<interact> 2020

27 and 28 February

Biomedical Center Munich

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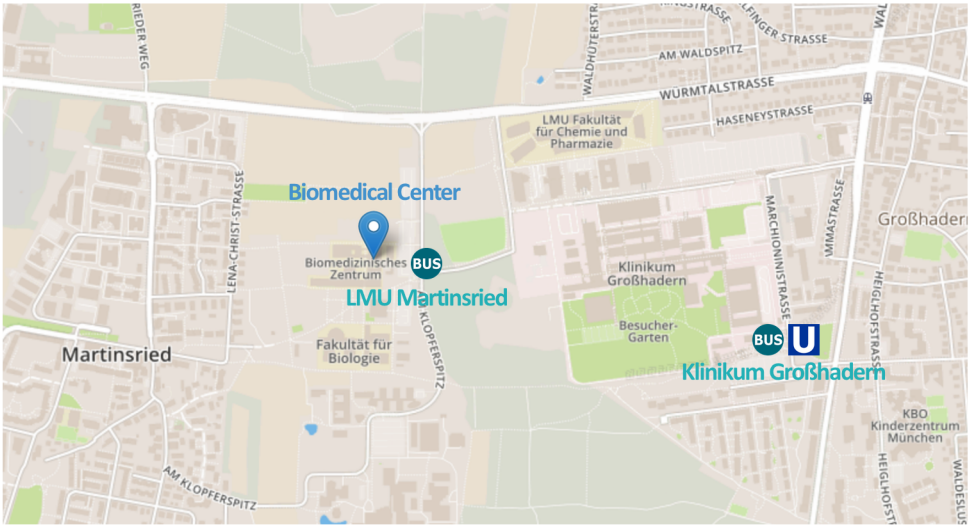
Location

Biomedical Center, LMU Munich

U6 Klinikum Großhadern (Endline)

BUS Line 266, Stop LMU Martinsried
Direction Planegg or Max-Planck Institute

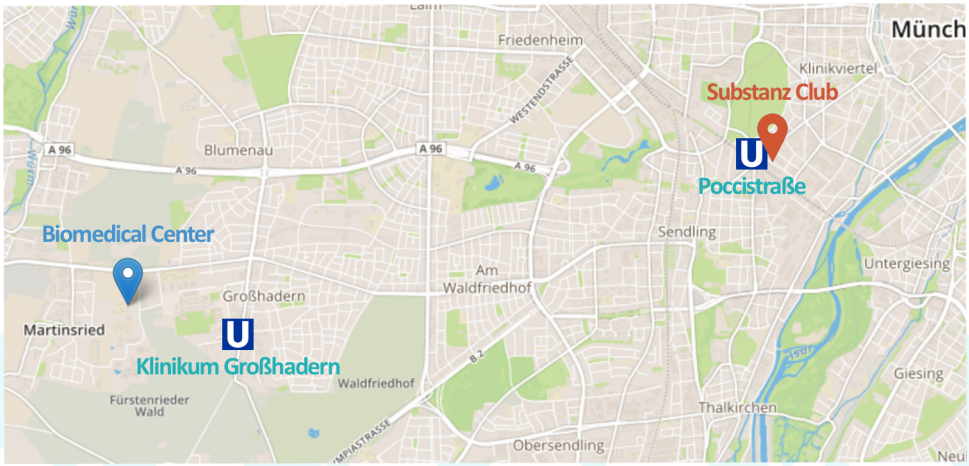
Großhaderner Str. 9
82152 Planegg-Martinsried



Substanz Club

U6 Poccistraße
Direction Garching-Forschungszentrum

Ruppert Str. 28
80337 Munich



Schedule

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Schedule

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Keynote Lecture I

27.02.
N02.040

Morgan Beeby

Imperial College
London, UK

Prof. Morgan Beeby leads an interdisciplinary research group at Imperial College London interested in understanding how molecular machines have evolved, such as the bacteria flagella motor, and how these complex machineries are fine-tuned for functioning in different environments. To achieve this, they image their structures in-situ using 3D electron microscopy, and contextualize that structural data against the evolutionary history of molecular machines by analyzing the sequences of their component proteins. In combination with biophysical methods and global collaborations, the group was able to describe for the first time how such motors function and the factors involved in driving their evolution.

Dr. Morgan Beeby represents the new generation of group leaders who were able to establish their groups in the modern competitive environment, alongside bringing novel and exciting ways of working, communicating and exploring ideas in addition to academic work. Having completed his PhD at UCLA and his postdoctoral training at CalTech, he became a group leader at Imperial College London's structural biology department at the age of 35. In addition to doing great science, Dr Morgan Beeby actively takes part in outreach schemes around the city by presenting science to the general public. The topics are usually communicated through creatively designed human sized lego models of bacteria flagella motors and injectisome, along with other creative art/digital projects. He is also known to let his curiosity take him into new directions, such as designing and coding a robot minion which conveniantly pours liquid nitrogen for you during long elecctron miscroscope measurement slots (this really happened!).

Keynote Lecture II

27.02.
N02.040

Tak Wah Mak

University of Alberta
Toronto, CA

Prof. Tak Wah Mak is a world-leading professor of Immunology from the University of Alberta in Toronto, Canada. He revolutionized the field by first discovering the T-cell antigen receptor structure in 1984, then going further into making many novel discoveries, including how neurotransmitters are able to affect and communicate with the immune system.

Professor Mak was born in Guangzhou, China and immigrated to the US to pursue a PhD at the University of Wisconsin in Madison before moving to Canada permanently. In addition to his academic career, he is also a founder of Agios Pharmaceuticals, a company that developed a first-ever compound approved by FDA to specifically target cancer metabolism in cancer treatment. Currently, his lab focuses on the mechanisms underlying immune responses and tumorigenesis.

Keynote Lecture III

28.02.
N02.040

Maria Carmo-Fonseca

University of Lisbon
Lisbon, PT

Prof. Maria Carmo-Fonseca is a full professor at the Faculty of Medicine, at the University of Lisbon, and Executive Director of the Institute of Molecular Medicine in Lisbon. She is most known for her work on understanding the role of mammalian splicing pathways and how their deregulation leads to diseases such as cancer. Her group's work focuses on understanding the fundamental mechanisms of gene regulation by RNA molecules, specifically the epigenetics and alternative splicing of immune T-cell differentiation and leukemogenesis, with the aim of developing novel therapeutic targets for medicine. Her group tackles these questions through collaborations and interdisciplinary research, using techniques in cell biology, genomics, structural biology, and bioinformatics, with the end goal of applying their findings to clinical settings.

After finishing her studies for MD in the medical school of Lisbon University, Portugal, Dr. Carmo-Fonseca went on to complete her PhD in Cell Biology at the Institute of Gulbenkian in Oeiras, Portugal. After this she worked as a post-doctoral researcher at the EMBL in Heidelberg, Germany, for several years before taking up her current position as group leader. Currently, Dr. Carmo-Fonseca is also a monitoring editor for the Journal of Cell Science and Director of the Harvard Medical School-Portugal Program.

Keynote Lecture IV

28.02.
N02.040

Karim El Kasmi

Boehringer Ingelheim
Ingelheim am Rhein, DE

Dr. Karim El Kasmi is currently a Laboratory Head of the Immunology and Respiratory Research Department at Boehringer Ingelheim in Biberach an der Riß, Germany. Before this he was pursuing a successful academic career in the USA at the University of Colorado, Boulder, as an assistant and associate professor, where his research has influenced our current understanding of macrophages and pathological innate immune signaling pathways.

Dr. Kasmi has recently made the transition to Boehringer Ingelheim where he leads numerous drug discovery research projects focused on first class new therapeutic concepts for the treatment of patients who suffer from severe tissue fibrosis and chronic inflammation. Currently, he investigates tumor microenvironments as well as hypoxial induced pulmonary hypertension in rodent models with a focus on drug discovery.

Karin Bodewits

Dr. Karin Bodewits is co-founder and owner of NaturalScience.Careers and author of the career fables in Science Magazine as well as several books, such as 'You must be very intelligent. The PhD Delusion'. Dr. Bodewits has obtained her PhD at the University of Edinburgh and now among other things, she gives career and soft-skill seminars to young scientists, helping them to navigate in the world outside of academia.

At the <interact>2020 symposium Karin will give an inspirational talk on career options for PhD in science.

Judith Bergner, a certified psychologist and director of Skillfactors, has obtained her Executive MBA focused in Change Management from the University of St.Gallen and has over 20 years experience in coaching, team building and entrepreneurship.

In her session at <interact> Judith will teach us how to reach an optimal work-life balance.

Judith Bergner

#breakingbarriers

What do we do science for? As young scientists, we are challenged to find research questions that are highly specialized, feasible, appealing to funding agencies and that can, ideally, lead to high impact publications. But, where do the needs of society come into play?

In this panel, we will discuss the driving forces that shape the way we do science: from the need of finding a research niche, to the future of scientific publications. In <interact> we want to not only discuss the future of science, but understand how we can help our fields to be more inclusive, to learn how to be fearless for ***#breakingbarriers***, and how to be better prepared for the current academic and outside world.

These and more questions will be addressed by our panelists: Dr. Karim El Kasmi, Dr. Maria Carmo-Fonseca, Dr. Tak Wah Mak, Dr. Karin Bodewits and Dr. Morgan Beeby. As a special guest, Children of Doom will be moderating the discussion.

Come and join us!

Abstract Finder - Student Talk Sessions

Abstract Finder - Poster Sessions

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Student Talk Session I - Chromatin Biology

27.02.
N02.040

01 Tajda Klobučar

Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Lisbon, PT

IMPLICON: a high-resolution method to uncover DNA methylation at imprinted regions.

Genomic imprinting is an epigenetic phenomenon leading to parental allele-specific gene expression. Its regulation is determined by differential DNA methylation between parental alleles at specific regulatory elements, known as imprinting control regions (ICRs). Correct dosage of imprinted genes is crucial for normal development and its dysregulation thus results in a number of human disorders. Although many approaches can be used for ICR methylation inspection, we lack an easy and cost-effective method to determine methylation at multiple imprinted regions at once. Here, we present IMPLICON, a high-throughput method targeted to measure DNA methylation levels at ICRs with base-pair resolution and over 1000-fold genomic coverage. Initially tested on samples from adult mice tissues, we further on validated the method in hybrid mice from reciprocal crosses for which we could discriminate methylation profiles in the two parental alleles. Finally, we developed a human version of IMPLICON, used to detect imprinting errors in human embryonic and induced pluripotent stem cells. IMPLICON provides a rapid, robust and cost-effective method, which we believe could become the gold standard for both imprinting research and diagnostics.

02 Haris Khan

Biomedical Center, Ludwig-Maximilians-Universität, Munich, DE

Want to control how much you eat? Story of a conserved transcription factor that regulates feeding behaviour in response to nutrients.

Understanding the regulation of metabolism is important to uncover the challenges behind nutritional disorders. Decades of advances in the field have identified many hormones that coordinate the response between organs and cell-types to achieve metabolic homeostasis. Little is known how the synthesis of these hormones is regulated. Using Drosophila genetics, we have identified the transcription factor CrebA, a homolog of the mammalian Creb3 transcription factor family, as a regulator of feeding behavior. Genome-wide sequencing techniques indicate that CrebA coordinates the expression of the classic early secretory machinery genes in response to nutrients. We also show that feeding regulates CrebA mRNA and protein levels. We are in the process of testing whether the co-regulation of the classic secretory machinery regulates the hormonal cell-to-cell communication of the nutritional status of an organism. Using mass spectrometry techniques we have identified signaling molecules in the circulating hemolymph that change with feeding hinting at their role in the regulation of feeding behavior. We are currently elucidating which of these signals are affected if CrebA levels are manipulated in the organism. Taken together, our findings indicate that the alteration in protein levels of CrebA in response to nutrients orchestrates a negative feedback loop which determines the nutritional state of the organism.

03 Ashish Kumar Singh

Molecular Biology, Biomedical Center, LMU, Munich, DE

The biogenesis and function of nucleosome landscape.

Nucleosome organization is essential for gene regulation and is established by a complex interplay of many redundant processes, including transcription and nucleosome remodeling factors. How individual processes contribute to nucleosome organization remains unclear. By yeast genome engineering, we establish a system to cleanly dissect the effects of transcription and individual nucleosome remodelers on the nucleosome landscape in vivo. We find that RNA Polymerase II-dependent transcription destroys nucleosome landscape genome-wide and overrides effects of nucleosome remodelers. By inhibiting transcription in a strain lacking all spacing remodelers known so far, we identify novel nucleosome spacing activity in the INO80 remodeler. We exploit our system to dissect the function of individual modules in the INO80 complex and identify Arp8 module as a critical regulator of nucleosome spacing function. Further, by systematically depleting individual factors, we identify conditions where nucleosomes attain DNA sequence dependent position on a genome-wide scale. Finally, we show that remodelers dependent nucleosome spacing is essential for maintaining genome integrity by protecting underlying DNA from double strand breaks, ectopic recombination, transposon integration and chromatin accessibility. Our results lead to a unifying model of the biogenesis of the nucleosome landscape and suggest that it evolved not only to regulate but also to protect the genome.

04 Mykhailo Tolkachov

Ludwig-Maximilians-Universität, Munich, DE

Design and development of the next-generation activating transposons for in vivo transposon screens.

The transposon-mediated insertional mutagenesis (TMIM) screen is a powerful tool for studying molecular biology underlying cancer. Following the forward genetics principle, TMIM introduces quasi-unbiased genetic perturbations resulting in a variety of phenovariants. The analysis of tumour samples and identification of common insertion sites (CISs) found within the genomes of malignant phenotypes led to the discovery of cancer drivers - genes which cause and maintain cancer either by a gain of function (GOF), i.e. oncogenes, or loss of function (LOF), known as tumour suppressors. The previous concept of transposon screens relied on random mutagenesis via activating/inactivating transposons (e.g. ATPs). Those screens, however, discovered almost exclusively tumour suppressors, and only a few oncogenes. While this might have biological reasons as well, we hypothesise that another cause is the high inactivation efficiency of bi-functional transposons. To address this, we propose a new generation of transposon constructs optimised for driving overexpression of genes. Here, we generate transgenic mouse lines containing novel transposons which utilise a CRISPRa based approach for gene activation. We complement these with an additional mouse line expressing both transposase and the CRISPR-SunTag activator as a single genotype to facilitate breeding of experimental animal cohorts.

05 Lin Chen

Max von Pettenkofer Institute, Virology, Ludwig-Maximilians-Universität, Munich, DE

Proteomic profiling of human CD4+ T cells within the first 24 hours of HIV-1 infection

Recent findings from proteome-wide analysis showed that human immunodeficiency virus 1 (HIV-1) reprograms the host target cell to facilitate viral replication. This reprogramming is supposed to be regulated by a plethora of post-translational modifications (PTM), adding another level of complexity to the human-HIV-1 interaction network. General low infectivity of full-length HIV-1 lab strains (resulting in a small population of infected cells harboring active replicating virus) combined with the low-stoichiometric nature of PTMs makes precise quantification of site-specific changes extremely difficult

Hence, we optimized a bottom-up mass spectrometry based proteomic approach for quantification of proteins and PTM sites from lysates of full-length HIV-1 infected cells. From a single time-course experiment, we were able to monitor changes in abundance of over 5000 proteins in a HIV-1 infected human T cell line. Simultaneously, we determined time-resolved quantitative data of over 3600 unique phosphorylation sites as well as more than 800 acetylation sites. The resulting proteomic profiles can be used (i) for prioritizing specific proteins or PTM sites for functional analysis, (ii) might support the construction of mechanistic models for a more comprehensive understanding of the HIV-1 replication cycle, and (iii) will help to identify new attack points for the cure of HIV infected individuals.

06 Sibgha Tahir

Klinikum der Universität, Ludwig-Maximilians-Universität, Munich, DE

Endothelial CD40 mediates von Willebrand factor-dependent inflammatory venothrombosis and leukocyte extravasation in the microcirculation

Background: CD40-CD40 ligand (CD40L) dependent von Willebrand factor (vWF) release plays an important role in platelet activation, which has been described in larger vessels but unknown in microcirculation. Here we studied whether CD40 is expressed in murine microvessels, whether CD40L induces platelet adhesion and leukocyte activation, and how deficiency of vWF cleaving enzyme ADAMTS13 affects these processes.

Methods and results: CD40L induced vWF-dependent platelet string formation was analyzed in murine cremaster microcirculation in vivo. CD40 and vWF expression was studied in isolated fixed cremasters. CD40 was expressed only in venous endothelium and under inflammatory conditions. CD40L treatment augmented the number of platelet strings exclusively in venules which was significantly increased in ADAMTS13 KO mice. Consequently, venules were subjected to extensive thrombus formation. Additionally, circulating leukocytes rapidly adhered to these platelet strings followed by their transmigration.

Conclusion: CD40-CD40L dyad plays an important role in microvascularvWF release, platelet adhesion and leukocyte transmigration but only in venules under inflammation. Lack of ADAMTS13 causes thrombus formation. Results suggest CD40 expression and ADAMTS13 activity to be important targets for preventing inflammatory thrombosis.

07 Ariane Schumski Institute for Cardiovascular Prevention, Munich, DE

Endotoxemia accelerates atherogenic monocyte recruitment through NET-resident H2A.

