



Functional Epigenomics Conference

May 27-28, 2024

Innovation Center, Saarland University

Saarbrücken

Programme

SUPPORTERS



IN COOPERATION WITH



AGENDA

MONDAY, 27 MAY 2024

09:00 - 09:30 *Registration*

09:30 - 09:35 **Welcome**

09:35 - 10:50 **Session 1: Epigenetic mechanisms**

09:35 - 10:00 **Human primordial germ cells: Specification and epigenetic resetting**

Azim Surani (University of Cambridge)

10:00 - 10:25 **Epigenetic memory: erasure, turnover and long term maintenance**

Petra Hajkova (MRC Laboratory of Medical Sciences (LMS), Imperial College London)

10:25 - 10:50 **The role of Tet3 oocyte specific isoform during preimplantation development**

Jörn Walter (Saarland University, Saarbrücken)

10:50 - 11:20 *Coffee Break*

11:20 - 12:10 **Session 1 – continued**

11:20 - 10:45 **The Role of DNA Cytosine Modifications in Eukaryotic Adaptation and Evolution**

Guoliang Xu (Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Fudan University Medical School, Shanghai)

11:45 - 12:10 **Non-canonical epigenetic regulation in early development**

Alexander Meissner (Max Planck Institute for Molecular Genetics, Berlin)

12:10 – 12:50 **Selected Short Talk and Rapid Fire Talks**

Short Talk (15 min)

Advancing SMRTseq-based DNA-Modification-Detection through Synergy of Chemistry and Artificial Intelligence

Pascal Giehr (Ludwig Maximilian University of Munich)

Rapid Fire Talks (5 min each)

Decoding tissue-specific epigenetic signatures orchestrating memory T lymphocyte residency

Jun Dong (German Rheumatism Research Centre Berlin)

Functional interaction between TET3 and MeCP2 controls DNA-hydroxymethylation in neurons

Franziska Traube (University of Stuttgart)

DNA replication speed is a physiological regulator of DNA methylation maintenance and cell differentiation

Dania Hamo (Berlin Institute of Health at Charité)

Evidence for compensatory evolution within pleiotropic regulatory elements

Zane Kliesmete (Ludwig Maximilian University of Munich)

Classification of epigenomic domains using a flexible joint DNA methylation-chromatin segmentation model

Nihit Aggarwal (Saarland University, Saarbrücken)

12:50 - 14:30 *Lunch & Poster session*

14:30 - 15:20 Session 1 - continued

14:30 - 14:55 The second dimension of the genetic code

Thomas Carell (Ludwig Maximilian University of Munich)

14:55 - 15:20 Epigenetic regulation of gastrulation in time and space

Amos Tanay (Weizmann Institute of Science, Rehovot)

15:20 - 16:10 Session 2: Computational epigenomics and functional interpretation

15:20 - 15:45 Studying heterogeneity in cell populations using epigenomic data

Maria Colomé-Tatché (Ludwig Maximilian University of Munich, Helmholtz Center Munich)

15:45 - 16:10 Dissecting the epigenome dynamics of human immune cells upon pathogen exposure at single-cell resolution

Fabian Müller (Saarland University, Saarbrücken)

16:10 - 16:40 Coffee Break

16:40 - 16:55 GHGA - The German Human Genome-Phenome Archive

Oliver Kohlbacher (University Tübingen)

16:55 - 17:45 Panel discussion: Advancing epigenomic data analysis: integration, archiving, sharing

Maria Colomé-Tatché, Martin Hirst, Oliver Kohlbacher, Joachim Schultze

17:50 - 19:05 Session 2 – continued

17:50 - 18:15 A systematic comparison of methods for the prediction of enhancer-gene interactions from epigenomic data

Marcel Schulz (Goethe University Frankfurt)

18:15 - 18:40 Programmed Cells? Epigenetics and Cell Engineering for Cancer and Immunity

Christoph Bock (CeMM Research Center for Molecular Medicine, Medical University of Vienna)

18:40 - 19:05 Heterogeneity in chromatin states define a disease spectrum in synovial sarcoma

Martin Hirst (University of British Columbia, Vancouver)

End of Day 1 Free Evening/ Speakers' Dinner

TUESDAY, 28 MAY 2024

09:00 - 10:15 Session 3: Healthy aging and disease

09:00 - 09:25 Human disease trajectories at single cell resolution: Sporadic Alzheimer as the example

Joachim Schultze (German Center for Neurodegenerative Diseases, University of Bonn)

09:25 - 09:50 Parkinson's disease at the nexus of genes, aging, and environment

Julia Schulze-Hentrich (Saarland University, Saarbrücken)

09:50 - 10:15 Epigenetic regulation of T Lymphocytes – Learnings to improve adoptive cell therapies?

Julia Polansky (German Rheumatism Research Centre, Charité Berlin)

10:15 - 10:45 Coffee Break

10:45 - 12:25 Session 3 - continued

10:45 - 11:10 Epigenetics and the Human Lifecourse

Michael Kobor (University of British Columbia, Vancouver)

11:10 - 11:35 Evaluating the Impact of Pro-Aging and Rejuvenating Interventions on Epigenetic Age and DNA Methylation

Steve Horvath (Altos Labs Cambridge Institute of Science)

11:35 - 12:00 Epigenomics in chronic inflammatory diseases

Philip Rosenstiel (Kiel University)

12:00 – 12:30 Selected Short Talk and Rapid Fire Talks

Short Talk (15 min)

Multiome single-nuclei analysis of sclerotic hippocampus reveals a novel pathological cell state with an atypical epigenome

Lasse Sinkkonen (University of Luxembourg)

Rapid Fire Talks (5 min each)

Identification of ZFX4 as a novel transcription factor controlling dopaminergic neuron differentiation

Elena Valceschini (University of Luxembourg)

Giant cell enriched IDH wildtype glioblastoma differs in metabolic phenotype, tumor microenvironment composition and DNA methylation age acceleration

Katharina Weber (University Hospital Frankfurt)

Epigenomic and transcriptional profiling identifies impaired glyoxylate detoxification in MAFLD as a risk factor for hyperoxaluria

Cristina Cadenas (Leibniz-Research Centre for Working Environment and Human Factors at the TU Dortmund)

12:30 - 14:10 Lunch & Poster session

14:10 - 15:25 Session 3 - continued

14:10 - 14:35 Developmental principles in reprogramming

Jasmin Taubenschmid-Stowers (Altos Labs Cambridge Institute of Science)

14:35 - 15:00 Targeting symmetric arginine methylation in cancer and immune cells

Dalia Barsyte-Lovejoy (University of Toronto)

15:00 - 15:25 Single cell multi-omics landscape of development and ageing

Wolf Reik (Altos Labs Cambridge Institute of Science)

15:25 - 15:45 Closing Remarks

ABSTRACTS – TALKS

SESSION 1: EPIGENETIC MECHANISMS

Azim Surani (University of Cambridge)

Human primordial germ cells: Specification and epigenetic resetting

Human primordial germ cells (PGCs) originate during gastrulation at approximately day 14 of development. Factors, including SOX17 and PRDM1 regulate the PGC fate and initiate the epigenetic reset for totipotency. Accordingly, PGCs migration to the gonads is accompanied by global 5mC erasure, together with 5hmC and histone modifications contributing to orderly germline development. Recent studies suggest that SOX17 and DMRT1 promote 5-hydroxymethylcytosine (5hmC) and expression of DAZL, representing DNA methylation-sensitive genes that are critical for genome defence and gametogenesis.

Petra Hajkova (MRC Laboratory of Medical Sciences (LMS), Imperial College London)

Epigenetic memory: erasure, turnover and long term maintenance

Using mouse embryonic development as an experimental model, our long term interest is how epigenetic information is propagated during development and erased in the context of the germ line (zygotic and germline reprogramming). My laboratory has contributed important insights regarding the mechanisms of global DNA demethylation, the connection to chromatin remodelling and the role of Tet enzymes in both zygotic and germline reprogramming events (Amouroux et al, Nat Cell Biol 2016, Hill et al, Nature 2018, Huang et al, Nature 2021).

Stability and maintenance of the epigenetic profile underpins the stability of cell fate. This is particularly pertinent in the case of long-lived post-mitotic cells, such as neurons or oocytes. Although our previous research was predominantly focused on developmental reprogramming (ie erasure of epigenetic information) we have become increasingly interested in the stability of epigenetic information across time scale.

I will present our latest results addressing stability and turnover of DNA modifications in cultured cells and contrast this with our findings in the context of developmental epigenetic reprogramming. I will also discuss the stability of epigenetic information in the context of long lived post-mitotic cells.

Jörn Walter (Saarland University, Saarbrücken)

The role of Tet3 oocyte specific isoform during preimplantation development

Narges Jafari, Konstantin Lepikhov, Kathrin Kattler, Anna Welle, Gilles Gasparoni, Walter, J.

25 years ago we and others discovered that massive epigenetic alterations occur in the zygote (1, 2), in particular affecting DNA-methylation and certain histone modifications. Following the discovery of 5mC oxidation by Tet enzymes (3, 4) several groups showed that the zygotic oxidation of 5mC to 5hmC and higher oxidised forms is mainly catalysed by Tet3 (5, 6 and 7). The impact of Tet3 5mC oxidation on the regulation of preimplantation development, however, is still debated and recent data suggest a combined loss of Tet3 and Tet2 may lead to developmental failure (8, 9).

Tet3 gene is transcribed and translated into various isoforms, but so far only full KO or KD experiments of Tet3 have been performed, eliminating all isoforms (10). To explore the differential functions of Tet3 isoforms we generated a set of isoform specific knockouts in mice, first focusing on the oocyte specific isoform Tet300. Our data demonstrate the effect of selective loss of Tet300 function on 5mC oxidation and gene expression profiles in oocytes and early cleavage stages. The loss of Tet300 leads to significant changes in gene expression despite of a rather normal development. By comparing

various Tet3 KO and WT crosses we observe maternal transmission effects on altered transcriptome profiles from the oocyte throughout the entire preimplantation development (11).

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Guoliang Xu (Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Fudan University Medical School, Shanghai)

The Role of DNA Cytosine Modifications in Eukaryotic Adaptation and Evolution

In sexually reproducing organisms, random meiotic recombination between homologous chromosomes is essential for maximizing genetic variation among offspring. However, the sex-determining region (SDR) does not undergo recombination, which is necessary for maintaining distinct alleles. The relative contribution of epigenetic versus genetic mechanisms to suppress recombination within SDRs and how the suppression mechanisms might have evolved remain poorly understood. We aim to investigate the mechanistic control of the mating-type locus (MT) in the green alga *Chlamydomonas reinhardtii*. We have identified a maintenance DNA methyltransferase, DNMT1, mutation of which leads to the depletion of 95% of 5-methylcytosines (5mCs) in the nuclear genome. While the 5mC deficiency causes no discernible alteration in haploid vegetative growth or sexual differentiation, the *dnmt1* homozygotes display substantially reduced spore viability and a lower frequency of 4-progeny tetrads. Remarkably, in *dnmt1* homozygotes, aberrant meiotic recombination occurs at MT, resulting in haploid offspring with a mixed mating-type containing both plus and minus markers. Despite the concurrent loss of repressive histone methylation H3K9me1 at MT in *dnmt1* strains, deleting the responsible histone methyltransferase SET3p alone does not induce anomalous recombination at MT. Thus, DNA methylation, rather than histone modification, mediates the recombination suppression at MT in *C. reinhardtii*. This finding suggests that in early eukaryotes, which probably lacked histone-based chromatin modification, DNA methylation might have been utilized to inhibit meiotic recombination between alleles responsible for distinct sexes.

Alexander Meissner (Max Planck Institute for Molecular Genetics)

Non-canonical epigenetic regulation in early development

Mammalian somatic cells share a canonical bimodal pattern of DNA methylation. Most of the genome is highly methylated at all times, CpG-rich regions remain free of methylation and dynamic changes are limited to cis-regulatory regions. In contrast, extraembryonic tissues display a highly divergent non-canonical landscape with DNA methylation simultaneously being reduced globally but elevated at hundreds to thousands of CpG islands. Our recent work finally sheds some light on this unusual form of epigenome regulation including a detailed mechanistic dissection of the key factors.

Thomas Carell (Ludwig Maximilian University of Munich)

The second dimension of the genetic code

DNA stores genetic information in the form of the sequence of the four canonical bases dA, dC, dG and dT. DNA contains in addition epigenetic information, which is established by the four modified cytidine bases 5-methylcytidine (mdC), 5-hydroxymethylcytidine (hmdC), 5-formylcytidine (fdC) and 5-carboxycytidine (cadC) (Fig. 1, left).^[1] Additionally, 5-hydroxymethyluridine may play a role as well. These bases are generated by Tet enzymes. The position and the kind of modified dC-base at a specific position in the genome establishes an unknown 2nd code in our genetic system (Fig. 1, left). Setting and erasing of these epigenetic bases controls the complete development process starting from an omnipotent stem cell and ending with an adult specialized cell (Fig. 1, right). I am going to discuss results about the function and the distribution of the new epigenetic bases hmdC, fdC and cadC in the genome.^[2] I am showing how metabolic states influence the chemistry by setting and erasing these modified bases. Synthetic routes to these new bases will be discussed that enable the preparation of oligonucleotides containing these bases embedded. Particularly, isotope dilution and isotope tracing mass spectrometry was used us to understand the chemistry that occurs on these bases in the genome.^[3] Interesting is the fact that base excision repair seems to play a central role during erasure of the bases and again mass spectrometry helped to quantify the repair processes involved epigenetics.^[4] Finally, I am dicussing potential präbiotic origins of modified bases.^[5]

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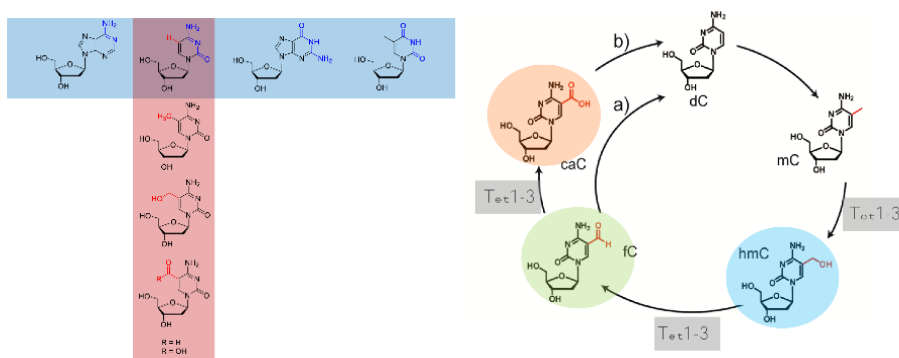


Figure 1. Illustrations of the 2nd orthogonal code that is present in DNA (left) and proposal of how the epigenetic bases are interconverted to establish dynamic changes of the epigenetic code during cellular development (right).

