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Book of Abstracts

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4th Danube Conference on Epigenetics

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BOOK OF ABSTRACTS

FEBS Advanced Lecture Course:
4th Danube Conference on Epigenetics
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Keynote Lecture
chair: **Petra Hajkova**

Epigenetic regulation of imprinted gene clusters

Marisa S. Bartolomei

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Imprinted genes, which are unique to mammals, are monoallelically expressed in a parent-of-origin specific manner. Most imprinted genes reside in clusters that are located throughout the mammalian genome. The clusters contain an imprinting control region (ICR), which harbors allele-specific methylation and governs the imprinting of the entire domain. Although most imprinted clusters use long non-coding RNAs to regulate imprinted gene expression, a few are regulated by CTCF and allele-specific insulator function. One such cluster harbors the *H19* and *Igf2* imprinted genes, and is controlled by an ICR that contains multiple CTCF binding sites. Gain of maternal methylation and loss of paternal hypermethylation of the *H19*/*IGF2* ICR are associated with the human growth disorders Beckwith-Wiedemann Syndrome and Silver-Russell Syndrome, respectively. A second imprinted locus, *Grb10*, also uses an ICR with CTCF binding sites to regulate an unusual allele and tissue-specific imprinting pattern. Using gene targeting and genome editing, we have generated mice to study imprinting mechanisms for both of these loci. As part of this work, we have identified a novel allele-specific insulator that regulates *Grb10* and the adjacent *Ddc1* gene in mesodermal lineages, forming a newly characterized imprinted cluster. This insulator is developmentally acquired after implantation through the imposition of allele-specific DNA methylation and CTCF binding. Finally, we have also studied the mechanism governing imprint establishment in the germline using mice harboring mutations in epigenetic regulators.

Session 1:
Carly career scientist workshop:
Data presentation best practices
chair: **Uschi Symmons**

Take a sad plot and make it better - Better figures in the biological sciences

Helena Jambor

Universitätsklinikum Carl Gustav Carus an der Technischen Universität Dresden, Germany

Visualizations are widely used, in figures, slides, or posters, to communicate about scientific data. Visual communication strategies are however not regularly part of the scientific curriculum. In the workshop participants learn how to recognise common fallacies of scientific plots and how to avoid these. We will discuss different chart types and their use and misuse, ethical data display, and design principles to make visualizations clear to wide audiences. Using exercises we will turn a few sad plot examples into better versions of themselves.

Session 2: Epigenetics and development
chair: **Petra Hajkova**

Epigenetic determinants of germline specification and protection

Deborah Bourc'his

Institut Curie, Paris, France

Acquisition of somatic DNA methylation patterns is an early process during mammalian development, coinciding with embryonic stem cells (ESCs) transitioning from naive to primed pluripotency. This precedes the emergence of the three somatic germ layers, but also of the primordial germ cells (PGCs) that undergo genome-wide DNA demethylation after their specification. DNA methylation is known to be dispensable for naive pluripotency, but necessary for development. When DNA methylation becomes critical and whether all lineages equally depend on DNA methylation is unclear. This is the question I will address, presenting recent findings obtained on *in vitro* culture of DNA methylation-free (TKO) ESCs.

Rapid evolution of non-coding regulators of X-inactivation across primates

Emmanuel Cazottes[1], Charbel Alfeghaly[1], Irène Aksoy[2], Cloé Rognard[2], Agnese Loda[3], Edith Heard[3], Pierre Savatier[2], Céline Morey[1] and Claire Rougeulle[1]

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What are the genetic sources of innovation for species to adapt to a constantly changing environment ? In contrast to its coding counterpart, the noncoding genome, which notably includes lncRNA genes and regulatory sequences, is less subject to selective pressure and could therefore represent a substrate for rapid species evolution. To tackle these questions, we study X Chromosome Inactivation (XCI), an essential epigenetic process, triggered early during mammalian development, which allows for dosage compensation of X-linked gene products between sexes. XCI is controlled by a network of non-coding actors, lying within the X-Inactivation Centre (*XIC*).which display species specific functions and modes of action.

To finely resolve the contribution of these actors to the divergence of XCI strategies between species, we focused on evolutionary close primates, rhesus macaques and humans, which diverged 35 million years ago. We use pluripotent stem cells (PSCs) as a working model.

We first showed that XCI is more stable in rhesus PSC compared to human PSC and less dependent on lncRNA genes. To unbiasedly probe for regulatory innovations in macaques, we characterized the network of topological interactions along the *XIC* at a high-resolution using capture-HiC.

We identified a rhesus specific regulation hub driven by the insertion of an HERVK transposable element which act as a topological domain boundary. This insertion is associated with the recruitment of upstream sequences into enhancer-like elements.

Altogether, these results illustrate the rapid evolution of non-coding effectors over short evolutionary timescales and possibly highlight adaptation of regulatory circuitries to species specificities.

Keywords: *Early Embryonic Development, Genome Architecture, Evolution*

Balanced gene dosage control rather than parental origin underpins genomic imprinting

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Mammalian parental imprinting represents an exquisite form of epigenetic control regulating the parent-specific monoallelic expression of genes in clusters. While imprinting perturbations are widely associated with developmental abnormalities, the intricate regional interplay between imprinted genes makes interpreting the contribution of gene dosage effects to phenotypes a challenging task. Using mouse models with distinct deletions in an intergenic region controlling imprinting across the Dlk1-Dio3 domain, we linked changes in genetic and epigenetic states to allelic-expression and phenotypic outcome *in vivo*. This determined how hierarchical interactions between regulatory elements orchestrate robust parent-specific expression, with implications for non-imprinted gene regulation. Strikingly, flipping imprinting on the parental chromosomes by crossing genotypes of complete and partial intergenic element deletions, rescued the lethality of each deletion on its own. Our work indicates that parental origin of an epigenetic state is irrelevant as long as appropriate balanced gene expression is established and maintained at imprinted loci.

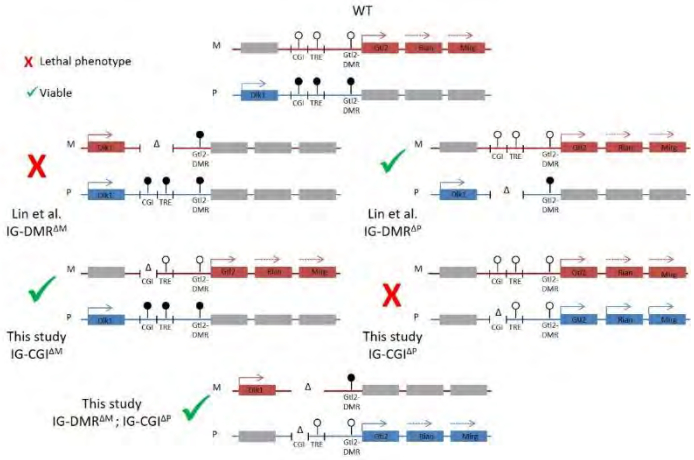

Figure 1: Balanced gene expression in the Dlk1-Dio3 locus, rather than parent-of-origin specific expression is required for proper embryonic growth and survival

Gene regulation in human spermatogenesis

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Embryonic development involves the fusion of highly specialised gametes into a totipotent zygote that gives rise to a whole organism. The production of gametes must therefore ensure that they can carry out their specialised roles while retaining the ability to be completely reprogrammed. During spermatogenesis the cell undergoes dramatic reorganisation, including replacement of histones by protamines, loss of most cytoplasm and reorganisation of mitochondria and the cytoskeleton. Errors in this process can lead to infertility, which affects 10-15% of couples worldwide. The exact cause is unknown in ~70% of male infertility cases.

Hence, to understand the gene regulatory networks that control human spermatogenesis, we have carried out joint analysis of gene expression and chromatin accessibility at the single nucleus level using testis biopsies that contain cells from every stage of spermatogenesis. We obtained more than 19,000 high quality nuclei from three biological replicates, representing all of the expected germline stages as well as supporting somatic cell types. Our multiomic approach allows us to understand the relationship between gene regulation and expression at the single cell level. Using this data, we have identified key transcription factors for each cell type, as well as their putative binding sites and target genes, revealing potential novel candidate infertility genes.

Pronounced sequence specificity of the TET enzyme catalytic domain guides its cellular function

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TET (ten-eleven translocation) enzymes catalyze the oxidation of 5-methylcytosine bases in DNA, thus driving active and passive DNA demethylation. Here, we report that the catalytic domain of mammalian TET enzymes favor CGs embedded within basic helix-loop-helix and basic leucine zipper domain transcription factor–binding sites, with up to 250-fold preference in vitro. Crystal structures and molecular dynamics calculations show that sequence preference is caused by intra substrate interactions and CG flanking sequence indirectly affecting enzyme conformation. TET sequence preferences are physiologically relevant as they explain the rates of DNA demethylation in TET-rescue experiments in culture and in vivo within the zygote and germ line. Most and least favorable TET motifs represent DNA sites that are bound by methylation-sensitive immediate-early transcription factors and OCT4, respectively, illuminating TET function in transcriptional responses and pluripotency support.

The chromatin landscape during early embryo development at single-cell resolution

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During embryonic development one cell divides and differentiates resulting in a complex organism made up of multiple cell types. Cell fate is largely determined by when and where specific genes are activated or silenced during embryonic development. This process of differential gene regulation can be achieved in several ways, including histone modifications and nuclear localization. For example, chromatin regions located at the nuclear periphery, termed lamina associated domains (LADs), are heterochromatic regions that impact genome organization and play a role in gene regulation. The very first cell fate decision occurs during the first few days of development and is crucial for correct embryonic development. The emergence of single-cell and low-input technologies has revealed heterogeneity and massive chromatin rearrangement occurring during the first stages of development. However, the role of these features in regulating gene expression as well as the interplay between the various chromatin marks remains elusive. In this study, we have employed DamID-based techniques to study LAD, chromatin accessibility and histone mark distribution in early embryos in single cells. Both epigenetic and gene expression readouts from the same cell were successfully obtained in several early developmental stages. This approach has revealed major LAD variability following the first cell division, while silencing histone marks and accessible chromatin remained remarkably similar among cells. Cell-cell LAD variability does not seem to affect transcription. In addition, a large number of genomic regions that tend to locate at the nuclear periphery in all cell types, are strikingly not located at the lamina in 2-cell embryos but are instead enriched in H3K27me3. This suggests an alternative silencing strategy during early development. Upon knock-out of maternal Eed and concomitant loss of maternal H3K27me3 during early embryogenesis, these regions relocate to the lamina and adopt a similar nuclear location as in ESCs and most other cell types. Thus, after fertilization, H3K27me3 appears to delay localization of genomic regions to the nuclear periphery. This study brings insight into the atypical regulatory mechanisms at play during early development by directly linking epigenetics and transcription in single cells.

Session 4: Epigenetics and transcription
chair: **Tibor Pankotai**

The related coactivator complexes SAGA and ATAC control embryonic stem cell selfrenewal through acetyltransferase-independent mechanisms

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SAGA (Spt-Ada-Gcn5 acetyltransferase) and ATAC (Ada-two-A-containing) are two related coactivator complexes, sharing the same histone acetyltransferase (HAT) subunit. The HAT activities of SAGA and ATAC are required for metazoan development, but the role of these complexes in RNA polymerase II transcription is less understood. To determine whether SAGA and ATAC have redundant or specific functions, we compare the effects of HAT inactivation in each complex with that of inactivation of either SAGA or ATAC core subunits in mouse embryonic stem cells (ESCs). We show that core subunits of SAGA or ATAC are required for complex assembly and mouse ESC growth and self-renewal. Surprisingly, depletion of HAT module subunits causes a global decrease in histone H3K9 acetylation, but does not result in significant phenotypic or transcriptional defects. Thus, our results indicate that SAGA and ATAC are differentially required for self-renewal of mouse ESCs by regulating transcription through different pathways in a HAT-independent manner.

SAGA saga or, how TBP is deposited on DNA

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The TATA-box Binding Protein (TBP) is universally required for the synthesis of RNA molecules encoded by eukaryotic genes. For RNA polymerase II (Pol II)-dependent genes, the interaction of TBP with the gene promoter initiates the assembly of a transcription Preinitiation Complex (PIC) that positions Pol II over the transcription start site in order to start mRNA synthesis. TBP delivery, turnover and dissociation from gene promoters are essential for PIC assembly and highly regulated. Yet, despite the importance of these steps in gene expression, we are still lacking a quantitative and dynamic understanding of TBP recruitment.

The multi-subunit TFIID and SAGA complexes share the capacity to load TBP onto Pol II gene promoters and enable transcription. The two complexes share several subunits implicated in the interaction with TBP. We solved the quasi-atomic model of SAGA in complex with TBP. The structure reveals the intricate network of interactions that coordinate the different functional domains of SAGA and resolves a deformed octamer of histone-fold domains at the core of SAGA highly homologous to nucleosomal histones. This deformed octamer is precisely tuned to establish a peripheral site for TBP binding, where it is protected by steric hindrance against the binding of spurious DNA. To date how SAGA and TFIID deliver TBP to promoters, the kinetics of TBP-DNA interactions and the modes of TBP reloading are not well understood.

To start transcription, TBP has to be released from SAGA. Here we demonstrate that DNA alone is not able to dissociate TBP from SAGA. We present structural and biochemical analyses hinting to a mechanism for TBP delivery and release from SAGA that requires the general transcription factor TFIIA and whose efficiency correlates with the affinity of DNA to TBP.

Transcriptional regulation at DNA double-strand break sites: A spotlight on Lysine Crotonylation

Enas R. Abu-Zhayia, Feras E. Machour, Laila A. Bishara, Bella M. Ben-Oz and Nabieh Ayoub

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Double-strand breaks (DSBs) at the vicinity of transcriptionally active genes trigger rapid and transient transcriptional silencing. Previously, we showed that CDYL1 is recruited to DNA doublestrand breaks (DSBs) to promote homology-directed repair (HDR) and foster transcriptional silencing. Yet, how CDYL1 elicits DSB-induced silencing is not fully understood. Recently, we identified a CDYL1-dependent local decrease in the transcriptionally active marks lysine crotonylation (PanKcr) and crotonylated histone residue H3K9cr at AsiSI-induced DSBs, which correlates with transcriptional silencing. Mechanistically, we revealed that CDYL1 crotonyl-CoA hydratase activity counteracts PanKcr and H3K9cr at AsiSI sites and triggers the eviction of the transcriptional elongation factor ENL to foster transcriptional silencing. Furthermore, genetic inhibition of CDYL1 hydratase activity blocks the reduction in H3K9cr and alleviates DSB-induced silencing, while HDR efficiency unexpectedly remains intact. Therefore, our results functionally uncouple the repair and silencing activity of CDYL1 at DSBs. In a broader context, we addressed a long-standing question concerning the crosstalk between HDR and DSB-induced transcriptional silencing, suggesting that they are functionally uncoupled and may occur independently.

FoxA pioneer factors and TET proteins establish a stable epigenetic signature in hepatocytes

Tzachi Reizel

The Technion's Faculty of Biotechnology and Food Engineering, Israel

Demethylation of thousands of tissue-specific enhancers drives the gene activation required for cell differentiation. This work aimed to study the kinetics and factors mediating tissue-specific demethylation in hepatocytes and their physiological role. For this purpose, we studied the main methylcytosine dioxygenases expressed in the liver, TET2 and TET3, and the FoxA pioneer factors known to be essential for liver differentiation. These genes were deleted at different time points during hepatic development and adulthood. Following gene's knock out, transcriptome, methylome, chromatin marks, and accessibility were analyzed. TET deletion at the time of liver differentiation and maturation prevented hepatic demethylation, dramatically affecting liver size and cholesterol and glucose metabolism. When FoxA pioneer factors were abolished during development, a significant fraction of TET-mediated demethylation was inhibited, indicating that the FoxA proteins recruit TET proteins to their targets in vivo. In sharp contrast, at the adult stage, FoxA pioneer factors and TET proteins were not required to maintain the hypomethylated state. Moreover, even when regulatory regions normally occupied by the FoxA proteins lost accessibility upon FoxA removal in the adult liver, no gain of DNA methylation was observed at these sites. This persistent hypomethylation demonstrates the stability of tissue-specific enhancers. An exception to this rule was found in a model for continuous liver regeneration, during which hepatic enhancers underwent epigenetic reprogramming and FoxA binding sites underwent de novo methylation. These observations demonstrate the stability of tissue-specific DNA methylation patterns in homeostasis and that epigenetic reprogramming accompanies liver regeneration.

