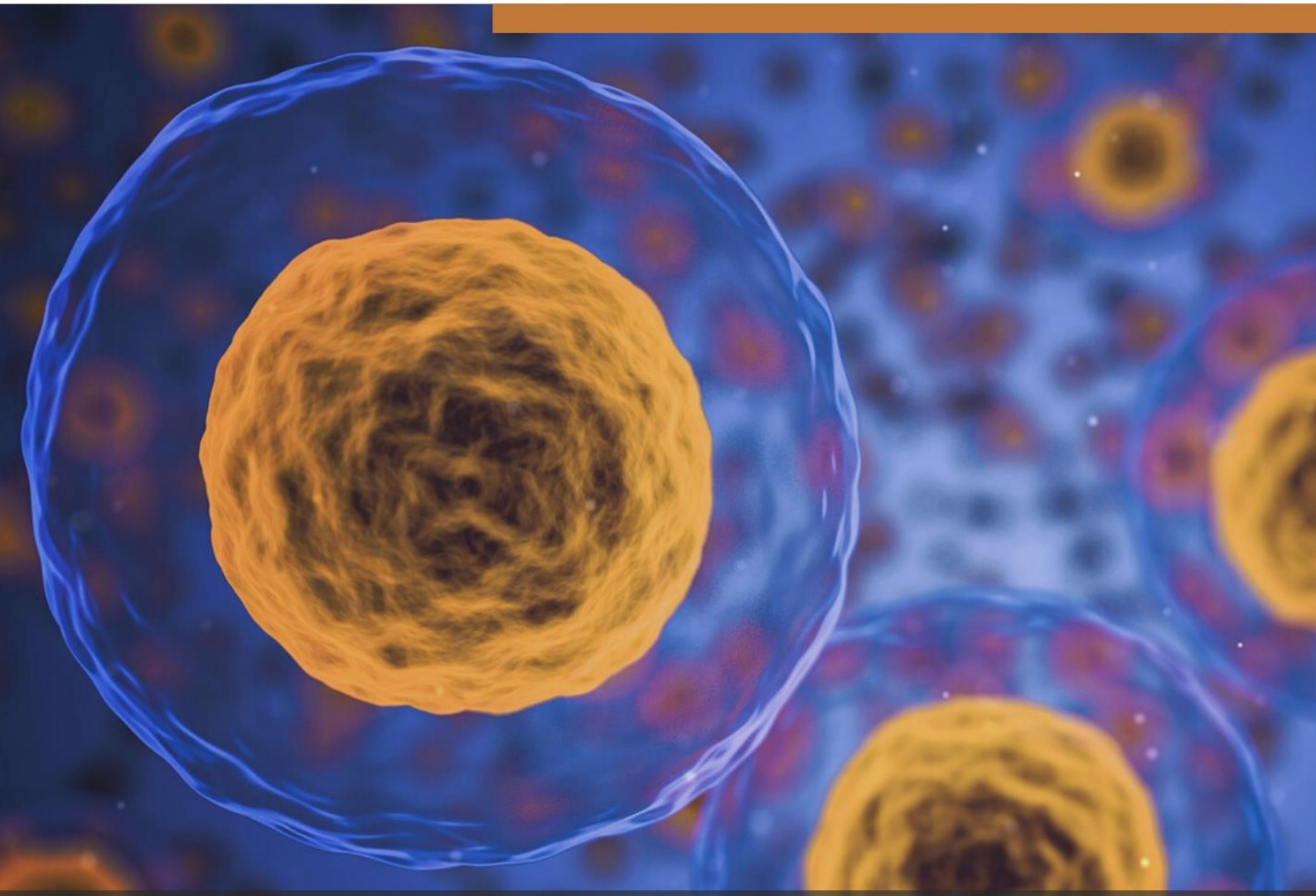
16TH GGL ANNUAL CONFERENCE

20TH - 21ST SEPTEMBER 2023

PHYSICS LECTURE HALLS
HEINRICH-BUFF-RING 14, GIESSEN



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International Giessen Graduate Centre for the Life Sciences

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Overview of GGL Sections

Participating in the 16th GGL Conference on Life Sciences

September, 20th and 21st 2023 at Justus Liebig University Giessen – Germany

CONTENT

Programme of the 16th GGL Conference on Life Sciences

Day 1: Wednesday, 20th September 2023

08:30 – 09:00 **Registration**

09:00 – 09:15 **Welcome Address**: Prof. Dr. Dr. hc. Martin Kramer, Vice President of the Justus Liebig University
Opening Remarks: Prof. Dr. Eveline Baumgart-Vogt, Speaker of the GGL

Section 9 – Ecology and Global Change **Chairperson: Eva-Maria Minarsch**

09:15 – 10:00 **Keynote: Dr. Martin Hartmann, ETH Zürich, Switzerland**
Organic and conventional agriculture promote distinct soil microbiomes with contrasting metabolic potential

10:00 – 10:15 **Kai Jansen**: Occurrence and dissipation of quaternary alkylammonium compounds in soils of Hesse, Germany

10:15 – 10:30 **Annalena Barth**: Black soldier fly as sustainable aquafeed for whiteleg shrimp

10:30 – 10:45 **Coffee Break**

Section 4 – Protein and Nucleic Acid Interactions **Chairperson: Daniel Bauer**

10:45 – 11:30 **Keynote: Dr. Andreas Diepold, Max Planck Institute for Terrestrial Microbiology, Marburg**
Dynamic protein interactions control toxin injection through the bacterial type III secretion system

11:30 – 11:45 **Laura Rehneke**: Analyses of symbiont effector candidates in redirecting phytohormone signalling and activating beneficial effects in Arabidopsis

11:45 – 12:30 **General Assembly**

12:30 – 13:30 **Lunch**

Section 6 – Reproduction in Man and Animals **Chairperson: Christine Rager**
Section 8 – Chemical Design and Analysis of Molecular Systems

13:30 – 14:15 **Keynote: Prof. Dr. Jorma Toppari, University of Turku, Finland**
Research on male reproductive health and toxicology

14:15 – 14:30 **Dingding Ai**: The role of resident macrophages in the immune response to bacterial infection of the murine epididymis

14:30 – 14:45 **Katrin Wiltschka**: Hydrodechlorination of Mine Water-specific Polychlorinated Biphenyls (PCBs) Using Palladium Catalysts

14:45 – 15:00 **Coffee Break**

Section 2 – Infection and Immunity **Chairperson: Monique Überall**

15:00 – 15:45 **Keynote: Prof. Dr. Friedrich Frischknecht, Universitätsklinikum Heidelberg**
Malaria research: from basics into parasite migration to new intervention methods

15:45 – 16:00 **Yukino Kobayashi**: Contribution of the actin-like-proteins Alp1 and Alp2b in Plasmodium transmission

16:00 – 16:15 **Franziska Maria Dort**: The role of autophagy and the related ER-phagy in coronavirus infected cells

16:15 – 16:30 **Svenja Gramberg**: Cutting-edge technologies in parasite research – How to create a molecular map of the liver fluke *Fasciola hepatica*

16:30 – 19:00 **Poster Session and Finger Food**

Day 2: Thursday, 21st September 2023

Section 10 – Clinical Sciences **Chairperson: Khaled Mahmoud**

09:00 – 9:45 **Keynote: Dr. Patricia Altea Manzano, Laboratory of Cellular Metabolism and Metabolic Regulation, Katholieke Universiteit Leuven, Belgium**
Metabolic Reprogramming in Cancer Progression

9:45 – 10:00 **Frederike Hagedorn**: Molecular Subtyping of Diffuse Large B-Cell-Lymphoma Using Panel-Sequencing

10:00 – 10:15 **Salisa Kruijning**: A novel regulatory link between EZH2 and HIF2α in breast cancer

10:15 – 10:30 **Yao Wang**: Orthodontic Compression Promotes Macrophage M2 Polarization via Histone H3 Hyperacetylation

10:30 – 10:45 **Coffee Break**

Section 1 - Nutrition and Metabolism **Chairperson: Rafael Castillo Negrete**
Section 5 - Neurosciences

10:45 – 11:30 **Keynote: Prof. Dr. Regina Verena Taudte, Philipps University Marburg**
Metabolomics in Biomedical Research – The influence of ω-3 fatty acids on the brain metabolome during systemic inflammation

11:30 – 11:45 **Leona Bähr**: Psychological and inflammatory stress in mice: investigating how neutropenia effects heart rate variability as a readout parameter

11:45 – 12:00 **Meng Meng Zhou**: Glutaredoxin 5 as a novel target for β-cell survival and regeneration

12:00 – 13:00 **Lunch**

Section 7 - Bioresources, Bioinformatics and Biotechnology **Chairperson: Daniel Kreft**

13:00 – 13:45 **Keynote: Dr. Angela Meccariello, Imperial College London, UK**
Making males: from medfly biology to medfly control

13:45 – 14:00 **Gözde Yildiz**: Understanding the impact of structural variations on gene expression using pangenome graphs

14:00 – 14:15 **Coffee Break**

14:15 – 16:45 **Poster Session and Finger Food**

Section 3 – Heart, Lung and Blood Vessels **Chairperson: Alejandro Egea Zorilla**

16:45 – 17:30 **Keynote: Prof. Jaya Krishnan, Institute of Cardiovascular Regeneration, Goethe University Frankfurt**
Modeling cardiopathologic TET2 CHIP in engineered cardiac ventricular tissue reveals therapeutics for disease resolution

17:30 – 17:45 **Esmeralda Vasquez Pacheco**: A unifying model to characterize lipofibroblast and myofibroblast differentiation

17:45 – 18:00 **Joshua Ayoson**: CD8+ T cells subpopulation "Tc17" induce a metabolic enriched microenvironment enabling lung cancer progression

18:00 – 18:15 **Vinita Sharma**: Exploring the early-stage pathomechanism of pulmonary hypertension in COPD: Insights into initial pulmonary vascular remodeling

18:15 – 18:45 **Closing Remarks and Award Ceremony**

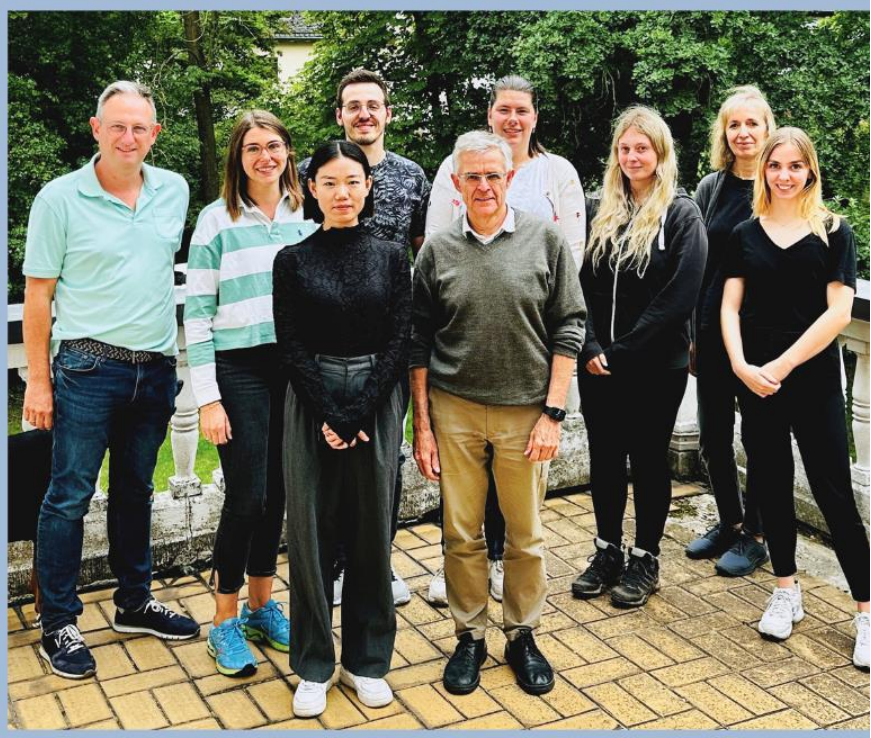
The 16th GGL Conference on Life Sciences is supported by:

SECTION 1

NUTRITION AND METABOLISM

ABOUT THE SECTION

This section is focused on translational research of the human food chain from food production to human nutrition. Doctoral research includes studies on plants and animals to improve food quality under environmental aspects as well as those investigating human metabolism and the functionality of organ systems in both healthy and diseased humans.



Day 2: Thursday, 21st September, 2023

CHAIRPERSON: Rafael Castillo Negrete

10:45

METABOLOMICS IN BIOMEDICAL RESEARCH - THE INFLUENCE OF ω-3-FATTY ACIDS ON THE BRAIN METABOLOME DURING SYSTEMIC INFLAMMATION

Prof. Dr. Regina Verena Taudte
Philipps University
Marburg

11:30

PSYCHOLOGICAL AND INFLAMMATORY STRESS IN MICE: INVESTIGATING HOW NEUTROPENIA EFFECTS HEART RATE VARIABILITY AS A READOUT PARAMETER

Leona Bähr

11:45

GLUTAREDOXIN 5 AS A NOVEL TARGET FOR β-CELL SURVIVAL AND REGENERATION

Meng Meng Zhou

T01

Glutaredoxin 5 as a novel target for β-cell survival and regeneration

Zhou M., Petry S. F., Linn T.

Clinical Research Unit, Medical Clinic and Polyclinic III, Center of Internal Medicine, Justus Liebig University, Giessen, Germany

Pancreatic β-cells are endocrine cells that synthesize, store, and release insulin, the only hormone capable of lowering blood glucose concentrations. Glutaredoxin 5 (Glrx5) is a mitochondrial enzyme of the group of thioredoxin proteins that exerts essential tasks for the respiratory chain and cellular iron homeostasis. Ferroptosis is a recently recognized form of non-apoptotic cell death caused by iron-dependent accumulation of lipid peroxidation. The present study was aimed to investigate the underlying mechanism of the potential protective benefit of Glrx5 on β-cells. MTT was used to study cell viability. ELISA and/or immunofluorescence were used to detect Glrx5 and insulin. FerroOrange, MitoferroGreen, and Liperfluo were used to visualize the cellular fluorescent images of intracellular Fe2+, mitochondria Fe2+, and lipid peroxidation, respectively. We found that oleic acid (OA) treatment not only promoted cell death, and decreased the Glrx5 and insulin, but increased lipid peroxidation and intracellular and mitochondria iron accumulation in MIN6 cells. Of note, treatment of the ferroptosis inhibitor liproxstatin-1 could rescue the OA-induced decline in glrx5 and insulin as well as lipid peroxidation and mitochondria iron accumulation but increased intracellular iron. Moreover, treatment with the ferroptosis inducer ML162 exacerbated most of the toxic effects induced by OA except intracellular iron accumulation. Collectively, our study indicated that OA induces Glrx5 deficiency and results in lipid peroxidation, and mitochondrial iron accumulation, which can be exacerbated by ferroptosis inducers and rescued by ferroptosis inhibitors. Further Glrx5-overexpressing mice presenting with uncontrolled diabetes will be assessed in terms of Glrx5 and insulin content of the islets, islet mass, insulin secretion, ROS production, and markers of ferroptosis.

P01

On-farm and innovative cow trait recording to derive genotype x feeding and genotype x emission interactions and effects on the offspring in organic dairy farming

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Organic dairy farming has become increasingly important in recent years and poses new challenges on cow's adaptation to environmental conditions. Therefore, it is imperative to identify cow genotypes within the most commonly used breed Holstein Friesian (HF) being robust and able to utilize less energy-rich feed. Interactions between genotype and different housing systems (organic vs conventional) have previously been identified in dairy cows. However, interactions between genotype and environment (feeding system, emissions) have been analyzed only to a limited extent. Hence, the project 'GreenDairy' aims to identify genotype x feeding (GFI) and genotype x emission (GEI) interactions for production and functional traits in dairy cows.

Data collection takes place on a research farm with ~126 HF cows. Cows were assigned to 2 different, equally sized feeding groups (low-input vs high-input) based on breeding values and lactation number by stratified randomization. Daily temperature and humidity are recorded with a data logger on the farm. Farm environmental emissions (e.g., CH_4, CO_2) are measured in 6-week intervals with climate gas sensors. Cow's individual methane emissions are measured every 6 weeks with a laser-methane-detector (LMD) and with a sniffer, which is installed in the automatic milking system. Back fat thickness (BFT) and body condition score (BCS) of the cows are determined at 3-week intervals, and weekly during the feed changeover. Further traits to be analyzed for possible GFI and GEI include daily milk production traits, milk fatty acids and ketosis measurements as indicators for metabolic diseases. Currently, the dataset includes 379 LMD records, up to 1294 observations for BCS and BFT, and 91 ketosis test results. Moreover, we analyze the effect of dam feeding on health and performance in the offspring. In this regard, we developed phenotypic and genetic-statistical models for diseases in a large dataset of ~10,000 cows with corresponding calves, which can be applied directly to the GreenDairy dataset in the end of the project.

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P02

Nutritional Interventions in *Drosophila melanogaster*: Impact on Lifespan and Health

Hof-Michel S., Wagner A.
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Drosophila melanogaster, commonly known as the fruit fly, has emerged as a powerful model organism for studying various biological phenomena due to its short lifespan, simple genetic makeup, and easy handling. Since the beginning of the 20th century, it has been extensively used to unravel fundamental biological processes. The high degree of genetic conservation between flies and humans allows a transfer of findings from *Drosophila melanogaster,* to a certain degree, also to higher organisms, including humans. Since recently, the fruit fly has also been introduced as a good model system to address physiological questions.

Nutrition plays a pivotal role in health and life expectancy in most organisms. Numerous studies have investigated the impact of a variety of nutritional interventions on the aging process and age-related diseases in flies. By altering the composition of their diet and supplementing the diet with specific nutrients such as antioxidants and vitamins, beneficial effects on longevity and age-associated pathologies has been demonstrated by other researchers.

In the present study, we have investigated the effect of potentially beneficial and detrimental nutritional compounds, respectively, on health and longevity of the fruit fly. To assess the compound-related changes, a variety of aspects have been considered including body composition, stress resistance, and epigenetic targets. Since epigenetic changes have been demonstrated to affect the process of ageing via methylation and acetylation patterns of the DNA, we have tested if our test compounds mediate its effects via epigenetic targets. Taken together, we have been able to show that selected nutritional compounds mediate its health-promoting effects by targeting various mechanisms related to the ageing process.

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P03

Oleoylethanolamide improves energy disposal in a cellular model of Alzheimer's disease

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Oleoylethanolamide (OEA) is an endogenous lipid mediator which is being discussed as a weight-loss drug for obesity. In addition to its homeostatic functions, OEA has neuroprotective and anti-inflammatory capabilities. To further investigate the properties of OEA against neurodegenerative diseases, we studied the influence of OEA on mitochondrial function with a focus on mitochondrial energy metabolism in a cellular model of Alzheimer's disease (AD).

SH-SY5Y-APP$_{695}$ cells were used as a model for an early stage of AD. Vector-transfected SH-SY5Y-MOCK cells served as controls. Using these cells, we investigated adenosine triphosphate (ATP) production, various glucose- and fat-metabolizing genes as well as fatty acid oxidation (FAO) and lactate/pyruvate levels in cells treated with OEA.

Incubation with OEA showed a significant increase in ATP levels in both cell lines. Pyruvate dehydrogenase 1 gene expression was significantly decreased in SH-SY5Y-MOCK cells, whereas FAO and lactate/pyruvate ratio significantly increased in SH-SY5Y-APP$_{695}$ cells.

Based on the increased ATP concentration, we conclude that incubation with OEA leads to a disease-specific higher energy availability in the cells. In SH-SY5Y-MOCK cells, this seems to result from the elevated conversion of pyruvate to acetyl-CoA, whilst in SH-SY5Y-APP$_{695}$cells it may be caused by an increased lactate level and more FAO.

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SECTION 2

INFECTION AND IMMUNITY

ABOUT THE SECTION

In this section, emphasis is put on the molecular pathogenesis of infectious diseases of man and livestock as well as on disorders of the immune system. Aspects of innate and adaptive immunity, replication and invasion of pathogens, gene expression induced by inflammatory triggers in the host, and the development of new detection methods for infections are the main topics of this section.

Day 1: Wednesday, 20th September, 2023 **CHAIRPERSON:** Monique Überall

15:00 **MALARIA RESEARCH: FROM BASIC INSIGHTS INTO PARASITE MIGRATION TO NEW INTERVENTION METHODS**
Prof. Dr. Friedrich Frischknecht
Universitätsklinikum Heidelberg

15:45 **CONTRIBUTION OF THE ACTIN-LIKE-PROTEINS ALP1 AND ALP2B IN *PLASMODIUM* TRANSMISSION**
Yukino Kobayashi

16:00 **THE ROLE OF AUTOPHAGY AND THE RELATED ER-PHAGY IN CORONAVIRUS INFECTED CELLS**
Franziska Maria Dort

16:15 **CUTTING-EDGE TECHNOLOGIES IN PARASITE RESEARCH - HOW TO CREATE A MOLECULAR MAP OF THE LIVER FLUKE *FASCIOLA HEPATICA***
Svenja Gramberg

Keynote 02

Malaria research: from basic insights into parasite migration to new intervention methods

Frischknecht F.

Heidelberg University Medical School, Center for Infectious Diseases, Professor for Integrative Parasitology, Heidelberg, Germany

Malaria parasites undergo a complex life cycle between mosquitoes and vertebrates. Yet, this complexity allows a number of intervention methods that can target mosquitoes and parasites alike. Current malaria control tools are insecticides and insecticide treated bed nets as well as drugs. No efficient vaccine is available but several are being tested and two have already been approved. These are based on a single antigenic protein, while work using attenuated parasites has also progressed to clinical trials. Our lab studies the way malaria parasites migrate with a main focus on the transmission to and from the mosquito. Parasite migration is essential for the parasite and based on an actin-myosin motor that is modulated by a number of proteins. To understand the basis of migration we employ reverse genetic methods, advanced microscopy and biophysics. In the talk I will highlight our motivation to study parasites in general, malaria in particular and guide through a number of recent studies focusing on specific proteins involved in different aspects of parasite migration. I will reflect on how these studies have led through serendipity to a novel approach using attenuated parasites that might aid malaria control.

T02

The role of autophagy and the related ER-phagy in coronavirus infected cells

Dort F., Heylmann D., Kracht M.
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The replication process of coronaviruses (CoV) is closely connected to the endoplasmic reticulum (ER) both functionally and structurally. Consequently, it was shown that CoV infection leads to a significant activation of ER stress and the unfolded protein response (UPR). Previous proteome analyses revealed several components deregulated by CoV involved in autophagy (including ER-phagy), ER quality control and ER-associated protein degradation (ERAD). Autophagy-related ER-phagy is a process to maintain ER homoeostasis or to remodel (patho)physiological ER expansion. The primary aim of this study is to uncover the mechanistic links between the CoV replication cycle and autophagy, in particular ER-phagy. For the assessment of autophagic flux a quantitive assay was established. The assay utilizes a fluorescent tandem reporter consisting of ER- (KDEL, RAMP4) or autophagosome (LC3)-associated proteins fused to mCherry/ssRFP-GFP proteins. Through the localization of the fusion proteins to lysosomes, a pH-sensitive quenching of the GFP fluorescence reports on active ER-phagy/autophagy [1]. Cancer Cell lines (Huh7, HeLa, A549) stably expressing the reporter proteins were created using lentiviral transduction. Validation of the successful transduction was done via Western Blot and fluorescence microscopy analyses. Flow cytometry was applied to monitor and quantify changes in fluorescence upon starvation, CoV infection, bafilomycin A1 and thapsigargin treatment.

An increase in active autophagy was observed upon starvation and CoV infection whilst the lysosomal fusion inhibitor bafilomycin A1 reversed this process. Western Blot analysis supported these results by revealing concentration changes in autophagy marker proteins (lipidated LC3B-II or p62/SQSTM1). A quantitative assay of cells that undergo autophagy/ER-phagy was established as well as validated and revealed a clear activation of autophagy due to CoV infection, while ER-phagy seems to be repressed. Further experiments with cell lines deficient in components of autophagy/ER-phagy according to our proteome analyses will be applied to clarify virus-specific mechanisms that account for differential regulation of autophagic flux.

References:

[1] Wulansari, Noviana et al., (2021), Neurodevelopmental defects and neurodegenerative phenotypes in human brain organoids carrying Parkinson's disease-linked DNAJC6 mutations, Science Advances, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7888924/, 2021-02-17

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T03

Cutting-edge technologies in parasite research – How to create a molecular map of the liver fluke *Fasciola hepatica*

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The introduction of "omics" technologies into parasite research has enabled the exploration of gene expression in whole organisms, different life stages and even single cells. However, the spatial organization of cells and tissues within multicellular parasites, including their function in parasitism, is still poorly understood. The cutting-edge technology "spatial transcriptomics" opens new avenues to study these pathogens, by enabling the visualization of gene expression in 2D according to the original morphological context. Here, we present the first spatial transcriptome of a parasitic flatworm, the common liver fluke *Fasciola hepatica.* This parasite, together with related species, is the causative agent of fasciolosis, a zoonotic disease affecting human and animal health worldwide.

By applying the Visium Spatial Gene Expression Solution (10x Genomics) on adult *F. hepatica* we were able to characterize eight different tissues, including intestine, tegument, and reproductive organs. Gene expression profiles and marker genes were identified for each of those tissues and subsequently validated by *in situ* hybridization. In addition, we performed gene ontology (GO) enrichment analysis, which revealed characteristic biological processes and molecular functions associated with each tissue.

The final dataset allowed us to visualize and explore the spatial expression of thousands of liver fluke genes. In this way, we identified several drug target genes (such as ß-tubulins and calcium channels) and drug resistance genes (e.g. ABC transporters and glutathione S-transferases (GST)) with tissue-specific expression. For example, we found that $TRPM_{PZQ}$ (transient receptor potential ion channel), a known drug target in related flatworms, was predominantly expressed in the subtegumental cell layer. Furthermore, the parenchyma of the parasite turned out to be particularly relevant for the detoxification of xenobiotics, as we detected an accumulation of various GSTs in this organ.

These results demonstrate how spatial transcriptomics can serve as a valuable tool for both basic research and drug development. It greatly improves our understanding of multicellular parasites and thereby supports the discovery of new therapies for these pathogens.

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T04

Contribution of the actin-like-proteins Alp1 and Alp2b in *Plasmodium* transmission

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[2]Institute of Parasitology, Giessen, Germany

Active motility of the malaria parasite *Plasmodium* is an essential factor for completion of its life cycle, which requires invasion of mosquito and mammalian host tissues. Actin-like proteins (Alps) are apicomplexan-specific relatives of actin, one of the central players of cellular motility. The primary structure of Alps is, however, highly divergent from actin and is characterised by unique insertions and deletions, which likely confer specialist functions in the parasite. Removal of half of the *Alp1* open-reading frame, or deletion of the complete *Alp2b* gene led to a drastic reduction of mosquito midgut colonisation at different steps of transmission. This project aims to elucidate the roles of Alps in the progression of *Plasmodium* and the contribution of unique protein regions to their functions.

A full knockout (KO) of *Alp1* was conducted in *P. berghei* to investigate the contribution of the full Alp1 sequence to parasite progression. Alp1 KO parasites developed normally until they reached ookinete stage where their gliding motility was completely stalled. This phenotype was fully rescued by complementing wild-type *P. berghei* Alp1, yet complementation with *P. falciparum Alp1* (PfAlp1) cDNA did not lead to phenotypic recovery. However, further experiments by C. Busse showed PfAlp1 can be functional when it is expressed from a gene including *P. berghei* introns. Consistent with a previous observation, Alp2b KO male gametocytes did not exflagellate after *ex vivo* activation. Mutation of two unique insertion regions of Alp2b also prevented exflagellation, implicating these insertion regions as critical contributors to Alp2b function. Despite the sensitivity of PbAlp2b to mutations, cross-species complementation with PfAlp2b partially rescued exflagellation. This complementation result indicates a degree of conservation and a possible structural significance of the unique regions of Alp2b.

My results so far emphasise that both Alp1 and Alp2b, the unique members of the apicomplexan actin superfamily, are critical factors for *Plasmodium* transmission. Alp1 is essential for ookinete motility and deletion of *Alp2b* completely blocked exflagellation and, consequently, fertilisation of the parasite. Furthermore, the two identified unique insertion regions of Alp2b could be targets for potential transmission-blocking compounds.

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P04

Mechanisms of transcript buffering in inflammation

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The inflammatory response is a complex and dynamic process that involves intertwined molecular mechanisms of intracellular signal transduction, all orchestrated through intricate feedback controls. In this research, we investigated the role of XRN1, a key cellular enzyme involved in cytosolic 5'-3' RNA degradation, in the regulation of the inflammatory response. Recently, we found that XRN1 depletion increases IL-1-NFk-B target gene mRNA stability, which apparently is sensed by the cell and leads to (compensatory) feedback modulations in chromatin and transcriptional processes in the nucleus by unknown mechanisms.

This project aims to identify XRN1-dependent factors involved in the transcript buffering of inflammatory mRNAs in order to unravel an additional layer of (post)-transcriptional control of the inflammatory response at the cellular level.

We employed *in-vitro* and *in-vivo* methods, including nascent RNA (5-ethynyl uridine, 5-EU) and protein (L-homopropargylglycine, L-HPG) labeling, RT-qPCR, and western blotting techniques, to assess inflammatory mediator mRNA and protein levels (steady-state and newly synthesized) in IL-1-treated control and XRN1-depleted HeLa cell lines.

Stimulation of cells with IL-1 led to a significant upregulation of mRNA synthesis for various inflammatory mediators, including *NFkBIA*, *IL8*, *IL6*, and *TNFAIP3,* but these mRNAs were highly unstable and underwent signal-induced decay within one to three hours. XRN1-deficient cells reduced the steady-state levels of these mRNAs, while the mRNA stabilities increased, but the corresponding protein levels remained unaltered. Additionally, the synthesis of these mRNAs was regulated in a target-specific manner. Furthermore, IL-1 induced the translocation of NF-kB p65 from the cytosol to the nucleus in an XRN-1-dependent manner. We also found significant amounts of XRN1, where its role is unknown.

Our study sheds light on the intricate nature of the inflammatory response and the involvement of XRN1 in this process, providing valuable insights into the molecular mechanisms underlying inflammation under XRN1-depleted conditions. Further investigations into XRN1-dependent factors involved in transcript buffering by LC-MS/MS will provide additional insights and potentially uncover new therapeutic targets downstream in the IL-1-NF-kB pathways that may be amenable for the treatment of inflammatory diseases in the long term.

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P05

Elicitation of antibodies broadly neutralizing the porcine reproductive and respiratory syndrome virus (PRRSV) using reverse vaccinology

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The porcine reproductive and respiratory syndrome virus (PRRSV) is one of the world's economically significant pathogens in pig populations. There can be significant losses from abortion storms following infection of pregnant sows and respiratory disease in piglets. Recently, PRRSV was re-classified taxonomically into two different species (PRRSV-1 and PRRSV-2), due to a nucleic acid sequence identity of less than 60%. Vaccination only provides limited protection even against the homologues species. Although vaccination mostly protects herds from severe disease, the field virus strains persist in the population. These field strains pose a constant risk of transmission and of new severe outbreaks due to mutation including recombination. The need for protective vaccines towards pathogens that remain elusive to classical vaccine design strategies has catalysed the adoption of innovative approaches. One attempt is reverse vaccinology, which aims to engineer immunogens to enhance focused antibody elicitation of specific neutralizing epitopes. One part of the project represents the determination of the repertoire of pig antibodies directed against PRRSV. A first step is the isolation of reactive B memory cells and the sequencing of their variable antibody regions. Additionally, we will immunize mice with virus particles of both species to produce antibodies that bind to highly conserved epitopes. Taken both immune responses, we will choose suitable epitopes, which were conserved and effective. We will further start the production of synthetic mimics of these epitopes. Nanoparticles presenting these "mimitopes" will be tested in mice and pigs to determine, if they induce the formation of the desired neutralizing antibodies. Subsequently, we will test protection of pigs with classical challenge experiments applying an established virulent field strain. These studies focus on relevant epitopes in the envelope proteins gp2 and gp4, which are the viral ligands mediating entry. In summary, we aim to solve the problem of unsatisfactory vaccine efficiency of PRRSV vaccines by applying reverse vaccinology. The term reverse vaccinology describes a new approach to develop vaccines for pathogens that cannot be tackled by a classical vaccine development approach. Synthetic immunogens that specifically induce antibodies against conserved neutralizing epitopes will be generated, hopefully providing superior protection against the porcine reproductive and respiratory syndrome.

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P06

Discovering the *Fasciola hepatica* kinome for the detection of novel drug targets and drug candidates

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Fasciolosis is a globally prevalent zoonosis and a neglected tropical disease caused by infection with liver flukes such as *Fasciola hepatica*. Triclabendazole (TCBZ) is a gold-standard drug used for its treatment; however, the mass treatment with TCBZ has led to development of TCBZ-resistant parasite strains.

Protein kinases (PKs) may serve as starting point for the development of new drugs, because they regulate a vast variety of cellular processes including cell growth, proliferation, differentiation and metabolism. PKs constitute the largest eukaryotic protein family and are druggable enzymes due to their catalytical process of transferring phosphate onto substrate proteins. Consequently, PK inhibitors are widely used in cancer therapies.

We pursue the hypothesis that inhibition of PKs represents a new therapeutic option for anti-parasitic treatment against the liver fluke *F. hepatica*.

To identify potential target kinases in *F. hepatica*, a genomic-bioinformatic approach using Kinnanote, OrthoMCL, Exonerate, pBLAT, and InterProScan programs was employed to generate a draft kinome dataset. The dataset will be used to prioritize potential target kinases and virtually screen compound libraries against homology models of these prioritized kinases. In a parallel approach, we selected PK inhibitors for which anti-parasitic activities have been described earlier and screened them against different life stages of *F. hepatica* in vitro.

A draft kinome dataset of *F. hepatica* contains 225 PKs in 9 (sub)families. Prioritized PKs are predominantly expressed in cells and tissues of therapeutic interest, such as neoblasts, the tegument, and neuronal cells, as revealed by single-cell RNAseq data that were recently obtained by our group. In total 11 PK inhibitors were tested that target PIM, TAO, VEGFR, PKCβ, FGFR, JAK/STAT and mTOR kinases. The inhibitors vandetanib, foretinib, ruboxistaurin, PAK-4 LCH 77499, PIM-CX6258, PIM-SG1776, tyrosine kinase-IN-1, and compound 43 were most potent and had lethal activity (after 24 h of incubation) against both immature and adult worms. Of note, the active concentrations of 25-50 µM were comparable to TCBZ.

Further investigations such as in silico target prioritization with the completed kinome, PamChip arrays on a PamStation for kinase activity profiling, and genetic validation of selected kinases by RNAi will assess the potential of re-purposing PK inhibitors and targeting PKs may be an effective approach to control *F. hepatica.*

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P07

Characterization of the zinc-metalloprotease activity of *Helicobacter pylori* HomB and its possible role in driving inflammatory responses

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A Gram-negative bacterium, *Helicobacter pylori* has evolved to invade the human stomach. This bacterium uses a variety of adhesins embedded in the outer membrane for attachment to the gastric epithelium. One of the putative adhesin is HomB and it has been linked to a higher incidence of stomach cancer and peptic ulcer disease. HomB has a metzincin motif found in numerous metalloproteases in its extracellular region. When the homology of the HomB sequences of various strains of *H. pylori* was compared, it was discovered that the metzincin motif is entirely conserved. This bacterium leads to the gastric inflammation, gastric diseases or even cancer. It has been previously demonstrated that HomB deletion *H. pylori* strains have a diminished ability to induce pro-inflammatory cytokine IL-8 from epithelial cells. The enzymatic activity of HomB may give rise to the inflammation by activating the protease activated receptor 2 (PAR-2) on epithelial cells.

Our initial attempts to recombinantly express the extracellular region of HomB in *E. coli* using various strains and expression strategies failed, as the protein was insoluble. Only expression as a fusion with MBP was successful. Analysis of the model allowed us to redesign the construct from Hom B_{19-511} to $HomB_{49-490}$, removing some hydrophobic regions which HomB uses for membrane anchoring which are probably the cause of the insolubility. In order to do that, $HomB_{49-490}$ was cloned into both pCOLDI and pCOLDI-GST vectors and the recombinant $HomB_{49-490}$-pCOLDI and $HomB_{49-490}$-pCOLDI-GST vectors were sent for sequencing to prove that the $HomB_{49-490}$ was cloned successfully. *E. coli* BL21 strain used for protein expression of $HomB_{49-490}$. However, $HomB_{49-490}$ protein was observed as insoluble protein like $HomB_{19-511}$. To overcome this insolubility issue, pMAL-c5X plasmid will be used for $HomB_{49-490}$ protein expression. Because it is known from our previous experiments, usage of this vector was succesful to express soluble $HomB_{19-511}$ protein. In the future studies of this project, the characterization of the metalloprotease activity of $HomB_{49-490}$ will be investigated with the help of metal chelators, protease inhibitors, and site-directed mutagenesis.

