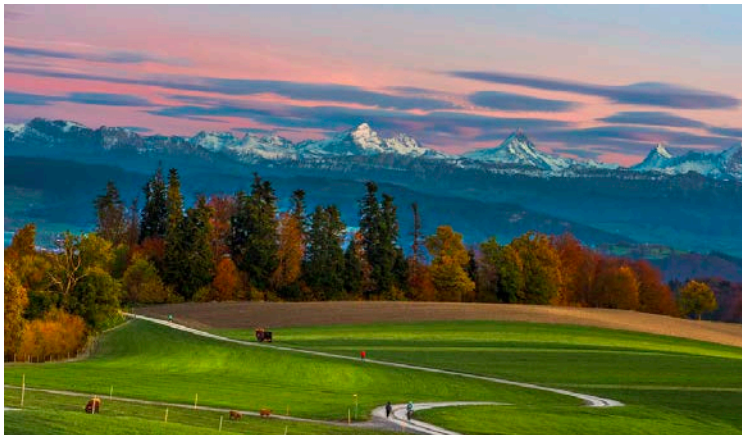
8th
SCRM PhD Students Retreat



Gurten Park
3 September 2021

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8th SCRM PhD Students Retreat
Gurten Park, Bern
3 September 2021

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: Chantal and Viviana		
09:10	09:30	Yuebing Li
09:30	09:50	Andreas Croft
09:50	10:10	Ioanna Tsioti
10:10	10:30	Franziska Langhammer
10:30	11:00	**Coffee Break**
11:00	11:20	Martina Minoli
11:20	11:40	Paola Bermudez
11:40	12:00	Elisa Rodrigues Sousa
12:00	13:00	**Mentor Talk: Prof. Jürgen Burger**
13:00	14:00	**Lunch Break**
Afternoon Session Chair: Cristina and Sandro		
14:00	14:20	Francesco Bonollo
14:20	14:40	Patricia Renz
14:40	15:00	Franziska Strunz
15:00	15:30	**Coffee Break**
15:30	15:50	Fatemeh Safari
15:50	16:10	Darya Karatkevich
16:10	16:30	Laura Jahnke
16:50	18:00	**Mentor Talk: Prof. Michal Schwartz**
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

5th Retreat, Paul Klee Center, 31th August 2018

6th Retreat, Gurten Park, 30th August 2019

7th Retreat, Gurten Park, 4th September 2020

Dear participants,

Welcome to the 8th 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. Jürgen Burger**, the director of the sitem Center and sitem School in Bern and **Prof. Michal Schwartz** from the Weizmann Institute of Science, Israel. 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

Viviana Rubino
Chantal Bachmann
Cristina Kalbermatter
Sandro Christensen

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.

Social distancing will be maintained as a limited number of people is participating at the retreat. In addition, use of masks is required in indoor premises.

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

Mentors

- Prof. Michal Schwartz, Weizmann Institute of Science, Israel
- Prof. Jürgen Burger, sitem Center and sitem School, Bern

Guests

The SCRM Steering Committee:

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

- Principal Investigators of the PhD students
- Rene Aeberhard

Laura Jahnke

Cross-species comparison of multicellular response during retinal gliosis

Purpose:

Degeneration in the human retina leads to visible remodeling processes, whereby the typical retinal layer structure gets lost. During the process, glial cells change morphology and establish a glial scar to contain degenerated tissue and to support remodeling of the retinal extracellular matrix (ECM). Fibrosis can develop in the course of the ensuing degeneration. So far, the mechanism responsible for limiting the regenerative capacity of mammalian glial cells nor the process of the retinal wound healing are well known. With that, there is no successful treatment imaginable.

Methods:

6-8 weeks old C57BL/6JRj mice underwent laser photocoagulation by a 532 nm diode laser (100 mW, 60 ms) to induce retinal fibrosis. Eight defined laser spots (diameter: 300 µm) per eye were set in the outer nuclear layer. Autofluorescence was employed to visualize the extent of laser damage, whereas the lesion size was quantified with optical coherence tomography (OCT) measurements. Hematoxylin and eosin staining (H&E) was performed on paraffin sections to follow degenerative changes over time. Additionally, confocal microscopy of vertical retina paraffin sections (5 µm). stained for gliotic markers glutamine synthetase (GS), glial fibrillary acidic protein (GFAP) and fibrotic markers such as fibronectin or type 1, 3 and 4 collagen after laser injury and during upcoming fibrosis from 3 h post injury (pi) up to day 49 pi. Sensory retinas with the laser spots were prepared for qRT-PCR analysis to quantify the gene expression of 84 different genes on day 7, 21, 35 and 49 pi.