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Amos Tanay (Weizmann Institute of Science, Rehovot)

Epigenetic regulation of gastrulation in time and space

DNA methylation is known to be essential for embryonic development. Many possible mechanisms underlying this phenotype were investigated, including de-repression of repetitive element, de-repression of pluripotent and germline genes, loss of imprinting and more. Nevertheless, many cellular systems were shown to function almost normally without methylation, and the direct linkage between methylation and repression in-cis was difficult to establish. We aim at bridging these gaps using a comprehensive single embryo/single cell temporal model for mammalian gastrulation and a series of mutants perturbing methylation in entire embryos or in mixed WT/mutant chimera. We show that

cells can differentiate into almost all lineages without DNA methylation or without the de-methylation machinery, when provided with normal signaling from WT host cells in chimeric context. Furthermore we show that the major chromosomal architectures at insulators, promoters and enhancers are conserved even without a functional DNA methylation system. Nevertheless, we also identify thousands of genes and regulatory elements that are mildly perturbed in a robust and CpG-dependent fashion when methylation is impaired. When embryos are developing entirely from mutant cells, these quantitative perturbations result in progressive loss of developmental coherence and embryonic collapse. Together our data explain both the cell-intrinsic and tissue-wide phenotypes of DNA methylation. The data quantify precisely the pervasive role of DNA methylation in stabilizing gene regulation, and show it is un-contradictory to the role TF-driven mechanisms in determining activity of the major TSSs and enhancers, independently of both the DNMT and TET systems.

SESSION 2: COMPUTATIONAL EPIGENOMICS AND FUNCTIONAL INTERPRETATION

Maria Colomé-Tatché (Ludwig Maximilian University of Munich)

Studying heterogeneity in cell populations using epigenomic data

Recent breakthroughs in high-throughput sequencing of single cells are revolutionizing the biological and biomedical sector. Among the different -omics layers that can be measured at the single-cell level, single-cell epigenomic measurements present a rich layer of regulatory information that stands between the genome and the transcriptome. These measurements can be obtained for large heterogeneous samples of single cells to profile tissues, organs and whole organisms, and to study dynamic processes like cellular differentiation, reprogramming or cancer evolution. These data types provide an unprecedented level of measurement resolution.

In this talk I will discuss how single-cell epigenomic data can be used to study different cell characteristics. I will introduce and compare multiple feature space constructions for epigenetic data analysis, and show how this allows to explore the different usage of the non-coding part of the genome for different cell groups.

Another level of genomic information that can be extracted from single-cell data are single-cell copy number variations (CNVs). I will present a novel algorithm, epiAneufinder, which exploits the read count information from scATAC-seq data to extract genome-wide CNVs for individual single-cells, and I will show how the obtained CNVs are comparable to the ones obtained from single-cell whole genome sequencing data. Thanks to epiAneufinder it is therefore possible to add a relevant extra layer of genomic information, namely single-cell copy number variation, to every scATAC-seq dataset without the need of additional experiments.

Studying single-cell DNA methylation heterogeneity using single-cell DNA methylation measurements is complicated, as experimental protocols are costly and difficult to implement. I will present an alternative strategy, which involves minION sequencing combined with deconvolution of single-molecule methylation signals to reconstruct cell-type methylation profiles. I will show how, using this method, it is possible to deconvolve the methylomes of different cell types from an in-silico mix of cells.

Fabian Müller (Saarland University, Saarbrücken)

Dissecting the epigenome dynamics of human immune cells upon pathogen exposure at single-cell resolution

Irem B. Gündüz, Bei Wei, Wenliang Wang, Manoj Hariharan, Joseph R. Ecker, William Greenleaf, Fabian Müller

Pathogen exposure leads to the activation of immune responses and changes in cell phenotypes. Our single-cell epigenomic atlas of PBMCs in healthy conditions and individuals exposed to HIV, SARS-Cov2,

influenza and organophosphates facilitates a detailed survey of cell states across the human immune system. Our analysis integrates data from 271,299 cells profiled by scATAC-seq and 75,458 cells profiled by snmC-seq2 and reveals cell type-specific signatures of gene regulation. Aggregating epigenetic profiles across putative binding sites of transcription factors (TFs), we define regulator activity based on DNA methylation and chromatin accessibility, and we observe consistent patterns between both epigenomic modalities.

We further characterized the epigenome dynamics associated with the immune response to pathogen exposure. While we observe limited differences in chromatin accessibility induced by influenza and organophosphate exposure, we report epigenetic changes associated with T cell maturation and exhaustion states during HIV infection. Furthermore, our analyses shows that epigenetic profiles in regulatory elements associated with TF binding (including the NF- κ B family) are significantly altered in monocytes from individuals exposed to SARS-Cov2 compared to controls.

Marcel Schulz (Goethe University Frankfurt)

A systematic comparison of methods for the prediction of enhancer-gene interactions from epigenomic data

Prediction of enhancer-gene interactions is an important task in regulatory genomics for studying non-coding variation and gene regulation by DNA-binding factors. Many different approaches have been developed that integrate a diverse combination of data for it, but generalization to unseen data is poor. We use the IHEC EpiAtlas for chromatin and expression data to benchmark existing and novel approaches for the purpose of predicting gene expression from epigenome data with established and novel approaches. We illustrate that model learning is influenced by different genomic features. We present novel machine learning approaches for the task and highlight differences between genes. Our new models provide a foundation for applications that link epigenome variation to gene expression in human cells.

Christoph Bock (CeMM Research Center for Molecular Medicine, Medical University of Vienna)

Programmed Cells? Epigenetics and Cell Engineering for Cancer and Immunity

Cell engineering is becoming widely useful for fundamental biology (e.g., cells as probes and recorders) and for therapy (e.g., CAR T cells). Our research combines biotechnology and bioinformatics to genetically program human and mouse cells, in order to execute complex biological functions *in vitro* and *in vivo*. We specifically focus on the many roles of epigenetic mechanisms as mediators of cellular memory and plasticity, connecting the developmental history of individual human cells to their future potential.

Our goal is to understand cells by programming them based on a quantitative understanding of epigenetic cell states. We pursue three synergistic directions: To map and analyze cell states by multi-omics, single-cell, and spatial profiling (READ), to model regulatory circuitries with deep learning (LEARN), and to build artificial biological programs into cells by genome engineering (WRITE). We develop wet-lab and computational methods for all three directions and pursue initial applications for cancer and immunity.

READ: We analyzed the single-cell and spatial landscape of autoimmune granulomas (Krausgruber et al. 2023 Immunity), epigenetic heterogeneity in solid tumors (Klughammer et al. 2018 Nature Medicine; Sheffield et al. 2017 Nature Medicine), the role of structural cells in immune regulation (Krausgruber et al. 2020 Nature), and organoids in the Human Cell Atlas (Bock et al. 2021 Nature Biotechnology).

LEARN: We inferred regulatory circuits from single-cell data using “knowledge-primed neural networks” (Fortelny et al. 2020), used knockout collections for JAK-STAT signaling in mouse (Fortelny et al. submitted) and B cell immunodeficiency in human (Zhao et al. submitted) to test for causality, and developed a strategy for biosimulation using Large Language Models such as ChatGPS (Schäfer et al. submitted).

WRITE: We developed concepts and assays for high-content CRISPR screening (Bock et al. 2022 Nature Reviews Methods Primers), including the CROP-seq method for pooled CRISPR screening with single-cell RNA sequencing readout (Datlinger et al. 2017 Nature Methods) and the scifi-RNA-seq method cost-effective single-cell RNA-seq in 100,000s or millions of cells (Datlinger et al. 2021 Nature Methods).

Combining these three directions, we developed a platform for epigenetic and gene-regulatory optimization of CAR T cells using high-content screens in human primary cells (*ex vivo*) and in mice (*in vivo*). We identified several regulators that boost the performance of CAR T cells in these screens, and we systematically validated the *in vivo* effects of these boosted CAR T cells in systemic *in vivo* tumor models.

In conclusion, the combination of high-throughput profiling (READ), deep neural networks (LEARN), and genome editing at scale (WRITE) enables rapid functional dissection of epigenetic cell states and gene-regulatory networks in human cells, and their rational programming for biological research and therapy.

Website: <https://bocklab.org>;

Twitter: <https://twitter.com/BockLab>. Bluesky: <https://bsky.app/profile/bocklab.bsky.social>

Funding: C.B. is supported by an ERC Consolidator Grant (n° 101001971) of the European Union.

Competing interests: C.B. is a co-founder and scientific advisor of Myllia Biotechnology (CRISPR screening technology and service) and NeuroLentech (precision medicine for neurodevelopmental disorders).

Martin Hirst (University of British Columbia, Vancouver)

Heterogeneity in chromatin states define a disease spectrum in synovial sarcoma

Synovial sarcoma (SyS) is an aggressive soft-tissue malignancy characterized by a pathognomonic chromosomal translocation leading to the formation of the SS18-SSX fusion oncoprotein. SS18-SSX associates with mammalian BAF complexes suggesting deregulation of chromatin architecture as the oncogenic driver in this tumour type. To examine the epigenomic state of SyS we performed comprehensive multi-omics analysis on 52 primary pre-treatment human SyS tumours. Our analysis revealed a continuum of epigenomic states across the cohort at fusion target genes independent of recurrent genetic changes. We identify subtypes of SyS defined by enhancer states and reveal unexpected relationships between H2AK119Ub and active marks. The number of bivalent promoters, dually marked by the repressive H3K27me3 and activating H3K4me3 marks, has strong prognostic value and outperforms tumor grade in predicting patient outcome. Finally, we identify defining SyS epigenomic features including H3K4me3 expansion associated with striking DNA hypomethylation of promoter regions in which SyS display the lowest mean methylation level of any sarcoma subtype. We explore this feature as a potential vulnerability in SyS cell lines and identify H3K4me3 inhibition as a promising therapeutic strategy.

SESSION 3: HEALTHY AGING AND DISEASE

Julia Schulze-Hentrich (Saarland University, Saarbrücken)

Parkinson's disease at the nexus of genes, aging, and environment

As the most common neurodegenerative movement disorder, Parkinson's Disease (PD) represents a major clinical and societal burden, leading to progressive locomotor deficits, and loss of dopaminergic neurons in the midbrain. The hallmark neuropathologic feature of this complex disease is the accumulation of α -synuclein (α Syn) protein aggregates in Lewy bodies in the PD brain. SNCA point mutations and genomic multiplications are linked to familial PD cases with high penetrance and therefore constitute major genetic risk factors for PD. However, the preponderance of PD cases is seemingly sporadic, suggesting an interplay between genetic predisposition, aging processes and environmental influences. The epigenome and transcriptome are important layers to study this interplay.

Using a multi-omics approach, we comprehensively profile the PD transcriptome and epigenome across multiple layers. Specifically, assessing epigenomic regulation including histone modifications, DNA methylation and hydroxymethylation in well-characterized transgenic SNCA mouse we interrogate the plasticity of epigenomic modifications under the influence of environmental factors using mouse models exposed to standardized and specific environmental factors.

Collectively, these efforts not only advance our understanding of the disease towards a systems-level regarding the interplay between environment, genetic predisposition, PD pathology, but also pinpoint targets in the pathomechanistic orchestration that could open up novel avenues to much-needed therapeutics for this still incurable disease.

Julia Polansky (German Rheumatism Research Centre, Charité Berlin)

Epigenetic regulation of T Lymphocytes – Learnings to improve adoptive cell therapies?

As a major therapeutic break-through in the last years, T lymphocytes can be used today as ‘living drugs’ for adoptive cell therapy to fight cancer. They also hold great promise as effector cells for other clinical situations, including antigen-specific immunosuppressive treatments to fight autoimmunity, chronic inflammation and complications after organ transplantations. Therefore, it is of utmost importance to fully clarify the regulatory mechanisms which control their function, but also drive their complex developmental pathways including their differentiation into memory populations which provide life-long immunity to reoccurring pathogens. Insights into these mechanisms, including the epigenetic layer, will reveal means for a targeted functional optimization of T cells for therapeutic use, by employing specific epigenetic editing techniques.

We generated genome-wide epigenomic profiles of mature T cells from human peripheral blood and defined epigenetic control elements which guide the differentiation into specific memory phenotypes. In addition, we observed that heterochromatic parts of the genome underwent progressive DNA demethylation during phases of cell division, which leads to a recording of the cellular proliferation history within the epigenome. Heterochromatic DNA methylation loss is driven by replication timing and can be prevented, but not reverted, by pharmacological slowing down of the DNA replication speed. During early development of T cells in the thymus, however, such a proliferation-driven loss of DNA methylation could not be observed, even though also developing thymocytes undergo episodes of strong proliferation. Furthermore, we could observe a de-coupling of DNA methylome remodeling and phenotypic and transcriptomic changes during thymic T cell development.

To make use of our epigenomic profiling efforts for clinical application, we used CRISPR-dCas9-mediated epigenetic editing techniques, to define key epigenetic switches, which allow the stable imprinting of a desired functional state. Similarly, we test the stabilization of the DNA methylome as a possibility to improve therapeutic T cell products. Both approaches are promising new avenues to further exploit the great potential of T lymphocytes as antigen-specific living drugs for precision medicine.

Joachim Schultze (German Center for Neurodegenerative Diseases, University of Bonn)

Human disease trajectories at single cell resolution: Sporadic Alzheimer as the example

Single cell multi-omics (SCMO) technologies including genome-wide assessment of chromatin landscapes and transcriptional regulation have been exceedingly informative for a better understanding of immune cell types and cell states, their differentiation, their physiological regulation during immune responses but also their involvement in disease-associated immune pathologies. We have previously illustrated that these technologies can be successfully applied to clinical questions such as the characterization of chronic diseases including chronic obstructive pulmonary disease or inflammatory bowel disease as well as infections such as COVID-19 or HIV. Common to all these diseases is the acceptance that the immune system plays a central role in the disease. Here, we asked, whether SCMO technologies would be suitable to answer a major open question in dementia research,

namely whether there is a systemic immune component that might contribute to disease trajectories over lifetime enhancing the risk to develop sporadic Alzheimer's disease, the most common form of dementia worldwide. I will present our newest findings concerning this medically important question, the requirements to apply SCMOs to large clinical cohorts, and the necessity to rethink, how we analyze SCMO data in clinical settings.