As a whole, this research will deepen the comprehension of the molecular mechanisms behind the suspected virulence of HomB and also may lead to the possible creation of novel treatment approaches with the goal of reducing inflammation caused by this bacterium.

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P08

Plasmodium falciparum and human chaperones, co-chaperones and their interaction as a target for drug development

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Upon entry into the human erythrocyte, the malaria parasite *Plasmodium falciparum (Pf)* rapidly and dramatically alters the physical and biochemical properties of the host cell. The host cell modifications are driven by parasite proteins that are exported into the host cell. Among them are a large number of *Pf*HSP40/J-domain protein (JDP) family members and a single exported *Pf*HSP70-x.

These molecular chaperones have been identified as potential drug targets. Based on recent study results, we would like to experimentally examine the hypothesis whether exported *Pf*JDP and *Pf*HSP70-x are essential for parasite pathogenicity and whether parasites can be targeted via protein export mechanisms. In order to test this hypothesis, this project aims to measure the functionel engagement of parasite and host-derived chaperones utilizing three independent *in vitro* assays. These medium-throughput assays also allow for screening of potential small molecule inhibitors of such protein-protein-interactions.

Initial milestones such as the establishment of expression and purification protocols for recombinant plasmodial and human HSP70 chaperones and co-chaperones as well as the establishment of medium-throughput protein-interaction assays have already been achieved. This includes all necessary full-length *Pf*HSP70 and *Hs*Hsp70 chaperones and also the full-length *Pf*HSP40 and *Hs*Hsp40 co-chaperones. To gain more information about critical domains during chaperone interaction, truncated versions and peptide-substrate fusion-proteins of the chaperones have been created. Chaperones with mutations in their functional domains as negative controls for interaction assays are in the making.

The progress of this project forms the basis for future characterization of the parasite-host interaction at the chaperone level. Furthermore, we plan to identify small molecules that inhibit this interaction using established medium-throughput screening methods.

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P09

Charcterization of deformed wing virus (DWV) leader protein

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The deformed wing virus (DWV) belongs to the family of *Iflaviridae* within the order *Picornavirales*. The positive-sense single-stranded RNA virus contains a single open reading frame encoding one large polyprotein. The polyprotein encompasses a leader protein, four structural proteins, and a non–structural protein cassette.

DWV is a feared infectious disease in beekeeping. Orally transmitted, it is not causing visible signs of disease in bees. However, in the presence of the ectoparasitic mite *Varroa destructor*, which is known to inject DWV during its feeding activities as a mechanical vector, the symptoms of DWV infection appear, typically characterized by wing deformities, and high brood mortality rates. Due to the worldwide spread of the Varroa mite taking place throughout the 20th century, DWV evolved from a pathogen of minor importance to a massive threat to the Western honey bee (*Apis mellifera*).

Historically, DWV persisted as swarm of different genome variants or quasispecies in the bee colonies. Following the emergence of the Varroa mite, the diversity of the genome variants was dramatically reduced. So far, four master variants or species of DWV are known (DWV-A, -B, -C and -D). All variants have been associated with disease and colony collapses, although it seems that DWV-A is the most virulent species, whereas DWV-B is less virulent. However, many basic research questions remain unanswered so far. The exact role of the Varroa mite in the viral lifecycle, the genetic determinants of virulence and the role of the leader protein, which's gene represents a genomic hot-spot of diversity, are certainly the most crucial issues.

In this project, I aim to elucidate the virulence factors of DWV genomes using a classical recombination approach. I applied an available, established model for a typical DWV-A strain and generated a novel molecular clone and model for a typical DWV-B strain. Using reverse genetics and DNA-clone inter-strain recombinantion, I tested the separate genomic regions of DWV with an *in vivo* model. Therefore, I generated eight inter-strain recombinants in total. Surprisingly, all recombinants were able to replicate and propagate in bees following the exchange of the functional cassettes between DWV-A and DWV-B. Ongoing studies will now elucidate the virulence of the recombinant viruses compared to the wild-type species focusing on virus growth and signs of disease in the pupal bee stage.

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P10

Neutrophil efferocytosis reprograms mitochondrial metabolism to lock alveolar macrophages in a pro-resolution phenotype at the expense of bacterial control

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Resolution of lung injuries is vital to maintain gas exchange. Concurrently, there is an increased risk of secondary bacterial infections. Alveolar macrophages (AMs) are the first to encounter incoming pathogens and to help orchestrate the initiation and resolution of inflammation. However, how environmental cues control these putatively opposing macrophage effector functions still needs to be discovered.

Based on transcriptomic in vivo and functional ex vivo analyses, we identified a transient incapacity of AMs to respond to bacteria during resolution of sterile inflammation. Importantly, efferocytosis of neutrophils impaired bactericidal properties of AMs. Mechanistically, upregulation of UCP2 by neutrophil-derived myeloperoxidase (MPO) fueled glutamine-dependent oxidative phosphorylation, which precluded an increase of the mitochondrial membrane potential (MMP), and, consecutively, a release of mitochondrial reactive oxygen species (mtROS) in response to bacteria. Instead, MPO-mediated upregulation of UCP2 enhanced the continued clearance of apoptotic cells, even in the presence of bacteria. Inhibition of UCP2 or canonical glutaminolysis and genetic deletion experiments confirmed our findings in vitro and in vivo in secondary bacterial infections after acid aspiration or influenza pneumonia.

Overall, clearance of apoptotic neutrophils switches AMs to prioritize resolution of inflammation over antibacterial responses and similarly affects murine macrophages at extra-pulmonary sites and human AMs.

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P11

Does plasma CRP induced by major surgery inhibit the ATP-mediated monocytic IL-1β release?

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During surgery, cellular damage can cause the release of cytoplasmic ATP, which activates the P2X7 receptor in monocytes and macrophages. This activation leads to inflammasome assembly, and subsequent release of the pro-inflammatory cytokine interleukin-1 beta (IL-1β). We recently showed that the IL-1β release can be inhibited by activation of nicotinic acetylcholine receptor (nAChR) subunits through conventional agonists, such as acetylcholine, but also by unconventional agonists containing a phosphocholine (PC) head group. A well-known biomarker of inflammation is C-reactive protein (CRP), which is produced in response to circulating cytokines. PC has been shown to act as a ligand for CRP. In recent studies, we demonstrated that CRP-PC complexes inhibit the ATP-induced IL-1β release by monocytic cells via activation of the nAChR.

In this study, we aimed to investigate the anti-inflammatory potential of endogenous CRP. Blood samples were collected from patients undergoing elective major abdominal surgery on day 0 (preoperative), day 2 and day 5 (postoperative). In a subset of experiments, endogenous CRP was depleted from their blood plasma via immunoprecipitation. Whole blood cells and peripheral blood mononuclear cells (PBMCs) were primed with LPS and stimulated with BzATP in the presence of plasma or CRP-depleted plasma. IL-1β release was quantified by ELISA. The results showed that ATP-induced IL-1β release by whole blood cells and PBMCs was inhibited by patient blood plasma in a dose-dependent manner, which positively correlated with the absolute CRP concentration. However, depletion of CRP-ligand complexes in the plasma did not affect the inhibitory effect.

The unexpected results of the clinical experiments could be attributed to a variety of different aspects. On the one hand, technical problems might explain the outcome. We are currently investigating potential sources of errors in the experimental setting. On the other hand, the results might also be explained by individual differences between endogenous CRP-ligand complexes and complexes formed with recombinant CRP. Therefore, we aim to further investigate potential endogenous CRP ligands regarding their effect on ATP-induced IL-1β release, to explain the contradictory results of the clinical experiments, and to provide new insights into the anti-inflammatory potential of endogenous CRP.

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P12

Is it possible for hepatitis D virus (HDV) to replicate intergenotypically using HDV small delta antigen (S-HDAg) from different HDV isolates of various genotypes?

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Hepatitis Delta virus (HDV) is causative of the most severe form viral hepatitis, affecting a global population of approximately 20 million individuals. It has been observed that HDV can superinfect patients already infected with HDV, as evidenced by the detection of chimeric HDV genomes in these patients. HDV encodes for only one open reading frame, resulting in the synthesis of small (S) and large (L) delta antigen (HDAg) which play crucial roles in replication (S) and secretion (L) of HDV. Yet, there is limited information on the ability of S-HDAg to transactivate or inhibit replication of other known HDV genotypes (Gt). Recently we discovered that, similar to Gt3, S-HDAg from Gt4 was unable to rescue replication of Gt1, but the protein domains responsible for HDV-replication and its mode of action are still unknown.

To further understand the role of S-HDAg, we used our "split deltaviral replication system" (SDRS), which is composed of a plasmid containing a full-length HDV Gt1 genome but is defective for HDAg expression and subsequent replication. Viral replication could be rescued in those cells by providing S-HDAg in trans by co-transfection of an S-HDAg expression vector. To access if the inhibitory effect of S-HDAg from Gt3 and 4 is isolate or genotype specific, we used plasmids expressing S-HDAg from 10 clinical isolates of those Gt and evaluated HDV replication by Northern blot. Most of S-HDAg of both Gt3 and 4 were unable to transactivate Gt1 replication. Surprisingly, only few isolates have shown transcomplementation of replication, suggesting isolate-specific effects of S-HDAg. To discover which S-HDAg motives are responsible for the HDV replication, intergenotypic chimeric HDAg between Gt1 and 3, and Gt1 and 4 were used. In sum, the N-terminus of S-HDAg of Gt1 was shown to be necessary for the replication of the viral genome.

Our study demonstrated that S-HDAg from all genotypes exhibits the capacity to transactivate Gt1 replication. However, a substantial proportion of Gt3 and Gt4 isolates failed to rescue HDV replication. Through intergenotypic recombination of S-HDAg, we were able to ascertain the significance of the N-terminus in viral replication. Additionally, we observed that certain combinations of genotypes displayed varying levels of transcomplementation, which directly influenced replication. These results allow a better understanding of the activation and inhibition effects of the S-HDAg on viral replication.

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P13

Re-evaluation of a hyperendemic focus of *Angiostrongylus vasorum* infections in gastropod intermediate hosts in Southern Germany

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Angiostrongylus vasorum is a metastrongyloid lungworm that causes severe cardiopulmonary disease in both wild and domestic canids. The life cycle of *A. vasorum* is heteroxenous, with gastropods acting as obligatory intermediate hosts. *A. vasorum* infections are distributed globally, with both hyperendemic and hypoendemic foci in close proximity. One of these so-called hotspot areas has been identified in 2018 in Obrigheim (Federal State of Baden-Wuerttemberg) in Southern Germany. To confirm this focus, 221 and 160 gastropods were collected on the hotspot meadow in Obrigheim in summer and autumn of 2022, respectively. After collection, the gastropods were cryo-euthanized and artificially digested. The gastropod remnants were microscopically analysed for the presence of lungworm larvae. In general, a high prevalence of *A. vasorum* infections in gastropods was confirmed. In total, 26.3% (58/221) of the summer samples revealed infected with *A. vasorum* larvae. Thus, the prevalence of *A. vasorum* infections in gastropods had almost doubled in 2022 when compared with data of summer 2018 (13.6%). Besides *A. vasorum*, the metastrongyloid lungworm *Crenosoma* spp. was also confirmed for 2022 gastropods, but at a much lower prevalence of 0.9% (2/221). For autumn samples, a total lungworm prevalence of 9.4% was found, being exclusively represented by *A. vasorum* infections. In this season, the prevalence revealed much lower than in the year 2018 (62.96%). Nevertheless, the current findings still represent a much higher prevalence for Obrigheim when compared to the mean prevalence in Germany (2.3%). Referring to slug species, metastrongyloid lungworm larvae were exclusively found in Spanish slugs (*Arion* sp.) and Leopard slugs (*Limax maximus*).

Overall, we here confirmed an *A. vasorum* hyperendemic focus in gastropods of Obrigheim. A sign informing pet owners of *A. vasorum*, its life cycle and the danger it poses on dogs was installed on the hotspot meadow. It included a QR code linking to a questionnaire for pet owners on potential lungworm infections in their dogs.

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P14

Extracellular vesicles derived from *Besnoitia besnoiti*- and *Toxoplasma gondii*-infected host cells and -exposed PMN induce NOX-independent NET formation

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Extracellular vesicles (EVs) are small membranous vesicles containing a complex mixture of proteins, RNA and lipids. EVs are actively released by various cell types including endothelial cells, granulocytes (PMNs) and protozoan parasites. EV functions include cell communication via molecular cargo transfer between cells. Moreover, EVs proved critical in pathological states like cancer or neurodegenerative disorders and also for responses to infectious diseases. Emerging evidence indicate that EVs also modulate neutrophil effector functions like NETosis. However, if EVs indeed play a role in parasite-mediated NET formation has not been studied, yet.

In the current project EVs were isolated from *B. besnoiti*- and *T. gondii*-infected bovine umbilical vein endothelial cells (BUVEC), from tachyzoite-exposed bovine peripheral blood neutrophils (PMN) and from *B. besnoiti* or *T. gondii* tachyzoites. EV nature was confirmed by analysing typical EV markers (CD9, CD63 and CD81) by Western blotting. The concentration and size of obtained vesicles was determined by protein quantification and Nano-Flow cytometry, respectively. We here tested the effects of EVs on PMN activation on the level of glycolytic and oxidative responses via Seahorse® instrumentation. We furthermore assessed NET formation by both extracellular DNA quantification and epifluorescence microscopy detecting early events of NETosis by nuclear area expansion assays.

Current data showed that EVs from *B. besnoiti*- or *T. gondii*-infected BUVEC (buEVs) and from tachyzoite-exposed neutrophils (nEVs) were positive for CD9 and CD81. A majority of EVs showed a size of 60-70 nm. Referring to PMN activation, exposure of PMN to any kind of EVs failed to induce glycolytic or oxidative responses. In contrast, stimulation of PMN with buEVs and nEVs both significantly induced extracellular DNA release as evaluated by picogreen-derived fluorescence intensities. Interestingly, buEVs had stronger effects on NET release than nEVs. This observation was further confirmed by epifluorescence microscopy showing that buEV and nEV exposure indeed led to an increased percentage of NETotic cells.

Overall, given that PMN exposure to buEVs and nEVs drove NET formation without changing oxidative responses in bovine neutrophils, we here demonstrated that EVs of different origin have the capacity to trigger NOX-independent NETosis.

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P15

Outer membrane vesicle of *Klebsiella pneumoniae* decrease bactericidal properties of alveolar macrophages

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Alveolar Macrophages (AMs) are the sentinel cells in the lung, which clear bacteria and initiate inflammation. Gram-negative bacteria release outer membrane vesicles (OMVs) into the extracellular environment and antibiotics increase OMV production. OMVs of bacteria contain different types of cargo such as proteins, lipids, and nucleic acids. As colonization with *Klebsiella pneumoniae (K. pneumoniae)* constitutes a risk factor for infection, we hypothesized that OMVs of *K. pneumoniae* might alter bactericidal properties of AMs to facilitate pneumonia.

K. pneumoniae were cultured *in vitro*, treated with different antibiotics, and the secreted OMVs were isolated. Murine AMs were harvested by bronchoalveolar lavage and treated with OMVs. Bactericidal properties were assessed in AMs infected with viable *K. pneumoniae ex vivo*. Reactive oxygen species (ROS) were quantified by flow cytometry. Cytokines were measured using a multiplex bead-based assay.

Preincubation with OMVs significantly decreased the killing capacity of AMs. In line, intratracheal instillation of OMVs facilitated bacterial outgrowth in a subsequent infection with *K. pneumoniae*. Whereas, OMVs did not alter cytosolic ROS production, they abrogated mitochondrial (mt) ROS release and decreased cellular respiration in AMs in response to *K. pneumoniae*. Specifically, OMVs isolated from *K. pneumoniae* treated with meropenem or piperacillin/tazobactam had the strongest effect. Inactivation of proteins and not DNA or RNA in permeabilized OMVs abrogated inhibition of mtROS release upon bacterial encounter.

In summary, we found that OMVs of *K. pneumoniae* dampen the killing capacity of AMs. Therefore, we suggest that OMVs might facilitate the transition from bacterial colonization to infection in the lung by decreasing bactericidal properties of AMs.

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P16

Assessing the metabolic requirements of MAIT cells during development.

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The thymus serves as a home for the step-wise development and maturation of conventional as well as unconventional T cells. Unconventional T cells such as γδT cells, invariant Natural Killer T cells (iNKTs) and Mucosal-associated invariant T (MAIT) cells share functions of both the innate as well as the adaptive immune system. The development and selection of iNKT cells is well studied, in contrast, less is known about MAIT cells mostly due to their sparse numbers in mice. The mode of selection of conventional T cells differs from that of iNKT and MAIT cells: weak TCR signaling facilitates the positive selection and strong TCR signaling results in clonal deletion of conventional T cells, whereas, in the context of iNKT cells it has been demonstrated that strong TCR signaling is required for cells to be positively selected. This is referred to as agonist selection. Post agonist selection iNKT cells proliferate rapidly and exit the thymus in pre-activated states, indicating distinct metabolic requirements. Albeit the developmental stages of MAIT cells being similar to that of iNKT cells, their metabolic requirements in the context of development are yet to be understood. In this study, we set out to reveal the metabolic requirements of MAIT cells during development by employing flow cytometry-based metabolic and functional analyses using expression of surrogate markers of metabolic states, metabolic dyes, and a novel approach - SCENITH. SCENITH enables us to study the metabolic profile of rare cell types at single-cell resolution by calculating the total energy consumption of each cell, using its capacity of protein synthesis deduced by the drug puromycin as readout. A combination of these three approaches provides insight into the metabolic profiles of MAIT cells at the single-cell level, during various stages of their development.

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P17

The proinflammatory cytokine Interleukin-1 attenuates global transcription rates

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Interleukin-1 (IL-1) initiates a fast but transient transcriptional activation and secretion of various chemokines and other interleukins such as IL6 and IL8 (CXCL8). While it is well established that IL-1-specific reprogramming of inflammatory genes is crucial in a wide range of acute or chronic inflammatory conditions, it is less clear to which extent IL-1 also affects basic or general cellular processes. While measuring global transcription rates using a metabolic labelling approach, we unexpectedly found a downregulation of global transcription rates in IL-1-stimulated cells.

Cells use uridine as a building block to produce messenger RNAs. The application of the uridine-analog 5-ethynyluridine (5-EU) to cell cultures leads to its incorporation into nascent RNAs. 5-EU carries an alkyne-group that we used to covalently attach a fluorescent molecule containing an azide group via a copper-catalyzed cycloaddition ("CLICK chemistry"). In this way, global transcriptional activity can be quantified based on the amounts of incorporated fluorophore as assessed by fluorescence microscopy. In five different cell lines, including non-transformed, diploid cells such as MRC-5 embryonic fibroblasts or hTERT-RPE-1 epithelial cells, we found that cells reduce their global transcription rate in response to IL-1 within the first hour of cytokine exposure.

Because IL-1 reduced mRNA biosynthesis by 9 % to 24% depending on the cell line, we also pursued an analogous approach, using O-propargyl-puromycin instead of 5-EU, to assess the translational rates within the same period of time. Surprisingly, protein biosynthesis remained mostly unchanged.

We then investigated if cellular transcription rates were reduced within the same cells that also showed a strong proinflammatory response to IL-1, by combining single-molecule RNA fluorescent-in-situ-hybridization (smRNA-FISH[K2]) with the metabolic labelling approach. As a proxy for the proinflammatory response, we measured *IL8* mRNA by smRNA-FISH. Interestingly, there was no significant correlation between global transcription rates and *IL8*-mRNA spots per cell.

The biological function of the IL-1-induced attenuation of global transcription rates is unknown, as is the question why (and how) this effect is balanced at the level of translation. The elucidation of the underlying mechanisms and their relevance for the inflammatory system await further clarification.

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P18

Characterization of fibroblast-mediated alteration of neovascularization *in vitro* and *in vivo*

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Rheumatoid arthritis (RA) is a chronic, systemic autoimmune-mediated disease primarily characterized by inflammation of the synovial tissue with pathological neovascularization. Chronically activated RA synovial fibroblasts (RASF) are key players in RA progression regarding cartilage destruction and underlying bone by producing matrix-degrading enzymes. Matrix degradation leads to the release of matrix proteins such as the angiogenic collagen-fragment canstatin, which may further activate RASF contributing to altered angiogenesis in RA.

2D tube formation assay was performed using HUVEC seeded on Matrigel®. 15% Calcein AM-stained RASF were added. RASF/HUVEC were treated with 0.5µg/ml canstatin. HUVEC were pre-treated with 0.2µg/ml canstatin. Tube thickness was measured. RASF were repetitively stimulated three times with 0.05ng/µl IL1ß every 24h starting at day 2. RNA was extracted and IL6 and CXCL2 were measured in supernatants by ELISA. Healthy cartilage was subcutaneously co-implanted with RASF +/- canstatin into SCID mice. Contralaterally, healthy cartilage without RASF +/- canstatin was implanted. Amount of Helix-like vessel (HLV) was evaluated after 3-45 days (d).

HLV were detectable at early time points in implants with RASF (d3: 8.2±1.0), increased until d30 (12.3±1.3) and reduced on d45 (9.5±1.8) in ipsilateral implants. RASF directly arranged into tubes and influenced tube formation *in vitro* by significantly reducing tube thickness from 22.9µm (*SD=6.3*) to 16.6µm (*SD=2.2*) (p=0.014) compared to HUVEC alone. After subsequent repetitive stimulation of RASF, a significant decrease in IL6 (1st stimulation: 5076±1730pg/ml vs. 3rd: 1890±758pg/ml, p<0.0001) and CXCL2 (1st stimulation: 656±300pg/ml vs. 3rd: 233±162pg/ml, p = 0,004) was observed. Co-culture of RASF with pre-treated HUVEC and addition of 0.5µg/ml canstatin significantly reduced tube thickness from 22.9µm to 14.6µm (*SD=1.4*) (p<0.001). Canstatin significantly inhibited the amount of HLV in SCID mice on d3 (8.2±1.0 vs. 2.8±0.5, p=0.008).

RASF specifically alter neovascularization in SCID mice by promoting helix-like vessel formation. RASF-induced functional effects on EC in the tube formation assay seemed to be mediated by altered expression of IL6 and CXCL2. Canstatin significantly attenuated the RASF-mediated HLV in SCID mice, but not in the tube formation assay, suggesting canstatin as novel therapeutically molecule for RASF-induced altered neovascularization in RA.

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P19

Biomechanics of the Platyhelminth Adhesion, Locomotion, and Reproduction

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"Physics of Parasitism (PoP)" describes the physics and mechanics of parasites interacting with their hosts. Two of the most common and major trematode species are schistosomes (blood flukes), which live in blood vessels, and liver flukes, which mature to live in the bile duct. *Schistosoma mansoni* and *Fasciola hepatica*, the two species being discussed, both have unusual characteristics, including migratory juvenile stages and lifelong residence in a variety of host niches (tissues). As is typical for trematodes (also known as "Saugwürmer" in German), these parasites also have head and ventral suckers that aid in locomotion and attachment activities. The regulation of biomechanical forces at an organ scale (suckers) and cellular scale (mechanotransduction) are crucial for the parasites' survival and the success of their infection. But the process of this regulation is still an unexplored field. The PoP initiative examines the processes of force transmission within parasites as well as the physical forces generated at the parasite-host interface by e.g., the action of suckers. Also, whether adhesion forces differ between fluke species, sexes, and developmental stages, and whether they are influenced by the environment of the parasite, which includes factors like substrate stiffness and flow stress. We will use fluorescence and traction-force microscopy to measure the suckers' in vitro attachment forces to several synthetic surfaces that resemble hosts. Biochips (flow chamber) will also be used to cultivate parasites under conditions resembling the natural environment. RNA interference and pharmacological agonists or antagonists have been used to functionally analyse cellular proteins implicated in mechanotransduction. Different formulations of hydrogels based on polyacrylamide have been the subject of preliminary tests. Parasite culturing in the biochips was successful for several days. Mechano-sensitive receptors such as Piezo and mechano-responsive transcriptional regulator proteins such as YAP have been found in the genome of *S. mansoni* and successfully knocked down to better understand force transduction at the cellular level. Optimisation of hydrogels with different material properties with fluorescent beads is under progress. For flow measurements, a flow chamber has been prepared and with the help of a peristaltic pump, parasites' physical forces under flow will be studied.

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P20

Insights into the molecular IgE-IPSE/alpha-1 interaction responsible for basophil activation

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IPSE/alpha-1 (IL-4 inducing principle of *S. mansoni* eggs) is a dimeric glycoprotein secreted by the eggs of the blood-trematode *S. mansoni*, the causative agent of schistosomiasis, an important neglected tropical disease. Natural IPSE has been shown to bind to IgE, resulting in the release of IL-4 and IL-13 from basophils and mast cells. The classical mechanism of IgE-dependent activation consists of cross-linking IgE by allergen binding to the antigen-recognition variable region of the corresponding immunoglobulin. Homodimeric IPSE appears to activate basophils by binding to IgE without any typical cross-linking. The aim of this study is to investigate the molecular details underlying this unique interaction between IPSE/alpha-1 and IgE.

Using site-directed mutagenesis, we created six mutants, based on the knowledge that neither IPSE monomers nor the T92Y/R127L mutant, are able to activate basophils. Proteins were expressed in HEK293-6E suspension cells, followed by affinity chromatography for purification. The ability of all IPSE forms to activate basophils by binding IgE was evaluated using humanized RS-ATL8 rat basophilic leukemia (RBL) reporter cells. Cells were sensitized with either IgE-containing sera or different IgE truncates and luciferase expression was measured after stimulation with IPSE. Ancillary ELISAs using similar truncated IgE forms and IPSE were performed to further determine the binding region. Our results show that all the mutations have an impact on IPSE's capability to interact with IgE, thus lowering the activation of the reporter cells. Only the double mutant T92Y/R127L hampers cell activation completely, leading us to the conclusion that both amino acids must be key residues involved in IgE interaction. Furthermore, we show that IPSE does not bind to all truncated forms of IgE, suggesting that IgE needs all heavy chain domains, with or without light chains, to be successfully bound by IPSE.

Ultimately, additional reporter assays including different IgE-forms, size exclusion chromatography and cryo-EM of the IgE-IPSE complex are expected to reveal a detailed model of interaction.

P21

Investigation of structure-activity relationships of sulfonamide-based inhibitors of carrier of the Solute Carrier Family SLC10

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Infections with the Hepatitis B (HBV) and D (HDV) viruses are the main cause of hepatocellular carcinoma and liver cirrhosis as consequence of chronic hepatitis [1]. Although an effective prophylactic vaccine is available, therapeutic options are highly limited, in particular for HDV. A promising novel drug target to block HBV/HDV virus entry into hepatocytes is represented by the hepatic bile acid carrier NTCP (Na^+/taurocholate co-transporting polypeptide, gene symbol *SLC10A1*) that has been identified as the high-affinity hepatic receptor for HBV/HDV [2,3]. While searching for NTCP inhibitors that block virus entry into hepatocytes, we systematically analyse a set of phenylsulfonamide derivatives. Apart from NTCP inhibition, we also determine potential cross-inhibition of the closely-related carriers ASBT (apical sodium-dependent bile acid transporter, gene symbol *SLC10A2*) and SOAT (sodium-dependent organic anion transporter, gene symbol *SLC10A6*), which together with NTCP belong to the Solute Carrier Family SLC10 [3]. ASBT and SOAT are established drug targets as well, so compounds from the phenylsulfonamide drug class could also be attractive new drug candidates for all three SLC10 carriers [4-6].

We developed a screening assay that is suitable for medium-scale screening of inhibitors that block (1) bile acids transport via NTCP and ASBT, (2) HBV/HDV preS1-peptid binding to NTCP and (3) transport of DHEAS via SOAT using stably NTCP-, ASBT-, and SOAT-transfected HEK293 cells. A set of 75 phenylsulfonamide-based compounds is currently tested in all these assays. In a first step, all compounds are screened at 10 µM and 100 µM inhibitor concentrations and subsequently full inhibition kinetics and IC_{50} values are determined for the most active compounds. Several compounds already proved to be very active against NTCP, ASBT, or SOAT. While some of these inhibitors showed equipotent inhibition of all three carriers, some others were more selective for only one of them.

Systematic structure-activity-relationship analyses of all phenylsulfonamid derivatives will help to increase the potency and carriers selectivity of this inhibitors class against NTCP, ASBT, and SOAT.

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P22

Biochemical characterization of a nucleotidyltransferase activity of equine arteritis virus nonstructural protein 9

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Arteriviridae are plus-strand RNA viruses in the order *Nidovirales*. Arteriviruses share with other nidoviruses a conserved array of protein domains in their replicase polyproteins (pp), including a putative nucleotidyltransferase (NiRAN) domain that is C-terminally combined with the RNA-dependent RNA polymerase (RdRp) domain in one of the nonstructural proteins (nsp) produced by proteolytic processing of pp1ab. To better understand the biochemical properties of nidovirus NIRAN domains, we characterized an arterivirus homolog, which is part of nsp9 of the prototype arterivirus, equine arteritis virus (EAV).

Using a combination of *in vitro* biochemical assays and native mass spectrometry analysis, we found that a recombinant His-tagged form of EAV nsp9 (nsp9-His_7) produced in *E. coli* is able to nucleotidylate (NMPylate) different EAV pp1a/pp1ab-derived proteolytic processing intermediates, such as nsp6-7-8-His_6, nsp7-8-His_6, nsp6-7-His_6 and nsp7-His_6, albeit with varying efficiency. Among the substrates tested, nsp7-8-His_6 proved to be the substrate that was NMPylated by nsp9 most efficiently. Nsp9-mediated protein NMPylation required the presence of Mn^{2+} ions and was found to be specific for UTP and GTP as nucleotide cosubstrates. Microscale thermophoresis data using fluorescence-labeled nsp9-His_7 and a range of protein substrates suggested that nsp9 binds most strongly to nsp7-8-His_6 followed by nsp6-7-8-His_6. Consistent with this observation, *in vitro* chemical crosslinking analysis using the lysine-specific crosslinker EGS revealed that nsp7-8-His_6 (followed by nsp6-7-8-His_6) binds to nsp9-His_7 more strongly than other test proteins. These and other data lead us to suggest that the nsp8 region in nsp6-7-8-His_6 and nsp7-8-His_6 facilitates interactions of the respective protein with nsp9, thereby promoting efficient NMPylation. Possible roles of NMPylated forms of nsp7-8 in virus replication remain to be established.

Taken together, our results provide important insight into the substrate specificity of EAV nsp9-mediated NMPylation activity, with nsp7-8 and nsp6-7-8 being preferred over other substrates. The data obtained so far and the protocols established in this study should allow us to identify the NMPylated amino acid residue(s) by mass spectrometric analysis and will form the basis for reverse genetic and biochemical studies aimed at understanding the biological relevance of this substrate-specific NMPylation activity.

P23

Establishment of a novel yeast-based reverse genetic system for Cavally virus using transformation-associated recombination cloning

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Cavally virus (CavV) was one of the first nidoviruses to be discovered in insects and prototypes a new virus family named *Mesoniviridae* in the order *Nidovirales.* With a genome size of approximately 20 kb, CavV fills an evolutionary gap in the evolution from small-sized nidoviruses, such as arteriviruses, to significantly larger nidoviruses with genome sizes of more than 30 kb. To date, there is nearly no information on the molecular biology of mesonivirus replication (1). To fill this knowledge gap, we aimed to establish a reverse genetic system for CavV that is suitable to investigate putative *cis*-acting RNA elements as well as functions of individual components of the mesonivirus replication-transcription complex.

To produce recombinant CavV, a recently established yeast-based system called transformation-associated recombination (TAR) cloning was used (2). In brief, 10 cDNA fragments with slightly overlapping sequences and covering the entire CavV genome were amplified by RT-PCR from RNA isolated from CavV-infected C6/36 cells. The 5’ and 3’ CavV fragments contained additional vector-derived flanking sequences facilitating recombination with the ppCC1BAC-his3 plasmid backbone. A mixture containing 75 ng of each of the 10 fragments and vector DNA was used to transform yeast cells which were subsequently grown on SD-his agar selection plates. Yeast transformants harboring the correctly assembled CavV genome-length cDNA were identified by multiplex PCRs spanning the junction sites of the various fragments. Correctly assembled plasmids were also examined by Sanger sequencing. Plasmid DNA containing full-length CavV cDNA was linearized and served as a template for *in vitro* transcription using T7 RNA polymerase to produce 5'-capped and 3'-poly(A)-tailed genome-length CavV RNA. To recover CavV infectious progeny, in vitro transcribed mRNA (or plasmid DNA) encoding the CavV nucleocapsid protein was mixed with in vitro transcribed genome-length CavV RNA and transfected into C6/36 cells.

In summary, we successfully established a reverse genetic platform suitable to produce recombinant CavV in cell culture. The system will be used to characterize CavV replicative proteins and *cis*-acting RNA elements and will help us to characterize and compare the molecular mechanisms involved in the replication of genetically diverse families of vertebrate and invertebrate nidoviruses.

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P24

Comparison of patients with active rheumatoid arthritis and patients in remission with respect to histological and molecular features in the synovial tissue

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Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease with a worldwide prevalence of 1%. RA leads to inflammation of the synovium, followed by the destruction of cartilage and bone. As a result, patients suffer from restricted movement, pain and swelling of the joints. Despite a variety of medication, RA cannot be cured, which is why remission is the therapeutic goal. Since the prognostic outcomes of the patients vary widely, a better characterization of remission is required.

After joint replacement surgery, RA patients (35-82 years) were classified into active RA or remission based on laboratory (e.g. ESR, CRP, leucocytes) and clinical (e.g. painful joints, pain, medication) parameters. The Krenn score was applied and immunofluorescence against CD90- and podoplanin-positive RA synovial fibroblasts (RASF) was performed. RASF were stimulated with IL-1ß and RNA sequencing was performed. Differentially expressed genes (DEGs) were analysed based on absolute expression, divergence and significance. Gene set enrichment analysis of DEGs was performed.

64 RA patients (29 active, 35 remission) were characterized using the Krenn score. Patients in remission showed reduced values for hyperplasia ($p=0.001$), infiltrates ($p=0.0001$) and overall synovitis ($p<0.0001$). Subanalysis of medication (biologicals, DMARDs, NSAIDs, glucocorticoids) confirmed differences between remission and active RA for all drugs and indicated a reduction of hyperplasia observed most strongly in the biologics group ($p=0.0008$). Podoplanin and CD90 were less expressed in remission indicating those patients have fewer numbers of activated synovial fibroblasts. Patients in remission showed lower expression of DEGs compared to active patients, including IL-36ß and its receptor antagonist. As those proinflammatory cytokines play a role in the RA progression, the impact of these factors on RASF of patients in remission compared to active RA are of interest.

Histological analysis of the synovium showed a lower degree of inflammation and, most prominent, hyperplasia for patients in remission, specifically in patients receiving biologics. RNAseq identified several genes showing altered expression pattern related to the pro-inflammatory and destructive processes during RA which gives insight into the molecular characteristics.

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P25

Conditional CRISPR/Cas9-based activation and depletion of individual cytokine genes

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Cytokines play a crucial role in mediating cell-cell communication during infection and inflammation, precisely regulating the recruitment, activation, and differentiation of immune cells and their interaction with local cells in inflamed tissues. However, controlling the local cytokine environment in a targeted manner has remained elusive. This study aims first to investigate the therapeutic targeting of cytokine-associated disease states at the mRNA transcriptional level by repressing or activating specific (cytokine) genes alone or in combination. Second, in addition to the research objectives outlined above, an intriguing question arises concerning the modulation of coronavirus-mediated cell responses by the same approaches. Given the significant impact of coronaviruses on infection and inflammation, it is worth exploring how these viruses might influence cytokine-mediated cell-cell communication and immune responses. Understanding the interplay between coronaviruses and cytokine regulation could provide valuable insights into the pathogenesis and therapeutic targeting of cytokine-associated infection systems. This inquiry opens up new avenues for investigating the specific mechanisms by which coronaviruses modulate cytokine gene expression and protein activity, and how such interactions could be manipulated using the CRISPR/Cas9-based systems developed in this study.

