Session 19: RNA deregulation in disease and RNA therapeutics

Lectures

L.19.1

RNA dysfunction and deregulation in polyglutamine diseases

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Huntington's disease (HD) is a fatal neurodegenerative disorder caused by the expansion of CAG repeats in the *HTT* gene, resulting in a long polyglutamine (polyQ) tract in the encoded protein. In this study, we searched for networks of deregulated RNAs contributing to initial transcriptional alterations in HD neuronal cells.
We employed RNA-Seq (including small RNAs) to analyze set of isogenic, iPSCs-derived neural stem cells (NSCs). We observed numerous changes in genes expression, as well as substantial dysregulation of miRNAs, in HD cell line and in *HTT* knockout (*HTT*-KO), as compared to control line. GO enrichment analyses of DEGs revealed that up-regulated genes in HD and *HTT*-KO cells were associated with DNA binding and regulation of transcription. For these both models, we reported substantial up-regulation of expression of transcription factors (TFs) and transcription regulators: *TWIST1*, *SIX1*, *TBX1*, *TBX15*, *MSX2*, *MEOX2* and *FOXD1* in NSCs and MSN-like cells. Moreover, we identified miRNAs: miR-214, -199a, and -9 as co-regulators of the level of specific transcripts together with TFs by a feed-forward regulatory loop. Based on the comparison of HD and *HTT* knock-out cell lines, we propose that transcriptional deregulation in HD results from a combination of both: gain and loss of huntingtin function.

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L.19.2

Deregulated expression of noncoding RNAs resulted from mutations in FUS protein as a potential molecular mechanism underlying amyotrophic lateral sclerosis

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FUS is a multifunctional protein involved in many aspects of RNA metabolism; it also mediates genomic maintenance, DNA recombination, and DNA repair. Numerous *FUS* mutations have been found in familial forms of amyotrophic lateral sclerosis (ALS). Mutations lead to the accumulation of the protein in cytoplasmic inclusions and the disruption of its nuclear activity. This, in turn, can abrogate the function of noncoding RNAs interacting with FUS.
We have reported that FUS interacts with U7 snRNA/snRNP and participates in replication-dependent histone gene expression (Raczynska *et al*., 2015) and furthermore we have shown that the function of both of them is disrupted due to ALS-FUS mutations (Gadgil *et al*., 2021). In addition, we have recently published that FUS can regulate the expression of numerous snoRNAs that guide the 2'-O methylation and pseudouridylation of rRNAs and snRNAs. The modification status at various rRNA positions was considerably changed in cells with the ALS-linked FUS mutation, which, in turn, can influence the ribosome heterogeneity and can underlie disease development and progression due to impaired translation efficiency/fidelity (Gawade *et al*., 2023). Moreover, according to our unpublished results, deregulated expression of several sdRNAs, lincRNAs, and transposable elements can be observed in cells with ALS-FUS mutations, suggesting their role in neurodegeneration as well.

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Oral presentations

O.19.1

MCPIP1 as a modulator of the RNA landscape in cutaneous squamous cell carcinoma

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Monocyte chemotactic protein-1-induced protein 1 (MCPIP1) is an RNase with strong anti-inflammatory properties. It negatively regulates the stability of transcripts coding for pro-inflammatory cytokines. We previously demonstrated that in mice loss of MCPIP1 specifically in keratinocytes ($Mcpip1^{EKO}$) aggravates the phenotype of chemically induced skin tumors. The aim of this study was to provide mechanistic understanding of the functional interaction between MCPIP1 and non-coding RNA molecules (ncRNAs) in the course of skin cancer.

We performed global analysis of total RNA including micro RNAs (miRNAs), circular RNAs (circRNAs) and long non-coding RNAs (lncRNAs) levels in chemically generated skin tumors in the control and $Mcpip1^{EKO}$ mice. Loss of epidermal MCPIP1 resulted in significant changes in the expression profile of all classes of ncRNAs. We identified 260 lncRNAs, 21 miRNAs and 222 circRNAs that were significantly upregulated (fold change >1.5) in $Mcpip1^{EKO}$ tumors compared to the control ones. Subsequent studies were focused on ncRNAs that are highly conserved in human. Comprehensive bioinformatic analyses indicated that 141 of the shortlisted mouse lncRNAs had human homologues. We next utilized several *in vitro* models of human keratinocytes to investigate molecular mechanisms driven by selected ncRNAs.

