Posters

P.1

Evolutionary analysis of the long non-coding transcriptome of the developing cerebral cortex

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The human outstanding cognitive abilities are computed on the outer surface of the cerebrum, a highly wired network of neurons, neuroglia, and other sustaining cells called the cerebral cortex. This mammalian-specific brain structure has been the place of massively biological innovation throughout evolution, displaying greater plasticity compared to other tissues. Over the past decade, transcriptional and evolutionary aspects of the developing cerebral cortex have been uncovered by comparing transcriptomes of different regions of the cerebral cortex of several mammalian species; however, they have been mostly focused on changes in the expression of protein-coding genes. In parallel, several neuronal-functional long non-coding RNAs (lncRNAs) have been identified. LncRNAs present greater tissue-specificity and evolutionary turn-over than protein-coding genes making them interesting candidates for genomic sources of cerebral cortex plasticity, evolution, and disease. How do changes in the expression of lncRNAs or the de novo expression of new lncRNAs have impacted the evolution of the cerebral cortex? Remains an open question. To characterize the evolutionary changes of the long non-coding transcriptome in the developing cerebral cortex, we used system biological approaches; first, to comprehensively annotate the long non-coding repertory of human, macaque, mouse, and chicken; second to identify the syntenic conservation of the cortical long non-coding repertory in human linage, classifying them into evolutionary groups in the function of the predicted minimal age. Those groups of lncRNAs showed differences in splicing efficiency, locus complexity, type of inserted transposable elements, the functionality of the closest protein-coding and small RNA genes, epigenetic marks, the number and type of regulatory proteins associated with promoters, and expression dynamics, pointing to a difference in their functionality. By combining single-cell and bulk RNA-seq analysis of human samples, the cellular context of the innovation of the lncRNAs expression was unveiled, older lncRNAs showed preferential expression in conserved early neurodevelopmental stages; while Human-specific lncRNAs showed preferential expression in synaptogenic neurons and were co-expressed in gene modules highly associated with the late stages of the cerebral cortex development and with neuropsychiatric disorders. Overall, our results point to a putative role of lncRNAs in the evolution and disease of the cerebral cortex.

P.2

Correction of FTDP-17-associated splicing mutations in MAPT gene *via* siRNA-induced Exon Skipping

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Tauopathies are neurodegenerative diseases marked by the abnormal processing of microtubule-associated protein tau and its accumulation as insoluble neuronal deposits. Tau, encoded by the MAPT gene, regulates several neuronal functions, such as neurite outgrowth, microtubule dynamics and axonal transport. The adult human brain contains equal amounts of tau isoforms with three (3R) or four (4R) repeats of microtubule-binding domains, originating from the alternative splicing of exon 10 (E10) in the MAPT transcript. Several tauopathies are associated with imbalances of tau isoforms due to splicing shortfalls.

Selective degradation of E10-containing MAPT mRNA isoforms is, in principle, possible using exon-specific siRNAs. However, very few examples of successful exon-specific siRNAs are available in the literature. This might be because secondary siRNAs can be produced by RNA-dependent RNA Polymerases in several organisms and cell types, which would generally silence all mRNA splicing isoforms. Here, we evaluated fourteen E10-targeting siRNAs for their efficiency in reverting the inclusion of E10 in MAPT transcripts via High Throughput Screening (HiTS) experiments carried out in immortalised cell lines, and we identified the best molecules. We validated the siRNAs in human and mouse cell lines that produce abnormal excess of 4R tau. Based on these results, we tested the efficacy of our best molecules in hiPSCs-derived neurons from an FTDP-17 patient (hiPSCs-FTDP-17), carrying point mutation E10+16 C->T, in comparison with the appropriate isogenic control (hiPSCs-ISO). Our results suggest a promising potential for the isoform-specific siRNAs employment in human neurodegenerative diseases.

P.3

Expression pattern of circRNA and their putative function in glioblastoma

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Glioblastoma (GBM) is one of the most lethal and the most aggressive malignant brain tumors. Recently, the world of non-coding RNAs, with special regard to circular RNAs (circRNAs), has become a field of intensive research. Their role has been extensively studied in various diseases, and a growing body of evidence shows that their disruption might play an important role in cancer development. CircRNAs can affect cellular processes associated with cancer at many levels. They can sponge miRNAs and proteins, regulate parental gene expression, and eventually, they can also be translated into proteins. The aim of our study was to identify circRNAs differentially expressed in GBM, that are potentially involved in GBM aggressiveness, invasiveness and tumor recurrence. We performed RNA sequencing on GBM primary and recurrent patient-derived tissues as well as on blood samples from GBM patients. We conducted differential analysis, distinguishing dysregulated circRNAs among primary and recurrent GBM samples, and we also managed to establish circular RNAs expression patterns among different GBM molecular subtypes, namely classical, mesenchymal, proneural, and neural. Moreover, we discovered circulating circRNAs with biomarker potential in blood samples. Consequently, we performed functional experiments to determine potential interactions between circRNAs, microRNAs and RBPs. The accomplished global-scaled analysis allowed us to select circular candidates that might serve as putative diagnostic or therapeutic targets in glioblastoma, which nevertheless need further investigation.

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P.4

Characterization of thyroid hormone signaling during early cortical neurogenesis in human cerebral organoids at single cell resolution

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Perturbations of thyroid hormone (TH) signaling during prenatal brain development cause severe neurological phenotypes. Here, we used human cerebral organoids (hCOs) to model the consequences of altered TH signaling for cortical neurogenesis and established strict quality control procedures to minimize experimental artifacts in hCO differentiation experiments (i.e. due to variable TH levels in culture media/supplements). We investigated the specific role of the biologically active hormone T3 for early neurogenesis by differentiating hCOs in the presence of low and high media levels of T3, applied continuously or in a pulsed fashion. hCOs were harvested at various stages and analyzed by single cell RNA sequencing. To control for batch effects and potential hiPSC line-inherent bias, all treatments were replicated in three different hiPSC lines, using lipid hashing of organoid cells for cost-efficient generation of multiplexed single cell libraries. Global analysis of 60k cells identified a large diversity of neural cell clusters, with more than 95% of all cells assigned to a major lineage of excitatory neurons. We also identified and removed a small cortical hem cell population in early stage hCOs and its derivatives (CR cells and choroid plexus) at later stages, as well as cells exhibiting signatures of ER stress and hypoxia. All transcriptome studies were accompanied by extensive validation work using IF staining and confocal imaging to control for proper differentiation and to capture cytoarchitectural changes.