Epigenetic and transcriptomic alterations caused by shattered chromosomes

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The epigenetic landscape is regulated by a myriad of factors. The underlying genome sequence contributes to this regulation, likely at a variety of scales, however the extent of this regulation is not fully understood. Chromothripsis is a localised catastrophic genome shattering event that can be seen in up to 32% of oesophageal adenocarcinomas. It can used to study how the underlying genomic sequence affects higher-order structuring and the epigenome. Since chromothripsis tends to affect only one allele, in every cell a direct comparison can be made between the wild-type chromosome and the chromothriptic chromosome. The wild-type chromosome represents the genome sequence and structure before reshuffling and the chromothriptic derivative chromosome can be used to query the direct effects of this reshuffling. Patient-derived oesophageal adenocarcinoma organoids with chromothripsis restricted to one allele were used to better understand genome regulation. Complex regions of structural variation between alleles and subclonal variants means haplotype-aware *de novo* assemblies are essential for contiguous cancer genome assemblies. We produced contiguous assemblies for 5 chromothriptic derivative chromosomes and the associated wild-type chromosomes, even when over 900 structural rearrangements are present. With these cancer-specific reference assemblies, the epigenome of the chromothriptic and wild-type chromosomes can be profiled in relation to structural variation. There are widespread differences between topologically associated domains, chromatin accessibility, histone modifications and gene expression in chromothriptic and wild-type chromosomes. This indicates that chromosome shattering has dramatic and direct consequences on gene regulation, far beyond what we see when comparing two wild-type alleles. It highlights that, while underlying genome sequence has a fundamental role in gene regulation, the epigenetic context of that sequence also has a profound impact. This work allows for unprecedented insight into the regulatory impact of structural variation.

Controlling transcription from within transcribed regions in plants

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Transcriptional regulatory sequences control when and where genes are transcribed. While plants and animals evolved the mechanisms for cell-type-specific gene expression separately, our understanding of transcription regulation in plants is mostly extrapolated from animal systems. In this work, we explore how transcription-regulatory sequences are organized in *A. thaliana*. We found that transcription factor binding sites and expression quantitative trait loci (eQTLs) are enriched downstream of the transcription start site (TSS). Using a massively parallel reporter assay, we directly demonstrate that sequences downstream of the TSS control transcription, and lose this ability when positioned upstream to the TSS. This position-dependent effect is unique to plants as opposed to animal enhancers, which are indifferent to relative position. Our study provides insights on the divergence of transcription-regulatory sequences between animals and plants.

Beyond motif: guiding sites of transcription factor binding by DNA modifications

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Transcription factors only bind a minority of their motifs in large mammalian genomes. One potential explanation is that many motifs are not accessible for binding due to the action of chromatin and DNA methylation. We are using mammalian stem cell models to understand this important interplay between gene regulation, chromatin structure and DNA methylation. We study the dynamics of the epigenome and test regulatory models by genetic perturbation and genome editing approaches.

I will discuss our recent efforts in understanding how DNA methylation represses regulatory regions and how the sensitivity to DNA methylation can limit transcription factor binding in the context of the cell.

Session 5: Epigenetics and differentiation

chair: Tamás Arányi

Genetic alterations precede epigenetic gene silencing

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Besides silencing gene expression at the post-transcriptional level, small RNAs mediate the formation of silent chromatin that is heritable across generations. Over the last two decades, fission yeast has been serving as an excellent model organism to elucidate the mechanism of small-RNA-mediated heterochromatin formation at repetitive DNA. More recently, work that we have performed with fission yeast revealed the existence of cellular activities that prevent small RNAs from triggering the formation of heterochromatin outside repetitive DNA. Because the list of experimentally determined alleles that allow small-RNA-mediated heterochromatin formation keeps expanding, we speculate that fission yeast's natural ecology may lead to the acquisition of silencing enabling genetic mutations as part of a biological bet-hedging strategy. We therefore advocate for the inclusion of non-laboratory strains or growth conditions in future research that aims at understanding the physiological relevance of small-RNA-mediated epigenetic gene silencing.

Molecular mimicry of SUMO to recruit Su(var)2-10 to the site of transposon repression

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Drosophila PIWI is a nuclear Argonaute (AGO) protein which is involved in the co-transcriptional silencing of transposons. After targeting by piRNA, the PIWI protein recruits a silencing complex and induces heterochromatin formation at the transposon locus leading to transcriptional repression. The E3 SUMO ligase Su(var)2-10 is an essential member of the silencing complex, however its initial recruitment by PIWI protein is poorly understood.

It has been demonstrated, that the AGO family proteins are able to directly bind to SIM motifs possibly through their SUMO-like structures. Therefore, we hypothesized that the *Drosophila* PIWI protein may possesses SUMO-like structures by which it is able to interact with the SIM motifs of Su(var)2-10. In this study, we demonstrated that the Su(var)2-10 directly interacts with PIWI in vitro. This interaction is mediated by the SIM-like structure in the CTD domain of the Su(var)2-10. Our results confirm, that the PIWI protein is not a sumoylation target, however it has SUMO-like structures in its MID and PIWI domains. Mutations in the predicted binding interface of the MID domain significantly decreased its Su(var)2-10 binding ability in vitro and resulted in transposon derepression in the ovary of flies.

Based on these results we suggest that the PIWI protein utilizes molecular mimicry of SUMO, which serves as an initial SUMO signal to recruit the Su(var)2-10 and the sumoylation machinery to the site of transposon repression.

***Keywords**: sumoylation, PIWI, piRNA, co-transcriptional repression*

Deciphering lineage specification during early embryogenesis using multi-layered proteomics

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The advent of mouse gastruloids, an *in vitro* model of embryonic development has opened new avenues to study key aspects of early embryogenesis, including germ layer formation. While single-cell sequencing technologies have been very popular to understand the cellular complexity and lineage trajectories in gastruloids and gastrula-stage embryos, proteome-based studies of early mammalian development are scarce. However, proteomic studies are extremely important, because proteins drive virtually all cellular processes. Generation of a triple fluorescent reporter embryonic stem (ES) cell line in which the germ layers are distinguishable by different fluorophores, allowed sorting and deep profiling of germ layer-specific (phospho)proteomes. We identified nearly 5000 proteins, including many differentially expressed proteins involved in signaling pathways and gene expression regulation. To further characterize lineage-specific chromatin proteomes, we employed p300 proximity labeling to identify the protein complexes bound to enhancers. p300 proximity labelling experiment in undifferentiated mouse ES cells and gastruloids yielded a treasure trove of enhancer associated proteins and transcription factors. Targeted protein degradation of selected transcription factors provided insights into the role for each of the targeted transcription factor in cell lineage specification and differentiation. For example, dTAG-based depletion of a zinc finger transcription factor causes a defective differentiation phenotype and single-cell RNA-seq revealed changes in cell populations. Collectively, integrative multi-omics and perturbation-based analyses provided novel insights into the establishment of lineage-specific transcriptional programs.

Keywords: *asgtruloids, proteomics, enhancers, transcription factors, interaction proteomics, dTAG system*

Single cell multi-omics landscape of development and ageing

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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 naïve 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 germ line and at zygotic genome activation. Using single cell multi-omics techniques, we are beginning to chart the epigenetic and transcriptional dynamics and heterogeneity during the exit from pluripotency and initial cell fate decisions leading up to gastrulation. 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 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.

Parental genetics and epigenetic programming of offspring phenotypes

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Individual genetics, environmental exposures, and their interaction are the three main determinants of an individual's phenotype. This picture has been complicated few decades ago when the Lamarckian theory of acquired inheritance has been rekindled with the discovery of epigenetic inheritance, according to which acquired phenotypes can be transmitted through fertilization and affect phenotypes across generations. These findings, together with the important degree of missing heritability in genetics highlighted by Genome-Wide Association Studies, suggest that not only acquired phenotypes, but also individual's genotypes may affect phenotypes intergenerationally through Indirect Genetic Effects. Here, we explored the genotype-phenotype association resource of the International Mouse Phenotyping Consortium with the aim of understanding whether Indirect Genetic Effects are detectable and how common they are in mammalian genetics, what are the underlying genetic determinants of Indirect Genetic Effects in mammals and which relevance they may have for human physiology and susceptibility to complex diseases. Our results demonstrate that Indirect Genetic Effects are common to mammalian genetics and influence intergenerational physiology across several layers spanning from metabolic to neurological and cardiovascular health. Interestingly, functional annotation of the underlying genetic determinants indicates a tight clustering to proteins involved in protein ubiquitination and neuroactive signaling, without clear genomic and topological clustering. Altogether, our results propose Indirect Genetic Effects as a new common feature of mammalian genetics, which controls physiology across generations in a gene-dependent, genotype-independent manner and highlight new functions for known genes and gene families.

Keywords: *Indirect Genetic Effects, Phenotypic programming, Complex physiology*

Layered epigenetic control of transcribed repetitive elements in human brain development

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Patient mutations in the chromatin-remodelling ATPase MORC2 cause severe neurodevelopmental disorders. MORC2 is required for epigenetic regulation of interspersed repeats by the HUSH complex through histone methylation mark H3K9me3. However, the functions of the MORC2-HUSH-H3K9me3 axis in human brain development are largely unknown and its relationship with DNA methylation – a key epigenetic regulator of repetitive elements in somatic cells – is poorly understood. Here we find that MORC2-HUSH controls H3K9me3 over hundreds of transcribed repeat sequences in human neural stem cells, and provide evidence that these sites are likewise regulated in human fetal forebrain tissue and maintained in adult neurons. Notably, loss of H3K9me3 does not deregulate transcription of targeted LINE-1 transposons, but we do observe pronounced epigenetic and transcriptional changes in clustered protocadherin repeat genes, whose combinatorial expression of synaptic adhesion proteins underlies complexity in neuronal networks. Loss of promoter DNA methylation through acute knockout of maintenance DNA methyltransferase DNMT1 activates transcription of both LINE-1s and protocadherins without affecting local H3K9me3, providing support for the model that H3K9me3 lies upstream of CpG methylation in certain genomic contexts. Together, our data demonstrate that MORC2 and HUSH are important chromatin regulators in neural lineages and begin to unravel mechanisms underpinning the layered epigenetic control of repetitive elements in human brain development, with important clinical implications.

Session 6: Epigenetics and physiology

chair: Lóránt Székvölgyi

Epigenetic underpinnings of metabolic disease heterogeneity

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Complex trait diseases afflict >2 billion people worldwide. The rapid rise in particular of early life disease carries long-term health burdened including heart disease, diabetes and stroke, making the issue one of the world's chief economic and health care challenges of the day. Whereas our understanding of the genetic framework for complex disease has expanded dramatically the last decades, our understanding of causal epigenetic mechanisms of disease remain poorly understood. Our focus has been to mine and understand the mechanisms underpinning non-genetic disease heterogeneity and thus to understand the spectrum of disease potential that lies within each individual. I will discuss how two recent directions within the lab that focus on how chromatin silencing mechanisms help shape unexplained phenotypic variation in complex disease traits at the organismal scale, and, at the cellular scale, how they appear also able to canalize the emergence of highly related yet distinct cell sub-types. The data suggest a highly regulated landscape of non-genetic phenotypic variation defines mammalian disease. They open the door to development of dietary or pharmacological regimens to manipulate epigenetic disease triggering and cell sub-type specific function.

Developmental plasticity as a predisposing factor for tumorigenesis

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Despite sharing the same DNA and similar rearing environment, monozygotic co-twins exhibit striking differences in susceptibility to complex diseases. For tumors, their concordance rate is as low as 0-20%. These co-twin discordances exemplify the "developmental plasticity" of genomes, i.e., how a single genotype can generate a range of alternative phenotypes during development. Epigenetic mechanisms likely underpin a significant portion of this plasticity. Nonetheless, little is known about how inter-individual epigenetic variation influences tumor susceptibility.

Our hypothesis is that epigenetic differences established in development predispose otherwise identical individuals to differential tumor susceptibility. To test this hypothesis, we took advantage of the first mouse line of epigenetically driven developmental plasticity. Isogenic animals from this line develop into two distinct morphs with different body composition, despite carrying the same mutation in the *Trim28* gene. We crossed this line with *Tp53* mutant mice, that are susceptible to a broad spectrum of tumor types. We monitored the animals before and after developmental bifurcation and tumor onset, and we collected longitudinal samples for metabolomics and epigenetics analysis to identify hallmarks and biomarkers of differential susceptibility.

Our data indicate that isogenic animals carrying the *Trim28* mutation (alone or with *Tp53*) not only develop into morphs with different body composition, but also exhibit specific and distinct metabolic signatures that precede the developmental bifurcation. Moreover, animals with a higher body composition score are protected from tumors, exhibiting lower incidence and succumbing more slowly than their isogenic lighter counterparts. Even the spectrum of tumor susceptibility appears to be different in the two morphs.

Our results show that inter-individual epigenetic differences established during development translate into differential risk levels for tumor susceptibility. Thanks to our newly established model we can dissect and ultimately target the mechanisms regulating this epigenetically driven differential susceptibility to a wide range of tumors.

Pharmacologic targeting of ARID1A for the non-estrogenic modulation of IGF-1 signaling

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Gaining pharmacologic access to the potential of ARID1A, a tumor suppressor protein, to mediate transcriptional control over cancer gene expression is an unresolved challenge. We hypothesized that synergistic drug interactions involving retinoid X receptor-selecive agonists, such as bexarotene, may induce global genomic responses affecting tumor cell progression, distinct from the effects of RXR agonists alone.

Our study identified a new regulatory mechanism of the IGF-1 growth pathway through the enrichment patterns of the chromatin remodeling factor ARID1A and histone H3 K27 acetylation in MCF-7 breast cancer cells under non-estrogenic conditions. The synergistic combination of two repurposed clinical drugs, bexarotene and the non-selective beta-blocker carvedilol, elicited a coordinated decrease in the expression of the IGF-1 receptor and IRS1 adapter protein through the upregulation and redistribution of ARID1A, and inhibited cell proliferation. In this model, ARID1A exerts its effect on the IGF-1 signaling pathway as a defining part of the SWI/SNF complex, reducing the accessibility of the affected chromatin segments.

This study offers the molecular interpretation of the interaction of bexarotene and carvedilol through the modulation of the ARID1A factor to down-regulate pro-tumorigenic IGF-1 activity, while modifying the notion that ARID1A exerts its activity mainly in the context of estrogenic action. Clinically, our results underscore the possibility of the pharmacologic control of a key mitogenic pathway targeting ARID1A in postmenopausal breast cancer patients undergoing aromatase inhibitor treatment.

Epigenetically-regulation of RNA-binding proteins underlies dormancy of relapsing malaria parasites

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Dormancy enables pathogens to survive unfavorable conditions and maximize their chance for transmission. Relapsing malaria parasites, such as *Plasmodium vivax* employ this strategy. After inoculation by the bite of an infected mosquito, the so-called hypnozoites stay quiescent inside liver cells for weeks to months before reactivating and establishing renewed blood stage infection. These dormant parasites can survive treatment of the primary infection and hence critically hinder malaria control and elimination. While they have been discovered more the forty years ago the mechanisms leading to hypnozoite dormancy and reactivation remain a mystery.

Here we integrate various omics approaches to explore the involvement of gene regulatory mechanisms in these processes. By profiling the genome-wide distribution of repressive and activating epigenetic marks we identified a small set of genes that are epigenetically silenced during liver stage development, a process that is conserved amongst relapsing parasites. Furthermore, by combining single-cell transcriptomics, chromatin accessibility profiling and fluorescent *in situ* RNA hybridization we show that these genes are exclusively expressed in hypnozoites and their silencing precedes parasite development. Intriguingly, the genes with clear hypnozoite-specific expression are almost exclusively encoding proteins with RNA-binding domains. Based on these findings we propose a model in which repressive RNA-binding proteins keep hypnozoites in a developmentally competent but dormant state and heterochromatin-mediated silencing of these genes enables hypnozoite reactivation. Detailed understanding of the mechanism that enables *de novo* heterochromatin formation over these genes could provide decisive clues for targeted reactivation and killing of these vicious pathogens.

Functional and mechanical response of epigenetic changes in leukemia cells

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During cell migration, the nucleus must alter its mechanical response in order to allow cell migration through confined spaces. As a result, the nucleus undergoes complex changes in the chromatin compaction and epigenetic changes, which in turn might affect cell transcription, migration and survival. However, how transient and persistent migration promotes permanent nuclear changes and their functional consequences remain poorly understood. Here, we use acute lymphoblastic leukemia (ALL) cells to describe the steps by which the nucleus alters its epigenetic signature and other molecular components that govern nuclear shape, stiffness and the invasion of tumor cells in vitro and in vivo systems. We focus on how confined cell migration through narrow spaces such as endothelial barriers and transwell filters promotes short and permanent changes in the nucleus of leukemia cells. These changes are connected to histone methylation, persistent modification of the transcriptional signature of migrated cells, DNA damage response and lamin B1 redistribution. Furthermore, we perform nuclear biophysical analyses using mechanical compression and optical tweezers to determine the implications of these changes for cell migration and nuclear stiffness. Mechanistically, we determined that actin polymerization balance is critical for the redistribution of nuclear components and the basal levels of DNA damage markers, with functional consequences for chromatin compaction. Altogether, our results suggest that cell migration influences the epigenetic signature and genetic instability landscape of migrating cells, which, in turn, might handle aging diseases and pathological states.

Keywords: *Cell migration, mechanobiology, nuclear deformability, lamin, histone methylation, DNA damage, acute lymphoblastic leukemia.*

Session 7: Genome architecture

chair: J. Andrew Pospisilik

Unravelling the genetic architecture of R-loops and chromatin loops

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Understanding of the molecular mechanism of genetic changes from point mutations to chromosomal rearrangements has been greatly facilitated by the discovery of R-loop structures (RNA-DNA hybrids with a displaced single-stranded DNA), which act as hotspots of genomic instability in many organisms. R-loops are tightly embedded with 3D chromatin architecture, therefore, it is essential to identify factors that regulate R-loop formation and long-range chromatin interactions.