To achieve this, CRISPR-based activation and inducible knockout systems using (dead) Cas9 enzymes, transcriptional activator domains, and inducible Cas9 wt versions were adapted and established [1,2]. The initial focus is on selectively expressing or suppressing the NF-kB-driven cytokines IL-8 and IL-6, along with the intracellular negative regulators of NF-kB *(NFKBIA, TNFAIP3*). Doxycycline-inducible lentiviral transduction vectors have been designed, cloned and shown to efficiently deliver the CRISPR activation and depletion systems to epithelial and liver cancer cell lines. Functionality of the vectors has been assessed at the mRNA and protein level using RT-qPCR, ELISA, and Western blot analysis in tumor cell lines. Furthermore, we plan to utilize these systems to modify human macrophage phenotypes in co-culture assays with genome-edited tumor cell lines. Ultimately, our aim is to optimize these CRISPR/Cas9-based systems to modulate key factors involved in (coronavirus) infection and inflammation, providing a new approach to control cell-cell communication in innate immune responses.

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P26

Toxoplasma gondii infections drive DNA damage in primary host cells which partially depends on the parasite effector protein HCE1

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Toxoplasma gondii is an obligate intracellular coccidian parasite, which is well-known for its extraordinary capacity to modulate its host cell. Recently, *T. gondii* infections were reported to affect host cell cycle progression, chromosome segregation and cytokinesis, thereby leading to polyploid host cells formation. To analyze whether these alterations are linked to host cell DNA damage and if they were directly driven by parasite protein, we here studied two *T. gondii* tachyzoite mutants previously described to be involved in host cell cycle modulation (*Tg*Δ*myr*1,*Tg*Δ*hce*1). MYR1 mediates the translocation of several parasite proteins from the parasitophorous vacuole into the host cell cytoplasm or nucleus and it helps to HCE1 binding to E2F. HCE1 induces an overexpression of host cellular cyclin E1, which regulates S-phase exit. Here, we studied host cell DNA damage by classical comet assays and by detecting $_{\gamma}$H2A.X, a classical marker of DNA damage foci indicating DNA strand breaks.

The results of comet assays and the quantification of DNA damage foci revealed that *Tg*RHwt significantly induces DNA strand damage after 12 h p.i. The downregulation of MYR1 and HCE1 displayed similar percentages of DNA strand damage (8.1% and 8.2%) which were 9.5% and 9.4% lower in comparison with *T. gondii* RH infections, respectively. Given that host cells physiologically respond to DNA damage, we also studied the activation of DNA damage response-related DNA repair pathways in *Tg*RHwt infections and stated a parasite-mediated activation of the homologous recombination pathway. Increased intracellular ROS production is a common cause of DNA strand breaks, thus we also quantified both intracellular and extracellular ROS levels in *Tg*RHwt-, *Tg*Δmyr1- and *Tg*Δhce1-infected host cells. However, no changes in ROS were found suggesting that oxidative responses are not related to parasite-driven DNA damage.

In conclusion, the current data suggest that *T. gondii* infections drive both host cellular double-strand DNA breaks and the activation of the homologous recombination pathway. These effects seem to be partially dependent on functional HCE1 expression in the parasite.

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P27

Role of a virus-encoded mono-ADP-ribosyl hydrolase activity in shaping the cellular response to human coronavirus infection

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Coronaviruses (CoVs) are plus-strand RNA viruses. To replicate and transcribe their genomes (~30 kb), they employ multi-subunit protein complexes including nonstructural protein 3, a large multi-domain protein containing a conserved macrodomain (MacD). Despite some recent progress in the characterization of MacD activities *in vitro*, the cellular and viral targets of CoV-MacDs remain largely unclear.

In this study, we aimed to characterize potential functions of CoV-MacDs in more detail. We sought to identify host genes being deregulated in cells infected with CoVs that express an active/inactive MacD.

Human coronavirus 229E (HCoV-229E) mutants encoding MacDs with active-site replacements were generated. Cells used for transcriptome analysis were pretreated with IFN-β followed by infection with HCoV-229E mutants. Recombinant HCoV-229E MacD proteins were tested for ADP-ribosyl hydrolase activity *in vitro*.

We were able to show that a recombinant form of HCoV-229E MacD has mono-ADP-ribosyl hydrolase activity *in vitro* and a mutational analysis revealed residues that are essential for this activity. The characterization of recombinant HCoV-229E variants containing these MacD mutations resulted in reduced viral titers (compared to WT) when propagated in IFN-β pretreated cells. Transcriptome analyses suggested that, among other genes, poly(ADP-ribose) polymerases are differentially expressed in cells infected with HCoV-229E WT and MacD knock-out mutants, respectively, suggesting potential cellular targets of this activity.

Taken together, the study identified residues that are essential for MacD activity *in vitro* and contribute to efficient virus reproduction. HCoV-229E expressing an inactive MacD were found to be more sensitive to type I interferon. Although the molecular details remain to be studied, it seems reasonable to suggest that the observed effects are linked to CoV-MacD-mediated mono-ADP-ribosyl hydrolase activity that removes ADP-ribose from cellular and/or viral proteins and thereby modulates protein functions. Further studies including proteome analyses are underway and expected to identify potential biologically relevant CoV-MacD targets.

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P28

Mucosal-associated invariant T cells and their involvement in the development of chronic obstructive pulmonary disease

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Chronic obstructive pulmonary disease (COPD) is one of the leading causes of death worldwide with many more being disabled by the disease, especially in low to middle-income countries. The disease develops over a long period of time and affects the small airways as well as pulmonary vessels. It is accompanied by increased inflammation and T cells have been implicated in the process of lung tissue remodelling.
One of the highly abundant T cell subtypes that can be found in the lung and other mucosal tissues are mucosal-associated invariant T cells (MAIT cells). MAIT cells recognize bacterial-derived small organic molecules of the riboflavin synthesis pathway complexed to major histocompatibility complex class 1-related gene protein (MR1) but can also be activated by IL-12 and IL-18 in concert. MAIT cells constitute around 4 % of lung T cells in humans and are therefore a highly relevant T cell subtype.
We hypothesize that MAIT cells contribute to the development and exacerbation of COPD by activation through cigarette smoke-derived neo-antigens and/or tissue destruction and inflammation.
We investigate whether cigarette smoke itself can alter or induce MAIT cell responses that can contribute to disease development in an *in vitro* antigen-presentation model of human peripheral blood mononuclear cells. Furthermore, we characterize how MAIT cell numbers and phenotypes change between the early onset of the disease and healthy lungs using animal models of COPD. Using MAIT-cell deficient mouse models we plan to investigate how MAIT cell absence affects disease development and progression by examining lung function, immune cell infiltration, and disease score.

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P30

P29

Development of diagnostic essay for Echinococcosis using IgE-based transgenic reporters and oncospheral antigens of *Echinococcus granulosus*

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Despite its importance as a widespread public health threat, *Echinococcus* sp. is one of the 20 Neglected Tropical Diseases (NTDs) targeted by the WHO for control and eradication. Echinococcosis has a global economic impact on both human and livestock health, as well as food security. The gold standard for echinococcosis diagnosis is the use of imaging technologies such as CAT, MRI, or Ultrasonography. However, such methods are not widely available in endemic countries. Therefore, cheaper test such as serology would be an advantage. However, such tests, usually based on IgG detection [1], are unreliable due to low specificity. Despite the central role of IgE responses in metazoan parasite infections, this isotype has not been used for diagnostic purposes of Echinococcosis. Therefore, we are developing a highly sensitive and specific serological assay based on humanised and caninised rat basophil leukaemia (RBL) IgE reporter cell lines [2]. The first part of study involved the development of NPY-mRFP RBL reporter cell line customized for binding of dog IgE. For this, we nucleofected NPY-mRFP RBL cells with 3 constructs, expressing the wild-type dog FceR1α, a chimeric Dog/Rat FcεRIα and a mutated wild-type dog FcεRIα chain . The cells were given several passages and stable transfection was verified by PCR. As the reporter essay detects antigen-specific IgE, we selected *E. granulosus* specific antigens that are target of an IgE response. For this, a bioinformatic approach was combined with a detailed study of published transcriptomic [3] and proteomic data of *E. granulosus*. Priority was given to the antigens expressed by tissue migrating stages as these are considered to induce an IgE response. The cDNA of the predicted IgE-immunoreactive antigens were cloned into pTT vector. For expression of the allergens, we used human embryonic kidney cells (HEK293-6E). We have expressed Calcineurin B, Cyclophilin, Ef1-beta, EgTeg, Eg19 and EgTPx. The expressed proteins were successfully purified using Immobilised Metal Affinity Chromatography (IMAC). These proteins will be used as allergens to bind with the dog-IgE sensitized caninized RBL cells to induce degranulation. Once developed, the performance of the expressed parasitic antigens in terms of their suitability, by determining sensitivity and specificity of the diagnostic assay will be assessed, and it will be compared with commercial serological assays.

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P30

The aldose reductase 1 of *Schistosoma mansoni* - expression and inhibitor testing for target evaluation

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Schistosomiasis, caused among others by the parasitic trematode species *Schistosoma mansoni*, leads to chronic inflammation and finally to liver fibrosis. If untreated, the disease can cause life-threatening complications. The current treatment of schistosomiasis is mainly based on a single drug, Praziquantel (PZQ). Due to the frequent use of PZQ, there is upcoming fear of emerging resistance. Therefore, it is necessary to find alternative drugs. Screening of potential drugs is currently based on *in vitro* tests against different stages of the parasite. An attractive alternative is the establishment of enzyme assays with potential target proteins found in the parasite. With the use of such assays, large compound libraries can be tested in high-throughput screenings (HTS) without the need for animal experiments and in a time- and cost-efficient manner. A potential target protein for such HTS, based on its role in detoxification processes in other organisms, is an aldose reductase (AR) orthologue in *S. mansoni* (Smp_053220, *Sm*AR). This assumption is supported by the ubiquitous and sex-independent expression in *S. mansoni* [1, 2] and by a study in *Schistosoma japonicum* where treatment with a specific AR inhibitor showed a clear decrease in worm activity [3]. *Sm*AR was recombinantly expressed in *Escherichia coli* strain BL21(DE3)pLysS and purified by immobilized metal ion affinity chromatography. The following buffer exchange was carried out performing size exclusion chromatography. Enzyme activity and IC_{50}-values for potential inhibitors were determined using the established enzyme assay. The enzyme was found to be active *in vitro*. A number of tested compounds, synthesized in the working group of Prof. Schlitzer (Philipps Universität Marburg), showed a clear inhibition of the enzyme and provide a solid basis for optimization and further development. The successful purification of *Sm*AR in sufficient amounts and the confirmed activity in the enzyme assay enabled first inhibitor screenings. Next steps will be further characterisation of the enzyme itself and possibly later on the performance of HTS.

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P31

Impact of obesity and T cell-mediated immune response on orthodontically induced inflammatory root resorption

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Orthodontically induced inflammatory root resorption (OIIRR is considered as one of the most severe and common complications of orthodontic tooth movement (OTM)), which is characterized by impaired function and metabolism of cementoblasts. Obese patients have increased risk of OIIRR, because the impairment of cementoblasts may be caused by changes in the level of adipokines and altered immune response. The common adipokines include leptin (pro-inflammatory) and adiponectin (anti-inflammatory). T cells are essential for immune responses, among which γδ T cells are a subset with tissue-resident characteristics. They are considered sensitive mechanoreceptors and play an important role in the process of OTM. Therefore, this study aims to reveal the association between obesity and γδ T cells with OIIRR, and to demonstrate the impact of adipokines, programmed death receptor ligand 1(PD-L1), and γδ T cells on OIIRR. Compressive force will be applied to cementoblasts to simulate the orthodontic process. Adipokines such as leptin and adiponectin will be added separately, and their effects on cell proliferation, migration, and osteogenic differentiation are evaluated using different methods including CCK-8 assay, scratch assay, and alkaline phosphatase activity. RT-qPCR, Western blot, and immunofluorescence are utilized to detect cellular reactions such as the PD-L1, tumor necrosis factor-α (TNF-α), interleukin (IL)-1, IL-6, IL-17, interferon-γ (IFN-γ) expression. Furthermore, cementoblasts will be co-cultured with γδ T cells and the mentioned stimuli will be applied as well. Subsequently, we will use the same approach to assess the cellular biological characteristics of cementoblasts and the changes in cytokine levels in the co-culture system. In addition, we will conduct an analysis of the corresponding signaling pathways involved in the process by using chemical inhibitors targeting signaling molecules and the generation of CRISPR/Cas9 knockout clones. The findings that may arise from this study can contribute to a more profound understanding of the association between obesity, inflammatory conditions, and OIIRR. Additionally, they may enhance our understanding of the mechanisms underlying immune orthodontics and obesity.

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P32

Site-directed mutagenesis of an *mcr-1*-encoding epidemic plasmid to understand its success strategies

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Plasmids are circular, autonomously replicating, double-stranded DNA molecules present in bacteria and archaea. They encode genes that are not essential for the bacteria to survive but confer advantages over their competitors. For example, they encode genes for antimicrobial resistance (AMR), which they can easily transfer to other bacteria. Many properties of plasmids are still unknown, in particular genes involved in the successful spread of epidemic plasmids. This work deals with an epidemic plasmid of the incompatibility group IncX4 (pV163M) that is wide-spread in Enterobacteriaceae worldwide and carries an AMR gene encoding resistance towards the last-resort antibiotic colistin (*mcr-1*). Our aim is to investigate and characterize this plasmid to understand the underlying success mechanisms.

Therefore, deletion mutants of pV163M were produced and characterized. One mutant (p9 mutant) was selected for deeper characterization because this mutant showed reduced growth and an increased conjugation rate in comparison to the wildtype (WT). The function of p9 is unknown, thus an *in silico* analysis via the program "ProDom" was performed to determine closely related proteins with known function. Our analysis revealed that the p9 protein has 68% amino acid identity to the CopG protein regulating the copy number of plasmids from Gram-positive bacteria. The p9 protein was compared with CopG-like proteins using Clustal Omega to determine amino acid residues important for its function. In a first experiment, the p9 mutant of pV163M was complemented with pUC19 encoding mutants of p9 in all possible combinations (single/double/triple mutants of residues 56 to 58). The amino acids were mutated to alanine. Growth analyses showed that six mutants had a changed growth behaviour as compared to the WT. The only exception was the mutant at residue 58 and thus this amino acid probably has no effect on the functionality of p9. In a second step, the mutations were inserted into the pV163M plasmid (approx. 36 kbp) by aqua cloning and confirmed by whole plasmid sequencing. Of the seven possible mutants, only three were recovered in pV163M (P58A, R57A/P58A, H56A/R57A/P58A), indicating that the remaining mutants might be harmful to the bacterial host.

In further investigations, the mutants of pV163M will be further characterised. For this purpose, growth analyses and conjugation experiments will be carried out. Furthermore, it is planned to carry out a copy number determination.

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P33

Fusobacterium nucleatum Induces PD-L1 Expression in Oral Epithelial Cells and Cancer Cells

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Fusobacterium nucleatum (*F. nucleatum*) is a Gram-negative obligate anaerobic bacterium that is highly prevalent in the oral cavity and gut. It has been reported that it likely plays a role in developing gastrointestinal cancer and oral cancer. The programmed death-ligand 1 (PD-L1), also known as the B7-H1 receptor, is an essential regulator of the cell-mediated immune response. The study aimed to investigate the potential of *F. nucleatum* to up-regulate PD-L1 expression *in vitro*.

Different strains of *F. nucleatum,* including ATCC 49256*,* ATCC 28856, and ATCC 10953, were incubated anaerobically. The human squamous cell carcinoma cell line SCC-25, human colon cancer cell line CL-11, and primary human gingival keratinocytes (PHGK) were stimulated with heat-killed *F. nucleatum* at the multiplicity of infection (MOI) (10, 50, 100, and 200) at different incubation times (4h, 8h, 24h, and 48h). Noninfected cells were used as the negative control. PD-L1 expression was analyzed using the western blot and quantitative reverse transcription polymerase chain reaction method. All strains of *F. nucleatum* induced up-regulation of PD-L1 protein expression in SCC-25 and CL-11 cells. Stimulating *F. nucleatum* ATCC 49625 with PHGK resulted in a slight increase in PD-L1 protein levels. However, *F. nucleatum* altered PD-L1 protein expression without affecting its mRNA levels.

Our findings indicate that infection with heat-killed *F. nucleatum* significantly induces PD-L1 protein expression without mRNA level alteration in SCC-25, CL-11, and PHGK cells. Furthermore, different *F. nucleatum* strains exhibit varying abilities to stimulate PD-L1 expression.

The authors wish to thank Prof. Dr. T. Chakraborty, M. Hudel (Institute of Medical Microbiology, Justus Liebig University of Giessen), and Prof. Dr. E. Domann (Institute for Hygiene and Environmental Medicine, Justus Liebig University of Giessen) for their kind support. This work was supported by a scholarship from China Scholarship Council (CSC) under grant CSC No.201908450033 and Forschungsgemeinschaft Dental e.V. (FGD) under grant Nr.02/2022.

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SECTION 3

HEART, LUNG AND BLOOD VESSELS

ABOUT THE SECTION

The blood circulation/vascular system is a unique communication network. One major research topic is concerned with the mechanistic understanding of functional aspects of the vascular endothelium in health and disease. Young scientists are focussing on cardio-pulmonary topics, e.g. related to infectious diseases, to pathogenic mechanisms in the respiratory system and respective new therapies for intervention in e.g. idiopathic pulmonary fibrosis.

Day 2: Thursday, 21st September, 2023 **CHAIRPERSON:** Alejandro Egea Zorilla

16:45 **MODELING CARDIOPATHOLOGIC TET2 CHIP IN ENGINEERED CARDIAC VENTRICULAR TISSUE REVEALS THERAPEUTICS FOR DISEASE RESOLUTION**

Prof. Jaya Krishnan
Institute of Cardiovascular Regeneration
Goethe University Frankfurt

17:30 **A UNIFYING MODEL TO CHARACTERIZE LIPOFIBROBLAST AND MYOFIBROBLAST DIFFERENTIATION**

Esmeralda Vasquez Pacheco

17:45 **CD8+ T CELLS SUBPOPULATION "TC17" INDUCE A METABOLIC ENRICHED MICROENVIRONMENT ENABLING LUNG CANCER PROGRESSION**

Joshua Ayoson

18:00 **EXPLORING THE EARLY-STAGE PATHOMECHANISM OF PULMONARY HYPERTENSION IN COPD: INSIGHTS INTO INITIAL PULMONARY VASCULAR REMODELING**

Vinita Sharma

Keynote 03

Modeling cardiopathologic TET2 CHIP in engineered cardiac ventricular tissue reveals therapeutics for disease resolution

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Generation of synthetic human tissue necessitates mimicry of native tissue cell composition, architecture and molecular fidelity. Here, we detail the establishment of developmentally-staged engineered iPSC-derived human ventricular cardiac tissue (iHCT) comprising of all cell-types of native human cardiac ventricles with its concordant molecular, metabolic, structural and physiologic characteristics. iHCTs exhibit the necessary vascularization and innervation to interrogate, at high resolution, the contribution of loss-of-function (LOF) TET2-mutant myeloid clonal hematopoiesis of indeterminate potential (CHIP) on cardiac inflammation and consequent heart failure. In challenging iHCTs with TET2-depleted myeloid cells, we demonstrate the causal role and sufficiency of myeloid TET2-depletion in driving cardiopathology. Combining this model with synthetic lethality screening, we identify the FDA/EMA-approved compounds for potential resolution of myeloid TET2-LOF driven cardiac inflammation and cardiopathology. Given the high unmet therapeutic need, our data suggests potential benefit in repurposing these identified compounds for treatment of cardiopathologic TET2 CHIP in patients.

T05

CD8+ T cells subpopulation “Tc17” induce a metabolic enriched microenvironment enabling lung cancer progression

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CD8+ T cells are important mediators of antitumor function, specifically recognizing and responding to tumour-expressing antigens. However, they are not as effective as expected. The dysfunctional state of subpopulations of CD8+ T cells in terms of antitumor activity may be associated with different transcriptional and metabolic states different from those of cytotoxic functional effector T cells (Tc1), that has been shown to induce apoptosis of cancer cells by releasing specific granules such as granzyme B and perforin. However, there are other subsets based on the associated cytokines, such as Tc17, Tc9, and Tc22. Our preliminary findings uncovered increased infiltration of Tc17 (IL-17+CD8+) T cells in lung tumor tissues compared to lung healthy tissues. Conditioned medium from Tc17 cell alone and Tc17 co-cultures with tumor cells displayed enhanced tumor cell proliferation and migration in vitro. Notably, the double knockout (DKO) of IL-17 A+F mouse isolated Tc17 cell conditioned medium and co-cultures with tumor cells restores CD8+ T cells tumor-inhibiting effects. Furthermore, metabolomics profiling of conditioned medium from wild type and IL-17 A+F DKO mice Tc17 cells indicate the explicit metabolic profile, most notably the TCA cycle, and metabolites including malate, aspartate, and pyruvate which are downregulated in the conditioned medium of IL-17 A+F DKO Tc17 cells. In addition, Tc17 conditioned medium activates the naive macrophages towards tumor-promoting macrophages by increasing M2 phenotypic markers (Fizz1, Arginase) inducing tumor cell proliferation and migration. In conclusion, we identified Tc17 as a novel pro-tumorigenic CD8+ T-cell subtype in lung cancer, which enables tumor growth. We also uncovered the intrinsic regulatory role of

IL -17 in CD8+ T cell metabolism, a gap in CD8+ T cell antitumor function that promotes lung cancer progression. Next, we will perform in vivo analyzes using transgenic mice to investigate whether the in vitro data can be replicated in preclinical settings. We also plan to investigate the re-education of CD8+ T cell subpopulations in in vivo and ex vivo settings by stimulation with key metabolites to regain anti-tumor potency. At the end of this project, a comprehensive understanding of the position of Tc17 as a subset of the CD8+ T cell subpopulation in the tumor microenvironment will allow us to develop novel immunotherapeutic strategies against the current problems in the treatment of lung cancer.

T06

Exploring the early-stage pathomechanism of pulmonary hypertension in COPD: Insights into initial pulmonary vascular remodeling

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Pulmonary hypertension (PH) is a common complication in chronic obstructive pulmonary disease (COPD), associated with elevated pressure in the pulmonary artery and remodelling of the pulmonary vasculature. Importantly, even mild PH can affect survival of the patients. Interestingly, pulmonary vascular alterations have been observed in smokers who had not developed COPD and therefore have been suggested to contribute to the development of emphysema. Nevertheless, the mechanisms involved are not yet fully understood. Previously, we demonstrated that chronic exposure to cigarette smoke (CS) in a mouse model leads to the development of pulmonary hypertension after three months, while emphysema is detectable only several months later. However, especially the early time points of disease development have not been investigated yet in detail. The attention towards early-stage alteration in COPD-PH can improve the diagnosis of patients as well as their survival rate.

Against this background, the present study aimed to observe the early alterations in the pulmonary vasculature that contribute to the development of PH in COPD. Accordingly, we exposed C57BL/6J mice to CS and room air (RA) for durations of 1 to 4 weeks. Our results indicated that 4 weeks of CS exposure induced the proliferation of pulmonary vascular cells, which might be the initial stage of vascular remodeling in the vessels. However, no differences were observed in the proliferation of pulmonary vascular cells after 1, 2, or 3 weeks of CS exposure when compared to the RA control.

Taken together, our observations indicate that the proliferation of pulmonary vascular cells may commence as early as 4 weeks after CS exposure. Our future aim is to study the specific transcriptomic signature of cells that drive the remodeling process of vessels using single-cell RNA sequencing.

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T07

“A unifying model to characterize lipofibroblast and myofibroblast differentiation”

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The majority of *in vitro* studies of myofibroblast/Lipofibroblast (MYF/LIF) differentiation are based on human primary fibroblast cultures (HPFc) derived from idiopatic pulmonary fibrosis patients. High variability among HPFc, availability and limited time represent major limitations of culture. MYF or LIF differentiation has been shown in HPFc models upon treatment with TGFB1 or Metformin (Met), respectively. WI-38 cells are a human embryonic fibroblast cell line that has been shown to differentiate to MYF when treated with TGFB1,

The aim of this work was to investigate the suitability of the WI-38 cells as a valid alternative to the HPFc to study MYF and LIF differentiation.

Treatment of WI-38 cells with TGFB1 or Met was used to induce MYF and LIF phenotypes, respectively. RNA-seq was performed on WI-38 cells and compared with published datasets of HPFc. Furthermore, organoid, wound healing, cell viability and cell cycle assay were carried out.

Comparison analysis confirmed the validity of the s-38 cells treated with TGFB1 or Met as an in vitro model to study MYF and LIF differentiation, as shown by the overlap in the MYF/LIF signatures from the HPFc and fresh lung tissue versus our model. Furthermore, organoids from Sftpc+ cells and LIF showed a significant increase in size. On another hand, MYF showed a significant decrease in the capability of wound closure while cell viability was not affected. Interestingly cell cycle distribution showed a higher percentage of cells in G2/M phase.

Consequently, the WI-38 cell line was demonstrated in this study to be a good model to study MYF-LIF differentiation in vitro.

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P34

Regenerative reprogramming in cardiac stromal cells post cardiac injury in zebrafish.

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Injury induced fibrosis and scar formation are a leading cause of heart failure and morbidity worldwide. Zebrafish, unlike mammals, have the ability to regenerate heart tissue even after severe myocardial lesions. Notably, epicardial derived cells (EPDCs) and endocardial cells (EndoCs) which are major contributors to fibrotic remodeling in mammals only show transient Matrix secretion and minor differentiation of scar forming myofibroblasts in zebrafish. In a previous study, we identified IL11/Stat3 signaling at the center of the injury response limiting scar formation while controlling the activation of regeneration specific gene programs. Consequently, *il11ra* mutant zebrafish fail to regenerate cardiac tissue post injury but induce a mammalian like scarring response. To better understand the mechanisms involved in regenerative reprogramming and scar formation, we now established scRNAseq data of cardiac stromal cells in response of zebrafish heart injury in wild type and *il11ra* loss of function mutants at different time points post injury. The combination of these datasets allows us to analyze Il-11 dependent gene programs during cardiac regeneration and scar formation. We find that cardiac fibroblast and endothelial cells rapidly undergo dedifferentiation including the transcriptional silencing of mature marker genes like *vsg1*, *ogna*, *mfap5*, *soul5* within the first 24 hours post injury while inducing a regeneration specific gene set including *fn1b, prdx1, rspo3, crlf1a* etc. In contrast, cardiac stromal cells isolated from mutants fail to undergo this response while directly committing towards a fibrogenic gene program. High level of pro-fibrotic markers expression such as *acta2*, *peristotin-b*, *col1a1* and *elastin-b* in mutant confirm this fibrotic event as early as 24hpci, which gets more pronounced over time. Together, our data suggests IL11/Stat3 signaling to act on top of the known hierarchy of scar free regeneration by driving cellular reprogramming, a process also observed in other models of scar free regeneration during blastema formation.

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P35

Tissues distiribution and subcellular Localisation of the proteins of D-family of the ABC transporters (ABCD1-4) in mice.

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ATP-binding cassette (ABC) are a superfamily of membrane proteins involved in the translocation of a wide range of molecules across cellular membranes.Among these, the D-family of ABC transporters plays a crucial role in various physiological and pathological processes .To date, four ABC transporters belonging to subfamily D have been identified: ABCD1-3 and ABCD4 . Understanding the tissue distribution and subcellular localization of D-family proteins is vital for elucidating their functional significance.A study showed that ABCD1-3 are peroxisomal transporters and ABCD4 is localized to Endoplasmic and in different organ system. ABCD1 and ABCD2 are involved in the transport of long and very long chain fatty acids (VLCFA) or their CoA-derivatives into peroxisomes with different substrate specificities, while ABCD3 is involved in the transport of branched chainacetyl coenzyme A (acyl-CoA) into peroxisomes.

In this study, we investigated the expression patterns of D-family ABC transporters (1-4) in various tissues of mice using immunofluorescence staining and confocal microscopy, and we also investigate in depth whether ABCD 4 is also located in the peroxisomal, especially since the three mentioned above are mainly located in the peroxisomal.

Our findings revealed differential expression profiles of ABCD-family transporters across different tissues, indicating their distinct roles in specific physiological contexts.

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P36

Mitochondrial T cell reprogramming in virus-induced COPD exacerbation and emphysema progression

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T cell dysfunction may contribute to increased susceptibility to infections and disease progression in patients with chronic obstructive pulmonary disease (COPD). Viral infections can trigger systemic inflammation and oxidative stress, which could further damage lung tissue and contribute to COPD exacerbations. Mitochondrial reactive oxygen species (mtROS) play an important role in immune function and increased mtROS levels may promote COPD. Cytochrome c oxidase subunit 4 isoform 2 (Cox4i2) regulates ROS production at complex III and thus may affect T cell function during virus-induced exacerbations of COPD.

Mitochondrial respiration and T cell characteristics were analysed in pulmonary or spleen T cells isolated from wild type (WT) and Cox4i2-/- mice after 2 weeks of in vivo cigarette smoke exposure with or without H1N1 influenza (PR8) infection, by respirometry, and flow cytometry, respectively. In vitro, mitochondrial respiration and ROS production were determined after the activation of spleen T cells by respirometry and flow cytometry, respectively.

Upon infection and exposure to smoke, the Cox4i2-/- mice exhibited a significantly lower amount of CD8+ T cells and albumin leakage in the lung compared to WT mice. Moreover, spleen T cells from the Cox4i2-/- infected mice showed a similar decrease in mitochondrial respiration and glycolysis. Accordingly, in vitro experiments revealed that spleen Cox4i2-/- T cells have reduced levels of ROS release, glycolysis and interferon γ release following activation, compared to T cells of WT mice

Cox4i2-/- mice show less accumulation of CD8+ T cells in the virus-infected lungs after two weeks of smoke exposure, and a lack of increase in respiration and ROS levels after in vitro activation. Thus, Cox4i2-dependent ROS release may regulate T cell activation and differentiation during virus-induced COPD exacerbations and may affect the progression of emphysema development

Funded by the German Research Foundation (KFO 309, Project 10)

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P37

Conversion of basal cells to the neuroendocrine trajectory

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Pulmonary neuroendocrine cells (PNECs) are innervated chemosensitive airway cells that contain secretory granules with bioactive compounds, hormones, and neuropeptides. During development, PNECs are the first differentiated cells that appear in the lung, and they are of endodermal origin. Achaete-scute homolog 1 (*ASCL1*) is the fate-determining transcription factor that leads to PNECs differentiation. Other transcription factors participating in this process are *NEUROD1* (Neuronal Differentiation 1) and *NEUROG3* (Neurogenin 3) (He et al., 2022). ScRNA-seq in human embryonic lungs aged 5–14 weeks post conception support that PNECs can be categorized in three populations based on differential expression of transcription factors, secreted peptides, and Notch signalling genes: the progenitor cluster, a cluster that express mainly the transcription factor *ASCL1*, and a cluster that express *NEUROD1* (Sountoulidis et al., 2023). The aim of this study is to generate PNECs from donor airway basal cells in an in vitro system, and unravel the transcriptional factor hierarchy that leads to PNECs heterogeneity in the adult lung. Here, we use the *in vitro* ALI culture to generate PNECs. At differentiation day 30, both RTqPCR and immunostaining showed that basal cells from donor bronchus were successfully differentiated into a polarised epithelium that contains basal, ciliated, goblet, and club cells. Generation of PNECs was confirmed at differentiation day 30 by immunostaining with characteristic PNECs markers. Till now, only PNECs belonging to ASCL1 expressing cluster have been confirmed in the culture. Next, we hypothesized that overexpression of *ASCL1* and *NEUROD1* in basal cells will lead to differentiation of basal cells to the respective cell state. We cloned these two transcription factors into two lentiviral plasmids: the constitutive pWPXL and the inducible pTRIPZ. ASCL1-GFP lentiviral particles were successfully transduced in primary cells, and the GFP-positive cells were present in the ALI culture throughout the 21 days of differentiation. In future experiments, *ASCL1* and *NEUROD1* will be overexpressed in basal cells and, PNECs markers expression will be assessed with RTqPCR and immunostaining. The identification of regulatory hierarchy of PNECs differentiation is of particular interest, since they are likely the cells of origin of Small Cell Lung Cancer (SCLC) and a potential therapeutic target.

P38

The impact of neonatal hyperoxia on iNOS signaling and consequences in adulthood

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Bronchopulmonary dysplasia (BPD) is a chronic lung disease of premature infants caused mainly by oxygen supplementation and/or mechanical ventilation. BPD is characterized by arrested alveolarization. Nowadays BPD is not fully understood and the long-term consequences in adulthood are not well known. Evidence suggests that inducible nitric oxide synthase (iNOS) may play a role in BPD and in the susceptibility to develop chronic lung diseases (CLDs) in adulthood. Therefore, we hypothesized that iNOS might be involved in the development of BPD and CLDs in adulthood.

C57BL/6J newborn mice have been exposed to room air (21% O2) and to hyperoxia (85% O2) from postnatal day (P)1 to P14. Gene expression analysis were assessed in P14 lung homogenates by real-time RT-PCR. Single-cell RNA sequencing (scRNA-seq) data of P14 mouse lung homogenates exposed to 21% O2 and 85% O2 were analyzed to establish the expression of Nos2 in specific cell clusters of the lung[1] .

Gene expression analysis showed an increase of Nos2 transcripts in the lung homogenate from P14 mice exposed to 85% O2 compared to mice exposed to 21% O2. scRNA-seq analysis revealed that Nos2 is expressed in Foxj1+ ciliated cells and in Car4+ aerocytes (aCap). Interesting, hyperoxia reduced the expression of Nos2 in Foxj1+ ciliated cells and increased Nos2 expression in aCap. Conclusion: These data reveal a hitherto unknown role of Nos2 in experimental BPD. Moreover, the impact of hyperoxia on Nos2 transcript levels in Foxj1+ ciliated cells and aCap might play a crucial role in arrested alveolarization with consequences in adulthood. Further studies are necessary to clarify the role of iNOS in Foxj1+ ciliated cells and aCap in vivo.

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P39

Adenylate kinase 4 pro-proliferative effect on pulmonary vascular cells in pulmonary hypertension

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Pulmonary hypertension (PH) is a life-threatening disease, characterised by a mean pulmonary artery pressure (mPAP) ≥ 20 mmHg at rest. Pulmonary vascular remodelling, such as hyper-proliferation of pulmonary arterial smooth muscle cells (PASMC), endothelial cells and fibroblasts, leads to the occlusion of the vessel lumen, resulting in elevated vascular resistance and the development of PH. On a molecular level, hypoxia-inducible factor 1-alpha (HIF1-α)-dependent metabolic shift in pulmonary vascular cells was shown to be one of the mechanisms driving PH development.

Previously, we identified the adenylate kinase 4 (AK4) as a hypoxia/HIF1-α-regulated gene and a possible regulator of the HIF1-α-dependent glycolytic shift in hypoxic PASMC. Furthermore, our findings showed that hypoxia induces AK4 upregulation in pulmonary microvascular endothelial cells (PMVEC). However, the role of AK4 in the pathogenesis of PH and the molecular mechanisms driving AK4-mediated regulation of HIF1-α are still unknown.

Against this background, we investigated kinome profiles upon AK4 loss-of-function using small interfering RNA (siRNA) in long-term hypoxic air (1% O2)-exposed PASMC and PMVEC. The analysis revealed that the activities of several kinases (e.g. Src family kinases (SFK), cyclin-dependent kinases (CDKs) and mitogen-activated protein kinases (MAPKs)) were altered upon AK4 silencing in hypoxic PASMC and PMVEC, suggesting the pro-proliferative role of AK4 in pulmonary vascular cells. Further study will investigate the in vivo function of Ak4-ablation in PASMC and endothelial cells, seeking insight into a novel therapeutic strategy for PH.

In conclusion, we suggest, that the potential AK4-HIF1-α axis promotes the pro-proliferative phenotype of vascular cells and thus contributes to PH development.

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P40

LC3B interactome in alveolar epithelial cells: novel insights of autophagy regulation in lung fibrosis

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Autophagy is a dynamic intracellular catabolic process and a quality control mechanism that maintains cellular homeostasis by degrading long-lived proteins or damaged organelles. It is facilitated by a concerted action of several autophagy-related proteins that convert the cytosolic form of Microtubule-associated protein 1 light chain, subunit 3 (LC3BI) to the membrane-bound LC3BII. Altered autophagy is implicated in several diseases including idiopathic pulmonary fibrosis (IPF) and animal models of lung fibrosis. We previously demonstrated the localization of LC3B to lamellar bodies of the alveolar epithelial type II cells (AECII). We here aimed to identify novel interactors for LC3B in the AECII. We performed shot-gun proteomics for endogenous LC3B in AECII and identified Syndecan-4 (Sdc4), a transmembrane heparin sulphate proteoglycan as a novel LC3B interactor. The LC3B-Sdc4 interaction was confirmed via co-immunoprecipitation. Immunofluorescence indicated cytosolic and membrane-bound staining for Sdc4 in alveolar epithelial cells. Interestingly, Sdc4 accumulated upon autophagy inhibition and it is also elevated in IPF lung homogenates where defective autophagy is well documented. Overall our data open up new avenues for LC3B & autophagy regulation in alveolar epithelial cells in eliciting cellular stress pathways and subsequently in the development of lung fibrosis.