Despite stark hiPSC line-inherent differences in growth rates, differentiation trajectories were highly reproducible across different hiPSC lines. The use of biological triplicates permitted very robust analyses of differentially expressed genes and biological pathways across T3 treatments. T3 media levels clearly affected the proportions of neuronal cell types in hCOs, with higher T3 media levels promoting enhanced generation of upper layer neurons. Higher T3 media levels also increased the number of intermediate progenitors in lieu of radial glia cells, an effect that might stem from T3 stimulation of OXPHOS activity in neuronal progenitors. This first atlas of the T3-responsive transcriptome will serve as a unique resource to propel the use of hCOs for studies on local TH action in

P.5

Determining the mechanisms underlying tanycyte/neuron communication

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Energy balance requires a fine-tuned crosstalk between the periphery and the central nervous system. In the hypothalamus, elongated glial cells called tanycytes line the walls and floor of the third ventricle and extend their processes into the brain parenchyma. This strategic location allows tanycytes to integrate peripheral signals about the metabolic state and modulate neuronal function in consequence. However, the molecular mechanisms underlying tanycyte/neuron communication for energy balance regulation remain unknown.
Using scanning electron microscopy 3D models, we highlight the presence of contacts between tanycytes and neurons. At the interface of tanycyte-neuron contacts, tanycyte processes are enriched in cytoskeleton in the side that contacts a soma, suggesting that filament-mediated transportation could be close to neurons. Tanycyte processes also contain endoplasmic reticulum and ribosomes, which are involved in protein synthesis, and mitochondria, which have been described as docking sites for local translation. Moreover, using *in situ* hybridization and immunohistochemistry, we observed the mRNAs of multiple genes that regulate energy balance, as well as translating ribosomes in tanycyte processes. Finally, preliminary results suggest that the nutritional status modifies tanycyte ultrastructure for local translation.
Our results suggest that tanycyte processes contain ultrastructure that could participate in local translation, paving the way to question if tanycyte local translation could play a role on tanycyte-neuron communication for energy balance regulation.
Keywords: tanycyte; hypothalamus; RNA; arcuate nucleus; energy balance human brain development.

P.6

FUS modulates the level of ribosomal RNA modifications by regulating a subset of snoRNA expression

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FUS is a multifunctional protein involved in many aspects of RNA metabolism. In this study, we show that FUS depletion results in differential expression of numerous small nucleolar RNAs (snoRNAs) that guide 2'-O methylation and pseudouridylation of specific positions in ribosomal RNAs (rRNAs) and small nuclear RNAs (snRNAs). Using RiboMeth-seq and HydraPsiSeq for the profiling of site-specific 2'-O-methylation and pseudouridylation of rRNA species, we demonstrated considerable hypermodification at several sites in HEK293T and SH-SY5Y cells with FUS knockout (FUS KO) compared to wild-type cells. We observed a similar direction of changes in rRNA modification in differentiated SH-SY5Y cells with the FUS mutation (R495X) related to the early-onset disease phenotype of amyotrophic lateral sclerosis (ALS). Furthermore, the pattern of modification of some rRNA positions was correlated with the abundance of corresponding guide snoRNAs in FUS KO and FUS R495X cells. Our findings reveal a new role for FUS in modulating the modification pattern of rRNAs, possibly contributing to generating ribosome heterogeneity, that may constitute a fine-tuning mechanism for translation efficiency/fidelity. Therefore, we suggest that a site-specific increase in the levels of 2'-O-Me and pseudouridylation in rRNAs from cells with the ALS-linked FUS mutation may represent a new translation-related mechanism that contributes to disease pathology.

P.7

A novel lncRNA, prone, regulates neural stem cell reactivation from quiescence *in trans* in the Drosophila central nervous system

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We searched for non-coding RNAs involved in the regulation of Drosophila neural stem cell (NSC) quiescence and proliferation. Using Targeted DamID, we compared genome-wide RNA polymerase II occupancy during neural stem cell reactivation and quiescence and identified a lncRNA we call prone that is strongly upregulated in reactivating NSCs. We found that prone is localised in multiple puncta in the nucleus, suggesting that prone might act in trans to regulate gene expression. We showed that prone plays a role in regulating the timing of NSC reactivation from quiescence. Using RNA-DamID, we identified genome-wide targets of prone in vivo. We correlated genome-wide prone binding with differential gene expression and chromatin accessibility, using a combination single-cell RNA sequencing and chromatin accessibility DamID. We identified genome-wide changes in the expression and accessibility of genes associated with neurogenesis, ribosome biogenesis and cell cycle regulation – key processes implicated in NSC reactivation. Our work demonstrates that prone acts in trans to regulate the expression of a suite of genes involved in orchestrating NSC reactivation, strengthening the evidence for functional roles for lncRNAs *in vivo*.

P.8

LOC339803 lncRNA regulates neuronal inflammation emerging as a novel target for multiple sclerosis

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Multiple sclerosis (MS) is an autoimmune disorder that affects the central nervous system. Immune disease-associated noncoding SNPs, which have been described to often locate in tissue-specific regulatory elements, are emerging as key factors in epitranscriptomic regulation. Indeed, *LOC339803* long noncoding RNA (lncRNA) is located in the MS associated region 2p15, with the MS associated SNP rs11498 positioned very close to an m6A motif.

In the present study, we found that the lncRNA *LOC339803* promotes the expression of the inflammatory cytokine *IL1B* in the neuroepithelial cells SHSY5Y by a tissue-specific m6A-dependent mechanism. We observed that in neurons *LOC339803* binds the C-termini of YTHDC1 reader protein, facilitating its nuclear export via SRSF3. Interestingly, the MS risk allele, presents lower methylation and reduced ability to bind YTHDC1. Additionally, we discovered that the reduction of cytoplasmic *LOC339803* induces the expression of neuronal *IL1B via* mitochondrial hexokinase 2 decrease. Accordingly, we observed a reduction of this lncRNA together with an increase of *IL1B* in demyelinated samples from MS patients, especially in individuals homozygous for the risk allele. Manipulation of *LOC339803* or m6A in an *in vitro* model of MS showed a reduction in the expression of *IL1B*.

