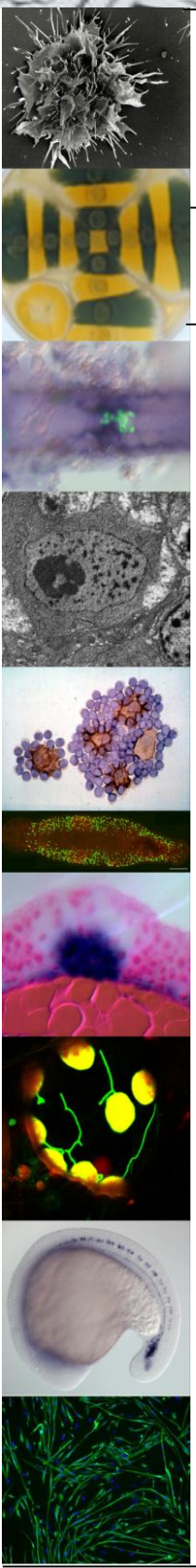
Symposium
Lab-Rotations WS 23/24



universität innsbruck

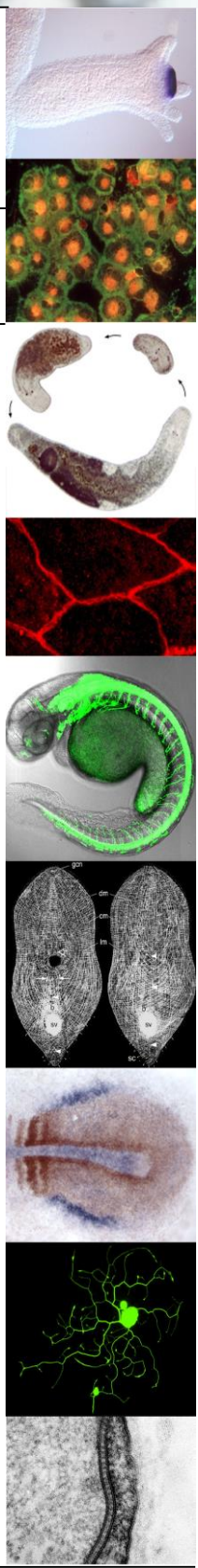
Master ‚Molecular Cell and Developmental Biology'

Monday, October 02, 2023, starting at 5 pm

in Lecture Hall F, Technikerstr. 25, upper floor

Reception:
17:00 - 17:15 welcome words by Alexander Weiss
Session 1: Model systems:
17:20 Feldmann, David (Rothbächer):
Functional analysis of Ciona intestinalis larval adhesion
17:30 Hellemans, Lore (Ladurner):
The role of Kringle domain proteins in the bio-adhesion of Macrostomumnov.sp.
17:40 Kolb, Philipp (Ladurner):
Annotation of clusters from single cell RNA sequencing data of a new Macrostomum species by screening of marker genes via in situ hybridization
17:50 Pötzl, Luis (Hautz-Neunteufel / Meyer):
Establishing assessment tools to monitor organ function and pharmacokinetics for a drug testing and development platform in ex-situ perfused livers
Session 2: Immunology:
18:00 Dieringer, Jonathan (Rocamora Reverte / Weinberger):
Identification of suppressive CD4+ Regulatory T cells in young and old age
18:10 Fleischmann, Anna (Hagen):
Characterization of CD4+/CD8+ double positive T cells
18:20 Rungger, Katja (Hackl):
Effects of intratumoral microbiota on antitumor immunity in ovarian cancer
18:30 **Coffee Break:** optional if we have time...
Session 3: Metabolism:
18:40 Campagnol, Sara (Edenhofer):
Bridging the gap between epigenetic changes and DNA damage repair: The role of PHF2 in the ageing brain
18:50 Giaquinta, Riccardo (Weiss):
Cell-based in vitro screening of FAHD1 inhibitors
19:00 Huber, Laura (Seretis / Jansen-Dürr):
Role of FAHD1 in senescence of Immune cells: establishing new methods
19:10 Löffler, Ferdinand (Kimmel):
Transcriptomic profiling of normal versus diabetic retina to uncover mechanisms of glucotoxicity and retinal regeneration
19:20 Mayer, Elena (Erlacher):
How mRNA modifications at the start codon affect translation initiation
Flash-Talk awards
19:30-19:45 as judged by anonymous vote

