Lectures

O.1

Cellular architecture of the human brain

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We have generated single-cell atlases of the adult and developing human brain using a combination of single-cell RNA sequencing and spatial transcriptomics. In the adult, we found an unexpectedly high diversity of neuronal types especially in the midbrain and hindbrain, with conservatively more than 3000 distinct types. In the developing human brain, we revealed highly refined trajectories of differentiation of cortical neurons. We further found that astrocytes and oligodendrocytes were regionally patterned, and that these region-specific types persisted in the adult. These findings may provide a basis for the brain region-specific occurrence of pediatric brain tumors.

O.2

Brain tumor microenvironment at single-cell resolution

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Dynamic interactions of tumor cells with their microenvironment consisting of stromal and immune cells and extracellular matrix components are essential for tumor progression, responses to therapy and disease outcome. Within the tumor microenvironment (TME) specific niches exist created by oxygen, nutrients and soluble factors gradients that attract distinct cells and strongly affect a given cell phenotype. Dissecting these cell type and spatial patterns has emerged thanks to single cell omics technologies revealing unexpected heterogeneity and complexity. Glioblastoma (GBM) is an aggressive, lethal brain tumor that is massively infiltrated by myeloid cells supporting invasion and creating the immunosuppressive microenvironment. It disables the anti-tumor immunity and immunotherapy. We employed Cellular Indexing of Transcriptomes and Epitopes by sequencing (CITE-seq) to reliably dissect myeloid components of TME, cell identities and states during progression of murine GL261 gliomas. Spatial transcriptomics to localize cells of interest within TME. Using computational approaches we identified fate trajectories and cell-to-cell interactions. We found that glioma-activated microglia are the major source of cytokines attracting other immune cells, whereas bone marrow-derived myeloid cells show monocyte-to-macrophage transition and immunosuppressive phenotypes. This transition is coupled with a phenotypic switch from the IFN-related to antigen-presentation and tumor-supportive signatures. Intriguingly, we found sex-dependent differences in transcriptional programs and composition of myeloid cells. Higher abundance of protumor macrophages in males correlated with greater tumor size. Re-analysis of single-cell omics data from human GBMs revealed the predominance of inflammatory monocytes in female GBMs and the abundance of protumor macrophages in male GBMs demonstrating higher expression of MCHII complex and PDL1. Our findings expand understanding of the complexity of anti-tumor immune responses in gliomas and may guide future therapies in consideration of patient sex.

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O.3

Multimodal profiling of the chromatin at single-cell resolution in the brain

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In the past years profiling of the epigenome with single-cell resolution has brought novel insights into functions of the regulatory chromatin, transcription factors and gene regulatory networks. These advances were mainly driven by single-cell ATAC-seq profiling of the accessible chromatin. However, the functions of regulatory chromatin elements such as enhancers or promoters is determined not only by DNA accessibility, but also by the histone modification status among others.

We have recently developed a method to profile histone modifications with single-cell resolution from thousands of cells at the same time – single-cell CUT&Tag. Now, we went one step further and developed a novel improved technology – nano-scCUT&Tag that make it for the first time possible to multimodally profile three epigenetic modalities including open chromatin and two histone modifications from thousands of single cells. Nano-scCUT&Tag uses a new set of nanobody-Tn5 transposase fusion proteins to target multiple epigenetic modalities at the same time. In addition to being multimodal, nano-scCUT&Tag has lower input requirements, yields more fragments per cells and improves clustering resolution over previous generation of scCUT&Tag.

We have applied nano-CUT&Tag to the juvenile mouse central nervous system and uncovered unprecedented epigenetic heterogeneity. The obtained multimodal profiles can be used to deconvolute the individual cell identities in the brain and generate sub-population level epigenetic profiles. Moreover, we used nano-CUT&Tag to uncover for the first time real-time dynamics of the chromatin during a differentiation process *in vivo*. We then leveraged the relationship between chromatin opening and enhancer activation to define chromatin velocity and predict the future cell state based on multimodal snapshot of chromatin state. Altogether, nano-scCUT&Tag provides unprecedented insights into chromatin regulatory landscape in the mouse CNS.

O.4

SIngle-cell and spatial transcriptomics characterisation of the mouse white-matter reveals adaptive immune system as driver of white matter aging.

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A hallmark of nervous system aging is a decline of white matter volume and function, but the underlying mechanisms leading to white matter pathology are unknown. Using spatial transcriptomics and scRNA-seq, we found that microglia and oligodendrocyte identities show age-related alterations in white matter. To further characterised these cell responses, we developed Spatial Transcriptomics-correlated Electron Microscopy (STcEM) which correlates large-area scanning EM and multiplexed error-robust fluorescence in situ hybridization (MERFISH) and links transcriptional identities of single cells with ultrastructural data. In summary, we provide evidence that CD8+ T cell-induced interferon-responsive oligodendrocytes and microglia are important modifiers of white matter aging and likely be involved in neurodegenerative disorders.

O.5

Modeling herpes simplex virus 1 infection in cerebral organoids reveals new potential therapeutic approaches for viral encephalitis

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Understanding how the human brain functions in health and disease is one of the greatest challenges of modern science, yet hindered by limited availability of human samples and ethical restrictions. The three-dimensional human brain organoid model has emerged as a cutting-edge, genetically-tractable experimental system to study human brain development and function in health and disease. Here, I will present one exemplary project illustrating how brain organoids can be employed to model human disease, specifically viral encephalitis.