Background: Acute infection is a well- established risk factor of destabilization of pre-existing atherosclerotic lesions. However, the nature of the underlying processes remains unclear. Of note, epidemiologic studies show that endotoxemia results in heightened lesion development as well as acceleration of atheroprogression. LPS is a potent activator of circulating immune cells including neutrophils, which foster inflammation through expelled chromatin (NETs).
Material and methods: To investigate the role of endotoxemia- induced NET- formation in atherosclerosis we studied arterial monocyte adhesion by intravital microscopy of the carotid artery. Furthermore, mechanisms of monocyte adhesion to NETs were studied in vitro adhesion assays and by atomic force microscopy.

Results: In endotoxemia neutrophils release NETs decorated with different granule proteins and as well as histones. Our data show, that NET- resident H2A causes charge dependent monocyte adhesion to NETs and accelerates atherosclerosis. If H2A is blocked, arterial monocytes adhere less and lesion formation is reduced.

Conclusions: This study provides a mechanistic link be-tween NETs and monocyte adhesion at the site of developing atherosclerotic lesions. By combining a mouse model of early atherosclerosis and in vitro studies, we identified that endotoxemia-driven NET formation accelerates atherosclerosis through increased monocyte adhesion at sites of atherosclerotic lesions, which can be limited by blocking NET-resident H2A.

08 Vidya P. Nair Institute of Virology, Helmholtz Zentrum, Munich, DE

Analysing the role of HERV-K(HML-2) in neuronal differentiation and brain development.

More than 10% of the human genome encompasses sequences of infectious retroviruses known as human endogenous retroviruses (HERVs), presumably integrated 2-40 m.y.a during primate evolution. Substantial scientific research on HERVs has been carried out in recent years. Here, we generated a CRISPR activation system in human embryonic stem cells to actively regulate the most recently integrated member of the HERV family, HERV-K(HML2) and attempted to delve into how these elements influence the neuronal differentiation and brain development. The HERV-K(HML2) overexpressing stable H9-dCas9VP64 embryonic stem cell line were established for active transcription of HERV-K(HML2) LTRs. Steady monitoring of the differentiation of these cells into cortical neurons showed considerable decrease in the overall MAP2 expression levels as well as less functionality of neurons, suggesting the potential role of these elements in neurodegeneration and related disorders. Interestingly, the differentiation into dopaminergic neuronal lineage showed no signs of neurodegeneration suggesting a cortical specific outcome. A whole genome RNA sequencing of HERV-K activated cortical neurons show enrichment in neuron-related genes, suggesting a clear correlation of HERV-K(HML-2) activation and neuron-related gene expression. Furthermore, analysis of forebrain organoids generated from these cells showed a difference in growth as well as a marked variation in the organisation of cell layers and expression of cell type specific markers. Taken together, these results point towards the active participation of HERV-K(HML-2) elements in remodeling the homeostasis of human brain development which could potentially translate into several neuron related dysfunctions.

09 Lisa Käshammer

Gene Center, Ludwig-Maximilians-Universität, Munich, DE

Mechanism of DNA end sensing and processing by Mre11-Rad50

DNA double strand breaks (DSBs) threaten genome stability in all kingdoms of life and are linked to tumorigenesis in humans. DSBs are caused by chemicals or radiation, but also occur during DNA replication and meiosis. They are chemically very heterogeneous and often need enzymatic processing to enable repair through homologous recombination or end joining pathways. The Mre11-Rad50 complex is an evolutionary conserved ATP dependent endo/exonuclease that detects and cuts diverse and obstructed DNA ends. However, the structural mechanism of ATP dependent DNA sensing and processing has not been resolved.

We solved the cryo-EM structures of the E. coli Mre11-Rad50 homolog bound to ATPS at 3.5 Å and bound to DNA and ADP at 4.2 Å resolution. The structures capture “resting” and “DNA cutting” states. In the resting state, the Rad50 ATPase domains block and inhibit Mre11’s nuclease active sites while the two coiled-coils form an open structure. DNA binding induces an extensive structural change. The coiled-coils close into a long rod and together with the ATPase domains form a clamp around a DNA duplex, narrow enough to sense DNA blocks. The Mre11 dimer moves to the side of the Rad50 nucleotide binding domains, generating a joint active site channel where DNA ends are bound and DNA is cleaved. The structures and biochemical analysis explain how the Mre11-Rad50 complexes process diverse DNA ends and suggest a chemo-mechanical gating function of the coiled-coils in DNA break repair.

10 Marijke Jansma

Gene Center, Ludwig-Maximilians-Universität, Munich, DE

Near-Complete Structure and Model of Tel1ATM from Chaetomium Thermophilum Reveals a Robust Autoinhibited ATP-State

Ataxia-Telangiectasia Mutated (ATM, Tel1 in yeast and fungi) is a central signaling kinase that responds to DNA double-strand breaks. In its resting state, ATM resides in the cell as an autoinhibited dimer, but once it is activated ATM phosphorylates hundreds of targets, thereby orchestrating the DNA damage response.

We used cryo-electron microscopy (cryo-EM) to solve the structure of endogenous dimeric ctTel1, anATM ortholog from the thermophilic eukaryote Chaetomium thermophilum, in complex with a nanobody. Our structure is the first complete model of any ATM kinase. Major parts, including the kinase domain with ATPγS bound in the active site, are resolved to a resolution of 2.8 Å. We also solved the structure of the regulatory N-terminal Spiral and Pincer domain in an open and a closed conformation to a resolution of 3.6 and 3.4 Å respectively, allowing us to build a side-chain model for nearly the entire polypeptide. The high resolution allowed us to have a detailed look at the active site and dissect the features of the autoinhibitory circuitry. Our structure provides the framework for future studies on the activation mechanism of this important kinase.

11 Hugo van den Hoek Max Planck Institute of Biochemistry, Munich, DE

Exploring the molecular landscape near the ciliary base of Chlamydomonas with in situ cryo-electron tomography

Cells accomplish the biochemical reactions of life by concentrating their proteins into a variety of subcellular compartments called organelles. Our group explores the relationship between the form of the organelle and the function of its resident macromolecules. How does organelle architecture direct molecular function, and reciprocally, how do macromolecules sculpt and shape organelles? To investigate these questions, we use focused ion beam (FIB) milling of frozen cells followed by cryo-electron tomography to image macromolecules within their native cellular environment. Through a combination of nanometer-precision localization and high-resolution structural analysis, we aim to chart the molecular landscapes of organelles. Thanks to its superb cryo-EM contrast and textbook organelle architecture, the unicellular green alga Chlamydomonas is an ideal specimen for this approach. We have taken a holistic approach to survey the whole integrated “planimal”, with in situ molecular studies of the nuclear envelope, ER, Golgi, basal body apparatus (centrioles), and chloroplast.

In this presentation, I will show the results of a selection of these studies. I will talk about cilia-related structures, showing new findings on centriole structure, the composition of the ciliary base, averaging intraflagellar transport train particles, as well as mapping their assembly.

12 Elena Davydova Institute of Structural Biology, Helmholz Zentrum, Munich, DE

NUFIP2, a new cofactor, which promotes recognition and regulation of ICOS mRNA by Roquin binding

In T cells, the paralogous proteins Roquin-1 and Roquin-2 repress post-transcriptionally target mRNAs of co-stimulatory factors, like ICOS and Ox40. Roquin interacts with CCR4-CAF1-NOT de-adenylation and mRNA decapping complexes after binding on the 3’UTR of the mRNAs and induce their decay. The Roquin proteins are essential for the immune cell function and postnatal survival of mice. Furthermore, the so-called sanroque mutation in the ROQ domain of murine Roquin leads to severe autoimmunity.

Because there was little knowledge of modulatory cofactors of Roquin-induced mRNA decay, an siRNA screen was performed. NUFIP2 (Nuclear FMRP Interacting Protein 2), an unstructured protein, was identified as a cofactor of Roquin-mediated ICOS repression. Using Surface Plasmon Resonance, we showed that Roquin binds directly and with high affinity to NUFIP2. In parallel, we showed that both Roquin-1 and NUFIP2 bind to tandem stem-loops, each of them separately, but also stronger together to the ICOS and Ox40 using EMSAs. Moreover, quantitative RT-PCR,after treatment with actinomycin D, showed that the downregulation of target transcripts was lower in cells with knocked down NUFIP2, indicating that NUFIP2 cooperates with Roquin to induce ICOS mRNA decay. All these data confirmed that NUFIP2 is a cofactor that contributes to mRNA target recognition by Roquin.Structural studies on the cooperative interaction of Roquin and NUFIP2 are underway to better understand their interaction.

13 Emilio Dorigatti Faculty of Mathematics, Informatics and Statistics, LMU, Munich, DE

Joint optimization of string-of-beads vaccines with variable-length spacers.

Modern vaccine research is focused on in-silico design of synthetic, epitope-based vaccines. String-of-beads vaccines are composed of an alternation of epitopes and spacers, whose purpose is to elicit correct cleavage of the vaccine and presentation of the epitopes by the MHC. Conceptually, vaccine design poses two distinct problems: selecting the best epitopes, and generating suitable spacers; current state-of-the-art tools focus only on one step, and different tools must be applied sequentially to design a vaccine. In this work, we present a linear program that optimizes immunogenicity subject to constraints related to both epitope selection and spacer design, enabling users to weigh the selection of a set of epitopes that have great immunogenic potential against their assembly into a string-of-beads construct that provides a high chance of recovery. We conduct Monte-Carlo cleavage simulations to show that, indeed, a fixed set of epitopes often cannot be assembled adequately, whereas selecting epitopes to accommodate proper cleavage requirements substantially improves their recovery probability and the effective immunogenicity of the resulting vaccines. We also explore the limits of these constraints, quantifying how immunogenicity decreases as cleavage requirements become more stringent.

14 Hiromune Eto Department for Cellular and Molecular Biophysics, MPI of Biochemistry, Martinsried, DE

3D printed lipid bilayer architectures for in vitro synthetic biology.

In bottom-up synthetic biology, one of the major methodological challenges is to provide reaction spaces that mimic cellular structures with regard to topography and surface functionality. Of particular interest are lipid membrane interfaces, as many protein functions take place in and on membranes of various shapes. With the advent of 3D printing by two-photon direct laser writing, we can fabricate arbitrarily shaped 3D microstructures with length scales relevant to cellular and biological processes, mimicking specific features of cells and organelles. Using such micron-scale structures, we are able to shape lipid membranes according to the imposed 3D architecture, from which we create environments and geometries relevant to in vitro synthetic biology. By employing appropriate surface modifications, we can manipulate charges on the polymer surface, which enables the formation of both negative and positive charged lipid membranes. On these 3D lipid architectures, we in vitro reconstitute dynamic protein systems that are geometry sensitive, and observe their self-organisation and pattern formation in response to the printed 3D geometries.

15 Christian Gebhardt

Physical and Synthetic Biology, Faculty of Biology, LMU, Munich, DE

Watching single proteins dancing: Angstrom precision distance measurements in dynamic protein structures with single-molecule Förster-resonance energy transfer.

Single-molecule Förster resonance energy transfer (smFRET) has evolved towards a mature toolkit for the study of distances, structures and dynamics of biomolecules in a physiologically relevant context in vitro and in vivo. There is, however, no generally accepted way to derive and use quantitative distance information from the FRET-ruler to derive structural models or constraints in the protein data base. Hellenkamp et al. recently presented a quantitative smFRET study of oligonucleotide ruler structures that revealed high precision, accuracy and reproducibility of FRET-derived distances in a worldwide comparative study of 20 labs with a distance uncertainty of less than 6 Å. While this establishes smFRET as a suitable technique for accurate distance measurements of static biological reference structures, we raise the question if smFRET is applicable for proteins with dynamic conformational motions or allosteric modulation of protein structure by an effector. Additionally, proteins are more challenging targets for site-specific fluorophorelabelling compared to oligonucleotides. We identified substrate binding proteins as a suitable model system that we used here to benchmark FRET-derived distance uncertainty in proteins for situations of (i) stochastic labelling and (ii) allosteric and dynamic modulation of the structure and show similar angstrom precision comparable to DNA.

16 Johannes Bruno Müller

Max Planck Institute of Biochemistry, Munich, DE

The Proteome Landscape of the Kingdoms of Life.

Proteins perform the vast majority of functions in all biological domains but their large-scale investigation has lagged behind for technological reasons. Since the first essentially complete eukaryotic proteome was reported, advances in mass spectrometry (MS)-based proteomics have enabled increasingly comprehensive identification and quantification of the human proteome. However, there are few comparisons across species, especially compared to genomics initiatives. We employ an advanced proteomics workflow, in which the peptide separation step is performed by a microstructured and extremely reproducible chromatographic system, for the in-depth measurement of 100 taxonomically diverse organisms. With two million peptide and 340,000 stringent protein identifications obtained in a standardized manner, we double the number of proteins with solid experimental evidence known to the scientific community. Our results provide a comparative view into the functional organization of organisms across the entire evolutionary range. A remarkably high fraction of the total proteome mass in all kingdoms is dedicated to protein homeostasis and folding. Likewise, a constantly high fraction is involved in supplying energy resources, although these pathways range from photosynthesis through iron sulphur metabolism to carbohydrate metabolism. Generally, however, proteins and proteomes are remarkably diverse between organisms.

17 Mihail Todorov Inst. for Tissue Engineering and Regenerative Medicine, Helmholtz Zentrum, Munich, DE

VesSAP: Automated analysis of whole mouse brain vasculature using machine learning.

Tissue clearing methods enable imaging of intact biological specimens without sectioning. However, reliable and scalable analysis of such large imaging data in 3D remains a challenge. Towards this goal, we developed a deep learning-based framework to quantify and analyze the brain vasculature, named Vessel Segmentation & Analysis Pipeline (VesSAP). Our pipeline uses a fully convolutional network with a transfer learning approach for segmentation. We systematically analyzed vascular features of the whole brains including their length, bifurcation points and radius at the micrometer scale by registering them to the Allen mouse brain atlas. We reported the first evidence of secondary intracranial collateral vascularization in CD1-Elite mice and found reduced vascularization in the brainstem as compared to the cerebrum. VesSAP thus enables unbiased and scalable quantifications for the angioarchitecture of the cleared intact mouse brain and yields new biological insights related to the vascular brain function.

18 Florian Noack Helmholtz Zentrum, Munich, DE

Deciphering the gene regulatory network of the developing cortex.

The mammalian neocortex consists of a broad variety of neuronal subtypes with different morphology, connectivity as well as function. All these cells are generated by neuronal stem cells in a very precise order throughout cortical development. However, our understanding of the molecular mechanisms, which allow neuronal stem cells to differentiate into various neuronal subtypes as well as regulate the precise timing of their production remain incomplete. In my project I want to tackle open question by different approaches:

First, we performed simultaneously scRNA-seq and scATAC-seq from cortices during different developmental timepoints (E12 to E18). This will allow us not only to analyse the heterogeneity of the neuronal stem cell population but also how they change during developmental time and during their differentiation into distinct neuronal subtypes. By performing a integrative analysis of both scRNA-seq and scATAC-seq data we identified potential enhancers and their associated transcription factors which are crucial for regulating these processes.

Second, we developed a novel technique which enable us to concurrently measure gene expression, DNA methylation levels, chromatin accessibility and 3D genome configuration with low numbers of cells (< 1000), potentially down to single-cells. Key advantages of this new method are the lower experimental cost, the reduction of required sample amounts and most importantly the generation of highly comparable datasets avoid of any batch effects. Using this technique on isolated cell populations of the developing cortex will give use insights how different epigenetic mechanism influence each other and collectively regulate neuronal stem cell differentiation.

Finally, we will functionally validate our results by performing knock-down/overexpression of candidate transcription factors as well as site-specific epigenetic engineering of identified enhancers in vivo using in utero electroporation.

19 Renee Vieira

Max Planck Institute of Neurobiology, Munich, DE

Subcellular localization of the glutamate receptor GluClα in the Drosophila elementary motion detectors.

In the fruit fly, Drosophila melanogaster, the motion vision pathway has been intricately dissected. The elucidation of neuronal connections and cell dynamics have led to the development of the current motion detection model. This model implements both preferred direction enhancement and null direction suppression at the level of T4/T5 dendrites; the first direction selective neurons. Recently, an EM study identified all the synaptic inputs to both T4/T5 neurons and described their spatial topography. However, implementation of direction selectivity at the molecular level on the dendrites of T4/T5 neurons is still largely unknown due to limitations in protein labeling techniques and optical imaging. To this avail, we have developed a UAS-driven GFP-tagged GluClα line that allows for the labeling of the receptor in a cell-type specific manner. Additionally, we have developed an endogenous protein tagging strategy to label and visualize membrane bound proteins in the cells of interest. Together, these tools will help reveal the distribution of excitatory and inhibitory neurotransmitter receptors along T4/T5 dendrites and elsewhere in the optic lobe, a feat that has rarely been achieved in any system so far.

20 Vini Tiwari

German Center of Neurodegenerative Diseases, Munich, DE

Lipid metabolism in microglia shares an epigenetic control during remyelination.

Microglia are the brain macrophages, which are confronted with large amount of myelin debris (lipids) in a multiple sclerosis (MS) lesion. Metabolism of lipid rich myelin involves a reverse cholesterol pathway in microglia, which is essential for successful remyelination. However, in conditions such as ageing, microglia are persistently inflamed as a result of accumulation of large amount of cholesterol esters, which overwhelm the efflux capacity of microglia. This is due to failure in lipid storage and metabolism. Epigenetic regulation of macrophages in lipid storage and metabolism is documented in atherosclerosis and other lipid disorders. However, it is unestablished in brain macrophages during remyelination. We wanted to know if there is a link between lipid metabolism and epigenetic regulation in Microglia. In our in vitro model of myelin injury on microglia, we found the first pattern of acetylation and methylation signatures on Histone3, which indicated the involvement of epigenetic regulation in response to myelin lipids. We further think, the modulation of epigenetic landcscape would induce reverse cholesterol pathway in microglia, which will be substantial for remyelination.