To evaluate the potential therapeutic role of *LOC339803 in vivo*, we used a k-mer based approach to find functionally convergent lncRNAs in rat brain. We found a rat lncRNA that presents a very similar k-mer content and function related characteristics with *LOC339803*. As we previously observed *in vitro*, manipulation of the rat lncRNA in primary cortical neurons exposed to excitotoxic agents resulted in a reduction of *IL1B* levels.

Thus, our observations in cell lines, human samples and *in vivo*, shed light on how a noncoding m6A-QTL influences MS phenotype, mediating predisposition to inflammation in a cell-type specific manner and open the door to novel therapeutic approaches for this disorder based on lncRNA and m6A regulation.

P.9

Spatial and temporal expression pattern of selected synapse-enriched circular RNA in the mouse brain

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Circular RNAs (circRNAs) are class of vital non-coding RNAs, that usually are generated from protein-coding genes during back-splicing. Previous studies showed that circRNAs are particularly enriched in the mammalian brain, differentially expressed in different mouse brain regions and often derived from genes specific for neuronal and synaptic function therein. There is a subset of circRNA that are particularly highly abundant at the synaptic areas as revealed in RNA-seq of synptoneurosomes. However, molecular roles and expression patterns of synapse-enriched circRNAs in mouse brain has not been revealed. The aim of this study is to evaluate spatial and temporal expression of synaptosome-enriched circRNAs in the mouse brain. Based on the RNA sequencing data of mouse cortical neurons, cortex and synaptoneurosomes, we selected 18 circRNA candidates for further analysis and here we present evaluation of four of them: 3 novel candidates and 1 already known from the literature. We analyzed their expression pattern in primary neural cells as well as brain tissues at different postnatal stages (P0, P11, P30 and adult) with qRT-PCR and in situ RNA hybridization. We observed that the expression of circRNAs progressively increased in cortex during from the early postnatal stages to the adulthood, yet with different peaks across different transcripts. We found that, circC1 and circC14 are higher expressed in cerebellum compared to cortex, whereas circC3 is higher in the cortex. In situ RNA hybridization showed that circC1 and circC3 can be found enriched in the Purkinje cells and certain cell types in the granular layer of the cerebellum. Using Allen Brain Atlas database, we also found that all analyzed circRNAs have different expression pattern in the mouse cortex and cerebellum as compared to mRNAs transcribed from the same host gene. Based on that characterization, we are moving on to functional studies of these circRNA in the mouse brain.

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P.10

A circular RNA expressed from the FAT3 locus regulates neural development

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Circular RNAs (circRNAs) are key regulators of cellular processes, are abundant in the nervous system, and have putative regulatory roles during neural differentiation. However, the knowledge about circRNA functions in brain development is limited. Here, using RNA-sequencing, we show that circRNA levels increase substantially over the course of differentiation of human embryonic stem cells into rostral and caudal neural progenitor cells (NPCs), including three of the most abundant circRNAs, ciRS-7, circRMST, and circFAT3. Knockdown of circFAT3 during early neural differentiation resulted in alterations in the expression of genes involved in insulin signaling and resistance. Single-cell transcriptomic analysis of 30 and 90 days differentiated cerebral organoids deficient in circFAT3 showed a loss of telencephalic radial glial cells and mature cortical neurons, respectively. Furthermore, non-telencephalic NPCs in cerebral organoids showed changes in the expression of genes involved in neural differentiation and migration, including FAT4, ERBB4, UNC5C, and DCC. *In vivo* depletion of circFat3 in mouse prefrontal cortex using in utero electroporation led to alterations in the positioning of the electroporated cells within the neocortex. Overall, these findings suggest a conserved role for circFAT3 in neural development involving the formation of anterior cell types, neuronal differentiation, and migration.

P.11

The lncRNA Snhg11 is required for synaptic function, neurogenesis and memory and is downregulated in the dentate gyrus of Down syndrome mouse models

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Down syndrome (DS) is the most common genetic cause of intellectual disability. The trisomy of human chromosome 21 (HSA21) underlying the syndrome causes a general perturbation of the transcriptome. This may derive in part from the overexpression of HSA21 transcription factors, chromatin modifying enzymes and non-coding RNAs (ncRNAs) on the gene expression regulatory landscape. However, the study of the particular mechanisms involved in this perturbation has been largely limited to bulk transcriptomic experiments from mixed cell populations while these mechanisms are highly cell-type specific. Here, single-nuclei transcriptional profiles of the hippocampus of a DS mouse model revealed that the trisomy results in a highly cell-type specific alteration of the transcriptome. Strikingly, we observe a major transcriptomic shift in trisomic granule cells from the dentate gyrus (DG), a brain region involved in the encoding of new memories, without important changes in other hippocampal regions. We identified the small nucleolar RNA host gene 11, Snhg11, specifically downregulated in the trisomic DG, as the main contributor to the observed shift. Knockdown of Snhg11 in the DG of wild type mice impaired synaptic function and adult neurogenesis, and was sufficient to recapitulate trisomic hippocampal-dependent cognitive phenotypes. Our work highlights the importance of this lncRNA in intellectual disability, and reveals its functional involvement in the DG. These findings suggest the DG as a main region for the memory deficits observed in DS, and indicate the relevance of new players such as long non coding RNAs in its etiology.

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withdrawn

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Post-transcriptional regulation of LINE-1 retrotransposition by enzymes modifying RNA ends

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LINE-1 retrotransposons are one of the major constituents of human genomic repetitive sequences. These retrotransposons might still create new genomic insertions in genomic DNA in modern humans by a copy-paste mechanism involving RNA intermediates. LINE-1 is tightly regulated by multi-layered regulatory processes to prevent the detrimental effects of LINE-1 insertions on the integrity of the genome. Although transcriptional regulation of LINE-1 expression have been widely studied, the specific influences of general post-transcriptional regulatory processes on LINE-1 remain less well understood. In this work we investigated the role of XRN1 and other factors including DCP2, TUTases, DIS3L2 and deadenylases involved in post-transcriptional regulation of RNA 5' and 3' ends on LINE-1 biology. By using multiple experimental approaches we demonstrate that LINE-1 unlike many other cellular mRNAs is very susceptible to 3' end modifications but less so to degradation. In result of the interconnected processes of deadenylation, uridylation, decapping and reduction of translation of retrotransposonal proteins, LINE-1 retrotransposition is grossly diminished. This mostly results from incapability of deadenylated, uridylated LINE-1 mRNA to initiate reverse transcription in the genomic DNA context, and likely reduction in retrotransposonal proteins' levels but not from decrease in LINE-1 mRNA abundance, which is not observed. We conclude that the general post-transcriptional processes shaping LINE-1 mRNA 5' and 3' ends play the major role in LINE-1 biology.