Herein, I will present two approaches that enable the characterization of R-loops in the context of 3D chromatin structure. The first approach focuses on the homeodomain protein NODULIN HOMEOBOX (NDX), which was previously described in *Arabidopsis* as a transcriptional regulator of the *FLC* gene, mediated thorough R-loop stabilization (Sun Q *et al.* Science 2013). Using high throughput 'omics' approaches and quantitative microscopy we show that NDX is primarily a heterochromatin regulator that functions in pericentromeric regions to control the transcription of het-siRNAs and deposition of repressive CHH/CHG methylation. NDX localization was antagonistic with R-loop structures that were prevalent in euchromatic chromosomal arms in wild type plants. Hi-C analysis showed significant chromatin structural changes in an *ndx* mutant, with decreased intrachromosomal interactions at pericentromeres where NDX is enriched in wild-type plants, and increased interchromosomal contacts between KNOT-forming regions, similar to those observed in DNA methylation mutants. The second approach employs a cross-platform method to the massively parallel screening of R-loop regulatory genes and long-range chromatin interactions in budding yeast, which can be used as a "proxy" to find human functional homologues to discover clinically relevant R-loop regulators.

Reference:

Karányi, Z. *et al.* NODULIN HOMEOBOX is required for heterochromatin homeostasis in *Arabidopsis*. *Nat Commun* **13**, 5058 (2022). https://doi.org/10.1038/s41467-022-32709-y

Regulatory and structural chromatin topology: Lessons from an imprinted domain

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Genome function is regulated temporally and tissue-specifically through the orchestrated interplay of genomic features, epigenetic states, and topology. Genomic imprinting provides a perfect paradigm for studying the relationship between epigenetics, conformation, and gene regulation as two genetically identical regions that share the same cellular milieu express a different repertoire of genes in a parental-origin-specific manner. Using the *Dlk1/Gtl2* imprinted domain as a model, we have identified a tissue and parent-of-origin specific sub-TADs (Topologically Associated Domain) in fetal brain. This conformation is demarcated by two differentially methylated CTCF binding sites situated in the first intron of the maternally expressed *Gtl2* gene and is lost upon paternalization of the maternal chromosome. Deleting the CTCF binding sites both separately and together has enabled us to dissect the relationship between epigenetics, topology, and gene expression in this domain. We find that removing the maternal chromosome-specific subTAD boundary causes a partial disruption of imprinting without affecting methylation of the imprinting control regions. This suggests parent-of-origin specific conformation acts beyond epigenetics to regulate imprinted gene expression.

Effect of ageing on cellular variability and transcriptional dynamics

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The dysregulation of transcriptional networks and the increase in transcriptional variability are crucial components of ageing. In order to investigate the role of cellular heterogeneity in response to a long-term perturbation and its impact on the transcriptional networks of individual cells, three hemizygous knock-out (KO) mouse strains were generated, two of them lacking one copy of a liver-specific transcription factor, *Hnf4a* and *Cebpa* respectively, and another missing *Ctcf* as ubiquitous factor. Here, we perform single-nucleus RNA-seq2 in young and aged mice showing higher abundance of polyploid hepatocytes and steatosis in aged C57BL/6J livers. We also observe an increase in transcriptional variability in most of the cell types, and differential transcriptional profiles between aged polyploid hepatocytes. Furthermore, hemizygous KO mice show increase in hepatocyte ploidy and complex transcriptional phenotypes due to the increase in cellular heterogeneity. Importantly, aged livers from all three hemizygous KOs present no relevant steatosis indicating a conserved transcriptional program that rescue liver function upon a long-term perturbation. Our results demonstrate that hepatocyte polyploidization constitutes a non-canonical mechanism that protects against transcriptional dysregulation and associated chronic liver diseases in the context of ageing.

Epigenetic modulation based on the fundamental role of the C-terminal tail of H2a.Z in determining nucleosome stability, accessibility of DNA in chromatin and nuclear architecture

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H2A.Z-nucleosomes participate in both euchromatin and heterochromatin and it has proven difficult to reveal correlation of the disparate roles with the stability features imparted by H2A.Z. Using an *in situ* assay of nucleosome stability and cells expressing engineered forms of the variant we show that a major fraction of H2A.Z is released from nucleosomes of peripheral heterochromatin at unusually high salt concentrations, a feature dependent on the C-terminal tail of H2A.Z. This was reproduced with reconstituted nucleosomes, and binding of the peptide was detected by fluorescence correlation spectroscopy, implicating tail-mediated internucleosomal interactions in increased stability. Upon addition of the tail peptide to control nuclei the H2A.Z-nucleosomes assumed canonical stability, the peripheral heterochromatin became dispersed and nuclease sensitivity increased, especially at promoters, similarly to cells expressing tailless H2A.Z. When introduced into live cells, the peptide induced chromatin reorganization and reduced myc expression. Effective epigenetic modulation is interpreted in terms of the fundamental role of the tail in H2A.Z function.

Using random scrambling to decode sequence determinants of nuclear organisation

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Lamina-associated domains (LADs) are large repressive chromatin domains anchored to the nuclear lamina (NL). The determinants in the DNA sequence of LADs that govern these interactions are still poorly understood. This requires systematic genome perturbation experiments, which are challenging because LADs are very large and CRISPR editing in LADs is inefficient.

To overcome these challenges, we developed a novel approach to generate systematic series of deletions and inversions in a megabase-sized genomic locus of interest, here applied to perturb the structure of LADs. This method randomly scrambles the locus of interest in two steps. First, we pseudo-randomly insert pairs of LoxP sites across the region by local "hopping" of a Sleeping Beauty transposable element. Next, we induce Cre-mediated recombination between the loxP pairs, resulting into precisely defined deletions and inversions, depending on the orientation of the LoxP sites.

We used this approach to study two neighboring LADs in mouse embryonic stem cells. In this locus we generated a total of 12 long-range deletions and inversions from 2.5kb to 2Mb in size, truncating and rearranging the LADs in various ways. Cre recombination was remarkably efficient, even over very long distances (~10% at 2Mb). In each clonal cell line we then mapped NL interactions and heterochromatic histone marks using our pA-DamID technology (van Schaik et al, EMBO Rep 2020). The results indicate that a LAD can expand over up to 150kb into a neighboring inter-LAD region when its border is deleted, and suggest cooperativity between neighboring LADs. Furthermore, we identified a putative region that may nucleate NL interactions.

We expect our hopping and scrambling technique to be widely applicable to study the architecture of megabase-sized genomic regions.

Session 8: Epigenetics and inheritance

chair: László Tora

Epigenetic regulation of 3D genome architecture in cell differentiation and development

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The eukaryotic genome folds in 3D in a hierarchy of structures, including nucleosomes, chromatin fibers, loops, chromosomal domains (also called TADs), compartments and chromosome territories that are highly organized in order to allow for stable memory as well as for regulatory plasticity, depending on intrinsic and environmental cues. Our lab has provided evidence suggesting that the formation of TADs and chromatin loops can assist gene regulation, both in *Drosophila* and in mouse cells. However, the physical nature of compartments, TADs and loops remain elusive and single-cell studies are critically required to understand it. We characterized chromatin folding in single cells using super-resolution microscopy, revealing structural features inaccessible to cell-population analysis. TADs range from condensed and globular objects to stretched conformations. Favored interactions within TADs are regulated by cohesin and CTCF through distinct mechanisms. Furthermore, super-resolution imaging revealed that TADs are subdivided into discrete chromatin nanodomains.

We also analyzed loops that depend on chromatin components that regulate the expression of a large number of genes, dubbed as Polycomb group proteins. Originally, these factors were shown to silence gene expression and we found that they induce the formation of chromatin loops, which may play instructive roles in gene regulation. Our progress in these fields will be discussed.

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Integrated multi-omics reveal polycomb repressive complex 2 restricts human trophoblast induction

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Human naive pluripotent stem cells have unrestricted lineage potential. Underpinning this property, naive cells are thought to lack chromatin-based lineage barriers. However, this assumption has not been tested. Here, we define the chromatin-associated proteome, histone post-translational modifications and transcriptome of human naive and primed pluripotent stem cells. Our integrated analysis reveals differences in the relative abundance and activities of distinct chromatin modules. We identify a strong enrichment of Polycomb Repressive Complex 2 (PRC2)-associated H3K27me3 in naive pluripotent stem cell chromatin, and H3K27me3 enrichment at promoters of lineage-determining genes, including trophoblast regulators. PRC2 activity acts as a chromatin barrier restricting the differentiation of naive cells towards the trophoblast lineage, while inhibition of PRC2 promotes trophoblast fate induction and cavity formation in human blastoids. Together, our results establish that human naive pluripotent stem cells are not epigenetically unrestricted, but instead possess chromatin mechanisms that oppose the induction of alternative cell fates.

Reiterative de novo methylation maintains methylation levels in somatic cells

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DNA methylation is a pervasive epigenetic mark in normal cells. DNA methylation abnormalities are a fundamental hallmark of cancer that can promote carcinogenesis. DNA methylation is lost specifically in heterochromatic regions in tumours. These hypomethylated regions are termed partially methylated domains (PMDs) and replicate during late S- phase. The late replication of PMDs has been proposed to play a key role in their hypomethylation. Specifically, it has been suggested that PMDs passively lose methylation due to incomplete maintenance of methylation after consecutive cell divisions. This model directly implicates DNMT1 as the maintenance methyltransferase and suggests that it does not have enough time to fully methylate late replicating regions. I aimed to elucidate how PMDs become hypomethylated during tumorigenesis and address this 'passive loss' model. I investigated the levels and patterns of DNA methylation in HCT116 colorectal cancer cells and their DNMT1 Knock-Out (DNMT1KO) derivatives. I identified that PMDs show distinct hypomethylation in HCT116 cells, depending on their heterochromatic state. Constitutive heterochromatic PMDs, marked by H3K9me3, showed more pronounced hypomethylation than facultative ones, marked by H3K27me3. In DNMT1KO cells, I observed global loss of methylation levels. However, hypomethylation was particularly prominent within PMDs, suggesting that hindering DNMT1 activity led to poorer maintenance of methylation, in agreement with the model. I also observed a subgroup of PMDs that were predominantly marked by H3K9me3 and bordered by H3K27me3 in HCT116 cells, which unexpectedly showed increased methylation levels in DNMT1KO cells. These hypermethylated PMDs remain late replicating in DNMT1KO cells despite their high methylation. However, these regions were no longer marked by H3K9me3 and H3K27me3 in DNMT1KO cells, indicating the loss of their heterochromatic state. Finally, using ChIP, I identified that DNMT3A and DNMT3B were not recruited in constitutive and facultative heterochromatic regions. DNMT3A, but not DNMT3B, recruitment was detected in these hypermethylated PMDs in DNMT1KO but not HCT116 cells, aligning with the loss of the heterochromatic marks in the hypermethylated PMDs. Taken together, my results suggest that hypermethylated PMDs in DNMT1KO cells could maintain high methylation levels, despite their late replication timing, due to the recruitment of DNMT3A, mimicking early developmental methylation processes. These findings suggest that de novo DNMTs play an important role in maintenance of methylation levels via reiterative de novo methylation, while highlighting that chromatin environment and its role in DNMT recruitment might play a more important role than replication timing in the hypomethylation observed in cancers.

Epigenomic organisation and vertebrate conservation of cis-regulatory landscape during zebrafish development

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The DANIO-CODE consortium created a central repository for zebrafish developmental functional genomic data. Its Data Coordination Center (https://danio-code.zfin.org) currently holds 1,802 unpublished and reanalysed published genomics datasets minable in genome browsers. Here we report exploitation of this multi-omics database for comprehensive enhancer annotation and novel biological observations on gene regulatory principles during embryonic development. We identified and annotated >140,000 cis-regulatory elements, which we classified into functional categories including novel classes and assigned to cell-type specificity by whole embryo single-cell ATAC-seq. Exploiting this integrated genome-wide atlas we studied the temporal dynamics of regulatory landscape formation in a subset of genes characterised by long range regulation within gene regulatory blocks (GRBs) characterised by colinear organisation and extreme conservation of cis-regulatory elements across vertebrates. In these GRBs the developmental dynamic of cis-regulatory topology organisation is characterised by promoter proximal regulatory interactions during an early phase from zygotic genome activation to somitogenesis. These interactions are overlapping and likely seeded by large like H3K27ac domains, we called H3K27ac ensembles. Long range regulation of target genes is established in a second phase during organogenesis and is decoupled from H3K27 ensembles and argue for dynamic function by these epigenomic features distinct from 'super-enhancer'. Despite collinearity of cis-regulatory element organisation in GRBs the vertebrate conservation the underlying epigenomic features (such as H3K27ac or H3K27me3) is challenging due to the inability to establish their equivalence in the absence of direct sequence similarity spanning large genomic distances. To meet this challenge, we developed a multispecies synteny-anchoring approach to massively boost synteny resolution, which led to detection of conservation of epigenomic domains between zebrafish and mouse. We found remarkable similarities in H3K27me3 coverage of subTAD epigenomic domains associated with orthologous Polycomb target genes, as well as similarities in architectures of epigenomic domain-associated regulatory elements. Our comparative epigenomic tool also demonstrated evolutionary conservation of H3K27ac ensembles suggesting their pan-vertebrate role in TAD and subTAD organisation.

Continuous transcriptome analysis of single embryos reveals novel early transcriptional programs in *Drosophila melanogaster*

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Early embryonic development is known to lay the foundation for long-term phenotype and adult body formation. Despite this understanding, its rapid progression and the limited material available are major barriers to studying the earliest stages of development. The size and accessibility of *Drosophila* embryos, however, overcome these limitations, and several studies characterizing early transcriptional events have been reported. Unfortunately, elaborate protocols and embryo staging make these techniques prone to human and technical errors and incompatible with routine laboratory use. Herein, we present a straight-forward and accessible methodology for studying early transcription (≤3 hours) in *Drosophila*. This method relies on single-embryo RNA-sequencing and transcriptome ordering along a developmental trajectory (pseudo-time), which avoids the need to visually stage the embryos. Further, the improved resolution of this method allows for the identification of an earlier transcription start for some zygotic genes, including the exact onset of transcription and degradation of transcripts. Degradation patterns indicated that maternally deposited mRNAs decayed proportionally to transcript abundance, suggesting that degradation is independent of mRNA levels. In addition, our method also allows us to study sex-biased transcription from the beginning of zygotic transcription, leading to the identification of 120 mRNAs that exhibited significantly different expression between males and females before gastrulation. 56% of these genes were in autosomes and rDNA. Using sex-specific transcription, embryos can be sexed directly, eliminating the need for Y-chromosome genotyping. Together, our results agreed with existing studies and provided previously inaccessible knowledge of early developmental processes.

Keywords: *single-embryo, RNA-sequencing, transcriptome, development, zygote genome activation.*

Dynamic changes in chromatin configurations in late oogenesis impact the epigenome of the early embryo

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A well-orchestrated program of oocyte growth and differentiation results in the generation of a developmentally competent oocyte. In late oogenesis, fully grown oocytes are subjected to large-scale epigenetic modifications with implications in transcriptional silencing. Oocytes with decondensed chromatin are transcriptionally active, whereas oocytes with dense perinuclear chromatin are transcriptionally silent. Depending on their chromatin state they differ in their developmental potential, where upon fertilization oocytes with dense chromatin are able to develop to term, while oocytes with decondensed chromatin arrest at the 2-cell stage. Although changes in histone modifications in fully grown oocytes have been characterized, changes in DNA modifications and their potential consequences for developmental competence have not been investigated yet. Here, we characterized the different types of fully grown oocytes in more detail and observed nuclear, molecular, and cytoplasmic differences. Our data suggest that a successful transition of chromatin configurations in late oogenesis is important for the establishment of a developmentally competent epigenome at the beginning of life.

Keywords: *oocyte, chromatin configurations, epigenome*

Stability, turnover and erasure of epigenetic information *in vivo*

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Using mouse embryonic development as an experimental model, our long term interest is in how epigenetic information is propagated during development and erased in the context of the germ line (zygotic and germline reprogramming). My laboratory has contributed fundamental 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 address the dynamics of DNA modifications during replication and the consequences of the findings for our understanding of epigenetic stability across cell divisions.

Poster abstracts

P-01
Investigating functional consequences of *Mcts2* during embryonic development

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Imprinted gene models are informative for understanding the interactions between genome and epigenome and their impact on mammalian development. Our lab has previously identified that genomic imprinting can contribute to isoform transcript diversity through alternative polyadenylation at the imprinting *Mcts2/H13* locus.