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O.19.2

lncRNA expression pattern in cell lines and in the TCGA model: searching of potential modifiers of response to radiation and biomarkers

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Radiotherapy is one of the main strategies of head and neck squamous cell carcinomas (HNSCC) treatment. In spite of knowledge about HNSCC mutations and new treatment approaches, no dramatic changes in patients' survival were achieved [1]. However, some epigenetic elements such as lncRNAs are promising targets for improvement of radiotherapy [2,3,4]. The aim of the project is to study the lncRNAs as potential radiobiomarkers.

FaDu and Detroit cell lines were irradiated and RNAseq was done. Next, the RNAseq results were validated using the TCGA-based model with a group of patients with response (RG) and no response (NRG) to radiotherapy.

Changes in the expression of lncRNAs are more stronger than protein coding genes and they are e dose dependent. Dysregulated lncRNAs were associated with cellular processes, such as cell cycle regulation, the p53 pathway, DNA damage and repairs mechanisms, cell death and autophagy, regulation of reactive oxygen species. Moreover, the RG group of patients displayed a different lncRNA expression pattern than the NRG group.

Observed differences in lncRNA expression are strictly associated with DNA damage pathway and response to this cellular stress. Description of these changes could help in the prediction of patients' response to radiotherapy in the future.

References

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O.19.3

Structural studies of small ligands targeting disease-related RNA molecules

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The presented work is a part of our crystallographic studies focused on structural analysis of interactions between disease related RNA and synthetic molecules. Although the recent progress in high-throughput screening of small molecule libraries resulted in discovery of a number of drug-like compounds their further improvement requires determination of three-dimensional structures unravelling the details of RNA-ligand interactions.
We will present crystallographic studies of complexes of small ligands and RNAs associated with neurodegenerative disorders called TREDs (Trinucleotide Repeat Expansion Disorders). The abnormal expansion of repeated sequences located within certain genes results in mutated mRNAs gaining pathogenic properties. The specific binding of small molecules to mutated RNA can block pathological pathways preventing disease progression. We analysed a series of small molecules recognizing unique pattern of nucleobases engaged in non-canonical pairing or located in single stranded regions of repeated RNA sequences. Structures of the complexes allowed detailed characterization of interactions between the ligand and RNA indicating how small compounds can be improved for future biomedical studies.

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Posters

P.19.1

Novel mouse models of Huntington's disease distinguishing transcript and protein toxicities

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Huntington's disease (HD), caused by CAG repeat expansions in HTT gene, belongs to the group of neurodegenerative polyglutamine (polyQ) diseases. The pathogenic gain-of-function effect of mutant polyQ protein has been already established. However, the contribution of mutant RNA to the pathogenesis of HD still remains ambiguous. To resolve this, we have generated two unique HD mouse models using a knock□ in strategy into the Rosa26 locus that allows for the separation of pathogenic effects caused by mutant RNA alone from overall pathogenesis. These models contain four first exons of human HTT gene and mutation of ~100 CAG repeats in non-translated version (HD/100CAG) and in translated version (HD/100Q). In addition, HA tag and MS2 aptamer are included for visualization of protein and transcript.The cohorts of animals were analyzed with a broad spectrum of molecular, behavioral and cognitive tests, every 4 months for 21 months. Behavioral testing showed a progressive phenotype in created models with more severe phenotype in the HD/100Q model. Rotarod, static rod and open-field tests performed at different time points revealed motor deficits during the light phase, while ActiMot indicated hyperkinesis during the dark phase. Both models also displayed molecular neuropathological changes in the striatum as indicated by nCounter analysis. In summary, using these novel in vivo models we demonstrate the significant involvement of CAG repeat-mutant RNA in the pathogenesis of HD.

