Session 6: Non-coding RNA and Gene Expression

Lectures

L6.1

Biogenesis and function of plant microRNAs

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MicroRNAs (miRNAs) are small RNA molecules that play a key role in plant gene expression regulation. The majority of microRNAs are encoded by independent transcription units transcribed by RNA polymerase II. Primary transcripts of miRNA genes undergo extensive co-transcriptional/post-transcriptional processing to generate mature miRNAs. The main player in plant pri-miRNA processing is the microprocessor, a multiprotein complex that cleaves pri-miRNAs in two consecutive steps, generating the stem-loop structure containing miRNA and miRNA* in the first step, followed by the excision of a duplex composed of miRNA/miRNA* in the second cleavage reaction. The RNA duplexes are exported from the nucleus to the cytoplasm, where they are incorporated into the RISC complex. miRNA guides RISC to cognate mRNAs leading to mRNA cleavage or translation inhibition.
The levels of miRNAs must be under tight control, since the main classes of mRNAs that are regulated by these molecules encode diverse types of transcription factors responsible for plant development and responses to environmental cues. The simplest way to regulate miRNA level is via transcriptional control. However, for many plant miRNAs it has been observed that under varying environmental conditions there is a broad response at the level of pri-miRNAs, whereas mature miRNA level is rather confined. These observations lead to the conclusion that in many instances, the level of a particular mature miRNA could not be predicted based on the level of its pri-miRNA. This points to an essential role of post-transcriptional events in miRNA expression regulation. Recent discoveries showing new layers of posttranscriptional regulation of microRNA level and function will be presented.

L6.2

Copy number variation and other types of genetic alterations in miRNA genes and their potential implications for miRNA function and human diseases

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Micro-RNAs (miRNAs) are short non-coding RNAs that post-transcriptionally regulate (usually downregulate) expression of a substantial fraction of protein-coding genes. Hundreds of miRNAs expression profiling studies have been performed, and it was convincingly demonstrated that particular miRNAs are involved in the regulation of many biological processes, including pathogenesis of human diseases, especially cancers. However, disproportionately little is known about the genetics (genetic variation) of miRNA genes. It is reflected in just a few causative mutations identified in miRNA genes associated with Mendelian diseases. To answer the reason for this gap, we will present current knowledge about genetic variation of miRNA genes, including mutations associated with rare Mendelian diseases, single nucleotide polymorphisms (SNPs), copy number variants (CNVs), and somatic variation occurring in cancer cells. We will also discuss how genetic variants may affect the function of miRNAs.

Oral presentations

O6.1

Arabidopsis thaliana mRNA adenosine methylase (MTA) is an important player in miRNA biogenesis regulatory pathway

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Methylation of adenosine at N6 position (m^6A) is the most abundant mRNA modification. Recently researchers have identified m^6A as a mark for further processing of pri-miRNAs in animals. In this study we aim to identify the role of MTA and m^6A methylation in plant miRNA biogenesis. Using NGS to sequence small RNA's (in a hypomorphic *mta* mutant), 37 miRNAs whose levels were significantly changed in the mutant were found (33 of which were downregulated). RT-qPCR for pri-miRNAs in the mutant showed that out of 298 pri-miRNAs more than 50% were upregulated. m^6A-IP followed by RT-qPCR revealed that the pri-miRNA methylation levels are lower in the hypomorphic *mta* mutant, indicating that the pri-miRNAs carry the m^6A mark. RNA-IP using MTA-GFP tagged Arabidopsis plants showed enrichment of pri-miRNA in the MTA-GFP line, indicating that MTA binds to pri-miRNAs. Using Yeast Two Hybrid system, interactions of MTA with several proteins involved in very early stages of miRNA biogenesis were identified. Interactions between MTA and and some of them were confirmed using FLIM-FRET. The results indicate that m^6A methylation and MTA affect miRNA biogenesis in plants. To study this role further, m^6A-IP and RIP will be followed by high throughput sequencing experiments to understand the influence of MTA/m^6A modification on plant miRNA biogenesis.