Results:

From day 1 to day 49 after laser injury the leakage observed in the fluorescence angiography decreased whereas the subretinal scar in the OCT images increased. Furthermore, the expression of GFAP-positive, activated glial cells did not decrease over time. However, the expression of the different collagens in the lesion area increased reciprocally, whereas the fibronectin content decreased. The retinal mRNA samples showed the expression of all tested mouse ECM and adhesion molecule types. Surprisingly, we show a downregulation of 84 genes on day 21.

Conclusions:

During retinal degeneration, collagens are seen to replace fibronectin in the damaged area. The results also indicate that glial cells are involved in the development of a fibrotic scar and thereby hamper the regeneration in the retinal tissue decisively. Surprisingly, we proof a genetically shutdown on day 21 of 84 different ECM and cell adhesion involved genes with a restart on day 35 which seems to be the key point of the scar formation in mammal retina.

Yuebing Li

Rho-kinase involvement in an animal model of subretinal fibrosis

Background:

Age-related macular degeneration (AMD) is the leading cause of adult vision loss in the developed countries. Subretinal fibrosis can evolve in the course of neovascular AMD. Until now, there is no successful treatment nor established animal model for subretinal fibrosis. Intravitreal anti-VEGF treatment can reduce choroidal neovascularization (CNV), but not subretinal fibrosis. Rho-associated, coiled-coil-containing protein kinases (ROCKs) are involved in cytoskeletal rearrangement, contractility, angiogenesis and inflammation. Rho kinases have multiple functions, including the regulation of smooth muscle cell contraction, cell migration, maintenance of cell viability and morphology, in part by regulating stress fibres and focal adhesions[1,2]. Rho-kinase inhibitors are compounds that target rho kinase (ROCK) and inhibit the ROCK pathway[3]. Rock has been implicated in fibrosis formation[4].

Purpose:

Investigate the impact of the ROCK inhibitor fasudil on subretinal fibrosis after CNV in AMD.

Methods:

To induce CNV-related fibrosis, C57BL/6J mice were anaesthetized, and pupils were dilated with 5% phenylephrine and 0.8% tropicamide. Using a 532-nm laser, a slit-lamp delivery system, and a cover glass as a contact lens, six spots (100 mW, 50 µm,100 ms) were placed in each eye. The mice were then treated intraperitoneally with fasudil 20 mg/kg every day from day 35 after laser injury for two weeks. The volume of CNV and fibrosis was quantified with optical coherence tomography (OCT) measurements and with choroidal flat mounts stained with isolectin B (CNV) and type 1 collagen (fibrosis) every week after laser injury (d7, d14, d21, d28, d35, d42, d49, n=6 per timepoint). Additionally, we performed autofluorescence and fluorescence angiography at every time point to document CNV and fibrosis changes over time.

Results:

The leakage in the fluorescence angiography decreased and the subretinal fibrosis in the OCT images increased over the investigated time. Correspondingly, from day 21 to day 35 after laser injury, the expression of collagen 1 increased, whereas isolectin B decreased. The pan-ROCK inhibitor, Fasudil, substantially reduced subretinal fibrosis in vivo. With fasudil fibrosis markers, such as collagen1 and α-SMA, in the subretinal fibrosis lesions decreased significantly compared to the control group (vehicle-treated mice) at day 49 after laser injury.

Conclusions:

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

1. Leun T, Chen XQ, Manser E, Lim L (October 1996). "The p160 RhoA-binding kinase ROK alpha is a member of a kinase family and is involved in the reorganization of the cytoskeleton". Molecular and Cellular Biology. 16 (10): 5313–27.
2. Liao JK, Seto M, Noma K (July 2007). "Rho kinase (ROCK) inhibitors". Journal of Cardiovascular Pharmacology. 50 (1): 17–24.
3. Liao, James K.; Seto, Minoru; Noma, Kensuke (July 2007). "Rho Kinase (ROCK) Inhibitors". Journal of Cardiovascular Pharmacology. 50 (1): 17–24.
4. Wang SK, Chang RT (2014). "An emerging treatment option for glaucoma: Rho kinase inhibitors". Clinical Ophthalmology. 8: 883–90.