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P.14

RNA subcellular mislocalization in myotonic dystrophy type 1 (DM1) patient iPSC-derived neurons

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Myotonic dystrophy type 1 (DM1) is a multisystem disorder caused by an expanded CTG triplet repeat in the 3' untranslated region of the dystrophia myotonic protein kinase (DMPK) gene. CUG repeat expansions in DMPK RNA transcripts form ribonuclear foci that dysregulate RNA-binding proteins. In muscles, this causes abnormal splicing, which gives rise to progressive myopathy and myotonia. However, DM1's cognitive symptoms do not appear to be fully explained by missplicing of neuronal transcripts. We hypothesized RNA mislocalization contributes to neuronal phenotypes in DM1. We differentiated neurons from DM1 patient-derived iPSCs and neurotypical controls. Then we utilized a version of subcellular fractionation modified for neuronal cells, followed by RNA sequencing, to characterize RNA subcellular localization. When comparing DM1 to control neurons, we observed hundreds of mislocalized transcripts, most of which became more cytosolic in DM1. These changes could not be fully explained by changes in overall gene expression. We observed that GC content is positively correlated with more nuclear enrichment in DM1. Nuclear-shifted transcripts included many genes important for muscle and/or cardiac development, as well as components of the extracellular matrix, a result that is consistent with DM1 phenotypes. In contrast, cytosolic-shifted transcripts were highly enriched for the "plasma membrane" GO cellular component term and included multiple families of cell surface receptors. Many of these are G protein-coupled receptors, whose translation and transport to the plasma membrane are known to be highly dependent on specific RNA localization, suggesting these changes may have functional consequences at the protein level as well. To our knowledge, this is the first report of receptor dysregulation in DM1 neurons. Furthermore, cell fractionation of iPSCs revealed distinct patterns of mislocalization compared to neurons, suggesting that these changes derive from a neuron-specific mechanism. Our results highlight neuronal RNA subcellular localization defects as an important layer of dysregulation in DM1.

P.15

Surgical aspirate as a new source for glioma stem cell isolation

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Glioblastoma multiforme (GBM) represents one of the most malignant and therapy-resistant tumors and it is the most frequent and aggressive malignancy of the central nervous system. GBM prognosis is generally devastating with a median overall survival of 15 months and a 5-year survival rate lower than 5%. The etiology of GBM is not well-known; consequently, patient's prognosis has not improved much in the last two decades. GBM harbors a small population of dormant but highly tumorigenic self-renewing glioma stem cells (GSCs), which decisively contribute to tumor initiation, maintenance, recurrence and therapy resistance.

GBM diagnosis is made based on molecular and histopathological analysis of resected tissue, which limits the availability of tissue for research purposes. Therefore, it is necessary to establish alternative sources to obtain tumor samples, such as the Cavitational Ultrasonic Surgical Aspirator (CUSA). CUSA is used by neurosurgeons and allows for gentle and precise removal of tumor by fragmenting the tissue whilst irrigating and aspirating continuously into a sterile waste bottle. Typically, this bottle is discarded making this a potential resource of valuable tumor material for research purposes.

In this study, we have explored the use of CUSA aspirate to obtain tumor sample, which recapitulates the molecular hallmarks of the original brain tumor. For that, we have isolated small tumor fragments from the CUSA aspirate, which we have both snap-frozen and dissociated for GSC isolation. Furthermore, a molecular characterization of both samples has been performed in order to validate this approach.

The aim of the study is to improve the procedure to obtain biopsies from GBM patients in the Basque Country in order to perform high-throughput transcriptomic studies that allow us to identify new therapeutic targets.

P.16

Abundance of sno-derived RNAs in patient-derived glioblastoma tissues, their subcellular localization and potential biogenesis

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In the last few decades, knowledge about the role of small nucleolar RNAs (snoRNAs) in tumor development as both suppressors and oncogenes in different cancers is still rising, peculiarly their involvements in gliomagenesis. Glioblastoma (GBM) is one of the most lethal malignant brain tumor in adults with poor median survival of patients constituting less than a year. Mature snoRNAs undergo processing into stable, shorter 20–30 bp fragments, so called sno-derived RNAs (sdRNAs). Research work involving sdRNAs changes in expression provides a range of new potential biomarkers and/or therapeutic targets, although the importance of sdRNAs expression pattern in glioblastoma (GBM) is still rarely known. The major aim of our study was to analyze the expression pattern of sdRNAs and their snoRNAs precursors in patients-derived primary GBM tissues seeking for potential suppressors and oncogenes. According to the snoRNAs potential processing into smaller fragments, we analyzed the secondary structures of snoRNAs and tested the hypothesis of the Dicer, angiogenin and FUS participation in sdRNAs origination. To determine localization of sdRNAs in purpose of their biogenesis and function, subcellular fractionation was performed. To conclude, the negative correlation between abundance of sdRNAs and snoRNAs expression pattern was observed, which might confirm the processing of the snoRNAs into shorter fragments. Also, secondary structures analyses allowed to remark structural elements most favored by enzymes tasking to generate sdRNAs. The localization of sdRNAs fragments confirms the lack of Dicer and angiogenin participation in sdRNAs biogenesis and suggest their nuclear origin and mechanism of action.

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P.17

Diving into the circRNAome of breast-to-brain metastatic cancer and glioblastoma stem-like cells

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Introduction. Breast-to-brain metastatic (BTB met) cancer constitutes a great challenge for modern medicine and science. As the research on protein-coding genes brought only incremental progress in developing therapy against the disease, much attention is now devoted to understanding the role of non-coding RNAs, including a relatively recently discovered group – of circular RNAs, known to shape cellular phenotypes of other malignancies. We thus aimed to determine the relationship between circular RNA signatures of BTB met and cells derived from a deadly primary brain tumor – glioblastoma (glioblastoma stem-like cells – GSCs), along with non-malignant brain cells – neural progenitor cells (NPCs).
Methods. Circular RNAs were identified using the Arraystar platform, and differentially expressed groups were selected for bioinformatics analysis (the cutoff of fold change ≥ 2 and *p*-value < 0.05). The microarray was performed using patient-derived BTB met cancer cells, GSCs of two distinct subtypes – proneural and mesenchymal (P and M GSCs, respectively), and NPCs as a non-malignant control.
Results. In all evaluated cell types, we detected 12,660 circular RNAs. Among these, 1625, 936, and 1352 were significantly deregulated in BTB met cells, P GSCs, and M GSCs, respectively. Interestingly, 547 of those circular RNAs had changed expression simultaneously in all cancer groups when compared to NPCs.
Conclusions. The analysis of the circular RNA landscape showed striking similarities between BTB met cells and GSCs, despite different tissue-of-origin. The circRNAome of BTB met clustered especially closely with mesenchymal GSC – the most aggressive and therapy-resistant subtype of GSCs. As metastasis is associated with the prevalence of cancer stem-like cells that are particularly resistant to chemotherapy, our results suggest circular RNAs' role in shaping these cells' oncogenic traits that effectively limit the treatment avenues.