O6.2

A possible direct involvement of Dicer ribonuclease in the post-transcriptional control of gene expression

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Dicer is the ribonuclease III enzyme that plays a fundamental role in the biogenesis of small regulatory RNAs (srRNAs), such as microRNAs (miRNAs) and small interfering RNAs (siRNAs). A number of recent reports have demonstrated that the activity of Dicer may be associated not only with the RNA interference pathway and production of srRNAs. For example, it has been found that Dicer may participate in chromosome fragmentation during apoptosis, chromatin structure remodeling or inflammation. Interestingly, results of our latest studies indicate that human Dicer (hDicer), by supporting base-pairing of complementary sequences present in nucleic acids, can act as a nucleic acid annealer [1]. Consequently, we addressed the issue of how RNA structure influences the annealing activity of hDicer. We found that hDicer facilitated base-pairing of a short RNA with the target site present within a fully complementary stem of the RNA hairpin structure, even when a short RNA constituted one strand of a miRNA duplex. However, no such activity was observed when a short RNA was one of the strands of an siRNA duplex. Moreover, we found that hDicer, guided by a miRNA, similarly as the Argonaute proteins could bind to the miRNA target sequences present in mRNAs. Overall, the results of our current studies suggest that Dicer might influence the fate of targeted transcripts and thereby be directly involved in the post-transcriptional control of gene expression.

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O6.3

TGF-β1 affects proliferation, viability and migration of renal cancer cells, possibly by targeting regulatory microRNAs

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Introduction: TGF-β1 is a cytokine that regulates cancerous progression. We recently demonstrated that TGF-β1 affected expressions of genes involved in cellular adhesion and ECM remodeling in RCC (renal cell cancer). The mechanism of this regulation is currently unknown. Here, we hypothesize that TGF-β1 effects on genes involved in adhesion and proliferation are mediated by microRNAs.
Material/Methods: RCC-derived cell lines (786-0, Caki-2, KIJ265T, and KIJ308T) were cultured in the presence or absence of TGF-β1, followed by microarray analysis and qPCR, analysis of cell proliferation and viability (BrdU and MTT assays, respectively), migration (scratch assay), and adhesion (ECM assay).
Results: TGF-β1 treatment of RCC cells induced ($p<0.05$) the expression of four microRNAs (miR-181a-5p, miR-181b-5p, miR-125b-5p and miR-181d-5p) and decreased ($p<0.05$) the expression of two microRNAs (miR-155-5p and miR-30c-5p). The genes bioinformaticaly predicted as potential targets of microRNAs regulated by TGF-β1 are known to regulate proliferation, migration and invasion. TGF-β1 treatment inhibited proliferation, viability and migration of RCC cells, and suppressed their adhesion to collagens (I, II, IV) and tenascin.
Conclusion: We found that TGF-β1 affects proliferation, viability and migration of RCC cells, as well as the expression of microRNAs predicted to regulate these processes.

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O6.4

U7 snRNA activity and histone gene expression is affected by ALS-linked FUS mutations

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U7 small nuclear RNA is a part of U7 snRNP, which is a major factor in correct 3'end processing of replication dependent core canonical histone pre-mRNAs. We recently showed that U7 snRNP interacts in the nucleus with FUS (Fused in sarcoma), a nuclear RNA-binding protein wherein mutations are observed in patients with familial Amyotrophic Lateral Sclerosis (ALS). Here FUS was observed to play a role as a positive regulator of replication-dependent histone gene transcription and 3'end processing of histone pre-mRNAs during the S phase of the cell cycle [1]. The fidelity of histone gene regulation is essential for genome replication and genome stability [2] and that FUS deficiency results in chromosomal instability [3].
In our microscopic results using undifferentiated and terminally differentiated neuroblastoma cells we show how ALS-linked mutations in FUS gene leads to mislocalization of U7 snRNA in the cytoplasm with diverse effects related to different mutations. As a result we also observed impaired processing of histone pre-mRNAs in cells transfected with FUS mutant as compared with wild type FUS. Together combined it shows how ALS-linked FUS mutations cause cytoplasmic re-localization of U7 snRNP and affect histone gene expression indicating further that disturbed repression or activation of histone gene expression resulting in genome instability or toxic effect of excess of histones may be the molecular mechanism underlying altered motor neuron homeostasis in ALS.

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Posters

P6.1

Crosstalk between miRNA production and transcriptional machinery *via* DRB1 protein

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DRB1 (Double-Stranded RNA Binding Protein 1, also known as Hyponastic Leaves 1 (HYL1)) protein was shown to play an important role in the efficient recognition of primary transcripts of microRNA as well as in further steps of the maturation of pri-miRNAs. DRB1 protein interacts with a DCL1 (Dicer-Like 1) protein, which is the main RNAse enzyme that releases mature microRNAs from their precursors. However, recently it was demonstrated that a DRB1 protein has to be dephosphorylated by a CPL1 (C-Terminal Domain Phosphatase-Like 1) protein for its optimal activity. On the other hand, CPL1 also dephosphorylates a CTD domain of RNA Pol II. These observations suggest that the DRB1 protein may be involved in the early steps of biogenesis of microRNA, probably in the initiation of transcription of *MIR* genes. To test this hypothesis, two reporter genes (GUS expression under the control of the *MIR* gene promoter) were introduced via crossing into *hyl1-2* mutant background. Results showed that expression of the reporter gene in lack of DRB1 is downregulated. Additionally, ChIP-seq experiments showed higher occupancy of total RNA Pol II at several *loci* (not only *MIR* genes) in *hyl1-2* mutant background. Presented data provide the first evidence of a crosstalk between DRB1 and the transcriptional machinery in plant cells.

