Session 6. Genomics and Epigenomics

Lectures

L6.1

Search for genetic variants influencing cardiac hypertrophy — an integrative genomics approach

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The left ventricular hypertrophy which is commonly observed in aortic stenosis develops as an adaptive response to pressure overload and itself is an independent major risk factor for morbidity and mortality. Previous studies have demonstrated a poor correlation between the degree of aortic stenosis or other clinical factors and the degree of left ventricular hypertrophy. The absence of a clear relation between the stenosis-dependent pressure load on the degree of left ventricular hypertrophy suggests that the left ventricular phenotype is dependent on a polygenic background. Familial predisposition of left ventricular hypertrophy is supported by several studies. In a recent genome-wide association study (GWAS) we found evidence for association between variation in left ventricular mass index and several chromosomal regions. We postulate that these chromosomal regions harbor genes that contribute to the development of heart hypertrophy in individuals with aortic stenosis. In this project we use an integrative genomics approach that combines GWAS results and informative SNP linkage disequilibrium maps with transcriptome sequencing data to identify novel genes and biological pathways affecting cardiac hypertrophy that could not be identified using GWAS alone.

L6.2

Epigenetic mechanisms behind plant phenotypic plasticity

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Plants are sessile organisms that complete their life cycles at the same location in which they grew from seeds. This requires the ability to constantly adapt to a changing local environment as well as to interact in both a negative (competition) and a positive (cooperation) way with other plants populating the area, in order to optimize access to basic resources (light, water, mineral nutrients). These needs have fostered – especially in angiosperm plants – the evolution of remarkable adaptive phenotypic plasticity and interactive capabilities. In response to the prevailing environmental conditions, a single plant genome is capable of generating a wide range of physiological and developmental phenotypes and supporting both competitive and cooperative (facilitative) interactions within plant communities. Phenotypic plasticity and cooperation enabled by facilitative interactions among plants have profound implications for plant evolution and are the subject of intensive study by theoretical and evolutionary plant ecologists. However, the mechanisms by which environmental conditions are communicated to the genome and affect transcription, enabling adaptive responses, are poorly understood. In particular, despite increasing evidence of the importance of epigenetic mechanisms in the dynamic interplay between environment and genomes underpinning adaptive plasticity, the link between these mechanisms and external cues remains enigmatic. We assessed the developmental, physiological and molecular role of the 'stress inducible' linker histone H1.3 in the adaptation of *Arabidopsis thaliana* to combined light limitation and drought and present evidence that H1.3, a member of a subfamily of plant H1 histones conserved in angiosperms but absent in mosses, ferns and gymnosperms, plays a key role in the *Arabidopsis* response to complex abiotic stresses. Our results give strong support to a notion that structural and *cis*-regulatory subfunctionalization that led to the evolution of 'stress-inducible' H1 variants may have helped to promote the rapid radiation of angiosperm plants on Earth.

L6.3

Computing local chromatin state: a supervised approach

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It is now well established that local changes in the state of the chromatin fibre are essential for establishment of proper epigenetic changes in development. In particular, changes in gene regulation require complex modifications of histone tails to be orchestrated both at the transcribed parts of chromosomes as well as in non-coding regulatory regions. Given the abundance of data on both histone modifications and histone modifying enzymes from ChIP-Seq or similar experiments it is now a good time to ask if or how well can we predict the function of regulatory elements such as enhancers or insulators on a genomic scale. In this talk I will present our recent results regarding prediction of regulatory elements using data from ENCODE and modENCODE projects to discover positions of enhancer and isulator elements in fruitfly and human genomes.

Oral presentations

O6.1

TCGA-based analysis of gliomas uncovers a putative role of miRNA-155 in regulation of gene expression

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Introduction: Molecular mechanisms of progression from lower grade gliomas to anaplastic, highly malignant forms are poorly known. The Cancer Genome Atlas (TCGA) project provides an opportunity to study molecular aspects of transcription regulation among histological subtypes of gliomas in large patient cohorts.
Materials and Methods: In a present study both mRNA and miRNA expression datasets from histological subtypes of WHO II and III grade gliomas were acquired from the TCGA website. It includes next generation sequencing data for mRNA and miRNA for 54 astrocytoma, 53 oligodendroglioma and 37 oligoastrocytoma tumors. Many miRNAs were associated with differential gene expression in gliomas, threfore miRNA and mRNA expression was analyzed to find relationship between a level of specific miRNA and regulation of gene expression. Best inverse correlations for astrocytomas (rho < -0.5 and Bonferroni corrected p-value 0.1) were identified and 4 algorithms (miRDB, microT4-CDS, Paccmit, Paccmit-CDS) were used for bioinformatics prediction of the possible target genes of miRNAs. Correlations confirmed by 2 out of 4 algorithms were reported. GSEA (gene set enrichment analysis) of Gene Ontology terms for biological function and CGP (chemical and genetic perturbations) enrichment was performed to investigate genes that were potentially regulated by miRNAs in gliomas.
Results: An integrated analysis of the observed miRNAs and mRNAs resulted in 100 highly correlated miRNA-mRNA pairs that fulfilled criteria of analysis. Strikingly 60% of them were putative regulations of miRNA-155, which is a very well known oncomir involved in glioblastoma development. GSEA analysis of putative targets of miRNA in astrocytoma revealed that amongst most enriched GO terms for biological functions ESTABLISHMENT AND OR MAINTENANCE OF CHROMATIN ARCHITECTURE and CHROMOSOME ORGANIZATION AND BIOGENESIS were most enriched functional groups. The most GSEA CGP enriched group consisted genes correlated with a proneural type of glioblastoma multiforme (WHO grade IV).
Conclusions: MiRNA-155 could be a key player in astrocytoma development. Main functional groups of miRNA targets are genes involved in chromosome organization and chromatin architecture. The expression pattern of genes that are putative targets of miRNA in astrocytoma is very similar to the expression patterns of the proneural type of glioblastoma multiforme.

Acknowledgements

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O6.2

The extent of gene copy number variation in natural populations of *Arabidopsis thaliana*

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Copy number variation (CNV) refers to genomic rearrangements resulting from gains (duplications) or losses (deletions) of DNA segments, typically larger than 1 kb in size. Increasing evidence confirms the pivotal role of CNV in shaping intraspecies genetic variation. Although majority of CNV regions are intergenic, variation of copy number is also observed for many protein-coding genes Thus, CNV may affect gene structure or dosage, thereby contributing to phenotypic diversity. In plants, it has been shown that CNV of specific genes affects important agronomic traits e.g.: plant height, fotoperiod sensitivity and flowering time in wheat, resistance to pathogenic nematode in soybean or resistance to glyphosate herbicide in multiple species to name a few. Still, the extent of CNV in plant genomes, as well as the rate and the mechanisms of copy number variants formation, are poorly recognized [1].
The 1001 Genomes Project [2] provides whole-genome sequence data of multiple *Arabidopsis thaliana* accessions, allowing for CNVs discovery and analysis in this plant. We used the next generation sequencing data of 80 *A. thaliana* accessions from eight different geographic locations [3] for CNV inference in this species. By combination of read depth and paired-end mapping methods we were able to identify over 1000 CNVs, each at least 1kb long. We used Multiplex Ligation-dependent Probe Amplification (MLPA) and droplet digital PCR (ddPCR) genotyping approaches for the validation of multiple gene-overlapping CNVs.
We also characterized a 10-kb CNV region on chromosome 3, where three protein-coding genes are located: (i) AT3G18524 (ATMSH2) encoding a DNA mismatch repair homolog of human MutS gene, (ii) AT3G18530 encoding ARM repeat superfamily protein and (iii) AT3G18535 encoding tubulin-tyrosine ligase. Detailed genotyping of each gene with ddPCR revealed heterogeneity of the analyzed region. Distribution of each gene copy number in individual samples suggested that at least two types of events contributed to the CNV pattern observed in *A. thaliana* population. One event is duplication/deletion of AT3G18530 and AT3G18535 genes, probably driven by perfectly identical 156-bp repeats, flanking this region. The second event involves amplification (and not deletion) of all 3 genes. Possible effects of gene CNV on their function and phenotype will be discussed.
In summary, our results indicate that CNV affects a substantial part of *A. thaliana* genome. Additionally, we show that MLPA and ddPCR are complementary approaches which can be successfully applied to precisely determine the copy number of individual genes in multiple plant samples.

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O6.3

Hybrid nature of pathogenic fungi

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Invasive candidiasis is the most commonly reported invasive fungal infection worldwide infecting over 400000 people every year. Although *Candida albicans* remains the main cause, the incidence of emerging Candida species, such as *C. parapsilosis* is increasing. The *Candida parapsilosis* species complex was recently subdivided into three different species: *C. parapsilosis* sensu stricto, *C. orthopsilosis* and *C. metapsilosis*. We have studied numerous isolates from all three species by means of genomics and transcriptomics.
It has been postulated that *C. parapsilosis* clinical isolates result from a recent global expansion of a virulent clone. Our analyses of additional three *C. parapsilosis* strains revealed unexpected patterns of genomic variation, shared among distant strains, that argue against the clonal expansion hypothesis. All strains carry independent expansions involving an arsenite transporter homolog, pointing to the existence of directional selection in the environment, and independent origins of the two clinical isolates. Furthermore, we report the first evidence for the existence of recombination in this species. Altogether, our results shed new light onto the dynamics of genome evolution in *C. parapsilosis* [1].
At least two clearly distinct subspecies seem to be present among clinical isolates of *C. orthopsilosis* (Type 1 and Type 2). To our big surprise, the analysis of *C. orthopsilosis* and *C. metapsilosis* stains revealed highly heterozygous genomes, which we showed to be the consequence of a hybridization event between both subspecies of *C. orthopsilosis*. This implicitly suggests that *C. orthopsilosis* is able to mate, a so-far unanswered question. The resulting hybrid shows a chimeric genome that maintains a similar gene dosage from both parental lineages and displays ongoing loss of heterozygosity. Several of the differences found between the gene content in both strains relate to virulent-related families, with the hybrid strain presenting a higher copy number of genes coding for efflux pumps or secreted lipases. Remarkably, two clinical strains isolated from distant geographical locations (Texas and Singapore) are descendants of the same hybrid line, raising the intriguing possibility of a relationship between the hybridization event and the global spread of a virulent clone [2].
All twelve globally-distributed clinical isolates of *C. metapsilosis* result from a single, common hybrization, followed by multiple events of loss of heterozygosity. As no homozygous strains were identified to date, we were unable to denote parentals and their gene dosage. Nevertheless, we showed all isolates are diploids with some anueploidies. Finally, we implicitly showed the donor for loss of heterozygosity is random, yet one parental is favored on small scales [3].
In addition, I will discuss methodological challenges coming with highly heterozygous genomes and solutions available to tackle at least part the these challenges.

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O6.4

hnRPK is involved in mRNA processing for significant fraction of transcriptome

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Heterogeneous nuclear ribonucleoprotein K (hnRPK), long known for its role in transcryption and signal transduction [1, 2], has been recently associated with RNA maturation [3] and translation silencing [4]. As two-way posttranscriptional expression modulation by hnRPK becomes more evident the genome scale assessment of the process is still lacking. Here we report a comprehensive characterization of hnRPK-bound mouse transcripts during their translocation from nucleus to cytoplasm.

We performed hnRPK immunoprecipitation followed by deep sequencing (RNA-Seq) to identify hnRPK-bound RNA isoforms in liver samples from two mice models of obesity and control.

Hundreds isoforms meeting strict criteria of binding strength and specificity were identified. We infer indirect hnRPK — transcript action mechanism. Because hnRPK is highly conserved (single aminoacid substitution between mouse and human) widespread engagement in posttranscriptional processing is likely to be universal feature.

References:

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O6.5

Influence of intragenic CpG islands on transcriptome diversity

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The Human Genome Project revealed that the number of human protein-coding genes is surprisingly small, only slightly greater than the number of genes in simple organisms such as the nematode C. elegans. However, the mammalian transcriptome and proteome achieve much greater complexity because a single locus can give rise to multiple isoforms. Transcript variants are generated through alternative promoter usage and mRNA processing, including alternative splicing and alternative polyadenylation.