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P41

Impairment of mucociliary clearance after experimental stroke

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Bacterial pneumonia is a major complication in patients with acute stroke, of which the causes are only poorly understood. Here, we addressed in an experimental stroke model whether mucociliary clearance, the primary innate defense mechanism of the respiratory tract, is affected after cerebral ischemia.

Mice (C57BL/6J; Adrb2-/-) were subjected to middle cerebral artery occlusion (MCAO) according to a standard procedure, sham-operated and naïve animals served as controls. Ciliary activity was assessed by measuring particle transport speed (PTS) in explanted tracheas. Cellular composition of the tracheal epithelium in the intercartilage spaces was quantified by confocal imaging of whole mounts, immunolabeled with cell type-specific antibodies. Expression of adrenergic receptors was investigated by RT-qPCR. Distribution of catecholaminergic nerve fibers in the trachea was visualized by immunohistochemistry.

Baseline PTS was significantly reduced by 63% and 39% 1 and 3 days after MCAO, respectively, and returned to control values at d14, while keeping responsiveness to the activating stimuli nicotine and ATP throughout. In parallel, the frequency of ciliated cells was reduced by 48% at day 3 after MCAO, and was back to control at day 14. The decline in ciliated cell frequency was detected mostly in the cranial (sublaryngeal) part of the trachea (intercartilage spaces 1 to 5). We hypothesized an involvement of the sympathetic system in mediating the effect, and first addressed distribution of catecholaminergic nerve fibers and expression of adrenergic receptor in the trachea. Immunolabeling showed a dense subepithelial network of catecholaminergic (tyrosine hydroxylase-positive) nerve fibers, and RT-qPCR analysis revealed Adrb2, encoding the beta2-adrenergic receptor, as the dominantly expressed receptor in the tracheal epithelium. In initial experiments with a cohort of Adrb2-/- mice, no significant decline in baseline PTS was observed after MCAO.

In the mouse MCAO model, cerebral ischemia induces rapid changes in cellular composition and function of the respiratory epithelium resulting in impaired cilia-driven clearance function, which is a potential cause of an increased risk of infection. First data suggest that these changes are mediated, at least in part, by beta2-adrenergic signaling.

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P42

P2X4 and P2X7 receptors in the expression and release of interleukin-1β by mononuclear phagocytes

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In mononuclear phagocytes activation of the ATP-sensitive P2X7 receptor (P2RX7) regulates the biosynthesis of pro-inflammatory cytokines in response to lipopolysaccharide (LPS). ATP, released from injured cells in the context of major surgery or trauma, can either enhance this response or induce tolerance. Further, the maturation and secretion of the inflammasome-dependent cytokine interleukin (IL)-1β can be triggered by activation of the P2RX7. There is some evidence that the P2RX4 contributes to this process. The aim of this project is to characterize the role of the P2RX4 in the expression and the release of IL-1β by mononuclear phagocytes.

Human monocytic THP1-cells, THP-1-derived macrophages (MΦ), human peripheral blood mononuclear cells (PBMCs), and PBMC-derived MΦ were primed with LPS. Thereafter, ATP or the pore-forming reagent nigericin were applied to induce IL-1β release. These experiments were performed in the absence or presence of different inhibitors of the P2RX4 (5-BDBD, PSB-15417) or the P2RX7 (A438079). The mRNA expression of pro-IL-1β (IL1B), and secretion of IL-1β were analyzed by real-time RT-PCR and ELISA, respectively.

The P2RX4 inhibitor 5-BDBD enhanced the LPS-induced mRNA expression of IL1B by human monocytic THP-1 cells and THP-1-derived MΦ. Accordingly, the secretion of IL-1β in response to ATP-independent nigericin was also increased. When the P2RX4 inhibitor PSB-15417 or the P2RX7 inhibitor A438079 were used, only a minimal increase in IL-1β secretion was detected. These data suggest that the P2RX4 down-modulates the LPS-induced biosynthesis of pro-IL-1β. More experiments are, however, warranted to confirm this conclusion and to explain the functional differences between 5-BDBD and PSB-15417. When LPS-primed mononuclear phagocytes were stimulated with ATP, A438079 fully inhibited ATP-dependent IL-1β secretion by monocytic THP-1 cells, THP-1-derived MΦ, and primary human and murine cells. Moreover, 5-BDBD and PSB-15417 dose-dependently blunted the ATP-dependent secretion of IL-1β, suggesting that the P2RX4 contributes to the signaling of ATP in this context.

Our findings that P2RX4 and P2RX7 regulate the expression and release of IL-1β by mononuclear phagocytes might be of eminent clinical relevance, because LPS-driven *IL1B* expression is essential for host defense against pathogens, while ATP-induced IL-1β secretion contributes to trauma-associated hyperinflammation.

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P43

A profibrotic Notch signal derived from alveolar epithelial cells is essential for the development of pulmonary fibrosis

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Epithelial - mesenchymal interactions are critically relevant in the pathogenesis of pulmonary fibrosis. In fact, alveolar epithelial cell dysfunction is considered a major trigger of fibrosis, revealing the importance of unravelling the mechanisms driving the complex crosstalk between the alveolar epithelium and the plethora of cell types in the neighbouring mesenchyme in the fibrotic lung.

We have previously identified a subpopulation of collagen-expressing cells showing Notch3 activity (N3ICD) implicated in the development of lung fibrosis[1]. Our findings demonstrated that Notch3 deficiency attenuates bleomycin-induced pulmonary fibrosis and impedes lung function decline through the regulation of fibroblast survival and myofibroblast differentiation. Here, we identify specific subsets of alveolar epithelial type 2 (AT2) cells expressing the Notch ligands Jag1 and Jag2 that contribute to the development of lung fibrosis.

We demonstrate that the genetic inhibition of one or the other ligand specifically in AT2 cells, results in less tissue damage following bleomycin administration and lower collagen content due to the decrease in αSMA+ myofibroblasts. These observations suggest that Jag-expressing AT2 cells promote fibrosis presumably through the activation of Notch3 in neighbouring fibroblasts inducing their differentiation into myofibroblasts.

Interestingly, a similar mechanism seems to occur in human idiopathic pulmonary fibrosis (IPF) where we previously found an expansion of N3ICD+ aSMA+ myofibroblasts[1]. Now, we provide evidence of Notch3 activation in a fraction of the CTHRC1+ pathologic fibroblasts localized within fibroblast foci[2]. Furthermore, we detect specific Notch ligands in aberrant epithelial cells that line the edge of fibroblast foci[3], suggesting that a Notch signal from epithelial cells to adjacent fibroblasts may drive the development and progression of human pulmonary fibrosis.

Altogether, our findings reveal a novel mechanism involved in lung fibrogenesis that may represent a novel therapeutic strategy for the treatment of IPF.

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P44

Identification of a potential Roquin-miR-34c-3p axis in post-infarct remodeling that cannot be affected by conditioning

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Ischemic pre- and post-conditioning affects infarct sizes but the impact on post-infarct remodeling is less clear. Post-Infarct remodeling is triggered by alterations in protein expression, depending on transcriptional activation generating mRNA that is translated into protein and protein degradation by ubiquitination. Therefore, mRNA stability is important for this process. mRNA stability is regulated by micro-RNAs (miRs). miRs bind to mRNAs at specific binding sites due to sequence homology and thereby induce mRNA degradation. However, the stability of miRs is regulated by proteins vice versa. In this context, roquins have recently been identified. Roquin bind to specific miRs and destabilize miRs. It is known from immune cells that Roquin targets miRs that control the activity of the AKT-Pathway that is mandatory for hypertrophic growth in cardiomyocytes. Alterations in Roquin expression should therefore affect post-infarct remodeling. Here, we investigated the effect of ischemia-reperfusion and conditioning on post-infarct mRNA expression of Roquin.

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P45

Mobilization of the mesenchymal niche in influenza virus induced lung injury

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Viruses such as Influenza-A virus (IAV) can infect the lung and be the main cause for severe pneumonia, respiratory distress and hypoxia. IAV infection can damage both the airway and the alveolar compartment of the lung resulting in acute respiratory distress syndrome (ARDS). In the recent years, multiple studies have been published investigating how alveolar epithelium regenerates upon IAV infection. These studies gave us insights on how the epithelial cells mobilize after infection and how epithelial stem cells aid the repair of the damage inflicted by it. In this context, not much is known about mesenchymal niches and their response towards IAV infection. Taking into account that the lung mesenchyme plays a crucial role in the repair of other lung injuries such as idiopathic pulmonary fibrosis (IPF), we hypothesized that a population of mesenchymal niche is mobilized after IAV infection and plays an important role on the repair of the caused damage. In this study, we used a combination of bulk RNA sequencing, single cell RNA sequencing (scRNA-seq) and a lineage-traced approach to investigate the distribution of the mesenchymal cell populations after IAV infection. We identified a smooth muscle actin positive (ACTA2+) population of fibroblasts that responds to immune stimuli and gains regenerative and possibly anti-viral properties, as well as exert a transient fibrotic response. Further experiments will determine the cellular heterogeneity within the ACTA2+ cell population that arises in response to IAV infection as well as the mode of interaction with inflammatory and epithelial cells.

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P46

Characterization of transcription factor RBPJ in pulmonary hypertension

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Pulmonary hypertension (PH) is a life-threatening disease, characterized by an elevated pulmonary arterial pressure due to pulmonary vascular remodeling. If left untreated, PH progresses to right heart failure and eventually death. Pulmonary vascular remodeling can be caused by chronic alveolar hypoxia triggering an uncontrolled proliferation of pulmonary arterial smooth muscle cells (PASMC) and dysfunction of pulmonary arterial endothelial cells (PAEC). Despite the already characterized involvement of Notch receptors in PH pathogenesis, involvement of downstream signaling is poorly understood. In this regard, we aim to assess the role of recombination signal-sequence-binding protein J (RBPJ; also known as CSL or CBF1) in PH pathogenesis, the central transcription factor in the Notch pathway.

The in vivo function of RBPJ for disease development will be assessed by using tamoxifen-inducible cell-specific gene-knockout mice in a model of chronic hypoxia-induced PH. Mechanistically, loss- and gain-of-function experiments will be performed to unravel the role of RBPJ for vascular cell dysfunction in PH. Our data revealed a hypoxia-dependent regulation of RBPJ in primary human pulmonary vascular cells. Furthermore, hypoxia-induced PASMC and PMEC (pulmonary microvascular endothelial cells) proliferation was reversed to baseline conditions following RBPJ silencing. Preliminary in vivo data in cell-specific Rbpj-knockout mice presume a role of RBPJ in chronic hypoxia-induced PH.

In conclusion, our data provide evidence for a role of RBPJ in chronic hypoxia-induced PH in mice by affecting vascular cell dysfunction.

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P47

Role of AKT isoforms in TGF-β1-induced EMT

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Transforming growth factor-β (TGF-β) as well as phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) signaling are implicated to induce central factors of carcinogenesis in late stages of cancer including tumor invasiveness and metastasis by induction of epithelial-to-mesenchymal transition (EMT). Interactions between pathways induced by TGF-β1 and PI3K/AKT pathway have been described, however exact mechanisms are unclear. Moreover, many studies do not differentiate between the three AKT isoforms (AKT1, AKT2, AKT3) despite their unique functions.

To investigate the role of each AKT isoform in TGF-β1-induced EMT, a shRNA-mediated knockdown of each AKT isoform was performed in human lung adenocarcinoma cell line A549. After stimulation with TGF-β1, EMT-protein markers were analyzed by western blotting in whole cell lysates. In addition, nuclear extracts were prepared for analysis of EMT-transcription factors (Snail1, Snail2, ZEB1, ZEB2, Twist). qRT-PCR analysis was performed to show effects on RNA level. To visualize the location of EMT-protein markers, cells were stained by immunofluorescence.

TGF-β1-stimulated A549 cells underwent EMT, which was observed by an increased amount in mesenchymal marker vimentin and a decreased amount in epithelial marker E-cadherin. Downregulation of AKT2 lead to a significant increase in N-cadherin, and AKT3-knockdown cells showed a significant increase in N-cadherin and vimentin after TGF-β1 stimulation indicating an inhibitory role of AKT2 and AKT3 in EMT. A significant reduction in Snai1 mRNA level after isoform-specific AKT downregulation indicates that all AKT isoforms are relevant for Snai1 transcription.

Future experiments will be needed to verify the exact role of each AKT isoform in TGF-β1-induced EMT. These include a cell migration assay of TGF-β1-stimulated A549 cells with isoform-specific downregulation of AKT to analyze the role of the AKT isoforms in cell migration. Moreover, a 3D cell culture will be used to establish a carcinoma-like situation.

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P48

Role of ubiquinol-cytochrome c reductase hinge protein and NADH dehydrogenase [ubiquinone] iron-sulfur protein 2 in hypoxic pulmonary vasoconstriction and pulmonary hypertension

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Acute hypoxia triggers hypoxic pulmonary vasoconstriction (HPV), which is crucial for systemic oxygenation optimization by adapting pulmonary arterial blood flow to alveolar ventilation. Chronic hypoxia induces pulmonary vascular remodelling and pulmonary hypertension (PH) development. Mitochondria mediate HPV by release of reactive oxygen species (ROS) from complex III (CIII) of mitochondrial respiratory chain (ETC). Chronic hypoxia-induced PH is independent of this mechanism. Ubiquinol-cytochrome c reductase hinge protein (Uqcrh) is a component of CIII. Deletion of Uqcrh impaired CIII function and electron flow through ETC. Mice with conditional deletion of NADH dehydrogenase [ubiquinone] iron-sulfur protein 2 (Ndufs2), an essential subunit of complex I, in glomus cells of carotid body, vanished hypoxic hyperventilation. Rats subjected to airway nebulization of Ndufs2-siRNA, resulting in Ndufs2 silencing, exhibited a blunted rise of mean pulmonary artery pressure (mPAP) during acute hypoxia. Thus, we aimed to investigate the role of Uqcrh and Ndufs2 in HPV and in PH development.

HPV was examined in isolated perfused and ventilated lungs from wild-type (WT) and Uqcrh-/- mice. The depolarisation of cellular membrane potential in mPASMCs during acute hypoxia was studied using patch clamp technique. mRNA and protein of Uqcrh and Ndufs2 were evaluated in lung homogenates from donors and patients with idiopathic pulmonary artery hypertension (IPAH), as well as in mouse pulmonary artery smooth muscle cells (mPASMCs) exposed to 1% O2 for 24, 48, and 72 hours. The proliferation of mPASMCs during chronic hypoxia was investigated by means of BrdU incorporation.

HPV was abolished in Uqcrh-/- mouse lungs in acute hypoxia, while vasoconstriction induced by U46619 was preserved. NDUFS2 downregulation by Ndufs2-siRNA inhibited acute hypoxia-induced cellular membrane depolarisation on mPASMCs. mRNA of UQCRH and NDUFS2 as well as protein of NDUFS2 were not altered in IPAH lung homogenates compared to donor lung homogenates. Moreover, mRNA and protein of Uqcrh and Ndufs2 remained unchanged in WT mPASMCs during chronic hypoxic exposure for 24, 48, and 72 hours. UQCRH and NDUFS2 downregulation by siRNA did not inhibit mPASMCs proliferation induced by chronic hypoxia. Chronic hypoxia-induced HIF-1α stabilisation was not altered in Uqcrh-/- mPASMCs.

Preliminary results suggest Uqcrh and Ndufs2 is essential for acute but not chronic response of pulmonary vasculature to hypoxia.

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P49

FGF10 treatment repairs lung alveolar and vascular structures in mice after established emphysema and pulmonary hypertension

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Chronic obstructive pulmonary disease (COPD) is a serious medical condition, comprising emphysema, chronic bronchitis and often pulmonary hypertension (PH). We recently identified impaired fibroblast growth factor (FGF) 10 signalling as an integral part of cigarette smoke (CS)-induced emphysema and PH development. Mice with impaired FGF10 signalling spontaneously developed emphysema and PH, associated with vascular pruning and remodelling. Moreover, FGF10 treatment reversed established CS-induced emphysema and PH in mice. However, the exact mechanism of FGF10-mediated repair of lung alveolar and vascular structures remains elusive.

In lungs of experimental mice, we investigated the response of epithelial and endothelial cells upon FGF10 treatment after established CS-induced emphysema and PH. Interestingly, following the treatment, an increase in alveolar type 2 and endothelial cell number was observed. This could be an indicator of possible reversion of the disease. Moreover, at protein level we found β-catenin as a potential molecular mechanism downstream of FGF10 treatment. These results from experimental animals were further verified in human COPD precision cut lung slices, which were treated in vitro with recombinant FGF10. Furthermore, using cell-specific knockout mice we aim to investigate the role of FGF10 in lung homeostasis and repair.

The lungs of the experimental animals were then subjected to laser-capture microdissection, and RNA from vessels or the septal wall compartment was collected for further transcriptomic analysis.

The analysis revealed TOX High Mobility Group Box Family Member 2 (TOX2) as a novel potential driver of FGF10-mediated reverse remodelling in pulmonary vasculature. Available single cell sequencing data indeed pinpoints TOX2 expression in pulmonary arterial endothelial cells. This was further verified in lung sections of experimental animals and using *in vitro* endothelial cell models. However, more research is needed to decipher the role of TOX2 in vascular remodelling/reversion.

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P50

lncRNA19 - a major regulator of cellular responses to hypoxia in the lung

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Hypoxia, a condition characterized by insufficient oxygen levels, plays a crucial role in various physiological and pathological events. The transcription factor hypoxia inducible factor alpha (HIFα), a key player in hypoxia, dysregulates multiple signaling pathways inducing a pathological phenotype. Recent studies have shown that several long non-coding RNAs (lncRNAs) are dysregulated under hypoxia. lncRNAs can regulate several signaling pathways by acting as RNA-decoy, miRNA sponge, and splicing modulator. In this project, we aim to delineate the role of lncRNAs in modulating the hypoxia-driven pathways and their contribution to the pathogenesis of chronic lung diseases.

Screening for dysregulated lncRNAs under hypoxia was done by performing RNA-sequencing (RNAseq) from human pulmonary arterial smooth muscle cells (hPASMCs) exposed to 24hrs of hypoxia (hox). The top ten most significantly upregulated ones were further validated at different time points of hox exposure. Functional studies revealed that the knockdown of LNC19 significantly reduced the proliferation and increased the apoptosis of hox-exposed hPASMCs. Analysis of the promoter region of LNC19 revealed that it lacked the hypoxia responsive element (HRE),the binding site of HIFα. However, based on knockdown experiments, HIFα seems to regulate the expression of LNC19. Furthermore, RNAseq showed that LNC19 regulated the translation mechanism in hPASMCs. As such, the contribution of LNC19 in the IRES-dependent translation of hypoxic-responsive genes will be further investigated. Screening of LNC19 in different lung diseases revealed that it was significantly upregulated in COPD. Further studies delineating the role of LNC19 in-vitro and ex-vivo in the hypoxia-driven pathways and its therapeutic potential in COPD will be performed.

P51

Precision cut lung slices: an in vitro model of acute lung injury? Evidence from epithelial changes

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Precision-cut lung slices (PCLS) are valuable tools for studying pathological mechanisms and drug responses in vitro, as they closely resemble the in vivo environment. In our recent research on mouse PCLS with lineage tracing of Sftpc+ cells, we identified PCLS as an acute lung injury model for mature AT2 cells. Building on this, we hypothesise that this model may help to identify lineage-resolved cells involved in the repair process.

PCLS were obtained from human donors and transgenic mouse lungs expressing fluorescent markers. Time-course qPCR analysis revealed dynamic changes in gene expression related to epithelial markers and repair processes. Live imaging of transgenic lung mice within the PCLS showed significant migration of TomSftpc cells near bronchial regions over 8 days. Notably, this migration was absent in the GfpSftpc PCLS, suggesting that lineage-traced cells near the bronchi lose Sftpc expression at later time points.

Pathway analysis of sorted TomSftpc cells after 8 days in culture showed an upregulation of genes associated with high motility and migration compared to control cells from two mice. Both human and mouse PCLS showed significant damage to the alveolar epithelial lineage over time, associated with a repair process that may involve FGF10 signaling. In addition, lineage tracing of Sftpc+ cells in mouse PCLS revealed an unexpected progenitor population that is currently being analysed.

Our study highlights the value of long-term PCLS culture in providing novel insights into lung epithelial biology and repair and sheds light on important cellular dynamics in lung tissue.

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P52

Role of MST1/2 and its therapeutic modulations in Pulmonary arterial hypertension

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Pulmonary arterial hypertension (PAH) is progressive disease with multiple pathologies, characterized by hyper proliferative, prosurvival and anti-apoptotic behavior of pulmonary vascular resident cells. The mechanisms underpinning the pathology of PAH is unclear. Mammalian STE20-like protein kinases 1/2 (MST1/2) are members of HIPPO signaling axis involved in cellular proliferation and survival. Our recent report on the pro-survival/hyper proliferative role of MST1/2 signaling in PAH confirms that MST1/2 can be a potential therapeutic target for the reversal of PAH in vivo. Interestingly, XMU-MP-1 inhibitor is known to selectively inhibit MST1/2 kinases. Thus, this study aims to elucidate the in vitro and in vivo pharmacological modulation of MST1/2 kinases in PAH.

Our in vitro functional study shows that treatment of IPAH-PASMCs with XMU-MP-1 inhibits BrdU incorporation, migration and wound closure. Interestingly at the molecular level, significant downregulation of Hippo signaling molecules associated with re-activation of BMPR2 and SIRT1 are observed upon XMU-MP-1 treatment as well as with MST1/2 knockdown. Furthermore, daily intraperitoneal administration of XMU-MP-1 to monocrotaline – induced PAH rats attenuated mean pulmonary arterial pressure and improved cardiac output. Also, XMU-MP-1 dysregulated hippo signaling molecules and significantly ameliorated pulmonary vascular remodeling and collagen deposition in pulmonary arteries as well as collagen deposition in the RV of XMU-MP-1 treated MCT-PAH rats. From our data gathered so far, pharmacological modulation of MST1/2 kinases could be a potential therapeutic strategy in PAH.

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P53

The role of SMYD2/RUNX2 axis in right ventricular hypertrophy and failure.

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Pulmonary hypertension (PH) is a life-threatening disease, characterized by excessive pulmonary vascular remodeling, leading to elevated pulmonary arterial pressure and right ventricular hypertrophy (RVH). The RV is the major determinant of functional state and prognosis in PH. The RVH triggered by pressure overload is initially compensatory but ultimately leads to RV failure. The mechanisms underpinning the development of PH and RV failure are still unexplored. Recent studies demonstrated that an osteogenic transcription factor Runt-related transcription factor 2 (RUNX2) plays a pathogenic role in cardiac hypertrophy and failure, and also contributes to the development of PH. In this study, we hypothesize the existence of the interplay of the SMYD2 (SET and MYND domain-containing protein 2) and the RUNX2 in RVH. SMYD2 is a methyltransferase, which targets histones, as well as a number of non-histone proteins. The impact of gene silencing and overexpression studies of RUNX2 and SMYD2 over one another was analyzed by using primary rat and human cardiac fibroblasts (CFs) by employing adenovirus-mediated and si-RNA-mediated overexpression and gene silencing approaches, respectively. In loss-of-function studies, RUNX2 silencing reduced CF proliferation and promoted the down-regulation of mRNA expression of osteogenic genes Osteopontin (OPN), Col1α1, Col3α1, Cysteine-rich acidic matrix-associated protein (SPARC), Alkaline phosphatase (ALP) and Cartilage intermediate layer protein (CILP). Notably, SMYD2 knockdown provided opposite effects in CFs. Our experiments on primary rat and human CFs indicated that SMYD2 overexpression inhibits the expression of osteogenic markers like OPN, Col1α1, Col3α1, SPARC, ALP, and CILP. However, our preliminary results suggest that SMYD2 is not directly involved in RUNX2 regulation. Thus, the main conclusion of our study is that SMYD2 although plays a negative role in osteogenesis by suppressing the osteogenic gene program, does not directly impact RUNX2 protein. We further aim to explore the mechanisms by which SMYD2 might contribute to osteogenesis and investigate whether the RUNX2 serves as a specific therapeutic target for RVH and failure.

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P54

Role of repair-supportive mesenchymal cells (RSMCs) in airway epithelialregeneration

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Stem and progenitor cells, as well as the surrounding niche, function in a coordinated and dynamic manner to promote tissue regeneration. The crosstalk between these cells within the lung tissue is often highly complex. Recent efforts have helped simplify such complexity, especially through the use of genetic lineage tracing. We have identified a novel cell population that we termed "repair-supportive mesenchymal cells" (RSMC), which is distinct from airway smooth muscle cells (ASMC) and is critical for regenerating the conducting airway epithelium by secreting fibroblast growth factor 10 (FGF10). We recently showed that RSMCs at least in part derive from glioma-associated oncogene 1 (Gli1)-expressing mesenchymal cells. In this project, we plan to delineate the complexity of the RSMC lineage and assess the contribution of other known mesenchymal subsets to this lineage. Our approach includes lineage tracing, single-cell RNA sequencing (scRNA-seq), and lung organoids. We will also focus on cell-cell communication networks between airway progenitor/stem cells and RSMCs.

The attained data might potentially shape novel treatment strategies to treat airway diseases.

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P55

Identification of the origin of pulmonary hypertension-associated ACTA2^{+} vascular smooth muscle cells

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Pulmonary vascular remodeling underlying pulmonary hypertension (PH) leads to the appearance of alpha-smooth muscle actin (ACTA2+) cells in normally non-muscularized distal pulmonary arterioles and expansion of the tunica media of the proximal arterioles. Although the origin of these newly formed vascular smooth muscle cells (VSMCs) has gained increasing interest in recent years as it contributes to the overall understanding of PH pathogenesis, the precise origin of these PH-associated VSMCs appearing in non-muscularized vessels in the context of pulmonary hypertension induced by chronic hypoxia (HOX) is still unclear.

Therefore, the aim of the present project is to investigate which population of ACTA2+ cells is the origin of the VSMCs proliferating during PH. In this experiment, 8-24 weeks old males and females ACTA2Cre-ERT2; tdTomatoflox mice were exposed to normoxia or hypoxia for 28 days. Hemodynamic measurements and right ventricular hypertrophy assessment conformed PH development upon HOX exposure. We have identified 9 different subclusters of ACTA2+ cells, two of which distinctly respond to hypoxia conditions. Combining the FACS and scRNA-seq data, we found that the pre-existing ACTA2+ cells are not the only source of newly formed ACTA2+ cells, and other cellular origins of those cells are highly probable.

Further studies will focus on the same analysis of the ACTA2+ cell population captured during the hypoxia injury. The turning point of this research will be the use of the Dre-ERT2 mice model to better characterize the ACTA2+ cell population.

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P56

Myocardial Perfusion and PKA Signalling in Right Ventricular Adaptation in Experimental Pulmonary Hypertension

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Right ventricular (RV) function is the most critical determinant for the survival of patients with pulmonary hypertension (PH). The PH will lead to decreased radial and anteroposterior shortening, while relatively preserved longitudinal function. Then the adaptive RV responds to increased pressure overload by augmenting contractility and maintaining ventriculoarterial coupling. However, prolonged pressure overload leads to RV dilation, maladaptive remodelling, ventriculoarterial uncoupling, and functional decline. This process involves various mechanisms such as capillary rarefaction, metabolic shifts, sympathetic hyperactivity, and fibrosis. Though the contribution of microvascular density to RV adaptation to pressure overload remains unclear. Adult Wistar-Kyoto rats were used for the SuHx-induced PH, while Sprague-Dawley rats were utilized for monocrotaline (MCT)-induced PH. Echocardiography, in vivo contrast microscopic computed tomography (µCT), and invasive hemodynamic measurement were performed., accurate, consistent, standardized, and predictable, to measure RV volumes and function. To explain the exact mechanism of the RV remodelling alteration, metabolomics analysis and western blotting on the PKA-CREB pathway in the RV of MCT-induced PH rats' model were conducted.

Data suggested that SuHx rats developed maladaptive cardiac function- an inadequate adaptation of increased afterload- and RV-pulmonary arterial (PA) uncoupling as assessed by the ratio of end-systolic to arterial elastances (Ees/Ea). The MCT rats exhibited adaptive RV function at days 14 and 21 and maladaptive function at day 35, limited adaptation to increased afterload or preload and impaired ability to generate higher SV and CO, associated with RV-PA uncoupling and reduced RV functional reserve. During the dobutamine stress test, the impairment degree of the PKA-CREB signalling pathway, the down steam of beta-adrenergic receptors, increases with the severity of the RV remodelling. Meanwhile, dobutamine fails further to increase right ventricular contractility during the RV maladaptive stage. Furthermore, the absolute RV blood volume significantly increased with RV hypertrophy development in both PH models, but relative blood volume reduction was associated with maladaptive RV function. RV-PA uncoupling in MCT-PH or SuHx PH is characterized by capillary rarefaction in relation to altered PKA-CREB signalling.

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P57

Estimated plasma volume status: Association with congestion, cardiorenal syndrome and prognosis in precapillary pulmonary hypertension

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Volume overload is often associated with clinical deterioration in precapillary pulmonary hypertension (PH). However, thorough assessment of volume overload is complex and therefore not routinely performed. We examined whether estimated plasma volume status (ePVS) is associated with central venous congestion and prognosis in patients with idiopathic pulmonary arterial hypertension (IPAH) or chronic thromboembolic PH (CTEPH).

We included all patients with incident IPAH or CTEPH enrolled in the Giessen PH Registry between January 2010 and January 2021. Plasma volume status was estimated using the Strauss formula.

In total, 381 patients were analyzed. Patients with high ePVS (≥ 4.7 mL/g vs < 4.7 mL/g) at baseline showed significantly increased central venous pressure (CVP; median [Q1, Q3]: 8 [5, 11] mmHg vs 6 [3, 10] mmHg) and pulmonary arterial wedge pressure (10 [8, 15] mmHg vs 8 [6, 12] mmHg), while right ventricular function was not altered. In multivariate stepwise backward Cox regression, ePVS was independently associated with transplant-free survival at baseline and during follow-up (hazard ratio [95% confidence interval]: 1.24 [0.96, 1.60] and 2.33 [1.49, 3.63], respectively). An intra-individual decrease in ePVS was associated with a decrease in CVP and predicted prognosis in univariate Cox regression. Patients with high ePVS without edema had lower transplant-free survival than those with normal ePVS without edema. In addition, high ePVS was associated with cardiorenal syndrome.

In precapillary PH, ePVS is associated with congestion and prognosis. High ePVS without edema may represent an under-recognized subgroup with poor prognosis.

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P58

Role of lipofibroblasts in the development and resolution of pulmonary fibrosis

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Idiopathic pulmonary fibrosis (IPF) is an extremely aggressive and fatal form of interstitial lung disease (ILD) and current antifibrotic therapies only slightly alleviate disease progression. Lung fibrosis is associated with repetitive injury to alveolar epithelial cells (AECs), recruitment of inflammatory cells, profibrotic cytokine signaling and aberrant repair responses that ultimately lead to respiratory failure. The precise cellular source and ultimate fate of myofibroblasts, which are the effector cells in IPF, have been a controversial topic. Fortunately, recent advances in lineage-tracing models have helped address lung myofibroblast precursors and revealed additional contributors to the heterogenous myofibroblast population. Identifying the primary sources of myofibroblasts could be a promising strategy to develop novel anti-fibrotic therapies. It has been previously shown that lipofibroblasts transdifferentiate into myofibroblasts during the development of pulmonary fibrosis. Such myofibroblasts can undergo apoptosis or redifferentiation into lipofibroblast-like cells during fibrosis resolution. Studying this phenomenon offers a promising prospect to identify signalling pathways that lead to accumulation of lipofibroblast-like cells and re-engagement of these cells to promote lung repair. In this work we used state-of-the-art techniques including lineage tracing, single-cell RNA sequencing (scRNA-seq) and alveolar organoids to characterize lipofibroblasts and lipofibroblast-derived cells during various phases of fibrosis formation and resolution. Our preliminary data highlight the heterogeneity of lipofibroblasts and their descendants during fibrosis formation and resolution, and shed light on the impaired alveolar niche activity in the fibrotic lung.

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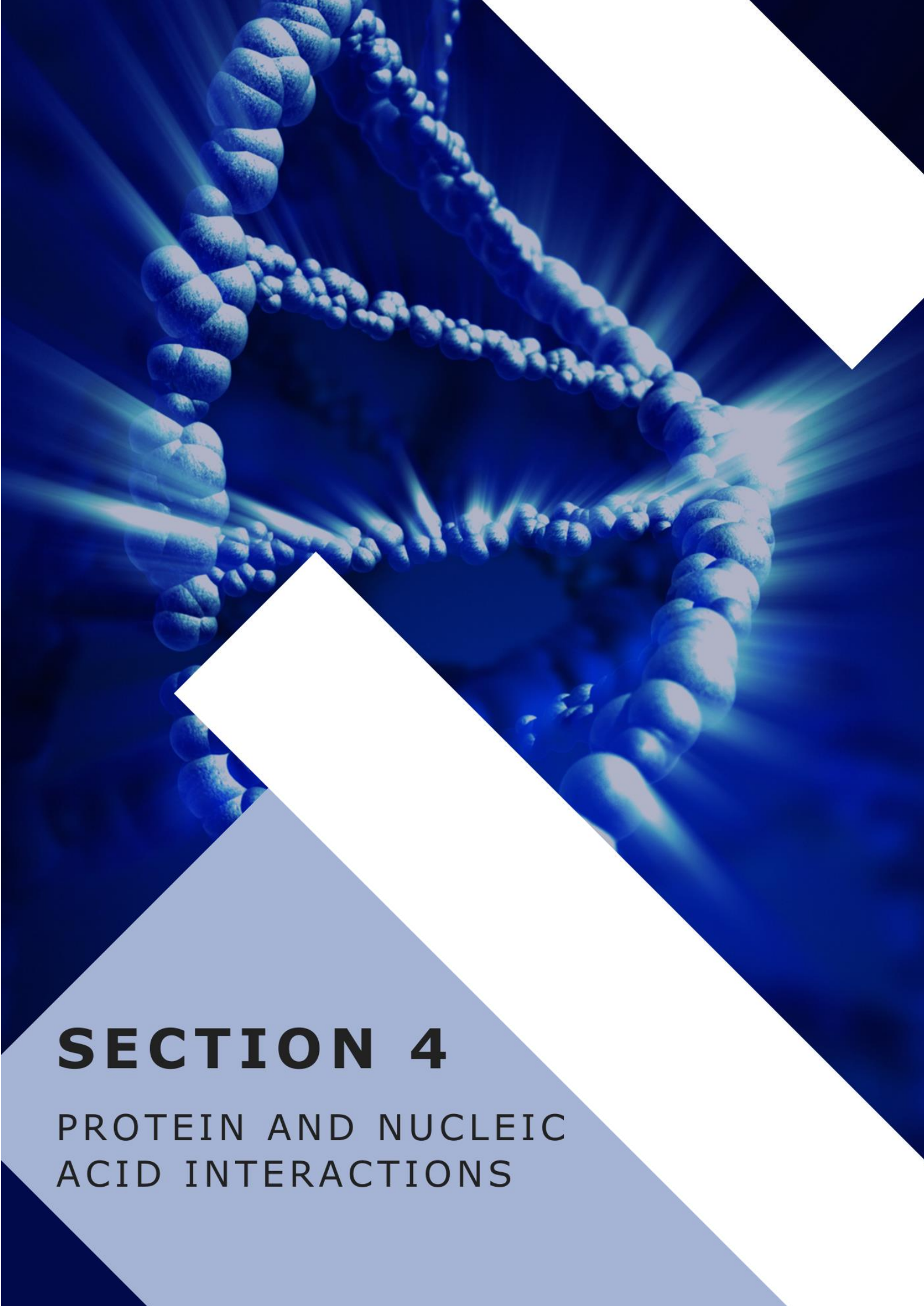

SECTION 4

PROTEIN AND NUCLEIC ACID INTERACTIONS

ABOUT THE SECTION

Research in this section employs molecular genetics and biochemistry to understand the regulation of genes and other cellular processes as well as development in a range of bacterial, viral and plant systems in addition to animals and humans. The interaction of proteins with nucleic acids, protein partners, lipids and other biomolecules is the common interest.