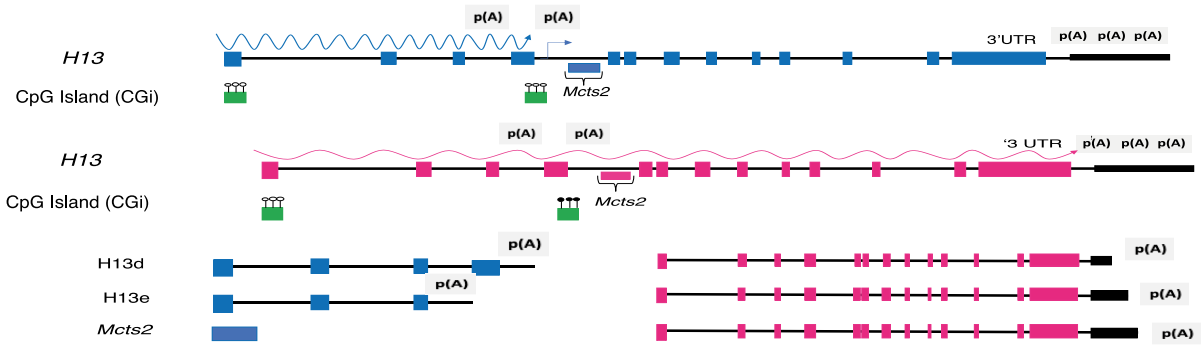

Figure 1: Mcts2/H13 locus in mouse (blue = paternal allele, pink = maternal allele)

The *Mcts2* retrogene is located within the intron of its host gene, *H13*. What is remarkable about this locus is how allelic expression of *Mcts2* influences the choice of polyadenylation sites utilised at *H13*. On the maternal allele, where *Mcts2* is silenced, *H13* uses canonical 3' polyadenylation sites and generates full-length isoforms. On the paternal allele, *Mcts2* is expressed, and this results in alternative polyadenylation site usage and short *H13* isoforms. We are interested in understanding the mechanisms generating this transcript diversity and the contexts in which they have biological relevance.

This project investigates the function of *Mcts2* and asks whether disruptions at this locus impact development since there are suggestions that defects in the chromosomal region of *Mcts2/H13* are associated with growth failure. My project aims to investigate the functional consequence of *Mcts2/H13* imprinted locus in a disease setting by:

1) Utilising an *Mcts2* knock-out mouse model
2) Examining a cohort of patients with variants at the homologous locus from Genomics England and other databases

This provides a comparative approach to understanding the genotype-phenotype relationships at this locus through mechanism and function.

Keywords: *Alternative polyadenylation, development, imprinting*

P-02
IMPROVE-RRBS tool Ignores MsPI site and sequencing Read 3' end OVErlap in RRBS methylation calling

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Reduced Representation Bisulfite Sequencing (RRBS) is frequently used to determine DNA methylation at the single nucleotide level in CpG-rich regions of the genome. During RRBS library preparation a non-methylated cytosine is added to the 3' end of MspI digested DNA fragments. Trim Galore cuts these end-repaired cytosines during computational analysis. However, Trim Galore fails to detect end-repair when it overlaps with the 3' end of the sequencing reads. These non-trimmed cytosines bias methylation calling, thus can be identified erroneously as differentially methylated sites. To circumvent this problem, we developed IMPROVE-RRBS, which efficiently identifies and hides these cytosines from methylation calling. We found biased cytosines in all datasets analyzed. We suggest therefore the implementation of IMPROVE-RRBS in RRBS pipelines (https://github.com/fotabel/IMPROVE-RRBS).

P-03
Fueling aberrant cell populations – Metabolic dysregulation drives the progression of pulmonary fibrosis

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Idiopathic pulmonary fibrosis (IPF) constitutes a major cause of morbidity and mortality worldwideand thus represents an enormous unmet medical need. Although the definitive causes of IPF remain unclear, it has become evident that aging is a major risk factor in the initiation and progression of IPF. Notably, maintaining metabolic homeostasis has been proposed to restore physiological conditions in multiple age-related diseases. This raises the plausible question of whether metabolic reprogramming affects IPF pathology. Using a lung epithelial-mesenchymal co-culture system, we identified that metabolic dysfunction alters intercellular communication; creating an environment vulnerable to injury that leads to fibrosis. Multiome analysis further revealed increases in the glycosaminoglycan biosynthesis pathway in the injury model. Perturbating this pathway, by inhibiting regulatory enzymes with pharmacological tools, attenuated the profibrotic response. These findings highlight the biological and therapeutic importance of metabolic reprogramming in determining the regenerative capacity and aberrant repair in IPF.

Keywords: *Metabolism, Glucosaminoglycan Biosynthesis, Idiopathic pulmonary fibrosis*

P-04
Dynamics and locus organization of enhancers regulating key factors in early zebrafish development

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Numerous long-range enhancers, which often overlap with highly Conserved Non-coding Elements (HCNEs) within genomic regulatory blocks (GRBs), control the expression of key genes regulating development. GRBs often contain other "bystander" genes that do not respond to those enhancers. The extent of GRBs coincides with those of topologically associating domains (TADs) around developmental genes. However, the exact influence of GRB spatial organization on gene regulation in the early stages of development is still poorly understood, which substantially limits our understanding of processes leading to differentiation and cell fate decision. To answer those questions, we exploited early zebrafish developmental datasets to characterize chromatin opening and interaction topology in those poorly understood loci and their regulatory role in TADs. In GRB TADs, characterized by a high density of extreme non-coding conservation, we found more promoter-proximal enhancers in early and distal enhancers in late developmental stages. We observed that enhancers in the late stages are numerous, short, and distributed throughout the entire TAD length. In contrast, fewer enhancers were active at the early stage, and they often occurred in clusters with uninterrupted H3K27ac signal connecting them, a histone mark found on active chromatin. We called those regions H3K27ac ensembles. We hypothesized that they might be associated with the lack of fully formed TADs in the early stages when enhancers are proximal to active promoters. We also investigated the relationship between the chromatin interactions and activity of H3K27ac-ensemble-associated genes during early vs late embryogenesis. We observed increased contacts within H3K27ac ensembles at an early stage which later spread throughout the entire TAD, arguing for their role in the timely opening of chromatin in their host TADs. We show that H3K27ac ensembles participate in the activation of early-acting developmental genes, including those later dependent on long-range regulation. Finally, using a novel projection method and comparison with mouse, we show that this phenomenon is conserved in mammalian development.

P-05
The association between cord blood DNA methylation of serotonin-regulating genes and body fat-related characteristics in newborns

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The signalling monoamine serotonin regulates many aspects of energy homeostasis including lipid metabolism (1). In the present study we investigated a possible association between cord blood DNA methylation in regulatory regions of serotonin-related genes (*SERT*, *MAOA*, *HTR2A*) and neonatal parameters related to lipid metabolism (ponderal index, fat mass estimate, serum triglyceride levels). The study included 100 full-term newborns (35 female, 65 male), all delivered by planned Cesarean section. Neonatal fat mass was estimated from neonatal length, weight, and abdominal skinfold thickness, using a standard formula (2). In venous cord blood, serum triglyceride levels were measured with Alinity (Abbott) enzyme-based assay and DNA methylation in circulating cells was quantified by bisulfite pyrosequencing. Partial correlation determined the relationship between methylation and metabolic parameters, whilst controlling for newborn's sex and gestational age. *SERT* methylation was negatively correlated with ponderal index ($r=-0.21$, $p=0.040$) and fat mass ($r=-0.22$, $p=0.033$), and positively correlated with triglyceride level ($r=0.38$, $p<0.0001$). *HTR2A* and *MAOA* methylation did not correlate with any of the metabolic parameters analysed. These results support a role of *SERT* gene, encoding the serotonin transporter, in contributing to regulation of energy homeostasis during human fetal development and suggest its epigenetic modifications as a potential biomarker of later-life disorders associated with neonatal metabolic parameters.

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P-06
Conditions of drug induced enzymatic DNA cytosine deamination and genomic uracil profiles

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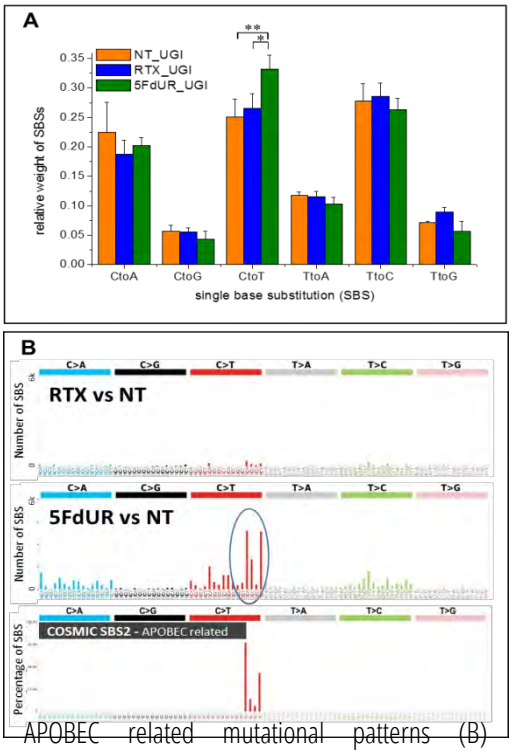
Widely used chemotherapy strategies relies on perturbation of cellular nucleotide pools, particularly the dUTP/dTTP ratio that causes cell cycle arrest and eventually cell death via massive thymine-replacing uracil incorporation and hyper activation of a futile uracil-DNA repair [1]. Recently, it has also been shown that several of these drugs can induce APOBEC3 DNA cytosine deaminases via DNA-damage response [2]. Cytosine deamination events within the DNA result in mismatches and stable C to T transition mutations after the first replication cycle, unless repaired by uracil-DNA glycosylase initiated base excision or mismatch repair pathways. Hence drug induced APOBEC3s might trigger mutagenesis leading to further cancer progression and/or emergence of drug resistance.



APOBEC related mutational patterns (B) selectively increases in mismatch repair and uracil-DNA repair deficient cells upon 5FdUR treatment. RTX treatment causes no changes as compared to the non-treated (NT) cells.

Recently, we have characterized the genomic U-DNA pattern in drug treated HCT116 derived cell lines with altered DNA repair capacities [3] using our previously developed uracil-DNA sensors [4]. Here, we present our new results focusing on differences between the treatments with two thymidylate synthase inhibitors, raltitrexed (RTX), and 5-fluoro-2'-deoxyuridine (5FdUR). Drug-specific differences between the genomic uracil patterns were observed that are also reflected at the level of genes. We also address correlated transcriptional changes induced by the two drugs. Interestingly, in case of 5FdUR treated U-DNA repair deficient cells, we could detect increased frequency of C to T transitions with mutational spectra characteristic for cytidine deaminases (Figure). In accordance, we could demonstrate induced APOBEC3B expression at both protein and mRNA levels in these drug treated cells. These drug specific differences in the genomic uracilation and mutational frequencies, as well as in gene expression profiles are accompanied with different phenotypes reflected in cytotoxicity and cell cycle arrest. Exploring the conditions of these drug specific differences especially the mutagenic processes, might contribute to personalized cancer treatments in long-term.

Keywords: *raltitrexed, 5-fluoro-2'-deoxyuridine, uracil-DNA, APOBECs, HCT116 cell line*

References' PMIDs: [1] 24732946, [2] 33323971, [3] 32956035, [4] 26429970

P-07
The roles of the transcriptional repressor BACH1 in regulating the differentiation of macrophages

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The inflammatory response of macrophages (Ms) is controlled at transcriptional level to provide the appropriate induction, magnitude, and resolution. Transcriptional activation has well-established roles in shaping M gene expression, but the roles of transcriptional repressors such as the heme-sensor BACH1 is less known in the changing tissue microenvironment. Our work on sterile regenerative inflammation identified BACH1 as a regulator of M differentiation, subtype specification, and inflammatory gene expression. Importantly, our data support the notion that BACH1 acts in an extensive and much broader capacity genome-wide than previously suspected and acts as a contextual regulator of both inflammatory and anti-inflammatory gene expression programs in a heme-dependent and heme-independent manner. Thus, we aimed to investigate the contribution of macrophage BACH1 in active repression and activation of genes. More importantly, the contribution of BACH1 was also measured in M-subtype specification, heterogeneity, and inflammatory responses in vivo. We employed single cell-RNA-seq approaches to simultaneously detect mRNA and chromatin accessibility from the same cell complemented by assays for cellular interactions, thus linking the effect of BACH1 regulation to gene expression and cellular phenotype during the inflammatory response. Our observations suggest that BACH1 is part of the core hardwired transcriptional circuit of Ms as a signal-dependent master regulator, but unlike linage determining developmental regulators it most likely acts as a signal-specific master regulator and integrator.

Keywords: *macrophage, BACH1, repression, differentiation*

P-08
Characterisation of molecular mechanisms of healthy spermatogenesis in humans

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Infertility affects 10-15% of couples worldwide, approximately half of which are cases of male factor infertility. However, while female infertility is well-studied, only about 30% of male infertility cases have a known cause. Understanding the process of spermatogenesis in fertile and infertile men will make it possible to provide diagnoses for a higher number of infertile couples, and open the way for future treatments for infertility and development of male contraceptives.

In order to better understand human spermatogenesis, we have used testis biopsies from men with normal sperm production to produce single-nucleus multiome data consisting of gene expression and chromatin accessibility measurements from the same cell. These datasets include more than 19,000 high quality nuclei from three biological replicates, representing all of the expected germline stages as well as supporting somatic cells. We compare our data to published single-cell studies on human testicular cells to systematically assess the contribution of single-nucleus RNA vs single-cell RNA in accurately capturing the heterogeneity of the tissue. Furthermore, we utilise our data to characterise the chromatin state and gene expression of each of these cell types and their dynamics across differentiation. This will allow us to investigate the molecular mechanisms of spermatogonial stem cell renewal, commitment to differentiation, passage through meiosis, and development of haploid spermatids.

P-09
Locus-specific genomic rearrangement of the transcriptional repressor BACH1 upon heme treatment supports transcription

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Upon severe cellular damage, the level of intra- and extracellular content and degradation products increases. Under these conditions, extracellular heme acts as a critical alarmin molecule and can activate innate immune cells and influence macrophage polarization. BACH1 (BTB and CNC homology 1) is believed to act primarily as a heme-regulated rheostat controlling heme metabolism, and it is known that heme is able to inhibit the DNA-binding capacity of BACH1 and promotes its nuclear export and degradation. However, the effect of heme in shaping the BACH1 cistrome and how it modulates the epigenomic environment is not fully understood.

We treated naïve mouse bone marrow-derived macrophages (BMDMs) with 100 μM heme for 1 hour and performed Chromatin Immunoprecipitation coupled with sequencing (ChIP-seq) experiments using an antibody for BACH1. Contrary to expectations, we found that besides the ~12,900 TF binding sites whose binding was significantly reduced or completely abolished ($P \leq 0.05$, $FC \geq 1.5$) upon heme treatment, 13,300 new BACH1 binding sites got occupied by this protein. We have identified clear differences between the enriched DNA sequence motifs: expectedly, the repressed peaks are enriched in BACH1-specific sequences (MARE and TRE), while the induced regions do not show the presence of these motifs, rather EGR, ZNF263, NFκB, and KLF motif enrichments.

We also found that upon heme exposure, BACH1 is able to redistribute from enhancers to promoters (within a ±250-kb frame relative to the primary BACH1 peaks), thus potentially activate them and change the chromatin architecture during its activation. These findings further suggest that nuclear BACH1 is not uniformly and immediately exported to the cytoplasm to be degraded but rather is still stable and part of nuclear chromatin complexes that bind to target promoters. Current efforts are focusing on using capture Hi-C (in order to map physical contacts between selected chromatin regions using paired-end sequencing) to provide evidence that the dynamic redistribution of BACH1 upon heme stimulation results in changes in the 3D-chromatin architecture by restricting the proximity of functional enhancer-promoter pairs that are increased upon heme exposure.

P-10
Unravelling the „inflammatory-chromatin“ epigenetic code using *Drosophila* genetics

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Cells live in constantly changing environments and need at any time to be able to adapt. Depending on the stimulus, a fast and specific response must be engaged to preserve not only the cellular, but also the organism's integrity. In general, this response is governed by the expression of inducible genes, leading to the activation of signalling cascades. Inducible genes, such as immune response genes need to be i) shut down in normal conditions, ii) activated upon stimulation and iii) rapidly inactivated after the stimulus ends. This implies highly dynamic changes at the molecular level, which involve epigenetic mechanisms.

Our team use the fruit fly *Drosophila melanogaster* as a model to study the molecular regulation of NF-κB innate immune response pathways. The discovery of the co-activator Akirin brought new insights in the regulation of these pathways (Goto *et al*., 2008). We showed that Akirin is implicated in the transcriptional activation of a subset of NF-κB target genes in both *Drosophila* and mammals through the recruitment of the chromatin remodeling complex SWI/SNF (Tartey *et al*., 2014). Also, these genes are characterized by acetylated histone 3 lysine 4 (H3K4ac) (Bonnay *et al*., 2014). These findings on a Akirin-mediated dichotomy within the NF-κB pathway open new perspectives to study the epigenetic control of the immune response. To describe the dynamic of these epigenetic events, we combine genetic approaches and next-generation sequencing techniques such as CUT&RUN, MNase-seq and RNAseq.