The regulation of these events is far from being fully understood. In the last few years, epigenetic modifications such as DNA methylation, nucleosome occupancy and histone modifications have been linked to alternative splicing, and evidence of chromatin modifications influencing alternative polyadenylation has emerged as well. Epigenetic control over alternative polyadenylation has been demonstrated in mouse at several loci where a host gene harbours an imprinted retrogene, e.g. Mcts2/H13 (Wood *et al.*, 2008). In these cases, poly(A) site choice of the host gene transcripts is influenced by the methylation status of intragenic CpG island (CGI) that serves as the retrogene promoter. Whether intragenic CGIs are part of a more general mechanism for the generation of transcriptome complexity is unclear. There are thousands of intragenic CGIs in the mammalian genome, of so far mostly unknown function. In contrast to typical, non-intragenic CGIs, they are frequently methylated in a tissue-specific way.

We developed an RNA-seq data-driven method to correlate tissue-specific intragenic CGI promoter activity with alternative processing of host gene transcripts. We utilised mouse ENCODE data from 30 tissues and developmental stages and identified 1427 loci genome-wide where intragenic CGI promoter activity likely influences host gene polyadenylation, independently of imprinting. Based on our analysis of ENCODE ChIP-seq data, H3K36me3 may be involved in a common regulatory mechanism at identified these loci.

References:

Wood A *et al* (2008) *Genes & Development* **22**: 1141–1146.

O6.6

Identification of novel non-coding RNAs in human cells infected with hepatitis C virus

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The spectrum of known non-coding RNAs has significantly expanded in the recent years with the discovery that various constitutively expressed RNA classes (including tRNA, rRNA and snoRNA) give rise to a broad repertoire of stable fragments. These fragments have been identified in all kingdoms of life and some of them were proven to have regulatory functions. It has been shown that in mammalian cells RNA fragments are involved in stress response and are capable of guiding the silencing of target gene expression. In view of these data, it seems plausible that such molecules can also modulate the course of viral infections. The current state of knowledge in this matter is very limited.

To gain insight into the role of RNA fragments in host-virus interaction, we focused on hepatitis C virus (HCV), which is a model human (+)RNA virus capable of inducing persistent infections. We characterized the non-coding RNA repertoire, which accumulates in HCV-infected human hepatoma Huh-7.5 cells 72 and 96 hours post infection. To this end, RNA molecules ranging from 15 to approx. 80 nucleotides were analyzed by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and next generation sequencing (NGS).

The data demonstrated that the fraction of high-copy number RNA in the examined pool did not change considerably upon viral infection. Interestingly, this fraction contained several types of RNA fragments, among which some were as abundant as miRNA and snoRNA. Non-coding RNAs that differentially accumulated in infected and non-infected cells occurred at much lower levels. Most of these molecules were up-regulated during the infection. Importantly, they included fragments derived from host cell RNAs. The analysis permitted the selection of candidate molecules derived from various RNA classes for further bioinformatic and biochemical studies of their functional potential.

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Posters

P6.1

Properties of fibrin clots formed from plasma of cystathionine β-synthase-deficient patients and their unaffected family members

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Homocysteine (Hcy), an emerging cardiovascular risk factor, arises from the metabolism of the essential dietary protein amino acid methionine. Severe hyperhomocysteinemia (HHcy) due to cystathionine β-synthase (CBS) deficiency is associated with vascular complications (thrombosis), which are the major cause of morbidity and mortality in untreated CBS-deficient patients. Although the causes of thrombosis in CBS-deficient patients have been studied, and Hcy-lowering therapy is known to improve vascular outcomes, the underlying mechanism is not understood. Modification of fibrinogen by Hcy-thiolactone generates pro-thrombotic *N*-Hcy-fibrinogen, which is known to affect fibrin clot structure *in vitro* and to accumulate in CBS-deficient patients (Jakubowski H., Homocysteine in Protein Structure/Function and Human Disease, Springer, Wien 2013). In the present study, using tromboelastometry (TEM), turbidimetric assays of clot formation and lysis, and scanning electron microscopy (SEM), we examined properties and structure of fibrin clots formed from plasma of CBS-deficient patients and their unaffected family members. CBS-deficient patients had elevated plasma total Hcy, relative to unaffected subjects (148+/-51.5 μM, n=6, *vs.* 14.6+/-4.8 μM, n=16). TEM analyses showed that maximum clot firmness, which reflects the absolute strength of the fibrin clot, is lower for clots formed from plasma of CBS-deficient patients compared to unaffected subjects. Plasma clotting times were similar for CBS-deficient patients and controls. Clot firmness after clotting was lower in CBS-deficient patients than controls. SEM pictures showed that fibrin networks had similar density in CBS-deficient patients and unaffected family members. Treatments with Hcy or Hcy-thiolactone of control plasma from unaffected subjects caused lengthening of the clotting time. In conclusion, our data suggest that HHcy due to CBS deficiency affects mechanical properties of fibrin clots, but not clot structure, and factors other than HHcy contribute to pro-thrombotic phenotype in CBS-deficient patients.

Acknowledgments

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P6.2

Inhibition of G9a histone methyltransferases enhances inflammatory response of human endothelial cells

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Activation of endothelial cells is a multilevel process of cell stimulation to release the agents crucial for cardiovascular system and whole organism functions. Recent years show a big impact of epigenetic processes, such as DNA methylation, histone post-translational protein modifications and miRNA, on the endothelium functions due to their effect of gene expression.
In presented studies we have focused on histone metyltransferases (HMTs), which are responsible for methylation of histone amino acids tails (lysine and arginine reisdues). In the center of our interest was G9a HMTase, which forms homo- or heteromeric complexes with GLP (methyltransferase G9a-like protein). The activity of enzyme comprises of mono- and di-methylation of H3K9, which is a marker of silent euchromatin. Moreover, G9a can methylate lysine residues (K26 and K20) of the histone H1 and H4, respectively. To detemine the role of G9a, we have used highly specific HMTase inhibitor BIX-01294, which inhibits G9a activity in a SAM-dependent manner.
Performed experiments have shown an impact of inhibition of G9a methyltransferase on endothelial cell functions. Human microvascular endothelial cells (HMECs) were incubated with substrate-competitive G9a inhibitor, at the range of concentrations 0.5–50 μM. 24 h treatment have shown that concentrations of BIX-01294 higher than 20 μM exert cytotoxic effect on HMECs proliferation, revealed in resazurin oxidation assay. We have also found that inhibition of G9a HMTase strongly affects redox homeostasis and inflammatory pathway of ECs. At the first point we have seen that 12 h incubation of HMECs with BIX-01294 (5–30 μM) decreases reactive oxygen species production, monitored *via* H2DCF oxidation. At the same time, gene expression analysis have shown elevated level of catalase and SOD (mRNA level, qPCR) after G9a inhibition. Induction of inflammatory response of HMECs by LPS treatment, completely reversed the response of ECs (ROS production was significantly elevated, as well as antioxidant enzyme expression). To determine the role of G9a in the inflammatory response of ECs, 12 h co-treatment with the inhibitor and LPS (50 ng/ml) was performed (with 4 h preincubation of cells with BIX-01294). Elevated gene expression level of molecules engaged in the analyzed process, e.g. IL-6, IL-8, MCP-1, points involvement of G9a in regulation of ECs activation and significantly enhances cell response.

Acknowledgements

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P6.3

The choice of "housekeeping gene" for analysis of gene expression after cryopreservation by quantitative RT-PCR method

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Cryopreservation is an effective way of long-term storage of biological objects. On the other hand - it is multifactor stress, the aggregate effect of physical and chemical factors, and a variety of structural and a consequential functional changes in the nature of cryopreserved material. As a result, modification of bioobject, which affects not only metabolic processes, but also the genomic level of their regulation, can occur. Moreover, different protocols of cryopreservation can affect the functioning of the genome of the same cell in different ways.
Nowadays polymerase chain reaction (PCR) in real time is the most precise method for quantifying gene expression changes in different types of cells. Relative estimation of the transcripts representation allows to compare the control and experimental samples. During this process the normalization of a total RNA in samples is performed, which allows to level differences in the quantity and/or quality of the starting material and in conditions of sample preparation. For this purpose, transcripts of "housekeeping genes" that have a relatively stable level of expression in all cell types, are used. However, according to the literature, there is no such a "housekeeping gene" which expression level was stable under various experimental conditions. Besides, there's no data on the effect of cryopreservation on the expression of "housekeeping genes", which does not exclude its change after the freeze-thawing procedure. Therefore, when using a particular reference gene, the experimental confirmation of constancy of its representation level in the samples is needed. The solution of this problem is the parallel determination of the level of transcripts representation and stability of several "housekeeping genes", what we did in our experiment. This research will give explanation to the choice of the reference gene, most appropriate for this study, specifically, for a particular mode of cryopreservation.

P6.4

Deamination of methylated and non-methylated cytidines by human AID

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Activation-**i**nduced cytidine **d**eaminase (AID) was first identified as an enzyme that initiates two processes of antibody diversification: class switch recombination (CSR) and somatic hypermutation (SHM). AID triggers SHM and CSR as a result of its ability to deaminate cytidine to uridine. Interestingly, some of the recent data showed that this enzyme can also play a role in a process of an active genome demethylation – heart of epigenetic gene activation and reprogramming. Most of the data indicate that AID initiates demethylation by deaminating 5-methyl cytidine (5mC) to thymidine. This reaction in cooperation with the subsequent excision repair of DNA can lead to the removal of the epigenetic mark.

The potential role of AID in DNA demethylation process has been an area of controversy. AID has been reported to be expressed in pluripotent tissues, which strongly suggests its role beyond the immune system. Moreover, *in vivo* data from the mice primordial germ cells, the heterokaryon system of human somatic cells and embryos of lower vertebrates provide evidence for the involvement of AID in the active genome demethylation. Nevertheless, some *in vitro* data suggest that 5mC is a poor substrate for human AID. It has been proposed that the mechanism responsible for the lower activity of human AID on 5mC (relative to non-methylated substrate) is a size restriction against the methyl moiety imposed by the enzyme's catalytic center. The postulated steric requirements for the deamination reaction support the opposite hypothesis that the involvement of AID in DNA demethylation process is highly unlikely.

To verify the current hypothesis regarding AID catalytic center discrimination against 5mC, we tested the activity of human AID and its mutants on 5mC *in vitro*. We found that a mutant, which is inactive on cytidine, exhibits a robust activity on 5mC. It suggests that other mechanism, besides size restriction of the catalytic center for a methyl moiety, is involved in 5mC discrimination. Employing molecular modeling allowed us to gain a new insight into the issue of methylated and non-methylated substrate positioning in the AID catalytic center. On the base of our results we conclude that there is no size restriction against the methyl moiety imposed by catalytic center of human AID, so the proposed mechanism of 5mC discrimination is probably incorrectly considered the evidence against AID involvement in demethylation processes.

P6.5

The frequency of A118G polymorphism in MOR gene and comparison to the expression in healthy and autistic children

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Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder which ranks with prevalence about 60–70 per 10 000. Data shows that enhancement of the opioid system function or administration of opiates may result in autistic-like symptoms. The most important components of the discussed system are opioid receptors.

Research has confirmed that BCM-7 (β-casomorphin-7) is an exogenous opioid released from β-casein of cow's milk, and reacts with the μ-opioid receptor (MOR). It can enter the child's circulation and exert an effect on the opioid receptors in the brain to produce autistic symptoms.

Although there is obvious genetic basis for autism, information about specific gene responsible for the disease is lacking. It has been suggested that mutations or single nucleotide polymorphisms (SNPs) in genes may influence the expression of proteins in the opioid system and affect balance of opioid system function.

Polymorphism of MOR in exon I (A118G) is the most frequent. Presence of the G allele reduces transcription of gene, which has the effect on the binding ability to opioids and may result in the decreased sensitivity to pain.

In our study, we examined the frequency of A118G SNP in MOR receptor gene in healthy and autistic children. We also correlated specific point mutation in these genes with the effect of β-casomorphin-7 on the expression of μ-opioid receptor (MOR) gene.

Frequency of allele G in MOR gene was 0.09 in research population. There was no statistical correlation between the occurrence of autism and examined SNP in MOR gene. There was also no statistical evidence of A118G SNP in MOR influencing expression of the gene.