Herpes simplex encephalitis (HSE) is a life-threatening disease of the central nervous system caused by herpes simplex viruses (HSV). However, with the standard anti-viral treatment, most patients still experience various neurological sequelae.

We employed human brain organoids to model acute HSV-1 infection in a complex neuronal tissue and preformed single-cell RNA sequencing, electrophysiology and imaging to characterize the molecular changes associated with HSV-1 infection. We observed strong perturbations of tissue integrity, neuronal function and cellular transcriptomes. Antiviral acyclovir treatment alone, which reflects clinical treatment, prevented viral replication, but did not rescue HSV-1-driven defects observed in organoids. Unbiased analysis of pathways deregulated upon infection revealed TNF activation as a potential causal factor. Therefore, we combined anti-inflammatory drugs with antiviral treatment to rescue the damages caused by the infection. Our data indicate that tuning the inflammatory response in acute infection may improve current therapeutic strategies.

O.6

Characterisation of alternative polyadenylation at single cell resolution in Alzheimer disease

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Alternative polyadenylation (APA) is a widespread mechanism of gene regulation that generates mRNA isoforms with distinct 3'ends. APA is well known to be regulated during cell differentiation and is a major source of gene regulation in the brain. Proliferating cells tend to have shorter 3'UTRs while differentiated cells have longer 3'UTRs. Changes in APA patterns are not only characteristic of cellular differentiation but also have been associated with pathological processes such as cancer or neurodegenerative diseases like Alzheimer's disease (AD). The rapid development of 3'tag-based single-cell RNA sequencing (scRNAseq) has enabled the study of gene expression at the individual cell level and the implementation of methods for describing APA sites at single cell resolution. Here we present PLAPA, a tool for characterising APA sites at single cell resolution using 10X Genomics or Dropseq scRNA-seq dataset. PLAPA allows quantifying RNA expression at isoform level and single-cell resolution and identifying changes in isoform usage across cell populations and conditions. We used PLAPA to study the changes in APA during the differentiation of induced pluripotent stem cells (iPSCs) to neuroprogenitor cells (NPCs).

The results from our analysis show clear changes in 3'end usage between iPSCs and NPCs. We project to use PLAPA to investigate the role of APA in neural differentiation and its role in the development of AD and how APA changes during neural differentiation and how these changes are altered in AD.

O.7

Spatio-temporal, optogenetic control of gene expression in organoids

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Organoids derived from stem cells become increasingly important to study human neurodevelopment and to model disease. However, methods are needed to control and study spatio-temporal patterns of gene expression in organoids. To this aim, we combined optogenetics and gene perturbation technologies to activate or knock-down RNA of target genes, at single-cell resolution and in programmable spatio-temporal patterns. To illustrate the usefulness of our approach, we locally activated Sonic Hedgehog (SHH) signaling in an organoid model for human neurodevelopment. High-resolution spatial transcriptomic and single-cell analyses showed that this local induction was sufficient to generate stereotypically patterned organoids and revealed new insights into SHH's contribution to gene regulation in development.
With this study, we propose optogenetic perturbations in combination with spatial transcriptomics as a powerful technology to reprogram and study cell fates and tissue patterning in organoids.

O.8

Roles of *Silc1* lncRNA in the peripheral and central nervous system

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Mammalian genomes encode tens of thousands of lncRNAs that closely resemble mRNAs on the molecular level, yet do not encode functional proteins. An increasing number of human and mouse lncRNAs have been implicated as key regulators in a variety of cellular processes, including proliferation, apoptosis and response to stress. Substantial effort has now been directed toward dissecting the function of lncRNAs *in vitro* and *in vivo*, mainly using genetic manipulations, however their mechanisms for regulating gene expression and cellular function are not completely understood.
The central and peripheral nervous systems (CNS/PNS) express a particularly rich repertoire of lncRNAs and the number of their implications in gene regulation keeps increasing. Neurons within the PNS can undergo axon outgrowth that may lead to substantial functional recovery, while this process is limited within the CNS. Understanding the regulatory program of the transcriptional response to injury, and how lncRNAs contribute to the intrinsic ability of PNS neurons to regenerate, is crucial for improving regenerative outcomes in both the PNS and the CNS. While we know quite well which lncRNAs are expressed in the nervous system, it is still unclear how those lncRNAs act, and which have an important functional impact in the PNS and CNS. Even lncRNAs that have been studied are yet to be determined how similar their functions are when they act in different physiological contexts.
Recently we profiled the gene expression following sciatic nerve crush in mice and identified long noncoding RNAs (lncRNAs) that act in the regenerating neurons using RNA-seq. The analysis of the RNA-seq data highlighted several well-expressed, tissue-specific and conserved lncRNA genes as candidates for follow-up. We show that two of these lncRNAs regulate the extent of neuronal outgrowth. We then focused on one of these, Silc1, and showed that it regulates neuroregeneration in cultured cells and *in vivo*, through cis-acting activation of the transcription factor Sox11 (Perry *et al., Mol. Cell* 2018). Next, we decided to test whether Silc1 activity is restricted only to the PNS or that it has a function in the CNS as well. In the adult brain and spinal cord, Sox11 expression is low, but Silc1 is expressed at considerable levels, which are comparable to the levels observed post-injury in the PNS. By analyzing public RNA-seq data, we found that Silc1 and Sox11 are both induced following activation of neurons in the mouse hippocampus.
Our main hypothesis is that Silc1 is required for Sox11 induction following adult hippocampal neurons activation. Sox11 in turn activates a neurite growth-associated transcriptional program that is important for learning.
Using the RNAscope method we found that Silc1 is expressed mainly in the CA3 region, Sox11 is expressed in the dentate gyrus, and both increase upon novel environment activation. We also used stereotaxic injections for Silc1 and Sox11 knockdown or gain-of-function ex-