Michael Kobor (University of British Columbia, Vancouver)

Epigenetics and the Human Lifecourse

Steve Horvath (Altos Labs Cambridge Institute of Science)

Evaluating the Impact of Pro-Aging and Rejuvenating Interventions on Epigenetic Age and DNA Methylation

I will cover recent studies that shed light on the efficacy of various interventions in modulating epigenetic age, as measured by DNA methylation clocks. Concentrating on mammalian samples—specifically mice, rats, and humans—the analysis aims to discern which interventions can accelerate or decelerate the aging process at the epigenetic level. Additionally, I will describe a framework to assess the impact of aging interventions on the epigenome.

Philip Rosenstiel (Kiel University)

Epigenomics in chronic inflammatory diseases

Jasmin Taubenschmid-Stowers (Altos Labs Cambridge Institute of Science)

Developmental principles in reprogramming

Jasmin Taubenschmid-Stowers, Ricard Argelaguet, Wolf Reik

Expression of the Yamanaka factors in adult somatic cells can change their cellular identity to an embryonic-like state, namely induced pluripotent stem cells (iPSCs). This process does not only revert cellular programs to a developmentally earlier time point, but also resets age-associated molecular properties, such as epigenetic clocks, to a younger state. Notably, shorter, as well as cyclic expression of the Yamanaka factors (partial reprogramming) has been shown to revert age-associated changes without the permanent loss of cellular identity, both *in vitro* as well as *in vivo*. Here, we are using an unbiased, data-driven approach to dissect the gene regulatory networks and chromatin changes in partial reprogramming of adult somatic cells. We identified the emergence of embryo-like programs and are now trying to dissect how developmental principles are linked to age-associated epigenetic phenotype reversal *in vitro*. With this, we aim to understand the molecular nodes that rejuvenating cells have to pass through and identify mechanisms resetting age-associated changes to a younger, healthier state in humans.

Dalia Baryte-Lovejoy (University of Toronto)

Targeting symmetric arginine methylation in cancer and immune cells

Dalia Baryte-Lovejoy. Structural Genomics Consortium, University of Toronto, Toronto, Ontario, Canada

Protein arginine methyltransferases (PRMTs) regulate gene expression programs by methylating arginine residues on a wide variety of substrates, such as histones and splicing factors. PRMT5 and PRMT9 are the members of the PRMT family that symmetrically dimethylate arginine in proteins. The expression patterns of PRMT5 and PRMT9 reflect their differential function in cancer and immune cells. We developed chemical probe inhibitors that bind cofactor and substrate binding pockets in PRMT9 and PRMT5 and can be used to modulate the activity of these enzymes in cells. Using the chemical probes to pharmacologically modulate the symmetric methylation in leukemia cells and monocyte-

derived macrophages, we uncovered the transcriptional programs regulating cancer cell survival and immune cell function. Understanding the dynamic nature of symmetric methylation in normal immune cells and its misregulation in cancer cells will provide new insights into the therapeutic targeting of PRMT enzymes.

Wolf Reik (Altos Labs Cambridge Institute of Science)

Single cell multi-omics landscape of development and ageing

Abdulkadir Abakir, Jasmin Taubenschmid-Stowers, Diljeet Gill, Aled Parry, Maria Rostovskaya, Stephen Clark, Fatima Santos & [Wolf Reik](#)

Epigenetic information is relatively stable in somatic cells but is reprogrammed on a genome wide scale in germ cells and early embryos. Reprogramming is essential for imprinting, the return to totipotency and pluripotency, the erasure of epimutations, and for the control of transposons. Following reprogramming, epigenetic marking occurs prior to and during lineage commitment in the embryo. The epigenome changes in a potentially programmed fashion during the ageing process; this epigenetic ageing clock seems to be conserved in mammals. Our work addresses the mechanisms and consequences of global epigenetic reprogramming in the early embryo, which is followed by epigenetic programming during lineage commitment. Using single cell multi-omics techniques, we are charting the epigenetic and transcriptional dynamics and heterogeneity during the exit from pluripotency and initial cell fate decisions leading up to gastrulation and organogenesis. We discovered priming of enhancers prior to lineage decisions as well as acute epigenetic remodeling of enhancers at the time of lineage commitment. We are also interested in the potentially programmed degradation of epigenetic information during the ageing process, how this might be coordinated across tissues and individual cells, and how this process potentially could be reversed to rejuvenate cells, tissues, and organisms.

ABSTRACTS – SELECTED SHORT TALKS AND RAPID FIRE TALKS

Pascal Giehr (Ludwig Maximilian University of Munich)

Advancing SMRTseq-based DNA-Modification-Detection through Synergy of Chemistry and Artificial Intelligence

The oxidative cytosine forms (oxdCs) 5-hydroxymethylcytosine (hmdC), 5-formylcytosine and 5-carboxylcytosine are formed through stepwise oxidation of 5-methylcytosine (mdC) in the DNA catalyzed by ten-eleven-translocation dioxygenases (Tets). These oxdCs serve as intermediates for the active removal of mdC, but presumably also have important gene regulatory functions. Despite recent advances in DNA methylation research, the exact biological significance of oxdCs remains elusive. This is partly due to technical limitations in the detection of non-canonical nucleic acids. To understand how oxdCs interact with other intrinsic epigenetic factors and to study their influence on DNA methylation patterns during cellular differentiation and aging, we postulate a simultaneous, strand-specific detection of multiple DNA modifications at single-molecule level. We utilize Single-Molecule Real-Time sequencing (SMRTseq), a sequencing-by-synthesis technology that leverages the real-time observation of individual DNA polymerase molecules as they incorporate fluorophore-tagged nucleotides along a native DNA template. When the polymerase encounters a modified base within the native DNA it is transiently stalled which is reflected by the kinetics of the recorded fluorophore signal. We apply selective chemistry to generate unique kinetic footprints of oxdCs (e.g. hmdC) during SMRTseq. Subsequently, we deploy deep learning algorithm to classify the individual kinetic profiles in unmodified, methylated and hydroxymethylated cytosines. Using small synthetic genomes, we demonstrate the feasibility of our approach for parallel detection of cytosine, mdC and hmdC on the same DNA molecule with high precision and accuracy. Our approach is the first to enable parallel detection of three DNA modifications using SMRTseq, it is extensible for fdC and cadC and thus represents a powerful tool to study oxdCs in context to mdC and to dissect their enigmatic role within the human epigenome.

Jun Dong (German Rheumatism Research Centre Berlin)

Decoding tissue-specific epigenetic signatures orchestrating memory T lymphocyte residency

Xiangyi Deng, Weijie Du, Gilles Gasparoni, Abdulrahman Salhab, Karl Nordström, Jinchan Li, Viktoria Wagner, Erping Zhang, Joachim Wachtlin, Juliane Bodo, Simon Reinke, Carsten Perka, Sebastian Hardt, Thomas Dörner, Christoph Holmer, Mario Tönnies, Hyun-Dong Chang, Julia Polansky, Jörn Walter, Pawel Durek, Andreas Radbruch, Jun Dong

The recent understanding that memory T lymphocytes, an essential part of immunological memory, persist as a nuanced landscape of tissue-resident memory T lymphocytes (Trm) remains elusive with respect to tissue-specific maintenance mechanisms. Here we report a global analysis of tissue-specific epigenetic imprinting of memory T lymphocytes. We have analyzed 58 “reduced representation bisulfite sequencing” (RRBS) datasets of 22 distinct tissue-derived and blood-borne human memory CD4+ and CD8+ T-cell populations, from bone marrow, intestine, spleen, lung, skin, and peripheral blood. The methylome profiles contain both tissue-specific and shared differentially methylated regions (DMRs). Trm in different tissues exhibit site-specific hypo- and hyper-methylated DMR encompassing genes encoding chemokine receptors, integrins, effector functions, and transcription factors. The correlation between DMR and gene expression is substantial, and may contribute significantly to tissue-specific recruitment and maintenance in steady state, as well as to effector gene expression upon reactivation, e.g. the memory for cytokine gene expression. Epigenetic imprinting of memory T lymphocytes thus qualifies as a molecular correlate of “memory”. Beyond that, it provides

a unique option to track Trm in secondary immune reactions. Using their tissue-specific DMR signatures, we could for the first time demonstrate the mobilization of Trm and their contribution to systemic secondary immune reactions.

Franziska Traube (University of Stuttgart)

Functional interaction between TET3 and MeCP2 controls DNA-hydroxymethylation in neurons

Franziska R. Traube, Gilles Gasparoni, Chiara Bernardini, Anna Winkler, Anjana Rao, Jörn Walter and Stylianos Michalakis

Ten-eleven translocation (TET) enzymes are α -ketoglutarate-dependent dioxygenases that oxidize the highly abundant epigenetic mark 5-methyl-2'-deoxycytidine (5mC) in DNA to 5-hydroxymethyl-2'-deoxycytidine (5hmC) and further on to 5-formyl- (5fC) and 5-carboxy-2'-deoxycytidine (5caC)¹. TET enzymes are important for developmental processes and especially in brain, 5hmC is also an important epigenetic mark, which is dynamically placed in synaptic genes depending on neuronal activity². Recently, loss-of-function mutations of TET3, which is the most abundant TET family member in somatic cells including neurons, were reported to cause the Beck-Fahrner syndrome, a global developmental disorder that is characterized by mild to severe intellectual disabilities³. Methyl-CpG binding protein 2 (MeCP2) on the other hand, is a 5mC and 5hmC reader protein and MeCP2 deficiencies also result in a severe developmental disorder known as the Rett syndrome⁴. Using a rapid neuronal differentiation model (iNGNs), we show now that TET3 interacts in post-mitotic neurons with MeCP2. Both proteins compete at 5mC loci in the genome and while knockout of TET3 (TET3KO) results in globally decreased 5hmC levels compared to the wildtype, knockout of MeCP2 (MeCP2KO) results in globally increased genomic 5hmC. However, despite the inversely correlated hmC pattern, the resulting phenotype of the TET3KO and MeCP2KO is very similar on the epigenome, transcriptome and proteome level with particularly proteins involved in synaptic function and organization showing the same deregulated expression compared to the wildtype. In summary, our data reveal that the functional interaction of TET3 and MeCP2 and not TET3 alone is important for establishing correct 5hmC patterns during neuronal differentiation processes and the interplay of TET3 and MeCP2 controls expression of neuronal genes during neuronal development and maturation with possible clinical implications.

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2. Khare, T. et al. 5-hmC in the brain is abundant in synaptic genes and shows differences at the exon-intron boundary. *Nat. Struct. Mol. Biol.* 19, 1037-1043 (2012).
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Dania Hamo (Berlin Institute of Health at Charité)

DNA replication speed is a physiological regulator of DNA methylation maintenance and cell differentiation

Dania Hamo, Anne Schulze, Kristy Ou, Cornelia Peitsch, Marcel Finke, Ghazaleh Zarrinrad, Paula Kramer, Pascal Giehr, Judith Gottfreund, Mingxing Yang, Frederik Hamm, Abdulrahman Salhab, Hanieh Moradian, Leila Amini, Verena Wolf, Jörn Walter and Julia K. Polansky

Partially methylated domains (PMD) make up a large fraction of the genome in almost all somatic cell types and overlap with the transcriptionally-silent heterochromatin. The progressive hypomethylation of PMDs in response to proliferation has recently been reported, but their molecular regulatory mechanisms and functional impact so far remain unclarified. Using a cell division tracking system in primary human T lymphocyte cultures, we were now able to show that the DNA replication speed during proliferation events was the driving force for PMD methylation loss and that maintenance of the DNA methylation profile in such heterochromatic domains could be achieved by interfering with

DNA replication speed. PMD methylation loss suffered during past proliferation events could not be regained later by slowing-down DNA replication speed, revealing that current PMD methylation levels reflect the proliferation history of a long-lived cell. Unexpectedly, prediction of DNA methylation modifying enzyme activities using a mathematical model clearly showed that the degree of proliferation-driven methylation loss in PMD is correlated to insufficient de novo methylation activity, rather than insufficient DNA methylation maintenance activity alone. Interestingly, we observed that increased loss of heterochromatic PMD methylation levels identified a population of long-lived, central memory T cell populations (TCMs) which preferentially undergo differentiation into effector memory T cells (TEM), indicating that past proliferation episodes predispose TCMs for TEM differentiation. Also, we were able to reduce the frequency of cells undergoing TEM differentiation by modulation of the DNA replication speed, indicating that the proliferation speed of T cells is causally involved in driving memory differentiation. Finally, our preliminary data suggest that this newly discovered mechanism might be exploited to improve expanded T cell populations for cell therapy approaches, as reducing the DNA replication speed during the manufacturing process improved functionality of therapeutic regulatory T cell products.

Zane Kliesmete (Ludwig Maximilian University of Munich)

Evidence for compensatory evolution within pleiotropic regulatory elements

Zane Kliesmete, Peter Orchard, Victor Yan Kin Lee, Johanna Geuder, Simon M. Krauß, Mari Ohnuki, Jessica Jocher, Beate Vieth, Wolfgang Enard, Ines Hellmann

Pleiotropy, measured as expression breadth across tissues, is one of the best predictors for protein sequence and expression conservation. In this study, we investigated its effect on the evolution of cis-regulatory elements (CREs) on six different functional levels. To this end, we carefully reanalyzed the Epigenomics Roadmap data for nine fetal tissues, assigning a measure of pleiotropic degree to nearly half a million CREs. To assess the functional conservation of CREs, we generated ATAC-seq and RNA-seq data from humans and macaques. We found that more pleiotropic CREs exhibit greater conservation in accessibility, and the mRNA expression levels of the associated genes are more conserved. This trend of higher conservation for higher degrees of pleiotropy persists when analyzing the transcription factor binding repertoire. In contrast, simple DNA sequence conservation of orthologous sites between species tends to be even lower for pleiotropic CREs than for species-specific CREs. Combining various lines of evidence, we suggest that the lack of sequence conservation for functionally conserved pleiotropic elements is due to compensatory evolution within these large pleiotropic CREs. Furthermore, for less pleiotropic CREs, we find an indication of compensation across CREs. This suggests that pleiotropy is also a good predictor for the functional conservation of CREs, but this is not reflected in the sequence conservation for pleiotropic CREs.