Day 1: Wednesday, 20th September, 2023 **CHAIRPERSON:** Daniel Bauer

10:45

DYNAMIC PROTEIN INTERACTIONS CONTROL TOXIN INJECTION THROUGH THE BACTERIAL TYPE III SECRETION SYSTEM

Dr. Andreas Diepold
Max Planck Institute for Terrestrial Microbiology
Marburg

11:30

ANALYSES OF SYMBIONT EFFECTOR CANDIDATES IN REDIRECTING PHYTOHORMONE SIGNALLING AND ACTIVATING BENEFICIAL EFFECTS IN *ARABIDOPSIS*

Laura Rehneke

Keynote 04

Dynamic protein interactions control toxin injection through the bacterial type III secretion system

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Bacteria manipulate eukaryotic target cells by injecting proteins through type III secretion systems (T3SS), large molecular machines. In contrast to the well-defined, engine-like structures that come to mind, many biological molecular machines are dynamic and adaptive.

To understand these dynamic protein interactions, which are difficult to study, we used proximity labeling, a new method especially suited for these transient interactions. Combining these studies with live cell microscopy, single particle tracking, proteomics and molecular dynamics modelling, we found that large parts of the T3SS, including the membrane-spanning core apparatus, exchange subunits, and that bacteria modulate these dynamics in order to optimize the assembly and function of the T3SS, and ultimately the outcome of the interaction with eukaryotic cells. In addition to these new findings, I will show how we can exploit protein dynamics by using optogenetics to control the activity of the T3SS, a new approach for the biotechnological and medical application of bacterial molecular machines.

T08

Analyses of symbiont effector candidates in redirecting phytohormone signalling and activating beneficial effects in Arabidopsis

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Fitness of land plants, particularly under stressful environments, relies on symbiotic interaction since the colonization of land. Beneficial symbioses are based on molecular communication between all partners, for example by secretion of small proteins (effectors) by microbes, which are transferred to the host to reprogram host signalling. Effectors were first described to supress plant immunity and therefore used to investigate plant immune systems. Later effectors were found to alter a vast number of signalling pathways in plants. We set out to explore their function in mediation of beneficial effects by the mutualistic fungus *Serendipita indica* (*Si*). The beneficial colonisation by *Si* enhances biotic and abiotic stress resistance as well as growth and yield in a large number of hosts including different crops with high agricultural value. Increased stress resistance and yield of crops is especially important under currently drastically increasing climate stress and regarding the growing world population.

We identified 106 *Si* effector candidates (SIECs) by RNA sequencing at different plant colonisation phases and revealed their complex interactions with *Arabidopsis thaliana* proteins in a yeast-two-hybrid screen. To understand differences and similarities between symbiont and pathogen effectors we created a comparative interactome (as entirety of effector-plant protein interactions). Functional cell-based analyses showed specific SIEC modulation of hormone and stress signalling networks which were subsequently confirmed in whole plants. SIEC functions in plants included increased plant growth, altered responses to hormones, and enhanced resistance to abiotic stresses and pathogen infection. Additionally, based on the functional and interactome data we discovered previously unknown *Arabidopsis* protein functions in hormone signalling indicating SIECs as tools for uncovering molecular mechanisms of driving host-symbiont interactions. Effector analyses thus represents a powerful device for investigating signalling pathways and stress resistance of plants [1].

References:

[1] Osborne*, Rehneke*, Lehmann* et al., (2023), Symbiont-host interactome mapping reveals effector-targeted modulation of hormone networks and activation of growth promotion, Nature Communications, https://doi.org/10.1038/s41467-023-39885-5

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P59

Functional characterization of the yeast RNA-binding proteins Sub2 and Tho1 and their interaction *in vivo*

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To ensure that nascent mRNA is processed and exported through the nuclear pore complex in a correct manner, a plethora of RNA-binding proteins (RBPs) is necessary. These RBPs are loaded already co-transcriptionally onto the nascent mRNA and form together a messenger ribonucleoprotein particle (mRNP). One key factor in packaging the newly synthesized mRNA into an exportable mRNP is the TREX complex, which consists of the heteropentameric THO complex (composed of Tho2, Thp2, Mft1, Tex1 and Hpr1), the two SR-like proteins Gbp2 and Hrb1, the DEcD-box helicase Sub2 and the mRNA export adaptor Yra1. Sub2 is highly conserved, ATP-dependent and has many functions in gene expression such as splicing, 3' processing, mRNA export and R-loop prevention. To determine the function of Sub2's RNA binding activity the amino acids binding to RNA *in vivo* were identified by UV crosslinking followed by mass spectrometric analyses. These potential RNA-binding sites were mutated, and the phenotypes of the resulting mutants were analyzed. Interestingly, the double mutant sub2-K202E-Y203E mislocalizes to the cytoplasm. In addition, the mRNP components Cbp80 (part of the nuclear mRNA cap-binding complex) and Tho1 (an RNA binding protein with unknown function) are also mislocalized. Besides finding the mechanism how the mutation leads to the mislocalization of the proteins, it is our goal to elucidate the role of Tho1 and its interaction with Sub2. Since the deletion of *THO1* does not cause a phenotype, we examine the functions of Tho1 in a *Δhpr1* strain. Tho1 can suppress the growth defect of *Δhpr1* cells at 37°C by overexpression. Like Sub2 it also reduces R-loop accumulation occurring under the lack of Hpr1. We compare these effects of overexpression of yeast Tho1 with its orthologues of *C. thermophilum*, *A. thaliana* (Mos11), *H. sapiens* (SARNP) and *S. pombe* (Mlo1) to investigate if the functions are conserved.

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P60

The phosphorylation status of the shuttle protein FHY1 regulates the nuclear translocation of the photoreceptor phytochrome A (phyA) in *Arabidopsis thaliana*

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Plants use photoreceptors to detect light intensity, duration and wavelength to adapt their developmental processes to their environment. Phytochrome A (phyA) is a red (R)- and far red (FR)-light sensing photoreceptor which is involved in important processes like germination, de-etiolation, shade avoidance and flowering. PhyA (Pr) is converted by R-light absorption into the physiologically active Pfr form, which is transported into the nucleus where it regulates gene expression. For the nuclear translocation of phyA the shuttle protein FHY1 (FR elongated hypocotyl 1) and its homolog FHL (FHY1-like) are required. Previous work showed that conserved serines which might be potential phosphorylation sites, are present in FHY1 and FHL. Additionally, it could be shown that FHY1 mutants in which conserved serines have been replaced by phosphomimic aspartate, are unable to transport phyA (Pfr) into the nucleus (Helizon et al., 2018). This findings suggesting that the phyA transport is regulated by the phosphorylation state of FHY1. In this work, FHY1 is purified from seedlings undergoing de-etiolation, to study the *in vivo* secondary modifications of FHY1 by ESI-MS. In addition, FHY1 and phyA are heterologously expressed and purified to study their interaction *in vitro*. The interaction of FHY1 with PP2AA2, which is a subunit of the protein phosphatase 2A, could be shown by bimolecular fluorescence complementation assays and yeast two- and three-hybrid assays (Helizon 2015, PhD thesis). These findings and the observation of a delayed phyA transport in *pp2aa2*-mutant protoplasts support the idea that the phyA transport is regulated through the change of the phosphorylation status of FHY1. The results presented will demonstrate the role and regulation of FHY1 on phyA nuclear translocation.

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P61

Unravelling the physical and functional interaction between the mRNP components Sub2 and Tho1 in nuclear mRNA export

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The conserved TREX complex functions in mRNA processing and packaging of the mRNA into an mRNP for nuclear export in eukaryotes. In *Saccharomyces cerevisiae*, TREX comprises the pentameric THO complex (Hpr1, Tho2, Mft1, Thp2 and Tex1), Gbp2, Hrb1, Yra1 and the DEAD-Box helicase Sub2. The THO complex also recruits the nuclear hnRNP Tho1 to transcribed genes. Overexpression of Sub2 or Tho1 suppresses phenotypes caused by deletion of THO components. While the human Tho1 ortholog CIP29 (SARNP) interacts with and stimulates DDX39 (UAP56), the ortholog of Sub2, our previous study uncovered an RNA annealing activity of Tho1, thus inhibiting nucleic acid unwinding by Sub2.

Here, we mapped the annealing activity of Tho1 to its non-conserved, basic C-terminal extension, and its interaction with Sub2 to the conserved C-terminal domain (CTD). Additionally, we discovered that the CTD not only enhances Sub2's RNA binding activity but also stimulates its helicase activity. Importantly, the CTD alone suppresses the thermosensitivity phenotype of Δ*hpr1* cells. Our findings provide insights into the interaction between Sub2 and Tho1 and their role in nuclear mRNP export.

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P62

Establishing the homosporous fern Ceratopteris richardii as a genetic model organism

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The homosporous fern Ceratopteris richardii (C-fern) belongs to the pteridophytes, the sister lineage to seed plants, making it an interesting organism for evolutionary studies. While important genetic resources, like a reference genome and a stable transformation protocol using the gene gun are already established, we aim to increase this genetic repertoire to establish C-Fern as a novel genetically tractable model organism.

We will present our progress on (1) generating a transcriptome atlas including sporophytic and gametophytic tissues essential to track transcriptional activity during C-Fern development, (2) Establishment of a CRIPSR/Cas12a system in C-Fern for multiplex gene editing. Further, we (3) provide recent developments for stable genetic transformation, by systematically testing transformation methods established in other species.

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P63

Stamen galore — occurrence and regulation of stamen-producing ring meristems

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Floral meristems are dynamic systems stem cell systems that generate floral organ primordia and often terminate while the gynoecium forms. However, many species develop additional ring meristems during their floral morphogenesis, that generate stamen primordia continuously while the floral meristem has already ceased its activity. This results in 'polystemous' flowers (include more than twice the number of stamens than petals or sepals), and decouples the timing of stamen initiation from carpel initiation. Polystemy has originated several times independently and provides a widespread mechanism to modify the male/female reproductive ratio. But nothing is known so far about the differential regulation of these two types of stem cells in very close vicinity. We use the polystmous species *Eschscholzia californica* (California poppy) a member of the Ranunculales, the sister lineage to the core eudicots, as model system to understand the molecular mechanism regulating polystemy. We use histological analyses and Laser Microdissection followed by RNAseq and differential gene expression analysis to identify the gene regulatory network orchestrating polystemy. Here, we provide a systematic overview of the occurrence of polystemy in dicots and summarize our first results from the differential gene expression analysis.

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P64

Early response to antibiotic exposure is characterized by induction of small genes in *Sinorhizobium meliloti*

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Bacteria respond to antibiotic exposure at all levels of gene regulation including the RNA level. However, information about very early steps in response to antibiotics exposure is scarce, although these steps could be decisive for bacterial adaptation. Soil and plant-interacting bacteria often have high intrinsic resistance or tolerance to antibiotics. Therefore, bacteria such as *Sinorhizobium meliloti* could be useful for discovery of novel adaptation mechanisms. In this study, we aimed to identify new genes involved in adaptation of *S. meliloti* to tetracycline (Tc) exposure. RNA-seq was used to identify genes with changed RNA levels 10 min after addition of subinhibitory Tc amount to exponential cultures. To validate Tc-induced genes and to study induction mechanisms, RT-qPCR, Northern blot, reporter fusions (analyzed by fluorescence measurements and Western blot) and suitable mutants were used. The RNA-seq revealed 273 genes with changed mRNA levels (at least two-fold change; max. p-value of 0.01). Among the 24 genes with highest induction, 13 encode proteins < 100 aa, 11 of them with unknown functions. Induction was observed already 3 min after Tc addition and mRNA levels increased in time. Increasing Tc concentrations also resulted in gradually increased mRNA levels. The highest induction was detected for the conserved gene of unknown function SM2011_RS13250 (83 aa). Its Tc-induction was detected at the level of mRNA and protein, and depends on the translation of a small upstream ORF (uORF, 29 aa), which was identified recently by ribosome profiling [1]. In short, the early transcriptome response of *S. meliloti* to Tc exposure is characterized by induction of small genes with unknown function. Posttranscriptional mechanisms are part of this response. Their importance for bacterial adaptation to antibiotic exposure is under investigation.

Reference: Hadjeras L, Heiniger B, Maaß S, Scheuer R, Gelhausen R, Azarderakhsh S, Barth-Weber S, Backofen R, Becher D, Ahrens CH, Sharma CM, Evguenieva-Hackenberg E. Unraveling the small proteome of the plant symbiont Sinorhizobium meliloti by ribosome profiling and proteogenomics, microLife, Volume 4, 2023, uqad012, https://doi.org/10.1093/femsml/uqad012

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P65

Comparative transcriptome analysis to identify deeply conserved carpel development gene networks

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The carpel is the most important autapomorphy of angiosperms. It protects the embryo from herbivory, ensures outcrossing success and is of high agronomically importance. After fertilization, the carpel tissue will develop into a seed-dispersal unit, the fruit. Most of our plant knowledge is based on the core eudicot *Arabidopsis thaliana*, member of the Brassicaceae family and is limited for studying carpel evolution. To broaden our perspective, *Eschscholzia californica* (California poppy), has been proven to be a suitable model organism for evolutionary developmental studies of the female reproductive tissue in plants. Here, I present preliminary analyses using high-resolution LMD-RNAseq data by Kimmo Kivivirta, Ph.D. (GGL alumni) of carpel development for following species: *Arabidopsis thaliana* (Brassicaceae), *Solanum lycopersicum* (Solanaceae), *Oryza sativa* (Poaceae, Monocot) and *Eschscholzia californica* (Ranunculaceae). We aim to identify the minimal set of highly conserved candidate genes required for carpel development and elucidate the dynamics of the underlying gene-regulatory-network (GRN) in *E. californica* using state-of-the art bioinformatic tools, such as OrthoFinder, Clust, DeSeq2 and GSEA. Furthermore, we aim to provide an interactome of those identified carpel regulators by the investigation of protein-protein interactions (PPI) using Y2H/BiFC screens to shed some light on puzzling questions, whether highly connective hub genes are more conserved than less connective genes or do evolutionary novel proteins, that has arisen after duplication events, preferentially bind to highly connective, existing nodes.

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P66

Molecular and mechanistic basis for spatiotemporal organization of polar flagella

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Motility through flagella is one of the most important modes of locomotion within bacterial populations. Bacteria can exhibit different flagellation arrangements: Flagella can either be located all over the cell body (peritrichous) or at one or both cell poles, where they occur as a single flagellum (monopolar) or a flagella bundle (lophotrichous). Our model organism *Shewanella putrefaciens* is a peritrichously flagellated bacterium with two distinct flagellar systems: the polar and lateral system. With regard to the polar flagella system, it is already known that the SRP-GTPase FlhF assumes the role of a polar marker and is responsible for the arrangement of one polar flagella. Together with the polar landmark protein HubP, FlhF initiates the recruitment of further components like FliG and FliF, building blocks of the flagellar basal body. Our current model suggests that only a few proteins are required to initiate flagella formation and that their recruitment to the cell pole in *S. putrefaciens* follows a specific order, starting with the polar landmark protein HubP, followed by FlhF, FliG and FliF. To investigate whether our model is complete or whether additional proteins are involved in this process, we analysed the arrangement in a heterologous host, *Escherichia coli*, that is unable to produce its own flagellar proteins. To this end, fluorescently labeled versions of HubP, FlhF, FliG and FliF were introduced into *E. coli* cells using a plasmid-based system and their localization behavior was analysed via fluorescence microscopy. In addition, the stability of the fluorescent fusions was verified by western blot experiments and the swimming behavior analysed using a motile *E. coli* MG1655 strain. We showed that in *E. coli* HubP localizes to both cell poles and the cell division plane, where it can also recruit FlhF. The presence of FlhF also allows FliG to localize to both poles as well as to the cell division plane. Furthermore, we demonstrated that FlhF plays an important role in the stability of FliG and FliF. Taken together, the results confirm our proposed model of flagellar protein recruitment.

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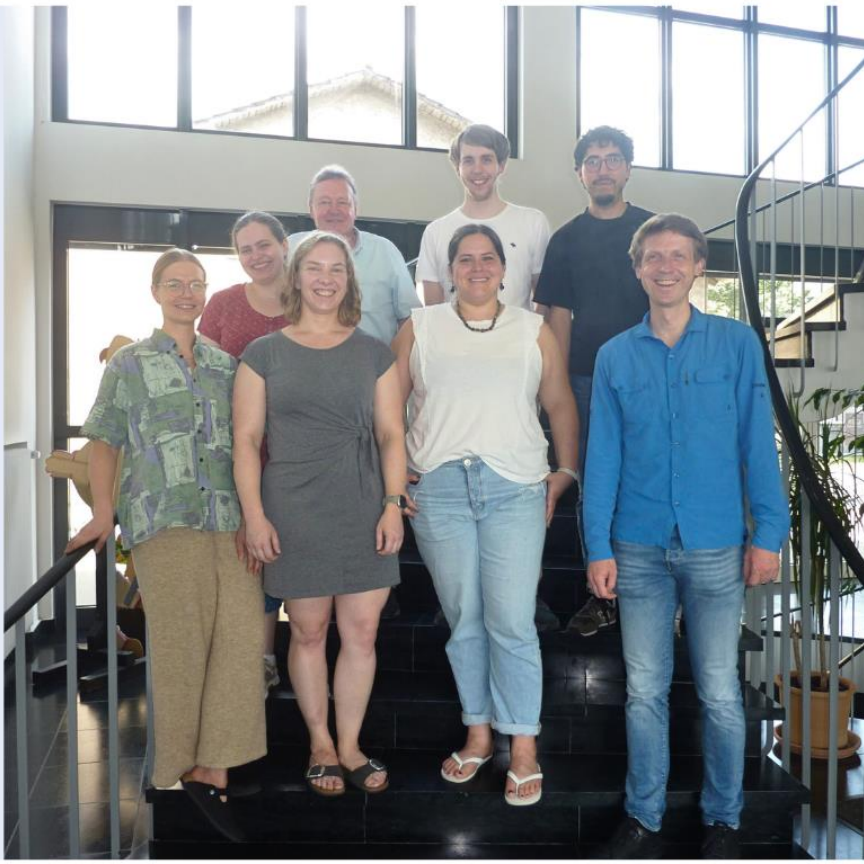
SECTION 5

NEUROSCIENCES

ABOUT THE SECTION

Research in this section includes a wide range of topics from molecular mechanisms of neural information processing to neurosurgery and neuropsychology. Overarching research areas are cerebrovascular disease, neuroimmunology and cognitive neurosciences, specifically pain, the interaction between the immune system and the brain as well as the neuronal basis of cognition and emotion.



Day 2: Thursday, 21st September, 2023

CHAIRPERSON: Rafael Castillo Negrete

10:45

METABOLOMICS IN BIOMEDICAL RESEARCH - THE INFLUENCE OF ω-3-FATTY ACIDS ON THE BRAIN METABOLOME DURING SYSTEMIC INFLAMMATION

Prof. Dr. Regina Verena Taudte
Philipps University
Marburg

11:30

PSYCHOLOGICAL AND INFLAMMATORY STRESS IN MICE: INVESTIGATING HOW NEUTROPENIA EFFECTS HEART RATE VARIABILITY AS A READOUT PARAMETER

Leona Bähr

11:45

GLUTAREDOXIN 5 AS A NOVEL TARGET FOR β-CELL SURVIVAL AND REGENERATION

Meng Meng Zhou

Keynote 05

Metabolomics in biomedical research – The influence of ω-3 fatty acids on the brain metabolome during systemic inflammation

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Omics fields such as genomics, transcriptomics, proteomics and metabolomics have revolutionized the study of biological systems. The corresponding holistic analyses of genes, RNA, proteins or metabolites enable a comprehensive understanding of biological systems and help to elucidate pathological mechanisms. Among the omics family, genomics, transcriptomics and proteomics are well-established, while metabolomics is a relatively young, however, rapidly growing technology. In metabolomics, small molecules that reflect the phenotype of a biological system as well as the interaction of an organism with its environment are studied.

Since the metabolome is highly dynamic, it changes considerably depending on different factors, for instance altered functions or concentrations of enzymes and associated product concentrations.

Lipid mediators play an essential role in systemic inflammations. Among these, ω-3 fatty acids have demonstrated to exert anti-inflammatory actions and moved into the focus of various research areas. In many animals, a ω-3 fatty acids desaturase enzyme converting ω-6 to ω-3 fatty acids is missing. Genetically altered FAT-1 mice however express this enzyme. Thus, these mice have increased ω-3 fatty acid concentrations and provide a valuable model to study the functions of these important mediators.

To further resolve the function of ω-3 fatty acids during systemic inflammation in the brain, we performed untargeted metabolomics via liquid chromatography high-resolution mass spectrometry on tissue samples taken from hypothalamus areas of FAT-1 and WT mice treated with LPS and controls. After normalization and comprehensive data processing, we identified significant metabolite differences between brain samples taken from FAT-1 and WT mice associated with higher or lower ω-3 fatty acid concentrations. In conclusion, untargeted metabolomics assisted in the characterization of brain tissue from FAT-1 and WT and allowed for a better understanding of the function of ω-3 fatty acids during systemic inflammation.

T09

Psychological and inflammatory stress in mice: investigating how neutropenia effects heart rate variability as a readout parameter

Bähr L., Hernandez J., Marks D., Roth J., Feldotto M., Rummel C.
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Stress affects the brain by immune cell trafficking, cytokines and the autonomous nervous system via neuronal signalling. Here, we investigated how psychological or mild systemic inflammatory stress alter heart rate variability (HRV) in relation to changes in inflammatory markers in the brain and periphery in mice. We used HRV as a quantitative marker for autonomous nervous system activity providing some insights in terms of the sympathetic and parasympathetic tone.

Mice were injected intraperitoneal with either normal rabbit serum (NRS) as a control or an anti-polymorphnuclear neutrophil serum (anti-PMN) to induce neutropenia. After 24 h, mice underwent a novel environment stress test (NES) for psychological stimulation or not as a control. For the inflammatory stimulus, we utilized a low dose of LPS (50 µg / kg) and PBS (phosphate buffered saline) as a control. Using the telemetric implant ETA-F10, we continuously recorded an ECG, body core temperature and locomotive activity throughout the experiment. Animals were perfused 24 h after the LPS/PBS injection or 4 h after the NES test and brain, pituitary, liver, spleen, retroperitoneal/inguinal fat, and blood were collected. This tissue and blood are going to be investigated regarding specific pro- and anti-inflammatory markers using bioassays, PCR, immunohistochemistry and Western Blot.

Our established experimental setup and the preliminary data from telemetric recordings show an effect of LPS that could be exacerbated by PMN, which is visible in a decrease of HRV in different parameters. New insights into the role of neutrophils regarding the autonomous regulation are currently assessed.

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P67

3-O-Sulfated Heparan Sulfate induces tau abnormal phosphorylation by Tgfb1

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Aging is the main risk factor for Alzheimer's disease (AD) characterized by a cognitive decline related to a synaptic dysfunction (Chen et al., 2019). In AD brains, tau abnormal phosphorylation coexists with heparan sulfates (HS), suggesting that HS could lead to the formation of neurofibrillary tangles (NFT) (Goedert et al., 1996). In vitro, tau aggregation is not possible in the absence of heparin, a highly sulfated HS-carrying 3-O sulfation (3S-HS). 3S-HS is the product of HS 3-O sulfotransferase enzymes (HS3ST) (Sepulveda-Diaz et al., 2015). In AD, Heparan Sulfate-Glucosamine 3-Sulfotransferase 2 (HS3ST2) expression is higher in tauopathy-vulnerable brain regions, such as the hippocampus and cortex (Huynh et al., 2019). Previously, it was shown in a cellular model that HS3ST2 induces cell-autonomous aggregation of tau (Huynh et al., 2022). However, the mechanism leading to an increased expression of HS3ST2, and therefore an increase in 3S-HS synthesis are unknown. Additionally, it is not known the effect of reducing the levels of HS3ST2 in tau oligomerization and aggregation in neurons.

In the brains of aged individuals, transforming growth factor TGFB1 is upregulated, modulating gene expression (Doyle et al., 2010). SMAD4 contains genome-wide recognition motifs that are present in the Hs3st2 promoter, suggesting a possible regulation (Martin-Malpartida et al., 2017). We showed that in human AD brains, there is an increase in the phosphorylation of Smad2/3. Interestingly, we found that Hs3st2 is under the regulation of Tgfb1, and blocking pSmad2/3 reduces its levels. In contrast, Hs3st2 expression increased with increasing pSmad2/3. The importance of co-regulation of Tgfb1 coregulation with Hs3st2 was assessed by blocking the expression of Hs3st2, leading to a decrease in tau oligomerization and aggregation. Finally, we found that the reduction of 3S-HS by Hs3st2 enhances synaptic size and presynaptic/postsynaptic connectivity.

Overall, we depicted the regulation of Hs3st2, and demonstrated its involvement in synaptic connectivity and tau aggregation, confirming the importance of 3S-HS in the physiopathology of AD.

P68

Deciphering neuroprotective effects of n-3 polyunsaturated fatty acids and related impacts of adipokines using organotypic hippocampal slice and primary neuroglial cell cultures

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Alzheimer's disease (AD) is characterised by amyloid-beta (Aβ) and tau neuropathology and progressive, debilitating cognitive decline. Early life stress leads to brain inflammation, changes in lipid mediators in AD and exacerbates amyloid pathology. Recent studies have shown that specialized pro-resolving lipid mediators (SPMs, derivatives of omega-3 polyunsaturated fatty acids, n-3) and metabolic sensors such as adipokines affect AD disease states.

The aim of the present study is to decipher neuroprotective properties of n-3 and their metabolites (SPMs) and how this effect is modulated by adipokines like C1q/TNF-Related Protein 3 (CTRP3). For this purpose, we use organotypic hippocampal slice cultures (slices) from male and female neonatal mice exposed to early life stress and incubate them with Aβ oligomer-enriched stocks. Here, we present the project methodology of our established slice cultures and first results after excitotoxic or inflammatory stimulation with either NMDA and glutamate or bacterial lipopolysaccharide (LPS). Moreover, we show that LPS increases SPMs release and CTRP3 inhibited LPS-induced IL-6 secretion in primary neuroglial cell cultures. We are further testing if stimulation of the slices in the way described above leads to changes in neuronal survival using propidium iodide, detection of inflammatory signalling (RT-PCR) and measures of cytokines/PUFAs/SPMs. To reveal the functional role of resolvin E1 (RevE1), we will also derive slices from Fat1-mice that lack resolvin receptors (ChemR23-deficiency), to model n-3 enrichment with and without functional RevE1-signalling. As a positive pharmacological control, slices from wild-type mice will be treated with either 18-HEPE, the direct precursor for RevE1, or RevE1 directly.

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P69

Measuring raptor prey-capture behaviour during natural flight

Junker K.[1,2], Sawinski J.[1], Voit K.-M.[1], Stahr P.[1], Cheekoti A.[1], Klioutchnikov A.[1], Matheson E. J.[1], Wallace D. J.[1], Kerr J. N. D.[1]

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Birds of prey are thought to use a wide range of behavioural strategies when capturing airborne prey during flight. While many studies have tracked bird behaviour during flight using a combination of ground-located camera-tracking approaches and computational modelling, a method for capturing flight behaviour with high resolution has not been developed. Here, we developed a light-weight tether-free camera and tracking system that could be mounted on the bird's head and body enabling high-resolution imaging and tracking of bird behaviour, the airborne target, and the surrounding environment. To test whether this system was capable of accurately tracking these features during prey capture, we trained a Harris's Hawk (*Parabuteo unicinctus*), a bird of prey, to capture airborne target objects during various flight tasks ranging from predictive airborne prey capture to prey location.

In order to maintain the behaviour as uninhibited as possible we used a combination of falconry techniques, training 'dummy' equipment with increasing dimensions and weights and developed a gradual behavioural training routine. Here we outline the training strategy used to maintain flight behaviour while capturing airborne prey. We show that we could maintain the bird's behaviour close to control conditions with the full tether-free tracking system in place. In addition, we were able to design the equipment to remain stable and unintrusive during high-speed object pursuit, allowing the birds to be trained to tolerate the equipment. Together this allowed generation of high-resolution measurements of head and body rotations of the Harris's Hawk during airborne prey-capture in a natural outdoor arena. We plan to use this high-resolution approach to generate a detailed understanding of the behavioural strategies that hawks undertake during airborne prey-capture.

P70

Intestinal organoids as a model for intestinal inflammation

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Intestinal organoids are 3D-structures proliferating from stem cells into distinct cell types, which are found in the intestinal epithelium. In comparison to other cell cultures, they have the advantage of being long-lived and having the same properties as the original tissue, e.g. secretory and epithelial barrier functions. Intestinal organoids can be used to study drug effects or different diseases, e.g. inflammatory bowel disease (IBD). IBD patients have elevated levels of proinflammatory cytokines which mediate symptoms like pain, abdominal cramps and diarrhea caused by an impaired epithelial barrier and altered transport mechanisms. Until now, research in this field has mainly been carried out in animal models, resulting in pain and suffering for the animals. This could be reduced by an organoid model.

In this project we want to establish a mice organoid model to study inflammatory processes in the gut. To achieve this, we incubate the organoids with proinflammatory cytokines (INF-γ, TNF-α and IL-1β; separately and mixed). When incubated with TNF-α (100 ng/ml) the organoids showed an increased secretion into the lumen (presumably due to an increased chloride secretion), a flattening of the epithelium and an increased number of apoptotic cells in the lumen. The organoids showed no changes after incubation with IL-1β (20 ng/ml).

We are using the Ca2+-imaging technique to see how the different cytokines affect the intracellular Ca2+ response evoked by carbachol (a stable acetylcholine derivative that induces a chloride secretion) in organoids. Therefore, we are conducting the measurements using a buffer with Ca2+ and without Ca2+ to distinguish between Ca2+ release from intracellular stores or a Ca2+ influx via Ca2+ channels in the cell membrane. The maximal increase of the cytosolic Ca2+ concentration in organoids was seen after 72 h of TNF-α incubation, whereas it was significantly diminished after 72 h of IL-1β treatment compared to the control group. These changes were only seen when measuring the organoids in Ca2+ containing buffer suggesting that changes in Ca2+ influx from the extracellular space rather than changes in the release from intracellular stores are responsible for the effects of the cytokines.

This will be further investigated by immunohistochemical staining and qPCR measurements of different epithelial barrier proteins or transporters (e.g. chloride channels) to see how proinflammatory cytokines modulate these.

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P71

An assessment of bacterial induction of mouse Neutrophil extracellular traps *in vitro* using LPS or Group B *Streptococcus*.

Hernandez J.[1], **Stoll J. S.**[1], Meurer M.[2], Harden L.[3], von Köckritz-Blickwede M.[2], Rummel C.[1]

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Neutrophil extracellular traps (NETs) are formed by activated neutrophil granulocytes (NG) and serve an important role in pathogen clearance. However, previous studies have found that NET overexpression can induce pro-inflammatory cytokine profiles that contribute to the pathogenesis of certain disease states as well as observed neurotoxic properties of the proteases and decondensated DNA released from NGs. Group B *Streptococcus* (GBS) is a leading cause of neonatal sepsis and meningitis worldwide and can have severe outcomes including neurodevelopmental impairments and even death. We aimed to investigate the inflammatory response of NGs to gram-negative (LPS) and gram-positive (GBS) bacterial mimetics to induce NETs *in vitro* and assess their potential to contribute to neuronal damage. Bone marrow derived NGs were isolated from the femur and tibia of aged mice (19-22 weeks). To induce NETosis NGs were then incubated for 3h with CXCL1 (50ng/ml) in combination with LPS (1, 10, or 20mg/ml) or GBS (MOI 100). After 3h the cells were fixed and analyzed by immunofluorescence for the NET markers myeloperoxidase and DNA/Histone1. Supernatants were collected and analyzed by bioassays for TNFa and IL-6. Preliminary results indicate that treatment with GBS alone but not LPS may significantly increase NET formation. Treatments performed in combination with CXCL1 did not increase NET formation in either GBS or LPS groups. Levels of TNFa and IL-6 appeared unaffected by treatment with GBS or LPS but further analysis is required. Together, our ongoing experiments suggest a possible role for NETs in contributing to the inflammatory response during GBS infection.

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SECTION 6

REPRODUCTION IN MAN AND ANIMALS

ABOUT THE SECTION

Male reproduction is the main topic of this research section which deals specifically with male factor infertility due to impaired spermatogenesis, thereby investigating the possible role of testicular structures and pathological mechanisms in infertility. Further research is carried out in the areas of female fertility and hormonal regulation in livestock.

Day 1: Wednesday, 20th September, 2023

CHAIRPERSON: Christine Rager

13:30

RESEARCH ON MALE REPRODUCTIVE HEALTH AND TOXICOLOGY

Prof. Dr. Jorma Toppari
University of Turku
Finland

14:15

THE ROLE OF RESIDENT MACROPHAGES IN THE IMMUNE RESPONSE TO BACTERIAL INFECTION OF THE MURINE EPIDIDYMIS

Dingding Ai

14:30

HYDRODECHLORINATION OF MINE WATER-SPECIFIC POLYCHLORINATED BIPHENYLS (PCBS) USING PALLADIUM CATALYSTS

Katrin Wiltschka

Keynote 06

Research on male reproductive health and toxicology

Toppari J.

University of Turku, Institute of Biomedicine, Turku, Finland

Epidemiological and experimental studies complement each other in research of male reproductive health. Most epidemiological studies are cross-sectional, retrospective case-control studies which may have a large sample size. These studies often suffer of inconsistent diagnostic criteria and recollection bias. Data may also be patchy, because it has not been collected systematically. Prospective cohort studies avoid many of these limitations but usually suffer of small sample size and high expenses. In birth cohort studies, the length of the study may also span the whole scientific career of the researcher yielding the most interesting results only at the end. In the era of epigenetic studies, transgenerational studies necessitate observations of multiple generations further lengthening the study time. Experimental reproductive toxicology includes both animal studies and *in vitro* and *ex vivo* studies. Protocols of reproductive toxicity testing are regulated by EU, United States Environmental Protection agency and inter-governmental organizations like OECD. Chemical industry is responsible for toxicity testing for approval of a new chemical to enter the market. Academic research goes beyond the regulatory testing and invents new methods that may not be ever applied in regulatory testing. We have used e.g., culture of rat or mouse seminiferous tubules at defined stages of the seminiferous epithelial cycle for testing genotoxicity of drugs and other chemicals. Staged tubules are isolated with transillumination-assisted microdissection, which allows strictly targeted analysis of specific events in spermatogenesis. Another important approach is the use of fetal testis cultures, either after *in vivo* or *in vitro* exposure. With the rapid technological advancement new possibilities open every day.

T10

The role of resident macrophages in the immune response to bacterial infection of the murine epididymis

Ai D.[1,2], Kreyling L.[1,2], Bhushan S.[1,2], Meinhardt A.[1,2], Pleuger C.[1,2]

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The epididymis faces contrasting immunological challenges, i.e. tolerance towards sperm and defense against pathogens. Accordingly, the epididymal regions (initial segment (IS), caput, corpus, cauda) show striking differences in their immune responses during bacterial infection. We have previously shown that resident immune cells are strategically positioned along the epididymal duct to shape distinct immunological environments. CX3CR1+ macrophages constitute the major resident leukocytes population and show region-specific specializations in phenotype and morphology. Based on their canonical function (tissue homeostasis and regulation of immune responses), we hypothesize that CX3CR1+ macrophages play a crucial role in epididymal immune regulation.

This study aims at depleting tissue-resident CX3CR1+ macrophages using Cx3cr1CreERT2Rosa26iDTR mice to analyze the phenotypical consequences under physiological and pathological conditions. Uropathogenic E. coli (UPEC) were injected into the vas deferens and disease progression of acute bacterial epididymitis was compared between macrophage-depleted and wild type (WT) mice. The extent of macrophage depletion, leukocytic infiltration, and concomitant histopathology were assessed by flow cytometry, immunofluorescence staining and Masson-Goldner-Trichrome staining.

Depletion of CX3CR1+ macrophages resulted in complete loss of intraepithelial macrophages, which recovered within 30 days. Interstitial macrophages remained unaffected. The loss of macrophages resulted in focal epithelial damage and extravasation of spermatozoa under physiological condition. After UPEC infection, immune responses and histological changes varied amongst regions in WT and macrophage-depleted mice. The distal region of WT mice showed leukocyte infiltrations, epithelial damage and fibrosis, which was not evident in the IS and caput. Contrastingly, macrophage-depleted mice showed inflammation also in the proximal regions in line with an earlier onset of inflammation and tissue damage in macrophage-depleted mice compared to WT.

We successfully established a mouse model for targeted depletion of intraepithelial CX3CR1^{+} macrophages within the epididymis allowing a comprehensive assessment of their function in immune homeostasis and defense within future studies. Our data strongly suggest a pivotal role of CX3CR1^{+} macrophages in maintaining epithelial integrity required for propel sperm maturation and in controlling the magnitude of immune response.