P.18

Bdnf UTRs regulate its translation and mRNA subcellular localization in neurons

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Brain-derived neurotrophic factor (BDNF) promotes neuronal survival and growth during development. In the adult nervous system, BDNF has important roles in synaptic function, governing several biological processes such as memory formation and food intake. In addition, BDNF has been implicated in development and maintenance of the cardiovascular system. *Bdnf* gene comprises several alternatively spliced untranslated 5' exons and two variants of 3' untranslated regions (UTRs). Using reporter and endogenous *Bdnf* mRNA analyses, we here show that commonly used 5' UTRs, but not 3' UTRs exert a repressive effect on translation. No activity-dependent translation was detected neither in reporter assays nor in analysis of endogenous BDNF protein. *In vivo* analysis using knock-in conditional replacement of *Bdnf* 3' UTR by recombinant 3' UTR from bovine growth hormone (bGH) reveals that *Bdnf* 3' UTRs are required for efficient *Bdnf* mRNA and BDNF protein production in the brain, but act in an inhibitory manner in lung and heart. Finally, we show that *Bdnf* mRNA is enriched in rat cortical and hippocampal synaptoneurosomes, with higher enrichment detected for exon I-containing transcripts. These results uncover two novel aspects in understanding the function of *Bdnf* UTRs. First, our data suggests that the long *Bdnf* 3'UTR does not act as a repressive element in regulating BDNF expression in the brain. Second, exon I-derived 5' UTR has a distinct role in subcellular targeting of *Bdnf* mRNA.

P.19

Identification of RNA binding proteins which influence translational efficiency of toxic FMRpolyG protein in fragile X-associated tremor/ataxia syndrome

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The uncontrolled expansion of short tandem repeats, which are common in human genome, may lead to inherited disorders. For example, in the 5'UTR of fragile X mental retardation 1 (FMR1) gene, healthy individuals possess typically between 5 and 54 CGG trinucleotide repeats, while premutation expansions (55-200 CGG repeats) causes fragile X-associated tremor/ataxia syndrome (FXTAS), and a full mutation (above 200 repeats) leads to fragile X syndrome (FXS).
The pathogenesis of FXTAS remains unclear. One of the possible mechanism is the repeat associated non-AUG initiated (RAN) translation. If present in mRNA, the expanded short tandem CGG repeats (CGGexp) can trigger the production of mutant proteins, without the canonical AUG initiation codon. Resulting aberrant proteins contain long monoaminoacid tracts (i.e. polyglycine, FMRpolyG), aggregate and accumulate in nuclear inclusions in the brain of FXTAS patients, leading to neuronal death.
Despite emerging reports about the possible factors playing role in RAN translation, still little is known about this process. With aim to identify proteins playing a role in RAN-dependent protein biosynthesis in FXTAS, we employed the CGGexp RNA-targeting pull-down approach combined with proteomic analysis. Potential candidates were further stratified based on gene silencing approach in cells expressing FMRpolyG protein. In these models, silencing of 116 kDa U5 small nuclear ribonucleoprotein component (SNRP116) and U3 small nucleolar ribonucleoprotein (IMP3) led to decrease in production of FMRpolyG. This suggests that SNRP116 and IMP3 could be novel regulators of RAN translation.

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P.20

Dysregulated expression of long noncoding RNAs in a novel SCA3 cellular model

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Spinocerebellar ataxia type 3 (SCA3) is a progressive neurodegenerative disorder caused by a CAG repeat expansion in the ATXN3 gene encoding the ataxin-3 protein. Despite extensive research the exact pathogenic mechanisms of SCA3 are still not understood in depth. A growing body of evidence suggest that long noncoding RNAs (lncRNAs) might be implicated in the pathogenesis of neurodegenerative disorder, including repeat expansion diseases. Thus, in the present study, to gain insight into the toxicity induced by mutant ATXN3, we examined the potential contribution of lncRNAs to the pathogenesis of SCA3. We developed a novel isogenic SCA3 models in SH-SY5Y cells using Flp-In T-REx system, stably expressing full-length ataxin-3 with expanded CAG repeats (120CAG, SCA3 model) and performed lncRNAs profiling through RNA-Seq analysis. A total of 63,335 lncRNAs were identified among which 633 lncRNAs (200 upregulated and 433 downregulated) were differentially expressed (DE) between control (expressing ataxin-3 with 20 CAG repeats) and SCA3 model (Padj <0.05; fold change >1.5; TPM ≥ 1). Of these 63% were classified as retained-intron transcripts, 12% as long noncoding intergenic RNAs, 9% as antisense lncRNAs and 15.3% represent other classes of lncRNAs, including processed-transcript, sense-intronic and sense-overlapping. Gene ontology analysis showed that DE lncRNAs were associated mainly with translation, ribonucleoprotein complex biogenesis, nucleocytoplasmic transport or autophagy. The cellular localization, the evolutionary conservation of DE lncRNAs as well as A to I RNA editing in DE lncRNAs were also determined. Based on these bioinformatic analyzes, BCYRN1, GAS5, H19, MALAT1, RMRP and SNHG14 appear to be promising lncRNAs candidates involved in SCA3 pathogenesis.