P6.6

The A118G polymorphism in MOR gene in patients with cancer

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In recent years, more and more attention is paid to the role of the opioid system in various disease processes. The most important components of this system are opioid receptors. MOR receptor is an important factor of action for clinically significant opioid analgesics.
Numerous point mutations have been identified in the MOR receptor gene, whereas polymorphism in its exon I (A118G) is the most frequent. The consequence of this mutation is the presence of aspartic acid (Asp) instead of asparagine (Asn) in its protein chain.
Molecular consequences of A118G MOR polymorphism which may explain the observed clinical effects remain unknown. However, the common fact is that the presence of the G allele at a polymorphic site of MOR receptor is associated with decreased ability to opioid binding and can results in the increased resistance to pain. The presence of opioids and opioid receptors has been demonstrated in many cancers, as well as tumor cell lines.
In the present study we included patients with breast cancer, and colorectal cancer. These cancers were selected primarily due to the fact that are some of the most common cancer types in Poland. Our choice of colorectal cancer was also dictated by the contact of the digestive tract with opioid peptides (β-casomorphin-7). Moreover in the milk of lactating women β-casomorphin-7 was also detected. Bioactive ingredients of food change the genetic expression in many cellular processes and thus may influence on the progress of carcinogenesis.
The aim of presented study was a comparison of the frequency of alleles A and G in polymorphic site of MOR receptor gene (A118G) in the group of patients diagnosed with breast cancer to a group of patients diagnosed with colorectal cancer. Both groups were compared to the frequency of this polymorphism in healthy people as a control group.
The obtained preliminary results suggest a strong association between A118G polymorphism in MOR receptor, with coexisted increase in occurrence of breast cancer (OR = 3.57, 95% CI: 2.26–5.65, P <0.0001). Our studies indicate that AA genotype in position A118G of MOR gene may have a protective effect against breast cancer.

P6.7

DNA damage and repair in depression

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Major depressive disorder (MDD, depression) is severe psychiatric illness affecting 350 mln people worldwide, which at its worst can lead to suicidal death. It is characterized by persistent low mood, loss of interest or pleasure, feeling of tiredness, loss of appetite and disturbed sleeping. Although pathophysiology of the disease is still not fully understood, increasingly number of reports indicate that inflammatory cytokines, together with other alternation such as oxidative stress and mitochondrial dysfunction may play important role in MDD. It was showed that patients with depression had elevated levels of 8-oxoguanine (an oxidative DNA damage marker), lipid peroxidation (an oxidative stress marker) and mitochondrial ROS production. Recently, increased expression of NLRP3 inflammasome was observed in the MDD patients. This protein complex is thought to activate pro-inflammatory pathways and contribute to the development of depression. Furthermore, it was revealed that NLRP3 is involved in DNA damage response and its knockout enhance efficiency of DNA damage repair. These findings encourage us to investigate the involvement of DNA damage and repair in depression.
In the present study we used alkaline version of comet assay to evaluate extent of endogenous DNA damage, the damage induced by hydrogen peroxide and kinetics of DNA repair. To assess the level of oxidative DNA damage, we used modified comet assay with two glycosylases of base excision repair pathway: hOGG1 and Nth. Study group consisted of 40 patients with diagnosed depression, and 40 healthy controls without depression episode. All experiments were performed on peripheral blood mononuclear cells isolated from blood samples by isopycnic centrifugation in gradisol. Our results not only validated the previous studies that patients with depression have elevated levels of oxidative DNA damage, but also indicated the presence of other DNA alternation, such as breaks and alkali label sites. Furthermore, for the first time we showed that the patients had lower efficiency of repair of oxidative DNA damage when compared to the controls.
Our findings suggest that lowering of the repair capacity may be one of the mechanism underlying pathogenesis of the disease. But the question how DNA damage or impairments of its repair are involved in development of depression is still open.

P6.8

TRIM28 transcriptional corepressor regulates breast tumor growth

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Cancer stem cells (CSCs) play an essential role in the development of various types of cancer and are largely responsible for resistance to chemotherapy and radiotherapy, metastases and relapse. Thus, various novel therapeutic approaches specifically targeting CSCs are currently being investigated by academic community and pharmaceutical companies. It has been documented that self-renewal of CSCs is controlled by epigenetic mechanisms that regulate the expression of CSCs-specific genes. Modulation of specific histone modifications and DNA methylation may be exploited to induce differentiation of CSCs and sensitize them to standard chemotherapeutics.
Here, we investigated the role of TRIM28 protein in the hemostasis of breast cancer stem cells. TRIM28 protein regulates expression of large group of genes through recruitment of histone methylases and deacetylases to specific genomic regions that leads to local DNA heterochromatization. As previously shown, TRIM28 depletion in mouse Embryonic Stem cells resulted in differentiation into non-pluripotent cells, therefore we hypothesize that TRIM28 knockdown in cancer would lead to reduction of CSCs population and would inhibit the tumor growth as well as sensitize the tumor to chemo- and radiotherapy.
In a first series of experiments, we have silenced TRIM28 gene expression in a panel of six breast cancer cell lines using lentiviral vectors carrying specific shRNAs. The knockdown efficiency was confirmed by RT-qPCR, Western blot and immunofluorescence using specific antibodies. Using FACS analysis we have evaluated the percentage and morphology of $CD44^{+}/CD24^{-/low}$ CSCs population in the $TRIM28^{KD}$ and WT breast cancer cells. Next, the modified and control cells were subjected to treatment with serial dilutions of doxorubicin, DNA intercalating drug that is a standard treatment for many types of cancer including breast tumors. Furthermore, $TRIM28^{KD}$ and WT breast cancer cells from the panel were subjected to radiotherapy. We have observed that TRIM28 depletion does not sensitize breast cancer cell to doxorubicin treatment and irradiation *in vitro*, suggesting the importance of cancer stem cell niche in maintenance of stem-cell properties and therefore, the essence for the observation of expected result.
Next, $TRIM28^{KD}$ and WT cells from two selected breast cancer cell lines from the panel were subjected for further studies *in vivo* in nude mice. Results obtained so far suggest, that the inhibition of tumor growth in TRIM28 depleted xenografts is due to differentiation of CSC population.
TRIM28 is overexpressed in more aggressive subtypes of breast cancer and its high level correlates with worse prognosis. Our current work is focused on investigating molecular mechanisms that mediates TRIM28-dependent proliferation and tumor growth. Ultimately, our findings may pave the way to novel and more effective therapies for breast tumors.

P6.9

Nencki Genomics Portal – a web-based platform for analysis of transcriptional co-regulation and function, starting from (epi-)genomic and expression data

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We present Nencki Genomics Portal (NGP) – a website integrating tools for analysis of gene transcriptional co-regulation and function, accessible to a broad biological community via a web browser at http://galaxy.nencki-genomics.org. The NGP tools are separated into four categories: genomic, expression, regulation and function, and closely integrated, so that the output of one tool can be an input for another (or can be stored).
The genomic tools leverage on Nencki Genomics Database, which extends Ensembl funcgen. The portal provides functionality of genome-wide refinement of regulatory regions, including mapping them to genes, intersecting with other types of regions, intersecting with TFBS motifs, and visualization of these data for specific genes. This makes public data from funcgen (and thus from ENCODE) immediately available to the user alongside own data.
The expression tools provide a typical workflow of analysis of transcriptomics data, from preprocessed gene expression data (genes x conditions) to identification of differentially expressed genes, clustering, and visualization.
The regulation section provides a specialized version of BNFinder that permits analysis of effects of interactions of several regulatory features on gene expression. This tool uses our mammalian model of cis-regulation, updated to take advantage of experimentally identified gene regulatory regions.
The function tools accept a (ranked) list of genes as inputs and provide a unified interface to established tools, including gProfiler, for analysis of functional annotations, such as Gene Ontology, KEGG and REACTOME.
In addition to the web browser, the local NGD tools are also accessible programmatically, via the standard SOAP/WSDL interface (http://webservices.nencki-genomics.org), permitting integration into automated analysis pipelines. The middle layer of NGP is based on Taverna Server, which allows us to seamlessly connect to webservices and command line tools, and to rapidly deploy new analysis workflows. The NGP architecture permits future tailoring of the portal to users' needs.

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P6.10

Role of the single nucleotide polymorphisms (SNPs) in bitter taste receptor TAS2R38 encoding genes in genetic predisposition to chronic rhinosinusitis (CRS)

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Background: Chronic rhinosinusitis (CRS) is one of the most common health complaints continues to await a new therapeutic approaches. There is little data on identifying specific genetic factors important for CRS. Bitter taste receptors (T2R38) may play a critical role in this process. They were recently shown to be expressed in cilia of sinonasal epithelial cells, suggesting that respiratory cilia may function as a chemosensory organelle, possibly to detect bacterial presence in the airway. T2R38 is encoded by the TAS2R38 gene. The single nucleotide polymorphisms (SNPs) in TAS2R38 gene may contribute to individual differences in susceptibility to CRS. So far is no evidence in Polish population for correlation between this SNP in TAS2R38 gene variants and higher risk to CRS.
Aim: The aim of this preliminary study was the identification of SNPs in bitter taste receptor TAS2R38 encoding genes in Polish patients with CRS to find potential correlation with CRS phenotypes.
Material and methods: The preliminary study concern 20 CRS patients undergoing functional sinus surgery (FESS). Fresh sinus mucosa (SM) was obtained during FESS from the osteomeatal complex in CRS patients. Patients were genotyped for TAS2R38 from blood, and the genotype frequencies of the medically recalcitrant CRS cohort was analyzed. Genotyping the TAS2R38 was performed using Sanger method. Analysis of TAS2R38 expression in SM of CRS patients was performed using immunohistochemistry (IHC).
Results: Our preliminary data showed that TAS2R38 is highly expressed in SM of CRS patients. We detected TAS2R38 in the nuclei and the cytoplasm. This location was found in all CRS patients. There were three frequently occurring amino acid polymorphisms (positions 49, 262, and 296) that gave rise to two haplotypes: a taster (proline, alanine, and valine = PAV) and non-taster (alanine, valine, and isoleucine = AVI). Our results showed that the frequency of AVI/AVI (nonfunctional genotype) (27.5%) and AVI/PAV (45%) was higher than the PAV/PAV (protective) (27.5%) genotype in the recalcitrant CRS patient. We observed that the staining intensity of TAS2R38 was stronger in PAV/PAV than in AVI/AVI and AVI/PAV genotypes.
Conclusion: TAS2R38 polymorphism may influence the susceptibility to CRS. The AVI/AVI genotype was an independent risk factor for CRS. However, we observed that both protective and nonprotective alleles (PAV, AVI) occurred at highest frequencies in the population and the susceptibility for CRS was changeable. It may be only in certain PAV/AVI patients that specific bacteria assault the airways and stimulate TAS2R38. The difference in genotype expression has raised our concern in terms of a question why heterozygote also suffer from CRS. Furthermore, we plan to compare the genetic results with selected immune responses and clinical data using statistical approach.

P6.11

Influence of O-GlcNAc transferase down-regulation on expression of Polycomb genes in breast cancer cells

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O-GlcNAc tarnasferase (OGT) is an enzyme which calalyzes the addition of N-acetylglucosamine moiety to serine/threonine residues of cytosolic or nuclear proteins. This dynamic and reversible modification called O-GlcNAcylation constitutes an important regulator of cancer growth and progression. In breast cancer the increased expression of OGT is correlated with increased invasion and metastatic potential. The results of recent studies concerning the role of OGT in the animal model systems showed, that this enzyme may link cell metabolic status with transcriptional repression caused by Polycomb proteins. It has been established that in Drosophila OGT is encoded by the Polycomb group (PcG) gene super sex combs (sxc) and genome-wide profiling revealed that GlcNAc-modified proteins are highly enriched at Polycomb response elements. Polycomb proteins regulate proliferation and differentiation of cells via epigenetic silencing of important growth regulatory genes. Deregulated expression of PcG proteins in particular BMI1 or EZH2 is associated with several human malignancies, including breast cancer. In this study we investigated the effect of OGT gene expression down-regulation by RNA interference in breast cell lines MCF10A, MCF7, MDA-MB-231 and Hs578t on expression of PcG genes, i.e. EZH2, SUZ12, RING1B and BMI-1 as well as histone modifications levels. Moreover, the expression of several Polycomb target genes involved in cell differentiation and epithelial mesenchymal transition was analyzed. The results showed that reduced OGT expression did not cause significant changes in mRNA level of Polycomb. However, OGT knockdown and decreased O-GlcNAcylation specifically down-regulated the EZH2 protein level. OGT down-regulation influence expression of some Polycomb target genes especially FOXC1 and CDH1. FOXC1, a member of the Forkhead box transcription factor family, plays an important role in differentiation. CDH1 encodes E-cadherin and its reduced expression is associated with metastasis and invasion. Both genes regulated by EZH2 showed increased expression in cells with OGT depletion. These results suggest that observed earlier correlation between increased breast cancer invasiveness and expression of OGT at least partially depends on OGT-PcG regulatory axis.