Nihit Aggarwal (Saarland University)

Classification of epigenomic domains using a flexible joint DNA methylation-chromatin segmentation model

DNA methylation and histone marks are key modifications which in combination form the cell type specific epigenomic program. Approaches for precise combinatorial analyses of both types of epigenetic marks mapped in EpiAtlas will provide new insights into the functional organization of the genome. So far excellent computational analysis tools such as ChromHMM or MethylSeekR have been used independently for interpreting either histone marks or DNA methylation alone hence neglecting the cross-dependence of these epigenetic marks. Here we describe the results of a combined and integrated analysis approach “EpiSegMix”, which uses a hidden Markov model combined with flexible read count distributions and state duration modeling. This allows us i) to robustly integrate results from DNA methylation and histone marks across EpiAtlas samples, ii) to more precisely identify the extend of broad (heterochromatic) domains and iii) to better define border transitions. While our approach can be used for a broad range of analytical questions, we here focus on partially methylated

domains (PMDs) and their distinct association with the heterochromatin marks H3K27me3 and H3K9me3. PMDs can be classified into heterochromatin subtypes showing distinct distributions of repressive marks and DNA-methylation. This EpiSegMix approach allows to more precisely define short and shallow PMDs previously missed by other approaches. The joint segmentation helps to classify the “quiescent state” comprising up to 50% of the genome into more biologically meaningful states and contributes to better determine cell type specificity and epigenetic disease causes

Lasse Sinkkonen (University of Luxembourg)

Multiome single-nuclei analysis of sclerotic hippocampus reveals a novel pathological cell state with an atypical epigenome

Danielle C.F. Brun³, Anhoula Gaigneaux, Jaqueline C. Geraldis; Tony Heurtaux, Marina K.M. Alvim, Clarissa L. Yasuda,⁴ Fabio Rogerio, Kamil Grzyb, Fernando Cendes, Thomas Sauter, Iscia Lopes-Cendes, Lasse Sinkkonen

Mesial temporal lobe epilepsy with hippocampal sclerosis (MTLE+HS) is often drugresistant, requiring surgical treatment. Understanding the complex mechanisms underlying MTLE+HS and those leading to different treatment outcomes is key to improving patient care. Multi-omics single-cell studies may provide a broad view of the biological processes underlying gene expression regulation, and valuable information about tissue cell composition.

We performed 10X Genomics Multiome analysis of single-nuclei, including ATAC-seq and RNA-seq, in surgical tissue of sclerotic hippocampus from patients with drugresistant MTLE+HS who underwent surgical treatment. We included 16 adult patients with similar ages and disease duration. After applying stringent quality control filters, and jointly analyzing chromatin accessibility and transcriptome data, we identified a total of 59 000 single cells with multiomic data. Overall, our analysis revealed at least ten major cell types, with oligodendrocytes and excitatory neurons exhibiting the highest number of cell subpopulations. Importantly, we uncovered notable structural differences in cell numbers and subpopulations when compared to the non-sclerotic control hippocampus. In particular, a reduction in excitatory neurons and an increase in oligodendrocytes were identified.

Intriguingly, a novel cellular state with atypical epigenomic features emerged, exhibiting signature characteristics of both microglial cells and oligodendrocytes. This cell type was not found in healthy hippocampus, and we hypothesize that it is derived from oligodendrocytes in response to pathological activation of SPI1 pioneer factor in the inflammatory microenvironment of the epileptic brain. The novel cell type expresses many microglial cytokines and genes associated with neuroinflammation, possibly contributing to the disease progression.

Our findings reveal previously unidentified cell populations associated with MTLE+HS. Furthermore, by identifying distinct cell types, gene expression patterns, and regulatory elements within the sclerotic hippocampus, we provide novel insights into its cellular composition and gene regulation landscape.

Elena Valceschini (University of Luxembourg)

Identification of ZFX4 as a novel transcription factor controlling dopaminergic neuron differentiation

Elena Valceschini, Borja Gomez Ramos, Jochen Ohnmacht, Raquel Machado, Anhoula Gaigneaux, Dimitrios Kyriakis, Kamil Grzyb, Anne Grünwald, Alexander Skupin, Thomas Sauter, Rejko Krüger, Lasse Sinkkonen

The selective degeneration of specific neuronal subtypes represents a pathological hallmark of several neurological diseases such as the degeneration of midbrain dopaminergic neurons (mDANs) in Parkinson’s disease (PD). Improving *in vitro* models of human neuronal subtypes that could recapitulate the heterogeneity of the human brain would have implications in the biomedical field as well as improve our ability to unravel the underlying molecular mechanisms of diseases. Transcription factors (TFs) and their co-regulators play a central role in the cell lineage commitment, acting in gene

regulatory networks (GRNs) to control gene expression and define specific cellular identities. Nowadays, high-throughput sequencing methods have paved the way for data-driven multi-omics approaches that allow unbiased identification of cell-type specific transcriptional regulators.

We have taken advantage of transcriptomic and epigenomic data from the International Human Epigenome Consortium (IHEC) to identify TFs with high and selective expression and regulation in human brain or during neurogenesis. Among the interesting TFs we identified ZFH4. Single nuclei RNA-seq of the whole-brain transcriptomic atlas from Allen Brain Atlas confirmed a high expression of *Zfh4* in neurons from several areas of the mouse brain, including dopaminergic neurons.

We have previously generated time-series transcriptomic and epigenomic profiles of purified human induced pluripotent stem cell (hiPSC)-derived mDANs. From the integration of the generated high-throughput data, ZFH4 was confirmed as one of the most prominent super-enhancer-controlled TFs in mDAN differentiation. We further investigated its role using RNA-seq analysis upon *in vitro* knockdown (KD) assays, as well as CUT&Tag analysis of ZFH4 enrichment in mDANs. The depletion of ZFH4 affected the mDAN differentiation while enrichment analyses on the downregulated genes resulted in an augmentation of cell-cycle related TFs and pathways. The RNA-binding protein LIN28A, involved in stem-cell maintenance, emerged among the most upregulated genes upon the ZFH4 KD while the LIN28A regulatory locus was enriched for the ZFH4 binding signal. Finally, the mDANs time-series analysis showed an anti-correlation of LIN28A and ZFH4 induction. Our analysis indicates a pivotal role of ZFH4 in the regulation of cell cycle, specifically in silencing pluripotency and proliferative programs while maintaining the mDANs in a post-mitotic state.

Katharina Weber (University Hospital Frankfurt)

Giant cell enriched IDH wildtype glioblastoma differs in metabolic phenotype, tumor microenvironment composition and DNA methylation age acceleration

Pinar Cakmak, Philipp Jurmeister, Iris Divé, Pia S. Zeiner, Joachim Steinbach, Karl H. Plate, Marcus Czabanka, Patrick N. Harter, Katharina J. Weber

Introduction: Besides their usage for CNS tumor classification, DNA methylation profiles can be exploited for tumor deconvolution to infer the cellular composition of the tumor microenvironment (TME). Furthermore, properties of the energy metabolism in glioma are deducible from epigenetic changes of metabolic genes. By use of epigenetic clocks DNA methylation data was recently evaluated also for its capability to serve as biomarker linked to tumor-induced age acceleration (MethAgeAcc).

Objectives: We therefore set out to analyse giant cell enriched glioblastoma, IDH wildtype (gcGBM) as this histologic variant is supposed to differ regarding the TME and rather harbours p53 mutations, which lead to accumulation of DNA double-strand breaks, potentially fueling epigenetic defects.

Patients & Methods: 310 epigenetically classified GBM were analysed including 26 gcGBM. We collected large-scale DNA methylation data and subjected it to tumor deconvolution and analyses of genome-wide and focused differential methylation of metabolic and mitotic genes. The cohorts were immunohistochemically characterized for expression of p53, glycolytic and mitochondrial proteins. Methylation-based age prediction was computed. 5 gcGBMs were subjected to transcriptome analysis.

Results: While glioblastoma without gc showed a mean MethAgeAcc of 12 years, gc-enriched tumors were characterized by a balanced chronological and epigenetic age. gcGBM showed lower proportions of intratumoral neurons and infiltrating CD4+ T cells. Expression scores of the anti-mitochondrial antibody MTCO2 were higher in gcGBM.

Conclusion: Gc might contribute to an altered TME with lower infiltrating T helper cells as well as promote oxidative phosphorylation. The observed differences in MethAgeAcc between glioblastoma with and without gc questions their association with DNA damage and sequel for prediction of patient survival.

Cristina Cadenas (Leibniz-Research Centre for Working Environment and Human Factors at the TU Dortmund)

Epigenomic and transcriptional profiling identifies impaired glyoxylate detoxification in MAFLD as a risk factor for hyperoxaluria

Kathrin Gianmoena, Nina Gasparoni, Adelina Jashari, Philipp Gabrys, Katharina Grgas, Ahmed Ghallab, Karl Nordström, Gilles Gasparoni, Jörg Reinders, Christian A Hudert, Georg Damm, Daniel Seehofer, Tobias S Schiergens, Maren Falk-Paulsen, Philip Rosenstiel, Rosemarie Marchan, Jörn Walter, Jan G Hengstler, Cristina Cadenas

The contribution of epigenetic modifications to metabolic dysfunction-associated fatty liver disease (MAFLD) and to its frequent extrahepatic complications, such as cardiovascular and kidney disease, are poorly explored. Using an integrated epigenome and transcriptome analysis of mouse MAFLD hepatocytes we identified alterations in hepatic glyoxylate metabolism. This pathway is responsible for oxalate release, a harmful waste product and kidney stone-promoting factor that may result in kidney damage. Particularly important was the downregulation, chromatin inaccessibility and hypermethylation of the key enzyme alanine-glyoxylate aminotransferase (Agxt), which detoxifies glyoxylate, preventing excessive oxalate generation. Low expression of Agxt in MAFLD was accompanied by increased oxalate formation from its precursor hydroxyproline. The relevance of Agxt to this process was investigated by genetic interventions. Viral-mediated Agxt transfer rescued excessive oxalate release in MAFLD. In human steatotic hepatocytes, AGXT was also downregulated and hypermethylated. In a cohort of adolescents with MAFLD, steatosis severity correlated with urinary oxalate excretion. Altogether, this work identified a reduced capacity of the fatty liver to detoxify glyoxylate, resulting in elevated oxalate formation, and provided a possible mechanistic explanation for the increased risk of kidney stones and chronic kidney disease in patients with MAFLD.

ABSTRACTS – POSTERS

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P01 Maria C Arroyo Lopez (Technical University of Darmstadt)**Isoform-specific and ubiquitination-dependent recruitment of Tet1 to replicating heterochromatin causes aberrant methylcytosine oxidation**

Maria C Arroyo-Lopez, Florian D Hastert, Andreas Zhadan, Florian Schelter, Susanne Zimbelmann, Cathia Rausch, AnneK Ludwig, Thomas Carell, M. Cristina Cardoso.

Oxidation of the epigenetic DNA mark 5-methylcytosine by Tet dioxygenases is an established route to diversify the epigenetic information and modulate gene expression, and overall cellular (patho-)physiology. Here, we demonstrate that Tet1 and its short isoform Tet1s exhibit distinct nuclear localization during DNA replication resulting in aberrant cytosine modification levels in human and mouse cells. We show that Tet1 is tethered away from heterochromatin via its zinc finger domain, which is missing in Tet1s allowing its targeting to these regions. We find that Tet1s interacts with and is ubiquitinated by CRL4(VprBP). The ubiquitinated Tet1s are then recognized by Uhrf1 and recruited to late replicating heterochromatin. This leads to the spreading of 5-methylcytosine oxidation to heterochromatin regions, LINE 1 activation, and chromatin decondensation. In summary, we elucidate a novel dual regulation mechanism of Tet1, contributing to understanding how epigenetic information can be diversified by spatiotemporal-directed Tet1 catalytic activity.

P02 Richard Bachmann (Saarland University, Saarbrücken)**Design and use of EpiEditors to mitigate alpha-synuclein overload in Parkinson's disease and related synucleinopathies**

Parkinson's disease (PD) is the most common neurodegenerative movement disorder worldwide [1]. A key hallmark of PDs are aggregates of α -Synuclein (aSYN) protein, encoded by *SNCA* gene, in Lewy bodies [2]. Increased cellular aSYN has been linked to DNA hypomethylation in Intron1 of the *SNCA* gene [3].

Epigenome Editing (EpiEditing) is an emerging technology allowing to change gene expression without changing the genetic code [4]. Utilizing a fusion protein (EpiEditor) consisting of CRISPR/dCas9 and DNMT3A could reintroduce methylation for *SNCA* providing a new therapeutic strategy for PD.

[1] T. Pringsheim et al., The Prevalence of Parkinson's Disease: a Systematic Review and Meta-Analysis. *Mov Disord.* **2019**

[2] Wassouf et al., Environmental Enrichment Prevents Transcriptional Disturbances Induced by Alpha-Synuclein Overexpression. *Front Cell Neurosci.*, **2018**

[3] Miranda-Morales et al., Implications of DNAN Methylation in Parkinson's Disease. *Front Mol Neurosci.* **2017**

[4] Kungulovski et al., Epigenome Editing: State of the Art, Concepts and Perspectives. *Trends in Genetics.* **2016**

P03 Nour El Kazwini (International School for Advanced Studies (SISSA), Trieste)**Share-topic: Bayesian interpretable modelling of single-cell multi-omic data**

Nour El Kazwini, Guido Sanguinetti

Multi-omic single-cell technologies which simultaneously measure the transcriptional and epigenomic state of the same cell, enable understanding epigenetic mechanisms of gene regulation. However, noisy and sparse data pose fundamental statistical challenges to extract biological knowledge from complex datasets. SHARE-Topic, a Bayesian generative model of multi-omic single cell data using topic models aims to address these challenges. SHARE-Topic identifies common patterns of covariation between different omic layers, providing interpretable explanations for the data complexity. Tested on data from different technological platforms, SHARE-Topic provides low dimensional representations recapitulating known biology and defines associations between genes and distal regulators in individual cells.

The paper is available with doi: [10.1186/s13059-024-03180-3](https://doi.org/10.1186/s13059-024-03180-3)

P04 Sven Beyes (University of Freiburg)**Metastatic cell identity is maintained by SOX9 function during mitosis in triple negative breast cancer**

Sven Beyes, Daniela Michelatti, Maria Luce Negri, Enrico Frigoli & Alessio Zippo

During tumor progression, cancer cells undergo substantial phenotypic changes to successfully disseminate and form distant organ metastases. Recent evidence links these changes to reversible non-genetic alterations, such as epigenetic alterations at distal *cis*-regulatory elements (CRE) and transcriptional reprogramming.