Andreas Croft

In situ cell signalling of the Hippo-YAP/TAZ pathway in reaction to complex dynamic loading in an intervertebral disc organ culture

Introduction:

Intervertebral disc (IVD) degeneration (IDD) is the main contributor to chronic low back pain. However, the exact mechanism of IDD is still not understood in its entity. Recently, the hippo pathway has been correlated with IDD, as it plays a key role in cell proliferation, differentiation, regeneration, and cell survival. Especially the inactivation and phosphorylation of the transcriptional co-activators yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ), which are a negative regulator of this pathway, have been associated with the induction and progression of IDD. Therefore, our aim was to investigate the influence of different mechanical loading profiles in 2 Degree-of-Freedom (2DoF), i.e., compression and torsion, on the induction and progression of IDD and in particularly the role and context of YAP/TAZ and the hippo pathway. In particular, we were interested to see how mechanical overloading in torsion would trigger the tis sue responses in the center and the periphery of the IVDs, which consist of two different tissue types.

Methods:

About one-year old coccygeal IVDs were isolated from bovine tails obtained from a local abattoir. All discs were excised and prepared for organ culture as previously established. The discs were randomly assigned to one of four different mechanical loading regimes for seven days, i.e., i) static, ii) low stress, iii) intermediate stress, and iv) high stress profile using a bioreactor that allows 2DoF loading. After one week of loading, the tissue and the culture medium of each condition was harvested and analyzed for their glycosaminoglycan (GAG) content, stress factors, i.e., the presence of nitrogen oxide (NO) radicals, relative disc height changes, relative gene expression of anabolic, catabolic, and inflammatory markers as well as the major players in the hippo pathway.

Results:

After one week of organ culture in the bioreactor, a significant loss of the disc height was observed in every mechanically loaded test condition: However, in particular for the intermediate stress condition (up to 27 ± 5% height loss). Furthermore, a significant decrease of GAG in the tissue (232 ± 48 vs. up to 314 ± 14 µg GAG / mg dry weight) and a significant increase of GAG in the culture medium was recorded for the high stress condition (97 ± 14 vs. 44 ± 22 µg GAG / cm^3 fresh tissue). Moreover, generally a higher upregulation of catabolic (MMP13 up to 1805 ± 2284-fold upregulation) and inflammation-related genes (MCP1 up to 121 ± 201-fold upregulation) was observed the more stressed the IVDs were. Finally, a significant upregulation of TAZ (up to 49 ± 73-fold upregulation) was found for the high stress condition.

Discussion:

Altogether, the experiments conducted for this study showed that the four different mechanical loading profiles lead to a decrease in IVD height, a shift from a predominant anabolic to catabolic state, and an upregulation of pro-inflammatory markers, which are all known indications for IDD. Furthermore, we were able to show that the hippo pathway reacted differently depending on the degenerated state of the IVD and the applied mechanical loading profile. Hence, this study showed the potential of targeting YAP/TAZ and the hippo pathway to counteract IDD.

Darya Karatkevich

Schedule-dependent treatment increases chemotherapy efficacy in malignant pleural mesothelioma

Background:

Malignant pleural mesothelioma (MPM) is a rare aggressive cancer, with insidious growth, and is associated with poor outcomes. Ethiology is strongly linked to exposure to asbestos. Prognosis with MPM is poor and median survival ranges from 8 to 14 months from diagnosis. Chemotherapy is the only treatment modality that has been shown to improve survival in MPM. Standard chemotherapy consists of combined pemetrexed and cisplatin treatment. Pemetrexed is blocking key enzymes of nucleotide synthesis, which leads to nucleotide pool depletion and subbsequent inhibition of DNA synthesis. Cisplatin treatment results in DNA inter- and intrastrand crosslinks. We hypothesized that pretreatment with pemetrexed will induce nucleotide pool depletion thereby increasing the anticancer efficacy of sequentially administered cisplatin.