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P72

Aspects of Caspase-8 biology in human ovarian cancer studied in an orthotopic mouse model

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Ovarian cancer (OC) is the deadliest gynecological malignancy in women due to late-stage diagnosis in an already metastatic phase. Therapy regimens relying on surgery and chemotherapy show good first response but frequent relapses. Novel therapies are eagerly needed addressing cancer cell biology with a focus on programmed cell death, with particular consideration of Caspase-8 as a key protein in the apoptotic cascade. We aimed to analyze the impact of Caspase-8 depletion in an *in-vivo* orthotopic mouse model of human OC as well as in different chemo-therapy regimes with Carboplatin (CPT) and Paclitaxel (PTX) in cell-based assays. As a model for OC, we used the human high-grade serous ovarian cancer cell line OVCAR8, in which Caspase-8 was knocked out using CRISPR/CAS9 technique.

Wildtype (WT) and Caspase-8 knockout (KO) OVCAR8 cells, stably expressing luciferase reporter gene, were injected into the bursa ovarica of n=4 NMRI nu/nu mice (n=2 WT, n=2 KO). Starting one week after surgery and until the mice were sacrificed, the luciferin signal, reflecting tumor cell proliferation and spreading, was measured weekly using an in vivo imaging system (IVIS). WT and KO-cells were treated with CPT and PTX at different concentrations and time points. A significant rise in luciferin signal was observed in the Caspase-8 KO group compared with the WT group, suggesting a substantial increase in tumor growth after Caspase-8 depletion.

In addition, In-vitro proliferation assays using CellTiter-Blue showed that Caspase 8-KO cells are more resistant to CPT, leading to a three times higher survival of Capase-8 KO cells compared to their WT counterparts. Furthermore, while the treatment of OVCAR-8 WT cells by CPT demonstrated a substantial and dose-dependent rise in the rate of both early and late apoptosis, Caspase-8 KO cells showed less sensitivity, indicating a strong resistance against CPT. Moreover, preliminary transcriptome data analysis showed a dysregulated expression of several genes involved in biological processes, exceeding the apoptotic functions of Caspase-8, where we now focused on Connexin-43. The involvement of Connexin-43 will be analyzed in-depth given its importance for OC growth and metastasis.

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P73

Characterization of immune cell populations in the male reproductive tract of the mouse

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The male reproductive tract is composed of the testis, epididymis, vas deferens, the accessory sex glands (seminal vesicles, prostate, ampullary, bulbourethral, and preputial glands), urethra, and penis. Microorganisms such as bacteria, viruses and parasites can infect the male reproductive organs, leading to inflammation and impaired fertility. Immune cells, particularly macrophages, play a crucial role in defending against invading microorganisms and maintaining tissue homeostasis. This study aims to characterize immune cell subsets with an emphasis on macrophages and their respective functions throughout the male reproductive tract in adult mice under normal and inflammatory conditions.

Flow cytometry analysis of single-cell suspensions derived from healthy male reproductive organs of 9-10 week-old C57BL/6J mice revealed that macrophages (F4/80+CD11b+ cells) constituted approximately 70% (testis), 55% (epididymis), 25% (vas deferens), 40% (seminal vesicles), 70% (prostate), 55% (urethra), and 10% (penis) of all CD45+ leukocytes. Notably, CD11bhiF4/80+ macrophages were present in all male reproductive organs except the seminal vesicles, while CD11blowF4/80+ macrophages were observed in the seminal vesicles, prostate and urethra. Granulocytes (Gr1+CD11b+ cells) comprise the predominant populations in the lower GU tract (penis ~30%). Macrophage heterogeneity was further elucidated based on the expression of CD206 and MHCII, identifying four distinct macrophage populations. Moreover, the impact of macrophage depletion was investigated by administering the colony-stimulating factor 1 receptor inhibitor (PLX5622) to mice in their food for 10 days. Initial analyses revealed collagen deposition in the testis and cauda region of the epididymis as well as a reduction in collagen staining in the urethra. These findings indicate the presence of distinct macrophage populations in the male reproductive tract, which likely contribute to organ-specific homeostatic functions.

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P74

The influence of cell culture on the expression and function of natriuretic peptide receptors in smooth muscle cells

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The second messenger cGMP mediates smooth muscle cell (SMC) relaxation and is generated by the natriuretic peptide (NP) receptors NPR-1 and -2 upon binding of their respective ligands ANP and BNP, or CNP. Besides cardiovascular signalling, the NP/cGMP pathway contributes to the regulation of SMC function in organs of the male reproductive tract.

We aimed to clarify the role of the NPs in vasculature and in interstitial SMCs of male reproductive organs comparing primary cells and the corresponding intact tissue, thus assessing potential effects of the isolation and culturing of these cells.

A cGMP-specific ELISA confirmed a predominance of NPR-1 in intact vascular tissue (aorta tunica media) at the level of receptor function. In cultured aortic SMCs however, more cGMP was produced in response to CNP suggesting a predominance of NPR-2.

Real-time quantitative PCR was selected to quantify the NP receptor expression in cultured aortic SMCs and the corresponding tissue of origin. This required the careful selection of a stably expressed reference gene, which was challenging since vascular SMCs seem to change drastically once isolated and in culture. First, the expression of beta-actin and Gapdh, two routinely used housekeeping genes, was investigated. Significant differences between their Ct values showed that neither can be used for such direct expression comparison of cultured cells and intact tissue.

We selected 15 different reference genes and tested them regarding their gene expression stability. Only the small ribonuclear protein U2 showed no significant differences between the Ct values of cultured aortic SMCs and the corresponding intact media and can therefore be used to compare the relative expression levels of NP receptors in SMCs in culture to those within their physiological tissue environment.

The expression of NPR-1 remained at a similar level, but the expression of NPR-2 was elevated by ~5 in cultured cells, thus exceeding NPR-1 significantly. This matched the reduced cGMP production in response to ANP compared to CNP in cultured SMCs.

Culturing alters cGMP/NP related gene expression and corresponding NP receptor activity in vascular SMCs. Possible explanations could be a general expression switch in all isolated SMCs or the predominance of a specific SMC subpopulation. Hence, total tissue or *in vivo* setups may be needed to reflect the NP related signalling more realistically. This remains to be investigated for SMCs in male reproductive tissue.

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SECTION 7

BIORESOURCES, BIOINFORMATICS AND BIOTECHNOLOGY

ABOUT THE SECTION

This section is focused on the application of advanced technologies in biochemistry, molecular and cell biology, biotechnology, drug discovery (e.g. novel insect-derived-compounds), agricultural and medical relevant pest control and preclinical research. Emphasis is put on the investigation of molecular responses of insects, microbes, plants, animals, and humans to environmental and pharmacological stressors.

Day 2: Thursday, 21st September, 2023

CHAIRPERSON: Daniel Kreft

13:00

MAKING MALES: FROM MEDFLY BIOLOGY TO MEDFLY CONTROL

Dr. Angela Meccariello
Imperal College London
United Kingdom

13:45

UNDERSTANDING THE IMPACT OF STRUCTURAL VARIATIONS ON GENE EXPRESSION USING PANGENOME GRAPHS

Gözde Yildiz

Keynote 07

Making males: from medfly biology to medfly control

Meccariello A.

Imperial College London, Faculty of Natural Sciences, Department of Life Sciences, South Kensington Campus, London, United Kingdom

Ceratitis capitata, commonly known as the medfly, is a Tephritid pest known best for its global distribution, vast economic impact and flexible host selection. The current golden standard for Tephritid population control is the sterile insect technique (SIT), which relies on mass release of males sterilized using radiation. Its biggest caveats are the reduced fitness of released males and the high rearing due to the need for sex sorting at adulthood. These limit the efficiency of SIT-mediated population suppression, as well as the frequency of its implementation globally. Genetic control offers a species-specific approach to tackle this problem by delivering novel traits into target populations via the release of modified insects. We developed a highly efficient CRISPR/Cas9 toolkit for use in the medfly to establish a fully-fledged precision-guided sterile insect technique (pgSIT). PgSIT, which utilizes CRIPR/Cas9 technology to simultaneously target genes vital for female development and male fertility generating a sterile male-only progeny,was recently developed as a replacement for the traditional, cost-ineffective SIT. To date, pgSIT has already been successful in *Drosophila* and *Aedes aegypti*, and in this project we showcase the feasibility of this approach in the medfly. Establishing a sex conversion-based pgSIT system in *C. capitata*, as proof of principle, will act as a steppingstone to control other Tephritid pests through the implementation of the same technology.

T11

Understanding the impact of structural variations on gene expression using pangenome graphs

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Structural variations (SVs) are large genomic alterations including deletions, insertions, and duplications of DNA segments (>50 bp). Due to their size, they can have a greater impact on traits than single nucleotide polymorphisms (SNPs) and smaller InDels, leading to changes in gene expression, protein function, and cellular behavior.

Some of the SVs have been shown to affect candidate genes associated with important agronomic traits. However, this extensive variation can lead to biased variant detection and gene expression quantification. Therefore, pangenome graphs, which capture species-wide genomic variation in a single data structure, provide an excellent framework for expression quantitative trait loci (eQTL) analyses, facilitating the association between SVs and gene expression. The primary aim of this project is to understand the impact of SVs on gene expression and transcriptional regulation using pangenome graphs to overcome the single reference bias in oilseed rape.

To construct the pangenome graphs, we used 57 long-read datasets from Oxford Nanopore sequencing (>5x coverage) and ~100 short-read datasets from Illumina (>20x coverage). Using Oxford Nanopore data for 57 winter oilseed rape genotypes we identified almost 100,000 structural variants including 46,428 deletions and 48,396 insertions, which were used for pangenome graph construction together with 1,975,171 SNPs identified from short-read Illumina data. Pangenome graphs were used for SV genotyping from short-read whole genome sequencing and gene expression quantification for 100 winter oilseed rape lines. Our analysis revealed that a substantial proportion of variants found in long reads could not be genotyped from short reads even using pangenome graph reference. We also found systematic differences between linear reference- and graph-based gene expression quantification. EQTL analysis revealed a subset of SVs associated with gene expression.

In this study, we constructed pangenome graphs as a framework for eQTL analysis in oilseed rape. We characterized the association between SVs and gene expression elucidating the impact of larger genomic variants on gene regulation.

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P75

sORFDB – A database for sORFs, small proteins, and small protein groups in bacteria

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Small proteins with fewer than 100 and, in particular, fewer than 50 amino acids are still largely unexplored. Nonetheless, they represent an important part of the genetic repertoire of bacteria that often remains neglected. In recent years, the development of ribosome profiling protocols has led to an increasing number of newly detected small proteins. Despite this, they are frequently overlooked in automated annotation, and often no functional descriptions can be assigned due to a lack of homologs of high confidence in public databases. To understand and overcome these limitations, the current state of small proteins in existing databases was evaluated and a new database specifically for small proteins and their possible function was created. To this end, small proteins were extracted from annotated bacterial genomes in the GenBank database. Subsequently, they were quality-filtered, compared, and complemented with proteins from the UniProt and smProt databases to ensure a specific identification and characterisation of small proteins. Potential small protein groups were created using bidirectional BLAST hits followed by Markov clustering. Analysis of small proteins in public databases showed that their number is still limited due to historical and technical limitations and that functional descriptions were often missing despite the presence of possible homologs. As expected, there was a strong taxonomic bias demonstrating an overrepresentation of clinically relevant bacteria. Despite these limitations, a comprehensive and feature-rich database has been built, which provides a search for small open reading frames (sORFs) and small proteins of high quality, Hidden Markov Models for small protein groups as well as information on taxonomic distribution and other physicochemical features. In conclusion, the novel small protein database sORFDB is the first specialized database that improves the searchability of small proteins and their functions in bacteria, thereby easing their future consistent identification and annotation.

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P76

Black soldier fly larvae breeding on by-products of the food industry and considering frass as fertilizer

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As a valuable source of proteins and lipids, the larvae of the black soldier fly (Hermetia illucens) are already used in poultry feed and aquaculture. In order to economically produce protein and lipid-rich biomass, the use of by-products from local industrial partners as feed for the black soldier fly larvae (BSFL) represents a promising solution.

This approach offers a cost-effective alternative to intricate composting or thermal recycling methods of by-products. In addition, the residues from BSFL rearing (frass) which consist of leftover feed and insect excrement, can serve as a valuable fertilizer. The composition of frass is highly influenced by the metabolic processes of the larvae and the feed composition.

In this study, we used potato peelings, potato pulp, apple pomace, and rapeseed press cake as feed for BSFL rearing and analysed the frass after larval development. Our findings underscore the remarkable variation in the chemical composition of frass depending on the feed used, particularly with regard to nitrogen, phosphorus, and potassium (NPK) content. Furthermore, we see that complying with the EU-mandated requirement of subjecting frass to a hygienisation process at 70°C not only reduces potentially harmful coliform bacteria, including E. coli, but also reduces the total number of viable microbes. However, this process also appears to affect the presence of bacteria crucial for improving soil quality, such as those involved in nitrogen fixation, phytohormone production, and organic acid synthesis, potentially compromising the effectiveness of frass as a fertilizer. Additionally, in our study, both cultivation-dependent and cultivation-independent (amplicon sequencing) methods were employed to assess the microbial composition and to identify strains capable to promote plant growth.

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P77

Overcoming the challenges in *Hermetia illucens* protein production fed with agricultural by-products

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The black soldier flies (*Hermetia illucens*) are widely popular as an insect used for waste management. In addition, their protein and fat can be used as feed ingredients for various animals including in aquaculture. The ability of these insects in devouring organic substances is well documented in the literature. The challenges in utilizing the by-products using this biological tool are rarely discussed. The challenges include identifying the local by-products (availability, current use, cost), processing of by-products (energy requirement, time requirement, shelf-life), preparation of an ideal diet mixture (particle size, substrate moisture), inoculation of larvae (age and size of the larvae, larval initial health). The other challenge is the lack of knowledge on the application part of the small-scale experiments. Several feed trials were conducted to optimize the black soldier fly larval growth. The diet mixture consisted of spent mushroom substrates (SMS), a byproduct of mushroom production, and chicken feed, a feed currently used in European black soldier fly production firms. With the feed trials, optimum larval density and optimum substrate were identified. Based on the feed trials up to 50% replacement of chicken feed using SMS was possible without compensating larval growth to a greater extent. An upscale of 10- and 25-times were executed to study the variations in larval performance, if any. For the 100 g DM feed mix, a larval density of 250 and substrate moisture of 75% gives the best larval yield in terms of survival and biomass. The chemical analysis results showed that as the proportion of SMS in the feed mix increases the crude protein content in the larvae also increases. The chosen diet composition, larval density, and substrate moisture were upscaled to check the validity of the small-scale experiments.

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P78

Genetic differentiation of Huacaya and Suri alpacas (*Vicugna pacos*) by their fleece type

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Alpacas (*Vicugna pacos*) are usually classified as two so-called breeds, Huacaya and Suri. However, the main distinguishing trait separating them is their fleece type. Huacaya fleece is crimped with blunt-tipped locks that resemble that of Coriedale sheep. Suris, on the other hand, have silky and cork-screwed type of fleece that resemble Angora goats. These uniquely different fleece types enable fibre from alpacas to be manufactured for specific textile products. The specific variants influencing the difference in Huacaya and Suri fleece types have not been identified so far, however, several models of inheritance have been proposed. The first genetic model is a single, autosomal dominant gene for Suri fleece type inheritance with an additional genetic mechanism that supresses the Suri phenotype in some animals. Another genetic model proposes two linked loci, both of which must be homozygous for recessive alleles to produce the Huacaya phenotype. The aim of this study was to analyse genomic regions of interest for the different fleece types. 91 alpacas (54 Huacayas, 37 Suris) from Germany (n = 29) and Switzerland (n = 62) were genotyped with the recently developed 76k alpaca SNP array. Re-mapping of the markers to the VicPac 3.1 reference assembly resulted in 59k chromosome-localized markers that were utilized for downstream analysis. After quality control filtering, 49,866 SNPs were retained for a genome-wide association study between Huacaya and Suri with GEMMA 0.98.5 and assessment of the population structure. Genome-wide significantly associated markers were observed in the scaffold region of chromosome 16 (NW_021964192.1), which contains a cluster of keratin genes. Haplotype analysis of SNP genotypes encompassing all 225 markers mapping to the scaffold NW_021964192.1 was conducted using fastPHASE software. Principal component analysis showed that the two fleece-type cohorts overlapped rather than formed two distinct clusters. Haplotype variance analysis revealed a haplotype predominantly found in Suri alpacas which supports the dominant inheritance of the Suri type. The findings from this study challenge the accuracy of classifying alpacas as two separate breeds and propose to classify them according to their fleece type instead.

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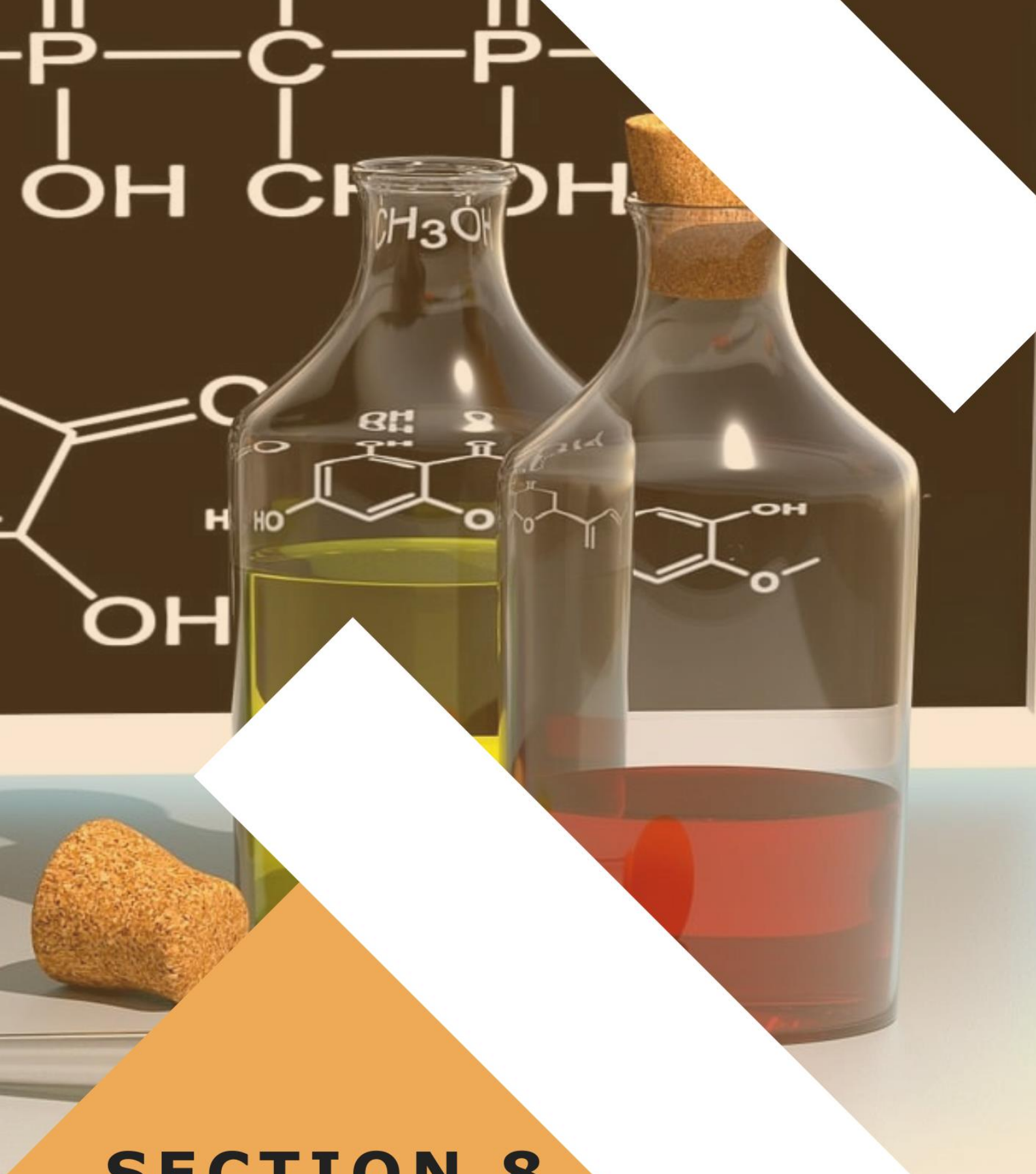
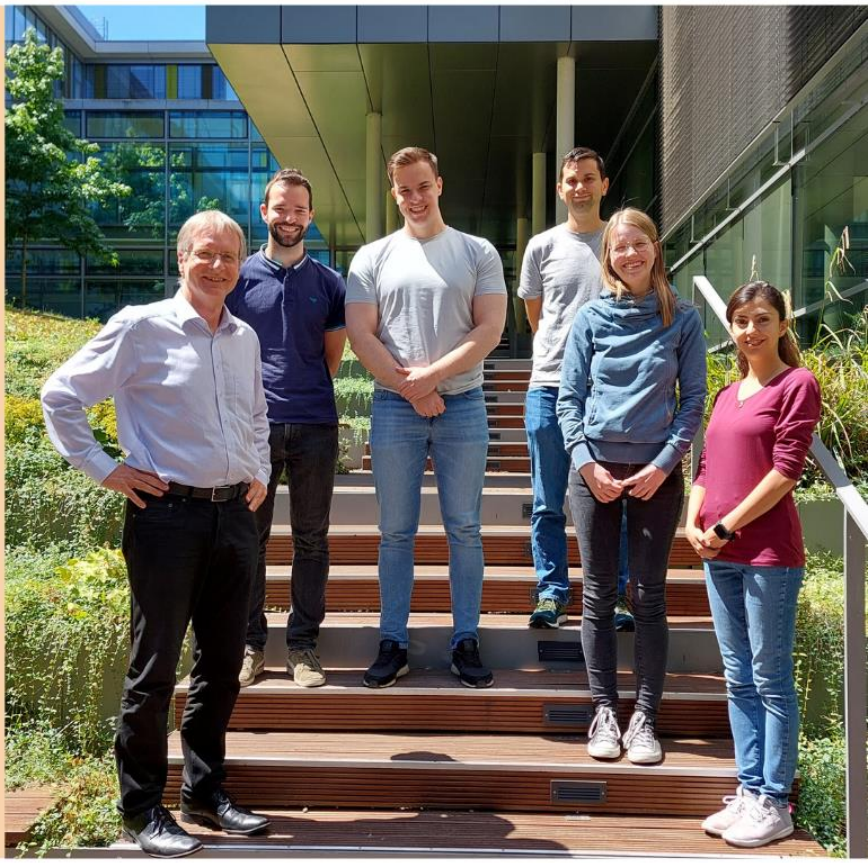


SECTION 8

CHEMICAL DESIGN AND ANALYSIS OF MOLECULAR SYSTEMS

ABOUT THE SECTION

The synthesis and analytics of biologically relevant molecules constitute the main topics of this section. Furthermore, the state-of-the-art analytics of biologically relevant molecules lies at the heart of this section, especially with respect to their distribution in natural systems as well as to their behavior at biological and artificial interfaces. In addition, man-made materials, involving nanostructures and implants, are synthesized and analyzed and their applications are studied.



Day 1: Wednesday, 20th September, 2023

CHAIRPERSON: Christine Rager

13:30

RESEARCH ON MALE REPRODUCTIVE HEALTH AND TOXICOLOGY

Prof. Dr. Jorma Toppari
University of Turku
Finland

14:15

THE ROLE OF RESIDENT MACROPHAGES IN THE IMMUNE RESPONSE TO BACTERIAL INFECTION OF THE MURINE EPIDIDYMIS

Dingding Ai

14:30

HYDRODECHLORINATION OF MINE WATER-SPECIFIC POLYCHLORINATED BIPHENYLS (PCBS) USING PALLADIUM CATALYSTS

Katrin Wiltschka

T12

Hydrodechlorination of Mine Water-specific Polychlorinated Biphenyls (PCBs) Using Palladium Catalysts

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Polychlorinated biphenyls (PCBs) were used, among other applications, in mining for fire protection reasons until they were banned worldwide. Even today, mines can still be contaminated with PCBs from leaks and abandoned equipment. Mine water must be continuously pumped out and discharged into surface waters. Unlike diffuse sources, efficient technologies can be applied to these point sources to reduce environmental impact.

A perspective promising approach is the catalytic dechlorination of PCBs using palladium nanoparticles. The aim of the present study was to determine the dechlorination rates of selected, mine water specific PCBs under optimal reaction conditions and to compare the dechlorination in matrix-rich mine water. Another goal is to protect the nanoparticles used as catalysts from deactivation by catalyst poisons. For this purpose, small amounts (0.15 mg L-1) of suspended palladium nanoparticles were added to the laboratory solutions containing PCBs and to the mine water. The experiments were conducted under anoxic conditions with hydrogen for hydrodechlorination. Simultaneous extraction and detection of reactants, intermediates and fully dechlorinated biphenyl were performed by SPME-GC-MS. Under laboratory conditions, the palladium particles showed high catalytic activities of up to 4400 L min-1 g-1 for mine water specific PCBs. This was not the case in the matrix-rich mine water. Here, the catalytic activity was strongly inhibited by the catalyst poisons present in the mine water (e.g. sulfur compounds).

To maintain the catalytic activity, experiments are underway to incorporate the palladium particles into a coating on the reaction vessels. The goal is to protect the catalysts from deactivation and to prevent the nanoparticles from being discharged into the environment. This additionally prevents the particles from entering the environment during on-site applications and causing further problems and costs. This embedding also results in higher degradation rates, as the catalyst poisons present in the mine water thus have less of an impact on catalyst activity. To further preserve catalyst activity, the application of an additional protective layer is planned to further shield the nanoparticles from the catalyst poisons and ensure a long catalyst life in in-situ dechlorination.

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P79

Mesoporous Silica Monoliths as Porous Scaffold for Heterogeneous Organocatalysis in Continuous-flow

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In organic chemistry for the synthesis of organic compounds, we need a lot of apparatus, reagents, catalysts, and specific conditions so that at the end we get a mixture of products, side products, and mixed catalysts and then we need to separate this mixture from each other. This complicated and time-consuming process is known as batch synthesis. To minimize all these flaws and synthesize products through a simple and straightforward way to get maximum yield that can be collected easily without further purification, the concept of "Synthesis in Continuous-Flow" is established and attracted great attention in chemical societies. In this project, we worked on tuning mesoporous space in silica monoliths that are being used as porous scaffolds for heterogeneous catalysis in continuous flow. We carefully tuned the hydrothermal temperature during the synthesis of silica monoliths and exclusive but detailed physisorption studies confirmed the orderly tuning of mesopore space from restricted mesopores to well-connected open mesoporous systems. The mesopore size is controlled from 11nm to 15nm with a difference of 1 nm by changing hydrothermal temperature from 80℃ to 95℃ by a gap of 3℃. The further change of mesopore size from 15nm to 27nm is controlled by using a hydrothermal temperature range of 95℃ to 110℃ with a change of 5℃. The resulting monoliths with known mesopore space were functionalized with thiourea catalyst and heterogeneous flow catalysis performed on a very classic reaction used for the protection of alcohols and phenols with 3,4-dihydro-2*H*-pyran. By using an immobilized thiourea catalyst on silica monoliths, very fine yields were achieved in a number of reactions by varying different alcohols protection by 3,4-dihydro-2*H*-pyran.

P80

MALDI mass spectrometry imaging for visualization of endogenous compounds in parasites and Vero cells

Mokosch A. S.[1], Li X.[2], Eckhardt D.[3], Ghezellou P.[1], Gerbig S.[4], Häberlein S.[2], Salzig D.[3], Grevelding C. G.[2], Spengler B.[1,4]

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Mass Spectrometry Imaging (MSI) is a powerful label-free analytical tool to map biomolecular compounds, especially in small samples like individual cells and parasites.

The parasitic flatworm Schistosoma mansoni causes schistosomiasis, which affects several hundred million people worldwide. In S. mansoni infection, a worm couple produces approximately 300 eggs daily, many of which lodge in host tissues and cause schistosomiasis pathology. Neurotransmitters appear to play an important role in the development of female worms. Investigating their occurrence and distribution in worms with different mating status is an important subject for research into potential drug targets.

The oncolytic measles virus is a promising candidate for cancer treatment, which can be produced in Vero cells. MSI-based metabolomic analyses of these cells are necessary to understand the influence of infection and the medium on the biomolecular composition of the cells.

We studied the distribution of neurotransmitters in mature S. mansoni couples, immature worms and genetically altered worms. Before measurement, samples were derivatised with 2,4,6-trimethylpyrylium tetrafluroborate (TMP) to enable the detection of neurotransmitters. Both samples were analysed by atmospheric pressure scanning microprobe MALDI mass spectrometry imaging (AP-SMALDI MSI). Sections were prepared using a cryotome (HM525, Thermo Fisher Scientific). Imaging experiments were performed using an AP-SMALDI5 AF ion source (TransMIT GmbH, Giessen) coupled to a high-resolution orbital trapping mass spectrometer (Thermo Scientific Q Exactive HF, Thermo Fisher Scientific (Bremen) GmbH).

Since neurotransmitter imaging is challenging due to poor ionisability, ionisation-enhancing derivatisation is an inevitable approach for MALDI-MSI. In the control worms, several neurotransmitters were detected after TMP derivatisation. Differences in signal intensities were observed within couples, with males having higher signal intensities than females. No neurotransmitters were detected in immature females. The results suggest that female schistosomes cannot produce certain neurotransmitters themselves and must obtain them from the males.

Preliminary analyses of Vero cells were successful, and individual cells could be distinguished from each other using a lateral resolution of 5 µm. Further research will include cells infected with the oncolytic measles virus and discrimination of Vero cells depending on infection status.

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P81

LC-MS/MS Determination of Altrenogest and further Steroid Hormones in Dust from Animal Husbandry

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Altrenogest is one of the few synthetic steroid hormones authorised in the EU, used for oestrus synchronisation as a zootechnical treatment in sows and mares.[1] A simultaneous oestrus cycle and the efficient integration of gilts into an existing production rhythm reduces the workload and facilitates a steadily produced number of piglets for fattening.[2] For this purpose, altrenogest is applied with 20 mg/animal/day over a period of 18 days via feed or orally. It can be assumed that altrenogest and its transformation products occur in barn dust through particles originating from application or faecal particles. Adsorbed to certain dust components, altrenogest and other pharmaceuticals may be respirable or enter the environment through ventilation/cleaning of the barn. Synthetic substances with hormonal effects such as altrenogest may act as endocrine disrupting chemicals and are an ongoing part of public discussions on environmental contaminants. One reason for this is the negative impact of these substances and their transformation products e.g. on aquatic life, even at low concentrations (< 0.4 ng/L).[3]

So far, and presumably without dispute, manure is considered the main pathway for hormones and other contaminants from intensive animal husbandry into the environment. However, do we currently underestimate the risks of barn dust borne contaminations?

Using a QuEChERS-based purification method with subsequent LC-ESI-LRMS detection, various dust samples from barns with and without altrenogest application will be analysed for altrenogest, its cycloaddition photoproduct and 13 other steroid hormones and presented on the poster. An in house validation is currently in progress to ensure representative measurements using deuterated standards for quantification.

[1] Council Directive 96/22/EC of 29 April 1996.

[2] Liesenfeld S et al. Chemosphere (2022) 287, 132353.

[3] Orlando, E F, Ellestad L E. General and comparative endocrinology 203 (2014): 241-9.

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SECTION 9

ECOLOGY AND GLOBAL CHANGE

ABOUT THE SECTION

Interaction of global change and ecological feedbacks are manifold and complex. This section accounts for the multidisciplinarity required to study and improve our understanding of the intrinsic complexity. Experts from agriculture, biology, environmental sciences, and geography contribute to the broad perspective needed to investigate the interactions on a mechanistic level.

Day 1: Wednesday, 20th September, 2023

CHAIRPERSON: Eva-Maria Minarsch

09:15

ORGANIC AND CONVENTIONAL AGRICULTURE PROMOTE DISTINCT SOIL MICROBIOMES WITH CONTRASTING METABOLIC POTENTIALS

Dr. Martin Hartmann
ETH Zürich
Switzerland

10:00

OCCURENCE AND DISSIPATION OF QUATERNARY ALKYLAMMONIUM COMPOUNDS IN SOILS OF HESSE, GERMANY

Kai Jansen

10:15

BLACK SOLDIER FLY AS SUSTAINABLE AQUAFEED FOR WHITELEG SHRIMP

Annalena Barth

Keynote 09

Organic and conventional agriculture promote distinct soil microbiomes with contrasting metabolic potentials

Hartmann M.[1], Müller R.[2], Lori M.[2], Widmer F.[3], Mayer J.[3], Mäder P.[2], Krause H.-M.[2]

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Aim: Soil microorganisms deliver numerous ecosystem functions essential for crop production. Changes in agricultural practices can alter soil microbial diversity and the underlying metabolic potential encoded in their collective genomes. This offers opportunities to harness microbial resources for developing sustainable cropping systems. Here, we explored how different organic and conventional farming systems shape diversity and functional potential of the soil microbiome.

Method: Soils were collected from the DOK long-term field trial comparing five different farming systems since 1978. The soil microbiome was characterized by DNA metabarcoding and shotgun metagenome sequencing. Extensive auxiliary data on soil properties, greenhouse gas emissions, and crop performance from decades of research allow for a comprehensive system comparison.

Results: Organic fertilization as an integral part of organic farming increased diversity and altered the taxonomic and functional structure of the soil microbiome compared to stockless systems. The plant protection regime was of subordinate importance. Organic fertilization promoted microbial guilds involved in degradation of complex organic compounds, whereas minerally fertilized systems were characterized by oligotrophic communities adapted to carbon-limited environments. Functional gene composition showed a gradual change based on the type of fertilizer inputs from organic to conventional and unfertilized systems. While conventional stockless farming systems were dominated by genes indicative of accelerated elemental cycles and molecule transport, the genetic capacity of organically managed soils were dominated by genes required for degradation of complex lignocellulolytic compounds and internal nutrient cycling.

Conclusions: These results add to the emerging evidence that long-term organic and conventional management can promote soil microbiomes with unique genetic capacities that might ultimately alter key biogeochemical processes in agriculturally managed soils.

T13

Occurrence and dissipation of quaternary alkylammonium compounds in soils of Hesse, Germany

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Quaternary alkylammonium compounds (QAACs) are cationic organic substances with amphiphilic properties that have a wide range of applications in households, industry and agriculture. Concerns have been raised about this group of substances because they are presumably involved in the co-selection of antibiotic-resistant microorganisms in the environment. Since data on the fate of QAACs in soils are scarce, a study was conducted in cooperation with the Hessian Agency for Nature Conservation, Environment and Geology to investigate QAAC contamination as well as dissipation in Hessian soils. A total of 65 soil samples of different land uses (arable land, grassland, forest, vineyard) and area types (agglomeration, rural area) were analyzed for alkyltrimethylammonium (ATMACs, chain lengths C8-C16), benzylalkyldimethylammonium (BACs, C8-C18) and dialkyldimethylammonium compounds (DADMACs, C8-C18) via HPLC-MS/MS after ultrasonic-assisted extraction with acidified acetonitrile. Four pairs of soils were formed from eight of the arable soil samples, each with different clay content. Within each pair, one soil was contaminated with QAACs (> 100 µg kg^{-1}) while the other was uncontaminated (< 5 µg kg^{-1}). After addition of 12 QAACs (each ATMACs, BACs and DADMACs with chain lengths C10, C12, C16 and C18), the samples were incubated for a period of 56 days under controlled environmental conditions. QAACs were detected in 97% of all soil samples. The highest total levels were found in floodplain soils impacted by suspended particles during flooding. The presence of QAACs in forest soils suggests a possible input via atmospheric deposition. High concentrations of long chain DADMACs indicate low degradation and accumulation over time. We expect dissipation to decrease with increasing alkyl chain length and increasing clay content, as QAACs are protected from microbial degradation by binding to clay minerals. In addition, we expect that a possible adaptation of microorganisms in soils contaminated with QAACs will lead to higher dissipation.