periments and demonstrated that upon Silc1 reduction Sox11 transcript and protein levels are also reduced. These results are similar to the results we received using Silc1$^{-/-}$ mice. Silc1 KO mice also exhibit significant reduction in spatial learning but not in long-term memory using Morris water maze and Barnes maze behavioral tests.

These days, we aim to identify regulatory regions affected by Sox11 induction during neuronal activity in the hippocampus, using ATAC-seq and CUT&RUN methods.

O.9

Small and circular non-coding RNAs in neural development and neurological disease

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Neurological disorders such as temporal lobe epilepsy (TLE) and amyotrophic lateral sclerosis (ALS) are characterized by changes in RNA biology. Both the expression and distribution of different RNA species is altered, as well as the proteins that bind and regulate RNA. In the past several years, we have shown that both the expression and distribution of microRNAs (miRNAs) is changed in hippocampal tissue from human TLE patients. We have shown that several of these miRNAs regulate neuronal connectivity and that counteracting these changes has seizure-reducing effects in experimental TLE *in vivo*. Further, a novel class of miRNA-binding molecules, circular RNAs (circRNAs), is also altered in TLE and marks selective stages of the epileptogenic process while also controlling neuronal morphology. More recently we have explored the aberrant nuclear localization of some miRNAs in human TLE. RNA-seq on subcellular fractions from human brain tissue identified a subset of miRNAs displaying a nuclear distribution. More specifically we found that miRNAs are aberrantly localized in the nucleolus where they bind nucleolar proteins and affect the biogenesis of snoRNAs. snoRNAs guide the modification of other RNAs, e.g. rRNA and tRNA, and thereby their deregulation could affect a wide range of cellular processes in TLE.

O.10

Brain isoforms in space and time

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Most mammalian genes encode multiple distinct RNA isoforms and the brain harbors especially diverse isoforms. Complex tissue, including the brain, often include highly divergent cell types and these cell types employ distinct isoforms for many genes. To untangle the distinct cell-type specific isoform profiles of the brain, we developed Single-cell isoform RNA sequencing (ScISOr-Seq[1]) for fresh tissues as well Single-nuclei isoform RNA sequencing (SnISOr-Seq[2]). To add spatial resolution, we developed Slide-isoform sequencing[3]. Collectively, these long-read approaches reveal a striking difference between coordinated pairs of exons with in-between exons ("Distant coordinated exons") and without in-between exons ("Adjacent coordinated exons"): The former show strong enrichment for cell-type specific usage of exons, whereas the latter do not in mouse1 and human brain[2]. Of note, coordinated TSS-exon pairs and exon-polyA-site pairs follow the same trend as distant coordinated exon pairs[2]. Simultaneously, autism-associated exons are among the most highly variably used exons across cell types[2]. Differences in isoform expression between hippocampus and prefrontal cortex are most often explained by differences arising between the two regions in one specific cell type (e.g., excitatory neurons), but for a smaller program of genes brain regions can override cell-type identity[3]. Spatially barcoded isoform sequencing revealed that often region-specific isoform differences correlate with precise boundaries of brain structures (e.g., from the choroid plexus to the hippocampus). However, genes including Snap25 go against this trend, using a steady gradient of exon inclusion as one traverses the brain[3]. Moreover, choroid plexus epithelial cells show a dramatically distinct isoform profile, which originates from distinct exon and poly(A) site usage, but most strongly from distinct TSS usage[3]. Most recently, we have made advances in understanding the error sources of Pacific Biosciences and Oxford Nanopore long-read sequencing technologies[4] and have implemented a highly accurate long-read interpretation pipeline[5].

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O.11

Regulation of translation in glia

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The regulated translation of mRNA provides an opportunity to precisely tune the amount, location, and isoforms of protein produced within a cell. For example, translation regulation has long been studied in neurons, where local translation near synapses is thought to support changes in synaptic strength. Glia have equally complex processes that are essential in efficient synaptic formation and pruning, yet the phenomena of local translation in astrocyte and microglial processes has received little attention. Likewise, alternative translation can enable regulated production of distinct proteins from the same mRNA, increasing the potential number of alternative isoforms for each gene in the genome. I will share recent results from the lab proposing a role for local translation in regulating microglial phagocytosis, as well as the production of new isoforms of proteins in astrocytes that enhance clearance of Alzheimer's associated proteins from the brain.