Methods:

MPM MESO-1 cells were treated for 72 h with pemetrexed. Three treatment schedules were evaluated by initiating 24 h of cisplatin treatment at 0 h (concomitant), 24 h and 48 h relative to pemetrexed treatment, resulting in either concomitant administration or pemetrexed pretreatment for 24 h or 48 h, respectively. Multicolor flow cytometry was performed to detect γH2AX marker (phosphorylation of histone H2AX) as a surrogate marker to detect the activation of the DNA damage response pathway, Mito Tracker staining to determine the mitochondrial mass. DAPI staining of DNA was used to analyze cell cycle distribution.

Results:

Pemetrexed pretreatment for 48 hours prior to cisplatin treatment significantly reduced long-term cell growth. Furthermore, senescence induction was significantly increased and cells were characterized by persistent S-phase arrest and persistent DNA-damage.

The present study indicates that optimization of the standard treatment schedule by including pretreatment with pemetrexed increases the anticancer efficacy of pemetrexed-cisplatin combination therapy in MPM. The observed benefits were associated with a persistence of treatment-induced DNA damage.

Fatemeh Safari

Introduction:

Src-homology (SH) 2 domain-containing inositol-5-phosphatase 1 (SHIP1) is a lipid phosphatase that acts as a negative regulator of the PI3K/Akt signalling pathway. SHIP1 is widely expressed in hematopoietic cells where it regulates cell proliferation, differentiation, and survival. Ship1-deficient mice are characterized by a reduced bone mass, which is associated with an increase in the number of osteoclasts (OC). This study aimed to investigate the underlying mechanisms through which SHIP1 contributes to the regulation of bone mass and architecture.

Methods:

For the generation of osteoclast progenitor cells (OPC), bone marrow cells from Ship1$^{-/-}$, Ship1$^{+/-}$ and wt (wild type), mice were incubated overnight in culture medium supplemented with CSF-1. To develop OC, OPC were cultured with RANKL and CSF-1. Osteoclastogenesis was evaluated using an XTT cell viability assay, TRAP activity (OC marker) and qRT-PCR. Micro-computed tomography (MicroCT) of vertebrae and distal femora were performed to characterize the structure of bone.

Results:

SHIP1 deficiency was found to affect the *in vitro* development of OC. Ship1$^{-/-}$ OPC showed a 1.5-fold increased proliferation compared to Ship1$^{+/-}$ and wt controls ($p<0.001$). Furthermore, the number of OC developing from Ship1$^{-/-}$ OPC was reduced when compared to Ship1$^{+/-}$ and wt OPC ($p<0.001$). Levels of transcripts encoding Calcr (calcitonin receptor) were downregulated in cultures of Ship1$^{-/-}$ OC ($p<0.001$). *In vivo*, MicroCT results revealed a reduction of BV/TV in vertebrae and femora of Ship1$^{-/-}$ versus wt animals (35% and 24%, respectively ($p<0.001$)). Trabecular number in Ship1$^{-/-}$ mice was increased by 26%, while thickness was decreased by 30% ($p<0.01$). In contrast, in femora from Ship1$^{-/-}$ mice, thickness of the trabeculae was decreased by 25% ($p<0.05$), whereas the trabecular number remained unchanged. These differences in bone micro-architecture in femora and vertebrae from Ship1$^{-/-}$ mice in comparison to wt animals suggest different paths to low bone mass in bones with primary and secondary spongiosa, respectively.

Conclusions:

Taken together, our data suggests a direct or indirect role for SHIP1-dependent PI3K/Akt signalling in bone remodelling. The low bone density phenotype in Ship1$^{-/-}$ mice may be caused by either less bone formation or an increase in bone resorption. Further investigation will address the contribution of osteoblasts to the development of osteoporosis in Ship1-deficient mice.

Ioanna Tsioti

Systemic inflammation has been previously suggested to trigger activation of microglia cells in the retina. The aim of this study is to investigate the effect of systemic lipopolysaccharide (LPS) exposure on the inflammatory status of the mouse retina, *in vivo*.