Poster Session:
20:00 21:00 poster session please, be present at your poster
21:00 Poster awards as judged by a jury
21:00 - 22:00 open poster session



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ABSTRACTS

Functional analysis of *Ciona intestinalis* larval adhesion

Speaker: *David **Feldmann**, BSc.*

Supervisor: *Ute Rothbächer, Assoc.-Prof. Dr.*

Affiliation: *Institute of Zoology, LFUI*

Abstract:

The tunicate papillae (palps), function as a sensory adhesive organ during larval settlement and serve as the initiation point for the transition to a sessile lifestyle. The adhesiveness exhibited during metamorphosis can pose challenges as a biofouler for the shipping and aquaculture sectors, while simultaneously garnering interest in the medical field as a biological adhesive. The papillae are made of various cell types, including central collocytes (CC) responsible for secreting the glue-like adhesive which can be further subdivided into two cell types, the inner CC (iCC) and the outer CC (oCC). Recent data from mass spectrometry (MS) and single-cell RNA sequencing were used to identify candidate proteins found both in the adhesive substance and those expressed within the papillae. Candidate proteins were chosen depending on their abundance and predicted functional domains. Using cloning, electroporation, and fluorescent microscopy techniques, we are able to visualize and localize candidate proteins in the collocytes. Of candidates that were abundant and localized, we designed CRISPR knockouts to assess adhesion by disrupting the function of promising candidate proteins. Additionally, we designed constructs for overexpression of DACH, Islet, and SP5/6/8 genes under the larval papillae promoters pFoxC and pFoxG, which may influence collocyte formation, in order to gain a better understanding of papillae cell functionality. Utilizing genetic cloning techniques and electroporation we aim to unlock both the composition of the adhesive glue and the mechanism of papillae attachment in Ciona intestinalis. In the long run, this research could potentially aid in the development of biomimetic adhesives for use in wet environments or environmentally friendly anti-fouling formulations.

The role of Kringle domain proteins in the bio-adhesion of *Macrostomum nov. sp.*

Speaker: *Lore **Hellemans**, BSc.*

Supervisor: *Peter Ladurner, Univ.-Ass. Dr.*

Affiliation: *Institute of Zoology, LFUI*

Abstract:

Bioadhesion is the ability to use biological molecules to attach oneself to a surface temporarily in fresh- as well as saltwater environments. Truly understanding the functionality of such adhesion mechanisms would enable the production of bioadhesives beneficial for medical, as well as industrial application. A few years ago, a new member of the *Macrostomum* genus was discovered, *Macrostomum nov. sp.*, which holds suitable characteristics for a model organism: the availability of a high-quality reference genome, karyotypical stability, a short reproduction time with a high number of offspring, and they can be kept easily in culture. For these reasons we selected this animal to work with in this project study. We took a closer look at the function of a tail-specific bioadhesion candidate which was previously discovered in Theama mediterranea[1], a kringle-domain containing protein, referred to as M022-kringle in this project. Using bioinformatical tools, we identified and isolated the m022-kringle gene and protein sequences in *Macrostomum nov. sp.*. We cloned three different regions of the gene into vectors, allowing us to synthesize *in-situ* probes to identify the location of expression using whole mount *in-situ* hybridization, confirming the gene is tail specific. In addition, we also identified a marker gene for anchor cells, and verified that another Kringle domain containing protein, with a similar sequence to m022-kringle, is not tail specific. To test the function of M022-Kringle, we used RNAi knockdown and observed a reduction of adhesion capabilities by means of visual observation of the animals, an adhesion assay, and whole-mount *in-situ* hybridization.

[1]: Bertemes, P, et al. Sticking Together an Updated Model for Temporary Adhesion. Mar. Drugs 2022, 20, 359.