P6.12

Angiostatic abilities of arginine/lysine histone methyltransferase inhibitors, AMI-1 and A(K)MI-5

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Post-translational modification of histones, such as methylation, cause the chromatin structure changes affecting gene transcription process and in consequence, whole organism functions. Histones are subjected to an intricate pattern of posttranslational modifications such as acetylation and methylation. Methylation of lysine and arginine residues is one of the most often modifications of amino acid histone tails, catalyzed by number of histone methyltransferases (HMTases).

In presented studies we have focused on the role of HMTases in regulation of endothelial cell functions, particularly – angiogenesis process. To achieve the objective set, we have inhibited HMTases activity with AMI inhibitors and then selected functions of human microvascular endothelial cells (HMECs) were analyzed. Both compounds characterize diverse specificity and mechanism of inhibition. AMI-1 competes for the arginine binding site of PRMT and inhibits type I but not type II PRMTs. AMI-5 is a competitive inhibitor of *S*-adenosyl-L-methionine binding and has been shown to inhibit not only arginine, but also lysine methylation.

Inhibition of HTMases with AMI-1 and AMI-5 at the rage of concentrations: 0–200 μM, revealed changes in proliferation of cells (resazurin oxidation assay). Slightly cytotoxic effect of HTMases inhibition was observed after 24 h treatment. Extended incubation of HMECs with AMIs, till 72h (with changing the medium every 24h) resulted in enhanced cytotoxicity — IC_{50} parameter was determined at 100 μM ± 7.33 μM for AMI-5. AMI-1 was less cytotoxic for HMECs, and used range of concentrations did not allow to reached IC_{50}. Analysis of migration, one of the steps of angiogenesis process, have shown that both used inhibitors affect the process, in a dose-dependent manner. The biggest changes found in of wound-healing assay were observed at the highest concentration of the inhibitors. Also ability of HMECs to capillary-like tube formation was significantly decreased, with total inhibition of the process at the concentration of 100 μM and 300 μM, for AMI-5 and AMI-1, respectively. Trying to elucidate observed modulation of angiogenesis process *in vitro*, we checked the level of protein, released by HMECs. Elisa test have shown significantly less amount of MMP-9, THBS-1 and MCP-1 in cell culture supernatant after inhibitors treatment.

Performed analysis clearly show involvement of arginine/lysine histone methyltransferases in the regulation of angiogenesis process.

Acknowledgements

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P6.13

Novel *THAP1* (DYT6) mutations in Polish patients with dystonia

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The dystonias are a group of movement disorders characterized by contractions of agonist and antagonist muscles leading to involuntary movements and abnormal postures of various parts of the body. Several genes associated with dystonia have been identified. One of these is *THAP1* gene coding for a DNA-binding transcription factor named thanatos-associated protein domain containing apoptosis-associated protein 1. Over 50 missense, nonsense and frameshift mutations within *THAP1* gene have been described in dystonia patients in various populations. The aim of this study was to assess the presence of *THAP1* gene mutations in Polish patients with dystonia. We analyzed *THAP1* exons and their non-coding boundaries in 108 dystonia patients and 150 healthy controls. DNA was isolated from blood leucocytes; screening for mutations was performed using PCR and DNA sequencing methods. In four patients single nucleotide substitutions were identified. The codon 80 Ile/Val substitution was found in two patients, and codon 56 Glu/Gly mutation, in one patient. In another patient, a novel C/A substitution in the 5'-near gene region upstream of exon 1 was identified. Neither of these substitutions were found in healthy subjects. Further studies are needed in order to establish a possible influence of these nucleotide changes on the *THAP1* protein expression or function.

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P6.14

EPHA1 as a new candidate gene for non-syndromic intellectual disability

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Over the past 15 years many single gene causes of non-syndromic intellectual disability have been identified. However, up to 60% of patients have no genetic identifiable cause.

We report a tie between the *EPHA1* gene and ID on Ukrainian family (healthy non-consanguineous parents and two affected children with intellectual disability and similar psychoneurological symptoms). Biochemical and CGH array investigations revealed no genetic abnormalities in children while whole exome sequencing performed in family members identified two non-synonymous variants c.1475G>A and c.1891G>A in *EPHA1*. Both affected siblings were compound heterozygotes while father and mother were heterozygous carriers for c.1891A and c.1475A respectively. The *EPHA1* gene belongs to the ephrin receptor subfamily of the protein-tyrosine kinase family and encodes for the ephrin type-A receptor 1 involved in mediating nervous system developmental events and not previously associated with ID.

C.1475G>A and c.1891G>A frequencies analyses in 300 healthy Ukrainian controls revealed that the c.1475T allele frequency was 1.2% while the c.1891A was not found at all. We then analyzed the domains of c.1475G>A (p.R492Q) and c.1891G>A (p.G631R) and found that the mutations were in the fibronectin type III repeat and tyrosine kinase domain of EphA1 protein, correspondingly. On screening the site orthologs we found that R492 and G631 amino acid are conserved across mammalian and bird species. To understand a possible effect of these substitutions the mutant EphA1 proteins' tertiary structures were predicted. As it turned out the substitutions are located in important functional domains of EphA1. The substitution of positive charged Arg492 to uncharged Gln492 (c.1475G>A) is in the fibronectin type III repeat of EphA1 ectodomain involved in signal transduction and binding with ligands or protein-partners. The substitution Gly631Arg (c.1891G>A) is in the glycine-rich region of EphA1 tyrosine kinase domain responsible for ATP binding. We assume the c.1475G>A and c.1891G>A mutations may cause changes in conformational flexibility and solubility of these EphA1 domains resulting in impaired Eph signal transduction. This might result in the pathological changes in neural (brain) topography that became the cause of ID in two affected siblings from Ukrainian family.

We propose new candidate gene for ID. Our study implicates *EPHA1* gene mutations as a cause of a non-syndromic form of intellectual disability.

P6.15

Histones are targeted for *N*-homocysteinylation in human endothelial cells

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Hyperhomocysteinemia (HHcy) is an independent risk factor for cardiovascular and neurodegenerative diseases, and leads to the accumulation of a chemically reactive metabolite, homocysteine (Hcy) thiolactone (HTL). HTL modifies ε-amino groups of protein lysine residues, which impairs protein structure and function. *N*-Hcy-proteins become susceptible to aggregation, amyloidal transformation, oxidative damage, and induce cellular toxicity, inflammation, and autoimmune responses that can lead to atherosclerosis (Jakubowski H *Homocysteine in Protein Structure/Function and Human Disease*, Springer, Wien 2013). Endothelium damage plays a central role in atherosclerosis and a contributing mechanism may involve protein *N*-homocysteinylation (Jakubowski H *et al.*, 2000, *Circ Res* **87**: 45–51). In particular, modification of histones by HTL would affect epigenetic regulation of gene expression and thus contribute to the pathology of HHcy. However, it is not known whether histones are targeted for *N*-homocysteinylation in endothelial cells.

To determine whether modification of histones by HTL is a plausible cellular mechanism, we examined *N*-Hcy-histone formation *in vitro* and *ex vivo* in human umbilical vein endothelial cells (HUVECs). We also studied effects of Hcy, HTL, and *N*-Hcy-protein on histone *N*-acetylation in HUVECs. Using MALDI ToF/ToF mass spectrometry we identified *N*-homocysteinylation sites in human recombinant histones modified with HTL *in vitro*. Six *N*-Hcy-Lys sites (K59, K69, K82, K85, K97, K103) were identified in modified histone H1, five (K6, K37, K75, K76, K96) in H2A, four (K47, K109, K117, K121) in H2B, two in (K24, K80) H3.1, and three in histone H4 (K6, K13, K80). We purified histones from HUVECs treated with HTL or Hcy, separated into individual histone species by SDS-PAGE, and subjected to Western blotting using antibodies against *N*-Hcy-protein or *N*-Ac-Lys9-histone H3. We found that histones H3 and H4 were *N*-homocysteinylated in HUVECs treated with HTL or Hcy in a concentration dependent manner. We also found that the level of *N*-Ac-Lys9-histone H3 was not affected by treatments of HUVECs with HTL or Hcy. However, *N*-Ac-Lys9-histone H3 was decreased by treatments with *N*-Hcy-protein in a concentration-dependent manner.

In conclusion, our results indicate that each of the individual histones is susceptible to modification by HTL *in vitro*, that histone H3 and H4 are targeted for *N*-homocysteinylation in endothelial cells, and that *N*-Hcy-protein reduces histone H3 acetylation. Our findings support a hypothesis that histone *N*-homocysteinylation plays a role in the pathology of HHcy.

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P6.16

Telomere Length and Blood Cell Counts in Polish elderly population — PolSenior substudy

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Background: Age-related decrease in bone marrow erythropoietic capacity is often accompanied by the telomere length shortening in peripheral white blood cells. However, limited and conflicting data hamper the conclusive opinion regarding this relationship. Therefore, the aim of the study was to assess an association between telomere length and peripheral blood cell count parameters in the Polish elderly population.

Material and methods: The substudy included 1573 of 4981 subjects aged 65 years or over, participants of the population-based PolSenior study. High-molecular-weight DNA was isolated from blood samples. Telomere length (TL) was measured as abundance of telomere template vs. a single gene copy encoding acidic ribosomal phosphoprotein P0 by QRT-PCR.

Results: There was a trend towards higher all blood cells counts but lymphocytes and monocytes with increasing TL. Results of univariate analyses showed a significant association of TL with white blood count (WBC) in all subjects ($p = 0.02$) and in men ($p = 0.01$). Only in men significant, but weak correlations were found between TL and WBC ($r=0.11$) and RBC ($r=0.08$). The multiple regression analysis models confirmed a week independent contribution of TL to both RBC and WBC.

Conclusions: In elderly telomeres shortening limit hematopoiesis capacity in a very limited extent.

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P6.17

Spatial chromatin organization within the 30 kb genomic fragment containing the human tyrosine hydroxylase gene

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Tyrosine hydroxylase (*TH*) is the rate-limiting enzyme for catecholamine synthesis, and loss of *TH* expression in the brain leads to a reduction of dopamine synthesis and neurological disorders. Thus, there is great interest in understanding how expression of the *TH* gene is controlled. The human *TH* gene is located on the short arm of chromosome 11 between the *Igf2* and *H19* genes. Both uni- and biparentally expressed genes and several sequences required for precise transcriptional control of these genes have been mapped within this evolutionarily well-conserved region (Onyango *et al.*, 2000). Several scaffold/matrix attachment regions (S/MARs) have also been identified in this region (Weber *et al.*, 2003). Attachment of chromatin to the nuclear matrix (NM) via S/MARs is cell type-dependent, changes dynamically with the phase of the cell cycle, and affects expression of neighboring genes (Chavali *et al.*, 2011, Ottaviani *et al.*, 2008). We previously demonstrated that the -2300/+2300 fragment of the human *TH* gene is anchored by NM proteins in a tissue-specific manner (Lenartowski & Goc, 2002), suggesting a mechanism by which *TH* expression can be controlled. The purpose of this study was to identify NM protein-associated sequences within the wider, 30-kb human genomic region containing the *TH* gene. We isolated nuclear matrices from two human cell lines with differing *TH* transcriptional status: neuroblastoma SH-Sy5y cells, in which *TH* is transcriptionally active, and HepG2 cells, in which *TH* is inactive. We mapped regions that are attached to the NM by treating the NM with DNAse I, which will only cleave sites that are not anchored to the NM. We then used the protected DNA as template in PCR reactions with five pairs of primers for the 15-kb *TH* promoter, three pairs for the coding region, and two pairs for the 7-kb downstream region. Only three regions, -14582/-13938, +2916/+3526, and +6527/+6997, were DNase I sensitive in the chromatin from SH-Sy5y cells. By contrast, all examined regions were DNAse I sensitive in the chromatin from HepG2 cells. We conclude that seven of the examined regions of the *TH* genomic sequence were attached to the NM in SH-Sy5y cells, whereas none of the examined regions were attached to the NM in HepG2 cells. These results implicate an important role for S/MARs in cell type-specific control of *TH* gene expression.