C57BL/6-Tg(Csf1r-EGFP-NGFR/FKBP1A/TNFRSF6)2Bck/J(MaFIA),B6(Cg)-Tlr4tm1.2Karp (Tlr4$^{-/-}$), TekCre^{+}Tlr4$^{loxp/loxp}$ and C57BL/6J wild type mice were used in this study. MaFia mice express green fluorescent protein (GFP) under the colony stimulating factor 1 receptor (csf1r) promoter, allowing the *in vivo* imaging of microglia and monocyte-derived macrophages in the retina. TekCre^{+}Tlr4$^{loxp/loxp}$ mice lack the Tlr4 expression in endothelial cells, allowing us to study the involvement of endothelial cells in the development of retinal inflammation. The mice were intravenously injected with 1 mg/kg LPS for four consecutive days and their retinas were monitored *in vivo* at baseline, day 4, day 7 and day 14 after the first LPS injection. Experimental choroidal neovascularization (CNV) was induced on C57BL6/6J and and Tlr4$^{-/-}$ mice. The wild type animals were injected with a single dose of LPS (1 mg/kg) after the CNV induction. All the mice were monitored at day 3, day 7 and day 14 after the CNV.

In vivo imaging was performed using spectral domain – optical coherence tomography, autofluorescence imaging and fluorescein angiography, as well as, flow cytometry using specific markers for microglia and monocytes-derived macrophages. Immunohistochemical experiments were also accomplished, using specific markers for microglia/macrophages and Müller cells. Electroretinography recordings (ERG) were utilized in order to investigate alterations in the retinal function.

LPS injections led to elevated numbers of microglia/macrophages population, accumulation of these cells around the retinal blood vessels and vasodilation at day 4 after the first LPS challenge in the wild type animals but not in the Tlr4$^{-/-}$ mice. ERG recordings revealed a trend of decreased a and b wave amplitudes in the LPS-challenged C57BL6/J mice, suggesting that LPS may affect retinal function. Furthermore, a single dose of LPS led to an increase of fluorescein leakage and enhancement of gliosis at day 3 after the CNV induction in wild type animals. Additionally, CNV induction showed a reduction of fluorescein leakage at day 14 after the laser lesion in Tlr4$^{-/-}$ mice compared to C57BL6/J mice. All these phenomena are prevented in Tlr4$^{-/-}$ mice, suggesting a role of this receptor in the LPS-induced phenotype. Our data suggest that systemic LPS-induced inflammation perturbs retinal homeostasis in a Tlr4-dependent manner.

Franziska Langhammer

Pathomechanisms of RHOBTB2- associated neurodevelopmental disorders

Missense mutations in the RHO GTPase RHOBTB2 have recently been linked to a variety of neurodevelopmental disorders, including epileptic encephalopathy and seizures. However, neither the underlying pathomechanisms nor the role of RHOBTB2 in neurodevelopment are fully understood. This phenotype appears to derive from an accumulation of mutant RHOBTB2 in the cells, correlating with the reported seizure susceptibility in flies overexpressing the Drosophila orthologue. We hypothesize that these altered levels of RHOBTB2 impair RNA splicing events, resulting in deregulated ion channel expression and seizures. During my PhD, I test this hypothesis by adopting two different approaches. First, I will use genetic interaction studies in Drosophila to verify potential targets of RHOBTB2 involved in RNA processing and to determine the functional relationship between RHOBTB2 and ion channel genes. Second, I will validate this data in a neuronal context by generating neuronal precursor cells with altered RHOBTB2 expression. Collectively, my findings will elucidate the pathomechanisms of RHOBTB2-associated disorders, laying a foundation for the development of further treatment.

Franziska Strunz

Repair of a critical size defect in osteoporotic mice

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 natural or synthetic bone grafts, often in combination with the osteoinductive growth factor Bone Morphogenetic Protein-2 (BMP2). L51P, an engineered BMP2 variant, which binds and blunts BMP antagonists, may cause an increase of the biological efficacy of BMP2 and thereby may potentially reduce the amounts of BMP2 required to stimulate bone formation and repair.

In the present study, it is hypothesized, that BP therapy interferes with biomaterial turnover. To test this, the turnover of β-tri-calcium phosphate (βTCP) implant ceramics was studied in a long bone critical-size defect in ovariectomized (OVX) mice treated with BP.

Eight weeks after induction of osteoporosis by OVX, and after detection of bone loss, treatment with alendronate (ALN), a commonly used BP, commenced. Sham operated animals were used as controls. Five weeks later, a critical size defect (3.5 mm) 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 BMP2/2.5 µg L51P and empty controls were fitted into the defects. The implantation site was rigidly fixed, using a commercially available titanium osteosynthesis system. Femora were collected six and twelve weeks after placement of the defect.