Annotation of clusters from single cell RNA sequencing data of a new *Macrostomum species* by screening of marker genes *via in-situ* hybridization

Speaker: *Philipp **Kolb**, BSc.*

Supervisor: *Peter Ladurner, Univ.-Ass. Dr.*

Affiliation: *Institute of Zoology, LFUI*

Abstract:

Certain species have the ability to adhere to biological surfaces, with some platyhelminths in the tidal zone demonstrating the capacity to release this adhesion bond multiple times per second. *Macrostomum lignano*, a model organism for bioadhesion, possesses 130 adhesive organs in its tail. The protein responsible for adhesion was recently discovered by Ladurner et al. and is expressed in the adhesive cell. The anchor cell and releasing gland cell complete the adhesive organ of *M. lignano*. However, the releasing gland protein/molecule remains unknown. In our study, we conducted an analysis of clusters derived from single-cell sequencing of *M. 022*, a novel model organism for platyhelminths, in an effort to identify potential releasing gland candidates. We observed that multiple genes highly specific to cluster 28 exhibited strong staining in cranial glands during *in-situ* hybridization experiments, while genes from cluster 3 showed staining in testis, ovaries, and developing eggs of *M. 022*. Our findings suggest that cluster 28 corresponds to pharynx glands, while cluster 3 may represent either the stem cell cluster of the organism or only the stem cells of the germ line. We anticipate our results as a step forward in the analysis of single-cell sequencing data of this new species, providing valuable insights into the search for the releasing gland cell. This work also serves as a starting point for further investigations into neoblasts in this promising model organism.

Establishing assessment tools to monitor organ function and pharmacokinetics for a drug testing and development platform in *ex-situ* perfused livers

Speaker: *Luis **Pötzl**, BSc.*

Supervisor: *Theresa Hautz-Neunteufel, Asst.-Prof. Dr.med.*

Supervisor: *Dirk Meyer, Univ.-Prof. Dr.*

Affiliation: *Visceral, Transplant and Thoracic Surgery, MUI*

Abstract:

Normothermic machine perfusion (NMP) allows ex-situ organ perfusion, hence mimicking an *in-vivo* situation by delivering oxygen and nutrients at a physiological temperature and therefore creating a physiological environment. *Ex-situ* normothermic organ machine preservation provides the unique opportunity to assess organ quality and function in detail and to perform organ repair or drug treatment. NMP will be utilized for the first time to study the pharmacokinetics and impact of an immunosuppressive agent – tacrolimus – in isolated perfused livers when administered as a bolus. This pilot trial will help to (i) establish and refine assessment tools to monitor organ function during drug testing (ii) to better understand the impact of the pharmacokinetic profile in the blood on organ function in an isolated organ perfusion system (iii) to assess for the impact of tacrolimus on microenvironment, drug tissue concentrations, inflammation, bioenergetics and tissue viability. Four livers (discarded human donor livers or porcine livers) subjected to NMP will be included in this pilot study. Tacrolimus will be applied as a single bolus and liver perfusion will be continued for 24h after tacrolimus start/administration. Serial perfusate samples will be collected at 0h (baseline) and hourly thereafter for 24h as well as before termination. Liver function parameters will be routinely measured at these intervals and correlated with tacrolimus perfusate concentrations. Tissue levels of tacrolimus will be measured at close intervals using Microdialysis for sampling. Liver biopsies collected at different time points will be assessed for structural changes of hepatic tissue/hepatocytes using hematoxylin & eosin (H&E) stains, while immunohistochemical staining will be used to characterize immune cell infiltrates and to evaluate structural changes and necrosis. Cell death and structural changes will be analyzed with Syto/PI stains using confocal microscopy. The bioenergetic function will be measured with high resolution respirometry (HRR). To the best of our knowledge, this will be the first trial studying pharmacokinetics of immunosuppressive agents in a close-to-physiologic model of isolated organs ex-situ. It will help to refine and adjust already established assessment tools at the organLife lab in order to adequately monitor organs during ex situ drug testing and development. Especially, the effect of tacrolimus on bioenergetics, viability characteristics and gene expression profiles will significantly improve the understanding on how tacrolimus is biodegraded in the human liver. Furthermore, it will be an important step to introduce this platform of ex situ liver perfusion as a model to further advance the development and application of novel, targeted organ and cell-type specific therapy.