21 Erika Gonzalez Helmholtz Zentrum, Munich, DE

Hyperoxia exposure induces age and time dependent changes in gene expression in healthy mouse lung

Introduction: Premature neonates and adults with chronic lung disease often require long term oxygen therapy. Although the consequences of oxygen therapy in the diseased adult lung remain unclear, in neonates, oxygen supplementation is known to drive the development of chronic lung disease. We aimed to determine distinct and common effects of clinically relevant doses of oxygen in the neonatal and the adult lung, which can potentially inform treatment.

Methods: In vivo, term born C57BL/6J mice were exposed to moderate hyperoxia (FiO2=0.4) at postnatal day (P) 5-7 and 82-week adult for 2h and 8h or were kept at room air (RA, FiO2=0.21), respectively (n=4/group). RNA was extracted from total lung tissue and processed for microarray analysis. In vitro, primary neonatal mouse lung ATII cells, fibroblasts and endothelial cells were exposed to 40% oxygen for 24h and subjected to scRNA-seq single cell analysis. Statistical significance for differentially expressed genes between groups was set at false discovery rate 0.05.

Results: In vivo exposure to moderate hyperoxia for 8h led to around 30 differentially regulated genes, whereas 2h of exposure did not reveal significant changes in the transcriptome pattern. In contrast, a more pronounced response with up to 1500 differentially regulated genes was found in all three cell types in scRNAseq. The most pronounced changes were observed in fibroblasts. In the adult lung, oxygen exposure for 2h led to a pronounced transcriptomic response marked by the downregulation of >2000 genes. Similar to changes observed in the adult lung, single cell analysis indicated involvement of pathways that play a role in DNA repair and DNA-damage cell-cycle regulation.

Conclusions: In vivo, moderate hyperoxia induces a distinct, time and age-dependent transcriptome response in lungs of newborn and adult mice. Furthermore, the pronounced downregulation of cell cycle progression pathways induced by hyperoxia in the adult lung and shared on the single cell level in neonatal cells points to common injury signatures but also to a mechanism by which cells protect themselves from oxidant genotoxic stress.

22 Christina Schmidt University of Cambridge, Cambridge, UK

RACER, a 3D-model to study the influence of microenvironment on FH-deficient cells

The loss of the mitochondrial enzyme Fumarate Hydratase (FH) leads to a highly metastatic form of renal cancer associated with poor clinical outcome. FH catalyses the hydration of fumarate to malate and its loss leads to profound metabolic changes and the accumulation of fumarate, which activates a series of oncogenic signalling cascades that drive transformation. The tumour microenvironment affects the phenotype of cancer cells, but its effect on FH-deficient cells' behaviour is currently unknown. Here, we used a Tumour Roll for Analysis of Cellular Environment and Response (TRACER), a 3D scaffold that develops oxygen and nutrient gradients akin to those observed in tumours, to investigate how environmental cues affect FH-deficient cells. Using CRISPR/Cas9-based genome editing, we generated FH-deficient human renal epithelial cell lines. Applying proteomics, metabolomics and transcriptomics approaches we show that this model faithfully recapitulates the biochemical and phenotypic markers of FH-deficiency. Interestingly, using TRACER we were able to show that various hypoxia markers exhibit distinct behaviours in FH-proficient and FH-deficient cells, indicating that FH loss might impact the response to low oxygen. Moreover, we show that the cells harbour a layer-dependent metabolic signature implying that in vivo FH loss could undergo additional compensatory metabolic changes, which could be essential to investigate novel targets and survival mechanisms of FH-deficiency.

23 Deepti Agrawal

Klinikum Rechts der Isar, Technische Universität, Munich, DE

RIPK3 suppresses Kras-driven pulmonary adenoma and adenocarcinoma formation

Cancer is formed by acquiring molecular capabilities that serve as milestones for carcinogenesis, also termed the hallmarks of cancer. Apart from apoptosis being the major form of programmed cell death, we are interested in investigating other immunogenic cell deaths i.e. necroptosis whose role remains elusive in lung adenocarcinoma (LUAD). Neoplastic lung epithelial cells under specific circumstances readily activate necroptosis which proceeds independent of caspases. This occurs in the downstream of death receptors such as tumour necrosis factor receptor (TNFR) or the Toll-like receptor (TLR) families, amongst others. One of the principal components of necroptosis, receptor-interacting protein kinase 3 (RIPK3), plays a crucial role in inflammatory signaling and cell death pathways. Here we report that Ripk3 is tumor suppressor in KrasG12D/+ driven LUAD mouse model as Ripk3 whole body knockout mice have larger tumors and eventually higher tumor burden as compared to only KrasG12D/+ mice. Analogously, the analysis of TCGA gene expression data of LUAD patients indicates that RIPK3 expression reduces compared to healthy controls, an observation also confirmed by immunohistochemistry of human LUAD patient samples from tumour lesion at protein level. To decipher the underlying mechanism, we find that Ripk3 leads to immunogenic tumor microenvironment as Ripk3 derives immune cells (B cells and T cells) infiltration to rescue the tumor tissue. Moreover, Ripk3 deficiency leads to increase in Arginase1+ macrophages at advanced stage. This may explain the increase in tumor burden (M2 pro-tumorigenic activity) as well as decrease in T cell infiltration (arginase inhibits T cell propagation) in the lesion.

24 Tobias Dreyer

Klinikum Rechts der Isar, Technische Universität, Munich, DE

The Chemokine CX3CL1 improves trastuzumab efficacy in HER2-low expressing cancer in vitro and in vivo

A crucial mode of action of trastuzumab is the labeling of HER2-positive tumor cells for the eradication of natural killer (NK) cells, a process called antibody-dependent cellular cytotoxicity (ADCC).However, only a fraction with a robust HER2 overexpression benefits from trastuzumab therapy. ADCC requires both a sufficient lymphocytic infiltration and a close binding of the immune cells to the tumor cells. We speculated that the chemokine CX3CL1 could improve both processes as it is synthesized as a membrane-bound, adhesive form which is cleaved into a soluble, chemotactic protein. Here, we show that CX3CL1 overexpression is a positive prognostic marker in breast cancer and attracts tumor-suppressive lymphocytes, most notably NK cells,and inhibits tumor growth and lung metastasis in the syngeneic 4T1 breast cancer model in vivo. In HER2-positive SKBR3, MDA-MB 453, and HT-29 tumor cells, CX3CL1 overexpression increased NK cell-mediated cytotoxicity in vitro, and acted synergistically with trastuzumab. In vivo,in the HER2 low expressingHT-29 model, CX3CL1 overexpression not only tripled survival but also overcame trastuzumab resistance. Together, these findings identify CX3CL1 as a feasible pharmacologic target to enable trastuzumab therapy in HER2 low expressing cancers and render it a potential predictive biomarker to determine therapy responders in these entities.

01 Jei Diwakar

Pioner Campus, Helmholtz Zentrum, Munich, DE

Transcription factor mediated rewiring of 3D chromatin architecture in cortical development.

Chromatin is organised within the nucleus in a hierarchical manner, with the 3D architecture representing an additional layer in the regulation of gene expression. As higher resolution Hi-C methods have been developed to study the chromatin architecture, cell-type specific contacts at the sub-Megabase scale have been identified. Recently, ultra-high-resolution chromatin interaction maps from purified mouse cortical progenitors and neurons recognised neural transcription factors (TFs), such as Pax6 and Neurog2, to be associated with dynamic and cell-type specific chromatin contacts. Yet, to date, limited evidence exists for the mechanistic link between TFs and chromatin contacts.

Therefore, we aim to explicitly study if TFs are necessary and sufficient to mediate cell-type specific chromatin architecture. For this purpose, we will use a dCas9 MS2-stem loop system to ectopically recruit candidate neural TFs to promoters and enhancers of target genes in mESCs - a system in which the gene is not normally expressed. The effects on local chromatin architecture and potentially transcription will be evaluated using 4C and RNAseq. A complementary degron tag system will also be implemented to perform rapid degradation of candidates in neural progenitors to further examine the impact on the architecture. Following such studies, in vivo manipulation will be performed to analyse the functional consequences of an altered chromatin architecture on cortical neuron diversity.

02 Vanessa Luzak

Biomedical Center, Ludwig-Maximilians-Universität, Munich, DE

An inter-chromosomal interaction with an RNA splicing locus regulates monogenic gene expression in T. brucei.

Highly selective gene expression is a key requirement for antigenic variation in several pathogens, allowing evasion of host immune responses and maintenance of persistent infections. African trypanosomes, parasites that cause lethal diseases in humans and livestock, employ an antigenic variation mechanism that involves monogenic antigen expression from a pool of >2500 antigen coding genes. In other eukaryotes, the expression of individual genes can be enhanced by mechanisms involving the juxtaposition of otherwise distal chromosomal loci in the three-dimensional nuclear space. However, trypanosomes lack classical enhancer sequences or regulated transcription initiation and the monogenic expression mechanism has remained enigmatic. Here, we show that the single expressed antigen coding gene displays a specific inter-chromosomal interaction with a major mRNA splicing locus. Chromosome conformation capture (Hi-C), revealed a dynamic reconfiguration of this inter-chromosomal interaction upon activation of another antigen. Super-resolution microscopy showed the interaction to be heritable and splicing dependent. We find that the two genomic loci are connected by the antigen exclusion complex, whereby VEX1 associated with the splicing locus and VEX2 with the antigen coding locus. Following VEX2 depletion, loss of monogenic antigen expression was accompanied by increased interactions between previously silent antigen genes and the splicing locus. Our results reveal a novel mechanism to ensure monogenic expression, requiring the spatial integration of antigen transcription and mRNA splicing in a dedicated compartment. These findings suggest a new means of post-transcriptional gene regulation.

03 Volker Nitschko

Gene Center, Ludwig-Maximilians-Universität, Munich, DE

Nuclear export of siRNA precursors by the dsRBD protein.

RNA interference is an important regulatory pathway in genome surveillance, gene regulation and virus defence in higher organisms. The processing pathway starting from a long double-stranded RNA precursor molecule that is cleaved by a Dicer protein into its functionally active 21 nt long product has been heavily studied over the past decades. One process that so far has not been clear is the fate of dsRNA precursors formed in the nucleus, specifically whether they can be processed by Dicer before nuclear export or if they are exported as long double-stranded precursors. Surprisingly, the factors involved in the nuclear export pathway of dsRNA have not been characterized in Drosophila. The Drosophila nuclear localized dsRNA-binding protein Blanks can shuttle between nucleus and cytoplasm. In addition, the formation of a large number of siRNAs originating from convergent transcription of genes is dependent on blanks expression. Blanks do not interact with factors from the canonical RNAi pathway like Dicer or Ago but we found it associated with factors from the nuclear export and import machinery. These results strongly imply a role of blanks in the nuclear export of dsRNA precursors forming from convergent transcripts allowing them to be processed by Dicer in the cytoplasm.

04 Wenjing Shi

Experimental and Molecular Pathology, Ludwig-Maximilians-Universität, Munich, DE

Linc00673 exerts Oncogenic Function in Cervical Cancer by Negatively Regulating miR-126-5p Expression and Activates PTEN/PI3K/AKT Signaling Pathway.

Recent studies have indicated the crucial regulator roles of long noncoding RNA (lncRNAs) in cancer pathogenesis and development. However, the clinical significance and functional effects of LINC00673 in cervical cancer remains unknown. The relative expression of LINC00673 was determined by qRT-PCR analyses. Kaplan-Meier survival plot was used to analyze the association between LINC00673 expression and the overall survival time of patients. Multivariate Cox analysis was also performed. CCK8 cell proliferation, cell colony formation assay, cell cycle analysis in vitro and xenografts model in mice in vivo were used to evaluate the effects of LINC00673 expression on cell proliferation of cervical cancer. Luciferase activity assay was performed to clarify the association between miR-126-5p and LINC00673.

In this study, we confirmed that the relative expression of LINC00673 was higher in cervical cancer tissues compared with its corresponding normal tissues. Higher LINC00673 expression associated with tumor size, lymph node metastasis, and FIGO stage. Survival analysis showed higher LINC00673 expression predicted poor overall survival (OS) of cervical cancer patients and Multivariate Cox analysis demonstrated that higher LINC00673 expression was identified as an independent risk factor for OS. Gain-function and loss-function assays showed that LINC00673 overexpression promoted cell proliferation and cell cycle progression. In addition, overexpression of LINC00673 negatively correlated with lower miR-126-5p expression in cervical cancer tissues. LINC00673 promoted cell proliferation by sponging to miR-126-5p in cervical cancer cells. Moreover, we demonstrated that LINC00673 significantly activated PTEN/PI3K/AKT signaling pathways in cervical cancer cells. Thus, these results provide evidence that LINC00673 may be a potential therapeutic target for cervical cancer.

05 Sabrina Hepner

Bavarian Health and Food Safety Authority, Oberschleissheim, DE

Comparative genomics of the tick-borne pathogen Borrelia burgdorferi sensu lato.

Borrelia burgdorferi sensu lato is a species complex of tick-transmitted bacteria, comprising 22 genospecies six of which can cause Lyme borreliosis. The genome of Borrelia is unusual for bacteria consisting of a linear chromosome and a large number (> 20) of circular and linear plasmids, known to encode genes that play important roles in host/vector adaption and pathogenicity. Due to the genome complexity and high plasmid sequence similarity, plasmid assembly pose a big challenge. Our previous projects showed that Borrelia plasmids are best assembled by combining short and long read sequencing technologies (Illumina and Pacific Biosciences SMRT technology, respectively). The aims of this project are twofold: 1) to generate a large number of completed Borrelia genomes; 2) to compare completed consensus genomes to identify factors involved in host/vector adaption and pathogenicity. So far, a total of 30 Borrelia strains isolated from patients and ticks from Europe and Asia were sequenced using PacBio and Illumina. Included Borrelia species/strains are known to differ in host association(bird or rodent), vector association (specialists or generalists) and human pathogenicity (virulence levels ranging from non-pathogenic to highly pathogenic). Genomes available via GenBank will also be included in the study. Initial data indicate that the chromosome, and two plasmids (cp26, lp54) have concordant phylogenies whilst in plasmid phylogenies isolates clustered differently.

06 Ramya Nair

Max von Pettenkofer Institute and Gene Center, Ludwig-Maximilians-Universität, Munich, DE

Lentiviral protein Vpx as a potential weapon to improve acute myeloid leukemia (AML) treatment efficiency.

AML, an aggressive cancer of the blood, still remains to be a challenge to treat. Despite recent advances, Cytarabine (Ara-C) and Daunorubicin are still the primary drugs used in AML treatment since decades. However, one major hurdle in treatment success is drug resistance. In 2017, it was reported that SAMHD1, a dNTPase, plays a role in Ara-C resistance, thereby serving as an attractive target for AML treatment. The lentiviral accessory protein Vpx, which is found in Simian Immunodeficiency Viruses (SIV) and Human Immunodeficiency Virus-2 (HIV-2), is known to sequester SAMHD1 for proteasomal degradation. In our study, we aim to use the Vpx protein to manipulate SAMHD1 in AML cells to improve Ara-C sensitivity. In order to effectively deliver the Vpx into cells, delivery systems, namely virus-like particles (VLPs), cell-penetrating peptides (CPPs), nanoscaled metal organic frameworks (nanoMOFs) and liposomes, will be investigated. For the generation of nanoMOFs and liposomes, we were able to purify functional His-tagged Vpx from E. coli and establish an in vitro assay to test its function. VLPs containing Vpx and two different CPPs coupled to a truncated fragment of Vpx were efficiently taken up by the AML cell line THP-1 and induced degradation of SAMHD1. With these observations, we have built the basis to further test the efficiencies of these delivery systems in combination with Ara-C and perhaps other drugs that are also affected by SAMHD1.

07 Lianyong Han

Helmholtz Zentrum, Munich, DE

By nanoparticle exposure triggered MAPK signaling contributes to gammaherpesvirus reactivation.

Environmental particle inhalation and persistent herpesvirus-infection are omnipresent and associated with chronic lung disease. In a previous study, we showed that carbon nanoparticles (CNP) induced the production of lytic virus from persistently murine gammaherpesvirus 68 (MHV-68) infected macrophages (Ana- 1/MHV-68) in vitro. In vivo, pulmonary exposure to CNP led to an increase in the expression of lytic viral proteins in the lung of latently MHV-68 infected mice. Gene expression and metabolome analysis revealed similar patterns as observed during acute infection with the expression of lytic proteins localized to CD11b+ macrophage like cells. However, the underlying mechanism related to the reactivation is still unclear. The mitogen-activated protein kinase (MAPK) signaling pathway is one of the major intracellular signal transduction pathways that play an important role in several cellular processes such as cell growth, proliferation, and cell death. MAPK signaling was also reported to contribute to viral infection. We therefore set out to study whether CNP reactivates herpesvirus infection via the MAPK signaling pathway. Here we found that even though no pro-inflammatory transcriptional response could be detected, ERK1/2, JNK and p38 MAPK are rapidly activated by CNP exposure in Ana-1/MHV-68 cells, followed by upregulation of viral gene expression and increased viral titers. Pharmacological inhibition of p38 activation reduced virus reactivation. Taken together, these finds suggest that, p38 MAPK signaling pathways contribute to CNP induced herpesvirus reactivation, and their pharmacological inhibition might alleviate particle exposure related disease exacerbations.