OVX led within eight weeks to a significant decrease of total bone density in tibiae and femora in comparison to a sham procedure. Micro-computed tomography analysis revealed that the ALN treatment of both sham and OVX mice caused an increase in bone growth. Moreover, OVX mice with and without BP therapy, which received implants loaded with 0.25 µg BMP2/2.5 µg L51P and 2.5 µg BMP2, showed a strong induction of bone growth both 6 and 12 weeks after surgery. Furthermore, bone formation at the defect site was stimulated in sham animals as well, both with and without BP medication, after receiving implants coated with 0.25 µg BMP2/2.5 µg L51P and 2.5 µg BMP2.

The results indicate combined effects of BMP2 and L51P on bone healing. Moreover, BP caused a reduction in implant turnover. Therefore, efficiency of healing of biomaterial-filled bone defects might be impaired in patients treated with BP due to impaired implant substitution.

Patricia Renz

Deciphering astrocyte polarization in acute perinatal white matter injury and its contribution to disease outcomes

Acute perinatal white matter injury (WMI) is the most common form of brain injury in preterm infants and is characterized at the cellular level by reactive microgliosis and astrocytosis and disrupted oligodendrocyte maturation. Recent studies on the astrocyte response to brain injury highlight the formation of diverse reactive astrocyte subtypes, some favoring brain repair and other «inflammatory» astrocytes (iA) contributing to neurodegeneration. The specific nature of astrocyte reactivity after WMI remains obscure. We now know that iAs form in response to reactive microglia-derived cytokines and lead to myelination failure. We, therefore, hypothesize that iAs play a central role in WMI and may be an exciting therapeutic target for this disease.

iA formation was analyzed across multiple rodent WMI models using a combination of hypoxic-ischemic and inflammatory insults. To confirm WMI, myelin deficits were evaluated using immunohistochemistry for MBP. iA formation was investigated through *in situ* hybridization (ISH) using a complement component 3 (C3)-specific probe. We further characterized astrocyte reactivity in WMI by performing qRT-PCR analysis on mRNA isolated from primary astrocytes acutely purified from injured and healthy brains. IL-1α/TNF/C1q knockout mice unable to generate iAs were used to investigate the necessity of iAs for WMI outcomes.

ISH demonstrates a significant increase of C3-positive iAs in subcortical white matter tracts across multiple rodent WMI models. Supporting this finding, qRT-PCR results suggest that purified astrocytes from injured brains exhibit a multi-gene inflammatory astrocyte signature at the transcriptome level. Ongoing experiments in mutant mice test whether iAs are central drivers of WMI pathogenesis.

Our experiments demonstrate the formation of iAs in multiple rodent WMI models, test these cells' ability to drive WMI outcomes, and work towards an in-depth characterization of astrocyte polarity in WMI. Guided by these results, we will evaluate the therapeutic potential of optimizing astrocyte polarization to improve WMI outcomes.

Jordan Pickles

Effects of Home-based Fitness Gaining on Cardio-Metabolic and Cognitive Health Markers in Individuals at Risk of Type 2 Diabetes

Type 2 diabetes is a global public health emergency with ~463 million cases worldwide, which is projected to reach ~578 million by 2030. This is associated with a huge financial burden, as 12% of global health expenditure is spent on diabetes treatment and it is associated complications. Regular physical activity slows the progression of type 2 diabetes and cardiovascular events due to the beneficial effects on many physiological parameters. Despite overwhelming evidence that an inactive lifestyle leads to chronic disease and premature death, many adults do not meet the minimum amount of physical activity required to reduce the risk of metabolic disease. Common barriers to physical activity include: lack of time, limited access to facilities and appropriate equipment, inadequate financial resources, bad weather, difficulty with transportation and lack of motivation. The aim of this project is to combine the expertise of an interdisciplinary team of game researchers, game designers, exercise physiologists and metabolic researchers to develop and evaluate a home-based exergame that improves markers of cardio-metabolic health and cognitive health while effectively removing barriers to exercise in individuals at elevated risk of diabetes. To achieve this, two projects will be conducted: Part 1 aims to evaluate the usability and feasibility of the home-based exergame under lab conditions. Part 2 aims to assess changes in markers of cardio-metabolic and cognitive health following 6 weeks of home-based exergame training compared to traditional exercise programme based on the American Diabetes Association guidelines in obese individuals at elevated risk of diabetes. In addition, we will evaluate adherence and compliance as well as overall experience to the 6-week training intervention.