O.12

A cell-type-specific role for long non-coding RNAs in depression

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Depression is a common and debilitating disorder, which strikes women twice more than men, yet the molecular mechanisms contributing to this sex difference are poorly understood. We explored the roles of long non-coding RNAs (lncRNAs), a class of epigenetic regulators, in depression in both sexes. To that end, we integrated molecular, bioinformatics, behavioral, and physiological approaches spanning both humans and mice. We found that lncRNAs are robustly regulated in a brain-site and sex-specific manner in postmortem brain tissue from depressed humans compared to healthy controls. Utilizing a genome-wide "guilt-by-association" bioinformatic approach, we identified sex- and cell-type specific lncRNAs linked to depression. We highlighted two specific targets with opposing regulation in the prefrontal cortex (PFC) of depressed women: the neuronal LINC00473 (downregulated) and the neuronal and oligodendrocyte FEDORA (upregulated). Next, we determined a causal role for these lncRNAs by virally expressing them in mice of both sexes' PFC followed comprehensive phenotyping. This approach mirrored the human sex-specific phenotype: expressing LINC00473 induced stress resilience, while FEDORA promoted increased depressive-like phenotype in females only. These behavioral changes were accompanied by opposing alterations in synaptic function and gene expression. LINC00473 promoted a decrease in PFC pyramidal neurons excitability and blunted the PFC transcriptional response to chronic stress, while FEDORA triggered increased excitability and a female pattern of the transcriptional response to chronic stress. In parallel, we utilized chromatin isolation by RNA purification (ChIRP) followed by either DNA sequencing or mass spectrometry to pinpoint the molecular interaction partners of LINC00473. We found it binds the DNA of genes implicated in depression, as well as mitochondrial proteins. Finally, we found that circulating FEDORA amounts reflect its brain expression profile: blood FEDORA levels are elevated in depressed women compared to controls and not in males, suggesting FEDORA as a potential sex-specific biomarker for a depression diagnosis. Together, this work establishes that lncRNAs play critical roles in depression and contribute to the sex differences of this disorder. These findings provide a new view of molecular adaptations contributing to depression and point to targets that may serve as foundations for novel depression diagnosis and treatment.

O.13

Circular RNA Cdr1as is dynamically regulated during the circadian rhythm and linked to light-induced phase shifts in the SCN

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Cdr1as is a conserved circular RNA (circRNA) enriched in the CNS and important for maintaining brain homeostasis. The loss of Cdr1as results in aberrant synaptic transmission and deregulation of immediate early response and several circadian clock genes. However, it is not known whether the expression of *Cdr1as* or circRNAs, in general, follows a circadian pattern in different tissues. Using newly generated and public RNA-Seq data, we monitored circRNA expression throughout circadian rhythm in various mouse brain regions. We demonstrated that *Cdr1as*, despite its stable character, has a highly dynamic expression during the circadian cycle in the mouse suprachiasmatic nucleus (SCN). Computational integration of different transcriptomic data suggests that *Cdr1as* is an important regulator of light-entrainment in the SCN. Additionally, we identified that another brain enriched circRNA, *mbl*, is also substantially deregulated upon light induction in the fly head. Our study highlights potential impact of abundant and conserved circRNAs on maintaining a healthy circadian cycle across species.

O.14

Growth Regulatory miRNAs in Neuroplasticity and development

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Post-transcriptional regulation critically shapes the cellular complement of proteins, but knowledge of molecular pathways for post-transcriptional coordination of gene programs in neurons or locally at synapses is incomplete. Growth regulatory small non-coding RNAs, including the let-7 microRNA (miRNA) family, have evolutionarily conserved roles in control of protein synthesis from pro-growth mRNAs. While functions of the let-7 family have been extensively characterized during development, pluripotency, and in oncogenesis, current work indicates that let-7 miRNAs can also undergo signal-responsive regulation in the healthy adult nervous system during plasticity and following injury. Research in our lab has shown that elevated MAPK-dependent signaling, such as occurs downstream of neurotrophin or excitatory activity, is sufficient to upregulate the Lin28 RNA binding protein from low basal levels in mammalian neurons to coordinately lower let-7 family miRNA levels, which can support the growth of neuronal processes and synaptic connections. At the molecular level, defects in neuronal protein synthesis are implicated in the cellular, synaptic, and cognitive features of several neurodevelopmental disorders, including Fragile X messenger ribonucleoprotein syndrome (FXS), a monogenic autism spectrum disorder (ASD). We demonstrate aberrant control of let-7 family miRNAs during activity-dependent responses in a mouse model of FXS and test the molecular mechanism

O.15

Accurate Isolation of Purkinje cell nuclei: a novel approach for investigating mechanisms of selective neurodegeneration

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Purkinje cells (PCs) are cerebellar neurons that play a pivotal role in controlling voluntary movements, coordination and motor learning. Many human neurological disorders, including multiple forms of spinocerebellar ataxia, are characterized by PC degeneration and loss. The relatively low number of PCs in the cerebellum, which accounts for less than 1% of total cell count, is a bottleneck in studying PC-related pathology. Here, we developed a protocol for selective PC nucleus isolation that combines genetic or immuno labeling of nuclear envelopes with cytometry sorting. First, we crossed SUN1_GFP reporter mice with PCP2-Cre animals to drive SUN1_GFP expression in PCs. We sorted the nuclear population with strong GFP and side scatter signals and confirmed its PC identity by observing strong expression of PC marker genes, small number of nucleoli and increased nuclear size. To improve the method and make it more adaptable to various sources of cerebellar tissue, we replaced genetic tagging with immunofluorescent labeling of a nuclear pore complex protein RanBP2. By analyzing PC marker expression, nuclear size, and nucleolar number, we determined that the population with the strongest RanBP2 signal represents a pure fraction of PC nuclei. To illustrate applicability of our method, we isolated PC nuclei from spinocerebellar ataxia type 7 mice and found transcriptional changes in the cyclic nucleotide signaling pathway. Access to the pure fraction of PC nuclei offers a unique opportunity to study the pathology of PC-related disorders, including the nature of selective neuronal vulnerability.