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P.19.2

Deciphering the molecular mechanisms underlying the sdRNAs synthesis mediated by FUS

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Small nucleolar ribonucleoproteins (snoRNP) are nucleolus-localized complexes involved in rRNA modifications, that consist of small nucleolar RNAs (snoRNAs) and associated core proteins. A growing amount of evidence indicates that snoRNAs are processed into shorter, stable and functional molecules, called sdRNAs - small RNAs derived from snoRNAs. However, the mechanism of sdRNA generation is still not completely understood. Recently, by RNA immunoprecipitation followed by high-throughput sequencing (RIP-seq), Fused in Sarcoma (FUS) protein was found to bind snoRNAs in human cells. Further analysis revealed that FUS negatively regulates the level of mature snoRNAs. FUS might compete with snoRNP proteins and induce the synthesis of sdRNAs, which in turn, leads to a decreased level of mature snoRNAs. The main goal of the project is to elucidate the molecular mechanisms of sdRNAs synthesis mediated by FUS.
During the conference, I will present the results of RNA antisense purification (RAP) of two selected endogenous, differentially expressed snoRNAs with corresponding, stably expressed, control snoRNAs using biotinylated complementary oligonucleotides and streptavidin magnetic beads, followed by protein identification by mass spectrometry. Experiments were performed in SH-SY5Y cells with FUS knockout in comparison to wild-type cells. Detailed analysis of the pulldown protein fraction will enable to distinguish proteins that are recruited to the snoRNP complex by FUS.

P.19.3

Alzheimer's disease biomarker candidate miR-200a-3p synchronizes the regulation of neurodegenerative pathways and the cell cycle

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Pathogenesis of Alzheimer's disease (AD), the most common incurable neurodegenerative disorder, is complex and involves microRNAs (miRs), short non-coding RNAs that bind target mRNAs and mediate their degradation by RNA interference. Some miRNAs can be released from cells to blood. Recently we identified hsa-miR-200a-3p as one of the most significantly upregulated miRNAs in blood plasma of early AD patients (doi: 10.18632/oncotarget.15109). The aim of the present study was to investigate functions of miR-200a-3p in AD. Using PubMed and bioinformatics (miRBase and TargetScan), we predicted target mRNAs of miR200a-3p and validated them with luciferase assay and RT-qPCR in human HEK293T cells transfected with miR-200a-3p mimic. miR-200a-3p downregulated in dose-dependent manner transcripts encoding key proteins associated with neurodegeneration such as β-secretase BACE1, Ataxin-1, FUS, and PANK3. Moreover, miR-200a-3p caused upregulation of the cell cycle kinase 2 (CDK2) and downregulation of proapoptotic tumor suppressor TP53, resulting in enhanced cell proliferation. These data indicate that miR-200a-3p orchestrates the regulation of neurodegeneration and of the cell cycle and apoptosis, and support the hypothesis that neurodegeneration may be a process similar to carcinogenesis. As miR-200a-3p seems associated with AD pathomechanism, it can be developed as an early AD diagnostic biomarker.

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P.19.4

lncRNAs in neoplasms of the head and neck area and their biological and diagnostic significance

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In the case of head and neck squamous cell carcinomas (HNSCC) common risk factors are alcohol consumption, tobacco smoking and human papilloma virus (HPV) infection [1]. Deregulation of lncRNAs in many diseases, including HNSCC was previously observed [2,3,4]. The aim of the project is to study the lncRNAs profile in HNSCC to determine their function and usefulness as diagnostic or prognostic disease biomarkers.
The TCGA and GEO database were analyzed to list the most up- and down-regulated lncRNAs in HPV- and HPV+ patients. Next, association between lncRNAs and clinical-pathological parameters were defined.
We have found that UCA1 was significantly higher expressed in the HPV(-) patients and ANRIL was correlated with the HPV(+). It was noted that SNHG6 and TTTY14 were connected with the active viral status group whereas correlation with the inactive viral status group for Dio3os was observed. Only lower expressions of Jpx and LINC00152 as well as higher expressions of TTTY14 and TTTY15 were correlated with better survival.
These results show how the role of lncRNAs in HPV infection is diverse and that these lncRNAs may be great potential biomarkers and targets for future therapies.

References

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