Identification of suppressive CD4$^+$ Regulatory T cells in young and old age

Speaker: *Jonathan **Dieringer**, BSc.*

Supervisor: *Lourdes Rocamora Reverte, PhD.*

Supervisor: *Birgit Weinberger, Univ.-Prof. Dr.*

Affiliation: *Institute for Biomedical Aging Research, LFUI*

Abstract:

With increasing age differences in the innate and adaptive immune system can be observed. Older adults are more vulnerable to infections due to a decline in immune response with aging (immunosenescence). Aging of the immune system involves many changes in human T and B cell immunity. The most prominent effects are thymic involution and decreased numbers and functions of T and B cells. Numbers of memory T and B cells increase whereas their response to new antigens decreases. To optimally protect older adults, it is important to understand the parameters which lead to a decreased immune response. One of the most prominent T cell changes that occur with age is the loss of the co-stimulatory CD28 and the progressive accumulation of highly differentiated CD28-memory T cell subsets. These cells are characterized by decreased proliferative capacity, shortened telomers, a reduced TCR repertoire, and enhanced cytotoxic activity. CD28 loss is associated with increased susceptibility to infections and a weakened immune response to vaccination in older people. Regulatory T (Treg) cells are the master regulators of immune homeostasis. CD28 is required for the development of Treg cells in the thymus and contributes to their survival and homeostasis in the periphery. However, CD28 role in aged Treg is to date not described. Preliminary results in our laboratory show differential differences in the expression of CD28 by circulating Treg cells. The aim of this study is to identify differences on CD28 expressing Treg between old and young donors. For this purpose, CD4$^+$ CD25$^+$ CD127^{low} Tregs from peripheral blood mononuclear cells (PBMCs) obtained from young and old donors will be stained and analysed using FACS (fluorescence activated cell sorting) in order to characterize their steady state conditions. Furthermore, we will perform cell death experiments in cultured PBMCs in order to study Treg sensitivity to apoptosis by the analysis of Caspase-3 and γH2AX. Additionally, we plan to purify Treg using the ARIA Fusion cell sorter and co-culture them with their correspondent PBMCs in order to measure their ability to suppress T cell proliferation. From these co-cultures, the production of the anti-inflammatory cytokine IL-10 will be analysed using ELISA.

Characterization of $CD4^+/CD8^+$ double positive T cells.

Speaker: *Anna **Fleischmann**, BSc.*

Supervisor: *Magdalena Hagen, PhD.*

Supervisor: *Birgit Weinberger, Univ.-Prof. Dr.*

Affiliation: *Institute for Biomedical Aging Research, LFUI*

Abstract:

These cells exhibit heterogeneity, with distinct subsets of $CD4^{high}/CD8^{low}$ and $CD8^{high}/CD4^{low}$ T cells. While $CD4^+/CD8^+$ double positive T cells are typically found at low levels in young, healthy individuals, their numbers can increase in various disease contexts, particularly viral infections. Disparities in the distribution of these cells have also been observed among age groups, with adults showing higher proportions compared to children. Despite their potential significance, a comprehensive characterization of $CD4^+/CD8^+$ double positive T cells and their subpopulations in physiological contexts remains lacking. This project study aimed to fill this knowledge gap by investigating and characterizing $CD4^+/CD8^+$ double positive T cells and their subpopulations in healthy donors, with a focus on age-related changes. Blood was collected from both young (20-35 years) and elderly (65 years and older) individuals of both genders. Peripheral blood mononuclear cells (PBMC) were isolated, and detailed phenotypic characterization of $CD4^+/CD8^+$ double positive T cells was performed. Functional analyses included assessing cytokine production through a cytokine production assay and evaluating proliferation using a proliferation assay. The findings from this project study contribute to our understanding of $CD4^+/CD8^+$ double positive T cells in healthy individuals, shedding light on their phenotype and functional attributes. Moreover, the investigation into age-related changes in these populations provides valuable insights into the potential impact of aging on immune cell composition and function.

Effects of intratumoral microbiota on antitumor immunity in ovarian cancer.

Speaker: *Katja **Rungger**, BSc.*

Supervisor: *Hubert Hackl, Asst.-Prof. Dr.*

Affiliation: *Institute of Bioinformatics, MUI*

Abstract:

Effects of intratumoral microbiota on antitumor immunity in ovarian cancer The human microbiome can impact multiple physiological processes and can play an important role in the development of diseases such as cancer1. The intratumoral microbiota may influence the development, progression, metastasis formation and treatment response of multiple cancer types including ovarian cancer[1,2]. Here we wanted to study the intratumoral microbiota and their role in immune reactivity against ovarian cancer. We identified intratumoral bacterial species and their distribution from the raw RNA sequencing data of the TCGA ovarian cancer cohort (n=321) using Kraken[2]. In order to investigate the role of the identified species in immune reactivity we performed various analyses including 1) an analysis of species associated with overall survival, 2) a regression analysis of bacterial abundance with immune parameters derived from enrichment analyses, and 3) a prediction of potential epitopes which can bind to the major histocompatibility complex I (MHC-I) based on the proteomes of identified bacteria using NetMHCpan 4.1. Although the distribution of the intratumoral bacteria was very heterogeneous (alpha diversity Shannon index=0.22-6.61) we could identify two promising species: Niallia circulans and Pseudomonas chlororaphis. These species could be associated with various immune parameters such as IFNG pathways, infiltrating $CD4^+$ and $CD8^+$ T cells and encode a number of 8-11 amino acid long epitopes potentially binding to respective MHC-I molecules. However, the mechanism on how these bacteria are influencing the immune system still needs to be assessed. One possibility would be through molecular mimicry of tumor-associated antigens, which we will investigate with our predicted epitopes. Overall our results contribute to a better understanding of the immunoregulatory effects of intratumoral microbiota, which could be used for immunotherapeutic approaches.

[1]: Cullin N, et al. Microbiome and cancer. Cancer Cell 39, 1317–1341 (2021).
[2]: Sheng D, et al. The Interaction between Intratumoral Microbiome and Immunity Is Related to the Prognosis of Ovarian Cancer. Microbiol. Spectr. 11, e03549-22 (2023)

Bridging the gap between epigenetic changes and DNA damage repair: The role of PHF2 in the ageing brain

Speaker: *Sara **Campagnol**, BSc.*

Supervisor: *Frank Edenhofer, Univ.-Prof. Dr.*

Affiliation: *Institute for Molecular Biology, LFUI*

Abstract:

Neurodegenerative diseases encompass a broad spectrum of conditions characterised by the progressive dysfunction and loss of neurons[1]. While ageing stands as the primary risk factor for most neurodegenerative disorders, their development is influenced by a multifaceted interplay of genetic, epigenetic, and environmental factors[2]. Brain organoids, three-dimensional structures derived from induced pluripotent stem cells (iPSCs), have emerged as a valuable tool for neurological disease modelling[3,4]. However, iPSC reprogramming results in the loss of age-associated features, posing a significant challenge for modelling late-onset and neurodegenerative diseases[5]. One strategy to induce ageing in iPSC-derived models involves the use of Progerin, a truncated form of the lamin A protein associated with Hutchinson-Gilford Progeria Syndrome[6]. Inducible overexpression of Progerin in iPSC-derived brain organoids recapitulates several aspects of ageing, such as a reduction in heterochromatin and an increase in DNA damage. The transcriptome of Progerin-overexpressing organoids reveals dysregulation of several genes associated with ageing, including downregulation of the Plant Homeodomain Finger Protein 2 (PHF2), a histone demethylase that has emerged as a potential player in the earliest brain ageing manifestations. PHF2 acts as a guarantor of genome stability and its downregulation in artificially aged brain organoids suggests a link between epigenetics and DNA damage playing a role in neuronal ageing[7,8]. However, the precise mechanisms and specific target genes regulated by PHF2 are still being investigated, and its involvement in age-related processes remains to be fully understood. This study aims to investigate the role of PHF2 through a set of functional validation experiments, including genome-wide profiling and genetic manipulation. Cross-correlation between the transcriptome of Progerin-overexpressing organoids and previously published PHF2 ChIP-seq data[7] aims to uncover direct targets of PHF2 and potential regulatory interactions involved in the ageing process. Moreover, gene knockdown will be performed in 2D neural progenitor cells and 3D brain organoids *via* lentiviral transduction of a vector expressing a short hairpin RNA (shRNA) against PHF2. The effects of PHF2 knockdown will be assessed by immunofluorescence using markers for heterochromatin and DNA damage, and by qPCR of downstream targets within the same DNA damage repair pathways. Gaining a profound knowledge about the functional relevance of PHF2 in the ageing process can advance our understanding of the regulatory landscape of ageing-associated genes and pathways, ultimately providing insights into the early molecular mechanisms underlying ageing and disease of the human brain.