In order to maintain metastatic cell identity, alterations have to be preserved during cell division. Recent evidence highlighted that this preservation of cell identity is achieved by a mechanism termed mitotic bookmarking. It has been shown that both, transcription factors (TF) and epigenetic marks, can serve as mitotic bookmarks for the propagation of cell identity by allowing a rapid restoration of gene expression patterns after completion of mitosis. However, the mechanisms and players involved in cell identity preservation of triple negative breast cancer (TNBC) during mitosis are not yet fully understood.

To understand the contribution of single TF during early mitosis, we analyzed the changes of chromatin accessibility and associated pattern of TF binding during early mitosis in metastasis (MD)-derived TNBC cells. Chromatin accessibility is globally decreased during early mitosis and rapidly restored at later timepoints. TF analysis of CREs revealed an enrichment of the transcriptional master regulator SOX9, a TF linked to TNBC progression and stem cell maintenance during early mitosis. SOX9 belongs to a small subset of TF whose genes are actively transcribed during mitosis as determined by nascentRNA-seq and SOX9 protein remains partially bound to mitotic chromatin and rapidly rebinds to chromatin after completion of mitosis.

To further elucidate the role of SOX9 in propagating MD cell identity through mitosis, we employed Proteolysis Targeting Chimera (PROTAC) technology to deplete SOX9 in a time-controlled manner prior to mitosis to analyze the immediate effects on the progeny cell identity. We could measure a decrease of chromatin accessibility at CREs during mitosis, changes in gene expression and a loss of metastatic characteristics after completion of mitosis.

Taken together, we could show that the action of a single TF, SOX9, during early mitosis is important for propagating metastatic cell identity to the progeny, which might open new opportunities for therapeutic approaches.

P05 Tong Ge (Chinese Academy of Sciences)**DNA cytosine methylation suppresses meiotic recombination at the sex-determining region**

Tong Ge, Xiuqi Gui, Jia-Xi Xu, Wei Xia, Chao-Han Wang, Wenqiang Yang, Kaiyao Huang, Colum Walsh, James G. Umen, Jörn Walter, Ya-Rui Du, Hui Chen, Zhen Shao and Guo-Liang Xu

In sexual organisms, random meiotic recombination between homologous chromosomes is vital to maximize genetic variation among offspring. The sex-determining region (SDR), however, does not undergo recombination, as required for the maintenance of distinct alleles. The contribution of epigenetic versus genetic mechanisms to suppress recombination in SDRs and how the suppression mechanisms evolve remain poorly understood. Here we describe the mechanistic control of meiotic recombination at the mating-type locus (*MT*) in the green alga *Chlamydomonas reinhardtii*. We identify a maintenance DNA methyltransferase, DNMT1, mutation of which leads to the depletion of 95% of 5-methylcytosines (5mCs) in the nuclear genome. While the 5mC deficiency causes no discernible alteration in haploid vegetative growth or sexual differentiation, in diploid, the *dnmt1* homozygotes display substantial reduction in spore viability and 4-progeny-tetrad frequency. Strikingly, in *dnmt1* homozygotes, anomalous meiotic recombination takes place at *MT*, generating haploid progenies with mixed mating-type harboring both *plus* and *minus* markers. Although the repressive histone methylation mark H3K9me1 at *MT* is lost concurrently with DNA methylation in *dnmt1* strains, loss of histone methylation alone by gene deletion of the corresponding histone methyltransferase SET3p

does not lead to anomalous recombination at *MT*. Thus, DNA methylation, rather than histone modification, mediates the recombination suppression at *MT* seen in wild type *C. reinhardtii*. This finding suggests that in early eukaryotes, which likely lacked histone-based chromatin modification, DNA methylation might have been opted to suppress meiotic recombination between alleles responsible for separate sexes.

P07 Irem Gunduz (Saarland University, Saarbrücken)

Dissecting the Epigenome Dynamics of Human Immune Cells Upon Viral and Chemical Exposure at Single-Cell Resolution

Irem B. Gündüz, Bei Wei, Wenliang Wang, Manoj Hariharan, Joseph R. Ecker, Fabian Müller, William J. Greenleaf

Pathogenic and chemical exposures can cause changes in the regulation and activation of immune responses, potentially leading to immune dysfunction and increasing susceptibility to inflammation. Here, we present a comprehensive epigenomic exposure atlas by performing single-nucleus sequencing-based profiling of chromatin accessibility samples of individuals exposed to Organophosphates, HIV, Influenza, COVID-19 exposures, and healthy controls. Our longitudinal profiling of chromatin accessibility following HIV exposure revealed the dynamics of T-cell exhaustion. Furthermore, we identified alterations in chromatin accessibility profiles of CD14 monocytes upon COVID-19 exposure as associated with the disease severity. Our integrative analysis of chromatin accessibility and DNA methylation identified cell state-specific transcription factor (TF) activity and pathogen exposure-associated TF signatures. Together, this single-cell epigenomic atlas provides a foundation for understanding the gene regulatory mechanism of human immune-cell populations upon exposure. Keywords Single-cell, pathogen exposure, chemical exposure, human immune cells, infections, chromatin accessibility, DNA methylation, gene regulation

P08 Cristina Cadenas (Leibniz-Research Centre for Working Environment and Human Factors at the TU Dortmund)

Epigenomic and transcriptional profiling identifies impaired glyoxylate detoxification in MAFLD as a risk factor for hyperoxaluria

Abstract available in Rapid Fire Talk section

P09 Dania Hamo (Berlin Institute of Health at Charité)

DNA replication speed is a physiological regulator of DNA methylation maintenance & cell differentiation

Abstract available in Rapid Fire Talk section

P10 Marie Catillon (University of Luxembourg)

Using Prime Editing for generation and analysis of regulatory genetic variants in induced pluripotent stem cell (iPSC)-derived dopaminergic neurons.

Marie Catillon, Deborah Gérard, Jochen Ohnmacht, Borja Gomez-Ramos, Nina Baumgarten, Aurélien Ginolhac, Marcel H. Schulz, Thomas Sauter, Rejko Krüger, Lasse Sinkkonen

Parkinson's disease (PD) is a neurodegenerative disorder that is characterized by motor dysfunction. PD is caused by the degeneration of midbrain dopaminergic neurons (mDANs) located in the substantia nigra (SN). Large-scale genome-wide association studies (GWAS) have identified variants of many candidate genes that contribute to PD, such as SNCA, LRRK2, and PARK7.

GWAS have also contributed to discovery of a large number of disease-associated single nucleotide polymorphisms (SNPs), that are enriched in gene regulatory regions and have the potential to affect the binding sites of transcription factors. In PD context, the expression of PD risk genes can be regulated by the presence of PD-associated variants.

In our current project, we are focusing on BAG3 gene which is also controlled by regulatory regions harboring a PD SNP (rs144914361). A multiomic analysis (RNA-seq and ATAC-seq) of iPSC-derived mDANs and glial cells has allowed the identification of the regulatory regions involved in control of BAG3 gene and harboring this SNP, predicted to alter BAG3 expression, possibly modifying susceptibility to PD.

BAG3 gene encodes BAG3 protein (BCL-2-associated athanogene 3) described as a multifunctional protein which is implicated in the regulation of a variety of cellular processes (apoptosis, development, cytoskeleton arrangement). In the context of age related-degenerative disorders, BAG3 is involved in the clearance of aggregated proteins by macro-autophagy. In PD, BAG3 regulates autophagy to clear the PD pathogenic protein SNCA (α -synuclein) in microglial cells.

For this study, our challenge will be to generate an induced pluripotency stem cell (iPSC) line containing the genetic variant BAG3 rs144814361: C \rightarrow T, by using Prime Editing engineering technologies. The established BAG3 SNP variant iPSC line will be differentiated into different neuronal and glial cell lines, used as PD-model cell lines.

P11 Thomas Hentrich (Saarland University, Saarbrücken)

warP'D: Integrating transcriptome and DNA methylome data to discern accelerated from delayed adaptations in the unfolding of Parkinson's disease

Thomas Hentrich, Elisabeth Schenk, Michael Kobor, Olaf Riess, Julia M. Schulze-Hentrich

In the still largely enigmatic etiology of Parkinson disease (PD), age arises as the strongest risk factor. Disease underlying molecular perturbations share key characteristics with advanced age. Yet, aging is not PD.

Our results from gene expression analyses in rodent models of PD indicate an intriguing bimodality of perturbations that may lead to the disease state: While there are genes that behave in an accelerated aging-like mode, others are seemingly delayed or fail to adapt expression over time, resulting in trajectories to diverge from natural aging. We and others found evidence for such divergences between age and PD in human, too. However, revealing a more complete picture of disease unfolding in human brain over time remains elusive.

Here, we propose (i) to complement our results on gene expression in rodent models by DNA methylation. Then, (ii) to link DNA methylation between model and post mortem patient brain, and lastly (iii) to correlate perturbations in brain with—in part longitudinal—DNA methylation data in blood from larger patient cohorts.

Through this work, we seek to reveal deviating DNA methylation trajectories in blood that may serve as proxy to infer disease-associated alterations in brain and may aid in arriving at a much-needed earlier diagnosis of PD.

P12 Jun Dong (German Rheumatism Research Centre Berlin)

Decoding tissue-specific epigenetic signatures orchestrating memory T lymphocyte residency

Abstract available in Rapid Fire Talk section

P13 Shariful Islam (Ludwig Maximilian University of Munich)

Parallel Sequencing of Distinct Cytosine Modifications using Single-Molecule-Real-Time Sequencing

Shariful Islam, Raheleh Salehi, Hanife Sahin, Thomas Carell, Pascal Giehr

Oxidative cytosine forms (oxdCs)- 5-hydroxymethylcytosine (hmdC), 5-formylcytosine, and 5-carboxylcytosine (cadC) play a crucial role in gene regulation, as well as in the propagation of methylation patterns. Current sequencing technologies, such as the gold standard Bisulfite Sequencing (BS), fall short of providing a comprehensive view. For the mechanistic epigenetic study of oxdCs, we are focusing on developing labeling strategies that selectively target individual cytosine modifications and increase sensitivity during third-generation sequencing. This will allow us to dissect the role of

oxdCs and ten-eleven translocation enzymes (Tets) in the context of DNA methylation inheritance and gene regulation. We propose an approach using Single-Molecule Real-Time Sequencing (SMRTseq), offering strand-specific, quantitative, and parallel detection of multiple cytosine forms (e.g., 5-methylcytosine- mdC, and hmdC) on the very same chromosome at the single-base resolution on native DNA. With our distinct Tet3 variants, which are stable and highly active, we generate comprehensive model genomes for mdC, hmdC and cadC. The combination of mild, selective chemistry with SMRTseq derives unique intrinsic polymerase kinetics for each oxdCs (e.g. hmdC). These footprints can be used to train sophisticated deep-learning algorithms that can classify the individual cytosine forms and permit their parallel detection with high accuracy. Our approach has the potential to considerably advance our knowledge of oxdCs and their function within the mammalian epigenome.

P14 Susanne Edelmann (University of Tuebingen)

Blood transcriptome analysis suggests an indirect molecular association of early life adversities and adult social anxiety disorder by immune-related signal transduction

Susanne Edelmann, Ariane Wiegand, Thomas Hentrich, Sarah Pasche, Julia Schulze-Hentrich, Matthias H. J. Munk, Andreas J. Fallgatter, Benjamin Kreifelts, Vanessa Nieratschker

Social anxiety disorder (SAD) is a psychiatric disorder characterized by severe fear in social situations and avoidance of these. Multiple genetic as well as environmental factors contribute to the etiopathology of SAD. One of the main risk factors for SAD is stress, especially during early periods of life (early life adversity; ELA). ELA leads to structural and regulatory alterations contributing to disease vulnerability. This includes the dysregulation of the immune response. However, the molecular link between ELA and the risk for SAD in adulthood remain largely unclear. Evidence is emerging that long-lasting changes of gene expression patterns play an important role in the biological mechanisms linking ELA and SAD.

Therefore, we conducted a transcriptome study of SAD and ELA performing RNA sequencing in peripheral blood samples. Analyzing differential gene expression between individuals suffering from SAD with high or low levels of ELA and healthy individuals with high or low levels of ELA, 13 significantly differentially expressed genes (DEGs) were identified with respect to SAD whilst no significant differences in expression were identified with respect to ELA. The most significant DEG was *MAPK3* ($p = 0.003$) being upregulated in the SAD group compared to control individuals. In contrary, weighted gene co-expression network analysis identified only modules significantly correlated with ELA ($p \leq 0.05$), not with SAD. Furthermore, analyzing interaction networks of the genes from the ELA-associated modules and the SAD-related DEG *MAPK3* revealed complex interactions of those genes. Gene functional enrichment analyses indicate a role of signal transduction pathways as well as inflammatory responses supporting an involvement of the immune system in the association of ELA and SAD.

In conclusion, we did not identify a direct molecular link between ELA and adult SAD by transcriptional changes. However, our data indicate an indirect association of ELA and SAD mediated by the interaction of genes involved in immune-related signal transduction.

Keywords: RNA sequencing, Gene Expression, Social Anxiety Disorder, Early Life Adversity, Transcriptome, Immune system, Signal Transduction

P15 Narges Jafari (Saarland University)

Effects of Tet3 Isoforms on Gene Expression and chromatin Architecture of Early Mouse Embryos

Changes in the DNA methylation landscape are controlled by Tet oxygenases which act as major modifiers of DNA-methylation. Tet3 is an important player during early development and in the brain, mediating the full oxidation from 5mC to 5hmC, 5fC and 5caC. Tet3 triggers replication independent (active) and replication dependent (passive) demethylation processes. Tet3 is expressed in different isoforms whose role during preimplantation development, in stem cells and in the brain are largely unclear. To better understand the role and dynamics of Tet3 mediated modifications we analysed the

expression of various isoforms of Tet3 and generated a series of isoform specific knockouts in mice. In this study we focused on Tet3 oocyte specific isoforms and their effect on expression, methylation and chromatin architecture of the preimplantation mouse embryo. Our observations show *in vivo* consequences, manifested at different degrees, caused by the loss of individual isoforms as seen by NGS based analyses, immunohistochemistry and phenotypes of the KO mice. We find that the loss of Tet3OO has consequences on gene expression in metabolism regulation and development related genes of preimplantation embryos and causes changes in the cytosine modification.