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P6.2

FUS mediates the processing of snoRNAs to shorter RNA fragments that can regulate gene expression

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FUS/TLS is a multifunctional protein involved in many pathways of RNA metabolism in human cells, including transcription, splicing, miRNA processing and replication-dependent histone gene expression (Raczynska *et al. Nucl. Acids Res.*, 2015). Recently our RIP-seq experiment revealed that FUS also binds small nucleolar RNAs (snoRNAs) in human cells, both C/D and A/HCA box. Further, we observed that FUS negatively influences the level of selected snoRNAs. However, neither the splicing efficiency of introns encoding snoRNAs nor the level of their precursors were affected. We figured out that FUS activates the processing of short RNA fragments called sdRNAs (snoRNA-derived RNAs), from snoRNAs. Such sdRNAs can act similarly to miRNAs in the regulation of gene expression. Indeed, using *in silico* approach we predicted putative targets that could be bound by FUS-dependent sdRNAs. Some of them may hybridize to the 3'UTRs of target mRNAs suggesting their role in posttranscriptional regulation of transcript stability and/or translation. Moreover, we have identified sdRNAs that can interact with non-coding, processed transcripts. Preliminary results suggests that sdRNAs prevent the synthesis of noncoding RNAs that might in turn stabilize the level of mRNA transcribed from the same genomic region. The role of FUS-dependent sdRNA in the regulation of gene expression will be discussed.

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P6.3

Circular RNAs in *Arabidopsis thaliana*

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Covalently closed single-stranded circular RNAs (circRNAs) have recently emerged as a new class of mainly non-coding, highly conserved and abundant molecules. Although some circRNAs have been shown to perform important regulatory role (e.g. microRNA sponges and transcriptional regulators in mammalian cells) for the vast majority their function and mode of action is unknown. The latter is especially true for plant circRNAs. The questions on circRNA biogenesis, functions as well as the levels and patterns of accumulation in plants remain unanswered to date. We also do not know whether the formation of circRNA is associated with particular stages of plant development. To address these issues we performed comprehensive analyzes of circRNA in 3 organs (root, leaf, flower) and in seedlings of a model plant *Arabidopsis thaliana*. To this end, we identified circRNA by the RNA-seq method. Then for the selected transcripts we determined the levels of accumulation of circRNAs and their linear counterparts using droplet digital PCR. As a result, we found organ-specific and constitutively expressed circRNAs. Our results indicated that the abundance of circRNAs is not a simple consequence of the levels of parent gene expression which implies that in the biogenesis of circRNAs could be involved other cis and trans-acting factors.

P6.4

The many faces of Dicer: RNA landscape in Dicer-deficient human cells

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Endoribonuclease Dicer is best known for its pivotal role in the biogenesis of short regulatory RNAs; the enzyme processes precursor molecules to yield small interfering RNAs (siRNAs) and microRNAs (miRNAs). However, extensive efforts over the last years have greatly expanded the functional repertoire of Dicer, highlighting the versatility of the enzyme. It has been shown that Dicer is involved in processing of diverse RNA classes, including tRNA and snoRNA, detoxification of repeat-element-derived RNAs, and maintenance of genome integrity. Moreover, a recent discovery of Dicer-binding passive sites in mRNAs and long non-coding RNAs (lncRNAs) pointed out to a novel, catalysis-independent regulatory role of the enzyme.

Due to the essential function of Dicer in RNA silencing pathways, reports describing Dicer-deficient cells have so far mainly focused on profiling miRNAs and their target genes. In this study, we characterized global RNA composition of wild-type and Dicer-knockout HEK293T cells. We provided the profiles of small RNAs, including stable RNA fragments accumulating in analyzed cell lines. We also determined changes in gene expression upon Dicer depletion and performed the enrichment analyses of processes and molecular functions associated with differentially expressed genes.

Altogether, our data reinforce the notion that Dicer regulates diverse aspects of RNA metabolism, and thus participates in multiple fundamental biological processes in mammalian cells.