[1]: Wilson DM, et al. Hallmarks of neurodegenerative diseases. Cell; 186(4):693–714.
[2]: Azam S, et al. The Ageing Brain: Molecular and Cellular Basis of Neurodegeneration. Front Cell Dev Biol; 9:683459.
[3]: Lancaster MA, et al. Guided self-organization and cortical plate formation in human brain organoids. Nature Biotechnology; 35(7):659–66.
[4]: Lancaster MA, et al. Cerebral organoids model human brain development and microcephaly. Nature; 501(7467):373–9.
[5]: Studer L, et al. Programming and Reprogramming Cellular Age in the Era of Induced Pluripotency. Cell Stem Cell; 16(6):591–600.
[6]: Miller JD, et al. Human iPSC-Based Modeling of Late-Onset Disease *via* Progerin-Induced Aging. Cell Stem Cell; 13(6):691–705.
[7]: Pappa S, et al. PHF2 histone demethylase prevents DNA damage and genome instability by controlling cell cycle progression of neural progenitors. Proc Natl Acad Sci USA; 116(39):19464–73.
[8]: Alonso-De Vega I, et al. PHF2 regulates homology-directed DNA repair by controlling the resection of DNA double strand breaks. Nucleic Acids Res; 48(9):4915–27.

Cell-based *in vitro* screening of FAHD1 inhibitors.

Speaker: *Riccardo **Giaquinta**, BSc.*

Supervisor: *Alexander Weiss, Asst.-Prof. Dr.*

Affiliation: *Institute for Biomedical Aging Research, LFUI*

Abstract:

Fumarylacetoacetate hydrolase domain-containing protein 1 (FAHD1) was identified as mammalian oxaloacetate decarboxylase (ODx). By contributing to the regulation of oxaloacetate levels in mitochondria, it also plays a crucial role in preserving mitochondrial respiratory function. Research conducted on HUVECs reported that depletion of FAHD1 inhibited the mitochondrial electron transport chain (ETC) and triggered premature cellular senescence through the regulation of the mitochondrial ETC within the context of mitochondrial dysfunction associated senescence (MiDAS). FAHD1 plays a crucial role in preserving mitochondrial respiratory function by preventing the accumulation of OAA, which acts as an inhibitor of the succinate dehydrogenase (SDH, complex II). Energy failure through inhibition of SDH has also been recognized to be linked with premature senescence in brain and heart tissues. Importantly, FAHD1 was found to be upregulated in certain malignant breast cancer tissues. Lentiviral knock-down of FAHD1 in BT-20 cells, a basal triple-receptor negative breast cancer cell line derived from an invasive ductal carcinoma, resulted in reduced SDH activity and attenuated levels of the enzyme glutaminase (GLS), leading to the activation of programmed cell death. Recently, glutaminolysis in cancer cells gained more attention as it is essential for the growth of triple-negative breast cancer cells. In this context, the amino acid glutamine can fuel the tricarboxylic acid (TCA) cycle and it is also an important precursor for the biosynthesis of nucleotides, proteins and lipids. In summary, these findings demonstrate the importance of FAHD1 in the functionality of SDH and highlight its involvement in mitochondrial glutaminolysis. A first-generation of FAHD1 inhibitors was developed by exploiting the proposed FAHD1 mechanism. Initially, drug-like scaffolds were deduced from FAHD1 substrates, like fumarylpyruvate and OAA oxalamic, three distinct scaffolds, oxalamic acid, N-pyridyl-oxalamic acid and 1-oxide congener. The rationale behind this design approach was to replace the cleavable carbon-carbon bond of the FAHD1 substrates with an uncleavable bond, while retaining the Mg^{2+}-binding motif and maintaining a small molecular weight that leaves room for further optimizations. As a result, several FAHD1i with activities in the low micromolar IC_{50} range were discovered. They either competitively bind to the metal cofactor or exhibit novel inhibitory mechanisms that are yet to be fully understood. Based on this data and first *in vitro* experiments with recombinant protein, we developed standard operating procedures to test first-generation FAHD1i in cellular assays with HUVECs and BT-20 cells. Additionally, our approach has revealed promising compounds with the potential to induce cell death or senescence. Successful cell-based experiments will pave the way for future efficacy studies in human tissue culture and xenograft models. This progression will foster translational research, aiming to introduce novel therapeutic strategies for cancer and human malignancies linked to mitochondrial dysfunction.