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T14

Black soldier fly as sustainable aquafeed for whiteleg shrimp

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The whiteleg shrimp (*Litopenaeus vannamei*) is one of the most important aquaculture species worldwide, with an annual output of about 6 million tons. Recently, attempts have been made to improve the overall sustainability of shrimp aquaculture. One of the targeted drivers is feed, which often uses fishmeal as the primary protein source. Since fishmeal contributes to global overfishing and increases the risk of marine pathogens in feed, non-marine protein sources such as insects can provide a safe and sustainable protein source. In a feeding trial, 25% of the standard compound feed was replaced with fresh larvae of the black soldier fly (*Hermetia illucens*) to test the performance of insects as aquafeed. During the trial, the shrimp growth rates (size and weight) and their amino and fatty acid profiles were recorded and the results were compared to a control group (compound feed only). While growth rates were significantly higher in the control group, survival rates were higher in the BSF larval group. As a result, total biomass was comparable in both treatments. The shrimp had significantly lower alanine, histidine, serine and tyrosine levels in their amino acid profile. There were no significant differences in the fatty acid profile of the shrimp between the treatment and control groups. These results indicate that fresh BSF larvae can successfully replace parts of the common fishmeal based compound feed to conserve natural resources. Furthermore, the usual grain-based black soldier fly (BSF) feeding substrate was replaced with a local side stream (apple pomace). Subsequently, these BSF larvae were fed to the shrimp to observe whether the BSF nutrient profile was altered through the substrate in a way that affected shrimp growth. The results showed that there were no differences in shrimp growth between the two treatments (apple pomace and cereal substrate). Therefore, apple pomace seems to be equally suitable as a feed substrate for BSF when used as shrimp feed. Furthermore, preliminary life cycle assessments show that the use of locally produced BSF can improve the carbon balance of shrimp aquaculture.

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A review on dendroecological studies within non-forestry systems

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Global climate change is already altering plant performance and thus vegetation composition, with many implications for the use and management of trees in agroforestry and forestry. Agroforestry systems consist of an upper tree layer and a ground layer, which is mostly managed as grassland. Both of these components respond to climate, and at the tree level, this response can often be recognized through the analysis of its rings. Studies in the area of dendroecology have been identifying the climatic response of tree species under different environmental conditions. Understanding these responses, especially in agroforestry systems, is crucial if we want to adapt the management of the cultivated species and the structure of their site to changing environmental scenarios. The objective of this study is to perform a non-systematic literature review on climate change-related dendroecological studies within agroforestry systems of the last twenty years and identify the sub-areas that are addressed in these studies. The literature search is been carried out with the keywords "dendrochronology", "dendroecology", "climate change", "agroforestry systems", "orchards", "fruit trees", "garden", and "silvopastoral system". We provide an overview of where most of the dendroecological studies are concentrated. When associated with climate change, the vast majority of tree ring studies were performed in forest ecosystems and only very few studies of agroforestry systems exist. Our preliminary results show that most dendroecological studies on non-forestry systems involve urban trees, gardens, and fruit trees. However, compared with other approaches, tree ring studies make up only a small proportion of climate change-related investigations on non-forestry systems. The next step of this ongoing research is to understand the reasons for this research pattern and to identify research gaps and future avenues of dendroecological research in non-forest systems

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Potential of multi-view geometry for training of convolutional neural networks

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The advent of unmanned aerial vehicles (UAVs) with affordable consumer-grade cameras and developments in deep learning have enabled automated plant species mapping across landscapes. The performance of deep learning models, which is often measured as precision and recall, depends largely upon the quality of the training dataset employed. Only few studies use the acquired UAV images directly, but make a deliberate effort to remove overlapping images, as to avoid object instances appearing both in training and testing datasets. Most other research processes the acquired images into an orthographic projection called an orthomosaic, where only 1 observation of each pixel is retained. In both approaches the imagery is used to generate annotated training and testing datasets. Often the datasets obtained this way are too small for training deep learning models, which have been shown to benefit greatly from large training datasets. Therefore, most studies employ subsequent steps of image augmentation to enhance these data. We ask whether using the original UAV images (including the overlapping ones) can improve network performance. The hypothesis is that the object of interest is seen from a slightly different perspective in each overlapping picture and using this variability for network training could be superior to traditional image augmentation techniques, where the perspective cannot be changed and only affine transformations to the whole scene are applied. Similar work has been done for object identification in 3D datasets, namely point clouds and meshes: there the authors generated 2D views of the 3D objects from various perspectives and used those projections to train convolutional neural networks (CNNs). Furthermore, plant identification in close-range photography has also been shown to benefit from multi-angle observations, but to our current knowledge, no such approach has been employed for UAV images in nature. We propose a method to quickly annotate entire datasets of UAV imagery and explore limitations and prospects of such an approach using a case study.

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The impact of different mulch materials on climate mitigation and adaptation in an organic vegetable system

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Climate change is one of the key challenges humanity is facing in the 21st century. Prolonged droughts, heavy rainfall events and other weather extremes have dramatic effects on human life, especially on the agricultural sector. Utilizing agricultural practices resilient to climate change is essential to ensure future food production. Mulching, applying plastic or organic material to the soil surface, is a practice with the potential to counteract many of the negative effects of climate change. Mulching has been shown to reduce evaporation, prevent soil erosion, increase nitrogen use efficiency and alter soil temperature. While mulching has received a lot of research in recent years, the effect of the practice on climate change adaptation and mitigation has received little attention. In a previous study, we found that rye straw as a mulch material not only increased soil water contents and reduced soil temperatures but also increased yields while not affecting greenhouse gas emissions. In order to evaluate the efficacy of different types of mulch material, we conducted an experiment in field cabbage at the research farm Gladbacherhof in Central Germany, investigating three organic mulches: rye straw (C/N-ratio: 46), rye-vetch-pea ley (C/N-ratio: 25) and alfalfa-grass (C/N-ratio: 16). Each material was applied to the soil surface at a rate of 15 t DM ha^{-1}. We evaluated the effects of these materials on yields, greenhouse gas emissions and soil parameters, such as water-filled pore space, soil temperature and soil mineral nitrogen. Our results show that each of the mulch materials used has benefits for the growing of cabbage compared to the non-mulched treatment. Plant available water was increased, soil temperatures were lower and yields were higher in all mulched treatments compared to the non-mulched control. Nitrous oxide (N_2O)-emissions increased with the application of rye-vetch-pea and alfalfa-grass mulch, but decreased with rye mulch. Emissions per yield, however, were lower for all mulch materials, compared to the control. These results suggest that the application of organic mulch materials is beneficial for the adaptation to climate change in field vegetables, as mulching reduces the vulnerability to prolonged droughts and can even mitigate the negative impact of vegetable cropping systems on the climate, with reduced N_2O emissions. Further research is needed to confirm these results in other climate zones and with different crops.

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Thermodynamics of mineral-associated organic matter formation

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Regarding the mineral-based stabilization of organic matter in soils, the most important questions are: i) how tightly does a molecule bind to a mineral surface and ii) how much energy is required to convert this formerly stabilized organic matter into a (dissolved) substrate. In order to address these questions, we developed an isothermal titration calorimetric method to assess the thermodynamics of adsorption of low molecular weight organic compounds differing in polarity and degree of oxidation to soil minerals. The poster presentation will outline isothermal titration calorimetry as a method for assessing sorption reactions and relate the thermodynamic parameters, i.e. sorption enthalpy and Gibbs free energy, of the sorption of low molecular organic weight compounds to their (de)sorbability and sorption hysteresis.

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Side streams from agricultural and food systems for Sustainable Mealworm Production in the Context of the Republic of Kosovo

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As the global population grows, the need for sustainable and eco-friendly protein sources is increasing. Insect farming has emerged as a viable solution to tackle these challenges. This research focuses on utilizing regional and seasonal side streams from agricultural and food systems in the Republic of Kosovo to enhance mealworm production. The study aims to contribute to sustainable agricultural practices and development goals.

Various dried feeding substrates were prepared by combining agricultural by-products sourced from the Republic of Kosovo, such as brewer's spent grain (BSG), brewer's spent yeast (BSY), apple pomace (AP), and grape pomace (GP), with wheat bran (WB). Wet side-streams of pepper, melon, and potato were added. Larvae of the yellow mealworm beetle (*Tenebrio molitor* L.) were reared using these substrates. Their growth performance and nutritional composition were evaluated. Proximate composition, fatty acid analysis, amino acid analysis, and mineral content analysis were conducted to assess the quality of the substrate, larvae and their by-products, such as frass.

The study identified a substrate consisting of a mixture of wheat bran and BSG with melon as a wet source as the most suitable for larval growth. Larvae reared on this substrate showed highest growth rates, most efficient feed conversion, and shortest development times. Analysis of general feed parameters as well as fatty acid and amino acid profile analysis revealed variations among substrates and larvae. Mineral content analysis revealed the potential of mealworm frass as an organic fertilizer.

The findings demonstrate the potential of mealworms as an alternative protein source and their ability to address environmental challenges in the context of the Republic of Kosovo. The optimized feeding substrate yielded promising results in terms of larval growth, while the composition analysis highlighted the nutritional characteristics of mealworm larvae and their by-products. This research contributes to sustainable agricultural practices and development goals in the Republic of Kosovo and beyond.

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Downstream Effects of the Pandemic: Spatiotemporal Trends of Quaternary Alkylammonium Disinfectants in Suspended Particulate Matter of German Rivers

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The SARS-CoV-2 pandemic caused a surge in the global use of disinfectants, many of which contain quaternary alkylammonium compounds (QAACs). While QAACs are popular broad-spectrum antimicrobials effective against coronaviruses, they increasingly raise concerns due to their wide-spread occurrence, eco-toxicological effects, and potential to induce resistance in microorganisms, possibly including antibiotic-resistance in pathogens. Elevated levels of QAACs have been documented in households and wastewater during the pandemic, but so far, little research has focused on the environment where humans and wildlife could also be exposed.

As QAACs enter the environment mainly via wastewater discharge, recipient rivers likely reflect changes in disinfectant use first. QAACs have a strong tendency to adsorb to particle surfaces, and therefore, they may be present in high concentrations at the suspended particulate matter (SPM) of rivers. To understand the environmental footprint of increased disinfectant emissions during the SARS-CoV-2-pandemic, the aim of this study was to investigate spatiotemporal trends in QAAC concentrations in SPM of German rivers.

To this end, SPM samples from the rivers Rhine, Saar and Mulde provided by the German Environmental Specimen Bank were analysed for QAAC residues. The sampling set included pooled annual samples from 2006, 2013 and 2017-2021 and monthly samples from 2018-2021. Combing a shaking-ultrasonic extraction with targeted multi-residue HPLC-MS/MS analysis, 29 different QAACs could be quantified.

QAACs were found in all investigated samples. The total concentrations ranged from 0.3–17 mg kg^{-1} and were around 10-fold higher in the Saar compared to Rhine or Mulde. Due to strong seasonal variation, no clear impact of the pandemic was visible in monthly samples. In the annual samples, compared to the average of pre-pandemic years, ∑ QAACs measured in 2020 and 2021 were hardly changed in the Rhine and decreased slightly in the Saar while for the Mulde, a 1.5-fold increase was observed. DADMAC10, a common ingredient of disinfectants, however, showed an increase between average pre-pandemic levels and 2021 of 1.3-fold, 1.4-fold, and 2.2-fold in Rhine, Saar and Mulde, respectively. The potential eco-toxicological implications will be investigated next. Our findings already highlight the importance of considering the environmental dimension of public health measures to prevent side-effects on human health or ecosystems.

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Antago-Senecio: Integrated control of *Jacobaea vulgaris* by different management methods

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Ragworts (*Jacobaea vulgaris* Gaertn.) are increasing significantly in numbers, especially on extensively managed grassland. The native plant contains pyrrolizidine alkaloids, which are toxic to grazing animals. Hay containing ragworts cannot be sold, nor is it approved for internal use as animal fodder. This makes it difficult for farmers to utilize the harvested crop. On sites with high ragwort abundance, farmers often change the site-specific management through intensification, use of herbicides or abandonment of land, which poses a major challenge to nature conservation. The aim of the project is to develop effective strategies of ragwort control while preserving the high plant species richness of the grassland sites. Three topics are addressed: 1) Monitoring / GIS-Analysis and characterization of factors for ragwort population growth 2) plant biology experiments in the greenhouse 3) Testing of selective and nature-compatible management methods in the study region High Westerwald (420-540 m of elevation). Monitoring and the assessment of the infestation degree is key to understand reasons of population changes. For this purpose, ragwort was mapped on affected grassland sites. To understand the biology of the plant, greenhouse trials were carried out with investigation of germination rate, reproduction potential and regenerative capacity. In order to identify measures showing the best effect under real-world conditions, long-term field trials were established. For this purpose, mowing equipment of different cutting heights, fertilizer, lime and seed mixtures were used. Additionally, selective industrial approaches were tested, such as laser and electrothermal treatments. The greenhouse trials have shown that competition to other plant species has a significant effect on ragwort seedling vitality. In the field trial, a reduction in population growth rate by increasing cutting frequency was observed after one year. The laser treatment could not achieve a lethal effect, but reduced ragwort growth. Also the treatment with electricity led to (multiple) resprouts of ragwort in the greenhouse after several weeks, but this was not observed in the field. Together with the results of the Julius Kühn-Institute concerning herbivore Antagonists, the findings of the project aim at providing integrative approach of biological and agricultural methods to manage ragwort. The results can foster further development of machines in the field of weed control in grassland.

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The soil microbiome in agroforestry systems – A meta-analysis

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Healthy soils depend on a diverse and functional microbiome, which plays a crucial role in nutrient cycling, organic matter degradation, and nutrient provision to plants, among others. However, agriculturally managed soils often suffer from an imbalanced and depleted microbiome due to intensive fertilizer use, pesticide and herbicide application, low plant diversity, soil compaction, and frequent tillage. Agroforestry systems offer a promising solution by combining the cultivation of arable crops and grassland with perennial woody plants on the same land unit. The perennial woody plants grow in strips alongside with an understory vegetation and a lack of soil tillage. The diverse array of different perennial plant species with their associated root microbiome and leaf litter fall during autumn contribute to improved soil health. Previous studies have demonstrated that agroforestry systems generally enhance microbial abundance, diversity, and activity compared to agricultural non-agroforestry systems. However, a comprehensive quantitative analysis examining the overall effect of agroforestry systems on the soil microbiome and on differences within the systems is still lacking.

To address this research gap, we are conducting a meta-analysis to investigate microbial abundance, diversity, and activity in agroforestry systems compared to non-agroforestry controls, as well as differences within agroforestry systems. We have identified over 900 relevant studies through a comprehensive search on the Web of Knowledge database, which are currently being screened for suitability for the meta-analysis. In this presentation, we will outline the underlying concept of the analysis and present preliminary results. Furthermore, we will discuss the significance of the soil microbiome in agroforestry systems and its relevance for other agricultural practices. By shedding light on the role of the soil microbiome, this research aims to contribute to the understanding and advancement of agroforestry systems and their potential for sustainable and resilient agricultural landscapes.

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Potential for improving of water resource management in data-poor regions through participatory monitoring, remote sensing and artificial intelligence

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The collection of reliable hydro-meteorological data is urgently needed to address the global impacts of climate change on water availability and distribution. A certain amount of data is essential for quantitative studies and the development of sustainable water management strategies. Away from the usual costly way of obtaining this data, a promising methodology is the use of participatory monitoring (PM) to collect the required data at relatively low cost. Here, people who are not experts in hydrology collect the data using easy-to-understand tools and methods. This could be an option especially in countries of the Global South, where there are often large gaps in relevant data sets and monitoring is often limited by budget. These countries also tend to be the most affected by climate change. Therefore, it is of interest whether PM is a reliable method to obtain hydro-meteorological data in these remote areas.

The HydroCrowd project, on which this thesis is based, aims to strengthen the acceptance of PM as a valid approach in hydrology. This will be done in three different countries of the Global South: Ecuador, Honduras, and Tanzania. Citizens will measure various hydrometeorological parameters such as rainfall, air temperature, humidity and water levels using low-cost methods and will be able to transmit the data via a smartphone application. There will be a two-step validation process of the PM data: A direct validation using images from the gauges and a comparison with professional gauges at the sites that continuously record the data. The data from these three countries will be used to feed hydrological models that will demonstrate the applicability of PM in different regions.

To support this PM approach, other methods are integrated: remote sensing (RS) and artificial intelligence (AI). Through RS, the growing amount of satellite data can be used to estimate meteorological data that is more difficult to measure, such as evapotranspiration, on a large scale. This can support the attempt of hydrological modeling with the collected PM data. On the other hand, AI can be applied to various things like gap filling in these datasets or improving models. This leads to the following research goals: On the one hand, the successful application of PM data for hydrological modelling in different regions. On the other hand, the potentials to improve water resources management in data-poor regions by using PM in combination with RS and AI should be explored.

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Phytoremediation potential of *Miscanthus x giganteus* in organochlorine pesticide contaminated soils using polysorbate 80 as a mobilising agent

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There are extensive land areas globally of pesticide-contaminated agricultural soils in need of remediation. In this global environmental challenge, phytoremediation stands as a sustainable, low-cost, biological remediation perspective. *Miscanthus sp.* is a C4 perennial rhizomatous grass and trials to evaluate its potential for phytoremediation of contaminated soils have been reported and are ongoing for sites in Asia and Europe. However, more research on the establishment of a phytoremediation technique for the removal of organochlorine pesticide (OCP) residues of agricultural soils is essential.

Knowing the slow degradation attribution and bioaccumulative nature of the OCPs as well as their low plant availability, the objective of this study was the evaluation of *Miscanthus x giganteus* (MxG) ability, in combination with polysorbate 80 - based microemulsion as a biodegradable mobilising agent, for dichlorodiphenyltrichloroethane (DDT) and hexachlorocyclohexane (HCH) uptake and remediation potential.

A first six-month greenhouse experiment for the optimisation of DDT and HCH uptake was performed. The randomised block design consisted of a control group and three different treatments (T1 – no microemulsion was added, T2 – 0.1 % and T3 – 0.5 % microemulsion concentration). DDX and HCHs contamination concentrations in the treated groups were 765 µg/kg and 105 µg/kg respectively. These concentration levels were achieved by mixing aged contaminated Georgian soil with soil obtained from a local site.

During this experiment, plant phenotypes such as shoot and root development, leaf colour diversity and biomass yield were recorded on a weekly basis. These first preliminary results presented unexpected phenotypic significant differences in the plant growth between control and treated groups. Results from chemical analysis on DDT and HCH uptake and translocation in the plant show that the bioaccumulation of the DDX isomers was higher in the root system, yet very little was translocated into the shoot system. ΣHCH bioaccumulation was overall low. Polysorbate 80 microemulsion did not show any significant effect in boosting phytoremediation efficiency.

An ongoing OCP spiked soil experiment will allow us to quantify the plant's fitness and OCP uptake when compared to the aged polluted Georgian soil, and the fate of OCP accumulated in plants will be studied thoroughly.

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Promotion of plant-pollinator interactions through fallow strips

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Agricultural intensification is a major cause for biodiversity loss in open landscapes. Intensive mowing regimes in grasslands result in homogeneous areas. The low structural diversity results in a decline in habitats and food resources for interacting species such as pollinators. Fallow strips, i.e. agricultural grasslands taken out of management, add an ecological contrast in agricultural grasslands. In this way, they could increase resources for wild bees and hoverflies while maintaining the majority of the land under cultivation.

We sampled eight different grassland sites across Hesse (Germany), each divided into a fallow strip and three control strips with constant management of 10 m x 40 m each. In two sampling campaigns five to eight weeks after mowing of the control strips, we sampled wild bees and hoverflies from visited flowers and recorded species identity for both interaction partners.

Most of the host plant species were found only in the control strips, while fallow strips had the fewest visited plants. An intermediate number of host plant species was found in both areas. While wild bee and hoverfly species followed this general pattern, the resulting number of unique interaction links in fallow strips between plants and pollinators add ecological complexity to managed grasslands. However, this pattern varies greatly from region to region. Local identity of plant species appears to modulate the distribution of unique links in grasslands with fallow strips. This resulted in pronounced differences in the contribution of wild bees and hoverflies to plant-pollinator network structures. Thus, fallow strips contribute little to grassland species richness, but provide novel interaction patterns that are otherwise lacking in poorly structured grassland systems.

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Can roadsides promote biodiversity? - Identifying the value of roadsides for floristic and faunistic diversity in a landscape context

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Roadsides may be an important tool for nature conservation in agricultural landscapes. These margins, next to the large network of roads (in Germany ~230.000 km), can show similarities to extensively managed grasslands and provide secondary habitats and refuges for generalist and specialist plant and insect species. Due to their linear shape, roadsides can function as corridors providing functional connectivity within agricultural landscapes. Additionally, they provide various ecosystem services, such as pollination, protection from erosion and increasing the recreational value of landscapes. By this, they help maintaining resilient agricultural landscapes and are a refuge for biodiversity.

Currently, little attention is focused on the role of roadsides for nature conservation purposes and a strategy for management is missing. Locally, practitioners are developing ideas for the conservation of biodiversity in roadsides, but there is a lack of communication and comprehensive solutions for reoccurring problems such as the implementation of measures. Thus, we want to assess the ecological status of roadsides in four cultural landscapes with varying land use intensities in Hesse concerning their biodiversity and management options for their conservation.

We gather practitioners' knowledge regarding management efforts in different landscapes through semi-structured interviews. Secondly, we analyse the landscape structure while mapping the historical and current land use with all existing roads and sides using a geographical information system and calculating landscape metrics. This will lead to the identification of key areas relevant for maintaining or increasing landscape connectivity. In the course of the project, we investigate the floristic and faunistic species composition within these landscapes with a focus on ground beetles, hover flies and wild bees.

The knowledge gained in the project will be key to evaluate the ecological status and function of roadsides as connecting habitats and to propose a prioritization scheme for the implementation of management strategies for nature conservation.

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Enhancing LSM predictions with stable water isotope transport model.

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Stable water isotopes such as deuterium (^{2}H) and oxygen 18 (^{18}O), are considered natural tracers in hydrological cycle due to their physical and chemical properties. Lighter isotopes of ordinary water ($^{1}H_2{}^{16}O$), have higher affinity in vapour phase and tend to
diffuse easily which results in distinct concentration of heavier isotopes in water bodies such as streams, lakes, or aquifers[3]. The origin and movement of water in liquid as well as vapour phase can be traced back by analysing its isotopic signature. Modelling the complex hydrological system involves a high amount uncertainty. Isotopic transport modelling is an effort towards minimizing those associated uncertainties. As an integral part of Land Surface Model (LSM), it can distinguish various runoff generation processes thus making it able to quantify the fluxes from various origins. The application of isotope transport models so far includes differentiation between evaporation and transpiration fluxes[2], tracing water movements in biotic and abiotic systems[1] among others.

The proposed module aims to simulate transport and fractionation of stable water isotope fluxes in multiple scales and account for flux exchanges between multiple pore regions. It will be implemented as an extended tool for the LSMs and be able to run simultaneous with the hydrological models.

The module applies finite volume method to numerically solve the isotopic mass conservation equation and will account for all the processes affecting isotope movements, i.e., convective, and diffusive fluxes, fractionation between liquid and vapour phases thus resulting in storage changes within the layers. Finally, the isotopically enabled hydrological models will improve its overall performance by allowing us to better constrain the model predictions, and to produce a more reliable estimation of the belowground water fluxes and storages.

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Influence of feeding intensity on the emotional state of dairy cows

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The influence of feeding intensity on health and the emotional state in dairy cows has not yet been investigated in a comprehensive, systematic study with a controlled experimental design. Within the framework of the "Green Dairy" project which compares high- and low-input organic milk production systems over three and a half years, we aim to fill this gap.

We hypothesize that (1) high-input feeding, in comparison to low-input feeding, has a positive effect on the short-term welfare of dairy cows and thus on their emotional state due to increased energy and nutrition supply. However, we predict (2) the increased cumulative metabolic stress of high-input feeding to negatively affect the emotional state as a consequence of enhanced disease rates in the long run.

Cows (German Holstein) are either fed to achieve an annual milk yield of 9000 kg (High-Input, n = 64) or 7200 kg (Low-Input, n = 64). Cows of both treatments are kept under identical housing and management conditions at Gladbacherhof (65606 Villmar, Germany). To investigate the effects of feeding intensity on the cows' emotional state, Judgement Bias Tasks (JBTs) will be performed on 26 cows from each treatment every six months after implementation of the two feeding intensities and whenever diseases occur. JBTs rely on the assumption that an individual's emotional state will influence his or her behavioural response to ambiguous stimuli. To test cows' reactions towards ambiguous stimuli, they are habituated to an experimental arena in four steps. This includes teaching cows to (1) enter the arena, (2) approach a positive stimulus, i.e. feed reward in one corner of the arena, and (3) wait in the startbox adjacent to the arena to (4) re-enter the arena for another trial. Following a successful training in which cows learn to discriminate between a positive and a negative spatial stimulus, they will be presented with ambiguous stimuli during testing. It is expected that an individual in a positive emotional state will evaluate an ambiguous stimulus more positively and therefore approach it, while an individual in a negative emotional state will judge the same stimulus more negatively and not approach it.

20 low- and 21 high-input cows are currently performing the last habituation step. The remaining cows have not yet started with habituation since they were close to calving when the habituation phase started. Due to the extensive habituation of the focal cows, results from the JBT cannot yet be reported.

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P96

Practitioner knowledge on grassland restoration – bridging the knowledge gap to science

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In Central Europe, species-rich grasslands have strongly diminished over the last century. The transfer of seed-containing plant material from donor sites with a desired species composition to restoration sites is a well-established method to restore species-rich grasslands. However, despite a plethora of available literature, restoration projects with plant material transfer often fail or do not reach the planned goals. Practitioners' knowledge is a highly important but underexplored source of information on factors deciding about success of restoration projects. At the same time, it is unclear to which degree scientific findings on success factors are known and considered by practitioners, and if science actually investigates the most relevant aspects for practice. To bridge the gap between practitioners' knowledge and restoration science, we conducted semi-structured interviews with 33 practitioners involved in plant material transfer projects. Using qualitative content analysis, we analysed the interviews for success factors, and compared them to success factors of plant material transfer as investigated in peer-reviewed European studies on the method. We found that science investigated a broad range of practical, technical, and ecological success factors, and that practitioners were generally well aware of this evidence, trying to make use of the knowledge. Failure of practitioners' projects often resulted from organizational obstacles, which were founded in lacking trust and low experience levels among the involved people. We advise unexperienced practitioners to involve more experienced practitioners in their projects if possible. Furthermore, we emphasize the importance of identifying relevant local stakeholders and building trust. Interdisciplinary scientific studies considering success factors beyond practical and ecological aspects are required to support widespread successful grassland restoration with plant material transfer.

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P97

Water extractable organic carbon as affected by crop rotation - Insights into the carbon dynamics in arable soil of a mixed dairy farming system

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Mixed dairy farming systems can differ in feeding, crop rotation, and thereby productivity. High-intensity farming systems are aiming for the highest possible yield. Thus, dairy cows are fed large amounts of protein-rich fodder, leading to higher N concentrations in the manure. At low intensity, roughage with low N content predominates, making room for crops for human consumption in the crop rotation. To find out the holistic impact of changes in feeding management and crop rotation on animals and the environment, the "GreenDairy" project was launched. It aims to find a compromise that meets the demand for organically produced food while minimizing environmental impacts.

The project takes place on the experimental farm Gladbacher Hof (Aumenau, Hesse). So far, its dairy farming system corresponds to the high-intensity system. In the project framework, half of the trial area on arable land is converted by low-intensity cultivation. The 8-year crop rotation consists of winter grains, oat, alfalfa, field bean as well as fodder maize for high- resp. potatoes at low-intensity. Within GreenDairy our subproject focuses on the impact of organic milk production under low- vs. high intensity on humus and nitrate leaching. To begin with, soils of the arable and grassland plots have been sampled extensively by core drilling. Soil cores were divided into three depth segments (0-30, 30 – 50 and 50 – 100 cm). Changes in C- & N- dynamics are expected to occur first in the water-extractable C- and N-fractions (WEOC/WEON). Composite samples of field-moist soil were extracted by shaking using 10 mM $CaCl_2$ with a ratio of 1:4 (w/w).

First results of field moist water-extracted organic C indicate that within the crop rotation influences of the respective preceding crops are reflected. Generally, WEOC levels were low, ranging between 2.1 – 1 mg L^{-1} in the topsoil and 1.85 – 0.7 mg L^{-1} in the subsoil. The lowest WEOC level could be found in plots with current legume vegetation, whereas the highest WEOC levels in the subsoils occurred in plots with legumes as pre-crops. However, future samplings are necessary to confirm if the pattern rotates with crop rotation. As soils are a heterogeneous medium influenced by a variety of factors, this underlines the importance of a detailed baseline characterization to identify and interpret changes in the C and N fractions due to differentiated manure application.

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P98

Analysis of soil and site heterogeneity to delineate Management Zones in Organic Agriculture

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Precision Agriculture aims to optimize crop management by taking into account spatial variability, and thus optimize use of farm inputs [2]. Management zones are usually delineated based on yield maps , soil and topographic properties , remote sensing data[2] or a combination of the above. Most automatic methods to delineate management zones have several disadvantages, especially related to accuracy and also, applicability. Yield data tend to have a lot of erroneous points from sources such as sensor errors, georeferencing and even operator and data processing errors [2]. The cost and time efforts to pre-process these georeferenced data, which indirectly or directly implicate physical or chemical properties of the soil, is high because of the complexity of programs and the expertise required. Due to this reason, automated procedures can be developed in the programming language python. Python is oftentimes preferred because it has a lot of in-built procedures for fast calculations and frameworks that makes reproducible models and method analyses more flexible. Resource efficiency is a rising concern in agriculture because of the stress on natural resources caused by the higher demand for food. Studies state that unused lands for cultivation are becoming rare and 25% of farmlands are already marked as degraded because of activities like deforestation, overcutting vegetation and inadequate fallow periods [1]. Erosion from farmlands by frequent transfers from fields into rivers and lakes are also causing serious environmental problems [1]. All of these landscape factors need to be taken into account while suggesting recommendations for managing the delineated management zones. The idea is to include all of the above mentioned data in the heterogeneity analysis, focusing on the indicators identified for crop growth, and the soil heterogeneity data that was used to classify the field into low-yield and high-yield potential zones.

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P99

What's for dinner? Feeding behaviour of reef-building corals on common microplastic polymers

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The pollution of the marine environment with microplastic (MP, i.e., plastic particles 1-1000 µm) is of increasing concern, as it is affecting marine life, including reef-building corals. The exposure to MP is expected to be energetically costly for corals due to handling and ingestion of the particles. Although previous studies suggested that reef-building corals can distinguish between natural food and MP, the rates of MP ingestion by corals and the underlying mechanisms are not well understood. Additionally, while the effects of single polymers on reef-building corals are increasingly unravelled, knowledge about the role of MP particle forms and polymer types, but also the influence of simultaneous food availability remains limited. Our study therefore aimed to assess the factors influencing MP handling and ingestion by reef-building corals. For this, we conducted a feeding experiment using six polymer types in the forms of fragments (polyethylene, polyvinylchloride, polystyrene) and fibres (polyamide, polyester, polypropylene) and two common reef-building coral species (*Stylophora pistillata* and *Pocillopora verrucosa)*. To assess the influence of food availability on the feeding behaviour on MP, we offered them in four conditions: I) pristine particles, II) biofouled particles, III) pristine particles together with a food stimulus (crushed copepods), IV) biofouled particles with a food stimulus. We found that fibres were ingested less frequently (< 11%) than particles (< 21%), yet the polymer type caused only minor differences in behaviour. The presence of a biofilm was observed to decrease corals' reaction rates to MP. The presence of food in the surrounding water had only minor impacts on the ingestion but enhanced the egestion process, particularly for fibres. These findings suggest that particle properties, food stimuli, and biofilms can have a complex influence on the feeding behaviour of coral polyps. Overall, our findings imply, that the feeding behaviour of reef-building corals is primarily influenced by biotic factors (e.g., biofilm or feeding stimuli), rather than the MP polymer itself. However, the form (e.g., fibres vs. fragments) may play an important role, especially influencing the ingestion and egestion of MP. This highlights the importance of considering biotic factors present but also incorporating fibres in experimental studies addressing the effects of MP.

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P100

Seed quality testing methods in soybean (*Glycine max*)

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Seed quality plays a crucial role in successful crop growth by establishing a closed crop stand, which minimise the light and space for weeds. To reach a target crop stand (no of plants/m²) farmers need to know the germination of the seeds used. In a project we used different methods to study the germination and emergence of five soybean varieties under different temperature conditions, as well as after different durations of seed storage. We used ISTA germination method between paper, emergence was determined in pods filled with sand and field soil, as well as the so called soak test (to analyse seed coat damages) and counted fungal infected seeds.

A germination capacity of 80 %, as required in the German Seed Regulation was reached in the germination tests in 2015 within all soybean varieties. The field emergence was lower than the germination test. Germination and emergence declines with increasing storage duration and after 17 month a germination capacity of 55 % was found, while another variety showed only 33 % emergence after 24 month of storage. The two varieties with higher share of seed coat damages and more fungal infected seeds showed slightly lower germination after 17 month of storage. The temperature also influenced the success of germination, best results were obtained under 25° C (the optimum for soybean) and 22 °C. Cold growing conditions (15°C and 12°C) showed reduced germination/emergence of seeds.

Soybean seeds are quite sensible to damage of the seed coat, which in turn reduces seed germination capacity. The storability of soybean is comparable low, so farmers and researchers should always use seeds from the last harvest. It is advised to use large seed number per replicate and use field soil or sand in order to get field relevant data and reduce the risk of fungal infection.

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P101

Soil greenhouse gas fluxes in high- and low-input organic farming systems

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Reducing emissions of the greenhouse gases (GHG) carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) from agricultural soils is an essential component of mitigating climate change. The amount of GHG fluxes is largely determined by fertilisation practice, but knowledge about GHG fluxes especially in organic farming systems is limited. This study is part of the LOEWE priority programme *GreenDairy*, which aims to compare the effects of two management intensities (high vs. low input) in organic dairy production systems on animal and plant productivity, animal welfare, and the environment. Thus, CO_2, CH_4 and N_2O fluxes are quantified in arable as well as grassland soil within the high and the low input system, respectively, by using static chambers and soil air probes in combination with cavity ring down spectroscopy and gas chromatography. In arable land, baseline measurements of six different crop rotation elements per treatment have been performed. To be able to quantify the effects of high vs. low input slurry on greenhouse gas fluxes further measurements have to be carried out after various slurry applications. In grassland, CH_4 emissions of both organically fertilised soils (high- and low-input) showed peaks in the first hours after slurry application, but decreased over time and resulted in higher CH_4 oxidation levels than soils treated with mineral fertiliser after five days. Due to high abundance of ants in the grassland plots, the measured fluxes are additionally compared with gas emissions and uptake resulting from ant nests. Preliminary results indicate that either CH_4 oxidation could be inhibited or production could be promoted by *Lasius flavus* as well as *Lasius niger*. In order to examine the effects of the different agricultural systems and the factors influencing the measured gas fluxes, further research will be carried out.

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SECTION 10

CLINICAL SCIENCES

ABOUT THE SECTION

This section includes young clinicians from the medicine and veterinary medicine faculties. In this section, the researchers and healthcare professionals work collaboratively to study various aspects of human health, animal health and their well-being. The research approaches in this section range from the development of statistical models over investigation on the cellular and tissue level to clinical studies to better understand diseases and to develop new treatments. Major focal points in this section are the areas of lung diseases, cancer treatment and tissue regeneration or replacement.

Day 2: Thursday, 21st September, 2023

CHAIRPERSON: Khaled Mahmoud

09:00 **METABOLIC REPROGRAMMING IN CANCER PROGRESSION**

Dr. Patricia Altea Manzano
Laboratory of Cellular Metabolism and Metabolic Regulation
Katholieke University Leuven
Belgium

09:45 **MOLECULAR SUBTYPING OF DIFFUSE LARGE B-CELL-LYMPHOMA USING PANEL-SEQUENCING**

Frederike Hagedorn

10:00 **A NOVEL REGULATORY LINK BETWEEN EZH2 AND HIF2α IN BREAST CANCER**

Salisa Kruijning

10:15 **ORTHODONTIC COMPRESSION PROMOTES MACROPHAGE M2 POLARIZATION VIA HISTONE H3 HYPERACETYLATION**

Yao Wang

Keynote 10

Metabolic Reprogramming in Cancer Progression

Altea Manzano P.

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As cancer progresses, tumor cells must dynamically alter their cellular phenotype in order to adapt to changing environments and situations. This in turn requires metabolic adaptations to meet their challenging demands. Moreover, both cell-intrinsic and microenvironmental factors intensify heterogenic metabolic reprogramming in tumor cells to fuel growth, invasion, and metastasis. In this regard, my research has contributed to the emerging view of tumor metabolism as flexible and context-specific. First, I will explain how tumor cells reprogram their metabolism to sustain a high proliferative state adapting to challenging metabolic conditions. Furthermore, within a tumor, cancer cells exist in different states that are associated with distinct tumor functions. Thus secondly, I will discuss how tumor cells also harbor heterogenic metabolic states that enable cell-state transitions, dissemination, and seeding in distant organs. Finally, once disseminated tumor cells reach new organs, they encounter new environments with different nutrient availability. I will present our recent evidence that disseminated tumor cells take advantage of the local nutrient palmitate, which is highly available in the lung, to activate metabolic and signaling pathways promoting metastatic growth. I will discuss how this modulation in cancer metabolism impacts cancer progression as well as its therapeutic implications.

