matrigel in cancer organoid medium (COM). Co-culture of colon organoids with ILC2s: we established a 7-day 3D coculture model of disrupted CRC organoids (1000-3000 cells) in combination with ILC2s (250-1500 cells) or ILC2s' supernatant.

Results:

CRC: in 3D co-cultures of mouse CRC-derived organoids and colonic ILC2s, the presence of ILC2s significantly decreases organoid formation. This antitumorigenic effect was proportional to the quantity of ILC2s and independent of cell-cell contact. Consistent with these results, the antitumorigenic effect of ILC2s was maintained when only the ILC2s' supernatant was present. In an inflammatory CRC mouse model (AOM/DSS model), the absence of ILC2s lead to a significantly higher tumor load. These results suggest that, in the context of inflammatory colorectal cancer, ILC2s have a direct antitumorigenic effect.
AML: ILC2s' phenotype and cytokine production are different in AML vs. naive mice. In colony-forming assays with bone marrow ILC2s and leukemic-GMPs (L-GMPs), the presence of ILC2s increased the colony-forming capacity in the first round of platting and decreased it in the second round. This suggests that ILC2s might drive leukemic stem cells' differentiation into progenitor cells.

Conclusions:

Overall, we believe these preliminary results indicate ILC2s have a relevant and direct impact upon CRC and AML's cancer stem cells.

Slavko Corluka

Development of a Rotator Cuff Bioreactor for Biomechanical Study of the Tendon-to-Bone Interface

Purpose:
Rotator cuff tear (RCT) is a common injury for individuals over 50 years old, with a prevalence greater than 20%. The tear most often results from an avulsion of the supraspinatus tendon from the humeral head at the insertion site. This tendon-to-bone insertion site is called enthesis.
Once torn, RCTs are treated surgically in most cases. Unfortunately, the surgery has an unsatisfactory failure rate of up to 94% due to poor enthesis healing. To better understand the healing capabilities of the enthesis, *ex vivo* organ culture models with additional mechanical stimulation have been developed in the past. However, all those models investigate the tendon mid-substance but not the rotator cuff as a connected organ, including the enthesis. Consequently, these models do not adequately represent the clinical issue and are not entirely suitable for translational research. Therefore, a standardized and more complete RCT model is required. In this project, we aimed to: i) develop and validate a bioreactor device for future study of enthesis healing on human samples and ii) use the developed bioreactor to perform a preliminary investigation on the influence of mechanical loading on cell viability in isolated entheses from freshly slaughtered sheep.
Methods:
i) For the linear-stage bioreactor, a stepper motor, load-cell, and linear guide were combined and mounted inside a standard incubator for cell culture. A microcontroller did the communication between the load cell and the stepper motor.
ii) For the cell viability assay, entheses from freshly slaughtered sheep (N = 2) were halved and cultured in high glucose Dulbecco's Modified Eagle Medium (HG-DMEM) containing 5% fetal calf serum in a normoxic environment at 37°C. Samples were either cultured in an unloaded state (= free-floating) or under mechanical load (= loaded) for up to eleven days. The cell viability was assessed on days 0, 4, and 11 for the free-floating samples and on day 4 for the loaded sample.
Results :
i) A bioreactor that controls ex vivo enthesis loading based on predefined strain or force values was developed. As output, the bioreactor can measure force between 15 – 200 N with an accuracy of ±10% of the measured value and repeatability of ± 0.2 N. The absolute position of the linear displacement shows a precision of ± 0.1 mm with repeatability of ± 0.05 mm. Position, force, and time are all stored for evaluation. ii) The free-floating enthesis samples showed a cell viability of 97.6% on day 0, 63.9% on day 4, and 16.5% on day 11. The mechanically stimulated sample showed a cell viability of 74.4% on day 4.
Conclusions:
i) The developed bioreactor has sufficient accuracy and repeatability to be used for research purposes. Therefore, it can be used to develop a standardized human bioreactor model to study enthesis healing. Such a bioreactor opens an unprecedented field to test new treatment strategies ex vivo on rotator cuff tissue to facilitate a successful clinical translation.
ii) The results show the tendency that the mechanical load in the ex vivo bioreactor culture of entheses from freshly slaughtered sheep has a positive influence on cell viability. However, the results need further evaluation in future studies with larger sample sizes. In addition, the culture conditions and mechanical loading patterns need to be further investigated better to understand the exact influence of mechanical loading on enthesis.

Chaonan Jin

Comparative analysis of proteome and lysine acetylome between NAFL and early NASH

Purpose:

Progression of non-alcoholic fatty liver disease (NAFLD) towards non-alcoholic steatohepatitis (NASH) is currently one of the most important challenges in hepatology, and for which no effective drug is still available. Although recent quantitative proteomics has been used to characterize biomarkers in plasma and liver tissue of NAFLD patients, pervious serum proteomics only found biomarkers related to liver fibrosis in NASH patients. Lysine acetylation as a post-translational modification, is a maladaptation of physiology to the modern lifestyle. Reversible protein acetylation is now the subject of many investigations as an adjustable adaption to a modern lifestyle by regulating the activity of metabolic enzymes. Recent report about fatty liver is association with reduced sirtuin 3 activity and mitochondrial protein hyperacetylation suggest sirtuin 3 as a valuable biosignature. Nevertheless, the contribution from non-mitochondrial protein acetylation hasn't been recognized completely. What's more, the dysregulation of histones, as traditional targets of SIRTs, in NAFL and NASH, hasn't been demonstrated well. We aimed at uncovering differentially expressed and acetylated liver proteins, and at revealing manifestations of acetylation differences in the systemic circulation as well.

Methods:

To achieve this goal, we employed acetyl-lysine immunoaffinity enrichment and label-free quantitative proteomics approach to characterize the proteome and lysine acetylome between NAFL and early NASH (F0-2).

Results:

We reported data on the protein expression changes between NAFL and NASH as well. In total, 2898 proteins were quantified in global liver proteome profiling, 44 of them being different between groups. In the liver acetyl-proteome profiles, 223 proteins were quantified, and 21 showed statistical difference. In plasma acetyl-proteome profile, 97 proteins were quantified, 1 with differential expression between NAFL and NASH.

Conclusions:

In conclusion we could identify potential biomarkers in progression from NAFL to early NASH, revealing novel acetylated lysine residues and enriched pathways related to this essential development. Ultimately our results further help understanding the pathophysiology of the transition from NAFL, and thus provide therapeutic perspectives for NASH regression.