P6.5

Bioinformatics analysis of circular RNAs in *Arabidopsis thaliana*

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Circular RNAs (circRNA) are a subgroup of non-coding RNAs. Many circRNAs are conserved and expressed in a specific manner in different tissues/cell types. Recently, to identify circRNAs a number of algorithms were developed based on back-splicing reads (reads overlapping circular splice junction formed by linking a downstream 5' splice site to an upstream 3' splice site) in total RNA sequencing. Most of them focus on the qualitative analysis of circRNA and neglects problems that occur while quantitative analysis.

In this study we focus on the quantitative analysis of circRNAs identified in flower, leaf, root and seedling of *Arabidopsis Thaliana*. In total we identified 2090 circRNAs supported by at least 2 back-splicing reads. To compare circRNAs abundances in different plant organs/seedling we applied a new normalization method by the reads that mapped to the ACT2 gene (housekeeping gene expressed on the similar levels in all plant tissues). The most popular normalization method, by the library size gave false results because of differences in transcriptome composition and consequently in performance of rRNA removal kits. Differential analyses of 168 most abundant circRNAs revealed organ-specific expression of circRNAs. Altogether we identified 268 circRNAs differentially expressed (min. 2 fold change) in two organs or organ and seedling. Finally, for the selected circRNAs differential expression was confirmed using ddPCR.

P6.6

mRNA expression of *SOD1*, *SOD2*, *GPx1*,*GPx4* in depression-chronic mild stress model

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Background: Depression (depressive disorder, DD) is considered to be the most common mental disorder. Estimations show that 350 mln people worldwide suffer from DD. The aetiology of this disease is not completely known. As oxidative stress has been implicated in the Psychiatric disorders, we hypothesize that the abnormal expression of *SOD1*, *SOD2*, *GPx1*,*GPx4* genes may be involved in mechanism of DD development. **Methods:** We determined the mRNA expression of *SOD1*, *SOD2*, *GPx1*,*GPx4* in brain tissue in animal model of depression-chronic mild stress (CMS). Rats were exposed to CMS for four weeks and subsequently to CMS in combination with escitalopram treatment (an antidepressant of the selective serotonin reuptake inhibitor class) for four consecutive weeks. Gene expression at the mRNA level was analyzed by TaqMan probe-based real-time PCR (RT-PCR) assay. **Result:** We observed that the level of *SOD1* expression was higher in DD group than control group. Moreover, group after escitalopram treatment was characterized by lower level of *SOD1* and *SOD2* expression than DD group without treatment. In the case of *GPx1* and *GPx4*, DD group was characterized by increased expression of these genes as compared to control group. Additionally, group after escitalopram treatment had lower level of *GPx4* expression than DD group without treatment. **Conclusion:** Our results support the hypothesis on the involvement of oxidative stress in pathogenesis of DD.

Keywords: depression, oxidative stress, nitrosative stress, expression

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P6.7

Single nucleotide polymorphisms in genes encoding TPH1 and TPH2 increases risk of acute coronary syndrome

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Introduction: Acute coronary syndrome (ACS) is a group of diseases characterized by partial or complete limitation of blood flow in coronary arteries. Despite growing consciousness about healthy lifestyle and limitation of typical risk factors, ACS is more and more often observed in young people. The "CONFIRM" study conducted between 2003 and 2009 shows that one of the best indicator for assessing risk of ACS is diagnosed in family interview myocardial infarction. It indicates the necessity of detailed analysis of the genetic basis in the pathogenesis of ACS to search the genetic predictive markers.
Methods: Material used in this study was extracted genomic DNA from whole blood of 40 patients with confirmed ACS in coronary angiography and from 40 healthy volunteers. The TaqMans SNP Genotyping Assay were used to genotype the studied SNP in Real-Time PCR Detection System.
Results: In this study we genotyped three polymorphisms: rs1800532 (*TPH1* gene), rs457625 and rs7963803 (*TPH2* gene). Analysis of gene and allele distribution show that heterozygous G/T in rs1800532 causes a six-fold reduction of ACS development; heterozygous G/T in rs4570625 causes almost seventeen-fold reduction of ACS development. However homozygous G/G in rs4570625 causes almost seventeen-fold increased risk of ACS development and heterozygous A/C in rs7963803 causes one hundred and thirty-three times higher risk of ACS development.
Conclusion: Polymorphisms of TPH1 and TPH2 genes affect on risk of ACS development.