P16 Deborah Gérard (University of Luxembourg)

Identification of Parkinson's disease-associated regulatory variant altering SCARB2 expression in human dopaminergic neurons

Deborah Gérard, Jochen Ohnmacht, Borja Gomez, Nina Baumgarten, Aurélien Ginolhac, Marie Catillon, Aleksandar Rakovic, Christine Klein, Marcel H. Schulz, Thomas Sauter, Rejko Krüger, Lasse Sinkkonen

Parkinson's disease (PD) manifests through the deterioration of midbrain dopaminergic neurons (mDANs) located in the substantia nigra, leading to recognizable motor impairments. While genome-wide association studies (GWAS) have linked a significant portion of PD cases to common single nucleotide polymorphisms (SNPs), the precise mechanisms remain largely elusive. SNPs associated with various traits and disorders tend to cluster within gene regulatory regions, potentially altering transcription factor (TF) binding within accessible TF binding sites (TFBS) and subsequently influencing gene expression. To delve deeper into how PD-associated SNPs might impact mDANs, we conducted time-series profiling of transcriptome (RNA-seq) and chromatin accessibility (ATAC-seq) in purified mDANs and astrocytes differentiated from human iPSC-derived neural precursor cells. Integrating these epigenomic profiles with 3400 PD-associated SNPs from a PD GWAS repository, we utilized the SNEEP approach to predict SNPs affecting TF binding in accessible chromatin in a cell type-specific manner. Notably, we identified a PD-associated SNP that introduced a TFBS for NR2C2 at the promoter of lysosomal membrane protein 2 (SCARB2) in mDANs. SCARB2 is a transporter for glucosylceramidase (GBA), a gene frequently implicated in PD. Luciferase assays in human neurons validated the reduced transcriptional activity associated with the PD-linked SCARB2 allele, which also exhibited diminished chromatin accessibility in mDANs. Furthermore, eQTL analysis of human brain regions demonstrated decreased SCARB2 expression in the presence of the PD-associated allele, particularly in the substantia nigra. Consistent with these findings, knockdown of NR2C2 led to elevated SCARB2 expression, accompanied by an increase in the number of mDANs within the neuronal population. In summary, our results suggest that NR2C2 may act as a repressor of SCARB2 during mDAN differentiation in the presence of a PD-associated regulatory variant.

P17 Midhuna Immaculate Joseph Maran (Saarland University, Saarbrücken)

Chromatin Accessibility and Gene Expression Dynamics of $\gamma\delta^+$ Cells on Exposure to Malaria

Kathleen Dantzler Press, Sandy Klemm, Fabian Müller, Midhuna I. Joseph Maran, Derek Chen, Prasanna Jagannathan

Malaria, a parasitic disease primarily transmitted by female Anopheles mosquitoes, poses a significant global health threat, particularly in tropical regions. Despite decades of efforts towards eradicating the disease on a global scale, malaria continues to be a major contributor to the world's health burden, with the African Region bearing the brunt. Approximately 500,000 deaths annually in Sub-Saharan Africa are associated with malaria, with about 80% of these deaths occurring in children under 5 years of age [1]. $\gamma\delta^+$ T-lymphocytes, a specialized subset of T-lymphocytes, play a crucial role in innate and adaptive immune responses. During acute Plasmodium falciparum infections, there is an observed elevation of these cells in peripheral blood, highlighting their crucial role in the early control of malarial infection [2]. Our analysis of accessibility data from a longitudinal study shows that $\gamma\delta^+$ cells' activity is driven by changes in chromatin accessibility, exhibiting tolerance or trained immunity upon repeated exposure to malaria, a phenomenon known as "T-cell exhaustion." Additionally, we identify that $\gamma\delta^+$

T-lymphocytes regain their activity upon minimal malaria exposure. We identified exposure-related signatures through integrative analysis using expression and in vitro accessibility data.

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P18 Jiannan Guo, Meng Wang, Haiping Wu (CytosinLab Therapeutics Co., Ltd.)

Synthetic lethality of targeting thymine DNA glycosylase in p53-deficient cancers

Jiannan Guo, Meng Wang, Jiaxin Zhou, Yao Cao, Yiqin Wang², Yilin Liu, Yuan Mi, Yarui Du, Guoliang Xu, Haiping Wu

Mutations or deletion of the tumor suppressor gene (TSG) *TP53* are prevalent in a broad spectrum of human cancers, representing the most malignant or refractory status and posing significant challenges for therapeutic intervention. Thymine DNA Glycosylase (TDG), a multifaceted protein involved in DNA mismatch repair, DNA demethylation, and transcriptional regulation, emerges as a critical player in this scenario. Here, we uncover that targeting TDG represents a vulnerability in multiple p53-deficient cancers, largely due to its role in regulating gene transcription. Mechanistically, TDG and p53 complement each other to promote the transcription of the RNA helicase *DHX9*, facilitating the resolution of double-strand RNA (dsRNA). Inhibition of TDG in p53-deficient cancer cells leads to *DHX9* downregulation and thus aberrant accumulation of dsRNA derived from short interspersed nuclear elements (SINEs). This triggers an antiviral response via the RIG-I/MDA5-MAVS axis, inducing tumor growth inhibition and enhancing the anti-tumor immune response. However, the lack of proper ligands or tool compounds impedes the exploration and development of TDG druggability and therapies for human diseases including cancers. Leveraging CytosinLab’s proprietary EpigenPLUS drug discovery platform, we identified a first-in-class, orally bioavailable, small-molecular inhibitor C-271 specifically targeting TDG. C-271 completely disrupts the key DNA-binding capability of TDG, and displays robust therapeutic efficacy in suppressing p53-deficient/mutated tumors across multiple preclinical mouse models, especially when combined with immune checkpoint blockade (ICB). Collectively, our study for the first time highlights the closely synthetic lethality between TDG and p53, providing a promising therapeutic strategy for patients bearing p53-deficient cancers and addressing unmet medical needs.

CytosinLab Therapeutics Co., Ltd. owns full intellectual property (IP) of C-271 and its series of small molecules targeting TDG.

P19 Zane Kliemete (Ludwig Maximilian University of Munich)

Evidence for compensatory evolution within pleiotropic regulatory elements

Abstract available in Rapid Fire Talk section

P20 Tony Heurtaux (University of Luxembourg)

Age- and sex-specific alterations in the midbrain of a Parkinson disease mouse model

Sergio Helguet², Tony Heurtaux, Alessia Sciortino, Yujuan Gui, Jochen Ohnmacht, Pauline Mencke, Ibrahim Boussaad, Rashi Halder, Pierre Garcia, Rejko Krüger, Michel Mittelbronn, Manuel Buttini, Thomas Sauter, Lasse Sinkkonen

Oxidative stress is widely recognized as a key player in dopaminergic neuron degeneration in Parkinson’s disease (PD). Among brain cells, glial cells (astrocytes, microglia and oligodendrocytes) express higher nuclear factor erythroid 2-related factor 2 (NRF2) levels compared to neuronal cells in both mouse and human genome. NRF2, a pleiotropic transcription factor, is described as a master regulator of antioxidant cellular response. Relationship between NRF2 and brain health has been clearly established in the literature. NRF2 deficiency also mimics the ageing phenotype by exacerbating neuroinflammation and cognitive decline in mice.

Protein deglycase DJ-1, encoded by *Park7*, is a multifunctional protein affecting different biological and cellular processes. Loss of function in *PARK7* can lead to early onset Parkinson's disease. DJ-1 is especially necessary for transcriptional function and stability of NRF2 transcription factor. Given the role of oxidative stress in PD development, our objective was to obtain insights into the early events triggered by DJ-1 absence *in vivo*. Since sex differences have been reported in the age-adjusted risk of developing PD, we also investigate the possible sex-specific differences over time.

To understand the molecular changes upon loss of DJ-1, we performed transcriptomic profiling of midbrain sections from 3- and 8-month-old mice. Interestingly, while at 3 months the transcriptomes of both male and female mice were unchanged, an extensive deregulation was observed specifically in 8-month-old males. Focal adhesion, extracellular matrix interaction, epithelial to mesenchymal transition as well as antioxidant response are signaling pathways specifically altered in the midbrain of 8-months male DJ-1-KO mice. Under these conditions, misregulated genes are known target genes of estrogen and retinoic acid signaling and exhibit sex-specific expression in wild-type mice. After performing single cell epigenomic profiling of midbrain sections of 8 months-old male mice, we found that altered chromatin accessibility in astrocytes is associated with gene expression changes. Most of the alterations determined *in vivo* were also observed in male astrocytes (primary murine cultures), as well as in iPSC-derived astrocytes (PD patient with *PARK7* mutation).

Taken together, our data indicate that loss of *Park7* leads to sex-specific gene expression changes specifically in males through astrocytic alterations. These findings suggest higher sensitivity of males to loss of DJ-1 and might help to better understand variation in the reported *Park7*^{-/-} phenotypes.

P21 Konstantin Lepikhov (Saarland University, Saarbrücken)

Deciphering the Role and Regulation of 5mC Oxidation in Preimplantation Mouse Embryos: Effector Enzymes, Implications for EGA, Developmental Potential, and Differentiation

The rapid yet controllable oxidation of 5mC in mouse zygotes is an essential part of epigenetic reprogramming in early mammalian embryos. It has been shown to influence the developmental program by regulating embryonic genome activation (EGA), overall genome stability, and subsequent differentiation. Tet3 oxygenase is a key component reshaping the DNA modification profile in developing zygotes, prompting us to investigate the potential mechanisms regulating its function. With several isoforms of Tet3, we employ genetic knockout approaches combined with forced protein expression in zygotes to elucidate the role of each isoform in oxidizing 5mC. These methods allow us to manipulate 5hmC/5fC/5caC patterns in zygotes and developing embryos, providing deeper insights into the functional and regulatory roles of these cytosine modifications. Another challenging question pertains to the mechanisms underlying Tet3's preferential targeting of paternal chromosomes and specific genome regions. By manipulating the structure of Tet3 oxygenase (e.g., deleting or modifying its Low Complexity Domain), we aim to understand which functional parts of the enzyme regulate its targeting and enzymatic activities.

P22 Elisa Israel (Leipzig University)

MBD2/3 lost its methyl-CpG binding ability in multiple families of Holometabola

Elisa Israel, Zoe Marie Länger, Joachim Kurtz, Sonja Prohaska

DNA methylation is sparse in insects, compared to vertebrates. This reduction is even more pronounced in Holometabola. In some Holometabola, DNA methylation is lost entirely. Methyl-CpG-binding domain (MBD) proteins bind methylated DNA and are therefore important readers of these epigenetic marks. We hypothesize that evolutionary reduction of genome-wide methylation results in changes to MBD proteins. Only one MBD family member, MBD2/3, is known in insects. Two isoforms have been identified in *Bombyx mori*, MBD2/3-L and MBD2/3-S. The long isoform MBD2/3-L contains a complete MBD domain spanning the first two exons, whereas the short isoform MBD2/3-S lacks the second exon and therefore half of its MBD domain. It has been reported that only the MBD2/3-L isoform is able to bind methyl-CpGs.

In this study, we analyzed transcriptomic and genomic sequence data from holometabolan orders to identify MBD2/3 genes and isoforms. Furthermore, we analyzed transcripts of representative species from each insect order. Overall, MBD2/3 is highly conserved in sequence and gene structure, with splice sites being identical within each order. Both isoforms are present in most insect orders. However, in Coleoptera and Hymenoptera, the longer isoform MBD2/3-L, capable of binding methylated DNA, has been lost entirely. This loss is also observed in several other holometabolan families and species.

The results suggest that MBD2/3 has lost its binding ability in Coleoptera and Hymenoptera. Furthermore, we propose that MBD2/3-L has been lost several times independently but exclusively in Holometabola. The reduced levels of DNA methylation may be linked to these losses.

P23 Siyuan Luo (ETH Zurich)

Benchmarking computational methods for single-cell chromatin data analysis

Siyuan Luo, Pierre-Luc Germain, Mark D. Robinson, and Ferdinand von Meyenn

Single-cell chromatin accessibility assays, such as scATACseq, are increasingly employed in individual and joint multi-omic profiling of single cells. As the accumulation of scATAC-seq and multiomics datasets continue, challenges in analyzing such sparse, noisy, and high-dimensional data become pressing. Specifically, one challenge relates to optimizing the processing of chromatin-level measurements and efficiently extracting information to discern cellular heterogeneity. This is of critical importance, since the identification of cell types is a fundamental

step in current single-cell data analysis practices. In this work, we benchmarked 8 feature engineering pipelines derived from 5 recent methods to assess their ability to discover and discriminate cell types. By using 10 existing or novel metrics calculated at the cell embedding, shared nearest neighbor graph, or partition levels, we evaluated

the performance of each method at different data processing stages. This comprehensive approach allowed us to thoroughly understand the strengths and weaknesses of each method and the influence of parameter selection. Meanwhile, we used diverse real datasets with different types of ground truth, including RNA, genotypes, FACS-sorting labels, and tissue of origins, to guarantee a more representative and unbiased evaluation. Our analysis provides guidelines for choosing analysis methods for different scATAC-seq datasets. Overall, graph-based spectral embedding methods (SnapATAC, SnapATAC2), and feature aggregation method outperform latent semantic indexing-based methods (Signac, ArchR). For datasets with complex cell-type structures, SnapATAC and Snap-ATAC2 are preferred. With large datasets, SnapATAC2 and ArchR are most scalable.

Keywords: benchmark · single-cell · ATAC-seq · preprocessing.

P24 Kathrin Kattler-Lackes (Saarland University, Saarbrücken)

Fibrosis associated loss of zonal transcriptional and epigenetic programs in hepatocytes in human MASLD

Kathrin Kattler-Lackes, Raksha Ganesh, Alexander Herrmann, Mario Brosch, Jochen Hampe, Jörn Walter

Understanding the molecular changes underlying liver fibrosis progression in metabolic dysfunction-associated steatotic liver disease (MASLD) is critical for developing targeted therapies. We investigate the impact of fibrosis on transcriptional and epigenetic hepatic zonation programs in human liver tissue.

Using laser capture microdissection we isolated hepatocytes from pericentral, intermediate, and periportal zones of healthy and fibrotic liver lobules from MASLD patients and performed RNA-seq and Reduced Representation Bisulfite Sequencing to assess transcriptional and epigenetic profiles. We observe a molecular reorganization of both layers in fibrotic liver tissue. Pericentral genes become progressively downregulated and periportal genes upregulated leading to the loss of transcriptional

zonation in fibrotic samples. For DNA methylation we observe a striking remodeling of the epigenetic landscapes, particularly in fibrosis-specific differentially methylated regions associated with pro-inflammatory pathways. Integration of gene expression and methylation data revealed a disruption of DNA methylation gradients at transcription factor binding sites (TFBS) for some key regulators, implicating a dysregulated TF signaling caused by fibrogenesis.