08 Miriam T. Kastlmeier

Helmholtz Zentrum, Munich, DE

Modelling epithelial-fibroblastic cross-talk in vitro: using hiPSCs derived lung cells targeting fibrotic regeneration.

Pulmonary fibrosis is characterized by the irreversible increase of activated fibroblasts producing excessive interstitial matrix, in turn leading to the remodeling and ultimate loss of the functional gas exchange area. Current studies show that a vicious cycle is initiated by injury of primary alveolar epithelial cells triggering the activation of fibroblasts into a pro-fibrotic state that in turn stimulates epithelial cell injury.

Directed differentiation of cells with stem-cell like potential, known as human induced pluripotent stem cells (hiPSCs), into distal lung epithelial cells will enable to study cell characteristics and epithelial cell injury mediators directed towards the lung fibroblast, thereby starting the self-perpetuating injury crosstalk. Dissecting this crosstalk using either healthy or injured fibroblasts will help to understand initiation and further development of disease. With the significant comparability of hiPSCs derived distal lung cells to primary alveolar epithelial cells, a cell source of high specificity with a higher longevity compared to cell lines, distal lung cell progeny can be used to mimic primary recurrent and nonresolving injury.

The project aim is the development of an airlifted, advanced human in vitro lung model, allowing studies on the pro-fibrotic crosstalk of alveolar epithelial cell towards fibroblasts in a co-culture model. Distal pulmonary epithelial cells derived from hiPSCs are used to create lung organoids of the alveolar region. Therefore, differentiated alveolar type II cells, characterized among others by surfactant protein C production, will be cultivated at the air-liquid interface (ALI) to achieve an organotypic experimental set up. Primary injury will be introduced to the lung cells using zinc oxide (ZnO) and hyperoxia-inducing oxygen (O2) exposure. Furthermore, co-culture with fibroblasts will be established to mimic structures comparable to in vivo conditions. To investigate realistic therapeutic strategies the in vitro ALICE-CLOUD system will be used for an efficient delivery of aerosolized drugs to cultivated lung epithelial cells. The realization of this translational project will provide new insights in injury and regeneration and identify mechanisms that reveal therapeutic applicability.

09 Ariane Hallermayr

Medizinisch Genetisches Zentrum, Munich, DE

Analytical validation of the Droplet Digital PCR system for the detection of the most frequent clinically relevant EGFR, BRAF and KRAS hotspot tumor variants.

Precision medicine targeting specific genetic variants in cancer can be used for prediction and prognosis of disease progression and outcome. This requires accurate molecular profiling of the tumor, which is traditionally performed using DNA extracted from tumor tissue. Liquid biopsies can overcome some of the challenges regarding tissue biopsies and offer several advantages like real-time monitoring of driver hotspot variants, although highly sensitive methods are required to identify these variants in ctDNA. The Droplet Digital™ PCR (ddPCR) technology requires low amounts of input DNA and enables quantification of low variant allele frequencies (VAF) of targeted variants, yet accuracy varies between assays and laboratory protocols. We conducted the analytical validation of five ddPCR assays, for the detection of the most frequent clinically relevant EGFR, BRAF and KRAS hotspot tumor variants and established precise thresholds between negative and positive results as well as VAF quantification. All assays showed very low limits of blank and detection ranging from 0% to 0.11% fractional abundance and 0.08% to 0.4% VAF of the targeted variants, respectively. However, striking differences were observed between ddPCR assays for the detection of low VAFs. In particular, the observed difference in performance in low VAF variant detection and quantification demonstrates the importance of specific analytical validation for each assay to obtain reliable results of ctDNA analysis.

10 Anna-Lena Amend

Helmholtz Zentrum, Munich, DE

New mouse models for rare forms of diabetes: Ins2C109G and Ins2V26D.

The most commonly known forms of diabetes are type 1, type 2 or gestational diabetes, which are considered multifactorial diseases caused by several genetic variations and environmental factors (IDF Diabetes Atlas, 8th Edition). Less common types are monogenic forms of diabetes such as maturity-onset diabetes of the young (MODY) and neonatal diabetes mellitus (NDM). Both are characterized by early onset of hyperglycemia, usually before 25 years of age for MODY and before 2 years of age for NDM. Often patients are misdiagnosed as T1D or T2D. The main objectives of this study are the identification and characterization of new mouse models that reflect the human phenotype of MIDY (mutant INS gene induced diabetes of youth), one form of NDM. Here, we present two insulin2 mutant mouse models (Ins2C109G and Ins2V26D) derived from the Munich ENU mutagenesis screen.

11 Aleksei Belyi

Faculty of Biology, Biocenter, Ludwig-Maximilians-Universität, Munich, DE

Regulation of expression of X-linked genes in Drosophila melanogaster.

In Drosophila, the ploidy of the X chromosome differs between males and females, which has led to the evolution of sex-specific regulation of X-chromosomal gene expression. In the somatic tissues of D. melanogastermales, X-linked genes are upregulated approximately twofold through a process mediated by the dosage compensation complex (DCC). However, the exact degree of dosage compensation may vary among tissues and be influenced by a gene's proximity to a DCC binding. Here we experimentally test how the, of a gene to a DCC binding site influences its level of expression in males and females. To do this, we generated over100 fly lines that each have areporter gene inserted at a unique X-chromosomal location. In order to exclude the influence of chromosome-, sex-, or tissue-specific regulation, we used a lacZ reporter gene (encoding b-galactosidase) from Escherichia coliand a minimal human cytomegalovirus promoter, both of which are foreign to theDrosophilagenome.For each of these fly lines we will measure the expression of the reporter gene in germline and somatic tissues of both sexes. We anticipate that this research will lead to an improved understanding of the regulation of X-linked gene expression.

12 Amelie Bauer

Institute of Virology, Helmholtz Zentrum, Munich, DE

Characterization of the transduction behavior of the novel Adenoassociated virus (AAV) vector variant AAV9P1.

Astrocytes are the most abundant cell type in the human central nervous system. They have key functions in the brain, including the maintenance of neuronal homeostasis and active contribution to the regulation of synaptic transmission. Astrocytic dysregulation is associated with a variety of neuropathologies. For treating astrocyte-associated conditions in vivo it is of great importance to generate vectors which transduce astrocytes with high efficiency and selectivity.

To address this issue we identified a novel viral vector AAV9P1 which was generated from the Adeno-associated virus (AAV) subtype 9 via peptide display. This AAV shows high efficiency in transducing neural progenitor cells, differentiated astrocytes and astrocytoma cell lines in cell culture models when compared to cell lines from potential off-target tissues. AAV9P1 is superior in astrocyte transduction and transgene delivery in comparison to naturally occurring serotypes. A study addressing in vivo applicability revealed that AAV9P1 was able to cross the blood-brainbarrier and transduce astrocytes selectively in the brain after systemic injection. In vitro studies indicate that astrocyte targeting by AAV9P1 involves interaction with several astrocytic surface molecules such as RGD-binding integrins alongside other factors. Furthermore, we are currently finishing a CRISPR knockout screen in an astrocytoma cell line to identify cell-surface molecules and molecular pathways involved in AAV9P1 transduction.

13 Verena Häfner

Helmholtz Zentrum, Munich, DE

Reactivation of latent virus infection – a link between the acute inflammatory response to air pollutants and chronic lung disease in a mouse model.

Particularly gammaherpesviruses are omnipresent in human society and almost every adult person is infected by at least one virus. The virus persists lifelong in the human host in its latent state, and switches to a lytic and replicative phase only upon reactivation. Previous work from our group has shown that pulmonary exposure to carbon nanoparticles can lead to the reactivation of latent murine gammaherpesvirus 68 in the lung of mice (Sattler et al., 2017). Boosting the production of lytic proteins and subsequent immunomodulation by particle inhalation might contribute to the exacerbation of chronic lung diseases like chronic obstructive pulmonary disease (COPD), pulmonary fibrosis and allergic asthma. To investigate this hypothesis, different environmental relevant particles and other ambient aerosols will be tested in a realistic in vitro system. For this approach, latently infected cells growing at air-liquid interface conditions are exposed to fresh emissions of combustion-derived particles for investigating their reactivation potential.

In a second approach, the relationship of the pulmonary expression pattern observed after particle-triggered virus reactivation and that of exacerbations of chronic lung diseases are investigated in a translational mouse model to identify potential target pathways of by environmental factors induced disease exacerbations. Two genes have been identified whose expression is not only induced specifically in the second hit condition in lungs of infected and particle exposed mice, but also in lungs of COPD and asthma mouse models. Further studies shall investigate the involvement of the two genes for disease exacerbation and virus reactivation.

14 Laura Sellmer

Thoracic Oncology, Medizinische Klinik V, Klinikum der Universität, Munich, DE

Immune cell profiles as predictors of 3-year metastasis-free survival (3YMFS) in surgically treated non-small cell lung cancer.

Background: Surgery is the treatment of choice for early and for some locally advanced non-small cell lung cancer (NSCLC). Ipsilateral lymph nodes are generally removed at the time of tumor resection. We assessed select immune cell markers in tumor tissue in patients with and without 3YMFS.

Methods: Internal hospital databases were screened for NSCLC patients fulfilling inclusion criteria and clinical details were obtained. FFPE tissue blocks of primary tumor and affected, unaffected mediastinal and unaffected hilar lymph nodes were collected. We determined tumor-infiltrating lymphocyte (TiL) score and type as well as PD-L1 status.

Results: We obtained clinical details on 40 NSCLC patients from the internal database of the Ludwig-Maximilians University: 20 patients with and 20 patients without 3YMFS. Investigation of TiL patterns showed significant differences between patients with and without 3YMFS (p=0,05). A preliminary investigation of PD-L1 status showed no difference between patients with and without 3-year metastasis-free survival (p=0,53).

Conclusion: Immune markers differ between patients with and without relapse. We are planning to extend these findings to more patients as well as investigate additional immune markers in tumor samples and locoregional lymph nodes.

15 Madeleine Müller

Institute of Molecular Immunology and Experimental Oncology, Technische University, Munich, DE

Elucidating the significance of TNF/TNFR2 driven inflammatory cell death in IBD.

Inflammatory bowel disease, such as Ulcerative colitis and Crohn's disease, typically manifest in early adulthood. However, a deficiency in the apoptosis-inhibitor XIAP accounts for about 4% of pediatric IBD.

Hence, we focused on the role of XIAP and how its deficiency leads to the development of very early onset IBD. We showed that XIAP deficient mice display Crohn's like symptoms in the small intestinal that correlated with inflammation and microbial dysbiosis. So far, the underlying mechanism seems to be driven by signaling via TNFR2 which induces pro-inflammatory cell death. This is surprising since TNFR2 does not carry a death domain. Furthermore, we have found the expression of TNFR2 to be upregulated in patients suffering from IBD.

Our findings lead us to believe that elucidating the role of TNFR2 in intestinal inflammation is important for understanding intestinal homeostasis. We therefore aim to decipher its mechanistic contribution to the development of IBD, with regard to both the hematological as well as the epithelial compartment.

16 Qiongliang Liu

Institute of Lung Biology and Disease, Helmholtz Zentrum, Munich, DE

Elucidating particle and leukocyte dynamics during the course of particle triggered pulmonary inflammation by in vivo imaging.

Chronic lung diseases have been associated with the exposure to various factors of air pollution including (nano)particles. In the past research investigating cell-particle interactions was mainly based on cultures cells or tissues. Such experimental methods cannot reproduce in full, the complex reactions of the immune system, which might be triggered by nanoparticles in vivo.

We aim to use intra-vital microscopy (IVM) to analyze immune cell dynamics in living animals in response to insults caused by nanoparticles. To visualize and measure in real-time elements of the pulmonary immune response as well as NP dynamics, we will apply state of the art intravital microscopy (IVM) on the alveolar region of the murine lung, in combination with ventilator-assisted NP aerosol inhalation. This novel approach will enable the study of (sub-)cellular dynamic events, which were inaccessible up to now. Therefore will be able to record particle and leukocyte dynamics during the course of particle triggered pulmonary inflammation. Methods: Nebulizer (Aerogen Pro) liberate aerosols which contain nanoparticle, such as Carboxyl Quantum Dots (cQDs, 18 nm diameter) - which serve as fluorescent model NPs – into C57/Bl6 mice lung. Thereby, we will establishe an in vivo imaging system that combines real time imaging of QDs and by intravenous injection of fluorescently labeled anti-Ly6G antiebodies, of neutrophils. In the research project we will monitor and correlate several parameters and physiological changes including the spread of QDs, pulmonary permeability, pulmonary perfusion speed, number of recruited neutrophils in infected lungs, and neutrophil motion in the lungs of live mice.

17 Matteo Napoli

Walter Brendel Center of Experimental Medicine, Biomedical Center, Ludwig-Maximilians-Universität, Munich, DE

The Role of MRP8/14 in leukocyte recruitment in vivo.

The leukocyte recruitment cascade is the well-defined process that allows leukocytes to overcome the endothelial membrane and transmigrate into the inflamed tissue.

Myeloid related protein 8/14 (MRP8/14) is a Ca2+ binding protein, which is actively secreted during this process and mediates β2 integrin activation and neutrophil adhesion in vivo. Despite representing 40% of cytosolic protein content in neutrophils, a putative intracellular role of MRP8/14 is still elusive. Within our study, we investigated leukocyte adhesion and extravasation in vivo in WT and Mrp14-/-mice, which are functional MRP8/14 deficient mice. We analysed MRP8/14 dependent neutrophil spreading, polarization, crawling and shear-resistant adhesion using WT and Mrp14-/-neutrophils in microflow chambers coated with E-selectin, ICAM-1 and CXCL1.Finally, we tested the ability of WT and Mrp14-/- neutrophils to phagocytose E.coli particles by flow cytometry and confocal microscopy. We found that the lack of MRP8/14 causes neutrophil adhesion deficiency and impaired extravasation in vivo. In line with these findings, Mrp14-/-neutrophils showed a defective spreading behaviour, were unable to polarize properly, crawled for longer distances and were more prone to detach at increasing shear stress levels. Moreover, phagocytosis in Mrp14-/-neutrophils was reduced. We postulate an intracellular function of MRP8/14 in mediating neutrophil post arrest modifications and effector functions, presumably by coordinating Ca2+signalling.

18 Christoph Stange

Clinical Research Unit, Department of Obstetrics and Gynecology, Technische Universität, Munich, DE

The protease DPP4 as a novel target to improve Olaparib treatment of ovarian cancer.

Recently, different groups have shown that Poly (ADP-ribose) polymerase inhibitors (PARPi) like Olaparib not only rely on mutation of DNA repair enzymes, but also provoke tumor cells to express the chemokine Cxcl10. This chemokine is able to attract immune cells into tumor tissue, which can then fight the tumor cells. Dipeptidyl peptidase 4 (DPP4) can cleave CXCL10, producing an antagonistic form that shows a reduced capacity to provoke immune cell recruitment. Tumors that overexpress DPP4 may mitigate the Cxcl10-mediated immune effects, thus showing reduced susceptibility against PARPi. Taken together, inhibition of DPP4 might be a feasible way to improve PARPi treatment. To evaluate the relevance of DPP4 in ovarian cancer, DPP4 expression was analyzed in 230 patients with high-grade serous ovarian cancer, revealing a distinct DPP4 tissue expression in 46% of patients. Patients with high DPP4 expression showed a significantly worse overall survival (median 16 vs. 44 months, p<0.001). In the immunocompetent ID8 ovarian cancer mouse model, treatment with the specific DPP4 inhibitor sitagliptin lead to a significantly longer survival compared to placebo-treated mice (p=0.048), while the PARPi Olaparib showed no significant effect. Combination of Sitagliptin with Olaparib, though, could overcome tumor resistance against Olaparib (median survival 54 vs. 49 days, p=0.006). In line with our hypothesis, the ascites of mice treated with both drugs contained significantly more Cxcl10.

19 Simon Geißen

Department of Cardiology, Heart Center, University Hospital Cologne, Cologne, DE

Vascular dysfunction in dilated cardiomyopathy is attenuated in MPO deficient mice.

Purpose: In an ageing population, heart failure (HF) ranks among the most common causes of morbidity and mortality. The dysfunction of resistance vessels and consecutive elevation of peripheral vascular resistance strongly contributes to disease progression. Polymorphonuclear neutrophils (PMN), upon interacting and binding to the endothelium, promote vascular disintegrity, but the exact role of PMNs in regulation of vascular resistance remains elusive. Myeloperoxidase (MPO), a highly abundant protein in PNMs, interacts with the endothelial wall by binding to the endothelial glycocalyx and facilitating leukocyte recruitment. Myeloperoxidase-deficient (Mpo-/-) mice show preserved aortic relaxation in models of systemic inflammation when compared to wild type (WT) mice. Importantly, flow mediated dilation was impaired by PMN activation in MPO-competent, but not in MPO-deficient human individuals.