P.21

Periodontal pathogen *Porphyromonas gingivalis* and its virulence factors gingipains as targets in new therapeutic approaches in Alzheimer's disease

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Epidemiological studies have identified an association between a periodontal disease and Alzheimer disease (AD). However, the nature of this association has been still unclear. Recent works suggest that brain colonization by *P. gingivalis* may link these two inflammatory and degenerative conditions. Evidence of *P. gingivalis* infiltration has been detected in autopsy specimens from the brains of people with AD and in cerebrospinal fluid of individuals diagnosed with AD. Gingipains, a class of *P. gingivalis* proteases, are found in association with neurons, tau tangles, and beta-amyloid in specimens from the brains of individuals with AD. The brains of mice orally infected with *P. gingivalis* show evidence of the oral pathogen infiltration, along with various neuropathological hallmarks of AD. Oral administration of gingipain inhibitors to mice with established brain infections decreases the abundance of *P. gingivalis* DNA in brain and mitigates the neurotoxic effects of *P. gingivalis* infection. Thus, gingipain inhibition, also by RNA-based therapeutics, could provide a potential approach to the treatment of both periodontitis and AD. In our poster presentation, we will summarize available data published in the field.

P.22

microRNA-mediated dysregulation of transcription in Huntington's disease

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Huntington's disease (HD) is a fatal neurodegenerative disorder caused by expansion of CAG repeats in exon 1 of the huntingtin gene (*HTT*), resulting in abnormally long polyQ tract in encoded protein. Transcriptional dysregulation is an early event in the course of disease and has important implications to HD pathogenesis. In this study, we aimed to describe specific molecular pathways leading to transcriptional alterations in HD.
We employed RNA-Seq (including small RNAs) to analyze previously obtained set of isogenic iPSCs (HD, control and *HTT* knockout lines; Dabrowska *et al*., 2020) and iPSC-derived neural stem cells (NSCs). We observed massive changes in genes expression, as well as substantial dysregulation of miRNAs, in HD cell line and in *HTT* knockout, as compared to control line. GO enrichment analyses of differentially expressed genes revealed that deregulated genes in HD cells were mostly associated with DNA binding and regulation of transcription. We reported significant up-regulation of expression of transcription factors (TFs) and transcription regulators in NSCs and neurons but not in non-differentiated cells. Moreover, we identified miRNA that may co-regulate the level of specific transcripts together with TFs by feed-forward regulatory loop. Based on these results we propose new molecular pathways implicated in HD pathogenesis.

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P.23

Generation of isogenic set of iPSC models in the context of dentatorubral–pallidoluysian atrophy

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Dentatorubral–pallidoluysian atrophy (DRPLA) is a rare inherited disease affecting the function of the central nervous system. It belongs to the group of polyglutamine (polyQ) diseases caused by the expansion of CAG repeats in specific genes (another member of this group is Huntington's disease). Patients' clinical findings strictly correlate with the number of CAG repeats in the *ATN1* gene, encoding atrophin-1 protein.
Induced pluripotent stem cells (iPSCs) are commonly used in research concerning the pathogenesis of neurodegenerative diseases, as they can be (I) obtained from patient-derived cell line, (II) additionally genetically modified, and (III) differentiated into neural cells. The goal of this project is to obtain a set of iPSC lines which will be isogenic (they will have the same genetic background), apart from *ATN1* gene that will be in normal, mutant or knock-out version.
Previously generated by us iPSC DRPLA line was used to produce isogenic normal and knock-out models. The genetic editing was performed with the use of the CRISPR-Cas9 system. Different strategies were tested: cultured iPSC cells were electroporated with either a plasmid containing a fragment of normal allele and puromycin selection cassette or RNP complex with proper sgRNA.
DNA sequencing of *ATN1* fragments and western blot with anty-atrophin-1 antibodies, confirmed generation of clonal lines in which mutant *ATN1* (from original DRPLA line) was corrected or knocked-out. Moreover, SNP-based RT-ddPCR assay is used for validation of expression of two alleles of *ATN1* at RNA level. Cell lines characteristics also includes pseudokaryotyping (detection of potential karyotypic abnormalities after genetic editing), as well as immunostaining and RT-qPCR for assessing the expression of pluripotency markers.
Next step of research will include differentiation of iPSCs into neuronal precursor cells and then neurons, especially cortical and striatal. It should give the best possible look into the earliest stages of DRPLA pathogenesis by identification of initial molecular pathways disrupted by the presence of mutant mRNA and mutant protein. Generated *ATN1* knock-out models also should advance understanding of the role of normal atrophin-1 in human cells.

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P.24

A KO mouse model for a lncRNA produces motor neuron alterations and locomotor impairment

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About 40% of long non-coding RNAs (lncRNAs) display brain-specific expression, playing relevant roles in nervous system development and physiology. However, the role of lncRNAs in spinal motoneurons (MNs) is largely unexplored. In this work, we identified a conserved MN-specific lncRNA, whose knockout in mice produces motor impairment and post-natal reduction of mature MNs in the spinal cord. The endoplasmic reticulum (ER)-stress response pathway resulted specifically altered with the downregulation of factors involved in the Unfolded Protein Response (UPR). This lncRNA was found to bind the ER-associated PDIA3 disulfide isomerase and to affect the expression of the same set of genes controlled by this protein, indicating that the two factors act in conjunction to modulate the UPR. Our work revealed new insights on how lncRNAs can contribute to the control of motoneuron homeostasis and function through the regulation of the ER-stress response.

P.25

Identification of irradiation-induced ATM-dependent lncRNAs

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Ataxia telangiectasia (AT) is a complex genetic neurodegenerative disorder. AT is attributed to the deficiency of the protein kinase encoded by the ATM gene, caused by various ATM mutations. ATM is a sensor of DNA double-strand breaks and triggers cell cycle checkpoints and DNA repair machinery. Apart from its role in DNA damage response (DDR), ATM is also involved in numerous phosphorylation cascades and cell signaling pathways, e.g. cellular and redox homeostasis and the regulation of mitochondrial function. ATM dysfunction in the abovementioned processes is the cause of progressive neurodegeneration in AT patients. Still, some aspect of ATM function, e.g. the role of ATM-dependent long non-coding (lnc) RNAs, is not well understood.

In our study, we aimed to verify the hypothesis that ATM-dependent lncRNAs are essential players involved in DDR. DNA damage was induced by ionizing radiation (IR) in immortalized lymphoblastoid cell lines derived from 4 AT patients and 4 healthy donors. Cells were collected 1h and 8h after IR to allow identification of lncRNAs involved in the early and late response to DNA damage. RNA-seq identified several lncRNAs and mRNAs induced 1h and 8h after IR in the control group, but not in AT patients. Gene Set Enrichment Analysis revealed delayed induction of key DDR pathways in AT patients compared to controls. Based on Transcription Factor ChIP-seq ENCODE data, we found 71 TFs with binding sites within 1kb from DE lncRNAs. The majority of TFs are involved in pathways connected with DNA repair pathways. Inhibition of ATM with the specific inhibitor KU-60019 proved that those lncRNAs are dependent on ATM. We observed that induction of lncRNAs after IR preceded changes in expression of adjacent genes involved in DDR. This indicates that IR-induced lncRNAs may regulate the transcription of nearby genes. Subcellular fractionation revealed that the majority of studied lncRNAs are localized in chromatin. In conclusion, we identified lncRNAs induced in response to DNA damage in an ATM-dependent manner. Next, we want to verify that detected lncRNAs can modulate the neurodegeneration process.