P-11
Time dependent expression of mmu-miR-195 in mouse heart

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miR-195 is a conserved small non-coding RNA, member of the miR-15 precursor family, known as a major regulator of cell cycle/proliferation and cell death/apoptosis, and shows a tissue-specific expression in ante-natal and post-natal mammals. miR-195 has been associated with developmental disorders as well as with various diseases such as cancer, heart failure, chronic obstructive pulmonary disease, Parkinson's disease.

Here, we used quantitative real-time PCR to evaluate the expression of the mature and precursor form of mmu-miR-195 in the heart of male and female, wild-type mice at different stages of pre-natal and post-natal life. Our analysis revealed discordant changes of the mature and the precursor (pri-miRNA) forms, suggestive for the existence of a post-transcriptional, time dependent mechanism for mmu-miR-195 homeostasis.

Next, we used a combined, machine learning algorithm for mmu-miR-195 target prediction, and a clustering and topological community detection (CTCD) approach followed by STRING gene ontology analysis to evaluate the impact of mmu-miR-195 changes on heart transcriptome. We show that mmu-miR-195 regulates multiple signaling pathways associated with cardiac maturation, sphingolipid metabolism and ubiquitin-mediated proteolysis, and provide data validating our predictions at both mRNA and protein level.

Keywords: *microRNA, heart, gene network*

P-12
Epigenomic changes upon expression of the transposase-derived SETMAR histone methylase in the human genome

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DNA transposons provide a recurrent supply of genetic mutation to give rise to novel gene functions throughout evolution. The transposase SETMAR is a naturally occurring fusion protein that consists of a histone-lysine methyltransferase domain and an HsMar1 transposase. To elucidate the biological role of SETMAR, it is crucial to identify genomic targets to which SETMAR specifically binds and link these sites to the regulation of gene expression. Herein, we mapped the genomic landscape of SETMAR binding in a near-haploid human leukemia cell line (HAP1) in order to identify on-target and off-target binding sites at high resolution and to elucidate their role in terms of gene expression. Our analysis revealed a perfect correlation between SETMAR and inverted terminal repeats (ITRs) of HsMar1 transposon remnants, which are considered as natural target sites for SETMAR binding. However, we did not detect any untargeted events at non-ITR sequences, calling into question previously proposed off-target binding sites. We identified sequence fidelity of the ITR motif as a key factor for determining the binding affinity of SETMAR for chromosomes, as higher conservation of ITR sequences resulted in increased affinity for chromatin and stronger repression of SETMAR-bound gene loci. Importantly, small RNA sequencing of SETMAR-overexressing and deletion cell lines revealed that SETMAR regulates the activity of non-coding RNAs and microRNAs involved in cell cycle control and cancer pathways. These associations highlight how SETMAR's chromatin binding fine-tune gene regulatory networks in human tumour cells.

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P-13
Examination of 3D genome conformation related to R-loops

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Recent research have shown the significance of R-loops in the regulation of gene expression, DNA recombination and repair, and chromosome segregation, however their function in 3D chromatin conformation remains unknown.

In this study we constructed budding yeast cells with modified endogenous R-loop levels and analyzed 3D genome conformation and R-loop formation in parallel by DRIP-sequencing and chromatin conformation capture (Hi-C) technologies. Spatial interactions and R-loop profiles of cells lacking RNaseH1 and RNaseH2 enzymes were compared with mutants overexpressing RNaseH1, demonstrating quantitative differences in the relative contribution of R-loops to the formation of long-range chromosome interactions. The identified R-loop-related long-range chromatin interactions, if confirmed in human cells, are expected to shed more light on the epigenetic basis of human diseases such as Angelman syndrome, Prader-Willi syndrome, or cancer.

Keywords: *R-loops, Hi-C, Yeast, RNaseH*

P-14
Paclitaxel treatment induces BRG1-dependent chromatin remodelling at the promoters of lysosome-localized ABC transporters and confers multidrug resistance

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Paclitaxel (PTX) is an essential first-line drug for treating breast cancer, advanced non-small cell lung cancer and ovarian cancer. However, the development of resistance to paclitaxel limits the use of this drug. Mechanisms of PTX resistance include overexpression of ABC transporters, decreased drug uptake, increased drug metabolism, changes in tubulin composition as well as, according to recent studies, increased lysosome activity. To date, the influence of the lysosomal ABCB1 transporter on the occurrence of chemotherapy resistance has been demonstrated. The ATP-dependent chromatin remodelling complexes are an important part of the epigenetic mechanism of transcriptional regulation. One complex described to date is SWI/SNF containing two ATPase catalytic subunits, BRM and BRG1, which are involved in the transcriptional activation and repression of specific genes by altering DNA-histone contacts within the nucleosome. Bearing in mind that ABCB1 and ABCC2 expression in breast cancer cells is associated with BRG1 activity, we aimed to determine whether BRG1 is responsible for the increased expression of lysosomally localized ABC transporters contributing to PTX resistance. Our results show that PTX exposure increases the expression of ABC transporters, thereby increasing resistance to drugs of various chemical structures and biological activity. ATPase BRG1 interacts with promoter sequences of genes for ABC proteins. The silencing of BRG1 expression as well as pharmacologically inhibition of the enzyme decreases lysosome-associated ABC transporters and reduces drug accumulation in lysosomes. The results suggest that BRG1 inhibitors may have potential applications in overcoming resistance acquired after paclitaxel therapy by reducing drug accumulation in lysosomes.

Keywords:*Multidrug resistance, ABC transporters, lysosomal trapping*

P-15
Metformin-induced changes in transcriptomic profile of MCF-7 hormone receptor positive breast carcinoma cell line

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Metformin is a widely used antidiabetic drug to treat type 2 diabetes mellitus. Metformin affects the metabolism through inhibition of the mitochondrial electron transport chain, but it also affects cell survival and autophagy-induced apoptosis by blocking the mTOR pathway. As it is known that the mTOR pathway is overstimulated in diabetes mellitus and blocking this intracellular network allows treating diabetes. Furthermore, proliferation, migration and invasion of tumour cells are also mediated by inhibition of the mTOR pathway. In our experiments, we aimed to verify whether this anti-tumour effect is also exerted on oestrogen-and progesterone-positive MCF-7 cell line and to elucidate the transcriptomic changes by RNA sequencing. Therefore, MCF-7 cells were pretreated with 3 different concentrations of metformin for 24 h and further cultured after washing with PBS. Based on our results 3 major classes of genes and pathways are altered by metformin. The first one is directly linked to gene expression control, the second one consists of genes that alter the cell cycle while the third group is related to various stress responses. Our results are in line with similar studies that verified the anti-tumour effect of metformin and we believe metformin can be a potential candidate for preventing certain types of cancer. In the future, we would like to examine the effect of selective mTOR inhibitor and metformin treatment in combination on MCF-7 cells to clarify our understanding of the anti-tumour effect of metformin.

Keywords: *metformin, RNA-seq, human breast carcinoma*

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P-16

Uncovering the complexity of zebrafish (*Danio rerio*) snoRNAome

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Small nucleolar RNAs (snoRNAs) are one of the most abundant and evolutionary ancient group of functional non-coding RNAs. They were originally described as guides of posttranscriptional rRNA modifications, but snoRNAs fulfill an impressive variety of cellular functions. These range from guiding site-specific chemical modifications in several RNA classes and affecting the nucleolytic processing of ribosomal RNA (rRNA) to their involvement in the regulation of chromatin architecture and alternative splicing. Previous analysis suggests that snoRNAs and the modifications they mediate are highly conserved across species.

Based on common sequence motifs and structural features snoRNAs are classified in two major families, box C/D and box H/ACA snoRNAs. The identification of snoRNAs based on homology search is often difficult due to the lack of overall sequence conservation, small size (60-300bp) and short sequence motifs. Here, we use sizefractionated RNA sequencing data from adult zebrafish tissues to define the snoRNAome for this species. Our approach allowed us to identify several hitherto unannotated snoRNAs in the zebrafish genome.

We created an analysis pipeline where the raw reads were aligned to the latest zebrafish genome assembly (GRCz11) using Bowtie2 aligner and we identified putative snoRNA sequences using the blockbuster algorithm. Using several snoRNA predictor methods we were able to confirm the presence of multiple previously predicted snoRNAs in the data and also identified ~70 new snoRNA-like sequences missing from the current Ensembl database (v99). Based on our preliminary survey our snoRNAome dataset represents the most reliable set of snoRNAs to date in this species.

To date, multiple mechanisms have been identified that explain the effect of snoRNAs on gene expression. Currently I used ~100 samples for my analysis. These and many additional datasets, will be available in the developing database of the zebrafish snoRNAome. Thereby, we can organize the zebrafish snoRNAs into a searchable database, where data will be available individually.

Finally, our update of the zebrafish snoRNAome has identified the dynamic expression of some snoRNAs during the early stages of zebrafish development and tissuespecific expression patterns for others in adults. We also present our results that show distinct characteristics of snoRNA abundance profiles during zebrafish development, suggesting an underappreciated dimension of gene regulation.

P-17
The role of methylation in the formation of pathological R-loops

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R-loops are nucleic acid structures formed during transcription, which can cause DNA damage and are more abundant in diseases such as cancer. It has also been reported that R-loops are influenced by, and further influence, the surrounding epigenetic environment, although exact mechanisms remain unclear. 5-Azacytidine (Aza) and 5-aza-2'-deoxycytidine (Dac) are DNA methyltransferase inhibitors used as chemotherapeutics for acute myeloid leukaemia and are in clinical trial for several other cancers. Aza demethylates both DNA and RNA, whereas Dac only demethylates DNA, however they are often used interchangeably. By combining data from DRIPc-Seq, CHIP-Seq, Immunostaining, RNA-Seq and Mass spectrometry we show how these drugs affect R-loop formation and the impact on the surrounding epigenome. The resulting data demonstrates that Dac treatment led to a significant increase in R-loop formation, markers of genomic instability and an increase in H3K27me3. In contrast, Aza treatment resulted in decreased R-loop formation and markers of genomic instability and an increase in H3K9me3. This suggests there are mechanisms to prevent damaging R-loop formation in response to changes in RNA methylation but not DNA methylation. Collectively, these insights further our knowledge of how cells recognise and respond to methylation changes.

P-18
Altered cell responses induced by two thymidylate synthase inhibitory drugs

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Many chemotherapeutic drugs perturb DNA integrity and/or impair DNA associated mechanisms. Thymidylate synthase (TS) inhibitors perturb the conversion of dUMP into dTMP, resulting in increased cellular dUTP/dTTP ratio. Under such conditions, DNA polymerases can more frequently incorporate uracil into the DNA, and uracil-DNA repair pathways might turn into hyperactivate futile cycles, leading to cell cycle arrest and finally cell death. However, in case of inhibited uracil-DNA repair, the resulted high genomic uracil content might also influence essential DNA regulatory processes ending in similar cell fates. We have been studying the effects of two TS inhibitors widely used in cancer treatments: the base analogue 5-fluoro-2'-deoxyuridine (5FdUR) that forms stable complex with TS and methyl-tetrahydrofolate (1), and the TS selective antifolate, raltitrexed (RTX) (2). We had already seen in our U-DNA-Seq data that in HCT116 cells with decreased uracil-DNA repair capacity, these drugs lead to somewhat different genomic uracil patterns despite inhibiting the same target enzyme (3). We have also detected elevated frequency of genomic C to T transitions selectively in 5FdUR treated, repair deficient cells (unpublished). To get deeper insight into the phenotypic effects of the two TS inhibitors, here, we analyzed the cell cycle phases and the cell viability in a time- and dose-dependent way (Figure). We found these cells are arrested in S-phase already within the first 24 h of treatments by applying as low dose as 0.1 μM in both cases. Interestingly, in case of 5FdUR, but not RTX, the arrest is somewhat weaker at high-dose (20 μM) than at low-dose (0.1 μM). In accordance, we measured somewhat reduced cytotoxicity of high-dose than low-dose 5FdUR treatment. In RTX treated cells, this phenomenon was not detected at all. We are looking for possible reasons of this strange dose-dependency of the 5FdUR effect. Here we present RNA-seq results and drug specific alterations in gene expression levels. We found that p53 related genes are more enriched among the significantly overexpressed mRNAs upon 5FdUR treatment than in RTX treated cells. This might be connected to the known mRNA regulatory function of TS targeting p53 mRNA (4), which might be differently affected by the binding of the two drugs (5). Dissecting the molecular determinants of this

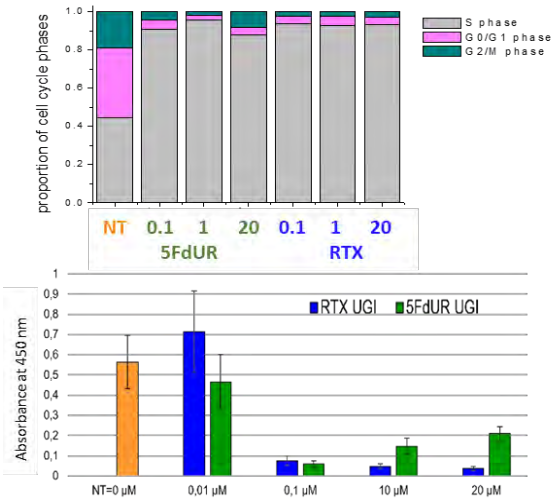

Figure: The effects of RTX (blue) and 5FdUR (green) on cell cycle (top panel) and cell viability (bottom panel) depending on the drug concentration.

behavior in long-term might shed light on processes related to drug resistance frequently occurring during TS inhibitory anticancer treatments.

Keywords: *thymidylate synthase inhibitors, uracil-DNA, cell viability, cell cycle arrest, p53*

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P-19
Single-nucleus multiomic analysis of gene regulation in human spermatogenesis

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Embryonic development involves the fusion of highly specialised gametes into a totipotent zygote that gives rise to a whole organism. The production of gametes must therefore ensure that they can carry out their specialised roles while retaining the ability to be completely reprogrammed. During spermatogenesis the cell undergoes dramatic reorganisation, including replacement of histones by protamines, loss of most cytoplasm and reorganisation of mitochondria and the cytoskeleton. Errors in this process can lead to infertility, which affects 10-15% of couples worldwide. The exact cause is unknown in ~70% of male infertility cases.

Hence, to understand the gene regulatory networks that control human spermatogenesis, we have carried out joint analysis of gene expression and chromatin accessibility at the single nucleus level using testis biopsies that contain cells from every stage of spermatogenesis. We obtained more than 19,000 high quality nuclei from three biological replicates, representing all of the expected germline stages as well as supporting somatic cell types. Our multiomic approach allows us to understand the relationship between gene regulation and expression at the single cell level. Using this data, we have identified key transcription factors for each cell type, as well as their putative binding sites and target genes, revealing potential novel candidate infertility genes.

P-20
ARID1A mediates the anti-proliferative effects of bexarotene and carvedilol in normal and transformed breast cells

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Selective estrogen receptor modulators and aromatase inhibitors showed effectiveness in treating ER-positive breast tumors. These agents are not effective in ER-negative cells independent manner. The rexinoid, bexarotene (Bex), in combination with the non-selective beta blocker, carvedilol (Carv), showed anti-proliferative effects in normal and transformed breast cells. ARID1A protein levels were upregulated upon the combination treatment in HME-hTert normal cells, but not in MCF-7 cancer cells. We studied ARID1A genomic occupancy upon Bex+Carv treatment in both cell types. In normal cells ARID1A was found to be enriched to regulatory elements assigned to genes involved in the TGF-b signaling pathway, including FOXQ1, BMP6, KLF4, and TGFBR2 associated with a change in their expression. Epithelial to mesenchymal transition marker expression, including fibronectin and N-cadherin, were downregulated upon Bex+Carv treatment. However, E-cadherin, the epithelial marker, was induced on transcript and protein expression levels upon the combination treatment. The results show that Bex+Carv treatment prevents the transformation of normal cells through suppressing the expression of mesenchymal markers and promoting epithelial cell characteristics. In MCF-7 breast cancer cells, ARID1A was enriched to regulatory elements related to the IGF-1R and IRS1 genes upon the combination treatment of Bex+Carv, associated with a downregulation in their protein expression levels. ARID1A silencing increased IGF-1R protein expression and stimulated cell proliferation. The findings suggest that Bex+Carv treatment inhibits MCF-7 cell proliferation through suppressing IGF-1R and IRS1 through the actions of ARID1A. Overall, the study revealed two novel tumor suppressive mechanisms based on ARID1A activity in normal and transformed breast cells.

Keywords: *ARID1A, Breast cells, Bexarotene, Carvedilol, IGF-1R/IRS1, TGF-beta*

P-21
Genome-wide expression profiling in colorectal cancer focusing on lncRNAs in the adenoma-carcinoma transition

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Introduction & Aims: Long non-coding RNAs (lncRNAs) play a fundamental role in colorectal cancer (CRC) development. We aimed to study whole genomic lncRNA expression patterns in colorectal adenoma–carcinoma transition.