P6.8

Differentially expressed gene transcripts in boar spermatozoa analyzed by RNA-Seq

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Variations in the freezability of boar semen have been associated with genetic markers, which could be useful to select boars prior to cryopreservation [1]. This study investigated the profiles of differentially expressed (DE) gene transcripts of spermatozoa from the Polish large white (PLW) boars differed in semen freezability using RNA-Seq. Total RNA was isolated from fresh spermatozoa of the PLW boars [2], classified as good and poor semen freezability (n=3, respectively), and was used for RNA-Seq (Illumina NextSeq 500 platform, USA). Library preparations were subjected to paired-end 75 bp sequencing on Illumina NextSeq 500 platform and sequences were mapped to the *Sus scrofa* genome reference. Transcript abundance was analyzed with different software packages, DESeq, EdgeR and DESeq2. KEGG pathway and Gene ontology (GO) terms were performed with the DAVID Tools. Using different software packages, varying numbers of DE transcripts were detected ($p < 0.05$) in spermatozoa, being markedly higher with the DESeq analysis. Functional enrichment of the DESeq-detected transcripts revealed that propionate pathway (*ACADM* and *LDHB*) was the major KEGG pathway. Both EdgeR and DESeq2 pipelines showed that MAPK signaling pathway (*FOS*, *NAFTC3* and *FGF-14*) was more prominent in the KEGG pathway. Furthermore, GO categories showed that most of the DE gene transcripts were associated with transcription activity (*TBIF1*), apoptosis (*FOS*), lipid metabolism (*CPT2*) and aberrant spermatogenesis (*CCDC85A*), and were up-regulated in spermatozoa from boars with poor freezability ejaculates.

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P6.9

Expression of soybean microRNAs and their target genes during chilling stress

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Soy is an annual legume grown for its edible bean. Exceptional nutritional value of soybean, with high protein (40%) and oil (20%) contents, make it one of the staple crops. Political position of EU greatly hampers cultivation of GM crops, meanwhile extensive meat production enforces the need for substantial import of soy meal, mainly from Argentina. Additionally, soybean in Polish climate is exposed to chilling stress, which impairs yield quality. Thus, in order to provide a sustainable source of soybean we must turn to other solutions, specifically breeding of a chilling resistant cultivar. We aimed to decipher the role of miRNAs and their target genes in plant chilling stress response, by determining the changes in their expression levels. Diverse soybean cultivars were employed for the comprehensive investigation of stress response molecular basis. Review of the literature data allowed us to select miRNAs associated with chilling response. Furthermore, gene ontology analysis offered candidates for target genes of studied miRNAs. Small RNAs were isolated from tissues of soy cultivated in stress conditions. Expression level of miRNAs was measured using novel technique called digital droplet PCR, which precisely defines the concentration of target sequence in each sample.

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P6.10

Metabolism of oxalic acid by *Abortiporus biennis* – transcriptomic analysis

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Oxalic acid plays an important role in fungal system involved in wood decomposition as a low-molecular-weight compound. It is also involved in many biological and geochemical processes in natural environment. Three enzymatic activities for decomposition of oxalic acid have been described: (1) a decarboxylation of oxalic acid catalysed by oxalate decarboxylase (ODC, EC 4.1.1.2) typical for fungi, (2) a decarboxylation of activated oxalic acid molecule (oxalyl-CoA) catalysed by oxalyl-Co decarboxylase (EC 4.1.1.8) typical for bacteria, (3) an oxidation of oxalic acid catalysed by oxalate oxidase (OXO, EC 1.2.3.4) which is widespread in plants and leads to formation of carbon dioxide and hydrogen peroxide [1]. An important indirect role in oxalate catabolism of basidiomycetous fungi is also assigned to formate dehydrogenase (FDH, EC 1.2.1.2), an NAD-dependent enzyme decomposing formic acid formed by ODC to carbon dioxide [2]. *Abortiporus biennis* was selected during our earlier study as the strain with unique oxalate metabolism [3, 4]. In presented study expression of genes involved in oxalate metabolism in *A. biennis* were monitored at transcriptomic level. Expression of oxalic acid decomposing enzymes were observed after induction by exogenic oxalate, but among them the genes for formate dehydrogenase (FDH) was up-regulated in the greatest extent. Three transcripts for FDH and one annotated for oxalate degrading enzymes were found. No transcripts for enzymes involved in oxalic acid synthesis were observed.

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P6.11

MAP kinases regulate RNA polymerase III activity in LPS-activated macrophages

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Macrophages are professional phagocytic cells of the innate immune system. They have many biological functions, such as antigen presentation, target cell cytotoxicity, phagocytosis and regulation of inflammation. Activation of macrophages with lipopolisacharydes (LPS), a major component of the outer membrane of most Gram-negative bacteria, induces rapid transcriptional changes and, within a few hours, transcription of several hundred genes is altered. LPS engage the Toll-like receptor 4 (TLR4) and induce various signalling pathways, including mitogen-activated protein kinases (MAPK), and activate several transcription factors such as nuclear factor (NF)-kappaB (NF-κB).