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P.26

Novel mouse models of Huntington's disease distinguishing mHTT transcript from protein toxicity

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Concept: RNA toxicity remains an unanswered question in pathology of Huntington's disease (HD). New aspects of HD pathology need to be determined to bring additional targets and leads for therapeutic approaches. Cell-specific expression of mutant huntingtin gene (*mHTT*) allows to study unique mechanism in determined environment. Our models allow for separation of pathogenic pathways caused by mutant RNA alone from overall pathogenesis and enable to carry out a number of precise molecular analyses.

Results: We have generated two HD mouse models that contain four first exons of *HTT* gene and mutation of 100 CAG repeats: in non-translated version (HD100CAG) and in translated version (HD100Q). The advantage of these models is the Cre/lox system. Additionally visualization of mutant RNA and protein is enabled with the use of specific tags. We have followed up our mouse models throughout 20 months and used broad spectrum of physiological, molecular, behavioral and cognitive methods every 4 months. Behavioral testing shows progressive phenotype of created models reflecting human condition. In different time points rotarod, static rod, open-field tests revealed motor deficits during light phase while ActiMot indicated hyperkinesis during dark phase. Furthermore, changes in organ weight, microbiome and immune phenotype have been found. HD100CAG and HD100Q show also some molecular hallmarks of HD.

Conclusions: We have generated two new unique mouse models of HD that distinguish between pathogenic roles of *HTT* transcript and protein. These models have been fully characterized using vast spectrum of behavioral and molecular methods. HD100CAG and HD100Q are also suited for investigation of cell- and tissue-specific HD initial pathogenic pathways. This will allow not only to understand relations within central nervous system but can further advance studies determining peripheral aspects of HD.

P.27

Understanding the course of ALS – identification of SOD, FUS, Lsm10 and Lsm11 proteins in *Dictyostelium discoideum*

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Amyotrophic lateral sclerosis (ALS) is an uncurable neurodegenerative disease which leads to degeneration of motor neurons and in consequence to muscle atrophy. ALS is treated only symptomatically. Patients usually live only about 3 years from noticing first symptoms. The disease can occur both in sporadic and genetic variant (when at least one family member was diagnosed with ALS)[1].

So far, a few genes which mutations play an important role in the course of ALS, have been discovered. Among them there is a gene encoding Zn-Cu superoxide dismutase (SOD[1,2] – a protein that catalyzes the conversion of superoxide into oxygen and hydrogen peroxide[3]. Another protein related to the course of ALS is fused in sarcoma (FUS)[4]. In multicellular organisms FUS interacts with U7 snRNA/snRNP and regulates the expression of histone genes by stimulating transcription and the unique 3' end maturation of their pre-mRNAs. Mutations of FUS lead to its relocation along with U7 snRNA/snRNP to the cytoplasmic aggregates. Lsm10 and Lsm11 proteins are a part of U7 snRNP complex[5].

The aim of this project was to identify and determine the expressions of Mn-SOD, FUS, Lsm10 and Lsm11 proteins in three stages of life of a slime mold *Dictyostelium discoideum*: unicellular, aggregation and streams phases. *D. discoideum* is a model organism that can be used for fundamental research, as its genes are highly homological to the human ones. It occurs both in the form of unicellular and multicellular structures[2].

The following methods were used: Bradford protein assay of samples containing mitochondrion (Mn-SOD) and nucleus fractions (FUS, Lsm10 and Lsm11), SDS-gel electrophoresis, Western blot and immunodetection with usage of Saccharomyces cerevisae antibodies against Mn-SOD and rabbit antibodies against FUS, Lsm10, Lsm11.

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P.28

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.

P.29

Identification of RNA-protein interactions in the mouse brain tissue

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The functions of RNA, including the various classes of non-coding RNAs, can be better understood by studying their interactions with proteins. RNA binding proteins (RBPs) can interact with RNA and form ribonucleoprotein complexes. RBPs are involved in the regulation of the RNA life cycle at the level of transcription, splicing, translation, modification, cellular transport and degradation, therefore key to cellular homeostasis. RBP malfunctions underly many diseases, which is especially recognized in neurodegeneration and other neurological conditions[1]. Providing insights into perturbed RNA:protein interactions hold promise to substantially enrich our understanding of these complex diseases and help to find new treatment options. In our project, we are interested in identifying mouse brain-specific RBPome. We apply XRNAX protocol[2], which is based on phase separation of RNA-protein complexes subsequent to UV crosslinking of RNA-protein contacts. We compared two different homogenization methods of brain tissue prior to UV crosslinking to capture RNA-protein complexes globally. We identified one of them as more reproducible and efficient in the enrichment for RNA:protein complexes as measured on both RNA and RBP levels. More detailed mass spectrometry analyses demonstrated the successful capture of >400 RBPs and the potential of the XRNAX method in combination with the appropriate preparation of mouse brain tissue for the identification of their association with RNA in the brain. Our results provide new insight into the brain's RNA interactome and await to be used to study mouse models of human neurological disorders.

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P.30

Correction of FTDP-17-associated splicing mutations in MAPT gene *via* siRNA-induced Exon Skipping

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Tauopathies are neurodegenerative diseases marked by the abnormal processing of microtubule-associated protein tau and its accumulation as insoluble neuronal deposits. Tau, encoded by the MAPT gene, regulates several neuronal functions, such as neurite outgrowth, microtubule dynamics and axonal transport. The adult human brain contains equal amounts of tau isoforms with three (3R) or four (4R) repeats of microtubule-binding domains, originating from the alternative splicing of exon 10 (E10) in the MAPT transcript. Several tauopathies are associated with imbalances of tau isoforms due to splicing shortfalls.