P6.18

Influence of CacyBP/SIP phosphatase on gene expression in colon cancer HCT116 cells

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CacyBP/SIP was originally discovered in Ehrlich ascites tumor cells (Filipek & Wojda, 1996) and was shown to interact with some proteins from the S100 family (Filipek *et al*., 2002) as well as with Siah-1 (Matsuzawa & Reed, 2001), Skp1, tubulin, actin, tropomyosin and extracellular signal-regulated kinase (ERK)1/2 (Schneider & Filipek, 2011; Kilanczyk *et al*., 2011). Analysis of the CacyBP/SIP level in different tissues showed that it is highly expressed in brain and spleen (Jastrzebska *et al*., 2000) and in cell lines such as neuroblastoma NB2a and SH-SY5Y, rat pheochromocytoma PC12 (Filipek *et al*., 2002) or human colorectal cancer HCT116cell line (Kilanczyk *et al*., 2012). In normal tissues eg. stomach or colon CacyBP/SIP is weakly or barely detected whereas in gastric or colon cancer its expression is much higher. Moreover, the level of CacyBP/SIP seems to be correlated with cell metastatic potential (Chen *et al*., 2008; Zhao *et al*., 2002). The effect of CacyBP/SIP on the multidrug resistance phenotype of gastric cancer cells was also studied and the data obtained showed that expression of this protein was negatively correlated with sensitivity of gastric cancer cells to vincristine, adriamycin and 5-fluorouracil.
Since some results suggest association of CacyBP/SIP with tumorigenesis and with multidrug resistance (Zhao *et al*., 2002), in this work we assessed functional consequences of the altered level of CacyBP/SIP in highly proliferating cells namely colon cancer HCT116 cells. In particular we investigated changes in gene expression in these cells after CacyBP/SIP overexpression or knock-down. Total RNA from cells transfected with plasmid encoding shRNA, which silence CacyBP/SIP expression, or from cells overexpressing CacyBP/SIP was isolated and subjected to microarray analysis. This allowed us to identify sets of genes, which were significantly up- or down-regulated due to change in CacyBP/SIP level. Based on functional analysis we found many genes the products of which are responsible for cell proliferation or involved in immune response. These observations point to CacyBP/SIP as an important player in signaling pathways whose disequilibrium induces relevant changes in cellular "transcriptome".

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P6.19

Transcriptional and epigenetic regulation of osteopontin (SPP1) expression in glioma cells

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Clinical and experimental studies show accumulation of microglia/macrophages in gliomas and their contribution to tumor progression. Re-programmed brain macrophages support tumor growth, invasion, induce immunosuppression and modulate response to cancer treatment. We identified osteopontin (known as secreted phosphoprotein 1 - SPP1) as one of the factors released by glioma cells which induces pro-invasive activation of microglia and creates the immunosuppressive tumor milieu. Osteopontin is overexpressed in many cancers including high grade gliomas and its expression inversely correlates with patient's survival. To investigate molecular mechanisms underlying regulation of osteopontin expression in glioma cells, we performed a computational analysis of the human *SPP1* gene promoter that revealed potential binding sites of transcription factor GLI1 (glioma-associated oncogene homolog 1), a Hedgehog signaling effector implicated in tumorigenesis. Using chromatin immunoprecipitation (ChIP) we confirmed binding of GLI1 to the *SPP1* gene promoter in U87MG, LN18 and primary glioblastoma WG4 cell, but not in nontransformed human astrocytes. Knockdown of *GLI1* expression in U87MG astrocytoma cells decreased the osteopontin expression at the mRNA and protein level. Previous studies on mouse embryos demonstrated binding of Oct4 (octamer-binding transcription factor 4), involved in maintaining stem cell pluripotency, in the first intron of *Spp1* gene. Using ChIP we showed that OCT4 binds to the first intron of the *SPP1* gene promoter in U87MG, LN18 and WG4 glioblastoma cells but not in normal human astrocytes. This finding implicates OCT4 in regulation of SPP1 expression in glioma cells. Accordingly, we found the increased level of osteopontin in populations enriched in glioma stem-like cells isolated by flow cytometry as side population or grown as cancer spheres. To investigate involvement of epigenetic mechanisms in regulation of osteopontin expression, we treated glioma cells with inhibitors of histone modifying enzymes (trichostatin A, 3-Deazaneplanocin A). Inhibition of histone deacetylases (HDACs) led to increase of the osteopontin expression in LN18 glioblastoma cells at the mRNA and protein level. Conclusions: Our results demonstrate re-establishment of the stem cell-type, transcriptional regulation of osteopontin expression in glioblastoma cells, in particular in glioma stem-like cells.

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P6.20

let-7d as a way to personalized therapy of head and neck cancers

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Introduction: miRNAs are proposed to be a good candidate as biomarkers for personalization of medicine nowadays. The main problem of miRbiomarkers is to define their physiological and pathological expression levels. We still don't know if two different over-expression levels have the same cellular effect or cause different cell behavior. let-7d has significant function in the biology of cancer. It acts as tumor suppressor and regulates expression proteins such as C-MYC, K-RAS, HMG2A proteins or Dicer enzyme and participates in epithelial-to-mesenchymal transition proces.
Purpose: Influence of different let-7d over-expression levels on behavior of hypopharynx squamous carcinoma cells (FaDu cell line) after irradiation and chemoexposure.
Methods: FaDu cell line with different let-7d over-expression levels have been achieved using lentiviral plasmids. The expression of let-7d and genes characteristic of the phenotypes of the cell, target protein levels and proliferation ratios have been measured in all models by qRT-PCR, western blot and 3H-thymidine incorporation respectively. The models have been irradiated using dose of 2 Gy and cells response has been described by clonogenic assay. The influence of 5-FU, cisplatin, doxorubicine and pacilitaxel has been measured by MTT assay.
Results: It was observed: 1) Decrease expression of genes characteristic for epithelial cells and increase of it for mesenchymal cells; 2) Corelation of cells survival after irradiation on miRNAs levels; in the case of low over-expression of let-7d, cell survival fraction (SF) was low (SF about 30%) and increased (SF about 70%) in the case of higher miRNAs over-expressions; higher levels of miRNAs caused strong radioresistance; 3) Cell response to cisplatin, doxorubicine and 5-FU depended on let-7d level but there was lack of correlation in the case of pacilitaxel exposure.
Conclusions: Over-expression of let-7d seems to be weak inhibitor of C-MYC and K-RAS proteins but these proteins may be not the first line of miRNAs inhibition under this genetic background. One miRNA regulates many proteins which have similar function or exist in the same pathway. The different overexpression levels have different cellular effect and cause different cell behavior after irradiation and chemoexposure. The cells survival depends on miRNAs level and it seems to be not linear corelation. We suspect existence of some miRNAs ranges when influence of miRNAs on cell is comparable and miRNAs thresholds when the cell phenotype highly changes. The mechanism of this phenomenon is unknown, but we suppose influence of changes in target gene regulation, which in turn modify cell phenotype.

P6.21

XRCC1 recruitment to endogenous DNA damage in replicating cells — studies by quantitative confocal and super-resolution microscopy

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Background: XRCC1 (X-ray repair cross-complementing protein 1) is a DNA repair factor involved in Base Excision Repair (BER) pathway. It takes part in repair of DNA single-strand breaks formed by exposure to ionizing radiation and alkylating agents. XRCC1 protein interacts with DNA ligase III, polymerase beta and poly (ADP-ribose) polymerase (PARP).
Goal: This work was focused on investigating a relationship between spontaneous induction of single-strand DNA breaks, DNA replication and formation of microfoci containing XRCC1.
Methods: Fluorescence live cell confocal imaging of mRFP-XRCC1 and eGFP-PCNA fusion proteins, structured illumination microscopy, single molecule localisation microscopy, and quantitative analysis of 3D data stacks and time-lapse sequences were used. DNA replication was also detected by incorporation of EdU (5-ethynyl-2'-deoxyuridine) followed by "click" reaction, imaged in fixed cells; γH2AX, as a marker of double-strand DNA breaks, was detected by immunofluorescence.
Results: In a population of transfected HeLa cells maintained in an *in vitro* culture a small proportion of cells develop spontaneously conspicuous XRCC1 microfoci. The foci are induced in S-phase and their number grows in time. The XRCC1 foci are found almost exclusively in the immediate vicinity of replicating DNA. Time-lapse recordings demonstrate that, following the formation of XRCC1 foci, replication is resumed close to these foci. Most cells exhibiting the numerous XRCC1 foci adjacent to replication regions eventually die, although some of these cells apparently survive the replication stress, complete replication, enter G2 phase and divide successfully.
Conclusions: Live cell imaging, confocal and super-resolution microscopy experiments suggest that spontaneously formed single-strand DNA breaks may be associated with replication forks. Although most cells that developed a high number of single-strand DNA breaks in S-phase are destined to die, apparently some are capable of repairing the damage and returning to the cell cycle.

P6.22

Polymorphism of selected opioid system genes in patients with acute pancreatitis

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Acute pancreatitis is a progressive inflammatory disorder which reveals from both genetic (mutations in genes) and environment factors (alcohol, cigarettes, chemicals, opioids) which can modify endogenous opioid system. Moreover, many researchers also proved the autoimmune ethiology of this disorder.

β-casomorphin-7 (BCM7) is a peptide released from amino acid sequence of β-casein. BCM7 demonstrates the opioid character and may influence on nervous, digestive and immune functions via μ-opioid receptors (MORs). BCMs are the substrates for proline dipeptidyl peptidase-IV (DPP IV, CD26, EC 3.4.14.5), the only enzyme responsible for degradation of these peptides. After intake of opioid peptides with dairy products both immune and digestive systems are stimulated, which may be the cause of many dysfunctions included food allergy, autism, pancreas disorders and diabetes type I. Moreover, the genetic factors of acute pancreatitis suggest that mutations in MOR and DPPIV genes may be also important informations to know the etiopathogenesis of this disease. Many authors proved that numerous point mutations have been identified in the MOR and the polymorphism in its exon I (A118G) is the most frequent of them. Occurrence of the G allele in the polymorphic site of the MOR is connected with lowering the transcription of the receptor and decreasing its ability to bind opioids. Consequently, there may be differences in the functioning of the endogenous opioid system, which is responsible not only for the feeling of pain, but also for the development of alcohol dependence for its prominent function in the central rewarding mechanism. Studies have shown that agonists of MOR can reduce alcohol consumption and it is associated with the above mentioned A118G polymorphism.

The aim of this study was to determine the frequency of alleles A and G in polymorphic site of MOR receptor gene (A118G) and rs7608798 of DPPIV (A/G) in the group of patients diagnosed with acute pancreatitis and among the healthy people as a control group.

In whole research population frequency of allele G was 0.07 for A118G SNP of MOR, and 0.63 for G allele of rs7608798 SNP in DPPIV gene. The obtained results also suggest an association between examined polymorphism rs7608798 in DPPIV gene, with coexisted increase in acute pancreatitis (OR = 1.91, 95% CI: 1.19-3.04, P <0.007). Our studies indicate that AA genotype in position rs7608798 in DPPIV gene may have a protective effect against acute pancreatitis.