We have previously shown that treatment of macrophages with LPS induces RNA polymerase III (Pol III) activity and that NF-κB is involved in this process. However, inhibition of the NF-κB pathway only partially precludes Pol III activation upon LPS treatment, and this suggests that other signalling pathways are involved in the activation of Pol III in these conditions.

MAP kinases have been previously shown to act on Pol III mainly through modulation of the levels of TFIIIB transcription factor components. While our results show that MAP kinases are involved in Pol III upregulation upon LPS treatment, we have found that this is not a result of increased levels of Pol III machinery. Instead, we propose that MAP kinases act directly on the Pol III transcription apparatus.

P6.12

The overexpression of AC19, a common subunit of RNA polymerases I and III affects autophagy in yeast

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RNA polymerases (Pols) I and III are multisubunit complexes that are responsible for transcribing ribosomal RNA (rRNA) and transfer RNA (tRNA), respectively. These RNAs are the most abundant transcripts in cells, comprising up to 80-90% of total cellular RNA. AC19 is a small 16 kDa protein that is a common subunit to Pol I and III. By using yeast as a model, we found intriguing connection between AC19 and the TOR (target of rapamycin) signalling pathway. TOR senses and integrates environmental cues to regulate eukaryotic cell growth. Importantly, TOR is a key regulator of autophagy, a catabolic process whereby intracellular are broken in order to help cells to survive adverse growth conditions.

In nutrient replete conditions TOR pathway positively regulates Pol I and III activity. Interestingly, it has been shown that AC19 interacts with Gtr1, a Ras-like small G-protein superfamily member that is implicated in TOR complex 1 activation in response to amino acid stimulation. The purpose of this interaction is not clear and we hypothesize that this interaction may be a part of the signalling feedback loop whereby yeast cells sense defects in tRNA and/or rRNA biogenesis in order to halt the translation.

Our data indeed show that overexpression of AC19 leads to changes in autophagy. Our work is currently focused on the elucidation of the molecular mechanism of this phenomenon and its biological relevance. The potential explanation for this interesting observations will be discussed.

P6.13

Influence of chemotherapeutics and irradiation on lncRNAs expression in HNSCC cell lines

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Introduction: Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cause of cancer mortality in the world. To improve the quality of diagnostics and patients' treatment efectiveness new biomarkers are needed. Recent studies have shown that different types of long non-coding RNAs (lncRNAs) are dysregulated in HNSCC and correlate with many biological processes. In this study, we examined changes in lncRNAs expression in HNSCC cell lines after chemo- and radiotherapy.
Materials and methods: SCC-40, SCC-25, FaDu and Cal27 cell lines were treated with doxorubicin and cisplatin and different doses of radiation (5, 10, 20 Gy). Effects of cisplatin and doxorubicin were analyzed by MTT test. lncRNAs' expression after chemo- and radioexposure was studied by qRT-PCR.
Results: The experiments with radiation showed:
1. Expression profile depended on type of a cell line and dose of radiation.
2. Dose of 5 Gy resulted in dysregulation of Hotair, HOXA3as, SNHG5, Zfhx2as; 10 Gy - CAR Intergenic 10, Dio3os, HAR1A, HAR1B, Zfhx2as; 20 Gy - HOXA6as, PTENP1, Zfhx2as. Common effect of radiation was observed only in Zfhx2as.
After cisplatin exposure 14 lncRNAs showed lower expression and only 2 higher. Doxorubicin resulted in lower expression of 8 lncRNAs and increased 4. Common effect of chemotherapeutics was observed in the case of anti-PEG11, BACE1AS, PCGEM1 and ST7OT.
Conclusions: Both chemotherapy and radiotherapy cause changes in lncRNAs expression in HNSCC cell lines. Further study will show if lncRNAs are useful tools in monitoring of patients' treatment.

P6.14

Quantification of long non-coding RNAs using qRT-PCR method– comparison of different cDNA synthesis methods and RNA stability

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Introduction: Long non-coding RNAs (lncRNAs), a class of regulatory RNA molecules, are over 200 nucleotides long and could be used as a new potential biomarker. Detection methods of lncRNAs such as qRT-PCR are still not validated, and the influence of RNA degradation on lncRNA quantification is not clear. In this study, commercially available cDNA synthesis kits were tested and the influence of RNA degradation was compared.
Material and methods: Total RNA from FaDu cells was isolated, next samples of high quality of RNA and highly degraded RNA were used. The reverse transcription was performed using three different commercially available kits and quantification was made using lncRNA Primer Plate and SYBR Green I Master and LightCycler 96.
Results: Lower lncRNA Ct values (61/90; 67.78%) after qRT-PCR quantification were observed for cDNA synthesized using random hexamer primers preceded by polyA-tailing and adaptor-anchoring steps. It was observed that 9/90 (10.00%) lncRNAs were not detectable using any of examined cDNA synthesis methods. In the case of 75/90 (83%) lncRNAs, RNA degradation weakly influenced lncRNA Ct values and no differences were observed between high quality and degraded samples. 17% of examined lncRNAs showed significantly different Ct values depending on RNA degradation.
Conclusions: cDNA synthesis kits with random hexamer primers preceded by polyA-tailing and adaptor-anchoring steps allowed to enhance lncRNA quantification specificity and sensitivity. In the most cases degradation of RNA samples did not affect lncRNA quantification probably because of their good stability.