withdrawn
Deciphering cell type specific molecular aberrations in ALS using human stem cell models

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O.16

RP-CONA (RNA Pull-Down Confocal Nanoscanning) detects small molecules targeting RNA-protein interactions, implicated in Parkinson's disease

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Parkinson's disease (PD) is an incurable neurodegenerative disease that affects all age groups but is most prevalent in elderly individuals, with more than 1% of the population older than 60 years of age suffer from it. The main cause of PD is the overproduction and accumulation of a protein called alpha-Synuclein (alpha-Syn) in the brain cells of affected individuals. There is ample evidence showing that lowering alpha-Syn protein levels can benefit PD patients, and several clinical trials are currently focusing on lowering alpha-Syn levels using experimental medical reagents or vaccines.

One cellular RNA (miR-7) has been shown to negatively regulate alpha-Syn production. We and others have shown that the HuR (ELAVL1) protein is a naturally occurring inhibitor of miR-7 production in human cells binding to miR-7 primary transcripts (pri-miR-7-1). Moreover, HuR directly binds and stabilises alpha-Syn mRNA and therefore further increases alpha-Syn levels in human cells. These findings suggest that disruption of the pri-miR-7/HuR and mRNA alpha-Syn/HuR complexes exerts a positive effect on miR-7 production and a negative effect on alpha-Syn mRNA production, respectively, resulting in a decrease in alpha-Syn protein levels.

Here, we developed a novel screening platform, RNA Pull-Down COnfocal NAnoscanning (RP-CONA), to identify RNA-protein interaction modulators. RP-CONA is based on fluorescent on-bead screening to identify small molecules that modulate the strength of RNA–protein interactions. Our method uses an ultrasensitive RNA–protein pull-down assay with cell extracts to detect RNA–protein complex modulators *via* confocal microscopy. Using RP-CONA, we identified small molecules that disrupt the interaction between HuR and the conserved terminal loop of pri-miR-7-1 as well as fragment of alpha-Syn mRNA. These molecules can upregulate cellular miR-7 levels and downregulate the expression of alpha-Syn. This brings new therapeutic avenues towards treatment of Parkinson's disease as well as provides a novel methodology to search for modulators of RNA-protein interaction.

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O.17

Harnessing the endogenous regenerative potential to 'rejuvenate' the Alzheimer's brain: from microRNAs to single-cell RNA sequencing

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The complex multifactorial and multigenic nature of Alzheimer's disease (AD) presents major challenges to drug development. Given the current scarcity of preventive or disease-modifying therapies, mapping the full mechanistic heterogeneity of AD (beyond β-amyloid and TAU, the two long-thought prime suspects for driving pathology) will be a critical first step to develop novel therapeutic targets. From such a therapeutic perspective, key mechanistic answers as to what renders an individual vulnerable or resilient to developing AD may be 'hidden' in the brains of a significant proportion of elderly individuals, who retain intact cognition despite substantial AD pathology. Such a 'cognitive reserve' can increase resilience towards developing dementia and delay disease onset. Yet, its molecular and cellular determinants are unknown. The hippocampus is one of the primary locations of memory formation and one of the most severely affected in AD. The birth of new neurons in the dentate gyrus of the adult hippocampus, referred to as 'adult hippocampal neurogenesis' (AHN), is one of the few cellular processes linked to ante-mortem cognition in AD patients, pointing towards an active role in the build-up of cognitive reserve, which can later on confer resilience to AD-related dementia. However, the lack of mechanistic understanding of how AHN cross talks with AD in humans remains a major bottleneck in the search for alternative therapeutic approaches to 'rejuvenate' the AD brain. We have identified a microRNA-dependent pro-neurogenic and neurotrophic mechanism, which can be targeted to restore AHN and, thereby, memory deficits in AD mice. Systematic profiling of cell type- and cell state- specific molecular signatures of the presumable adult neurogenic niche in healthy, AD and resilient human brain will shed further light into the mechanistic determinants of vulnerability and resilience to AD.

O.18

Novel RAN translation modifiers of RNA harboring expanded CGG repeats in Fragile X-associated syndrome

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Short nucleotide repeats are robustly distributed in human genome and contribute to pathogenesis of multiple neurodegenerative disorders such as Fragile X-associated tremor/ataxia syndrome (FXTAS). Pathogenesis of FXTAS is driven by expansion of CGG repeats (CGGexp) in the 5'UTR region of the FMR1 gene. One of the proposed molecular mechanism involved in disease progression is repeat associated non-AUG (RAN) translation, which results in the production of toxic glycine rich protein (polyG) derived from expanded CGG repeats. Mechanistic insights of RAN translation remain elusive, therefore we aimed to identify novel RAN translation modifiers. We applied RNA-tagging system and conducted mass-spectrometry (MS) based screening which allowed to capture proteins natively bound to FMR1 mRNA containing CGGexp. MS screening revealed a pool of proteins interacting with this RNA in cellulo and among them we selected two alternative, small ribosomal subunits, RPS25 and RPS26, and verified their regulatory properties in the context of CGG-related RAN translation. Both proteins appeared to selectively regulate the level of polyG in mammalian cells and became promising candidates for targeting repeats-associated toxicity in FXTAS.