Our findings demonstrate that both the progressive loss of transcriptional hepatic zonation and the overall remodeling of DNA methylation in fibrotic liver tissue cause a portalization phenotype of pericentral hepatocytes, which can be seen as a major hallmark of progressed liver fibrosis in MASLD.

P25 Quirin Manz (Technical University of Munich)

Revisiting evidence for epigenetic control of alternative splicing

Quirin Manz, Markus List

Alternative splicing is a crucial mechanism for increasing eukaryotic cell transcriptome and proteome diversity. Changes in alternative splicing play a key role in cell differentiation and tissue development, and aberrations in this process have been associated with diseases. Despite its importance, the exact mechanisms for regulating alternative splicing are currently poorly understood. Several epigenetic marks, such as histone modification H3K36me3, have previously been associated with changes in alternative splicing. Here, we leverage the EpiATLAS data set to systematically re-evaluate evidence for epigenetic control of alternative splicing.

We used SUPPA2 to calculate percentage spliced-in (PSI) values for skipped exons and retained introns and integrated this information with histone ChIP-seq and DNA methylation data. In addition to global association analysis with partial correlation and machine learning, we perform local PSI modeling on individual alternative splicing events. The latter represents a new contribution enabled by the unprecedented number of uniformly processed datasets in EpiATLAS.

Our results confirm previously reported global associations of DNAm and H3K36me3 for exon inclusion and emphasize the importance of intrinsic features for genome-wide associations.

On a local level, trying to identify cotranscriptionally spliced events with epigenetic influence, we show that overall gene expression biases local analyses. Further, we show that epigenetic signal can predict PSI value in cis and trans, indicating sample-specific epigenetic fingerprints that distort across-sample analyses.

Overall, our study demonstrates that epigenetic marks are associated with the alternative inclusion of exons and introns. However, we recommend prudence when interpreting these connections. Our findings encourage further, more detailed research into the relationship between epigenetic changes and transcriptome diversity.

P26 Dirk Prawitt (University Medical Center Mainz)

Establishing Nanopore based diagnostics for Imprinting Defects

Renée Koch, Dominik Wappner, Thorsten Enklaar, Christiane Krämer, Dirk Prawitt

Maternal and paternal genomes do not contribute equally to a phenotype in mammals. This is essentially due to genomic imprinting, i.e. the affected genes are only transcribed by one defined parental allele, while the other allele is inactive. Disorders of genomic imprinting lead to complex syndromes called imprinting diseases (IDs) that manifest themselves in early childhood. The molecular basis for the allele-specific transcription of genes that are subject to genomic imprinting are regulatory regions exhibiting parent-specific DNA methylation and consequently regulate transcription allele-specifically. Therefore, these differentially methylated genomic regions (DMRs) are specified as imprinting control regions (ICRs). IDs are usually not caused by gene mutations, but by changes in the methylation rates of individual ICRs due to early embryonic defects, which then lead generally to a somatic mosaicism in the patients with a correspondingly variable phenotype. This results in difficult-to-define methylation limits for diagnostics, as the mixture of normal and aberrantly methylated ICR

regions in the underlying tissue samples naturally poses a sensitivity problem for the detection technique. For diagnostic purposes, ICR methylation is generally determined using pyrosequencing, MS-MLPA or MS-PCR techniques. However, these techniques only allow the analysis of short informative DNA sections, often without the possibility of assigning the sequences to a parental allele. With the advent of new long-read sequencing techniques, in particular the Oxford Nanopore system of direct DNA sequencing, it is now possible to sequence larger sections of the genome and thus assign the sequences to the paternal or maternal allele by haplophasing and simultaneously determine the DNA methylation status of the parental alleles. Focusing on selected DMRs in the DNA isolated from primary fibroblasts of selected ID patients, we aim to clarify, 1) whether methylation determination using Oxford Nanopore technology is also suitable for determining mosaic methylation states, 2) whether the sensitivity can be significantly increased using a CRISPR-Cas9 enrichment strategy and 3) whether the data obtained by Oxford Nanopore technology give similar results to that obtained by classical methylation diagnostics using pyrosequencing analysis.

P27 Sonja Prohaska (Leipzig University)

Simulation of histone modification dynamics during state recovery after replication

Nico Herbig, Daniel Mayer, Michel Krecké and [Sonja J. Prohaska](#)

A crucial step for the maintenance of cell identity is the recovery of the parental histone modification state after DNA replication and cell division. While many of the players are known to date their kinetic parameters and therefore the dynamics of the system is less well understood. Thus, we developed a rule-based stochastic simulation tool to model chemical modification reactions and simulate histone modification dynamics. In a toy example the task of the system is to reestablish the parental modification pattern after lossy replication during cell division. The main model idea of our approach is based on two observations: first, certain types of histone modifications change the physico-chemical properties of the interaction between DNA and histone, other marks seem to be neutral. It logically follows that neutral modifications can be viewed as informational bits, which do not exert an effect by themselves but associate with the appropriate reader domain-containing proteins that exerts a function. Second, reader and writer domains of the same and different types of modifications can co-occur in a protein or protein complex. Consequently, we equate the modification enzyme with a rewrite rule and the educts and products with the left and the right side of the rewrite, respectively. As a result, different combinations of reader and writer domains within histone-modifying enzymes implement different local rewriting rules. This way, histone modification dynamics can be viewed as a computation. To grant chemical accuracy, we based our simulation tool EpiDynast (Herbig et al. unpublished) on Gillespie's approach, which models the master equation of a chemical system. Together with a suite of scripts to explore and analyze a large amount of simulated data we were able to systematically sample a toy system with 14 rules, 28 parameters and to characterize the general behavior of a surprisingly complex system.

P28 Günter Raddatz (German Cancer Research Center Heidelberg)

Estimating the Differentiation Status of human epidermal Keratinocytes with a tailored single-cell Methylation Clock

Saßmannshausen, Zoe, Lyko, Frank, Raddatz, Günter

Since their introduction a decade ago DNA methylation clocks have become an integral part of the analysis of epigenetic data, contributing a wealth of insightful results about the factors driving biological aging (Horvath, 2013, Horvath & Raj, 2018). An area that has been little researched so far is the application of methylation clocks to single cell data. This may be due to the fact that the biological processes that take place over long time scales, such as the lifespan of an organism, are usually described with sufficient accuracy by bulk data. Temporal processes that are adequately described by resolution at the single-cell level should be found on short time scales. In addition, in single cells there is strongly reduced coverage of CpGs, which also varies greatly between individual cells. This

represents a fundamental obstacle to the use of methylation clocks on individual cells, since the probability of finding the CpGs defined by the clock in a single cell is low. Consequently, the combination of single cell methylation data and methylation clocks requires further elaboration, as well as further application examples.

In this study, we now describe how the usual training algorithm for methylation clocks can be extended for the application to single cell data by exploiting the specific properties of methylation clocks. We show that the redundancy present in methylation data can be used to replace missing clock CpGs in individual cells with sufficient accuracy and quantify the quality of the resulting prediction. As an example we apply the single-cell methylation clock developed in this way to a single-cell methylation experiment of differentiating human epidermal keratinocytes based on the high-throughput sci-MET protocol (Sole-Boldo, L., et al., 2022). We analyze the distribution of predicted "differentiation ages" of single cells and show that the differentiation status of individual keratinocytes can be predicted in this way. This not only enables the assignment of cells to different subtypes of keratinocytes, but also allows to estimate the time associated with the various differentiation processes. Finally, we propose future applications which could be tackled by the introduced single-cell methylation clock.

P29 Raheleh Salehi (Ludwig Maximilian University of Munich)

A Convolutional Neural Network for Classification of multiple DNAModifications following Single Molecule Real Time Sequencing.

Raheleh Salehi, Shariful Islam, Hanife Sahin, Thomas Carell, Pascal Giehr

The stepwise oxidation of 5-methylcytosine (mdC) leads to the formation of 5-hydroxymethylcytosine (hmdC), 5-formylcytosine and 5-carboxycytosine. Like mdC these oxidative cytosines play crucial roles in pivotal processes such as embryonic development, pathogenesis, and aging. Particular hmdC, which is present in several cell types, has been implicated to impact several biological processes. However, the molecular mechanism by which hmdC impacts gene expression remains elusive. This is also due to shortcomings in current detection methods.

Single-Molecule Real-Time Sequencing (SMRTseq) is a sequencing by synthesis technology that follows individual DNA-polymerase molecules while incorporating fluorophore-tagged nucleotides along a native DNA-template. The presence of DNA-modifications within the native DNA template leads to transient stalling of the polymerase. These kinetic signals can be used to determine the mdC in the DNA without the need of chemical treatments. However, until now SMRTseq cannot distinguish between mdC and hmdC. Here, we propose a Convolutional Neural Network (CNN) model to classify the individual kinetic profiles in unmodified, methylated, and hydroxymethylated cytosines on the same DNA-molecule with high accuracy. Our CNN model is the first deep learning algorithm that permits parallel detection of three DNA modifications by utilizing SMRTseq. Furthermore, our algorithm is not limited to mdC and hmdC but can be trained for any DNA modifications assuming they exhibit unique kinetic footprints during SMRTseq. Thus, our CNN provides a powerful tool to dissect combinatorial effects of multiple DNA modifications within the epigenome.

P30 Paniz Rasooli (Humboldt University Berlin)

Investigation of the Contribution of DNA Sequence to Epigenetic Regulation by Polycomb/Trithorax Response Elements

Paniz Rasooli, Lisa-Sophie Huschenbeth, Bjørn André Bredesen, Natalia Chadala, Marc Rehmsmeier, Leonie Ringrose

While the past decades have brought tremendous advances in deciphering the genetic code and understanding how DNA sequence encodes proteins, our knowledge about DNA sequences that interact with epigenetic regulatory systems to establish and maintain genome regulation remains limited. It is becoming more and more clear that non-coding parts of DNA contain a wealth of hidden information that can be uncovered if we know how to look for it. These non-coding elements include Polycomb/Trithorax response elements (PRE/TREs), which help the cell to acquire and remember its

correct identity. The highly conserved Polycomb and Trithorax groups of proteins work antagonistically to regulate and maintain epigenetic regulatory information via PRE/TREs. A deeper understanding of the PRE/TRE code could fill in large missing pieces of the puzzle of gene regulation and provide insights into how the Polycomb/Trithorax epigenetic regulatory system contributes to the development and maintenance of cell identity. The primary aim of my project is to gain a better understanding of the role of DNA sequence in epigenetic regulation via PRE/TREs. To test the hypothesis that PRE/TREs have different “personalities”, we differentiate mouse embryonic stem cells to neural lineage to study the regulatory effects of specific PRE/TREs on a GFP reporter gene. GFP expression is captured by microscopy and quantified by image analysis. The data serves as the foundation to build mathematical models, enabling us to further explore how reporter gene expression is regulated by specific PRE/TRE sequences, and how the PRE/TRE behaves differently upon different promoter inputs.

P31 Johanna Elena Schmitz (Saarland University, Saarbrücken)

Discovering chromatin states using a flexible distribution hidden Markov model with duration modelling

Johanna Elena Schmitz, Nihit Aggarwal, Lukas Laufer, Jörn Walter, Abdulrahman Salhab, Sven Rahmann

Motivation: The combinatorial enrichment or depletion of histone marks plays an essential role in regulating chromatin dynamics and altering DNA accessibility. For instance, H3K27ac (acetylation of lysine (K) at position 27 in the amino-acid sequence of the histone protein H3) is mainly enriched in active promoters and enhancers, while H3K9me3 labels heterochromatic regions, thereby characterizing chromatin states which correspond to genomic elements with different functional roles.

The availability of genome-wide histone profiles allows us to automate chromatin state discovery and subsequent segmentation of the genome using a probabilistic model. A popular probabilistic model for chromatin segmentation is the hidden Markov model (HMM), which captures the combinatorial patterns of multiple histone marks using state-specific multivariate emission probabilities and the spatial relations via transition probabilities.

However, existing methods have two limitations. First, they use a fixed probability distribution type to model the read counts of all histone marks, although the read count distributions show different levels of overdispersion and skewness. Second, they have a limited flexibility to model chromatin domains of varying lengths, e.g., short promoters in comparison to long genes.

Method: To address the above limitations, we developed EpiSegMix, a new chromatin segmentation tool based on a more flexible HMM. Since previous studies have shown that the read counts of broad and narrow histone marks often show different levels of overdispersion and skewness, we support the selection of a different distribution for each mark. We support several distribution types to fit the read count distributions, including the commonly used 2-parametric Negative Binomial distribution and the more flexible 3-parametric Beta Negative Binomial distribution. In addition, we propose to use extended-state HMMs, which enable a more flexible state duration modeling by changing the internal HMM topology.

Results: We show in a comparison with ChromHMM, EpiCseg and Segway that the increased flexibility of EpiSegMix allows us to more accurately discover chromatin states that are predictive of cell biology.

P32 Claudia Rube (Saarland University Medical Center, Homburg)

Overexpression of histone variant H2A.J promotes radioresistance and oncogenic transformation

Benjamin M. Freyter, Mutaz A. Abd Al-razaq, Markus Hecht, Christian Rube, Claudia E. Rube

Background: Cellular senescence in response to ionizing radiation (IR) limits the replication of damaged cells by causing permanent cell cycle arrest. However, IR can induce pro-survival signaling pathways that reduce the extent of radiation-induced cytotoxicity and promote the development of radioresistance. Differential incorporation of histone variant H2A.J has profound effects on higher-order chromatin organization and on establishing the epigenetic state of radiation-induced

senescence. However, the precise epigenetic mechanism and function of H2A.J overexpression in response to IR exposure still needs to be elucidated.

Methods: Primary (no target, NT) and genetically modified fibroblasts overexpressing H2A.J (H2A.J-OE) were exposed to 20Gy and analyzed 2 weeks post-IR for radiation-induced senescence by immunohistochemistry and immunofluorescence microscopy. Transcriptome signatures were analyzed in (non-)irradiated NT and H2A.J-OE fibroblasts by RNA-sequencing. Since H2A.J plays an important role in epidermal homeostasis of human skin, the oncogenic potential of H2A.J was investigated in cutaneous squamous cell carcinoma (cSCC). Tissue microarrays of cSCC were analyzed for H2A.J protein expression pattern by automated image analysis.