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P6.15

Isoforms of ER translocon protein Sec61 α – differential expression and involvement in ERAD pathway

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In the endoplasmic reticulum (ER) many proteins of eukaryotic cells undergo folding and modifications. Proteins that misfold in the ER need to be transported back to the cytosol for degradation. Selective recognition of misfolded proteins and dislocation by retro-translocation is known as ER-associated degradation (ERAD). Currently, two types of ER channels are known – Sec61p complex and Derlin family proteins. Selection of channels by ERAD substrates is still unclear. The heteromeric Sec61p complex is composed of α, β and γ subunits, where α subunit is a central component. Previous studies have shown strong interaction of Sec61α protein with ERAD substrates while reduced level of Sec61α results in reduced retrotranslocation efficiency. Data reports identified two isoforms of Sec61α subunit – α1 and α2. It is believed that α1 isoform is dominant in most cell lines. The aim of this study was to determinate the presence of Sec61α isoforms in different cell lines and to investigate the effect of Sec61α downregulation on cell viability and transport of ERAD substrates from the ER to cytosol. We determined the ratio of the α1 and α2 isoforms in different cell lines and found efficient downregulation of Sec61α isoforms with esiRNA. We have shown that gene encoding isoform α1 of Sec61α is expressed at a significantly high level, while both splicing forms may play an important role in the transport of polypeptides associated with the ER.

P6.16

MALAT1, MEG3 and UCA1 lncRNAs as potential biomarkers of surgery assessment in head and neck cancers

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Introduction: In spite of progress in treatment methods, HNSCC belongs to the group of poor prognosis and high mortality cancers. To improve patients survival there is the need to use molecular biomarkers to estimate success of treatment procedures such as clarity of surgical margins. Deregulation of lncRNAs is shown in many diseases, including cancer. lncRNAs potentially can be used as new biomarkers and can lead to treatment personalization.
Methods: Expression of MALAT1, MEG3 and UCA1 lncRNAs, their targets and clinical-pathological parameters was performed using the TCGA data based on cBioPortal. Next, the cancer, margin and adjacent matched healthy samples originated from 22 patients were used as validation cohort. Total RNA was isolated and expression levels of selected lncRNAs were validated using qRT-PCR reaction.
Results: The TCGA analysis of MALAT1, MEG3 and UCA1 indicated their diagnostic potential for assessment HNSCC patients. The validation patients' cohort indicated differences in the expression of MALAT1 (p=0.0408), MEG3 (p=0.0020) and UCA1 (p=0.0073) between cancer tissues and normal samples. The receiver operating characteristic (ROC) curve analysis showed that the MALAT1, MEG3 and UCA1 can discriminate samples as healthy and cancer tissues. In margin samples, diffrent expressions of MALAT1, MEG3 and UCA1 (up- or down-regulated) for the same patient were observed what could help to estimate the surgical margine samples as tumor or healthy.
Conclusions: Our observations show that lncRNAs are involved in the process of tumorigenesis and they can serve as indicators to distinguish tumor and healthy tissues. Morover, MALAT1, MEG3 and UCA1 could be used for assessment of the clarity of surgical margins.

P6.17

miRNAs set expression profiles in whole blood during prostate cancer patients treatment

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Background: Changes in expression profiles of the 5 selected miRNAs were analysed in a group of PC patients before treatment, after hormonotherapy and radiotherapy.
Objective: Whether the expression profiles of the miRNAs may be useful for monitoring prostate cancer treatment.
Methods: The initial study was carried out on 44 advanced prostate cancer patients and 41 healthy volunteers. The target group consisted of 39 PC patients. Blood for miRNA analysis was taken before treatment, after hormonotherapy and radiotherapy. The miRNAs were analysed by real-time PCR, followed by statistical analysis.
Results: For the target group, the statistically significant differences in the expression level were found after radiotherapy: for miR-21 only in the group of patients above the cut-off value designed in the preliminary study (p=0.0369) and miR-100 for the whole group (p=0.0413) and for the above cut-off value group (p=0.0140). The differences between the levels of each miRNA between the high and low expression groups were statistically significant. The designed groups were stable during treatment. Inclusion to the high and low expression group levels did not influence the treatment result.
Conclusion: The miRNAs studied in this work could not serve as biomarkers for the effectiveness of therapy for prostate cancer patients.
Key words: prostate cancer, miRNAs, expression profile, hormonotherapy, radiotherapy