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O.19

Precise and accurate allele-specific quantitation of ATXN3 and HTT transcripts in polyQ disease models

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Background: Assessing the expression level of individual alleles is crucial in the context of dominant genetic disorders. Polyglutamine (polyQ) diseases are caused by the expansion of CAG trinucleotide tracts within specific genes. Spinocerebellar ataxia type 3 (SCA3) and Huntington's disease (HD) patients harbor two alleles that differ in the length of CAG tracts: normal (< 30 CAG) and mutant (> 40 CAG). Because CAG repeats are quite common in the human transcriptome, it is challenging to design allele-specific methods and therapeutic strategies that selectively engage CAG sequences in the mutant transcripts.
Results: To precisely quantify expression in an allele-specific manner, we used SNP variants that are linked to either normal or CAG expanded alleles of the ataxin-3 (*ATXN3*) and huntingtin (*HTT*) genes in selected patient-derived cell lines. We applied a SNP-based quantitative droplet-digital PCR (ddPCR) protocol for precise determination of the levels of transcripts in cellular and mouse models of SCA3 and HD. For HD, we showed that the process of cell differentiation can affect the ratio between endogenous alleles of *HTT* mRNA. Additionally, we reported changes in the absolute number of the *ATXN3* and *HTT* transcripts per cell during neuronal differentiation. We also implemented our assay to reliably monitor, in an allele-specific manner, the silencing efficiency of mRNA-targeting therapeutic approaches for HD. Finally, using the humanized Hu128/21 HD mouse model, we showed that the ratio of normal and mutant *HTT* transgene expression in brain slightly changes with the age of mice.
Conclusions: Using allele-specific ddPCR assays we observed differences in allele expression levels in the context of SCA3 and HD. Our allele-selective approach is a reliable and quantitative method to analyze low abundant transcripts and is performed with high accuracy and reproducibility. Therefore, the use of this approach can significantly improve understanding of allele-related mechanisms, e.g., related with mRNA processing that may be affected in polyQ diseases.

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O.20

Identification of circRNAs linked to Alzheimer's disease and related dementias

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder that causes dementia in patients. Even though changes in the mRNA transcriptome is well studied for AD, little is known about the changes in the non-coding RNA. Circular RNAs (circRNAs) are particularly notable because of their selective expression in the brain and differential regulation in neurological disease conditions, including AD. Using RNA sequencing data generated from the hippocampus and cortex, we identified 48 circRNAs significantly associated with AD condition. These circRNAs also exhibited changes in association with indices of tauopathy and cognitive loss suggesting that they affect neuronal biology. We also examined circRNA expression across multiple types of dementia and show that circRNA expression differs by dementia subtype. Out of 12 highly expressed and differentially regulated circRNA, we validated 8 in the cortex, and 5 in the hippocampus. Further, to recapitulate tau-mediated neurodegeneration in AD, oTau toxicity was induced in neural precursors cells. Treatment with oTau downregulated many of the AD-associated circRNAs. Importantly, circAPP and circPSEN1 were downregulated disproportionately to their corresponding linear mRNA. Downregulation of circMAPK9 was particularly striking because expression of the linear mRNA remained unchanged. These results point to tau toxicity as a driver of many changes in circRNA metabolism in AD.

O.21

Intra-tumor heterogeneity and cellular hierarchies in glioma

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O.22

A molecular map of the stress response in the brain tumor glioblastoma

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Objectives: Brain tumors (glioblastomas), are characterized by robust survival of a subpopulation of glioblastoma stem-like cells (GSCs) in a hypoxic tumor microenvironment, and poor infiltration of immune cells. Thus, a recent promising oncolytic herpes virus (oHSV)-based immunotherapy meets inefficacy barriers due to a prevalence of stress-resistant GSCs. We hypothesized that gene signature separating GSC in the context of hypoxic tumor microenvironment would reveal relevant targets of oHSV efficacy, providing clinically-relevant cues on how GSCs thrive in the hypoxic niche being resistant to oHSV immunotherapy. As a proof of concept for co-targeting hypoxic signaling effectors, we singled out an RNA molecule (HIF1A-AS2) that is a vital sensor for the response and adaptation of GSCs to hypoxia.
Methods: We compared (by gene microarray) transcriptomes in the intracranial xenograft tissue in vivo and ex vivo single-cell culture using a heterogeneous patient-derived GSC model. The HIF1A-AS2 targeting (by shRNA) was tested for survival benefits of tumor-bearing animals upon oHSV immunotherapy.
Results: Transcriptome data revealed that the presence of the brain microenvironment shapes two distinguishing characteristics of GSCs: increased cell-to-cell communication with immune response cells and metabolic shift toward hypoxic adaptation, both with signatures predictive of glioblastoma patent survival. HIF1A-AS2 silencing augmented sensitivity to oHSV immunotherapy and improved overall survival of tumor-bearing animals.
Conclusions: The intertwined influence of the tumor microenvironment diminishes therapeutic efficiency by enabling cell adaptation to hypoxia. Thus, co-targeting hypoxia response by the attenuation of HIF1A-AS2 rearranges gene expression, which boosts glioblastoma's sensitivity to therapy.