Results: In response to radiation-induced DNA damage, overexpression of H2A.J impairs the formation of senescence-associated heterochromatin foci (SAHF), thereby inhibiting SAHF-mediated silencing of proliferation-promoting genes. Dysregulated activation of cyclins and cyclin-dependent kinases disturbs cell cycle arrest in irradiated H2A.J-OE fibroblasts, thereby overcoming radiation-induced senescence. Comparative transcriptome analysis revealed significantly increased WNT16 signaling in H2A.J OE fibroblasts after IR exposure, promoting fundamental mechanisms of tumor development and progression, including activation of the epithelial-mesenchymal transition. Quantitative analysis of cSCCs revealed that undifferentiated tumors are associated with high nuclear H2A.J expression, related with greater oncogenic potential.

Conclusion: H2A.J overexpression induces radioresistance and promotes oncogenic transformation through activation of WNT16 signaling pathway functions. H2A.J-associated signatures may improve risk stratification by identifying patients with more aggressive cSCC who may not benefit from adjuvant radiotherapy.

Keywords: Histone variant H2A.J; ionizing radiation; radiation-induced senescence; senescence-associated heterochromatin foci (SAHF); radioresistance; WNT signaling; oncogenic transformation

P33 Franziska Traube, Chiara Bernardini (University of Stuttgart)

Functional interaction between TET3 and MeCP2 controls DNA-hydroxymethylation in neurons

Abstract available in Rapid Fire Talk section

P34 Perla Saoud (University of Luxembourg)

Epigenetic Response of Colorectal Cancer Cells to Fusobacterium Nucleatum Exposure

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Colorectal cancer (CRC) is considered a significant global health concern, being the third most common and second most lethal type of cancer in 2020. Recent studies underline the role of the gut microbiome in influencing CRC pathogenesis, highlighting the need for continued investigation in this evolving field. While there has been extensive exploration into the role of the gut microbiome in colorectal cancer initiation, progression and chemotherapy resistance, the understanding of the epigenetic responses of CRC cells remains incomplete. Attention has been directed into specific microbial species, such as *Fusobacterium nucleatum* (*Fn*), due to their already known influence on CRC pathogenesis. In this study, we investigated the epigenetic response of CRC cells to *Fn* secretome exposure using RNA-seq and ATAC-seq, aiming to address this knowledge gap.

In our investigations, *Fn* secretome showed to be able to trigger profound transcriptomic alterations in CRC cells along with changes in chromatin accessibility after only four hours exposure. From the RNA-seq an upregulation of different genes involved in inflammation and autophagy processes emerged. Moreover, through an integrated analysis of RNA-seq and ATAC-seq data, we were able to identify ELF3, CEBPB, and PPARG as some of the key transcription factors regulating the transcriptomic response of CRC cells to *Fn* secretome exposure.

Our research findings reveal how *Fusobacterium nucleatum* (*Fn*) secretome stimulates the upregulation of ELF3 and CEBPB expression, potentially through PPARG-mediated regulation. Our goal is now to uncover the mechanism through which this occurs, by working on shELF3 cell line and a PPRE luciferase reporter cell line and investigate their response to *Fn* secretome exposure. Furthermore, we acknowledge the importance of understanding the specific composition of the *Fn* secretome. Through the analysis of its components, we also aim to pinpoint the specific metabolites responsible for the observed effects.

In conclusion, our study emphasizes the intricate interplay between *Fusobacterium nucleatum* (*Fn*) secretome and CRC cell epigenetics, underlining the potential involvement of ELF3, CEBPB, and PPARG in mediating the transcriptomic response. Our future investigations will focus on elucidating the underlying mechanisms and identifying the responsible metabolite, aiming to further advance in the comprehension of the field.

P35 Irem Tüysüz (Ludwig Maximilian University of Munich)

Identify patterns in mRNA to predict transcript half-lives in Trypanosoma using Saluki

Irem Tüysüz, Anna Danese, T. Nicolai Siegel, Stefan H. Stricker, Maria Colomé-Tatché

Introduction: The stability of the mRNA is essential for the regulation of transcript levels. It is often measured as half-life. In *Trypanosoma*, mRNA stability is especially important as all genes in a polycistronic transcription units (PTUs) are expressed the same during transcription. Regulation takes place on the post-transcriptional level. Saluki, a hybrid neural network, detects the patterns in the mRNA that have an impact on the transcript half-life. Saluki uses this information to predict the half-life of the transcript. In the light of the Saluki findings, positioning of splice sites, codons, and RNA binding motifs have a big effect on mRNA half-life in human and mouse. Saluki also performs in silico mutagenesis and calculates the effect of mutation on stability.

Project aim: Apply Saluki to identify patterns in mRNA to predict transcript half-life in *Trypanosoma*. Our goal is to identify binding sites of RNA binding proteins and different codon usage that can explain the half- life of transcripts in *Trypanosoma*, especially the ones originating from the same PTU.

P36 Magdalena Szewczyk (SGC Toronto)

The inhibition of symmetric arginine dimethylation in macrophages

Magdalena M. Szewczyk, Xin Zhang, Victoria Vu, Sumera Parveen, Levon Halabelian, Lei Shen, Evianne Rowes, Ludwig Bauer, Matthieu Shapira, Kilian Huber, Yanhzong Yang, Tracy McGaha, Dalia Barsyte-Lovejoy

Protein arginine methyltransferases (PRMTs) regulate various biological processes and are increasingly being recognized for their potential as drug targets. Here, we report the discovery of a potent, selective dual chemical probe for Type II PRMTs – MRK990, which inhibits PRMT9 and PRMT5 in cells at sub micromolar concentration. PRMT9 was reported to regulate neuronal physiology and our data indicates its role in immune cells. PRMT9 is largely upregulated during monocyte to macrophage differentiation, that denotes the critical functional transition of these cells. Our data indicates that MRK990 treatment resulted in the regulation of gene sets involved in signaling pathways such as macrophage cell migration. In addition, as PRMT9 methylates the splicing factor SF3B2, a large number of alternative splicing events are elicited by MRK990. We demonstrate that perturbation of these pathways affects bone marrow-derived macrophage migration as well as phagocytosis.

P37 Yin Wang (Fudan University Shanghai)

Novel insight into the spatiotemporal specificity for the DNA methylation of early embryonic development

Tet3-mediated active DNA demethylation and DNA replication mediated passive demethylation has long been considered to result in global DNA reprogramming during early embryonic development.

However, Amouroux et al. [1] showed evidence for active DNA demethylation independent of Tet3 during early zygotic development. Here we profiled genome-wide methylation pattern of single pronucleus and demonstrated that the active demethylation independent of Tet3 existed in both maternal and paternal genome from fertilization to PN3 stage.

In addition, during zygotic epigenetic reprogramming, sperm genome with 80% CpG sites methylated undergoes rapid demethylation after fertilization. To define the role of 5mC in paternal genome during embryonic development, we applied CRISPR/Cas9-mediated Dnmt1/3a/3b deletion and restoration system to sperm-like androgenetic haploid embryonic stem cells (AG-haESCs), and successfully generated mice from the sperm-like cells completely devoid of DNA methylation.

With these rare models, we aim to illustrate the spatiotemporal specificity of DNA methylation during embryonic development.

[1] Amouroux, Rachel et al. "De novo DNA methylation drives 5hmC accumulation in mouse zygotes." *Nature cell biology* vol. 18,2 (2016): 225-233. doi:10.1038/ncb3296

P38 Sascha Tierling (Saarland University)

Characterisation of epigenetic heterogeneity in glioblastoma based on local 5-mC and 5-hmC profiling of the extended MGMT promoter region

S. Tierling, W.M. Jürgens-Wemheuer, J. Becker-Kettern, W.J. Schulz-Schaeffer and J. Walter

Glioblastoma multiforme (GBM) are the most frequent human brain tumors in adults and represent the most aggressive form of tumors deriving from astroglia. Besides genetic plasticity, DNA methylation of the O⁶-methylguanine-DNA methyltransferase (*MGMT*) promoter is a major local epigenetic classifier of GBM cells. DNA methylation-induced down-regulation of *MGMT* inhibits the DNA repair mechanism of alkyl group removal, thereby making tumor cells sensitive to cytotoxic alkylating agents like temozolomide which improves patients' overall survival. Local deep bisulfite sequencing (LDBS) revealed that CpGs located in exon 1 are suited best to discriminate methylated from unmethylated samples. Based on LDBS data of approx. 200 GBM cases we propose an optimized MSP primer pair with 83% and 85% concordance to pyrosequencing and LDBS data. In addition, a region upstream of the TSS (*MGMTup*), with an overall higher 5-mC level compared to exon 1 and intron 1 of *MGMT*, is also able to discriminate the methylation status. Moreover, the methylation level of single CpG sites within *MGMTup* can serve as an improved predictor of progression-free survival. Currently, a quantitative pyrosequencing assay covering the respective CpG sites is developed and evaluated. It was shown by several studies that global level of 5'-hydroxymethylcytosine (5-hmC) in GBM is an excellent predictor of patients' overall survival. Very recent ACE-Seq experiments on low numbers of GBM cases suggest that (5-hmC) is also locally present in *MGMTup* (up to 40% at single CpG sites), its loss, however, seems not to be correlated with progression-free survival. Our data catches a glimpse towards the importance of high-resolution sequencing of epigenetic marks in GBM diagnostics and prognostic estimations in GBM patients.

P39 Anna Winkler (Ludwig Maximilian University of Munich)

The impact of Tet3 isoform-specific KO on the murine retina and brain

Anna Winkler, Maike Däther, Nina Dudáková, Constanze Scheel, Franziska Traube, Stylianos Michalakis

Active DNA demethylation by the conversion of genomic 5-methylcytosine (5mC) into 5-hydroxymethylcytosine (5hmC) and further oxidized forms (5-formyl-, 5-carboxycytosine) correlates with higher DNA accessibility and gene activation. This oxidation process is catalyzed by dioxygenases of the Ten-Eleven Translocation (TET) protein family. *TET3* encodes the most abundant TET isoenzyme in the central nervous system and is linked to neurodevelopmental retardation in humans. Due to alternative splicing or alternative promoter usage, *TET3* exists in various isoforms that differ N-terminally in their sequence. The *TET3* long isoform contains a DNA-binding CXXC domain, while the short isoform lacks that domain. In the neuronal context, *TET3* short shows higher expression compared to

TET3 long. However, distinct roles and contributions of each isoform in the process of neuronal development remain unclear.

Here, we generated and characterized Tet3 long and Tet3 short isoform-specific knockout (LKO/SKO) mice using CRISPR-Cas9 technology and used them to examine the potentially different roles of each isoform in the retina and the brain. We found that there is a compensatory upregulation of the remaining isoform, when the other one is fully knocked out. Nevertheless, at the phenotypic level we identified isoform-related differences. In the retina and the cerebellum of the SKO mice 5fC levels were not detectable, while in LKO the levels corresponded to wildtype mice. We saw genotype-independent indications for isoform-related influences in the retinal morphology and cellular composition, that could be caused by an impact on the cell fate of cones and bipolar cells. Our findings point to an isoform-specificity in the 5mC-5hmC-5fC turnover and indicate that there might be a certain Tet3 short/Tet3 long ratio required for a normal retinal development. In conclusion, we observed first indications for Tet3 isoform-specific effects in the neuronal context.

P40 Elena Valceschini (University of Luxembourg)

Identification of ZFHx4 as a novel transcription factor controlling dopaminergic neuron differentiation

Abstract is available in Rapid Fire Talk section

P41 Andreas Zhadan (Technical University of Darmstadt)

Role of Mbd1 in embryonic stem cell pluripotency maintenance

Andreas Zhadan, Peng Zhang,, Maria Arroyo, Cathia Rausch, Maruthi K. Pabba, Christopher B. Mulholland, Sebastian Bultmann, Heinrich Leonhardt and M. Cristina Cardoso

The methyl-CpG binding domain protein 1 (Mbd1) is the largest member of the MBD protein family. The name-giving MBD-domain recognizes and binds methylated CpGs. Mbd1 possesses at its C-terminus the transcriptional repression domain (TRD) and three zinc-finger domains. Particularly in neurons and adult neural stem cells (aNSCs) in the brain, *Mbd1* is highly expressed. Neurogenesis in *Mbd1*^{-/-} mice was shown to be impaired, resulting in decreased learning ability and autism-like behavior. *Mbd1* was shown to have a major role in neurodevelopment by repressing lineage specific genes, thus maintaining stem cell identity of aNSCs.

To investigate the potential role of Mbd1 in the establishment and maintenance of pluripotency, we utilized CRISPR/Cas9 gene editing to knock out Mbd1 in J1 mouse embryonic stem cells (mESC). *Mbd1*^{-/-} mESC exhibited an accelerated transition out of naive pluripotency. Analysis using RNA-sequencing and qRT-PCR revealed a decrease in the expression of genes linked to naive pluripotency. Moreover, reduced levels of pluripotency factors were detected in the *Mbd1*-deficient cells via immunostaining. Our current results indicate that Mbd1 has a potential role in the maintenance of pluripotency in mESCs.

P42 Yanhao Yu (Chinese Academy of Sciences)

APE1 promotes lung adenocarcinoma through G4-mediated transcriptional reprogramming of urea cycle metabolism

Yanhao Yu, Chaochao Cen, Zhenyu Shao, Chaohan Wang, Meiling Sun, Chao Wang, Zongjie Miao, Qing Xu, Kaiwei Liang, Jiaxin Zhou, Dan Zhou, Hongbin Ji, Yarui Du, and Guoliang Xu

Lung adenocarcinoma (LUAD) remains as the leading cause of cancer deaths worldwide. Apurinic/apyrimidinic endonuclease 1 (APE1), an enzyme integral to DNA repair and redox signaling, is notably upregulated in various cancers, including LUAD. Our study delves into the specific role of APE1 in LUAD progression, an area previously not fully understood. Here we found that the majority of APE1 mutations were concurrent with allele amplification, and elevated APE1 expression associated with poor prognosis in LUAD patients. Functionally, APE1 deletion hampered the proliferation of human

LUAD cell lines and impeded tumor progression in a KRAS-driven genetically engineered mouse model (GEMM), highlighting its critical role in tumorigenesis. Importantly, the enzymatic activity is necessary for APE1 to exert its tumor-promotive function. Mechanistically, APE1 facilitated the transcription of urea cycle genes *CPS1* and *ARG2* via directly binding to their promoters and stabilizing the formation of G-quadruplex (G4) structures. Loss of APE1 induced the reprogramming of urea cycle metabolism, characterized by ammonia accumulation, suppression of amino acid biosynthesis and disruption of pyrimidine metabolism. Furthermore, *CPS1* restoration alleviated the impaired cell proliferation of APE1^{-/-} LUAD. These findings provide insights into the transcriptional regulation of APE1 in urea cycle metabolic reprogramming and hold therapeutic implication for LUAD patients with elevated APE1 expression.