Methods and Results: Based on these findings we investigated the role of MPO in a HF model of dilated cardiomyopathy (muscle LIM protein (MLP)-deficient mice, Mlp-/-) by cross-breeding with Mpo-/- mice. Ultrastructural electron microscopy imaging, heart weight, left ventricular end-diastolic volume assessment and histological analysis of fibrotic remodelling displayed no differences between Mlp-/- and MlpxMpo-/- mice. Functionally however, echocardiography and pressure-volume (PV-) loops revealed preserved systolic function in MlpxMpo-/- mice (EFMlp-/- vs MlpxMpo-/-, 29.86 6.53% vs. 40.32 9.32%, p<0.0001 and preload-recruitable stroke work MLP-/- vs. MLPxMpo-/-, 40.9 2.2 vs. 59.8 4.6 mmHg, p<0.05). Intriguingly, PV-loops showed an increase of vascular resistance inMlp-/- mice, whereas no such increase was detected with an additional deficiency of MPO (wt vs. Mlp-/-, 0.35 0.04 vs. 0.56 0.04 mmHg*min*ml-1, p<0.01; MlpxMpo-/- 0.36 0.04 mmHg*min*ml-1; Mlp-/- vs. MlpxMpo-/- p<0.01). This was accompanied by impaired vascular nitric oxide-bioavailability and aortic relaxation in Mlp-/- mice, which was likewise improved by MPO deficiency (% of relaxation to 100 nMACh treatment, wt vs. Mlp-/- vs. MlpxMpo-/-, -67.22 1.92 vs. -37.51 3.83 vs. -53.98 3.11, p<0.001). To narrow down the molecular role of MPO in endothelial cells, MPO treated human umbilical vein endothelial cells (HUVECs) were analysed by an immunoprecipitation for MPO. Proteomic analysis by mass spectrometry of the precipitate revealed MPO binding to the enzyme endothelial NO synthase (eNOS). Adverse regulation of that enzyme, a crucial source of nitric oxide, had previously been linked to hypoclorous acid (HOCl), an important product of catalytically active MPO. To evaluate the amount of dysfunctional, uncoupled eNOS, dihydroethidium (DHE) staining with and without eNOS inhibitor L-NAME treatment was performed. Increased amount of eNOS uncoupling was observed in Mlp-/- mice vs Mpo-/- vs. WT (p<0.05 n=6), but not in MlpxMpo-/-mice.

Conclusion: Our data strongly suggest a crucial role of MPO in the onset and progression of endothelial dysfunction during heart failure.

20 Tobias Beyer

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Glial co-inhibitory signaling in autoimmune inflammatory diseases of the central nervous system.

Multiple Sclerosis (MS) is an autoimmune inflammatory disease of the central nervous system (CNS). Astrocytes and microglia are two resident cell types within the CNS expressing a variety of factors that promote or suppress the local immune response. Apart from secreted mediators, microglia present antigens during acute and progressive stages of MS to re-activate infiltrated immune cells, thus contributing to disease progression. The role of astrocytes as antigen presenting cells is under current scientific debate, but several studies suggest antigen presentation function of astrocytes in autoimmune model systems. Programmed Death Ligand 1 (PD-L1) is a co-inhibitory factor, which modulates immune cell activation in MHC dependent and independent cell-cell interactions, and is expressed on both astrocytes and microglia. Its activities lead to the inhibition of proliferation and to the apoptosis of pathogenic immune cells. However, the role of PD-L1 on astrocytes and microglia is unknown, but may play an important role in the re-activation of CNS infiltrating and resident immune cells. Given its inhibitory effects, understanding the role of PD-L1 in relapsing and chronic stages of MS may lead to the development of novel tools to dampen autoimmune inflammation and may guide future therapeutic approaches to target hence untreatable forms of MS.

21 Tabea Eser

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Histological characterisation of HIV-1 RNA harbouring cells and their microenvironment in lymphoid tissues.

Lymphoid tissues (LT) are the primary anatomical site for HIV replication and persistence and thus play an important role in the pathogenesis of HIV. The analysis of cells derived from LT by flow cytometry and molecular methods has yielded valuable insight into the cell types involved in the immune response against HIV, as well as into the virus' preferred target cells. However, to study the spatial localisation of HIV-1 harbouring cells and their direct interaction with immune effector cells such as CD8+ cytotoxic T cells or T- regulatory cells, histological approaches such as in situ Hybridisation (ISH) and immunohistochemistry (IHC) are required.

To histologically characterise viral reservoirs within their native tissue context, we established a dual protocol for the detection of total HIV mRNA by ISH and cellular markers by fluorescence IHC. We applied the novel ISH technique RNAscope to formalin-fixed sections of mesenteric lymph nodes (LN), spleen and gut associated lymphoid tissue (GALT) of chronically HIV-1 infected humanised mice and human Ileum biopsies under ART. Total HIV-1 RNA was detected using a subtype B specific probe and visualised fluorescently. ISH was followed by multiplex fluorescence IHC for cellular lineage markers such as CD3 and FoxP3, or functional markers such as Granzyme B. Primarily results show that the majority of HIV-1 RNA+ cells co-stain for CD3 but not FoxP3 in mouse LN, spleen or GALT. This study into the microanatomical distribution of HIV-1 and immune cells will deepen our understanding of the viral reservoirs in LT, the major obstacle for HIV eradication and cure.

22 Valeria Viteri Alvarez

Investigating Myeloid-derived suppressor cells (MDSCs) homing and function in Idiopathic Pulmonary Fibrosis.

Introduction: Idiopathic pulmonary fibrosis (IPF) is a chronic and proliferative lung disease with a progressive loss of lung function and ominous prognosis. Myeloid-derived suppressor cells (MDSCs) are immature myeloid cells, which are increased and might play a role in the progress and development of organ fibrosis, including IPF. There are two different MDSC subtypes monocytic (M-MDSCs) and granulocytic (G-MDSCs).

Methods and results: Flow cytometry analysis of blood and tissue from control and IPF patients, was performed to analyze the presence and differential subset abundance of MDSC. Circulating MDSCs were increased in IPF patients compared with controls, being monocytic MDSC the predominant subset. FACS and immunohistochemistry analysis confirmed the presence of MDSC in IPF lungs. Proteomic analysis from sorted circulating IPF MDSC revealed the migratory and fibrotic program expression of MDSC in IPF, including the expression of CD263. FACS analysis from both blood and tissue, confirmed the presence and increase of CD263 in IPF M-MDSC. The invasiveness potential was assessed in sorted MDSCs subsets from IPF patients and controls, through collagen gel invasion. After 48 hours, we observed that M-MDSCs have a higher invasiveness potential than G-MDSCs, and that M-MDSCs from IPF patients were more invasive compared with controls.

Conclusions: Our study confirmed the presence and increased number of circulating MDSCs in IPF patients, being M-MDSC the predominant subset. CD263 is highly expressed in M-MDSCs from tissue and plasma, and it might influence the migratory and anti-apoptotic ability of this population in IPF. M-MDSCs have high invasive potential, suggesting that they may exert an in-situ role in fibrosis. Further studies are necessary to understand their interaction and determine their precise role in IPF.

23 Mehwish Ishaque

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The Uptake of Poly Lactic-co-glycolic acid (PLGA) Particles by Alveolar macrophages and epithelial cells depends on the Exposure scenario.

Due to its large surface area, the alveolar epithelium represents a major target for drug interaction and absorption. In this context nanoparticle-mediated drug delivery is an emerging therapeutic technique to deferent respiratory diseases, and predictive in vitro models are accordingly needed. The aim of this study was to investigate the cell specific uptake of different sized fluorescent labelled poly lactic-co-glycolic acid (PLGA) particles as carrier surrogates in different models. To this end we exposed cocultures of alveolar epithelial and alveolar macrophage cell lines, either at conventional submerged or at the air liquid interface (ALI) setup, to different sized PLGA particles, and compared the uptake efficacy to that observed in mice. Submerged exposures are experimentally simpler and more convenient to perform, but ALI exposures are even if costlier, physiologically more realistic and hence potentially biologically more meaningful. Additionally, while the cell delivered nanocarrier dose is usually rather obscure for submerged conditions, then on the cell surface deposited dose can be determined using the ALICE Cloud setup featuring a quartz crystal microbalance. 0.1µm, 0.5µm and 1µm PLGA particles were studied. For in-vitro experiments, the murine cell lines LA-4 (epithelial, type II-like mouse lung adenoma cells) and MH-S (immortalized mouse alveolar macrophages) as mono-cultures, were exposed to PLGA particles for 24 hours in submerged and ALI conditions. For in-vivo experiments, C57BL/6J mice were exposed to PLGA particles (0.1µm & 1µm) by intratracheal instillation and analysed after 24 hours. The uptake was monitored and quantified by confocal microscopy and flow cytometry. Under submerged conditions, flow cytometry revealed for epithelial cells the highest uptake for the largest particles, whereas macrophages showed more effective uptake of 0.5µm followed by 1.0µm particles. Uptake of 0.1µm particles was barely detectable at all. Under ALI conditions, significant particle uptake was only detected in macrophage and independent of particle size. Flow cytometry of particle exposed mouse lungs showed a significant uptake only for 0.1µm particles by alveolar macrophages, compared to little uptake by epithelial, type II cells, independent of size. Finally, our current results support the idea that the more elaborate ALI exposure setup represents a more realistic model when studying particle uptake at the air tissue interface. Currently performed histological investigations shall confirm the flow cytometry based findings.

24 Stefanie Galinec

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Phenotypic and (phospho-)proteomic characterization of Gemcitabine-based combination treatments of pancreatic cancer cell lines using kinase inhibitors.

Pancreatic ductal adenocarcinoma (PDAC) is one of the most devastating types of cancer, with 5-year survival rates below 10%. Most commonly, the nucleoside analogue Gemcitabine is used as first-line chemotherapy in pancreatic cancer but chemo-resistance is frequently observed. This project aims to characterize kinase inhibitors for their ability to sensitize human PDAC cell lines for Gemcitabine treatment.

Therefore, a representative set of 15 PDAC cell lines will be assessed for their phenotypic response to Gemcitabine using a live-cell imaging platform. Then, more than 140 clinically relevant kinase inhibitors will be screened in combination with Gemcitabine for their chemo-sensitizing effect using an automated liquid handling system. Using LC-MS/MS, selected drug combinations will be studied for their effect on the (phospho-)proteome of treated PDAC cell lines to elucidate the molecular mechanism underlying the observed phenotypic effect. Finally, most promising drug pairs will be tested for efficacy in clinically more relevant settings including 3D cellular and animal models of PDAC. In conclusion, through phenotypic and proteomic characterization of Gemcitabine-based combination therapies, this project may lead to the identification of more effective treatment options for pancreatic cancer.

25 Lisa Seufert

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The role of the Musashi RNA binding protein family in acute kidney injury.

Acute kidney injury (AKI) describes the acute onset of renal damage that leads to a –potentially reversible - loss of kidney function within days. Although AKI affects 13.3 million people worldwide and accounts for 1.7 million deaths per year diagnostic tools for early detection are limited and a treatment beyond renal replacement therapy is still missing. Consequently, most studies have focused on the prevention of AKI in high-risk patients. In this context so-called pre-conditioning strategies (e.g. dietary restriction, hypoxia) in animal models are utilized to protect mice from developing AKI (e.g induced by nephrotoxic substances like cisplatin). To shed light on the underlying molecular mechanisms of these renoprotective strategies our group performed large-scale transcriptomic and proteomic analysis of kidneys derived from preconditioned and AKI-damaged mice. Interestingly, we found a row of RNA-binding proteins to be differentially regulated by these interventions, with Musashi-1 (Msi-1) being one of the most significantly regulated genes in cisplatin-damaged mice. Msi-1 and its homolog Musashi-2 (Msi-2) have been extensively studied in context of different types of cancer and have been shown to be involved in oncogenic transformation. To dissect their putative role in acute kidney injury we identified the expression patterns of Msi-1 and Msi-2 in undamaged and AKIdamaged mouse kidneys. To get further insights into the role of the Musashi proteins in AKI the exact RNA targets which are bound by Msi-1 and Msi-2 in undamaged and AKI-damaged kidney cells will be determined by performing enhanced Crosslinking and Immunoprecipitation (eCLIP) and phenotyping of conditional knockout mice.

26 Marcel Proske

Institute of Structural Biology, Helmholtz Zentrum, Munich, DE

Structural and functional aspects of a new neurodevelopmental disorder – PURA Syndrome.

Pur-alpha (purine-rich element binding protein A) is a ubiquitously expressed multifunctional DNA and RNA binding protein, which is encoded by the PURA gene (5q31.2). Since the protein is involved in many crucial cellular processes like transcription, mRNA transport and translation during neuronal development, it is no surprise that mutations in the human PURA gene have severe consequences. Patients with the corresponding PURA Syndrome show several pathophysiological symptoms, including epileptic seizures, hypotonia, feeding difficulties, lack of speech and mental retardation.

First, we solved the crystal structures of human Pur-alpha in its wild-type form and with a patient related mutation. This knowledge was used to generate a DNA/RNA non-binding Pur-alpha mutant. Subsequently, overexpression cell lines for this mutant, for Pur-alpha wt and for three patient-related mutations were generated. Since there were several indications that Pur-alpha is located in stress granules and P-bodies we characterised the localisation of Pur-alpha and its mutants in immunostaining assays. Furthermore, we conducted individual-nucleotide resolution Cross-Linking and Immuno Precipitation (iCLIP) and proximity-dependent biotin identification (BioID) experiments in order to identify RNA and protein interaction partners respectively.

Together these results will eventually lead to a better understanding of the mechanisms that cause the symptoms associated with PURA syndrome. Ultimately, we want to provide information that can lead to the development of treatment strategies for PURA syndrome patients.

27 Ameirika

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Isw1c – A new ISWI chromatin remodeler complex from Saccharomyces cerevisiae.

Chromatin remodelers are well known to play important roles in the regulation of chromatin accessibility. Remodelers have the ability to move, assemble, eject or restructure nucleosomes. These mechanisms are very important for processes such as transcription, replication, DNA repair and recombination. ISWI-type remodelers represent one of four families of remodeler complexes, and they are highly conserved from yeast to humans. In Saccharomyces cerevisiae, there are two ISWI homologues, Isw1 and Isw2, forming a number of multi-subunit complexes. So far, two Isw1- containing complexes have been identified and characterized. The Isw1a complex consists of two subunits, Isw1 and Ioc3, while the Isw1b remodeler contains Isw1, Ioc2 and Ioc4 as subunits. Functionally, both remodelers have been linked to the establishment of nucleosomal arrays as well as gene transcription. We have identified a new variant Isw1 complex using TAP-tag purification and mass spectrometry. We term this complex Isw1c. The Isw1c complex consists of two subunits, Isw1 and Esc8. Also, we showed that Isw1c indeed works as a remodeler and has the ability to slide nucleosomes in vitro. In the future, we plan to further characterize Isw1c function both in vivo and in vitro.

28 Monika Witzenberger

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Establishing the tRNA methyltransferase TRMT2a as a novel drug target for treatment of PolyQ diseases.

Huntington's disease (HD) belongs to the family of Polyglutamine (PolyQ) diseases. These neurodegenerative disorders share an expanded CAG repeat stretch in their coding region that is translated into expanded PolyQ tracts in the disease-linked protein. In the case of HD, the Huntingtin protein harbors the pathologically elongated polyQ tract. Presence of polyQ Huntingtin causes neuronal decline predominantly in basal ganglia. Loss of these neurons result in the cardinal symptoms of HD: jerky movements and cognitive decline. Until now, no widely applicable, approved HD therapy is available. An RNAi screen in Drosophila melanogaster has shown that the inhibition of tRNAmethyltransferase homolog A (TRMT2a) reduces PolyQ-induced aggregate formation and toxicity in yeast, flies and HEK cells (Aaron Voigt's lab), establishing TRMT2a as a potential novel drug target. TRMT2a is a tRNA methyltransferase that converts uridine to 5-methyl uridine at position 54 in tRNAs. The protein is predicted to contain an RNA recognition motif (RRM) and a catalytic domain (CD). In our lab we achieved to purify and crystallize the predicted RRM domain of TRMT2a. With this structure in hands an in silico drug screen was performed using molecular simulation and chemoinformatics (Giulia Rossetti's lab) resulting in a set of potential RRM-TRMT2a inhibitors. With biophysical techniques such as ITC and SPR, binding properties of those inhibitors to the RRM-TRMT2a were tested. Functionally, the inhibitory effect of those compounds on the enzymatic activity of TRMT2a was dissected with a newly established methyltransferase assay.

29 Tanja Holzknecht

Structural and Chemical Biology, Centro de Investigaciones Biológicas, Madrid, ES

Glutathione Reductase Ligand Selection by an Adaptive Chemical System.

Malaria is a vector-born parasitic disease that is transmitted to human beings through the bite of a female Anopheles mosquito. Despite the efforts made to eliminate the disease, it remains an enormous global challenge, due to the appearance of drug resistance and the lack of effective vaccines. In the search of alternative treatment strategies, glutathione reductase (GR) has become a relevant therapeutic target. The main role of this flavoenzyme is to provide the organism with high levels of the antioxidant glutathione (GSH). Since the malaria-causing parasite requires an efficient thiol metabolism to shield itself from oxidative stress, an inhibition of GR could permanently harm the parasite's growth.

Using target-directed combinatorial chemistry (td-DCC) we identified a GR binder with an apparent dissociation constant in the micromolar range. Besides, we elucidated the structural molecular recognition event of our hit-compound to GR by molecular docking and dynamic simulation studies. In vitro essays of the identified GR-binder are currently carried out to evaluate its antimalarial activity.