O.23

A long road from the discovery of oncogenic non-coding RNAs in the brain to glioblastoma therapy development

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The human genome is transcribed to diverse classes of non-protein-coding RNA (ncRNA). However, little is known about their functional interactions in health and disease. The work in the Krichevsky Laboratory focuses on diverse classes of regulatory RNAs in the human brain and brain pathologies, including neuro-oncologic and neurodegenerative diseases such as glioblastoma and Alzheimer's disease. In this talk, I will highlight an ncRNA circuit comprised of miRNA, enhancer-associated RNA, promoter-associated RNA, and snRNA that is activated in the process of astrocyte transformation to glioma brain tumors. In the center of this circuit is miR-10b, a unique miRNA silent in the brain cortex and uniformly upregulated in gliomas, where it assumes an essential tumor-promoting role. miR-10b locus is derepressed in glioma via the mechanism involving 3D chromatin reorganization and CTCF-cohesin-mediated looping. This mechanism requires two interacting lncRNAs, HOXD-AS2 and LINC01116, one associated with the miR-10b promoter and another with the remote enhancer. The interplay of the two lncRNAs in the chromatin folding and concordant regulation of miR-10b and cotranscribed genes, normally silenced in astrocytes, trigger neoplastic transformation toward malignant brain tumors. Once induced, miR-10b becomes essential for glioma cell viability: gliomas exhibit striking dependence on this miRNA. However, despite the therapeutic promise of miR-10b targeting, this miRNA's largely unconventional properties hamper the clinical translation. Using a combination of biochemical and imaging approaches, we discovered that miR-10b localizes in the cell nucleus and binds to U6 snRNA, a core component of the spliceosomal machinery. Our experiments provide evidence of the direct binding between miR-10b and U6, miR-10b-mediated regulation of U6 binding to splicing factors SART3 and PRPF8, and U6 stability, conformation, and levels. These pleiotropic effects on U6 result in global splicing alterations and downstream regulation of cell proliferation and viability. The data suggest an unexpected intersection of lncRNA-mediated chromatin organization, miRNA, and splicing machinery, and a new nuclear function for a critical cancer-associated miRNA. Finally, I will describe our efforts in advancing miR-10b-targeted therapies for malignant brain tumors.

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O.24

Molecular mechanism of action and clinical relevance of circCLIP2 in glioblastoma

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Gliomas have always been one of the greatest challenges for medicine due to their location, significantly impeding the use of conventional methods of diagnosis and treatment. Nowadays, much effort has been made to explore how malignancy develops and to identify key players and mechanisms. Circular RNAs (circRNAs), a type of regulatory RNAs, have been found to play an important role in a variety of biological processes, including cancer. To establish their role in gliomagenesis and GBM progression, we performed RNA sequencing of GBM tissue, which revealed the disruption of several circRNAs in tumor samples. Moreover, with these results, we provided circRNAs expression patterns of specific subtypes of GBM, as a step forward to personalized treatment of GBM patients. One of the differentially expressed circRNAs - circCLIP2, emerges more often in the mesenchymal subtype of GBM, which is considered the one with the poorest patient prognosis. Our results also indicated decreased migration and invasion potential of GBM cells upon circCLIP2 knockdown. Furthermore, we indicated the impact of circCLIP2 knockdown on the EMT process and ECM rearrangement of GBM cells.

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O.25

Epigenetic modulation leads to the decrease of H3K27M oncohistone and transcriptomic changes in pediatric high-grade gliomas

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Pediatric high-grade gliomas (pHGG) are amongst the most challenging cancers to treat, with 5-year survival rates ranging from 1 to 28%. The majority of midline pHGG tumors confer a mutation in histone 3 variants (H3.3 or H3.1) with lysine to methionine substitution (K27M), leading to a global decrease in H3K27 trimethylation, increased H3K27 acetylation and consequent oncogenic changes in gene expression. Chromatin modifying agents, including histone deacetylase (HDAC) inhibitors, have been identified as promising candidates against DIPG. In addition, combinatorial epigenetic treatments involving HDAC inhibitors showed new directions in targeting H3K27M-expressing cells. However, the precise correspondence between the efficient treatments and chromatin response is not completely understood. The aim of our work is to understand the chromatin alterations induced by candidate HDAC inhibitors and particularly the role of histone variants in response to these therapies.

Using multiple cellular models expressing H3K27M histone variant we identified several new drugs with sub-micromolar efficacy in killing H3K27M-expressing cells. We found significant alterations in gene expression of multiple histone variants, including H3F3A, H3F3B, H2AFY and H2AFZ in response to these drugs. In addition, we found surprising modulation of the H3K27M oncohistone protein levels in response to some of the epigenetic drugs, which was dependent on the H2A.Z levels. RNA-seq and H3.3K27M ChIP-seq analysis shows HDAC-dependent deregulation of H3K27M oncohistone and consequent H3K27M-dependent transcriptomic changes.

In summary, direct analysis of the chromatin landscape and particularly expression of histone variants in pHGG expressing the oncohistone H3K27M provides new insights into chromatin response to specific epigenetic treatments. Manipulating the levels of H3K27M becomes a novel aspect in understanding the response of these tumors to epigenetic treatments.