Methods: LncRNA expression of 20 CRCs, 20 adenomas (Ad), 20 healthy (N) biopsies were analyzed with Human Transcriptome Array (HTA) 2.0. MiRNA targets of lncRNAs were predicted and their expression was analyzed on miRNA3.0 Array data. MiRNA-mRNA target prediction was performed using miRWALK and c-Met protein levels were analyzed by immunohistochemistry. Comprehensive lncRNA-mRNA-miRNA co-expression pattern analysis was also performed.

Results&Discussion: CRC-associated lncRNAs showed remarkable expression changes in precancerous lesions. In Ad vs. N and CRC vs. N comparisons 16 lncRNAs, including downregulated LINC02023, MEG8, AC092834.1, and upregulated CCAT1, CASC19 were identified showing differential expression during early carcinogenesis that persisted until CRC formation (FDR-adjusted $p < 0.05$). The intersection of CRC vs. N and CRC vs. Ad comparisons defines lncRNAs characteristic of malignancy in colonic tumors, where significant downregulation of LINC01752 and overexpression of UCA1 and PCAT1 were found. Two candidates with the greatest increase in expression in the adenoma-carcinoma transition were further confirmed by qRT-PCR and by ISH. In line with aberrant expression of certain lncRNAs in tumors, the expression of miRNA and mRNA targets showed systematic alterations. For example, UCA1 upregulation in CRC samples occurred in parallel with hsa-miR-1 downregulation, accompanied by c-Met target mRNA overexpression ($p < 0.05$). In conclusion, the defined lncRNA sets may have a regulatory role in the colorectal adenoma-carcinoma transition.

P-22
Exploring the impact of clonal mosaicism in the blood system on cardiac inflammation

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Massive DNA sequencing profiles have enabled to detect somatic mosaic mutations in the blood system, demonstrating such clonal hematopoiesis of indeterminate potential (CHIP) deteriorates with aging, and increases the risk of progression to myeloid malignancies, as well as for cardiovascular disease (CVD). However, it remains unclear the impact of CHIP mutations on transcriptional readouts associated with hyperinflammatory responses. To understand how CHIP mutations disrupt the cardiac transcriptional landscape, here we explore the link between cardiac gene expression profiles with somatic mutations using whole transcriptome sequencing of 472 heart tissues and matched blood samples. A robust pipeline of detecting expressed somatic mutations allowed us to recapitulate an age-related increase in mutational burden, and in incidence rate of CHIP mutations. We observed such relationships were particularly augmented in donors who have CVD. Notably, immunosenescent-like phenotypes were evident in cardiac gene expression profiles of donors with CHIP mutations. Expanding to single-cell RNA-seq data from 14 adult heart tissues, we identified cell-type specific changes of cardiac cell proportion with dysfunctional immune gene expression in an age-dependent manner. Altogether, our results provide insights into the impact of CHIP mutations in cardiac transcriptional architecture.

P-23
Role of epigenetic regulator RYBP in cardiac lineage commitment

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The mammalian heart is composed of many cell types including cardiomyocytes, endothelial cells, and neurons. Our laboratory focuses on the early onset of cardiac development where the cell fate determination occurs. We have previously showed that in the lack of the Polycomb Group (PcG) member RYBP (RING1 and YY1 binding protein) mouse cardiomyocytes (CMCs) are not able to contract. RYBP, as core member of the non-canonical Polycomb complex 1 (ncPRC1) was also shown to deposit epigenetic modification at promoter region of lineage specific gene expression therefore playing important role in suppression or activation of alternative lineages. To further dissect the role of RYBP in cardiac lineage commitment we **(i)** characterised the expression of key cardiac and endothelial genes in the *wt* and *Rybp*$^{-/-}$ cardiac cultures, **(ii)** compared the co-occupancy of RYBP and its Polycomb partner RING1B at the promoter region of key cardiac and endothelial genes **(iii)** we analysed the active and repressive marks in stem and cardiac mesoderm stages and finally **(iv)** we investigated the proportion of CMCs and non-cardiomyogenic cell types in the *wt* v.s. *Rybp*$^{-/-}$ cardiac cultures. Our results indicated that RYBP is important for cardiac, endothelial, and neural cell development, but not essential for smooth muscle cell formation. We showed PcG dependent repression of cardiac progenitor genes in stem cells stage and their activation at cardiac mesoderm stages highlighting the versatile epigenetic regulatory functions of ncPRC1s.

Keywords: *stem cells, RYBP, cardiac differentiation, Polycomb complexes, lineage commitment*

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P-24
Deciphering the functions of the lateral modifications during early developmental process

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The post-translational modifications (PTM) on histones, such as acetylation, phosphorylation or methylation as well as chromatin remodeling are two main pathways for cells to alter chromatin structure and to control gene expression. Histone PTMs can be deposited on both protruding tails and globular domains. This project is addressing the PTMs on histones, especially acetylation on the lateral surface of histone octamers such as H3 lysine (K) 56, K64, and K122. We want to investigate the combinatorial action of those acetylations and the mechanistic effect of these PTMs on nucleosome stability as well as their role in controlling gene expression. To illustrate this, we use the combination of in vitro and *in vivo* assays. To investigate the role of lateral modifications in the developmental process, we utilize mESC lines in which those three lysines on histone H3.3 were substituted with glutamine (Q) or arginine (R) mimicking acetylated or unacetylated state, respectively. To interrogate the dynamics of lateral modifications, we perform CUT&RUN time-course experiments. Together those will provide us a novel insight into the function of individual and combinations of PTMs within histone core.

P-26
Analyzing the methylation pattern of cell-free DNA for monitoring the chemotherapy response of colorectal cancer patients

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The early detection of colorectal cancer (CRC) recurrence and the monitoring of therapeutic response are crucial steps in the determination of treatment strategies. Therefore, we aimed to establish a liquid biopsy-based approach for tracking tumor dynamics in post-operative CRC patients focusing on the DNA methylation of cfDNA.

Blood samples were collected from CRC patients during chemotherapy, and finally, they were classified according to disease outcome. Longitudinal investigations of the amount, global and local (*SFRP2* and *SDC2* genes) methylation of cfDNA were performed. The global cfDNA methylation was determined by bisulfite pyrosequencing of long interspersed nuclear element-1 (LINE-1). The concentration of homocysteine was also determined, as it is one of the main components of DNA methylation.

The average cfDNA amount was significantly higher ($p<0.05$) in patients with recurrent cancer (30.4±17.6ng) and progressive disease (PD) (44.3±34.5ng) than individuals who achieved remission (13.2±10.0ng). The average global cfDNA methylation was significantly lower ($p<0.05$) in the PD group compared to people with remission (71.0±6.7% vs. 78.9±2.0%). Methylation level changes between study beginning and end indicated a decline (75.5±3.4% vs. 68.2±8.4%) in PD; in contrast, we found a reverse alteration in remission. The mean relative change of homocysteine concentration revealed an opposite trend with the DNA methylation in all groups. Regarding local DNA methylation, *SFRP2* and *SDC2* revealed higher promoter hypermethylation in the PD set compared to patients with remission.

Our study offers the possibility to monitor the therapeutic response during chemotherapy combining the analysis of cfDNA, global and local DNA methylation pattern, and homocysteine level.

P-27
Chronic morphine treatment prevents the X-chromosome inactivation (XCI)

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X chromosome inactivation (XCI) is an important epigenetic process, where one of the X chromosomes in females is inactivated for dosage compensation. The XCI involves several steps, such as the initiation of chromosome-wide silencing by XIST, the formation of a repressive compartment through Polycomb complexes and the maintenance of the stable repression by DNA methylation and other epigenetic regulators such as SMCHD1. Although XCI is part of normal development, changes induced by environmental factors might affect embryo development and lead to health problems. As morphine can easily pass through the placental barrier and reach the embryo, our aim is to elucidate whether morphine causes epigenetic regulation on XCI process. H3K27me3 ChIP-Seq, WGBS-Seq and RNA-Seq approaches proved that chronic morphine treatment prevents the XCI process in mESCs maintaining the cell in an activate state. Although chronic morphine treatment does not modify *Xist* levels in mESCs, ChIP-sequencing and WGBS-sequencing analysis confirmed a decrease of H3K27me3 enrichment and DNA methylation at global level and on the overall coverage of the X chromosome. Furthermore, morphine modifies the end of the initiation phase and the whole maintenance phase, altering PRC2 complex subunits, *Dnmt1* and *Smchd1* gene expression through H3K27me3 enrichment and DNA methylation changes. In conclusion, morphine alters normal X chromosome wide silencing. Our results provide insights into the epigenetic mechanisms induced by morphine and establish the bases to understand how environmental factors can cause epigenetic changes that origin health problems or diseases, since a skewed XCI pattern is behind the higher incidence on different neurological diseases such as Alzheimer or Autism.

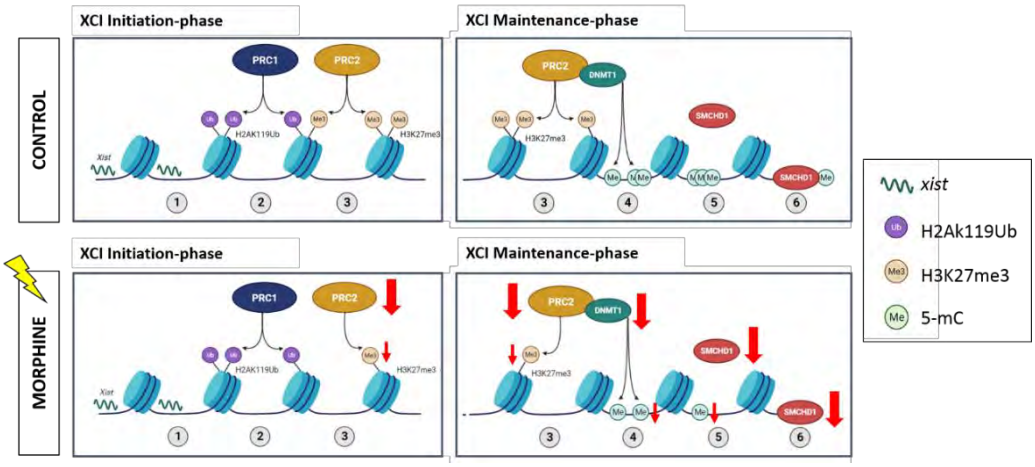

Figure 1. Morphine induced epigenetic changes on the XCI process.

Keywords: *X-chromosome inactivation, morphine, epigenetic changes, embryo development*

P-28
Integrated dataset of methylome data of colorectal normal, adenoma and adenocarcinoma tissues enables ranking of methylation changes in distinct gene-associated regions

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Background: As part of the nonmutational epigenetic reprogramming, genome-wide methylation changes contribute to the development of features associated with cancer. Colorectal cancer is a leading cause of cancer-related deaths and has been the focus of numerous studies focusing on methylation changes.

Aim: Our goal was to assemble an integrated database containing available methylation and clinical data and to rank methylation pattern changes present at the gene-region level.

Methods: The GEO (Gene Omnibus Database) and the GDC (Genomic Data Commons) repositories were mined to assemble raw Illumina HumanMethylation450 or HumanMethylationEPIC array data from 2,413 samples obtained from 17 studies. CpG and CNG sites were annotated to six gene regions. Alterations were compared using Kruskal-Wallis-test and ranked by the difference between normal and adenocarcinoma tissues.

Results: Genes BMP3, and NDRG4, the promoter methylation of which are established diagnostic biomarkers, showed hypermethylation 0-200 bases upstream of the transcription start site. In the promoter region, the genes with the most significantly increased methylation were *PMS2* ($p<0.0001$, $\Delta\beta=0.4$) and *RNF114* ($p<0.0001$, $\Delta\beta=0.38$). 73% of the promoters were already significantly hypermethylated during the normal-adenoma transition. The genes most affected in the gene body region were hypomethylated (e.g. *SNORA9* $p<0.0001$, $\Delta\beta=0.43$ and GATAD2B $p<0.0001$, $\Delta\beta=0.42$).

Conclusion: We established an integrated database of genome-wide methylation data derived from colorectal normal, adenoma, and adenocarcinoma samples. The cross-project analysis results can be the base of novel methylation-based colorectal cancer biomarker discoveries.

P-29
Dissecting the cistromes determining bone marrow-derived macrophages

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Mouse bone marrow-derived macrophages (BMDMs) are a widely used model to study gene expression regulation; therefore, a large number of various next-generation sequencing (NGS) results are available from them (such as bisulfite-, ATAC-, ChIP-, and GRO-seq). Using these NGS data, we aimed to study how the interaction of *cis*- and *trans*-regulatory elements contributes to transcription regulation in macrophages.

We collected 42 cistromes from BMDMs including those of bZIP, ETS, bHLH, and MEF2 family members, and our first goal was to identify the direct binding elements of the major transcription factors (TFs) participating in the steady-state gene regulation of BMDMs. Because of the similar DNA sequence preferences of members of a given TF (super)family, binding sites of related TFs were merged to a "super-cistrome", and this was divided into clusters of genomic sites with similar TF binding pattern to identify the direct binding elements.

Using the DNA sequences enriched in different clusters, it became clear that methylatable but non-methylated sequences and their specific binding TFs are the most potent transcription initiators in the lead with the promoter-specific ETS- and E-boxes. Based on ATAC-seq results, additional promoter-specific motifs (like SP1 and NFY) could be mapped, of which presence rather contributes to chromatin openness. Non-methylatable ETS- and E-boxes are also good "openers" and initiators relative to the bZIP and MEF2 binding sites. Our results provide insight into the initial step of gene regulation, and the exact knowledge of promoter building blocks may help one understand how the expression of individual genes is regulated.

Keywords: *macrophage, gene regulation, cistromes*

P-30
Folic acid combined chemotherapy has an immediate effect on the C1-methionin cycle and consequential DNA methylation in liquid biopsy samples of colorectal cancer patients

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The function of the single-carbon metabolic cycle is maintaining nucleotide pool and DNA methylation which is highly influenced by chemotherapy. Study aimed to evaluate the ultrashort effect of reduced folate combined anticancer treatment on peripheral blood parameters of CRC patients.

Post-operative CRC patients were treated with chemotherapy (oxaliplatin, 5-FU, leucovorin and capecitabine). Blood samples were taken until the beginning of treatment and after therapy. Plasma fractions were separated and levels of SAM, SAH and nucleotides (A,C,T,G,U) were detected by HPLC-MS/MS. Homocysteine was determined from plasma. DNA was isolated from plasma and PBMC cells and DNA methylation was analyzed by LINE-1 bisulfite pyrosequencing.

HCY level of plasma was decreased after oxaliplatin+capecitabine and with 5-FU+leucovorin. CfDNA level was decreased significantly as well as SAM level after XELOX and FOLFOX adjuvant therapy in plasma after treatment. Mostly elevated SAM/SAH ratios were observed in parallel with reduced SAH level after therapy. Significant mean LINE-1 hypermethylation was detected in cfDNA of plasma (81.84%) compared to PBMC (73.76%), furthermore, slightly DNA hypermethylation was noticed in CpG1 position of LINE-1 after chemotherapy in both mononuclear cells and cfDNA. Lower level of plasma nucleotides (A,C,U) was identified after treatment.

Chemotherapy results in a decrease in cfDNA immediately after treatment. The amount of the methionine cycle was reduced, and only a moderately increased DNA methylation could be detected even in such a short period of time. Co-administration of high-dose leucovorin as a folate derivative and a methyl donor with further chemotherapeutic agents may actively contribute to genome alterations.

P-31
SMCHD1 is a key regulator of epigenetic cellular memory induced by morphine

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Epigenetic cellular memory induced by morphine, implies the existence of mitotically heritable changes, as only those epigenetic changes that persist in a stable manner in differentiated cells might produce phenotypic changes. To evaluate if morphine can induce cellular epigenetic memory, we analyzed the dynamic transcriptional changes over the time by RNA-Seq, in the absence of morphine. RNA-seq has shown that morphine chronic treatment produces a transcriptional deregulation of 932 genes in P1 just after the treatment and this amount increased after treatment withdrawal (1196 genes, P2 and 2138 in P3), proving that mESCs can memorize morphine exposure and induces changes that can persist over the time, even in the absence of morphine. Integrative analyses identified *Smchd1* gene as a potential regulator of epigenetic cellular memory induced by morphine. SMCHD1 is known to be an important epigenetic repressor, inducing chromatin silencing throughout the genome. Morphine reduced the gene expression of *Smchd1* in mESCs and hiPSCs, which is consistent with an increase of H3K27me3 and DNA methylation at promoter. Crisper-Cas9 silencing analyses confirmed that *Smchd1* is a morphine-sensitive target gene. This cellular epigenetic memory was also observed upon embryo development, as morphine-induced *Smchd1* decrease was maintained during the mEpiLCs *in-vitro* differentiation and blastocyst development, after morphine withdrawal. In conclusion, SMCHD1 is a key regulator of epigenetic cellular memory induced by morphine, whose deregulation can be memorized by the cell, providing an epigenetic mechanism that is maintained into cell-to-cell memory and might cause diseases or health problems during the adulthood.