30 Saikat Chakraborty

Max Planck Institute of Biochemistry, Munich, DE

Integrated view of cytoskeletal architecture and mechanics: An in situ cryo-electron tomography study.

With millimeter long persistence length, microtubules (MTs) are well-placed to influence cell shape and mechanics based on their ability to resist large-scale compressive forces. However, highly curved MTs are often located inside cells. Reductionist approach of studying MTs outside their native environment could not adequately justify such behaviors. Therefore, we sought to visualize cellular MTs preserved at their near-native state at molecular resolution and investigate their bending behavior using an integrated view of cytoskeleton.

Recent developments in cryo-electron tomography enabled us to visualize subcellular ultrastructure within large eukaryotic cells by cryo-focused ion beam micro machining, establishing correlative cryo-fluorescence for the localization of organelles and their ultrastructural characterization. Using this workflow, we performed a molecular resolution survey of MT-cytoskeleton across cell-cycle stages and cell-types. It revealed native organization of MTs intertwined with the composite network of other cytoskeletal filaments. We show that presence of actin-cytoskeleton in the vicinity of the MTs has a substantial effect on the curvature of MTs. Remarkably, higher curvature induces accumulation of nanoscale defects on the MT lattice in situ, which further contributes to the MT curvature. Therefore, our data reveals more integrated realistic picture of cytoskeletal mechanics, linking molecular structure to physical properties across length-scales.

31 Timon Nast-Kolb

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In-vitro modulation of VASP mediated actin bundling on lipid membranes.

The diverse actin structures within the cell are formed by a different composition of few key nucleators and elongators and various actin binding proteins (ABPs). The Ena/VASP elongator family is involved in the enhanced formation of lamellipodia-like branched actin-networks, formation of dorsal stress fibers and stiff filopodia. The latter are composed of parallel actin bundles, which create thin membrane protrusions. The behavior of VASP on the formation of an actin network structure is investigated in a bottom-up approach using supported lipid bilayers or giant unilamellar vesicles. On the membrane of the vesicles, VASP is locally bound to induce unbranched actin elongation. Contrary to VASP-mediated polymerization on solid substrates, clustering of VASP promotes a connected network of bundles on lipid membranes. These actin filaments can be further affected by added ABPs. Myosin motor proteins, crosslinkers and turnover enhancing ABPs are added to elucidate their effect on size and structure of the actin networks.

32 Lena Molitor and Melina Klostermann

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graspPURA – Understanding the neurodevelopmental disorder PURA syndrome.

The purine-rich element binding protein A (Pur-alpha) has been described to localize to nucleus and cytoplasm as well as to interact with DNA and RNA in a similar manner. Apart from basic functional information and in-depth structural data very little is known on its cellular function. Recently a sporadic neurodevelopmental disorder was associated with heterozygous mutations in the Pur-alpha coding gene PURA (5q31.2). Several different mutations in the PURA gene cause neurodevelopmental delays, resulting in a lack of speech, difficulties with motion and for some patients severe epileptic seizures. Overall the PURA syndrome phenotypes vary tremendously. The reasons for these variations are unknown.
In an attempt to decipher the cytoplasmic role of Pur-alpha we generated data sets for protein-protein and protein-RNA interactions in HeLa cells. Here we report details on the in vivo binding of Pur-alpha to various 3'UTRs without observing the previously reported bias of CGG repeats. By validating these binding events in vitro we could only observe a moderate preference for the identified binding motifs, indicating that additional features such as cofactors might be important.

33 Christina Bebber

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Redox pathway plasticity allows ferroptosis escape of neuroendocrine small cell lung cancer.

Small cell lung cancer (SCLC) belongs to the most aggressive forms of cancer. Bi-allelic loss of p53 and Rb1 in chemo-naïve SCLC suggests selection against cell death prior to therapy. Tumour selection may involve extrinsic and intrinsic apoptosis, necroptosis, pyroptosis and ferroptosis. Yet, cell death selection landscapes of chemo-naïve cancers have remained unexplored. Here, we show that chemo-naïve SCLC shows signs of selection against apoptosis and necroptosis. Importantly, we find that ASCL1- (A-) but not neuroendocrine (NE) A+ SCLC responds to GPX4 inhibition or glutathione (GSH) depletion with induction of ferroptosis. NE-A+ SCLC, in turn, acquires dependency on the thioredoxin (TRX) pathway. Interestingly, A-SCLC switches from GSH to TRX pathway usage upon suppression of glutathione cysteine ligase (GCL) allowing for ferroptosis escape. Strikingly, combined induction of ferroptosis and inhibition of the TRX pathway prevents redox pathway plasticity resulting in effective killing of SCLC. Moreover, patient-derived chemo-naïve and refractory NE-A+ SCLC is selectively killed via this regime. In SCLC patient data, combined low expression of GPX4 and Thioredoxin reductase 1 (TXNRD1) identifies an SCLC patient subset with significantly improved overall survival underscoring a fundamental role for redox pathway plasticity in ferroptosis escape of NE-A+ SCLC.

34 Leonie Feik

Department III for Internal Medicine, Heart Center at the University of Cologne, Cologne, DE

The role of PI3 kinase γ signaling in pulmonary hypertension.

Pulmonary hypertension (PH) is a pulmonary vascular disease, characterized by chronically increased mean pulmonary arterial pressure, increased pulmonary vascular resistance, and right ventricular (RV) hypertrophy and failure. The pathogenesis is mainly based on increased proliferation and reduced apoptosis of both vascular smooth muscle cells and endothelial cells, as well as immune cell infiltration. Previous studies have shown that class IA phosphatidylinositol-3-kinases (PI3K) are involved in these changes. Recent findings suggest that the class IB PI3Kγ isoform could play a role too.

The role of PI3Kγ in PH was analysed in vivo using the hypoxia-induced mouse model of PH. Mice with a catalytically inactive form of PI3Kγ (PI3KγKD/KD) were kept at 10%O2 for 21 days (hypoxia HOX vs. normoxia NOX). Subsequently, right ventricular systolic pressure (RVSP) was measured with a pressure catheter. Contrary to our expectations, HOX PI3KγKD/KD mice showed a significantly increased RVSP and RV hypertrophy compared to wild type controls. Further, cell migration and proliferation of PI3Kγ inactivated vascular cells was analysed. The inactivation had no effect on proliferation but migration was reduced significantly.

Contrary to our expectations, the results indicate that kinase inactivation of PI3Kγ both in vitro and in vivo is not able to attenuate the pathogenesis of PH, but surprisingly tends to increase it. Thus, our results indicate an unexpected protective effect of PI3Kγ in PH.

35 Vera Manelli

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Molecular Mechanisms of 3D Chromatin Loop Formation.

Gene regulation is extremely multi-layered and relevant to the initiation of cell developmental programs. Enhancer-promoter interactions and the subsequent chromatin loop formation are thought to be part of the mechanisms that modulate cell differentiation and determine cell identity. Despite enormous advances in the field, how these loops are established and maintained is still poorly understood. Specifically, the causal relationship between recruitment of chromatin-modifying complexes or ncRNAs and the formation of the individual loops is still under investigation. Furthermore, due to technical challenges, many of these regulatory factors still await discovery. During my PhD, I will dissect the molecular mechanisms that underlie chromatin loop formation focusing on neural differentiation as a model system. In particular, I will employ a combination of CRISPR-based genome targeting, state-of-the-art proximity labelling and proteomics to identify, for the first time, factors associated with dynamic regulatory loops. I will then study how novel and candidate transcription factors and cofactors control the generation of enhancer-promoter interactions and how they influence the 3D genome architecture.

36 Sabrina Bacher

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Understanding the role of Pur-α in neural development.

PURA Syndrome is a rare genetic disorder occurring due to mutations in the PURA gene. Only around 320 people have been reported with this condition worldwide. Patients suffer from a moderate to severe degree of learning disability and neurodevelopmental delay as well as from diverse other symptoms, like epilepsy and neonatal hypotonia. The PURA gene encodes for the Pur-α (purine-rich element binding) protein that is highly conserved from bacteria through humans and can bind single stranded DNA as well as RNA. To date, the exact functions of Pur-α in the cell are still not well understood, but it is thought to be involved in many important cellular processes.One aim of this project is to understand the role of Pur- α during early neural development. For that Pur-α localization and protein levels in human neural stem cells (HNSCs) maturation towards astrocytes were investigated. No significant change in Pur-α protein expression could be detected during this differentiation period. Pur-α mainly localized in the cytoplasm but can also be detected in the nucleus and there was no re-localization upon differentiation, which could confirm its importance in many cellular processes during development. Pur-α was detected in granules suggesting its involvement in RNA transport and processing.Another aim of this study is to model PURA Syndrome in vitro for studying gene expression and function of Pur- α. For that, a PURA knock-out mutation was introduced in induced pluripotent stem cells (iPSCs), which then can be used for comparative studies, such as investigating metabolomics.

37 Janet Tait

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Invesigating H4K20 methylation-mediated regulation of cytoskeletal architecture using Xenopus culture systems.

Histone post-translational modifications greatly influence gene expression and different methylation marks have distinct functional connotations. H4 Lysine 20 (H4K20) can be unmethylated, mono-, di-, or trimethylated and these methylation states are present in different abundances on the chromatin. H4K20 methylation is regulated by a series of cell cycle-dependent methyltransferases. Set8, suv4-20h1, and suv4-20h2 write the mono, di and tri methyl marks, respectively. Histone demethylases may also play a critical role in regulating H4K20 methylation state, however they are not well studied. Knockdown of suv4-20h1 causes an increase in the H4K20me1 state which leads to defects in ciliogenesis and neurite outgrowth. Recent evidence from our lab suggests that this defect is caused by repression of cytoskeletal genes by increased H4K20me1. Xenopus embryos present an excellent opportunity to study cytoskeletal dynamics because of their large size, ease of manipulation and microinjection, and the ability to generate organoid culture systems. Using whole embryos, neural and animal cap explant culture systems, and newly established Xenopus cell lines, this study aims to determine how H4K20 methylation state regulates cytoskeletal dynamics at multiple levels of biological organisation.

38 Iris Langstein-Skora

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Uncovering the determinants of function in Abf1's IDR.

In Saccharomyces cerevisiae, general regulatory factors (GRFs), like Abf1, Reb1 and Rap1, hold a crucial role ingenomic processes such as transcription, replication and DNA repair. It may be a shared feature among these roles that GRFs are also key players in nucleosome positioning. They act as barriers for ATP-dependent remodelers while these set up nucleosomal arrays, for example, at the 5'-end of active genes. The underlying mechanism of GRFs in the context of nucleosome positioning remains to be elucidated. Besides sequence-specific DNA-binding domains, GRFs only contain intrinsically disordered regions (IDRs), which are present in at least30% of all proteins. IDRs lack a well-defined 3D structure, and despite their abundance, IDRs are still poorly understood. We strive to delineate which IDR features are decisive for GRF function, especially as nucleosome positioning barriers. Random mutagenesis genetic screens regarding the IDRs provided us with functional and non-functional GRF versions. These are used to learn the biophysical key features of an IDR required for a functional GRF. These features can be validated via rationally designed IDR mutants.

39 Vera Kleene

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Deciphering the Role of the Proteasome during Chromatin Assembly.

The proteasome plays an essential role in multiple key processes within cells. While its function is well studied in the cytosol, our knowledge of the role of the proteasome in the nucleus is still widely unknown. The assembly of chromatin structure after replication, transcription and DNA repair is a vital process that requires high fidelity to ensure chromatin stability. To decipher the role of the proteasome during chromatin assembly, we use in vitro chromatin reconstitution assays in Drosophila embryo extracts and in vivo NCC (nascent chromatin capture) methods in human cells in combination with cutting edge mass spectrometry. Thereby, we have found proteasomal proteins to interact with DNA during chromatin assembly in vitro and in vivo. Furthermore, we can show that the proteasome in Drosophila embryo extract can be efficiently inhibited by Epoxomicin, using chemoluminescence based proteasome activity assays. Exploiting these tools and findings, we are currently investigating how in vitro chromatin assembly is affected by proteasome inhibition. Collectively, we aim to shed light on a putative essential role of the proteasome in ensuring the fidelity of chromatin assembly.

40 Lena Bergmann

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H3K36 methylation and DNA-binding are both critical for Ioc4 recruitment and Isw1b function.

In eukaryotes DNA is organized in chromatin. Its smallest repeating unit, the nucleosome, represents a barrier for RNA polymerase II (RNAPII). So access to DNA needs to be regulated. This is done by chromatin remodelers, histone chaperones and histone modifying enzymes.

In yeast the Isw1b chromatin remodeler consists of Isw1, Ioc2 and Ioc4. Isw1b maintains chromatin organization during transcription by preventing trans histone exchange. This occurs when new, acetylated histones are incorporated in place of pre-existing H3K36 methylated histones. Thereby, Isw1b ensures that chromatin remains tightly organized which leaves cryptic, promoter-like elements unavailable to RNAPII and thus suppresses cryptic transcription. The remodeler is recruited to set2-mediated H3K36me3 in vivo which is a mark of actively transcribed genes and mainly found over gene bodies.

While Isw1 is the catalytic subunit of Isw1b, the Ioc subunits are thought to be responsible for correct targeting in vivo. Ioc4 harbors a PWWP domain. They mostly recognize H3K36me3.We found that deletion of the Ioc4 PWWP domain results in reduced association with nucleosomes and lowers remodeling activity.

We have recently obtained a high-resolution structure of the Ioc4 PWWP domain,which exhibits two basic patches thatmediate binding of the domain to DNA. It is also essential for PWWP binding to nucleosomes and full Isw1b remodeler activity in vivo and in vitro.

41 Cláudia Gil

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Cut the silence. A way to understand X-chromosome inactivation.

X-chromosome inactivation is a remarkable example of epigenetic silencing that occurs during female mammals' embryonic development. This process is regulated by a lncRNA called Xist which is monoallelically upregulated from the X chromosome chosen for inactivation, coating the entire chromosome in cis and inducing transcriptional silencing and heterochromatin formation. Xist possesses six tandem-repeated regions, known as the A to F repeats, believed to act as regulatory modules through the interaction with specific proteins. However, the relative contribution of each one of these RNA modules, and their protein partners, is not well understood.

With this work we aimed to dissect the role of each Xist's repeat in the initiation of XCI. So, we deleted every single tandem-repeated region of an inducible Xist allele at its endogenous locus in mESCs, using CRISPR/Cas9. Afterwards, we characterized the different cell lines in terms of cell lethality, transcripts stability and gene expression. Xist and X-linked gene expression was analyzed by two complementary techniques (RTqPCR and RNA-FISH) allowing the evaluation at population and single-cell level. Heterochromatin formation was also analyzed, by immunofluorescence combined with RNA-FISH, for different heterochromatin marks typical of the inactive X chromosome. Additionally, we wanted also to study the influence of several known protein interactors on this process, through their knock-down in our different cell lines.

42 Ming Toh

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The transcription factor, CrebA, regulates appetite in response to nutrient state.

Food availability has a significant impact on our metabolism. For instance, refeeding postfasting induces metabolic changes like increased fatty acid and glycogen synthesis, which are mediated partly by transcriptional regulation. The nutritional status of different organs is communicated to the central nervous system through appetitive hormones to guide foraging, but the mechanisms that regulate hormone secretion are not well understood.

Recently, CrebA was identified as the key regulator of cell secretory capacity in response to nutrition. It upregulates genes that target proteins to the endoplasmic reticulum (ER) upon feeding. We propose that CrebA is part of a negative regulatory loop which downregulates appetite after feeding, because first, its transcript and protein levels increase transiently within 2 hours of re-feeding. Second, CrebA binding to the promoters of secretory machinery genes is increased in the fed state. Third, modulating CrebA level also affects their food intake. Flies overexpressing CrebA eat less than the wild-type postfasting as it mimics the “fed state”. Altogether, we hypothesize that CrebA upregulates the secretion of some appetitive hormones in the hemolymph to coordinate a systemic response.

Interestingly, many of the genes that respond to feeding are only transiently upregulated. Hence, we speculate that they are regulated by RNA turn-over, which might be mediated through CrebA because it binds two RNA-binding proteins (RBP), one of which is a RNA nuclease. We have begun to investigate the role of CrebA in regulating these RBPs and their role in the response to nutrition.

In summary, we aim to elucidate the physiological role of CrebA in response to nutrients. Our knowledge of Drosophila CrebA is relevant for human physiology, because CrebA has five mammalian orthologs some of which are also regulated in a nutrient-dependent manner.

43 Thomas Retzer

Structure and Dynamics of Molecular Machines, Max Planck Institute of Biochemistry, Munich, DE

Single Molecule Studies of the Replisome coping with topological barriers.