Selective degradation of E10-containing MAPT mRNA isoforms is, in principle, possible using exon-specific siRNAs. However, very few examples of successful exon-specific siRNAs are available in the literature. This might be because secondary siRNAs can be produced by RNA-dependent RNA Polymerases in several organisms and cell types, which would generally silence all mRNA splicing isoforms. Here, we evaluated fourteen E10-targeting siRNAs for their efficiency in reverting the inclusion of E10 in MAPT transcripts *via* High Throughput Screening (HiTS) experiments carried out in immortalised cell lines, and we identified the best molecules. We validated the siRNAs in human and mouse cell lines that produce abnormal excess of 4R tau. Based on these results, we tested the efficacy of our best molecules in hiPSCs-derived neurons from an FTDP-17 patient (hiPSCs-FTDP-17), carrying point mutation E10+16 C->T, in comparison with the appropriate isogenic control (hiPSCs-ISO). Our results suggest a promising potential for the isoform-specific siRNAs employment in human neurodegenerative diseases.

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Oxidative stress implications in glioblastoma multiforme after purine derivatives treatment

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Glioblastoma multiforme (GBM) is the most widespread and major invasive brain tumor in adults[1]. Due to their infiltrative nature, limited drug uptake in tumor cells, and tumor resistance to chemotherapy show poor prognosis in glioma patients[2]. Thus, there is an immense need to develop new therapeutic agents that affect the metabolism of GBM cells to enhance their responsiveness to drugs[1-4]. Recently, small compound-based therapies have provided new insights into the treatment of glioblastoma multiforme (GBM) by inducing oxidative impairment[1]. Thus, there is a pressing need to design new therapeutic small molecular weight compounds that can modulate the redox status of GBM cells and induce cell death through oxidative stress and the apoptosis pathway.

Kinetin riboside (N6-furfuryladenosine; KR) and its newly designed derivatives (8-azaKR, 7-deazaKR) selectively affect the molecular pathways crucial for cell growth by interfering with the redox status of cancer cells. Previously we confirmed the complexity of the mechanism of action of KR, and we determined its effect on mitochondrial bioenergetics in HepG2 cells[5]. KR exerts a powerful anticancer effect and has an impact on molecular pathways that are crucial for cell increase, proliferation, and induction of cell death[5, 6]. It is also a member of the purine analogue family, in which every compound may show an inimitable mechanism of action in neoplastic cells.

Here, we show the effect of KR and its derivatives on the redox status of T98G GMB cells in 2D and 3D cell cultures. The use of spheroids of T98G cells (3D) which offered an *in vitro* system that mimicked the cancer cell environment enabled us the selection of one derivative-7-deazaKR-with comparable antitumor activity to KR. Both compounds induced ROS generation and genotoxic OS leading to apoptosis. Our results demonstrate that KR and 7-deazaKR are effective anticancer agents and they might serve as a potential alternative in oxidative therapy by focusing on the cellular redox environment of GBM cells[7].

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A CAG repeat-targeting artificial miRNA lowers the mutant huntingtin level in the YAC128 model of Huntington's disease

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Background. Among many proposed therapeutic strategies for Huntington's disease (HD), allele-selective therapies are the most desirable and the most challenging at the same time. We have previously demonstrated that RNA interference (RNAi) tools that target CAG repeats selectively reduced the mutant huntingtin level in cellular models of HD.

Aim. The purpose of this study was to test efficacy, selectivity and safety of two vector-based RNAi triggers in an animal model of HD.

Methods/Techniques. CAG repeat-targeting short hairpin RNA (shRNA) and artificial miRNA (amiRNA) were delivered to brains of YAC128 mouse model in two doses via intrastriatal injections of AAV5 vectors. Vector genome copies, protein and transcript levels in the striatum, hippocampus and cortex were analyzed four months post injection. Behavioral tests were performed every five weeks post injection. Activation of toxicity markers and protein aggregates were analyzed by immunohistochemical staining of brain tissues.

Results/Outcome. Molecular tests demonstrated that both shRNA and amiRNA reduced the level of mutant huntingtin to 50% without an influence on endogenous mouse huntingtin. We observed concentration-dependent reduction of HTT aggregates in the striatum and an improvement of motor performance using a static rod test. Expression of mutant huntingtin has previously been shown to increase organ weight. Interestingly, a treatment with amiRNA reduced the spleen weight to values characteristic of healthy mice (WT). In contrast to shRNA, amiRNA was well tolerated and did not reveal any signs of toxicity during the course of the experiment.

Conclusions. We confirmed that vector-based RNAi molecules targeting CAG tracts can be used to lower mutant huntingtin levels *in vivo* in an allele-selective manner. The amiRNA molecule has been shown to be effective, selective and safe. Therefore, this strategy could make an original and valuable contribution to currently used therapeutic approaches for HD.

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In vivo investigation of microRNA expression and function at the mammalian synapse by AGO-APP

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MicroRNAs (miRNAs) are short 20-23 nucleotide long non-coding RNAs, there are 2605 miRNA in Humans and 1936 miRNA in Mouse in total (miRBase). Nervous system express most abundant miRNA and most diverse. MiRNAs play role in many steps during neurogenesis like cell proliferation, differentiation, neural patterning and axon pathfinding etc. Moreover, *in vitro* studies suggested a role in the regulation of local translation at the synapse thus controlling neuronal plasticity. However due to the specific structure of miRNAs molecules, an *in-vivo* confirmation of the general role of miRNAs in the control of neuronal plasticity is still pending. For example, their small size and their high level of sequence homology make difficult the analysis of their cellular and sub-cellular localization *in-vivo* by *in-situ* hybridization. Moreover, it was found that only 40% of the expressed miRNA molecules in a cell are included in RNA-Induced Silencing Complexes (RISC) and therefore involved in inhibitory interactions while the rest is silent. Definitively, development of new tools is needed to have a better understanding of the cellular function of miRNAs, in particular their role in neuronal plasticity. Here we describe a new technique called *in-vivo* AGO-APP designed to investigate miRNA expression and function *in-vivo*. This technique is based on the expression of a small peptide derived from the human RISC-complex protein TNRC6B, called T6B, which binds all known Argonaute (Ago) proteins with high affinity allowing the efficient immunoprecipitation of AGO-bound miRNAs. We have generated 2 transgenic mouse lines conditionally expressing T6B either ubiquitously in the cell or targeted at the synapse. Comparison of the repertoire of miRNAs immuno-precipitated from mature neurons of both mouse lines will provide us with a list of miRNAs showing a specific activity at the synapse. The physiological role of these miRNAs will be subsequently addressed through gain and loss of function experiments.