Paola Bermudez

Exploring cell signalling in 3D organoid-like models that mimic intervertebral disc degeneration

Lower back pain (LBP) is a highly prevalent, chronic and costly medical issue in modern society. LBP is multifactorial, among the different possible causes for LBP, Intervertebral Disc Degeneration (IDD) is thought to account for around 40% of chronic back pain. IDD is a chronic process that results in structural and biomechanical changes in the intervertebral discs (IVD). An IVD is situated between two adjacent vertebral bodies and is responsible for motion, compressive loads, flexibility and protection of the neural anatomy of the spine. Under physiological conditions, the central region of an IVD consists of gelatinous nucleus pulposus (NP) including large amounts of proteoglycan in particular aggrecan, which is responsible for an osmotic swelling pressure within the disc due to the fixed negative charge of the associated glycosaminoglycans. Peripherally to the NP, the annulus fibrous (AF) is located. Finally, two hyaline cartilage endplates (superior and inferior) interface with the vertebral bodies regulating the nutrient diffusion in the disc. During degeneration, the NP region becomes more fibrous tissue with decreased water content due to loss of proteoglycans, and the AF shows an accumulation of structural defects. Therefore, IVD ageing is accelerated leading to a progressive and cell-mediated cascade of molecular changes. Moreover, disc degeneration is closely linked to genetics, environment, co-morbidities and lifestyle. However, back pain treatments both surgical and conservative, focus on palliating the symptoms rather than targeting the degeneration process.

In the last decades multi-dimensional biological data related to discogenic pain has been acquired from gene expression, signaling, secretory activity and metabolomics to advanced imaging using Magnetic Resonance Imaging (MRI). Similarly, a wide range of cytokines such as TNF-α or IL1-β have been applied *in vitro* to IVD cells in order to induce a degenerative phenotype. However, current studies have investigated the effects of those cytokines under standard culture condition. On the other hand, previous evidences have shown that IDD could be mimicked by adjusting glucose concentration, pH, osmolality and/or oxygen levels, remaking the importance of disc environment. Thus, a multidisciplinary approach could be a promising solution to establish a rational map of the most important degenerative processes. In the case of the IVD, it is evident that nutritional factors are crucial to understand the microenvironment. In terms of experimental models, 3D constructs with oxygen and nutritional perturbations could be proposed in order to study the transcriptome of IVD cells under these conditions. Thereby, a better understanding of the degenerative pathways behind IDD could be achieved.

Francesco Bonollo

Elucidating the stromal contribution of androgen deprivation therapy-resistance in prostate cancer

In my PhD project, I will investigate the role of Cancer-Associated Fibroblasts (CAFs) in promoting castration-resistant and metastatic prostate cancer (PCa) to support the idea that not only tumor cells but also components of the tumor microenvironment (also known as ‘reactive stroma’) can be targeted to halt tumor progression. To purse this aim, three different PCa patient-derived xenograft (PDX) models will be used, representing androgen-dependent soft tissue metastasis (PNPCa model), androgen-dependent bone metastasis (BM18) and androgen-independent bone metastasis (LAPC9). Gene expression profiling of the tumor and stromal components of these PDXs in androgen intact and castrated settings will be analyzed. Furthermore, PDX-derived tumor cells will be combined with PDX-derived fibroblasts, as well as patient-derived CAFs, to establish 3D co-cultures (tumor-stromal organoids). These in vitro models will be used to characterize the role of CAFs in promoting tumor organoid growth and drug resistance, as well as gene expression alterations occurring in tumor and stromal cells. The ultimate goal of my project is to identify stromal genes/pathways that could represent novel therapeutic targets in PCa.

Elisa Rodrigues Sousa

The role of CRIPTO signaling in lethal prostate cancer

Introduction & Objective:

Prostate cancer (PCa) is the most frequently diagnosed malignancy and the second leading cause of cancer-specific deaths in men in Western countries. Early stages of PCa can be treated with surgery and androgen deprivation therapy, but cancer can become castration-resistant (CRPC), possibly due to pre-existing stem cell-like PCa cells that survive castration and re-initiate tumor growth leading to metastasis.

Human tumors express high levels of CRIPTO, an oncofetal protein with multiple oncogenic effects both *in vitro* and *in vivo*. We hypothesize that CRIPTO is involved in tumor initiation and late progression of PCa and aim to investigate its oncogenic role in organoids derived from genetically engineered mice models (GEMMs) of PCa.