P6.23

Influence of global genome hypermethylation on mRNA level in sperm of mice with deletion in the Y-chromosome long arm

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Microarray analyses carried out in our laboratory revealed, that DNA of sperm produced by mice with large deletion in the Y-chromosome long arm (B10.BR-Y^{del}) is strongly hypermethylated and has different methylation pattern than DNA of sperm of control mice having the intact Y chromosome (B10.BR). In the present study we compared mRNA levels of 175 genes in B10.BR-Y^{del} and B10.BR spermatozoa to find out if the caused by the deletion aberrant DNA methylation has functional significance and affects gene expression. For the experiment we chose genes which play important role during spermatogenesis and simultaneously exhibit different methylation status in sperm of B10.BR-Y^{del} males in comparison with sperm of control males (137 hypermethylated and 38 hypomethylated genes). Transcription levels were assessed with RT real-time PCR method using custom designed TaqMan Array 96-Well Fast Plates. Most of the examined genes appeared to have very similar expression in the both sperm groups. For some of them we revealed, however, significant change of mRNA level in B10.BR-Y^{del} spermatozoa in relation to B10.BR spermatozoa: *Neurog3*, *Cfd*, *Chx2*, *Dnahc8*, *Tex11*, *Nutm1*-decreased mRNA level and *Ceacam2*, *Spata3*, *Fancd2*-increased mRNA level. Although majority of the investigated genes is similarly expressed in the compared groups of gametes, as many as 144 of them (82,3%) have slightly lower mRNA level in B10.BR-Y^{del} sperm. We postulate that global hypermethylation of genome in B10.BR-Y^{del} spermatozoa is accompanied with slight underexpression of numerous spermatogenic genes. Changes in expression levels are most often negligible in the context of single genes, but collectively they may considerably affect spermatogenesis and contribute to the fact that males charged with the broad deletion in the Y-chromosome long arm produce spermatozoa of definitely lower quality than control males with the intact Y chromosome.

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P6.24

Development of interferon alpha response in primary hepatocytes of rat

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Background: Interferon alpha (IFNα) is a pleiotropic multifunctional cytokine that has a key role in early immune events. It is produced at the minimal level in healthy liver and up-regulated by viral RNA, LPS and damage-associated molecules. Knowing how a cell responds to IFNα in dynamics is important for understanding its function. Herein for the first time we examined the gene expression profile in primary rat hepatocytes treated during two time periods — 3 and 6 hours, with quasi-physiological dose of IFNα similar to that locally produced in regenerating rat liver after partial hepatectomy.
Methods: We incubated primary rat hepatocytes with 250 u/ml of rat IFNα. We used gene expression profiling with Affymetrix rat genome array 230 2.0 (Affymetrix, USA) followed by computational analysis of promoter regions of differentially expressed genes to identify the signaling pathways that are engaged by IFNα.
Results: We identified 28 and 124 differentially expressed up-regulated genes which reveal the cell-specific traits of response and distinctive dynamics. From the beginning the up-regulation of genes responsible for autophagy, ISGylation, inhibition of translation and transition of mitochondrial dNTP synthesis to salvage pathway and chemokine CXCL10 production comes to the fore. Altogether they manifest the transit from the initial mode of cellular activity to the resource-conserving one, the usage of preexisting proteins and the attraction of the cells of putative hepatic environment for the development of response. The later changes are associated with the expansion of spectrum of differentially expressed genes, increasing magnitude of response and emerging interplay between activating and inhibiting factors which regulate the signal transduction, transcription and the activity of putative cells of hepatic environment. The classical Jak/STAT/ISGF3, Jak/STAT, PI3K and p38 signaling pathways are engaged in IFNα induced response of hepatocytes.
Conclusion: The short-term treatment of hepatocytes with quasi-physiological dose of IFNα induces the self-autonomous changes in hepatocytes and those referring to the hepatocytes potential input to the whole hepatic response. The interplay between activating and inhibiting factors of immune response is a characteristic feature of hepatocytes reaction to specified IFNα treatment.

P6.25

Predictive biomarkers in the development of heart failure after acute myocardial infarction

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Heart failure (HF) is the most common cause of morbidity and mortality in the developed countries, especially considering the demographic tendencies in their populations. The development of HF after acute myocardial infarction (AMI) is a result of left ventricular (LV) remodeling. This complex process, which is strongly associated with adverse outcome, involves changes in cardiac morphology affecting both cellular and extracellular elements of the myocardium. Early prediction of LV remodeling and development of HF after AMI is a challenge and may potentially be improved by the identification of novel molecular biomarkers associated with this process.
In our study we identify biologically relevant transcripts that are significantly altered upon HF and are associated with its progression. Blood samples were collected from 111 patients with AMI and from 41 patients from a validation cohort. The patients underwent comprehensive clinical evaluation on admission and throughout the follow-up period of 6 months. Total RNA isolated from peripheral blood mononuclear cells (PBMCs) was used for microarray analysis (Affymetrix Human Gene 1.0 ST microarrays). We obtained transcription signatures soon after AMI and throughout the follow-up, and found that in the acute phase of AMI dozens of genes from several pathways linked to lipid/glucose metabolism, platelet function and atherosclerotic plaque stability showed altered expression in PBMCs. On the basis of parameters commonly used for clinical diagnosis of patients with HF, such as plasma NT-proBNP and ejection fraction, the AMI patients were divided into quartile groups. We have identified a set of genes whose expression differed significantly between HF *vs.* non-HF patients on the 1st day of AMI. Validation by RT-qPCR was carried out for selected genes expressed significantly differently between HF vs. non-HF patients on admission: *FMN1* (Formin 1), *TIMP1* (Metalloproteinase inhibitor 1), *RNASE1* (Ribonuclease, RNase A family, 1), *JDP2* (Jun dimerization protein 2). Additionally, the analyses were performed on the validation cohort and these results confirmed the prognostic value of the chosen genes.
In summary, the identified changes in gene expression over time that differentiated HF patients from non-HF ones may serve as a novel tool contributing to prognosis and diagnosis of HF.

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P6.26

Pyrosequencing of *NDRG2* promoter region in central nervous system tumors reveals higher levels of methylation as compared to normal brain tissue

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Downregulation of *NDRG2* expression was found in various human cancer cell lines and tissues, including central nervous system cancers. Aberrant methylation of the *NDRG2* promoter region was reported as a likely cause of transcriptional silencing in primary glioblastomas and atypical and anaplastic meningiomas, but these suggestions were not confirmed on large patient cohorts. The aim of this study was to assess the methylation status of *NDRG2* promoter and to correlate the results with histological tumor grade, age, gender, progression free and overall survival time of central nervous system cancer patients. One hundred and seven tumor samples of CNS cancers were analyzed: 82 gliomas and 25 meningiomas. MSP technique was used for qualitative evaluation of the methylation status of 4 CpG dinucleotides and pyrosequencing for quantitative analysis of the methylation level of other 5 dinucleotides located 643 nt downstream of the region analysed by MSP. Pyrosequencing revealed significant number of samples with methylation level higher than that observed in normal non-cancerous brain tissue, namely 70.73% (58/82) of gliomas and 84.00% (21/25) of meningiomas had average methylation level of 5 analyzed CpGs higher than 11%, which was the cut-off level determined on the basis of non-cancerous brain tissue. Both in gliomas and meningiomas, the highest methylation level was observed in CpG4 (31.27% and 27.60%, respectively), whereas the lowest in CpG1 (14.23% and 8.17%, respectively). Pyrosequencing did not differentiate tumors of different grades, however in meningioma subgroup, the average methylation of 5 CpGs correlated with the age of patients (older patients had higher average methylation levels). Methylation in CpG2 was correlated with gender (women had higher methylation levels than men). Moreover, methylation level in CpG5 correlated with progression free and overall survival time of meningioma patients. MSP assay did not detect any methylated sample in both subgroups of patients, which can potentially be explained by the fact, that two different fragments of the promoter were analyzed, indicating that the one analyzed by pyrosequencing is a target of aberrant methylation. We conclude, that *NDRG2* methylation as assessed by pyrosequensing, is a hallmark of central nervous system cancers and could be considered as potential prognostic biomarker for meningioma patients.

P6.27

Epigenetic mechanisms in microglia polarization

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Classical inflammatory (M1) and alternative, anti-inflammatory (M2) phenotype are the two extreme states of microglial activation that can produce either detrimental or beneficial effects in the central nervous system. Recent studies indicate that glioma associated microglia polarize into anti-inflammatory phenotype and support invasion, angiogenesis and suppress the adaptive immunity. Transcriptome analysis of microglial primary cultures exposed to glioma-conditioned medium (GCM) or lipopolysaccharide (LPS) revealed activation of distinct signaling and metabolic pathways resulting in different patterns of gene expression. As these two phenotypes are relatively stable we sought to determine if changes in gene expression could be mediated by epigenetic mechanisms such as histone modifications. Chromatin immunoprecipitation with four antibodies that recognize common activating and repressive histone modifications revealed the decrease of histone acetylation in microglia exposed to GCM for 6 h. This alteration correlated with the increased histone deacetylase (HDAC) activity in the nucleus of GCM treated microglia. Changes in repressive histone modifications after GCM or LPS were delayed and correlated to transcription downregulation that suggests their role in consolidation of the phenotype towards which the cell is polarized. To investigate the role of histone modifications in microglia polarization, cells were exposed to GCM or LPS in the presence of selected inhibitors. HDAC inhibitors blocked morphological changes associated with GCM or LPS treatment and decreased the expression of GCM up-regulated genes to the level observed in control microglia. Furthermore, HDAC inhibitors restored the expression of LPS-inducible genes inhibited by GCM pretreatment. These results show that epigenetic modifications are crucial for polarization of microglia and inhibition of histone modifying enzymes blocks acquisition of a specific phenotype.

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P6.28

Polymorphisms of *RAD51*, *RAD51B*, *XRCC2* and *XRCC3* repair genes and prostate cancer

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Although prostate cancer is one of the most common cancer in men genetic defects underlying its pathogenesis remain poorly understood. DNA damage repair mechanisms have been implicated in human cancer. Accumulating evidence indicates that fidelity of double strand break response to DNA damage is critical for maintaining genome integrity. RAD51 and proteins that function with RAD51, ie. RAD51B, XRCC2 and XRCC3 are central players in double strand breaks repair *via* homologous recombination and its alterations may confer to increase the risk of cancer. The aim of the present work was to evaluate association between prostate cancer risk and polymorphisms of genes encoding proteins participating in homologous recombination repair: *RAD51* (rs1801320, c. -98G>C, G135C and rs1801321, c. -61G>T, G172T), *RAD51B* (rs10483813, c. 1037-29918T>A and rs3784099, c. 757-8674G>A), *XRCC2* (rs3218536, c. 563G>A, p. Arg188His*)* and *XRCC3* (rs861539, c. 722C>T, p. Thr241Met). Genomic DNA form peripheral blood of 101 prostate cancer patients and age matched 216 cancer-free men was obtained using the phenol-chloroform extraction and AxyPrep Blood Genomic DNA Miniprep Kit (Axygen Biosciences). Polymerase chain reaction combined with a restriction fragment length polymorphism (rs1801320, rs3218536, rs861539) and TaqMan® SNP Genotyping Assay (rs1801321, rs10483813, rs3784099) were used for genotyping.
No significant differences in the genotypes distribution and allele frequencies between prostate cancer patients and controls, except the rs1801320 polymorphismin the *RAD51* gene were found. Prostate cancer risk was significantly increased for carriers of the C allele of the rs1801320 polymorphism in the *RAD51* gene (OR=2.04, 95% Cl=1.30-3.19, P=0.002). No statistically significant correlation of the rs1801320 polymorphism in the *RAD51* gene with age of prostate cancer patients and PSA level was observed.
The rs1801321 of *RAD51*, rs10483813 and rs3784099 of *RAD51B*, rs3218536 of *XRCC2* and rs861539 of *XRCC3* polymorphisms seems to have no impact on the risk of prostate cancer development while rs1801320 of *RAD51* appears to be important in prostate tumorigenesis.