DNA stores the genetic information which it is essential for the proliferation of life. The helical structure of DNA bears great advantages when it comes to storing genetic information in a compact way but to access, DNA has to be unwound which leads to unwanted superhelical strain ahead (positive supercoils) and behind the fork (precatenanes). If the cell does not deal with the accumulating strain the whole process will stop and the cell dies. When it comes down to individual molecules not much is known about how the topology effects replication in a time-resolved manner. There are special proteins dedicated to deal with the stress in order to avoid severe consequences. So called topoisomerases mediate DNA topology. Most studies characterise how fast these enzymes can change the state of topology. DNA gyrase responsible for resolving positive stress ahead of the replication fork and recent studies showed that the number of molecules which are located close to the replication fork is not sufficient to keep up with the high speed of the replisome. This hints at a highly dynamic and ever changing process. Furthermore the machinery faces different situations depending on the state of replication. In the early phase the stretch of unreplicated DNA offers enough space for multiple gyrase molecules to get rid of positive twists while later when the replisome approaches the end of a topological domain or the end of the chromosome not enough space is left to change topology. This opens up the question of how the replisome can cope with the stress on its own. It would also be interesting to know how the replisome behaves for different numbers of enzymes. We try to simulate how the machinery behaves at different stages of replication. For that we designed a DNA substrate similar to the situation in a cell to investigate the dynamics in more detail. The DNA will be a topological constrained fork substrate which gives us a multifarious insight into the dynamics of individual molecules. A total internal reflection microscopy will give us the possibility to observe the dynamics of the fork as well as the composition of the replisome changing over time due to different stress situations. To asses protein exchange of the different components of the replisome FRAP (fluorescence recovery after photobleaching) experiments will be carried out. For that different proteins will be labelled and bleached to see how fast the fluorescence signal is recovering over time. We want to decipher the mechanism of the replisome coping with the topological stress without help of other proteins and shed some light on the interplay of the replication machinery and topoisomerases in different situations.

44 Bernardo A. Arús

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Multicolor, fast imaging of biological processes in awake and unrestrained mice using the shortwave infrared (SWIR).

High resolution, multiplexed experiments are a staple in biological imaging, however, existing methods are insufficient for complex animals due to significant scattering and autofluorescence in tissue at visible (VIS, 350–700 nm) and near-infrared (NIR, 700–1000 nm) wavelengths. Here, we enable real-time, non-invasive multicolor imaging experiments in mice through complementary advances in imaging technologies and optical contrast agents for the shortwave infrared (SWIR, 1000–2000 nm) region. We built an imaging system with modulated excitation and SWIR detection, facilitating video-rate multicolor in vivo imaging. We also developed novel organic dyes absorbing at 890 nm, 984 nm and 1061 nm that were used, along with indocyanine green, for real-time, four-color imaging with high spatial and temporal resolution in mice, as well as imaging at 300 fps in a single color. We demonstrate the capabilities of this novel technology for non-invasive imaging of physiological processes in mice.

45 Korneel Ridderbeek

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Measuring Rapid, Transient Nano-Bio Interactions using Plasmon Resonance Scattering Photon-Correlation Fourier Spectroscopy.

The unique properties of nanoscale materials have led to growing interest in biological applications. However, the complex interactive interplay between these nanomaterials and biomolecules in their environment has remained difficult to study. Predictive relationships are often lacking, thereby limiting efficiency and applicability. We present an approach for unveiling the fast, transient interactions between biomolecules and nanoparticles that have been, to date, particularly difficult to resolve. Our optical method combines the high temporal and spectral resolution inherent to photon-correlation-based interferometry with the local environment sensitivity of nanoparticle surface Plasmon resonances. Hence, transient interactions can be probed across different timescales (10-12-102 s), with low-intensity excitation, and without selection bias, in solution. Here, we show a proof-of-concept of this approach that captures controlled dynamic behavior on short timescales, proving that this optical method can be applied towards measuring interactions between biomolecules and nanoparticles.

46 Elena M. Bonke

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The Effects of Repetitive Head Impacts on Postural Control: A Systematic Review.

Purpose: The purpose of our study was to investigate the association between repetitive head impact (RHI) exposure and postural control.

Study design: Systematic review

Methods: PubMed, Embase and PsycInfo were searched using a self-developed term including the keywords balance OR postural control AND repetitive OR sub-concussive head impacts. Twenty-one studies were included for further analyses. We excluded non-peer-reviewed, secondary, cross-sectional, and animal studies, as well as studies with subjects who sustained a concussion. Level of evidence and quality were rated using three different assessments.

Results: All included studies were grouped into Category I or II studies. Category I included trials examining the effects of controlled soccer heading on postural control (n=8) mostly using instrumented postural assessment tools; Category II studies were prospective cohort studies investigating on-the-field pre-postseason changes in postural control (n=13) using instrumented as well as non-instrumented tools. Findings were mixed, with only 3/8 studies in Category I and 3/13 in Category II showing alterations in postural control after RHI exposure.

Conclusion: Due to mixed findings, future studies aimed at investigating the effects of RHI on different athlete populations are needed. Furthermore, the combination of objective clinical balance measures may be a promising approach to accurately measure how, and to what degree, postural control may be affected by RHI.

47 Xiaoyan Du Institute of Urban Environment, Chinese Academy of Sciences, Xiamen, CN
Molecular Exposomics, Pioneer Campus, Helmholz Zentrum, Munich, DE

Effect of chronic arsenic exposure via drinking water on DNA epigenetic response in the cortex and hippocampus.

The neurotoxicity of arsenic is a serious health problem, especially for children. DNA epigenetic change may be an important pathogenic mechanism, but the molecular pathway remains unclear. In this study, the weaned male Sprague-Dawly (SD) rats were treated with arsenic trioxide via drinking water for 6 months, simulating real developmental exposure situation of children. Arsenic exposure impaired the cognitive abilities, and altered the expression of neuronal activity-regulated genes. Total arsenic concentrations of cortex and hippocampus tissues were significantly increased in a dose-dependent manner. The reduction in 5- methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) levels as well as the down-regulation of DNA methyltransferases (DNMTs) and ten-eleven translocations (TETs) expression suggested that DNA methylation/demethylation processes were significantly suppressed in brain tissues. S-adenosylmethionine (SAM) level wasn't changed, but the expression of the important indicators of oxidative/anti-oxidative balance and tricarboxylic acid (TCA) cycle was significantly deregulated. Overall, arsenic can disrupt oxidative/antioxidative balance; further inhibit TETs expression through TCA cycle and alpha-ketoglutarate (a-KG) pathway, and consequently cause DNA methylation/demethylation disruption. The present study implies oxidative stress but not SAM depletion may lead to DNA epigenetic alteration and arsenic neurotoxicity.

48 Zhong-Min Li Helmholtz Zentrum, Munich, DE

Associations between Persistent Organic Pollutants and Thyroid Hormones in Human Breast Milk.

The thyroid-disrupting effects of persistent organic pollutants (POPs) at low levels are of interest since most of current studies were conducted in high-exposure populations. Here, we assessed the associations of background exposure of POPs with thyroid hormones (THs) using human breast milk samples (N=99) collected from the LUPE cohort (2015–2016, Bavaria, Germany). POPs including polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins and furans (PCDD/Fs), and polybrominated dibenzo-p-dioxins and furans (PBDD/Fs) were determined using GC-MS. Fourteen PBDEs, 17 PCBs, and 5 PCDD/Fs had quantification rates of > 80%. THswere determined using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Total L-thyroxine (TT4), total 3,3’,5-triiodo-L-thyronine (TT3), and total 3,3’,5’-triiodo-L-thyronine (TrT3)) were measured in all samples. We observed nonmonotonic associations. In adjusted single-pollutant models, (1) TT4 was inversely associated with BDE-99, -154, and -196; (2) TT3 was inversely associated with BDE-47, -99, -100, -197, -203, -207, and OCDD; (3) TrT3 was inversely associated with BDE-47, -99, -183, and -203. Multipollutant analysis using principal component analysis and hierarchical clustering revealed inverse associations of PBDEs (BDE-28, -47, -99, -100, -154, -183, and -197) with TT4 and TrT3. These results proved that POPs at low levels might be related to reduced THconcentrations.

49 Michael Kien Yin Ting Max Planck Institute of Molecular Plant Physiology, Munich, DE

uORF mediated translational regulation of the plant circadian clock.

Circadian clocks are an endogenous timing mechanism that allows an organism to fine tune diverse cellular processes in anticipation of external stimuli. In plants, circadian rhythms are generated by a complex network of negative and positive feedback loops that occur at the transcriptional, translational, and post-translational levels. Since most plant circadian studies have focused on transcriptional control, the genes that are “translationally” regulated by the clock are largely unknown. A mechanism that can fine tune levels of translation are upstream open reading frames (uORFs), which are short sequences located in the 5’UTR. Generally, uORFs act as translational repressors of the main open reading frame and have previously been implicated in regulating various metabolic pathways. However, the extent of uORF regulation in the plant circadian clock remains to be explored. Interestingly, core clock genes of the model plant Arabidopsis thaliana, including CCA1, PRR7, GI and TOC1, contain numerous uORFs within their leader sequence. Using luciferase reporters, we aimed to assess the functionality of these uORFs by mutating their “ATG” ribosome initiation site to be non-functional. By monitoring luciferase signals under free running conditions, circadian rhythms of these four genes were examined in detail, revealing interesting contributions of uORFs toward the rhythms of protein translation.

50 Verena Leitz

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The role of thioredoxins and regulation of starch metabolism in acclimation of plants to low temperature.

Cold acclimation requires a rapid stimulation of starch synthesis for storage of fixed carbon to generate stromal sugars for membrane protection, ROS scavenging and retrograde signaling. But the cold-responsive adjustments of chloroplast metabolism and especially the role and regulation of starch metabolism to generate stromal sugars for acclimation to low temperatures are poorly understood.

The ferredoxin-dependent thioredoxin f1 and the NADPH-dependent thioredoxin reductase C are two important chloroplast thiol-redox systems, which are able to regulate key enzymes of the Calvin-Benson cycle and starch synthesis in response to light (Thormählenet al. 2015). I will not only show that missing starch or redox-regulation has tremendous effects on the metabolic state of the cell but also that they're determining plant survival.

51 Silvia Vangelisti

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Functional importance of 3D genome rewiring for brain evolution.

One of the most important species-specific changes during human evolution is the expansion of the cerebral cortex, which likely contributed to human higher cognitive functions compared to the other primates. Structural variations (SVs) are largely responsible for the genetic diversity of the human genome and they can lead to the rewiring of regulatory interactions and changes in gene expression. Moreover, SVs account for 3-4 times more sequence divergence between the chimp and the human genomes than single-base-pair mutations. In order to shed light on the mechanisms behind this large expansion of the brain, we propose to systematically study how 3D genome organization has changed during primate evolution and how it might have influenced cortical evolution. We will use cerebral organoids derived from different primate iPSCs to determine the 3D genome architecture, exploiting Hi-C experiments, and relate the changes in topology to SVs. We will then explore how alterations in the 3D genome organization are linked to changes in chromatin accessibility, gene expression and transcription factor binding. Finally, we will test the functional consequences of specific chromosomal rearrangements for cortical development using genome engineering approaches. Taken together, this project will establish a new paradigm for studying and quantifying chromatin topology during primate evolution and may identify new mechanisms that have contributed to the evolution of the human cortex.

52 Robert E. Rollins

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Variable prevalence of Borrelia bacteria in ticks collected in southern Germany.

Lyme borreliosis (LB) is the most common arthropod transmitted disease in Europe and North America and is caused by certain genospecies of Borrelia bacteria. These bacteria are transmitted to humans through tick bites, primarily, from adult and nymphal ticks. Human LB risk is directly related to the prevalence and distribution of Borrelia genospecies within their tick vectors throughout the wild. These distributions vary in time and space and to understand LB risk fully, up to date information on prevalence and distribution of Borrelia is required. The last survey of Borrelia in southern Germany was completed in 2008 and new data is needed. Ticks were collected in seven plots located in and around Munich, Germany, from March-July 2019 and were screened for Borrelia bacteria. Two of the seven plots were also sampled in 2008. In total 158 adult and 525 nymphal ticks were collected with Borrelia positive ticks found in all plots. Adults and nymphs did not differ in their overall Borrelia prevalence. The percentage of Borrelia positive nymphs did vary significantly between plots whereas adults did not. In total, eight Borrelia genospecies were found with human pathogenic genospecies dominating. Borrelia prevalence differed significantly in nymphs in one plot from 2008 (12.3%) to 2019 (43.4%) but adults did not vary significantly. Our results highlight the variability in Borrelia prevalence including genospecies distribution both over time and short geographic distances.

53 Andrea Lukacs Ludwig-Maximilians-Universität, Munich, DE

A chromatin protein complex drives the evolution of species in Drosophila.

The rise of new species is key to the formation and maintenance of biodiversity. Heterochromatin for its fast evolving landscape is a strong driver of species isolation and heterochromatin-binding proteins must evolve accordingly to cope with their changing environment. In the two species Drosophila melanogaster and Drosophila simulans a heterochromatic complex involving the proteins HMR and LHR is responsible for the lethality of the hybrids that result from their breeding. Despite recent efforts, the molecular mechanisms involved in hybrids lethality are poorly understood. By molecularly and functionally dissecting the HMR/LHR protein complex, we revealed the importance of HMR/LHR interaction and heterochromatic localization in the development of hybrid lethality.

54 Francesca Luoni Technische Universität, Darmstadt, DE

Deep space exploration: characterizing and testing innovative radiation shielding materials.

Our century could be remembered by future generations as the era of mature space exploration and colonization. Nevertheless, galactic cosmic rays (GCR) and their effects on humans during longterm deep space missions have been classified as the number one possible “showstopper” for human space exploration. Shielding material will be added to the spacecraft structure with the aim of reducing the deleterious effects of space radiation on humans aboard the spacecraft, through nuclear fragmentation of GCR within their interaction with the shielding material. Due to the severe mass constrains that characterize space missions, the quantity to maximize when shielding materials are chosen is the nuclear fragmentation cross section per unit mass: this aim can be achieved with low atomic number materials. The lightest easy-to-handle material is high density polyethylene. However, lithium hydride and lithium boron hydride have been proved to be even more promising, which is why new strategies with the objective of making them less chemically hazardous are under development. The aim of the presented project is to deepen the current understanding of the shielding properties of the above-mentioned candidate materials and different possible shielding configurations, through strategized experiments. A first experimental campaign has been conducted at GSI measuring the absorbed dose attenuation delivered with a 1GeV/u Fe 56 beam as a function of the shielding thickness and configuration.

55 Flavia Söllner

Biomedical Center, Ludwig-Maximilians-Universität, Munich, DE

The ADP-ribosyl-hydrolase MacroD1 regulates mitochondrial DNA replication.

Mammalian mitochondria, the double-membrane-bound organelles found in most eukaryotic cells, contain multiple copies of mitochondrial DNA (mtDNA). The maintenance and regulation of the mitochondrial genome is essential for normal cellular function and metabolism, as the human mitochondrial genome codes for 37 genes, including rRNAs and tRNAs required for the mitochondrial translation of mRNAs encoding for vital subunits of the electron transport chain. The presence of ADP-ribosylation – a post-translational modification of proteins –in mitochondria was described over 40 years ago , yet little is known about its target(-s) and function(-s). ADP-ribosylation is the transfer of ADP-ribose from NAD+ to the amino acid side chains of proteins. Mono- and poly(ADP-ribose) moieties can be added, recognized and removed from specific substrates. It is generally thought that the dynamic addition and removal of ADP-ribose moieties induces alterations in protein function, enzyme activity or interaction with modified substrates. We and others have shown that human enzymes capable of fully reversing cellular mono-ADP-ribosylation, specifically the human ADP-ribosyl hydrolase MacroD1, localizes exclusively to mitochondria. In our project, we have used this knowledge to decipher the biological role(-s) of mitochondrial ADP-ribosylation. We have used MacroD1 to generate a macrodomain-based mitochondrial ADP-ribosylome, which allowed us to expand the list of mitochondrial ADP-ribosylated proteins, and to generate the first endogenous mitochondrial ADP-ribosylome. Using this approach, we identified core factors essential in mtDNA replication and mtDNA repair. MacroD1-deficient and MacroD1-overexpressing mammalian cells show that MacroD1 and mitochondrial ADP-ribosylation affect the maintenance and regulation of the mitochondrial genome and cellular homeostasis, identifying ADP-ribosylation as an important PTM of this organelle.

56 Paul Stolz

Center for Integrated Protein Science, Ludwig-Maximilians-Universität, Munich, DE

Crosstalk of TET1 and Chromatin during embryonic development.

Whereas all cell types harbor the same genetic information, they display highly divergent and specialized functions. The enormous diversity in cell types is regulated by a complex interplay of chromatin organization, epigenetic modulators and transcription factors. Upon implantation of the blastocyst, the epigenome undergoes a massive reprogramming including a dramatic increase in DNA modifications.

Only recently, TET proteins have been described to successively oxidize methylcytosine to hydroxymethylcytosine, formylcytosine and carboxylcytosine. It was shown that Tet1 KO mice are not viable and have severe gastrulation defects, illustrating TET1s important role in regulating developmental processes. Accumulating evidence indicates that TET1 has extensive functions as a transcriptional regulator independent of its catalytic functions. Interestingly, we found that TET1 associates with the BPTF/NURF complex. BPTF has been shown to associate with chromatin only when both H4K16ac and H3K4me3 are present on a single nucleosome. Interestingly, we see a massive reduction of H4K16ac in Tet1 depleted mESCs and mEpiLCs. Further, we can show that TET1 depleted naïve mESCs exhibit a more accessible chromatin phenotype independent of the catalytic function of Tet1.

To identify how TET1 and BPTF contribute to transcriptional regulation during embryonic development, gastrulation and lineage commitment we will make use of an Auxin-inducible protein degradation system. This strategy will for the first time shed light on the interplay of TET1 and the BPTF/NURF complex. Further, using the AID Auxin degron system we hope to get first insights on the direct and joint transcriptional targets of TET1 and BPTF/NURF.

57 Laura Prieto Clemente

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Dynasore blocks ferroptosis through suppression of ROS production and radical trapping.