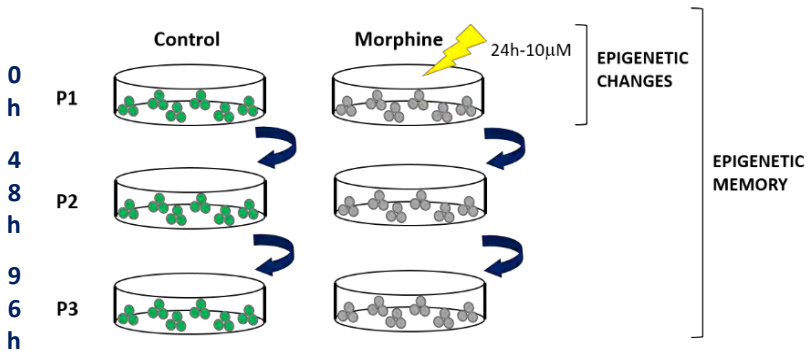

Figure 1. Scheme of mESC culture for in-vitro epigenetic memory determination. Three time points were analyzed: P1, cells collected just after 24h morphine treatment, P2 cells collected 48h after the treatment termination and P3, cells collected 96h after the treatment end.

Keywords: *morphine, epigenetic changes, embryo development, epigenetic cellular memory, SMCHD1, transcriptomics*

P-32
Hijacking of transcriptional condensates by endogenous retroviruses

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Most endogenous retroviruses (ERVs) in mammals are incapable of retrotransposition; therefore, why ERV derepression is associated with lethality during early development has been a mystery. Here, we report that rapid and selective degradation of the heterochromatin adapter protein TRIM28 triggers dissociation of transcriptional condensates from loci encoding super-enhancer (SE)-driven pluripotency genes and their association with transcribed ERV loci in murine embryonic stem cells. Knockdown of ERV RNAs or forced expression of SE-enriched transcription factors rescued condensate localization at SEs in TRIM28-degraded cells. In a biochemical reconstitution system, ERV RNA facilitated partitioning of RNA polymerase II and the Mediator coactivator into phase-separated droplets. In TRIM28 knockout mouse embryos, single-cell RNA-seq analysis revealed specific depletion of pluripotent lineages. We propose that coding and noncoding nascent RNAs, including those produced by retrotransposons, may facilitate 'hijacking' of transcriptional condensates in various developmental and disease contexts.

Keywords: *endogenous retroviruses, transcriptional condensates, RNA*

P-33
Morphine leads to a global hipometylation via active and pasive demetilation mechanisms in mESCs

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Epigenetic changes are essential for normal development and ageing, but there is still limited understanding of how environmental factors can cause epigenetic changes that lead to health problems or diseases. Morphine is known to pass through the placental barrier and impact normal embryo development by affecting the neural tube, frontal cortex and spinal cord development, and, as a consequence, delaying nervous system development. In fact, in-utero morphine exposure has shown alterations in anxiety-like behaviours, analgesic tolerance, synaptic plasticity and the neuronal structure of offspring. However, how morphine leads to abnormal neurogenesis and other physiological consequences during embryo development is still unknown. Considering that DNA methylation is a key epigenetic factor crucial for embryo development, our aim is to elucidate the role of methylation in response to morphine. By MS/MS approaches, we observed a decrease in methylation, together with an increase in hydroxymethylation global levels in mESCs after chronic morphine treatment (24 h, 10 µM). WGBSeq identified 13329 genes sensitive to morphine, which are involved in embryo development, signalling pathways, metabolism and/or gene expression, suggesting that morphine might affect methylation levels at developmental genes. Integrative analyses between WGBSeq and RNASeq identified *Tet1* as a morphine sensitive gene. Morphine increased the gene expression of *Tet1*, modifying the methylation levels at the promoter. On the other hand, RNASeq and qRT-PCR analyses revealed that *Dnmt1* gene expression decreased after morphine treatment. In conclusion, morphine induces a global hypomethylation in mESC through different mechanisms that involves passive demethylation and a self-regulatory mechanism via active demethylation.

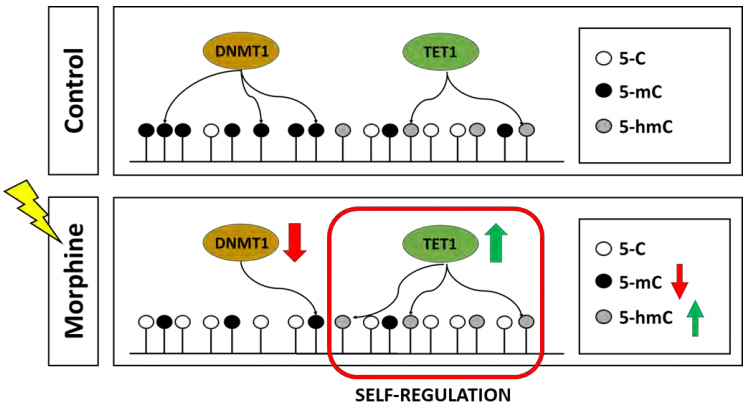

Figure 1: Chronic morphine treatment in mESC induces global hypomethylation through passive demethylation and self-regulated active demethylation.

Keywords: *morphine, development, global hypomethylation, self-regulation mechanism.*

P-34
Exploring the effects of drug exposure on the developing epigenome using *in vitro* models

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Reproductive toxicity is an important part of drug safety and, in particular, the potential effects of drug exposure on the epigenome during germline development are of extreme importance, though they are not thoroughly understood. Drug exposure during pregnancy can impact multiple generations but the mechanisms of such inheritance are still not fully established. Using the currently available models, variability of effect and partial phenotypic penetrance hampers these studies in identifying pharmacological risk. Here, we utilise a novel protocol of *in vitro* gametogenesis (IVG), alongside mouse embryonic stem cells, to investigate the effects of drug exposure on the epigenome in the developing germline. Using Reduced Representation Bisulphite-Seq, we show low-dose methotrexate, a known teratogen used to treat rheumatoid arthritis, causes selective hypomethylation in mESCs, in the absence of inducing stress or apoptotic markers. Treatment of the IVG model with methotrexate identifies a variable sensitivity of the early and late stages of differentiation from mouse embryonic stem cells to primordial germ cell-like cells. We present this model as a preliminary investigative tool *in vitro* to explore the epigenetic toxicity of currently prescribed medication on the germline. In addition, fertilisation of exposed gametes will determine the scope of embryo viability and will provide further information towards the understanding of generational inheritance.

P-35
Correlation between uracilation and transcriptional activities in drug treated colon cancer cells

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We have been investigating the genome-wide uracil incorporation profiles in HCT116 colon cancer cell lines with inhibited uracil-DNA repair upon treatment by two different thymidylate synthase inhibitors, namely 5-fluoro-2'-deoxyuridine (5FdUR) and raltitrexed (RTX). Using a uracil-DNA sensor protein developed in our lab [1], genomic uracil-DNA fragments are enriched and analyzed by next generation sequencing in a DNA-IP-seq application. Previously, we showed correlation of drug-induced genome-wide uracil patterns with early replication timing, active transcription and euchromatin regions [2]. The two drugs resulted in similar patterns, with characteristic differences that might indicate drug-specific alterations in the molecular mechanisms.

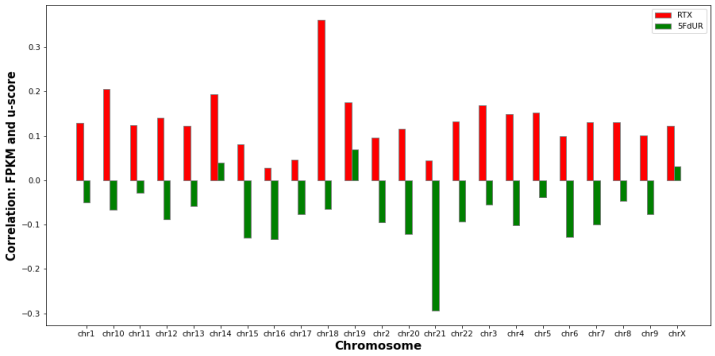

Figure: Spearman's correlation between transcription levels and U-scores calculated for protein coding transcripts by invidual chromosomes in UGI expressing HCT116 cells treated by RTX (red) or 5FdUR (green).

To measure the extent of uracil incorporation at the level of genes, we introduced a probability score termed U-score. Using this measure, differently uracilated genes were identified between RTX and 5FdUR treated samples. To get deeper insight how the level of uracilation correlates with transcriptional changes, we also performed whole transcriptome sequencing. Relative expression values, and their changes induced by the two drug treatments were calculated. We matched the U-score and the transcription level data, but the correlation between them is not straightforward. Spearman correlation coefficients calculated for individual chromosomes were quite small, however showed interesting drug specific tendencies: positive and negative correlation in case of RTX and 5FdUR treated samples, respectively. We also aimed at clustering genes using unsupervised machine learning algorithms (K-Means clustering, PCA) according to their transcriptional level, drug induced U-scores and transcriptional changes, also considering other factors like replication timing that might influence both uracilation and transcription activities. In long-term, we aim at identifying genes and their corresponding regulatory transcription factors sensitive or resistant for uracil content, which would provide deeper insight into the possible epigenetic role of genomic uracil.

Keywords: *uracil-DNA, transcription, U-DNA-Seq, RNA-seq, anticancer drugs*

References' PMIDs: [1] 26429970, [2] 32956035

P-36
Whole genome transcriptomic changes in the placenta suggests epigenetic origins of recurrent pregnancy loss

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Introduction: Nearly 50-70% of all conceptions, 15-20% of clinically recognized pregnancies are lost. Recurrent pregnancy loss (RPL), defined as the loss of two or more consecutive pregnancies before 20 weeks of gestation, affects 1-5% of clinically recognized pregnancies. In spite of its high prevalence and severe consequences, the etiology and molecular-pathways are poorly understood, so reliable screening tools or effective preventive treatments are not yet available.

Methods: In order to identify molecular pathways of RPL, we utilized a hypothesis-free systems biology approach. Placental samples were obtained from patients with RPL (n=8) and gestational age-matched controls (n=8) between 8-12 weeks of gestation and were investigated using whole-genome microarray. Gene modules, perturbed molecular pathways and hub genes were assessed, while DNA methylation analysis of the same placentas is under way.

Results: We identified 1,537 differentially expressed genes in RPL. Among these, the enrichment of placenta-specific (n=113, OR: 31.8, $p=4.4*10^{-88}$) and villous trophoblast differentiation-related (n=398, OR: 3.8, $p=5.6*10^{-80}$) genes was observed. The 98% and 83% of these genes were downregulated, respectively, suggesting that trophoblastic functions were strongly impaired in RPL. There was also an enrichment of immune associated genes along with the activation of pathways in allograft rejection and autoimmune processes. Perturbed gene modules and their hub genes were identified. Importantly, several enzymes of the DNA methylation machinery were found differentially expressed.

Conclusions: Our results suggest that the profound transcriptomic changes in the placenta in RPL may have an epigenetic origin and result in placental failure, severe immune maladaptation and the disruption of maternal-fetal immune tolerance.

P-37
Biologically informed deep learning for explainable epigenetic clocks

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Aging is characterized by progressive accumulation of damage, and is one of the biggest risk factors for chronic diseases. Epigenetic mechanisms, like DNA methylation may play a role in organismal aging, but if these processes are active drivers, or just byproducts of growing older is still not understood. In recent years, a number of age predictors based on DNA methylation, called epigenetic clocks have been developed, which accurately estimate the biological age of an individual. Despite these predictors getting more and more accurate, these models act as black-boxes, having limited ability to explain why they produced a given prediction and what possible underlying biological processes could play a role in it.

Recently, P-NET, an explainable deep learning model utilizing multi-omics data was developed to stratify patients with prostate cancer was developed, and it was shown that this approach can identify novel biomarkers extracted from the latent layers of the model. Based on this method, here we present XAI-AGE, a biologically informed, explainable deep neural network model for accurate biological age prediction across multiple tissues. We show that the model has better performance when compared to first-generation age predictors like the Horvath clock, and similar results to deep-learning based models, while opening up the possibility to infer biologically meaningful insights of the activity of pathways and other abstract biological processes directly from the model. This method surpasses the current techniques aiming for explanability, which usually involve correlating, or taking the linear combination of the transformed input data. We also show that this inference can be performed compared between tissues, or across time to identify specific biomarkers of aging. To make it easier to test hypotheses, we developed an online visualization tools for the model, accessible online.

P-38
Therapy induced microRNA modifications in Parkinson's disease

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Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder in the elderly and is characterized by a gradual loss of dopaminergic neurons in the substantia nigra pars compacta. The diagnostic of PD is entirely clinic, involving the presence of the four cardinal motor symptoms and a positive response to substitution therapy. LevoDopa (LD) replacement therapy is regarded as the main treatment, for it relieves the motor symptoms. Yet, its pharmacokinetics shows substantial inter- and intra-individual variations, and long-term use can trigger several associated complications. Moreover, regardless of the research attempts, there are currently no diagnostic, monitoring, and prognostic tests or biomarkers in PD.

Due to their remarkable stability in biological fluids, microRNAs are ideal biomarker candidates. Here, we used qRT-PCR array to screen for PD-associated plasma microRNAs and Taqman qRT-PCR to validate hsa-miR-19a, hsa-miR-19b and hsa-miR-195 as potential candidates. Analysis of naïve vs. LD-treated patients showed that hsa-miR-19b is a potential response biomarker to LD and dopa-decarboxylase inhibitors (DDCIs) treatment, a result validated both *ex vivo* (in human dopaminergic neurons exposed to LD) and *in vivo* (in a mouse model). STRING functional analysis of PD-related transcriptomes showed that miR-19b might regulate ubiquitin-mediated proteolysis. Moreover, we performed an analysis of the contribution of LD and DDCI treatment on the three microRNAs expression levels in brain and peripheral organs in a mouse model.

Keywords: *Parkinson's disease, LevoDopa, miR-19b*

P-40
Paclitaxel-loaded casein nanoparticles induces apoptosis in breast cancer cells via MAPK pathway

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Cancer is considered the second cause of death globally, surpassed only by cardiovascular disease. Breast cancer is one of the most common malignant tumors in the world causing death in women aged between 20 and 50 years with an incidence rate of 10.4% of all cancers. Various approaches were proposed to tackle the disease, including chemical-based therapies. The main obstacle of these dugs is that they have low availability, solubility and high toxicity rates. Nanocarriers are recently considered a reliable tool to solve the aforementioned problems. In the present study, we treated MAD-MB-231, MCF-7, and 4t1 breast cancer cells long with HSF (human fibroblast cells) with paclitaxel and cyclophosphamide with as free drug or loaded on casein nanoparticle as a carrier. Cells were incubated with drugs for 24h before harvesting. MTT assay data revealed a significant increase in cell cytotoxicity in PTX- and CYC-treated cells, with the higher levels was observed in the loaded drugs. Meanwhile, colony forming assay, wound healing, trypan blue tests were carried out and confirmed the same profiles. qPCR was performed to assess the expression profiles of several cancer-related genes, including BAX, Bcl-2, MAPK, P21, RASSF1, PTEN, CDK11, and CDH1. Data indicated that the apoptotic promoting genes were generally upregulated, while those related to cell cycle progression were downregulated. Furthermore, cell cycle analysis and apoptosis detection were performed using flow cytometry. Data indicated that treating cells with the loaded drugs has resulted in arresting the cell cycle at G2/M phase compared with the free drugs. Moreover, total apoptosis level was increased in the loaded drugs treatment compared to control and the free dugs. These data indicated the efficacy of the nanocarrier to deeply deliver paclitaxel and cyclophosphamide to exert their functions. This might endorse the involvement of nanocarriers in the clinical setting, however this study like others related needs further investigation.

P-41
CRISPR/Cas9-mediated activation of CDH1suppresses metastasis of breast cancer in rats

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Background: Cancer is a life-threatening disease that affects approximately 18 million individuals worldwide. Breast cancer is the most common female neoplasm globally with more than 276,480 new cases of invasive breast cancer expected to be diagnosed in women in the U.S. alone in 2020. Genetic and epigenetic factors play role in the carcinogenesis and progression of this disease. In this study, MCF-7 adenocarcinoma cells were transfected with CRISPR/Cas9 plasmid to either knock out CDK11 or to activate CDH1. Treated cells were allografted into the mammary glands of female rats (150–190 g, 6–8 weeks) to evaluate the capability of these cells to control cancer progression and metastasis. Results: qPCR data revealed a significant downregulation of CDK11 and upregulation of CDH1. Cell cycle analysis and apoptosis assays indicated the knockout of CDK11 and simultaneous activation of CDH1 resulted in cell cycle arrest at G2/M phase and accumulation of cells at G2. Meanwhile, the percentage of cells that underwent late apoptosis increased in both genome editing hits. Histopathological sectioning data indicated that untransfected MCF-7 cells were capable of developing tumors in the mammary gland and initiation g angiogenesis. Transfected cells significantly restricted cancer cell infiltration/invasion by minimally localizing tumors and inhibiting angiogenesis. Conclusions: Although further investigation is needed, the present data indicate the potentiality of using CRISPR/Cas9-based therapy as a promising approach to treat breast cancer. Impact: these data indicate targeting cancer-related genes via any genome editing tool might represent a novel approach to combat cancer.