Material & Methods:

The impact of high/low CRIPTO expressing cells on disease progression was measured in a PCa tissue microarray (TMA) from the EMPACT cohort (N=210).

To assess the role of CRIPTO in early and late PCa we triggered oncogenesis in NP ($Nkx3.1^{CreERT2}$; $Pten^{flox/flox}$, $R26^{LSL\text{-}YFP/LSL\text{-}YFP}$) and NPK (for $Nkx3.1^{CreERT2}$; $Pten^{flox/flox}$; $Kras^{LSL\text{-}G12D/+}$, $R26^{LSL\text{-}YFP/LSL\text{-}YFP}$) animals. NPY animals develop high-grade PIN/carcinoma lesions with local invasive epithelium while NPKY animals develop invasive prostate adenocarcinoma with lung and liver metastasis. Single cells were isolated from the prostate tumors, the $YFP^{+/-}$ population recovered by FACS sorting and cultured as organoids. To test the tumorigenic potential of the organoids derived from NPY and NPKY GEMMs, we will modulate levels of CRIPTO protein.

Results:

We measured the expression of CRIPTO in PCa TMA and we showed that an elevated number of CRIPTO positive cells within the primary tumor correlates with PSA (p=0.002) and local/metastatic (p=0.00037) progression.

NPY and NPKY animals were induced with tamoxifen both in intact and castrated/regenerated settings. Prostate tissue was collected, and organoids derived. Organoid morphology varies from solid to cystic structures, diameters range between 40 to 250 µm and positively express luminal (CK8) basal (p63) and proliferative markers (Ki67).

We have successfully generated NPCrY and NPKCrY ($CRIPTO^{flox/flox}$) GEMMs.

Conclusion:

High CRIPTO expression correlates with poor patient outcomes. Our preliminary data support the role of CRIPTO in PCa progression. We are currently characterizing the phenotype of NPCrY and NPKCrY animals and working on CRIPTO modulation in the organoids derived from NPY and NPKY GEMMs.

Martina Minoli

Bladder Cancer: Patient-Derived Organoids as a Tool for Precision Medicine

Introduction:

Bladder cancer (BLCa) is the 10^{th} most frequent cancer worldwide. Non-muscle invasive (NMBC) and muscle invasive BLCa (MIBC) are the two major categories used to define BLCa. This classification however underscores the molecular heterogeneity and clinical complexity. A more personalized characterization of the BLCa phenotype and its drug sensitivity may be crucial to promote both better patient stratification and tailored therapeutic treatments. Pre-clinical models as patient-derived organoids (PDOs) are useful tools to screen drugs and to tailor medical care to a patient's individual genetic background.

Our study is aimed in the investigation of PDOs to determine drug sensitivity profiles of BLCa patients to personalize therapy accordingly. We aim to correlate drug responses of PDOs to their molecular and genomic profiles.

Materials & Methods:

To generate PDOs, BLCa samples were digested and single cells seeded in suspension. The drug sensitivity was evaluated by viability assay after 48 hours of treatment. DNA was extracted from PDOs, parental tumors (PT) and blood, and whole exosome sequencing was performed. PDOs were characterized by immunofluorescence staining and parental tumor by immunohistochemistry.

Results:

We successfully generated PDOs from 14 NMIBC and 7 MIBC tissues. The genomic profile of the original tumor tissue and the matching PDOs is highly conserved. Mutations relevant for BLCa were found in both PT and matching PDOs. By further stratifying PDOs in luminal and basal according to specific molecular markers, we confirmed that PDOs retained BLCa heterogeneity in culture and that basal PDOs are more sensitive to cisplatin/gemcitabine combination, compared to luminal PDOs. In order to identify novel drug candidates, we evaluated the efficacy of a panel of FDA-approved drugs compared to standard of care on PDOs. Sensitivity of PDOs to the standard of care reflected the clinical course observed in patients. Moreover, we are performing longitudinal studies by collecting tissue from patients overtime. Our preliminary results support the concept that PDOs recapitulate the original tumor in terms of drug sensitivity and acquired resistance.

Conclusions:

Our findings strongly suggest that PDOs are a reliable *in vitro* model to provide clinically meaningful drug sensitivity profiles but also to investigate the correlation between drug sensitivity and genomic/molecular profiles.