Ferroptosis is a form of cell death which critically depends on labile ferric iron. Iron is required to catalyse a Fenton reaction generating hydroxyl radicals which attack membrane lipids followed by plasma membrane rupture. Thereby, iron import mediated via transferrin receptor and radical formation are thought to be two crucial prerequisites for the execution of ferroptosis. Intriguingly, the Dynamin inhibitor Dynasore, which has been shown to block transferrin receptor endocytosis, was shown to protect from ischemia/reperfusion injury as well as neuronal cell death following spinal cord injury. Yet, it is unknown how Dynasore exerts these cell death protective functions. Here, we uncover that Dynasore protects non small cell lung cancer cells from ferroptosis. Surprisingly, this activity was independent of dynamin 1 and 2 but instead involved Dynasoremediated inhibition of mitochondrial respiration and ROS production feeding into lipid peroxidation. In addition, Dynasore acts a direct radical trapping agent in cell-free assays. Thus, Dynasore can function as a ferroptosis inhibitor via two distinct activities highlighting important implications for the interpretation of studies observing protective effects of dynamin inhibitors.

58 Ali Doryab

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Next-generation biomimetic bioreactor for the lung with cyclic mechanical stretch.

Chronic respiratory diseases are among the leading causes of death worldwide, but only symptomatic therapies are available for terminal illness. This in part reflects a lack of biomimetic in vitro models that can imitate the complex environment and physiology of the lung. Here, we introduce the next generation of cell stretching bioreactor system, mimicking the physiologic conditions of the alveolar lung tissue. This bioreactorimplies not only the use of air-liquid interface cell culture conditions and aerosolized substance delivery but also a shift from the currently very rigid and static cell culture conditions (Transwell® Insert technology) to physiologically realistic conditions in terms of substrate elasticity and mechanical strain, mimicking the conditions experienced by alveolar lung tissue during breathing activity.

Compared to the current and conventional models, the proposed system could provide a novel platform to more efficiently study various chemical, biological and mechanical effects on lung epithelial cells in vitro.

59 Luise Rauer

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Characterisation of the skin microbiome in patients with atopic dermatitis in relation to demographic variables.

Background: Atopic dermatitis (AD) is a chronic inflammatory skin disease linked to an altered skin microbiome with reduced bacterial diversity and elevated frequencies of Staphylococcus aureus. We aimed to characterise the yet-incompletely understood relation between the skin microbiome, AD severity and demographic cofactors in more detail.
Methods: The composition and diversity of the lesional skin microbiome of 49 adult patients with moderate to severe AD was investigated using next-generation deep sequencing of the bacterial 16S rRNA gene (V1-V3 region).

Results: AD severity correlated with S. aureus frequencies ($rS = 0.53$, $p < 0.001$), but slightly better withmmicrobiome Evenness ($rS = –0.56$, $p < 0.001$). The association between S. aureus and AD severity was significant in African-American ($rS = 0.65$, $p = 0.001$) and Caucasian-American ($rS = 0.55$, $p = 0.04$) subjects, but not in Asian-American participants ($rS = 0.35$, $p = 0.3$). Overall, as confirmed by multiple regression, AD severity was associated with microbiome Evenness ($p < 0.001$), race ($p < 0.001$), and IgE levels ($p = 0.006$), but not with age, gender and BMI.

Conclusion: This study proposes the use of skin microbiome Evenness as an indicator for AD severity. Finding racial differences in the association between AD severity and the skin microbiome, this study provides for the first time a link between the skin microbial dysbiosis and documented race-based AD endotyp es, further highlighting the heterogeneity of AD.

60 Carla Glassl

Ludwig-Maximilians-Universität, Munich, DE

Virtual 3D staining of a mouse brain using the deep learning method cycleGAN.

Getting histological images from tissue samples is a multistep procedure. Fixated tissue needs to be cut into micron thin sections, which are then individually stained and imaged under the microscope. There a various approaches to omit sectioning of tissue in order to save time and also to sustain the tissue for further analysis. One approach is optical sectioning, a microscopic method where autofluorescence images of focal planes of the tissue can be acquired. However, this approach only works for unstained tissue and therefore replaces classical sectioning only to some extent. This limitation could be overcome by virtually staining the images of optical sectioning.

In this approach virtual staining is performed with a modified generative adversarial network (GAN), the cycleGAN containing a deep learning architecture, which is trained with unpaired data. This approach is applied on autofluorescence mouse brain images in order to create a virtually stained 3D mouse brain based on optical sectioned images. Supporting optical sectioning with virtual staining could decrease preparation time and minimize biopsy tissue volume per patient since the tissue is not sectioned with a microtome anymore but could be used for further analysis instead.

61 Alisha Nabel

Ludwig-Maximilians-Universität, Munich, DE

Development of Specific Functional Axon and Myelin Morphology in Auditory Brainstem Circuits.

Precise timing of signal transmission is a fundamental prerequisite for neuronal processing. Myelination patterning of axons defines action potential conductance speed and temporal precision, and it has recently become clear that myelination patterns may exhibit functional specializations to facilitate neural circuit processing. However, it is unclear how myelination patterns are regulated during development and to what extend their refinement is experience dependent. To address this question we analyzed axon morphology and myelination patterns at different time points during development in brainstem axons of Mongolian gerbils involved in sound localization. These circuits exhibit remarkable physiological and anatomical specializations to ensure fast, stable and precise synaptic transmission. Specifically, axons responding best to low frequencies show a unique myelination pattern compared to high-frequency axons. Our data reveal no difference of diameter size of axons tuned to low and high frequencies before hearing onset. Furthermore, only a few days after hearing onset the axon caliber increases to a mature like diameter for both high-, and low-frequency axons. Interestingly, the internode length seems to be already determined before hearing onset and only increases slightly. Thus, the length of internodes is determined before the axon caliber increases, indicating a combination of experience dependent and innate factors contributing to myelination patterns of axons.

62 Julien Klimmt

Institute for Stroke and Dementia Research, Ludwig-Maximilians-Universität, Munich, DE

Human stem-cell-derived brain tissue models to investigate Alzheimer's disease.

Alzheimer's disease (AD) is the most common dementia with hallmarks such as Aβ plaques, tau tangles and neurodegeneration. Current AD models,however,lack central pathologies such as tau tangles and/or rely on overexpression of disease-relevant genes, causing non-physiological artefacts. This impedes elucidation of pathomechanisms and translation of results to human patients.

Therefore, we aim to develop new, human in vitroAD models based ondisease-relevant brain cell typesderived from human induced pluripotent stem cells (hiPSCs). Inthese models, we aim to elicit late-stage disease phenotypes including plaques and tangles, without the use of geneoverexpression or non-AD mutations. To create these models, we (1) generated AD hiPSC lines using genome editing; (2)optimized protocols to differentiate theseiPSCs into highly pure cultures of cortical neurons, astrocytes and microglia;and (3) currently establish a human brain tissue model by 3D co-culturingall cell types, to elicit AD pathogenesis in a physiologically more relevant system.

We observed typical early-stage disease phenotypes in our AD cultures such as increased Aß secretion and phospho-Tau levels, Aß accumulation and microglial activation. Currently, we are optimizing the model to find factors necessary to elicit later-stage phenotypes such as Aß plaque formation. Our model will form the basis for studies elucidating novel pathomechanisms and developing novel drug screening approaches.

63 Angelika Dannert Institute for Stroke and Dementia Research, Ludwig-Maximilians-Universität, Munich, DE

Elucidating the role of Tau isoform expression in human iPSC-derived Tauopathy models.

Malfunction of the protein Tau is a hallmark of neurodegenerative diseases such as Alzheimer's Disease and Frontotemporal Dementia (FTD) with Tau mutations causing familial FTD. Tau expression and splicing are highly regulated and misregulation of the two classes of splice isoforms, 3R and 4R Tau, leads to FTD. Despite its importance in physiology and disease, the biological and pathological role of Tau isoform expression is poorly understood. This is also due to a lack of models recapitulating adult human Tau expression.

We hypothesize that adult human 3R:4R splicing is essential to recapitulate key pathological phenotypes. We have therefore developed a CRISPR/Cas9 genome editing strategy to alter endogenous Tau isoform expression in induced pluripotent stem cell (iPSC)-derived cortical neurons, and observed that our edited neurons express 3R and 4R Tau in an adult human Tau ratio of 1:1. We culture these neurons in single 2D culture as well as in 3D culture in combination with other major brain cell types, such as astrocytes and microglia, to mimic physiological cell-to-cell and cell-to-matrix interactions. We will use this model to investigate isoform-specific differences of Tau biology and disease.

64 Judit González-Gallego Institute for Stroke and Dementia Research, Ludwig-Maximilians-Universität, Munich, DE

A human blood-brain-barrier in vitro model to investigate the role of FOXF2 in cerebral smallvessel disease.

The human Blood-Brain-Barrier (BBB), composed of brain microvascular endothelial cells, astrocytes and pericytes or smooth muscle cells, is responsible for regulating the transport of substances into the brain. Most of the current BBB in vitro models are based on a two-dimensional monolayer of cells that fail to reproduce a characteristic tubular morphology naturally observed at the human BBB. Here we propose a 3-dimensional BBB model using organ-on-a-chip technology to better recapitulate theanatomical complexity of the human BBB.

Using microfluidics chambers, we generated a fully human iPSC-derived BBB model formed by endothelial cells, mural cells and astrocytes. The endothelial cells formed tight monolayers expressing specific brain vasculature markers such as VE-Cadherin, PECAM-1 and Cadherin-5. Moreover, we show that co-culture of the three cell types induces more complex and interconnected tubular structures.

Organisers

Elena Nikonova

My grandma still thinks that I am a medical doctor, while my friends are wondering why do I "collect virgins" at work. Drosophilist, biochemist, and just a person who can give you a hundred reasons of why being a scientist rocks. I am a PhD student in Dr. Maria Spletter group at the Biomedical Center of the LMU, working on splicing regulation during muscle development.

Isa-Maria Gross

Originally, I come from Hamburg but during my studies, I was always drawn to the south of Germany. Currently, I am working as a PhD student at the MPI of Neurobiology in Tobias Bonhoeffer's department investigating social learning in mice. Besides studying natural behaviors, I love to go hiking and do motorbike trips through the Alps.

Masood Aziz

I am a PhD student at TUM in the structural biology/biophysics department, where I mainly use NMR spectroscopy/other biophysical methods to study the atomic details of disease relevant protein:protein and protein:RNA interactions.

Alja Podgornik

A hopeless idealist who, if given an opportunity, will go on hours long tangents about human psyche, problems of western medicine's closed-mindedness and saving the world. You've been warned. I am a second year PhD working in the Gogolla lab at the MPI for Neurobiology. In my lab, we are trying to better understand mouse (and hopefully, that way, human) emotions, as well as the brain regions involved in their generation and processing. We basically get peed on by our mice. A lot. It's fun!

Vanessa Luzak

I love to bring people together! Good food, yummy drinks and the exchange of creative ideas is what keeps me going. I am currently spending most of my time studying how 3D genome architecture allows little trypanosomes to be such deadly parasites.

Claudia González-Leal

Passionately curious and serious taco eater. I enjoy talking about science and Mexican food. I am a PhD student in the Ladruner Lab at the Biomedical Center of the LMU, where I study the recruitment of non-canonical proteins to UV-induced DNA damaged sites. Don't forget to wear your sunscreen!

Zeynep Irem Günes

In my free time, I like to do improv theater, participate in science communication events and cook! I am doing my PhD in the Insitute of Clinical Neuroimmunology at the BMC. My research focuses on circuitmechanisms involved in Amyothrophic Lateral Sclerosis (ALS), the disease Stephan Hawking also suffered from.

Organisers

FINANCE TEAM

Laura Kuhn

Scientifically, I am interested in the evolution of pre-germinal center B cell lymphoma. Outside of the lab I love dancing salsa, cooking or playing board games with friends.

Laura Lindenthal

In my free time I delight my neighbors practicing my skills playing the violin. I am a PhD student in the lab of Peter Murray at the Max Planck Institute of Biochemistry, studying macrophage polarization after exposure to diseased cells.

Geri Rodschinka

I love traveling around the world and eat a lot, unfortunately one has to come back to reality at some point... Currently, I'm a PhD student at the Max Planck Institute of Biochemistry, investigating qualitative protein synthesis in metazoans.

Lena Molitor

I work in probably the most fun lab! In parts of our group at the Helmholtz Center Munich we try to understand PURA syndrome, a rare neurodevelopmental disorder that originates from de novo mutations in the PURA gene. The resulting protein is a DNA/RNA binding protein that we think is involved in tons of cellular processes – that makes it even more fun!

Irina Shcherbakova

I am originally from a small village in the north of Russia but already had time to live in 5 different cities. For now I settled down in Munich and I am happy about it! I am a PhD student in Gunnar Schotta lab at the Biomedical Center (LMU), where I am investigating how the activity of endogenous retroviruses is regulated in our genome.

Shuaijun Wang

I am a PhD graduate from the Department of Pharmacy of the LMU. I investigate actin binding natural compounds in vitro, both on a cellular level and on a protein-protein-interaction level, through co-crystallization structure determination. My colleagues named me "Actin Queen". I like traveling around Europe, and challenging myself with all kinds of sports.

Organisers

MARKETING TEAM

Barbara Šafarić

Growing up I always wanted to be an astronaut. Actually, I still want to be an astronaut! In the meantime, I am a PhD candidate studying nucleosome reorganization with single molecule approaches at the Max Plank Institute of Biochemistry in Munich.

Jasmin Weber

I hope teleportation is invented soon because I'm lazy and it's better for the environment. Until then, I'm a PhD student at the Walter Brendel Centre of Experimental Medicine (LMU) dealing with how the sympathetic nervous system governs circadian leukocyte trafficking in vivo.

Leonie Zeitler

No matter if it's summer or winter, I love spending time in the mountains. I am a PhD student in the Murray Lab at the Max Planck Institute of Biochemistry working on immunoregulation through amino acid metabolizing enzymes.

Myriam Ripphahn

"Viel zu lernen du noch hast/Much to learn, you still have." Following the advice of Jedi Master Yoda, I am doing my PhD at the Walter Brendel Center (LMU), investigating how Listeria monocytogenes overcomes the placental barrier during pregnancy.

Sergi Masgrau-Alsina

I love travelling but Greta makes me feel bad about it, so I am on the hunt for a new hobby... any ideas? Currently I am a doctoral candidate in medical research in the Biomedical Center of the LMU, trying to find out which is the role of the protein Mst1 in leukocyte recruitment.

Valentyna Inshyna

I'm a second year PhD student at Ludwig Maximilian University Munich in Functional Morphology Group. I study eight-legged micro-animals, called tardigrades, which can withstand just about anything, from boiling and freezing to the vacuum of outer space and radiation 1,000 times stronger than humans can handle. During my free time I like to hike and look for other interesting animals and plants and bake some cakes for friends.

Organisers

LOGISTICS TEAM

Sabine Helmrath

During my free time, I love gardening and like to teach myself photography skills. As a PhD student, I am now working in the immunology laboratory of Prof. Marc Schmidt-Supprian at the Klinikum rechts der Isar, Munich investigating the role of RNA-binding proteins in B lymphocyte development.

Anna Kiss

In my free time I practice Capoeira, a Brazilian martial and cultural art, which not only strengthens the body but also your team spirit, music understanding and Portuguese skills. Axé! My focus in research is integrating biochemistry and structural methods to find cool 3D structures of multimeric protein and RNA-protein complexes.

Annet Glas

I am Annet, I'm a PhD student in the lab of Tobias Bonhoeffer in the MPI of Neurobiology and I study learning and memory! The best part of my job is that I have access to a 3D-printer and laser cutter, which I use for super-scientific projects like making Christmas ornaments for extended family.

Isabel Weisheit

I am a PhD student in the Paquet Lab, where I am establishing human in vitro models of vascular diseases using CRISPR-edited stem cells. When I don't have to feed my cells, I try to spend a lot of time outdoors in the mountains going hiking or climbing, or at the Isar.

Anna Kolz

If I ever win the lottery, I will move to Florida, eat key lime pie and save manatees. Until then, I am a PhD student in the lab of Anneli Peters at the Institute of Clinical Neuroimmunology (LMU) investigating the interaction of adaptive immune cells in the pathogenesis of CNS autoimmunity.

Shao-Yen Kao

Once in my life I thought I would be a NBA player but in the end I fell into here as a PhD and dedicated my youth to science! I still love playing basketball and I enjoy traveling different places to experience adventures. Currently I am working on exploring the functional role and molecular mechanism RNA-binding protein in muscle and nervous system during animal development in the BMC.

Rodaria Roussou

I am a biologist by degree, a gardener by hobby, and a foodie by habit. I have been studying ageing in yeast (yes, they do age!) and the changes tha chromatin undergoes with time. Very soon though my research focus will shift to the maintenance of mtDNA integrity, trying to figure out why and how the daughters (almost) always get the good quality mtDNA. Apart from my beloved yeast, my plants are my greater passion, on such level where my place resembles a "green oasis".

Niklas Eggers

My name is Nick, and I am a PhD student in Peter Becker's lab at the BMC, working on transcription factor targeting and chromatin biology. When I am not in the lab you will find me on the slopes in Winter and at the Isar in Summer.

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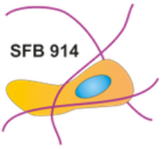
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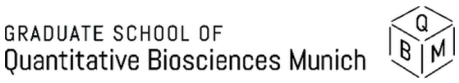
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