P-42
H3K14ac is decreased through genome in Slovenian male suicide completers

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With more than 800,000 deaths every year, suicide is one of the most important health problem worldwide. Slovenia ranks among countries with the highest suicide rate (number of suicide completers/100,000 citizens). The complexity of suicidality and suicide can be seen through numerous factors affecting it. Besides social and economic factors, biological factors play an important role. In the last decade, genetic and epigenetic studies became important contributors to understanding suicidality. In previous studies, we have already investigated DNA methylation. Now we are presenting our results from chromatin immunoprecipitation and nextgeneration sequencing (ChIP-seq) experiment.

In our study, we investigated posttranslational modification of histone tail on *post mortem* brain tissue of Slovenian male suicide completers who died by hanging. For this purpose, chromatin immunoprecipitation and next-generation sequencing (ChIP-seq) method was used. We have investigated H3K14ac modification, in the group of suicide completers.

Sequencing analysis showed that there is reduced acetylation profile of the H3K14ac mark through the genome in a group of suicide completers compared to a control group. Genes with different H3K14ac mark within two groups are classified into biological processes, cellular components, and molecular functions.

Histone acetyltransferases add a negatively charged acetyl group to the lysine of histone tails, open chromatin, and enable transcription. Results of lower acetylation levels in the group of suicide completers are in accordance with our expectations. Namely, studies of histone tail modifications in depression, which is one of the common comorbidities of suicide, showed lower levels of H3K14ac in depressed subjects compared to controls.

Keywords: *H3K14ac 1, ChIP-seq 2 suicide 3*

P-43
Amoeboid transition in polyploid breast cancer cells induced after genotoxic treatment is essential for clonogenic resistance

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The change of tumour cell gene expression profile towards unicellularity associated with polyploidisation through mitotic slippage (MS) has been reported, while the ameboid phenotypic transition was found as typical for the invasion stage. How ameboidisation is involved in cancer reproduction and resistance is unclear.

For experimental studies, the breast cancer MDA-MB-231 cell line was treated with 100 nM DOX (doxorubicin) for 24 h, and cells were sampled up to the appearance of escape clones (~3 weeks). DNA cytometry revealed the reciprocal relationship between 2C/G1 and >4C (polyploid) fractions with two critical points: (1) on day 8 – maximum of polyploidy/minimum diploidy with the absence of normal mitosis; (2) on day 16-18 recurrent reciprocality: polyploids go down, while diploids raise up, accompanied by restitution of mitosis. On/after day 8, the activation of the YAP1-TEAD1 nuclear network along with differentiation of subnuclei for remaining and budded one was observed indicating dichotomy of cell fate change. While till day 8 there prevailed genome multiplication, afterwards a powerful ameboid cytoskeleton enriched in ACTIN, CDC42 and RAC1was developed. The budding spore-like cells were enriched in these components; further, they formed multiple pseudopodia indicating motility. The hyperploid (>20C) amoeboids served for feeding and homing for initially scarce mitotic offspring. Further, these supergiants degraded. Inhibition of CDC42 decreased the clonogenicity of DOX-treated cells 3-fold. The data testify that ameboid transition, directly and indirectly, cooperatively supports resistant invasive growth of tumour cells.

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References: DOI:10.3390/ijms21082779; doi.org/10.1016/j.semcancer.2020.12.009

P-44
Integrated biological and chemical epigenetic-targeting platform to improve cancer therapy

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Cancer is still affecting the lives of too many patients. Aberrant epigenetic modifications are strongly correlated with cancer prognosis providing not only new biomarkers, but also new therapeutic approaches. However, drug resistance is an important limitation. Among the mechanisms, upregulation of the gene encoding the drug's target is common for chemotherapeutic drugs. Thus, inhibition at the protein level induces compensatory gene upregulation, resulting in loss of potency. Innovative approaches are needed to prevent this gene expression induction.

We developed an innovative co-treatment approach based on the envisioned synergic effect of gene expression silencing, by epigenetic editing, and protein inhibition by epi-drugs. Epigenetic editing by CRISPR/dCas9 has led to numerous successes in preclinical cancer models modulating selectively the gene expression. However, scientists have not yet explored the combination of chemical drugs with epigenetic editing. Preventing the compensatory gene upregulation induced by some epi-drugs will lower pharmacological dosages and limit toxicity. We targeted KDM4A, an epigenetic eraser overexpressed in several cancers, involved in carcinogenesis, tumor progression and migration. Cancer cell lines treatment with 16 epi-drugs confirmed the compensatory mechanism: three KDM4 inhibitors induced KDM4A upregulation, while 13 other epi-drugs did not. Epigenetic editing reduced the KDM4A expression to 50-70%, using KRAB and DNMT3A/L as effector domains fused with dCas9 (CRISPRoff-v2.1). The synergic effect of CRISPRoff-v2.1+drugs was validated in HEK293T, MCF7 and HCT116 cells. The 2-fold increase in KDM4A expression after KDM4i (QC6352) treatment was completely prevented by epigenetic editing. Moreover, epigenetic editing +drugs work synergistically in preventing cancer cell proliferation by blocking upregulation and inhibiting protein activity. Therefore, this approach allows to obtain greater pharmacological activity, leading to decrease in toxicity and an increase in selectivity. Applicable to a wide range of epi-drugs, this approach will lead to a huge shift in cancer research to increase effectiveness while reducing chemoresistance in different tumor.

Keywords: *Epigenetic editing, cancer, epi-drugs*

P-45
Altered circulating hsa-miR-195-5p level predicts Covid-19 severity in hospitalized patients

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Covid-19 is a heterogeneous, multisystem disease, ranging in severity from asymptomatic to acute respiratory distress syndrome and severe, multi-organ failure. The SARS-CoV-2 infection triggers tissue-specific damages that associate particular transcriptional profiles, including changes in host microRNA profiles.

Dysregulation of hsa-miR-195-5p (miR-195) expression is a relatively common response to viral infections, including HIV-1/HIV-2, enteroviruses, and coronaviruses. Furthermore, miR-195 is a possible interactor with all members of the Coronavirus families, including SARS-CoV-2.

Here we quantified the plasma miR-195 at the onset (first 48 hours upon hospital admission) of Covid-19 disease, analyzed its correlation with clinical and paraclinical parameters of Covid-19 patients, and estimated its transcriptome impact in lung, heart, lymphatic nodes, liver, and kidneys.

We show that miR-195 is downregulated in severe Covid-19 plasma samples (FC=0.16; P<0.0001), inversely correlates with SARS-Cov-2 RNAemia (r= - 0.52, P=0.011), and efficiently discriminates (AUC=0.913; P<0.0001) between severe and mild forms of Covid-19 disease. STRING functional enrichment analysis of transcriptome data links miR-195 to cardiac and muscle tissue and identifies mitochondria and cellular respiration as primary targets of mir-195 deregulation.

Our data suggest that the myocardial impact of SARS-CoV-2 is a rather early phenomenon, and we speculate that the switch to a severe course of the disease depends on the ability of the cells to maintain mitochondrial homeostasis.

Keywords: *hsa-miR-195, severe Covid-19, mitochondria*

P-46
DNMT1 specific covalent tracing of DNA methylation in mouse embryonic cells

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In mammals, methylcytosine plays crucial epigenetic roles in the harmonious maintenance of cellular functions during organism development. However, undesirable changes in DNA methylation may occur, causing various molecular processes to become unbalanced and promoting the manifestation of various pathologies. In cells, genome methylation is performed by the combined action of three AdoMet-dependent DNA methyltransferases, the catalytic interactions and temporal interplay of which are still unknown. Therefore, the development of molecular tools to evaluate DNA methylation precisely and selectively during development or the emergence of disease remains critical.

To gain a better understanding of DNA methylation patterns in mammalian cells, we engineered AdoMet-dependent mouse DNA methyltransferase Dnmt1 to catalyze the transfer of extended moieties onto DNA in vitro using a synthetic cofactor analog Ado-6-azide. The engineered codon was then installed in mouse embryonic cell lines using the CRISPR-Cas9 genome editing system. We were able to selectively covalently tag and precisely map catalytic Dnmt1 targets in mammalian cells using engineered Dnmt1 and pulse internalization of the Ado-6-azide cofactor by electroporation. Finally, we successfully differentiated mouse embryonic cells into adipocytes bearing engineered Dnmt1, and we were able to monitor Dnmt1 selective activity during the differentiation using the proposed approach.

P-47
Decreased methyl-donor content could be linked to global DNA hypomethylation in colorectal cancer progression

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Global DNA methylation loss is an early epigenetic alteration of colorectal cancer (CRC). Abnormality of DNA methyltransferases (DNMT), demethylases, or deviation in methyl-donor (folate and S-adenosylmethionine) availability can contribute to the development of genome-wide hypomethylation. Detection of global DNA hypomethylation in cell-free DNA (cfDNA) obtained from blood samples can expand the opportunities for early CRC recognition. Following the investigation of genome-wide methylation patterns in tissue (n=183) and cfDNA samples (n=48) along the colorectal normal-adenoma-carcinoma sequence and in inflammatory bowel disease (IBD), we aimed to explore possible underlying mechanisms of genome-wide hypomethylation formation in 12 colorectal tumor sections. Using LINE-1 pyrosequencing, significantly reduced global DNA methylation was detected in line with cancer progression in tissue (normal: 77.5±1.7%, adenoma: 72.7±4.8%, carcinoma: 69.7±7.6%, $p \leq 0.0001$) and in cfDNA as well (normal: 82.0±2.0%, adenoma: 80.0±1.7%, carcinoma: 79.8±1.3%, $p \leq 0.01$). However, no significant methylation changes were found in IBD. Analyzing microarray data *in silico*, altered mRNA expression of certain methylation-, and one-carbon metabolism-related genes were detected in tumorous vs. healthy biopsies, from which *DNMT1* was upregulated, and folate receptor 2 (*FOLR2*) was downregulated. Furthermore, significantly reduced folate and S-adenosylmethionine content was observed in parallel with diminishing 5-methylcytosine levels in adenoma and carcinoma sections compared to normal adjacent to tumor tissue areas by immunolabeling ($p \leq 0.05$). Our results suggest that intraindividual monitoring of genome-wide hypomethylation may assist in the recognition of adenoma formation, cancer progression, or remission as well. Moreover, lower global DNA methylation level could be connected to decreased methyl-donor availability with the contribution of reduced *FOLR2* expression.

P-48

Mesenchymal stromal cells as a tool to unravel the epigenetic mechanism underlying the developmental origins of disease

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Lifelong health is thought to be partially set during intrauterine life by persistent epigenetic changes induced by the prenatal environment. To evaluate this hypothesis, we initiated a prospective longitudinal study in monochorionic (MC) twins: The Twinlife study. MC twins are monozygotic, thus genetically identical in origin, and share a single placenta. In one-third of the MC twin pairs, one fetus has significantly less access to nutrients and resources during pregnancy than its co-twin often resulting in a significant discordance in prenatal growth. Hence, MC twins constitute a natural experiment to study the influence of the prenatal environment on health. In Twinlife, we will chart intrapair differences in DNA methylation and transcription focusing on mesenchymal stromal cells (MSC) isolated from umbilical cord as an advanced proxy of epigenetic dysregulation relevant for long-term health consequences. To allow a comparison between the MSCs of these twin pairs, we developed a standardized and reproducible procedure to obtain and characterize the MSCs. Using this procedure, we have now isolated MSCs from >200 twins. Moving forward, we will assess the epigenetic, transcriptomic, and functional differences between the genetically identical MSCs, to gain more insight into the mechanism underlying the long-term health impact of an adverse intrauterine environment.

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P-49
Generation and characterization of hepatocyte-specific DNA methyltransferase (Dnmt) 3a and Dnmt3b double knockout mice

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Our knowledge about the role of DNA methylation in the development of liver is limited. It is known that global loss of DNA methylation due to mutation of DNA methyltransferase 1 (Dnmt1) does not interfere with hepatocyte differentiation but blocks hepatic outgrowth. Furthermore, regarding *de novo* DNA methyltransferases, Dnmt3b has lately been reported to play a protective role against hepatocellular carcinoma. Our aim in this study is to explore how the elimination of the entire *de novo* DNA methylation apparatus can influence the development of liver and hepatocarcinogenesis.

For this purpose, mice carrying Albumin-Cre (*Alb-Cre*) transgene together with $Dnmt3a^{Flox/Flox}/3b^{Flox/Flox}$ were generated to abolish the expression of Dnmt3a and Dnmt3b specifically in hepatocytes. Regardless of the *Alb-Cre* transgene genotype ($Alb\text{-}Cre^{+/+}$, $Alb\text{-}Cre^{+/-}$, and $Alb\text{-}Cre^{-/-}$) mice were viable without apparent phenotypic difference. The *Alb-Cre* transgene distribution followed the Mendelian rule regarding the total number of offspring; however, $Alb\text{-}Cre^{+/+}$ genotype was twice as frequent in males as in females.

Since the liver is fully differentiated by postnatal day 21 (P21) in mice, we investigated gene expression changes between P1 and P21 by RT-qPCR and western blot. $Alb\text{-}Cre^{+/+}$ and $Alb\text{-}Cre^{+/-}$ mice were indistinguishable regarding the expression of all investigated genes. In contrast, by comparing with wild-type ($Alb\text{-}Cre^{-/-}$) littermates, knockout mice showed a significantly decreased expression for Dnmt3a and Dnmt3b at each time point. Furthermore, P1 double knockout mice showed significantly lower expression for Dnmt1, Tet2, Tet3, and Hnf4α.

These preliminary results suggest reduced DNA methylation in the liver of mice lacking *de novo* DNA methyltransferases which apparently does not cause a major effect on normal liver function and viability. However, the lack of Dnmt3a and Dnmt3b lead to gene expression alterations in the early postnatal development of the liver that will be further investigated in the future.

P-50
Convergent impact of schizophrenia risk genes

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Genome-wide association studies (GWAS) continue to identify significant risk loci for schizophrenia (SCZ). These common variant risk loci are enriched for genes expressed in pyramidal excitatory neurons, particularly synaptic pathways, and are expressed during fetal cortical development. We previously integrated human-induced Pluripotent Stem Cell-based models with CRISPR technologies to study putative SCZ-GWAS target genes, resolving specific pre/post-synaptic perturbations. However, an unanswered question is whether individual risk variants sum linearly or are influenced by epistatic interactions. Here we ask how the neuronal impact(s) of risk genes converge in a common genetic background. We applied two independent pooled single-cell RNA-sequencing CRISPR activation-based experiments (Expanded CRISPR-compatible CITE-seq: ECCITE-seq) to resolve the genome-wide transcriptomic consequences of activating expression of 12 genes with strong evidence of up-regulation by transcriptomic and epigenomic imputation at two developmental timepoints in human NGN2-induced glutamatergic neurons.

Querying the shared neuronal impacts across risk genes uncovers a convergent effect concentrated on pathways of brain development and synaptic signaling. Our analyses reveal shared and divergent downstream effects of these twelve genes. General convergence increases with increasing polygenicity, while the specificity of convergence increases with functional similarity. Convergent networks show brain-region, developmental period, and disorder-specific enrichments and were drug-able, representing potential therapeutic targets.

Overall, convergent signatures were observable in two distinct ECCITE-seq experiments and resolvable in post-mortem brain. That convergent networks were enriched for risk genes associated with multiple disorders suggesting that convergence may partially explain shared endophenotypes and cross-disorder pleiotropy, supporting the hypothesis that common and rare disorder-associated variants converge on the same biological pathways.

P-51
Epigenetic reprogramming of colorectal cancer cell lines by S-adenosylmethionine treatment

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S-adenosylmethionine (SAM) is a universal methyl donor that is involved in the DNA methylation process; thereby, it has a favorable effect on genomic stability and inhibition of proto-oncogene expression. In addition, it was reported to selectively reprogram pathways connected to carcinogenesis.

We aimed to analyze the effect of SAM treatment on the metabolic functions, genomic stability, DNA methylation, and gene expression of two colorectal cancer cell lines (HT-29, SW480) with distinct molecular features.

Cells were kept in SAM-free medium or treated with 0.5 and 1 mmol/L SAM for 48 hours. Metabolic functions were analyzed with SRB and AlamarBlue assays, as well as flow cytometry. Micronucleus scoring and comet assay were used to assess genomic stability. DNA methylation was detected with LINE-1 pyrosequencing and RRBS method. Finally, we used HTA 2.0 microarray to detect gene expression alterations.

Proliferation level was significantly ($p \leq 0.01$) decreased for SAM treatment along with an S phase arrest in both cell lines. Genomic stability was significantly ($p \leq 0.01$) higher in treated SW480 samples compared to controls. The number of hyper- (HT-29: 8333, SW480: 6407) and hypomethylated (HT-29: 8753, SW480: 6407) genes were almost equal in both cell lines, mainly affecting carcinogenesis-related pathways. Moreover, SAM could effectively lower the expression of EMT genes in both sample types and activate repair processes in SW480, opposite to HT-29.

Our results were in accordance with previous studies as SAM is a potent molecule in cancer inhibition by acting as a methylome modulator. Additionally, it can increase the genomic stability of SW480 cells.