[1]: Petit M, et al. Depletion of oxaloacetate decarboxylase FAHD1 inhibits mitochondrial electron transport and induces cellular senescence in human endothelial cells. Exp. Gerontol. 92, 7–12 (2017).
[2]: Weiss AKH, et al. Regulation of cellular senescence by eukaryotic members of the FAH superfamily – A role in calcium homeostasis? Mech. Ageing Dev. 190, 111284 (2020).
[3]: Holzknecht M, et al. The mitochondrial enzyme FAHD1 regulates complex II activity in breast cancer cells and is indispensable for basal BT-20 cells *in vitro*. FEBS Lett. 596, 2781–2794 (2022).
[4]: Weiss AKH, et al. Inhibitors of Fumarylacetoacetate Hydrolase Domain Containing Protein 1 (FAHD1). Molecules 26, 5009 (2021).

Role of FAHD1 in senescence of Immune cells: establishing new methods

Speaker: *Laura **Huber**, BSc.*

Supervisor: *Athanasios Seretis, PhD.*

Supervisor: *Pidder Jansen-Dürr, Univ.-Doz. Dr.*

Affiliation: *Institute for Biomedical Aging Research, LFUI*

Abstract:

The tricarboxylic acid (TCA) cycle is the main pathway responsible for the generation of energy, in the form of ATP, in eukaryotic cells. When it comes to the immune system, proliferating T and B lymphocytes, switch metabolic pathways upon activation, preferring glycolysis over the TCA cycle, while some subsets, for example memory T and B lymphocytes, mainly rely on the TCA cycle for their metabolic needs. In addition, there is accumulating evidence that mitochondrial fitness can contribute to overall immune cell function. The fumarylacetoacetate hydrolase-containing protein 1 (FAHD1) plays an important role in the TCA cycle. This protein catalyses the decarboxylation of the TCA substrate, oxaloacetate (OAA) into pyruvate and CO_2. This reaction regulates the levels of OAA in the mitochondrion, making FAHD1 an important regulator of TCA cycle. Loss of FAHD1 activity has been shown to lead to a Complex II defect and promote cellular senescence, as a result of mitochondrial stress. In order to determine whether or not FAHD1 may be influencing the activation and differentiation of i.e. B lymphocytes, we tried to establish protocols to reliably localize this protein in different immune cell subsets. For this purpose, we extracted spleen samples from both wildtype and FAHD1 knock-out mice, enabling us to perform various experiments involving one or both types of splenocytes. These experiments involved techniques such as immunostaining, western blot, flow cytometry, and qPCR, among others. The primary aim of these experiments was to develop reliable protocols for the precise localization of FAHD1 in different cell types. However, this goal proved challenging, due to the low levels of the FAHD1 protein within the cells and the limited existing knowledge about FAHD1 in general. The absence of well-established antibodies and primers, coupled with the lack of comparative data in the literature, created significant challenges in our work with FAHD1. Nevertheless, the conducted experiments seem to support to the presence of FAHD1 in B-cells, even though conclusive evidence remains yet to be found. Our efforts to establish new protocols and improvement of existing ones will hopefully pave the way for the successful identification and localization of FAHD1 in different immune cell subsets.

[1]: Etemad, S, Petit, M, Weiss, AKH, et al. Oxaloacetate decarboxylase FAHD1 – a new regulator of mitochondrial function and senescence. Mechanisms of Ageing and Development 177, 22–29.
[2]: Sharma, R, et al. Distinct metabolic requirements regulate B cell activation and germinal center responses. Nat Immunol 24, 1358–1369.
[3]: Weisel, FJ, et al. Germinal center B cells selectively oxidize fatty acids for energy while conducting minimal glycolysis. Nat Immunol 21, 331–342.

Transcriptomic profiling of normal versus diabetic retina to uncover mechanisms of glucotoxicity and retinal regeneration

Speaker: *Ferdinand **Löffler**, BSc.*

Supervisor: *Robin Kimmel, Asst.-Prof. Dr.med. PhD.*

Affiliation: *Institute for Molecular Biology, LFUI*

Abstract:

Diabetic retinopathy (DR) is the leading cause of vision loss in working-age adults, affecting over 3.2 million people worldwide[1]. When diabetes causes high levels of glucose for a long time, it can damage the retinal vessels and neurons, which are hallmarks of DR. DR was initially thought to only affect the microvasculature of the retina. However, this does not consider the possibility that damage to the neural retina may occur prior to the appearance of clinically observable DR[2]. Recent evidence suggests that early retinal neurodegeneration occurs independently, and potentially contributes to, vessel pathology. This is proposed to be a consequence of stress and damage in the neural retinal and the subsequent immune response[3]. To learn more about mechanisms of DR, this project used the model organism zebrafish, which has retinal and metabolic physiology that is similar to humans. The research group of Dr. Kimmel previously established the pdx1-mutant as a model of human diabetes in zebrafish[4]. It survives into adulthood with persistent hyperglycemia and develops retinal vascular and neuronal pathologies as seen in human DR patients[5]. The aim of my project study was to identify dysregulated genes in the retina of diabetic zebrafish and to localize expression changes to specific cells of the neural and vascular compartments. To discover genes involved in DR, I analyzed the retinal transcriptome from pdx1-mutant zebrafish compared to age-matched healthy controls. Candidate genes that showed significant differences in transcription were investigated using immunohistochemistry and *in-situ* hybridization to determine spatial expression patterns. The cell types that expressed the candidate mediators were further analyzed by qRT-PCR of dissected tissues. Through these studies we hope to learn about molecular mechanisms of the onset and progression of DR. Different from humans, DR in the pdx1-mutant zebrafish does not progress to blindness, as was shown by Dr. Kimmel's research group[5]. To study this retinal regenerative capacity in zebrafish could be helpful for understanding the molecular mechanisms that underlie this difference.

[1]: Ning Cheung, et al. DiabeTc reTnopathy. The Lancet. 2010;376(9735):124-36.
[2]: Sun JK, et al. UpdaTng the Staging System for DiabeTc ReTnal Disease. Ophthalmology. 2021;128(4):490-3.
[3]: Sachdeva MM. ReTnal NeurodegeneraTon in Diabetes: an Emerging Concept in DiabeTc ReTnopathy. Current Diabetes Reports. 2021;21(12).
[4]: Kimmel RA, et al. DiabeTc pdx1-mutant zebrafish show conserved responses to nutrient overload and anT-glycemic treatment. ScienTfic Reports. 2015;5.
[5]: Ali Z, et al. Photoreceptor DegeneraTon Accompanies Vascular Changes in a Zebrafish Model of DiabeTc ReTnopathy. InvesTgaTve Ophthalmology & Visual Science. 2020;61(2).

How mRNA modifications at the start codon affect translation initiation

Speaker: *Elena **Mayer**, BSc.*

Supervisor: *Matthias Erlacher, Univ. Prof. Dr.*

Affiliation: *Institute of Genomics and RNomics, MUI*

Abstract:

RNA modifications can impact numerous biological processes, including transcription, splicing, RNA export, mRNA translation, and RNA degradation. The cellular transcriptome and consequently the proteome are shaped by each of these molecular processes. Due to their ability to exert a variety of effects on the fate of mRNAs, the impact of RNA modifications on gene regulation became a focus of recent research. However, the functions and mode of action for numerous modifications have still not been clarified. In order to reveal the impact of modified mRNAs on translation, we aimed to establish an experimental system that allows introducing single modifications site-specifically in and around the start codon, as this aspect has not yet been addressed. Using site-directed mutagenesis and cloning, a bacterial plasmid with two overlapping reading frames of a luciferase reporter was generated. Firstly, the Shine-Dalgarno sequence had to be altered to adjust the initiation at both reading frames, allowing both ORFs to be translated. In addition, we tested different start codons (GUG and UUG) for the suitability for this approach. As next steps, introducing other types of modifications can be achieved by integrating synthetic RNA oligonucleotides into the coding sequence of the reporter mRNA. As the size of synthetic oligonucleotides is restricted in length, full-length mRNAs need to be generated by ligating two or more fragments. The modified reporter mRNA is translated and the respective protein product analyzed. This will provide insights into how certain modified RNA nucleotides affect translation initiation and thereby the efficiency of protein synthesis. In this project study, we generated a bacterial plasmid with two overlapping reading frames of a luciferase reporter and altered its Shine-Dalgarno sequences to facilitate balanced competition between the two reading frames for the ribosome initiation complex. Further, we saw that the modification of one start codon affects not only the respective ORF but also the translation of the overlapping ORF. This provides a more sensitive and accurate read out for translation initiation. The established system serves as a starting point for more sophisticated *in vitro* models of mRNA modifications and how they affect translation initiation. The system can be varied for mRNA sequences and types of modifications. This will give us a new understanding about the regulatory potential of RNA modifications and the recognition of the start codon by the ribosome.

LIST OF ATTENDEES

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Talk	Data	Presentation	Speaker	Overall
1				
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