P6.29

The influence of DNA methyltransferase inhibitors on the methylated suppressor genes

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Transcriptional silencing of tumor suppressor genes, caused by excessive or incorrect methylation of CpG islands located within the promoter regions, is associated with multistage carcinogenesis. DNA methyltransferases (DNMT's), which are responsible for an addition of a methyl group at the carbon 5 position of the cytosine ring in CpG dinucleotides, are involved in an epigenetic inactivation of tumor suppressor genes. The methylation of DNA is regulated by activity of few enzymes belonging to DNA methyltransferase family, however an important role in tumorigenesis play DNMT3A and DNMT3B which are responsible for *de novo* methylation. Overexpression of that methylotransferases are observed in ovarian carcinomas. It is established that DNA methyltransferase inhibitors can reduce the tumors formation.
The aim of the study was to verify the influence of DNA methyltransferase inhibitors (DNMT'is): the nucleoside analogue and the non-nucleoside inhibitor, on the methylation and expression level of two suppressor genes related to ovarian cancer *OPCML* (*Opioid Binding Protein/Cell Adhesion Molecule-Like*) and *DIRAS3* (*DIRAS Family, GTP-Binding RAS-Like 3*). *OPCML* is epigenetically inactivated and has tumor-suppressor function in epithelial ovarian cancer, while *DIRAS3* appears to be a putative tumor suppressor gene whose function is abrogated in ovarian and breast cancers.
Analyses of methylation level were carried out on three ovarian cancer cell lines A2780, TOV-21G and OVP-10. Cells were grown under standard conditions in complete growth medium (TOV-21G – mixture of MCDB 105 medium and Medium 199 (1:1) with 15% FBS and 10μg/ml gentamycin, A2780 and OVP-10 – RPMI-1640 medium with 10% FBS and 10μg/ml gentamycin) at 37°C and 5% CO_2. Stock solutions (100mM) of 5-Aza-2'-deoxycytidine and RG108 were prepared by dissolving in DMSO, diluted in cell culture media at different concentrations (1–25 μM) and were added into cultured cells for 1–7 days. After cells harvesting DNA was isolated, and MSP analyses were made after the bisulfite conversion of DNA. Simultaneously total cellular RNA were extracted and the gene expression was examined by RT-PCR.
Methylation-specific PCR revealed changes in the methylation pattern of analysed genes. Our results demonstrated correlation between genes expression and the methylation in the promoter regions chosen genes in tested cancer cell lines.

Acknowledgements

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P6.30

Comparison of the effect of polymorphism rs7608798 in the dipeptidyl peptidase IV gene and the serum concentration of DPP IV enzyme in patients with autism

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Dipeptidiyl peptidase IV (DPP IV) is serine protease which is involved in proteolysis of peptides with proline or alanine in the penultimate position. Decreased expression or mutation in the DPP IV gene may contribute to changed concentration of enzyme. Autism is neural development disorder characterized by social impairment. Increased level of biologically active peptides could be crucial in development of autism disorder. According to one of hypothesis intronic polymorphisms in DPP IV gene may result in overexpression of the gene. In our study we decided to investigate the relationship between SNP rs7608798 in the DPP IV gene and the concentration of enzyme in autism, compared to healthy people.

Blood samples were collected from children with autism and healthy people. DNA was isolated using commercial Kit. Polymorphism was determined using PCR-RFLP. Single nucleotide polymorphism was compared with previously determined DPP IV concentration in patient serum using commercial Kit. The correlation between rs7608798 polymorphism and serum concentration of DPP IV was analyzed using the ANOVA test. Independent sample *t* test was used to compare serum DPP IV concentration between genders.

The results show increased concentration of DPP IV enzyme in autism group compared to healthy people. There was statistical significant difference between concentration of DPP IV enzyme levels and GG genotype between autism and healthy people. All polymorphisms were in Hardy–Weinberg equilibrium.

Our results suggests, that allele G in DPP IV rs7608798 polymorphism may be involved in development of autism disorder, but on the other hand, low frequency of A allele in population makes it difficult to investigate its influence on concentration of DPP IV enzyme.

P6.31

N-homocysteinylation as a mechanism of connective tissue impairment in hyperhomocysteinemia due to cystathionine β-synthase deficiency

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Genetic hyperhomocysteinemia due to cystathionine β-synthase deficiency (CBS) leads to connective tissue abnormalities. However, mechanism by which excess of homocysteine (Hcy) causes these abnormalities is not fully understood. One possible explanation of Hcy toxicity is post-translational modification of proteins by a reactive Hcy metabolite, Hcy thiolactone. This reaction occurs on ε amino groups of protein Lys residues and is called *N*-homocysteinylation (Jakubowski H. *Homocysteine in Protein Structure/Function and Human Disease*, Springer, Wien, 2013). Because Lys residues are involved in maturation of connective tissue proteins, we assume that *N*-homocysteinylation of collagen and elastin, if it occurs *in vivo*, would prevent generation of cross-links that stabilize filament structures. Here we examined susceptibility of collagen to *N*-homocysteinylation *in vitro* and *in vivo* using the C57BL/6J Tg-I278T $Cbs^{-/-}$ mouse model (Gupta et al., *FASEB J* 2009). The study was conducted on tissues of $Cbs^{-/-}$ mice (n=6) and $Cbs^{+/+}$ littermates (n=5). Total homocysteine (tHcy) and N-Hcy-protein levels were measured using HPLC-based assays (Jakubowski, 2008, *Anal Biochem*) on 1260 Infinity UPLC instrument (Agilent). Site-specific *N*-homocysteinylation of rat tail collagen lysine residues was analyzed using LC-MS/MS. We found that *N*-linked Hcy was present in bovine Achilles tendon collagen at 60 pmol/mg, equivalent to 0.006 mol *N*-Hcy/mol collagen, similar to *N*-Hcy content of human serum albumin. We identified 10 sites of *N*-homocysteinylation in rat tail collagen modified with Hcy thiolactone *in vitro* (K266, K1085 in collagen α-1 (I), K1070 in collagen α-2 (I), K963 in collagen α-1 (VI), K975, K1001, K1014, K1910, K2024 in collagen α-3 (VI), K2601 in collagen α-1 (XII)). Inactivation of the *Cbs* gene resulted in a 4- and 16-fold increase in plasma and urinary tHcy levels, respectively. Liver, lung and heart tHcy was elevated 5-6-fold in *Cbs*-deficient mice. Plasma and liver *N*-Hcy-protein was elevated 2 and 1.5-fold in *Cbs*-deficient mice, respectively. There was 18- and 12-fold increase of Hcy *S*- and *N*-linked to collagen and elastin in $Cbs^{-/-}$ mice relative to $Cbs^{+/+}$ animals. These findings provide evidence that collagen is susceptible to *N*-homocysteinylation *in vitro* and that collagenand elastin undergo both *S*- and *N*-homocysteinylation *in vivo* and thus are major targets for modification in hyperhomocysteinemia.

Acknowledgements

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P6.32

Next generation sequencing technology — application to whole miochondrial genome analysis and molecular diagnostics of human diseases

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Variants of mitochondrial DNA (mtDNA) and mitochondrial haplogroups have been reported to associate not only with mitochondrial diseases, but also with neurodegenerative disorders, cancers, aging and some complex lifestyle diseases, such as diabetes, obesity and atherosclerosis. mtDNA is highly polymorphic, thus a method allowing fast, efficient and precise analysis of whole mitochondrial genomes is required in molecular diagnostics of human diseases. Here we present the results of verification of next generation sequencing (NGS) applicability to whole mtDNA analysis. This was performed using Illumina NGS technology and the MiSeq platform. We also present the preliminary results of mtDNA analysis obtained with the elaborated NGS method in a group of Polish patients with mitochondrial disorder, glaucoma or lifestyle disease. The long-range PCR (LR-PCR) method was elaborated to enrich whole mitochondrial genomes from total DNA samples. A single primer pair was designed to anneal "back-to-back" on a circular mtDNA molecule. This procedure eliminates to a large extent the interference of SNPs and multiple nuclear sequences that are highly homologous to mtDNA. Secondary sequencing data analysis was performed using CLC Genomics Workbench bioinformatic software.

High-throughput properties of NGS allowed obtaining deep mitochondrial genome coverage, which is essential for precise determination of the heteroplasmy level of mtDNA variants as NGS is an appropriate method for detection of variants with low heteroplasmy levels. Moreover, along with LR-PCR it is applicable for mtDNA deletion identification – it provides analysis of mtDNA deletion extent, level and breakpoints. Illumina technology of sequencing by synthesis (reversible termination of polymerization) is also suitable for sequencing of such "problematic" DNA regions as homopolymers, GC-rich regions and short tandem repeats that are also present in mtDNA. Altogether, we conclude that NGS is a powerful tool and desirable method for whole mtDNA sequencing and mutation analysis.

P6.33

Epigenetic regulation of *STAT3* gene activity in cancer cell lines

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Introduction: The epigenetic mechanisms include methylation of DNA to regulate gene expression in cancer cells. Hypermethylation of normally unmethylated tumor suppressor genes could be associated with gene silencing in cancer cell lines. On the other hand, hypomethylation of proto-oncogenes promotes cancer formation. The transcription factor STAT3 is a key signaling molecule that is activated by many cytokines, growth factors and oncoproteins. Constitutive STAT3 activation has been found in a variety of human malignancies, including ovarian, prostate and bladder cancer.

Aim of the study: The aim of studies was screening the methylation pattern of *STAT3* gene and comparison of this pattern to *STAT3* mRNA level, in cancer cell lines.

Material and methods: Two different human cell lines were used in our experiments: A2780 (human ovarian cancer cell line) and T24 (human urinary bladder cancer cell line). Both cells line were cultured in RPMI-1640 medium supplemented with 10% FBS and gentamycin (10 μg/ml) at 37°C in a 5% CO2 atmosphere. In the next step DNA was isolated from cultured cell lines by using GeneMATRIX Cell Culture DNA Purification Kit (EURx). Simultaneously the total RNA was extracted with TRIzol® Reagent (Invitrogen). DNA was modified by sodium bisulfate treatment with the EZ DNA Methylation Gold Kit (Zymo Research). Bisulphite-modified DNA was amplified by MSP (Methylation-Specific PCR). The changes in methylation level of *STAT3* were determined by Q-MSP technique (Quantitative Methylation-Specific PCR). After total RNA isolation the changes in expression level of *STAT3* were estimated by Real-Time™ RT-PCR.

Results: The methylated CpG islands are located in the promoter region of STAT3 gene. Methylation-specific PCR revealed demethylation of the promoter STAT3. Our results demonstrated correlation between the STAT3 gene expression and demethylation in the promoter region of STAT3 gene in examined cancer cell lines.

Conclusion: The present study suggests that STAT3 gene expression could be involved in the epigenetic regulation of carcinogenesis. We conclude that correlation of hypomethylation and expression of STAT3 gene has been recognized as an important mechanism for regulation of gene expression in cancer. Finally, results of our study suggest that STAT3 is good target for the development of anticancer treatments.

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P6.34

Potential relevance of epistatic effects in the genetic susceptibility to clear cell renal cell carcinoma

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Kidney cancer is the third fastest increasing carcinoma with the 30% growth noted in years 1993–2009. Among kidney cancers, clear cell renal cell carcinoma (ccRCC) is the most often type, responsible for approximately 80% of cases. Although several genes have been already associated with renal cell carcinoma through candidate and genome-wide association studies, the current data are still inconclusive in terms of a genetic component involved in susceptibility to RCC. The role of epistasis, that is gene-gene interactions is more and more often emphasized as an important factor influencing determination of human complex traits. It has been even suggested that the role of genetic interactions is even more significant than the main independent effect of any susceptibility gene. Our aim was to evaluate the significance of particular candidate single-nucleotide polymorphisms (SNPs) and interactions between them for potential association with RCC based on an independent sample set of renal cell patients. 102 patients with clear cell renal cell carcinoma subjected to surgical treatment and 500 healthy controls were genotyped with a single base extension method to elucidate main independent effects and interactions between 33 candidate SNPs in determining susceptibility to RCC. Logistic regression was used to search for independent effects as well as gene-gene interactions. Genetic interactions were also evaluated using multifactor dimensionality reduction method (MDR). *GNAS1* was implicated in a strong synergistic interaction with *BIRC5*. This effect was a part of a model suggested by MDR including also a synergy between *EPAS1* and *VEGFA* (P=0.03). Pair-wise analysis with logistic regression indicated involvement of *GNAS1* in additional interactions with *EPAS1* (P=0.006), *MC1R* (P=0.001) and *VDR* (P=0.013) as well as revealed epistatic effect between *SCARB1* and *VDR* (P=0.046). Overall, six genes previously implicated in RCC including *GNAS1* involved in RCC prognosis and *EPAS1, BIRC5* and *VEGFA,* overexpressed in ccRCC, were found to be a component of genetic interactions indicating important role of this phenomenon. Further studies are necessary to confirm whether epistasis is a valid factor determining susceptibility to renal cell carcinoma.