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O.26

Compounds which alleviate the pleiotropic toxicity of RNA harboring expanded CGG repeats in the Fragile X–associated syndrome

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Fragile X-associated tremor/ataxia syndrome (FXTAS) is an incurable neurodegenerative disorder caused by expansion of CGG repeats in the FMR1 5'UTR. The RNA containing expanded CGG repeats (rCGGexp) causes cell damage by interaction with complementary DNA, forming R-loop structures, sequestration of nuclear proteins involved in RNA metabolism and initiation of noncanonical translation of polyglycine-containing protein (FMRpolyG), which forms nuclear insoluble inclusions. During the lecture we will discuss the therapeutic potential of short antisense oligonucleotide steric blockers (ASOs) and small compounds targeting directly the rCGGexp. In nuclei of FXTAS cells ASOs affect R-loop formation and correct miRNA biogenesis and alternative splicing, indicating that nuclear proteins are released from toxic sequestration. In cytoplasm, ASOs significantly decrease the biosynthesis and accumulation of FMRpolyG. Delivery of ASO into a brain of FXTAS mouse model reduces formation of inclusions, improves motor behavior and corrects gene expression profile with marginal signs of toxicity after a few weeks from a treatment. We also identified small compounds, CMBLs, which bind to RNA structure formed by rCGGexp and attenuates translation of toxic FMRpolyG and formation of nuclear inclusions in FXTAS cells. Our results indicate that CMBL4c can reduce FMRpolyG-mediated cytotoxicity and apoptosis. Importantly, its therapeutic potential is also observed once the inclusions are already formed.

O.27

CRISPR-based approaches for the treatment of polyQ diseases

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The combined use of gene transfer and editing technologies has pushed the boundaries of precise genome modification and promoted the development of promising strategies to treat genetic diseases. In the CNS, an increasing number of genetic mutations and susceptibility loci have been identified. Among these, polyglutamine (polyQ) diseases represent a family of autosomal dominant diseases due to CAG triplet expansions in the coding region of various genes. Proof-of-principle experiments in Huntington's disease (HD) have demonstrated that contraction/deletion of CAG expansion in the huntingtin (HTT) gene as well as transcriptional repression or gene inactivation are associated with improved molecular, histopathological and behavioral features. In this presentation, we will describe the development of a second generation optimized AAV-KamiCas9 system and summarize *in vivo* studies of HTT genome editing.

O.28

Gene therapy for the treatment of CNS disorders: miQURE technology

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Central nervous system disorders present a large clinical unmet need. At uniQure, we develop miRNA-based gene therapies to treat various CNS disorders, including Huntington's disease, temporal lobe epilepsy, amyotrophic lateral sclerosis, synucleinopathies and Alzheimer's disease. Our miQURE technology is based on adeno-associated virus (AAV)-mediated delivery of engineered miRNAs, which are designed to specifically knock down disease targets. In addition to single miRNAs, we have recently expanded our portfolio of enabling technologies to include a combination of multiple miRNAs (linQURE), miRNA and protein (goQURE) and vectorized antibodies (AbQURE). In this presentation, I will discuss these innovative technologies as they apply to the various diseases, ranging from preclinical development to phase 1/2 clinical trials.

O.29

Idiosyncrasy of antisense oligonucleotide targeting protein-coding gene embedded with non-coding RNA *in vivo*

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Antisense oligonucleotides (ASO) are powerful tools to alter gene expression and ASO's are even under clinical use and clinical trials to treat human diseases. Whether ASO's targeting protein-coding genes embedded with non-coding RNA's affect non-coding RNA expression or function is relatively unknown. While studying how glial cells regulate axonal morphogenesis in larval zebrafish, we made a serendipitous observation that ASO targeting a splice site of one of our candidate protein-coding genes led to defects in axonal morphogenesis. We observed ASO-induced intron retention events and increased gene expression. The splice site-targeted ASO-induced phenotype was not rescued by candidate gene translation-blocking ASO's, thus potentially ruling out the role of truncated protein. However, the phenotype was rescuable by knockdown of embedded non-coding RNA. We believe our results highlight a blind spot in ASO-based research and calls for a careful evaluation of results from ASO studies when targeting protein-coding genes embedded with non-coding RNAs.

O.30

Manipulation of RNA-misprocessing with CRISPR-dCas13 as a new therapeutic approach in neurodegenerative disorders

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RNA-processing is the molecular mechanism by which precursor messenger RNAs (pre-mRNAs) are capped, spliced and polyadenylated. It is a fundamental process that ensures a correct gene expression pattern and, as such, its misregulation has been implicated in a variety of human diseases. In the lab, we are currently investigating RNA-misprocessing in cellular models and tissues of patients with neurodegenerative diseases in order to develop personalized antisense RNA-based therapeutic strategies using RNA-targeting CRISPR-dCas13 system.

In Frontotemporal dementia, we have combined bioinformatic and experimental approaches to characterize the molecular mechanism of a non-coding mutation in GRN gene, which leads to an aberrant splicing pattern that causes GRN mRNA degradation and progranulin haploinsufficiency. We are currently exploiting CRISPR-dCas13 RNA targeting system in order to identify sequences in GRN pre-mRNA whose targeting using antisense RNAs would restore GRN reading-frame. Based on this concept, we would like to develop a future personalized therapeutic approach for patients with this mutation.