T15

Molecular Subtyping of Diffuse Large B-Cell-Lymphoma Using Panel-Sequencing

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Diffuse large B-cell-lymphoma (DLBCL) is the most common type of B-cell non-Hodgkin lymphoma in adults. Several technical developments have enabled the subclassification of DLBCL into distinct molecular subgroups using gene-expression profiling and next-generation sequencing. However, the heterogeneity of existing assays and algorithms has hampered broad implementation and use in clinical practice. Therefore, our aim was to develop a simplified and optimized model for DLBCL subclassification to be used in clinical routine.

We studied more than 200 formalin-fixed, paraffin-embedded DLBCL specimens using hybrid capture based high throughout panel-sequencing, including genes that are frequently mutated in this disease. Identified variants were used to define the cell-of-origin (COO) and to determine the genetic subgroups of DLBCL. For COO classification we refined a modelling algorithm relying on Bayes' theorem that was initially developed by Scherer et al., 2016. Results were compared with other gene expression-based classifiers of DLBCL. Classification of genetic subgroups was performed using the LymphGen Classifier described by Wright et al., 2020.

Our panel-based high throughput sequencing analysis was able to determine the COO and genetic subgroups of the DLBCL samples analysed. Compared to other methods of molecular subclassification, our technique shows a high degree of overlap to the standard methods currently used in clinical routine.

Panel-based high throughput sequencing is well suited for molecular subclassification of DLBCL. It is simple, cost-effective, highly reproducible, accurate and objective**.** We believe, that in the near future a molecular classification will be routinely applied to identify those patients with a high-risk genetic profile while others can be protected from unnecessary therapy toxicity.

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T16

A novel regulatory link between EZH2 and HIF2α in breast cancer

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Reduction of oxygen availability, known as hypoxia, is a common consequence of rapid tumour growth. Hypoxia activates key hallmarks of cancer, including tumour cell plasticity, metabolic reprogramming, and proliferative signalling, which are closely linked to epigenetic processes. Currently, many details about the connection between hypoxia and epigenetic regulatory pathways remain unknown.

A loss-of-function screening study of histone methyltransferases and histone demethylases in our laboratory revealed a novel regulatory link between the H3K27-specific histone methyltransferase enhancer of zeste homolog 2 (EZH2) and hypoxia-inducible factor-2 alpha (HIF2α). We aim to discover how EZH2 regulates HIF2α and what consequences this regulation might have for cancer progression.

Our results show that depletion of EZH2 in breast cancer cells leads to a reduction in HIF2α protein and mRNA levels. EZH2 is the catalytic subunit of Polycomb Repressive Complex 2 (PRC2), which trimethylates H3K27 but may also exert PRC2-independent functions. To determine whether enzymatic function is required for HIF2α regulation, we used the EZH2 catalytic inhibitor GSK126 and knocked down SUZ12 and EED, two subunits of the PRC2 complex. Neither experiment resulted in a decrease in HIF2α protein and mRNA levels, suggesting that EZH2 regulates HIF2α levels independently of the PRC2 complex and its enzymatic function. To determine whether EZH2 directly regulates HIF2α levels, ChIP-qPCR assays were performed, and the results suggest binding to the EPAS1 (HIF2A) gene by EZH2. In future experiments, we will investigate a possible combinatorial effect of EZH2 with other transcriptional activator mechanisms on EPAS1 gene regulation and their impact on tumour progression in different cancer types.

Altogether, we have discovered a novel regulatory link between EZH2 and HIF2α in breast cancer cells and uncovered that regulation depends on a non-enzymatic, PRC2-independent function of EZH2, which can be exploited as a potential new target for cancer therapy.

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T17

Orthodontic Compression Promotes Macrophage M2 Polarization via Histone H3 Hyperacetylation

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Orthodontic compression can initiate localized immune response characterized by a sterile inflammation, which in turn causes tooth movement and conditionally root resorption. Macrophages are mechanically sensitive immune cells, but their role in orthodontic tooth movement is unclear. We hypothesized that orthodontic compression leads to macrophages activation associated with orthodontic root resorption. After force-loading and/or adiponectin application, the migration function and inflammatory genes (nitric oxide synthase 2 (Nos2), interleukin 1b (Il1b), arginase 1 (Arg1), interleukin 10 (Il10), apolipoprotein E (ApoE), and Serum amyloid A-3 (Saa3)) expression of macrophages were tested via scratch assay and qRT-PCR. H3 histone acetylation was measured using an acetylation detection kit. The specific inhibitor of H3 histone, I-BET762, was used to investigate its effect on macrophages. In addition, cementoblasts were treated with macrophage-conditioned medium or compression force, and osteoprotegerin (OPG) production and cellular migration were assessed. We further detected regulations of hypoxia inducible factor 1 alpha (HIF1α), vascular endothelial growth factor (VEGF), angiopoietin 1 (ANGPT1), angiopoietin-like 4 (ANGPTL4) and mechanoreceptor Piezo1 in cementoblasts by compression via qRT-PCR and Western-blot. The effects of the Piezo1 inhibitor were also analyzed. Compressive force significantly inhibited macrophage migration. Nos2 was up-regulated 6 h after force-loading. Il1b, Arg1, Il10, Saa3, and ApoE increased after 24 h. Higher H3 histone acetylation was detected in the macrophages subjected to compression, and I-BET762 impaired the expression of M2 polarization markers (Arg1 and Il10). The activated macrophage-conditioned medium showed no effect on cementoblasts. In contrast, compressive force directly impaired cementoblast migration and mineralization but enhanced HIF1α and downstream vascularization-related genes. These effects were accompanied with up-regulation of Piezo1 and could be suppressed by the Piezo1 inhibitor. Compressive force induces M2 polarization of macrophage via H3 histone acetylation in the late stage. Compression-induced orthodontic root resorption is macrophage-independent, but involves the activation of Piezo1 in cementoblasts.

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P102

In vitro investigation on connexin 43 with regard to osteoarthritis

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Osteoarthritis (OA) of humans and horses is characterized by elevated Connexin 43 (Cx43) levels compared to healthy cartilage *[1]*.

The present *in vitro* investigation was conducted to test the hypothesis whether the parameters 'aging and inflammation' increase the synthesis rate of Cx43. As OA affects mainly the elderly, we used senescent cells and continued to use cells, which were incubated with IL-1β – one of the most important inflammation factors of the osteoarthritic cartilage. Furthermore, cells were treated with shock waves, as it is used for OA therapy.

We used mesenchymal stem cells (MSCs) because they are the precursor cells of the chondrocytes. MSCs were isolated from adipose tissue of horses and cultured in standard medium. The senescent cells were harvested from passage 19. For the stimulation of inflammation 1µl/ml IL-1β was added to the supernatant once a day over a period of three days. The treatment with shock waves was carried out using PiezoVet 100 (Richard Wolf). Cx43 protein levels were determined by quantitative Western Blot (LICOR Odyssey Software) and were represented on a qualitative level by immunofluorescence. Untreated cells were used as negative control.

The senescent cells and the cells incubated with IL-1β as well as the shock waves treated cells had higher Cx43 protein levels compared to negative controls.

The elevation of Cx43 levels of the senescent cells and the IL-1β treated cells might be caused by a high number of gap junctions and hemichannels. These findings refer to a high cellular communication potential, which is more typical for undifferentiated cells. Therefore, an undifferentiated status of the cells may also underlie the high Cx43 values of the damaged cartilage affecting structural and functional integrity of the tissue.

Treatment with shock waves also resulted in an increase of Cx43. The causal effect of this therapy is therefore probably not due to a decrease in the values of Cx43 in the damaged cartilage.

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P103

Links between oral and general health

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Is there a connection between an altered oral flora and the occurrence of a general disease?

The bacterial colonisation of infectious oral diseases, such as dental caries, differs from the healthy oral flora [1]. There is also evidence of a pathological interaction between oral inflammatory diseases and immune diseases or systemic diseases [2]. The purpose of this study was to compare the oral bacterial colonisation of children (up to 18 years old) with and without general diseases, dental caries, and additional odontogenic infections.

Following the evaluation of the medical history, a clinical dental examination was conducted. Using the dmft (DMFT) index, the number of caries-affected areas (teeth and tooth surfaces) per child could be determined. Additionally, the oral cavity was inspected for odontogenic infections such as fistulas and/or abscesses.

Using a cheek swab, saliva samples were collected after an oral examination. The saliva was then frozen at -80 °C in a sterile preservative solution until further analysis. To capture as much of the bacterial microbiome as possible qualitatively and quantitatively at the DNA level and in live culture, a multistep approach combining short read next-generation sequencing, long read third-generation sequencing, and culturomics was employed.

Recruitment of test subjects, sample collection, preservation of saliva samples, and further analysis are currently ongoing.

Literature resources:

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P104

The role of socs1 mutations in the pathogenesis of hodgkin lymphomas and diffuse large b cell lymphomas

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Most B cell lymphomas derive from germinal centre (GC) B cells. GC are histological structures in the secondary lymphoid organs in which B cells undergo somatic hypermutation (SHM) and class switch recombination to generate high affinity B cell receptors. A gene frequently mistargeted by SHM is SOCS1, coding for a negative regulator of the JAK/STAT signalling pathway. Inactivating mutations of SOCS1 were identified in more than 90 % of paediatric and 60 % of adult Hodgkin lymphoma (HL), as well as 15 % of diffuse large B cell lymphoma (DLBCL), indicating an important role for SOCS1 mutations in the pathogenesis of GC derived B cell lymphomas.

In this study, in-vitro cultured, non-malignant GC B cells are used as a model system to study the role of SOCS1 in the development of HL and DLBCL. Applying CRISPR-Cas9 technology, changes in the clonal composition, proliferation, apoptosis resistance, signalling pathways and gene expression of SOCS1-depleted B cells are studied. Furthermore, in reconstitution experiments SOCS1-WT or different mutation variants are overexpressed in lymphoma cell lines harbouring inactivated SOCS1 to investigate cell toxic effects.

We show that the cultivation of GC derived B cells is well suited to mimic the effects of SOCS1 depletion in the germinal centre. The knock-out of SOCS1 in these cells led to an increased proliferation rate compared to non-treated control cells. Contrary, the overexpression of SOCS1 in different HL and primary mediastinal B cell lymphoma cell lines showed a distinct toxicity.

Further work will focus on the analysis of signalling pathways that act synergistically to SOCS1 and changes in gene expression that are the result of SOCS1 inactivation. This system may further be used to analyse the effects of SOCS1 reconstitution in primary human GC derived B cells with inactivated SOCS1 alleles.

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P105

Finite element simulation of callus biomechanical properties and statistical modelling

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In cases of diseases or injuries the reference depends on the normal healing considering the time needed and the sequence of events. Unfortunately, in experimental studies and preclinical practice it is more preferred to compare healing events between diseased and normal condition due to the lack of such reference. To reduce animal testing, simulation scenarios are used.

The aim of this project is to simulate a whole bone organ culture where materials can be tested and to develop, analyze and test new bone substitute substances and implantation material for systemic bone disease. The prediction of the simulation should reduce the experiments with real animals. The part of simulation will crystallize several parameters for the physical material tests.

In the first part µCT scans from discrete time points will be assembled and simulated. In the second part of the project, the statistical modelling will give a better understanding of the physiological process of cell migration measurement and modelling. The third aim and final goal of this project is to correlate between the simulation and statistical model to be more conducted and more targeted in further experiments.

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P106

Self-Expanding Hydrogel and Bone Marrow Aspirate Bioink for Tissue Regeneration

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Bone defects with limited intrinsic regenerative potential represent a considerable surgical challenge and are associated with high socialeconomical costs and severe reduction on patients' quality of life. Bone tissue engineering involving the use of scaffolds, cells, and bioactive factors is promising to meet this tremendous need.

For traditional pre-formed scaffolds to fit in a bone cavity, surgeons need to machine the graft or carve the surgical site, leading to increases in bone loss, trauma and surgical time. These scaffolds for cell delivery have drawbacks including the difficulty of seeding cells deep into the scaffold, and inability for injection in minimally invasive surgeries. Therefore, there is a need for the development of injectable bone substitutes suitable for cell delivery and minimally invasive surgeries.

More and more emerging synthetic polymers owe superior mechanical properties, while hindered by their poor biocompatibility and bioactivity. Natural polymers possess good biocompatibility and bioactivity due to their resemblance to extracellular matrix. However, their poor mechanical properties and structure instability have brought us a conundrum.

In the present study, we intend to combine polyurethane (PU) and bone marrow-derived stem cells (BMSC)-loaded methacrylated gelatin (GelMA) to invent a kind of injectable hydrogel. This hydrogel could be crosslinked under visible light and prolong the retention of the delivered cells at the graft site and offer a physiological three-dimensional environment for the seeded cells. We will investigate cell viability, proliferation and osteogenic differentiation in this hydrogel.

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P107

Fluorescent-label-based monitoring of multipotent mesenchymal stromal cell pools

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Mesenchymal Stem Cells (MSCs) are considered as highly promising for clinical application in numerous areas. Progress and success in the development of new drugs, including stem cell therapeutics, are highly dependent on the validity of preclinical cell culture studies. However, studies often do not sufficiently consider the individuality of cells from different donors. Instead, a common practice is to combine MSCs from different donors into a common cultivated cell pool and to perform experimental replicates with this cell pool.

The overall goal of the project is to validate whether these cell pools remain equally composed of MSCs from different donors over a relevant time frame of cultivation and thereby adequately reflect the cell properties of all donors included. Here, we aimed to establish a protocol to monitor the composition of cell pools over time.

Adipose-derived MCSs were labeled with different fluorescent dyes and cultured either in individual dishes or as cell pools composed of cells with different labels. Dyes tested included CellTrace™ Cell Proliferation Kits: CellTrace™ Violet, CellTrace™ CFSE, CellTrace™ Yellow, CellTrace™ FarRed, and Cytopainter Cell Tracking Dye Kit: CytoPainter Orange, CytoPainter Green. MSCs were seeded at 3000 cells/cm^2 and 6000 cells/cm^2, they were then cultivated and monitored under standard conditions for up to seven days.

Proliferation and viability were assessed regularly, while flow cytometry and fluorescence microscopy were used to trace the labeling in different channels. Dyes such as CellTrace™ Violet, CellTrace™ Yellow, CytoPainter Green, CytoPainter Orange, and CellTrace™ FarRed labeling had little impact on cell proliferation and viability as compared to unlabeled control cells, whereas CellTrace™ CFSE impaired the cell properties. Cell tracing with discrimination of different labels was possible over a period of seven days.

The here established method for cell tracing allows monitoring and comparing the proliferation properties of MSCs labeled with different cell dyes in direct, pooled co-culture. This is currently being implemented to validate the composition of cell pools from different donors over time, which will deliver crucial insight into whether they could properly reflect the different donor individuals.

P108

Function of urethral brush cell deciphered by genetic models allowing cell type-specific activation

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Brush cells are found in the epithelium of the mammalian respiratory, gastrointestinal and urinary tract. They are sensors for hazardous substances, and they release acetylcholine and other mediators (e.g. cysteinyl leukotrienes and interleukin-25), which activate various cell types including immune cells and nearby sensory nerve fibres. In nose and trachea, these nerve fibres then release substance P (SP) leading to neurogenic inflammation. In the urethra, previous work of our laboratory also indicated a link of brush cells to neurogenic inflammation using the bitter tasting substance denatonium as stimulus. However, denatonium is a broad stimulus activating more than only brush cells. Thus, this study aims to establish a model for specifically activating brush cells and investigating their impact on neurogenic inflammation. We chose an optogenetic approach, using a mouse line (*Chat*-ChR2-EYFP) expressing a fusion protein of the blue light-sensitive ion channel channelrhodopsin-2 (ChR2) and enhanced yellow fluorescent protein (EYFP) expressed under the control of the choline acetyltransferase (*Chat*) promoter. Additionally, we used wildtype and mice lacking brush cells due to genetic deletion of the transcription factor *Pou2f3*. Explanted urethrae from these mouse models were stimulated by blue LED light (optogenetic model) or denatonium and capsacin, a specific nerve fibre stimulus serving as positive control (*Pou2f3*-deficient mice), and the supernatant was analyzed for content of SP and the vasodilatory neuropeptide calcitonin gene-related peptide (CGRP) by ELISA. The expression of the transgene in *Chat*-ChR2-EYFP mice was validated by immunofluorescence with antibodies against the brush cell marker DCLK1 (Double-cortin like kinase 1), showing extensive colocalization (90%, 47/52 cells, from 5 animals). In the supernatant taken from wildtype urethrae stimulated with denatonium and capsaicin, increased release of CGRP was measured by ELISA. Up to now, the data validate *Chat*-ChR2-EYFP mice as a suitable model for studying urethral brush cell function and the explanted urethra as a model to study CGRP release from nerve terminals. Future use of *Pou2f3*(-/-) mice will show the role of brush cell activation in this denatonium-induced release, and we will continue working with ELISA using LED light to specifically activate brush cells in *Chat*-ChR2-EYFP mice.

P109

Influence of peroxisomes in macrophages on inflammatory and pro-fibrotic processes in the lung

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Peroxisomes are an essential intracellular metabolic compartment enclosed by a single unit membrane responsible for the processing of reactive oxygen species (ROS) and various bioactive and pro-inflammatory lipids, which activate signaling pathways like nuclear receptors of the PPAR-family. Despite the presence of peroxisomes in macrophages, their specific roles during inflammatory and pro-fibrotic processes remain largely unexplored. This study aims to a) establish well-defined protocols for studying peroxisomes in macrophages, b) explore how pro-inflammatory stimuli impact the peroxisomal compartment, and c) understand the consequences of peroxisomal dysfunction on the macrophage inflammatory response using primary cultures of murine Pex13 KO MΦ. Initial data from our group indicates an unclear but potentially significant role of peroxisomes in modulating the macrophage inflammatory response and contributing to inflammation resolution. To address this knowledge gap, we will employ the Cre-LoxP-technology to generate macrophage-specific Pex13-KO mice lacking peroxisomes and subject them to inflammation-inducing agents like LPS or TNF-*α*. Functional experimental studies will also be conducted using human THP-1 cells with CrispR/Cas-induced Pex13 KO. A comprehensive set of analytical techniques, including FACS, Western Blots, immunofluorescence, RNAseq, and qRT-PCR, will be utilized to analyze alterations in the macrophage inflammatory response and changes in pro-inflammatory gene transcription caused by peroxisomal dysfunction. The results will be further confirmed through appropriate functional pathway analyses.

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P110

Development of a Novel Immunotherapeutic Approach for Effective Elimination of Ovarian Cancer Cells by Inducing Immunogenic Cell Death

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Ovarian cancer is one of the most lethal gynecological malignancies. Surgery and standard cancer treatment strategies failed to treat local and long distant micro-metastases. Near-infrared photoimmunotherapy (NIR-PIT) is a newly developed molecular targeted cancer treatment, which selectively kills cancer cells and induces therapeutic host immune responses. Ovarian cancer expresses different levels of epidermal growth factor receptor (EGFR), epidermal growth factor receptor 2 (Her2), and folate receptor (FR α). To increase the therapeutic efficacy and specificity of NIR-PIT, we developed novel EGFR, HER2 and FR-α-targeted NIR-PIT agents by conjugating benzylguanine modified IRDye700 (BG-IR700) to EGFR, Her2 and FR α-specific SNAP tagged single chain antibody fragment. The flow cytometry and fluorescence microscopy results confirmed the binding specificity of NIR-PIT agents. After exposure to NIR light, all three NIR-PIT agents led to EGFR, HER2 and FR α-specific cell death, the consequent release of DAMPs biomarkers including calreticulin, Hsp70 and Hsp90 and the rapid release of immunogenic signals including ATP and HMGB1 in vitro. Our results suggest that EGFR, Her2 and FR α-specific NIR-PIT agents could be the potential therapy for ovarian cancer treatment that warrants further preclinical studies.

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P111

Cobalaminstatus in healthy dogs: reference intervals and age relation

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In contrast to humans, parameters evaluating cobalamin status in dogs are not routinely commercially available.

Aim was to establish reference intervals (RIs) for commercial routine canine cobalamin status evaluation comprising homocysteine, serum methylmalonic acid (sMMA), and urinary MMA-to-creatinine-ratio (uMMA:crea).

Between November 2021 and May 2022, reportedly healthy dogs were examined by questionnaires, haematology, biochemistry, urinalysis, and urinary tract ultrasound.

Of 169 dogs, 120 (76 females, 53 males) were eligible with a median age of 3.5 years (range 1-10.5) and a median body weight of 21.5 kg (3.8-53.9). Mixed breed dogs comprised 48% (58/12).

RIs of cobalamin (CLIA) and homocysteine (LC-MS/MS) were 173.6-841.1 pmol/l and 5.9-28.5 µmol/l, respectively. RI of sMMA (LC-MS/MS) was set as 45.3-165.1 µg/l and right sided RI for uMMA:crea <86.1 mg/g. Because of extreme uMMA:crea data distribution with 90% of dogs displaying values <30 mg/g, the 12 dogs with uMMA:crea >30 mg/g underwent a follow-up. A clinical re-check was available in 10/12 dogs performed 10-16 months after initial examination. One dog was reevaluated by a questionnaire only and 1/12 dogs was lost to follow-up. In 7/11 dogs, owners described development of continuous or intermittent chronic gastrointestinal signs and therefore, RI of uMMA:crea was adjusted to 33.2 mg/g. Cobalamin status displayed age related changes with a significant age-related decrease in cobalamin ($p=0.0035$) and increase in sMMA ($p=0.0017$) starting at six years of age.

This study suggests uMMA:crea as an early marker for developing chronic gastrointestinal disease and thus, cobalamin status evaluation is superior to sole cobalamin concentration.

P112

Microenvironmental control of lung cancer metastasis

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Lung cancer (LC) is the overall leading cause of cancer death in humans and the main cause of mortality is metastasis. Non-small cell lung cancer (NSCLC) accounts for 85% of LC and 25-30% of NSCLC patients develop brain metastases.

Increasing evidence has linked LC with other chronic lung diseases such as chronic obstructive pulmonary disease (COPD), which is an independent risk factor for LC and the mortality is correlated with the co-existence of COPD. On the other hand, the connection between pulmonary hypertension (PH) and LC is less well understood. Hypoxia and inflammation in local tumour microenviroment (TME) influence pre-metastatic niche formation and tumour plasticity for the adaptation to the metastatic organ microenvironment. However, the link between COPD/PH-related systemic hypoxia and inflammation-induced microenvironmental changes and the severity of LC and metastasis is unclear. Therefore, we aim to discover the mechanisms by which chronic lung diseases that frequently co-occur with LC promote a (pre)metastatic niche permissive for lung and brain colonization through intra- and inter-organ communication with using PH and COPD disease models.

First, we established an *ex vivo* brain slice co-culture model to investigate the effect of different brain microenvironments on tumour cell invasion *in vitro*. Furthermore, we developed TME mapping approaches for cellular and molecular characterisation of the early and pre-metastatic niches in experimental PH/COPD settings, including immunostaining analyses to detect systemic hypoxia-induced changes in the brain microenvironment. Our results suggest that systemic hypoxia can reduce vascularisation in the brain in a mouse COPD model, while it increases vascularisation and macrophage/microglia content in distinct brain regions in a mouse model of PH. In a complementary approach, we investigate intrinsic molecular mechanisms of cancer cells that promote adaptation to hypoxic conditions and metastasis in a KRAS-mutated lung adenocarcinoma model system.

Collectively, this work extends our understanding of the contribution of chronic lung diseases and intrinsic factors to LC metastasis to the brain and within the lung.

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P113

An epithelial defense program of the gall bladder epithelium orchestrated by tuft cells

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The gallbladder epithelium is composed of two main cell types: columnar cells (frequent) and tuft cells (rare). Gallbladder tuft cells sense the microbial metabolite propionate and respond with release of acetylcholine, which triggers mucin secretion, an innate defence mechanism, from columnar cells. It remained unclear, however, how the spatially limited release of acetylcholine from a rare epithelial cell type translates into a mass release of mucins all over the mucosal surface. Here, we hypothesized that mucin release is accompanied by ion transport and that the spread of information might operate through gap junctions or release of purines through hemichannels.

Mucin release and transepithelial ion transport were investigated in explanted mouse gallbladders with a glycoprotein dot blot assay and Ussing chamber experiments, respectively. Expression of connexins and purinergic receptors was analysed *in silico* utilizing publicly available scRNA-seq data. This guided selection of ligands and inhibitors for further use in mucin release and ion transport experiments. Immunofluorescence is used to validate expression data.

In silico analysis identified *Adora1*, *P2rx4* and *P2ry2* as the main purinergic receptors expressed in the epithelium. First preliminary experiments showed opposing effects of the ADORA1 agonist N6-cyclopentyladenosine and of ATP on short circuit current in the Ussing chamber. The ATP effect was blocked by the P2rx4 antagonist 5-(3-Bromophenyl)-1,3-dihydro-2H-Benzofuro[3,2-e]-1,4-diazepin-2-one (5-BDBD). Sunny.py, a program written in python language, was developed to ease data interpretation of Ussing chamber data. Dot-plot analysis confirmed that 5 mM propionate stimulates mucins release in the gallbladder. *In silico* analysis identified *Gja1* (Cx43), *Gjb1* (Cx32), and *Gjb2* (Cx26) as most expressed connexins. Preliminary immunofluorescence showed a discrete expression of Cx43 in the gallbladder epithelium.

The first data indicate involvement of purinergic signalling in regulating transepithelial ion transport in the gallbladder. Computer analysis showed a specific pattern in the expression of connexins for tuft and columnar cells in the gallbladder. To validate this *in silico* model, additional immunohistochemical and functional analyses have to be made. In a second step, the relevance for propagation of tuft cell-triggered signalling will be investigated.

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P114

Computational approach for modeling a 3D vertebrate body by fusion of various imaging technologies

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Bone is a unique organ that undergoes continuous remodelling throughout life. The detailed understanding of healthy bone and osteoporotic bone relies majorly on deciphering structural, cellular, and molecular changes. The advancement in computational hardware and software allowed the researchers to develop several 3D simulations, virtual, and interactive models. 3D modelling and experiments became more closely integrated in understanding the mechanism underlying severe human diseases. While the structurally unique microenvironment of bone presents many challenges for the development of 3D models. As scientific and clinical researchers continue to explore different therapeutic interventions to tackle osteoporosis and understand bone cellular environment, the establishment of 3D models has become important. The established 3D models of bone so far were able to replicate some properties of bone but not the complete complex architecture. Importantly, bone research relies on the radiological data (Micro-CT) and histological data to unravel the underlying complexity of diseases like osteoporosis. Therefore, virtual, accurate, and more realistic model of bone can be developed using both 3D radiological data and 2D histological data. Previous established virtual models of other organs were developed only through in vivo and ex vivo imaging techniques. Inclusion of other imaging data like histology and immuno-histochemistry are essential for the development of virtual model of bone. Therefore, this proposed project aims to set up a 3D anatomical modelling framework for creation of the v-Bone model in which various 3D (Micro-CT) and 2D (histology, immunostaining) images representing different physiological states and anatomical structures of the vertebral bodies of preclinical diabetic and osteoporotic models are fused. The aim of this project is to provide a 3D model of vertebral bodies to investigate and compare diabetic and osteoporotic relevant features.

Preliminary work on segmentation was carried out using dental implants as an example to investigate segmentation methods. The implant coating plays an important role in the healing phase. Model-free segmentation of bone volume at the implant-bone interface was investigated on implants with different coatings showing significant mineralization for the calcium phosphate mixed-coated implants. Model-free segmentation proved to be efficient in the micro range for segmentation given a high resolution of Micro-CT data.

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P115

Evaluation of Nanobody Anti-Mouse and Anti-Rabbit IgG as Secondary Antibodies in Immunoassays

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As a part of diagnostics or immunotherapeutic, antibodies and antibody fragments have several important functions, including their specificity, serum half-life, immunogenicity, folding-stability, and especially the binding affinity. In immunoassays, Immunoglobulin G(IgG) can detect the primary antibodies which bind to target molecules, then amplify the primary signals. Thus, IgG is the common secondary antibody. Because of difficult recombinantly, IgG modified antibodies must be obtained from living animals or mammalian cell culture systems, high manufacturing costs and time consumption are also challenging. Heavy-chain antibodies (HCAbs), have been described in species derived from camelid. Since they are composed of two identical heavy chains and are devoid of light chains, their antigen-binding part is composed of only one single immunoglobulin (Ig) variable region (VHH or Nanobody), this makes nanobodies are merely 1.5 × 2.5 nm and 15 kD in size. Moreover, nanobodies also can be easily expressed functional and high yields in bacteria and mammalian cells, even easily modified at the genetic level for labeling purposes in many ways. Additionally, our group has established an innovative enzymatic site-specific conjugation strategy based on the SNAP-tag technology, so we are aiming to extend the application of anti-IgG nanobodies and SNAP-tag technology to develop a sustainable alternative secondary antibody in immunoassays.

We have generated anti-IgG-SNAP and anti-IgG-HRP nanobodies fusion proteins as secondary antibodies, then labeled anti-IgG-SNAP nanobodies with fluorescence dyes. From our results, anti-IgG-SNAP-BG-647 nanobodies exhibit specific binding as the same as conventional IgGs in breast cancer cell lines. Moreover, anti-IgG-HRP nanobodies perform well in immunoassays such as Western blot and immunohistochemistry (IHC), and also confirm the application possibility of tyramide signal amplification (TSA)-based immunofluorescent multiplex (mIF).

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P116

Tracking extracellular vesicle uptake in equine chondrocytes from mesenchymal stem cells using CFSE Labeling

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Osteoarthritis (OA) is a degenerative multifactorial joint disease that commonly causes lameness in recreational and sport horses. There is growing interest in utilizing mesenchymal stem cells (MSCs) derived from bone marrow or adipose tissue to potentially treat osteoarthritis by regenerating and restoring damaged articular cartilage. MSCs release trophic factors and small extracellular vesicles (sEVs) that can stimulate regeneration. To study the uptake of sEVs by target cells, like chondrocytes, it is important to use bright labels that can be detected without creating any false signals.

sEV from equine donors (n=3) were purified, ultrafiltrated and then incubated with the 5-(and-6)-Carboxyfluorescein Diacetate Succinimidyl Ester (CFSE) dye for 2 h. Afterwards, the labeled sEVs were separated from free CFSE dye by obtaining the relevant fractions using size exclusion chromatography. These were transferred to chondrocytes previously seeded on glass slides in a 24 well plate and incubated for additional 24 h. After fixation of these cells, Hoechst staining of the nuclei was performed to counterstain chondrocytes and sEVs were detected within cells by fluorescence microscopy.

Following the administration of sEVs, fluorescent microscopy reveals the presence of small green dots within the target cells. These dots are evenly distributed throughout the cell body, with a more intense signal observed near the nucleus. No fluorescent signal is detected outside of the cells.

By utilizing CFSE staining, it is possible to accurately locate purified sEVs within the cell, ensuring successful transfer to target cells. Size exclusion chromatography is a reliable technique that prevents any artifacts and preserves the biological function of sEVs. This method is not only compatible with other tracking modalities like flow cytometry but can also be easily implemented across different laboratories.

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P117

The Role of CD155 in Hodgkin Lymphoma

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Hodgkin lymphoma is a B-cell derived malignancy diagnosed in young and adolescents, a key feature is a very strong involvement of the immune system in this disease.

Here we investigated the role of CD155, also known as the Poliovirus receptor in Hodgkin lymphoma. CD155 is a cell surface protein expressed on dendritic cells, macrophages, and malignant transformed cells including Hodgkin lymphoma (HL) cell lines. CD155 has been attributed to cell-intrinsic functions such as cell proliferation, survival and migration (*1).* Also, it interacts with distinct immune receptors such as CD226, TIGIT and CD96 on the surface of NK and T cells (*2*), thereby modulating immune responses. We hypothesize, that these immune-inhibitory signals may lead to pro-tumor functions in Hodgkin lymphoma (HL).

In this project, first, the expression of CD155 was analyzed in various HL and non-HL cell lines. Cell lines such as L-540, HDLM-2, Karpas 422 and Karpas 299 showed high expression of CD155. Second, it was investigated whether the expression of CD155 is regulated in HL, NHL, monocytes and B cells through cytokines, toll-like receptors (TLRs) or Interleukin-22 (IL-22). However, no significant modulation was observed after the respective treatment.

Future experiments aim to delete CD155 protein in HL cell lines. The generated $CD155^{-/-}$ and $CD155^{+/+}$ ("control") pairs will be compared to understand the intrinsic functions. Moreover, free expression of distinct variants (CD155-ED, CD155-Y398F and CD155-soluble) will further enlighten the role of CD155. Having established those model lines will also allow for interaction studies with immune cells.

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P118

A novel pre-targting drug delivery system for the treatment of breast cancer

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Female breast cancer is the leading cause of cancer incidence and fifth leading cause of cancer mortality worldwide with 2.3 million new cases and 685,000 deaths in 2020. To date, surgery, chemotherapy and radiotherapy are still the mainstream treatment of breast cancer. However, the traditional chemotherapy confronts the obstacle that the drug is inclined to aim at fast-dividing cells rather than targeting tumor cells specifically. The application of monoclonal antibody (mAb) has achieved the targeted therapy, but not all of them exhibit enough cytotoxicity so could be translated to clinical use. The antibody drug conjugate (ADC) which is the combination of mAb and small cytotoxic agent allows to use mAb as vehicle to transport the cytotoxic agent to tumor site specifically. All the approved ADCs now are based on full-length antibodies, and are using the lysine or cysteine residues for direct conjugation which leads to heterogeneous products, and long circulation time due to the big size of the intact antibodies. In this study, we developed a pre-targeting drug delivery system based on EGFR, EpCAM, Her2 and Trop2 as targets, scFv as backbone, Val-Cit dipeptide as cleavable linker, MMAE as payload and SNAP-tag technology as conjugation method to establish the novel ADCs. Rapid and efficient conjugation was achieved by SNAP-tag technology. The binding, internalization and colocalization properties of the pre-targeting system were confirmed by flow cytometry and fluorescence microscopy. The dose-dependent cytotoxicity was evaluated in cell lines expressing different levels of antigens. All ADCs showed specific cytotoxicity to antigen-expressing cell lines via inducing apoptosis at a nanomolar concentration. Our study demonstrated that the pre-targeting system could be promising ADCs for the treatment of breast cancer.

P119

3D Printed Bone Constructs: Combining Polyurethane and Methacrylated Gelatin with Bone Marrow-Derived Stem Cells

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Bone tissue engineering offers a promising approach to address critical-size bone defects that cannot be adequately repaired by the body's natural healing processes. In this study, we explore the potential of 3D printed scaffolds for bone tissue engineering using a combination of polyurethane (PU) and bone marrow-derived stem cells (BMSC)-loaded methacrylated gelatin (GelMA).

Traditional treatment methods for critical-size bone defects involve autografts, allografts, and xenografts, but they come with limitations. To overcome these challenges, we employ additive manufacturing techniques based on computer-aided design/computer-aided manufacturing (CAD/CAM) systems to produce complex structures with tailored properties. Customized patient-specific scaffolds can thus be prepared, particularly suitable for irregularly shaped defects such as craniofacial defects.

Bioinks, which are hydrogels capable of carrying live cells, play a crucial role in the 3D printing process. While synthetic polymers offer superior mechanical properties, their biocompatibility and bioactivity are limited. On the other hand, natural polymers exhibit excellent biocompatibility and bioactivity due to their resemblance to the extracellular matrix but lack sufficient mechanical strength.

To address these challenges, we propose a hybrid scaffold composed of PU and BMSC-loaded GelMA. The hybrid scaffold aims to offer mechanical strength suitable for cartilage and non-load-bearing bone tissue, thanks to the presence of PU. Simultaneously, BMSC-loaded GelMA enhances cell affinity and osteoinductivity, contributing to improved tissue regeneration potential.

By combining the strengths of both synthetic and natural materials, this study seeks to advance bone tissue engineering and pave the way for innovative treatment options for critical-size bone defects. The results obtained from investigating the properties of the PU/GelMA hybrid scaffold holds significant promise for the field of bone tissue regeneration.

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bold = first author

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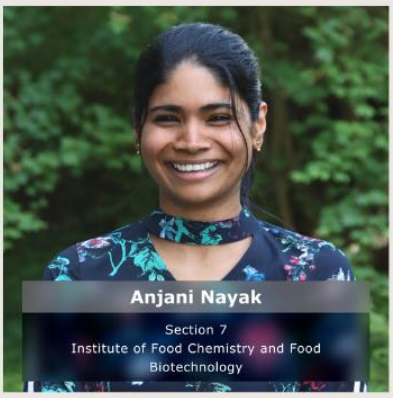

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