P6.18

EDEM3 overexpression in HEK293 cells – its role in ricin cytotoxicity and transport from the endoplasmic reticulum to the cytosol

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ERAD-*ER-associated degradation* is a part of a protein quality control system operating in the endoplasmic reticulum (ER), which has an impact on process determining the proper functioning of all eukaryotic cells. Many of all newly synthesized proteins are produced in the ER. Some of them may fail to attain their native structure and have to be degraded. ER do not possess its own degradation machinery, proteins have to be transported to the cytosol for proteasomal degradation (ERAD). The major group of chaperones which recognize terminally misfolded proteins is EDEM family (EDEM1, EDEM2, EDEM3). Ricin is a protein toxin that utilizes the ERAD pathway in its transport from the ER to the cytosol where it acts. Due to high toxicity, it is considered as a biological weapon. On the other hand, this toxin can be used as a component of modern immunotoxins and vaccines. Ricin is heterodimeric holotoxin composed of an A-chain (RTA) connected to a cell binding lectin B-chain (RTB). RTA contains hydrophobic C-terminal region. Substitution of proline into alanine in position 250 (P250A) of this region alters the secondary structure of ricin and decreases its cytotoxicity. In contrast to wild-type RTA, P250A transport to the cytosol appears to be EDEM1- and EDEM2-independent. Moreover, RTA P250A do not interact with EDEM1 and EDEM2. Thus, recognition of proteins by EDEM1 and EDEM2 may be determined by the structure of the ERAD substrate. The role of EDEM3 in this process is unknown.

P6.19

ER degradation-enhancing α-mannosidase-like proteins (EDEMs) can regulate amyloid precursor protein (APP) level in HEK293 cells

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Alzheimer's disease (AD) is a neurodegenerative disorder that manifests itself in behavioral disturbances and short-term memory loss. The pathophysiology of AD is characterized by the formation of brain senile plaques from a peptide amyloid-β. Amyloid-β is generated after sequential cleavage of amyloid precursor protein (APP). APP is a transmembrane glycoprotein which serves a variety of functions related to cell adhesion and migration. Like most plasma membrane proteins, APP is synthesized and N-glycosylated in the ER and transported to the Golgi for maturation, before being transported to the cell surface. Aβ can be generated in the ER or trans-Golgi network. Both APP and β-amyloid can be retrotranslocated from the ER to the cytosol and degraded by the 26S proteasome in a process called ER-associated-degradation (ERAD). ER chaperone proteins assist in proper folding or recognition of incorrectly folded proteins. The major group of chaperones which recognize terminally misfolded proteins is EDEM family (EDEM1, EDEM2, EDEM3). Our research focus on studies of the regulation of ER chaperones-dependent transport of APP from the ER to the cytosol and the impact of overproduction of EDEM proteins on the APP protein level in HEK293 (*Human embryonic kidney cells 293*) cells. Our studies strongly suggest that APP level can be regulated by EDEM proteins.

P6.20

Non-AUG translation generates new protein isoforms with mitochondrial localization

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As semi-autonomous organelles mitochondria have their own DNA and translational machinery, but mitochondrial DNA encodes only for a few polypeptides, while the majority of mitochondrial proteins are nuclear-encoded, translated in the cytosol and imported into mitochondria. The best-characterized mechanism of mitochondrial targeting involves specialized translocases, which deliver proteins containing the N-terminal mitochondrial targeting signal (MTS) as unfolded precursors.

Utilization of non-AUG alternative translation start sites is most common in bacteria and viruses, but it has been also reported in other organisms. This phenomenon increases protein complexity by allowing expression of multiple protein isoforms from a single gene. A few described cases concern proteins that are translated from upstream non-canonical start codons as N-terminally extended variants that localize to mitochondria. Using bioinformatics tools, we provide evidence that non-AUG translation initiation is much more prevalent than previously anticipated. Several hundreds of candidates are predicted to gain MTS, generating a hidden cache of mitochondrial proteins. We confirmed mitochondrial localization of a number of proteins previously not recognized as mitochondrial. Our data highlight the potential of non-canonical translation initiation in expanding the capacity of the mitochondrial proteome and probably also other cellular features.