P6.35

Response of head and neck cancer cell lines after radio — and chemoexposure

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Introduction: Head and neck squamous cell carcinoma (HNSCC) is one of the worst prognosis cancers and it is remarkable propensity for metastasis. Because of its chemoresistance, the main methods of treatment in HNSCC are surgery and radiotherapy. Chemotherapy in the treatment acts as an adjuvant. It is believed that microRNAs (miRNAs) pretend to be a good candidate as biomarkers for personalisation treatment in head and neck cancers. In this study we focused on behaviour of three different HNSCC cell lines after irradiation and chemoexposure. Examined miRNAs are strictly connected with cancer methastasis and epithelial-mesenchymal transition proces (EMT). It prompted us to define if the estimation of expression levels of examined miRNAs would be useful tool to predict tumor progression.
Purpouse: Expression analysis of miRNAs and genes characteristic of epithelial and mesenchymal phenotype in lymph nodes and other sites of metastasis of HNSCC patients.
Analysis of influence of the most commonly used chemotherapeutics on FaDu, SCC-25 and SCC-40 cell lines.
Methods: The cells were irradiated using dose of 2 Gy in water phantom by Clinac 2300-4. The total RNA was isolated using TRI reagent in 9 points of time after irradiation. TaqMan MicroRNA Assay was used. The data obtained were compiled in accordance with the $2^{-\Delta\Delta CT}$ method.
The same way of conduct was used to analise expression of EMT markers. To establish IC_{50} dose of chemiotherapeustics the MTT assay was performed.
Clonogenic assay was made in order to compare deifferences in reaction among FaDu, SCC-25 and SCC-40 lines reaction to irradiation. Schratch Wound Healing Assay was made to show the ability of cells migration.
Results: Overexpression of examined miRNAs in studied lines was observed. The same tendency of overexpression levels of miRNAs associated with cancer methastasis was observed. We noticed differences among examined lines in tendency to form colonies. Results of scratch wound assay showed differences in ability to migration of analised cancer cell lines.
Conclusion: The high mortality of HNSCC patients determines to look for adequate biomarkers of cancer progression and patients‘ prognosis. Selected miRNAs and EMTs markers seem to be ones. Different reactions to radio- and chemoexposure of examined HNSCC cell lines should provoke us to consider treatment personalisation to reduse the invasiveness of therapy and consequently increase its effectiveness.

P6.36

Identification of specific *N*-homocysteinylated lysine residues in human plasma albumin and fibrinogen from cystathionine β-synthase-deficient patients

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Elevated levels of homocysteine (Hcy) are an independent risk factor for atherothrombotic disease. Hcy arises from the metabolism of the essential dietary protein amino acid methionine (Met). Hcy is a non-coded amino acid that normally does not participate in ribosomal protein biosynthesis. However, at the end of 1990s mechanisms have been discovered by which Hcy can be incorporated into proteins. In one mechanism, Hcy is first metabolized to the thioester Hcy-thiolactone in an error-editing reaction during protein biosynthesis, when Hcy is erroneously selected in place of Met by methionyl-tRNA synthetase. Hcy-thiolactone is chemically reactive and forms isopeptide bonds with protein lysine residues in a process called *N*-homocysteinylation. The incorporation of Hcy into isopeptide bonds alters protein's structure/function and can contribute to pathologies associated with hyperhomocysteinemia (Jakubowski H *Homocysteine in Protein Structure/Function and Human Disease*, Springer, Wien 2013). Identification of lysine residues in proteins that are targeted for *N*-homocysteinylation *in vivo*, important in understanding their role in human health and disease, has been challenging.

Here we report methods for the identification of *N*-homocysteinylation sites in plasma proteins. Human plasma or purified fibrinogen is subjected to trypsin digestion and analysis of *N*-Hcy-peptides by liquid chromatography/mass spectrometry. Human fibrinogen is isolated from the plasma by the glycine precipitation method. Identification of *N*-Hcy-Lys-peptides in tryptic digests of *in vivo*-derived samples is facilitated by the use of *N*-Hcy-albumin and *N*-Hcy-fibrinogen synthesized *in vitro* from commercially available human proteins. Using these methods we identified *N*-Hcy-residues at Lys4, Lys12, and Lys525 in albumin directly in trypsin-digested human serum samples from normal and cystathionine β-synthase (CBS)-deficient subjects. *N*-Hcy-Lys137 was present in albumin only in CBS-deficient subjects. We also identified *N*-Hcy-αLys562, *N*-Hcy-βLys344, and *N*-Hcy-γLys385 in human fibrinogen from CBS-deficient patients. The fibrinogen αLys562 site of *N*-homocysteinylation is located in an unstructured region of the aC domain that is involved in tissue plasminogen activator and plasminogen binding, which suggests that *N*-Hcy-αLys562 contributes to the prothrombotic properties of *N*-Hcy-fibrinogen in CBS-deficient patients (Sikora *et al*., 2014, *Amino Acids*).

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P6.37

DNA methylation in the Epidermal Differentiation Complex (EDC) as a potential regulatory mechanism of epidermal differentiation

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The epidermal differentiation complex (EDC) located on human chromosome 1q21 comprises a syntenic and linear cluster of 4 gene families encoding the S100 proteins, the small proline rich proteins (SPRRs), the late cornified envelope proteins (LCE) and the S100-fused type proteins (SFTPs), all involved in keratinocyte differentiation process. We examined whether epigenetic factors affect the regulation of EDC gene expression. The employed human epidermal keratinocytes (primary HEKa cells) were analyzed as either undifferentiated (when grown in low calcium concentration) or differentiated cells (when grown in higher calcium concentration). Experiments were performed by means of real time PCR, genomic DNA isolation, bisulfite conversion and conventional sequencing. First, we examined the expression pattern of several S100 genes during the differentiation process. Next, we verified whether the observed changes in mRNA level were a result of alternations in DNA methylation. We performed the CpG methylation analysis for S100A6, S100A7, S100A8, S100A13 genes, for the NICE1 gene located within the LCE subcluster, and also for genes encoding involucrin and loricrin - the established markers of keratinocyte differentiation. In addition, we investigated methylation pattern within a recently identified conservative non-coding element of enhancer properties. Gene areas harboring considerably CpG rich regions, defined as CpG islands, along with relatively CpG poor regions were selected for analysis. The analyzed DNA fragments were localized in the proximity of transcription start sites as well as in both exons and introns. The obtained data, considering both initial and final stage of keratinocyte differentiation, did not indicate pronounced changes in CpG methylation. Furthermore, the actual methylation state did not preclude the gene expression level. Other EDC fragments are to be investigated to identify differentially methylated regions (DMRs) of potential regulatory significance.

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P6.38

Functional genomics analysis of rat myocardial infarction model in heart failure biomarkers prediction

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Myocardial infarction (MI) often results in left ventricular (LV) remodeling followed by heart failure (HF). LV remodeling is characterized by complex structural alterations involving scar formation, wall thinning and progressive chamber dilation. Interestingly, only a small percentage of patients develop HF immediately after MI, but with time many more progress to cardiac dysfunction. Currently, biomarkers like N-terminal *pro*-B-type natriuretic peptide (NT-proBNP) and cardiac troponins are used in clinical practice to evaluate long-term risk of adverse cardiac events. However, their prognostic value is limited due to the fact that their circulating levels fluctuate appreciably just after MI and can also be influenced by hepatorenal function. Thus, it is of great clinical importance to develop new tissue-specific and readily applicable biomarkers with high sensitivity and specificity for accurate diagnosis of HF.

To meet this challenge we analyzed changes in the gene expression level in rats with a wide range of myocardial infarct size two months after MI induction (Tulacz D *et al.,* 2013, *BMC Medical Genomics* **6**: 49). We determined 840 transcripts significantly altered (fold change>1.3, $P<0.05$) in heart tissue specimens from a group of rats with full-blown HF in comparison to control group and a group of rats with stable compensated LV injury. Cellular component classification showed that proteins encoded by the differently expressed genes were predominantly localized in the extracellular region (GO:0005576) and associated with the extracellular matrix (GO:0031012). Using the Ingenuity Pathway Analysis software (Qiagen) we distinguished 172 proteins differentially expressed in HF that potentially exist in the plasma. Of these, 57 are associated with the extracellular matrix whose remodeling is essential for development of HF. Our approach allowed to obtain, inter alia, the well-known BNP marker, which indicates that this strategy can be also effective in finding new cardiac-specific markers circulating in the plasma. It is the first step in selecting the best candidates and performing Western blots or ELISA tests.

We believe that functional genomics analysis of rats after myocardial infarction is valuable in prediction of heart failure biomarkers. To the best of our knowledge this is the first attempt to apply global gene expression profiling of heart specimens to predict differences in the level of suitable proteins that may occur in the plasma.

P6.39

Changes in DNA methylation profile in maize under herbicide stress conditions

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DNA methylation and chromatin modifications has been shown to be involved in gene silencing at both transcriptional and posttranscriptional levels. Transcriptional gene silencing is associated with hypermethylation of promoter sequences, while post-transcriptional gene silencing is linked with hypermethylation of transcribed or coding sequences. DNA methylation (both asymmetric and symmetric) plays a crucial role in the regulation of gene expression, in the activity of transposable elements, in the defense against foreign DNA, and even in the inheritance of specific gene expression patterns.

It has been observed in the fields that some maize lines display higher resistance to herbicides than others but to this day the molecular mechanisms of such resistance remain unknown. The link between stress exposure and sequence-specific changes in DNA methylation was hypothetical until recently, when it was shown that stresses can induce changes in gene expression through hypomethylation or hypermethylation of DNA.

Our initial experiments indicated that there are changes in global DNA methylation levels under herbicide stress conditions in maize. To reveal more detailed changes in DNA methylation in two maize lines displaying different susceptibility to RoundUp® we used *Methylation Sensitive Amplified Polymorphism* (MSAP). It is a technique where isoschizomers Hpa II and Msp I are used to determine the differences in DNA methylation due to enzyme's differential sensitivity to DNA methylation. We observed differences in methylation profiles between the two tested lines under herbicide stress conditions. Differentiating DNA bands were eluted from PAA gels and sequenced. Analyses of 197 DNA fragments using Blastn and Maize GDB databases allowed us to divide them into several groups representing genes encoding for transporter proteins, transferases, methyltransferases, genes involved in stress responses but also transposons. For more detailed analyses (DNA methylation, chromatin modifications) we chose 4 genes encoding for proteins that could potentially be involved in herbicide stress response.

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P6.40

Inactivation of the bleomycin hydrolase (*Blmh*) gene affects the expression of glyoxalase domain-containing protein 4 (*Glod4*) gene in mice

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The function of glyoxalase domain-containing protein 4 (Glod4, C17orf25) is not fully understood. Amino acids sequence comparison using *Blast* in *UniProt* reveals that Glod4 is widely expressed from insects to human and an homologous amino acid sequence is also found in the alga *Chlamydomonas reinhardtii* (Albee AJ *et al*., 2013, *G3* **3**: 979–991). Glod4 could have a detoxifying activity of glyoxalase I in the mitochondria. In fact, Glod4 interacts with mitochondrial NUDT9 protein (Zhang HT *et al*., 2003, *Acta Biochim Biophys Sin* **35**: 747–751). *Glod4* mRNA is detected in the human heart, brain, liver, kidney, pancreas, and placenta but not in the liver and skeletal muscle (Qin WX *et al*., 2001, *Cell Research* **11**: 209–216). Murine Glod4 protein has three isoforms varying in molecular weight and pI.
We found that Glod4 interacts with bleomycin hydrolase (Blmh), an enzyme that participates in homocysteine metabolism. Proteomic analyses revealed that a novel Glod4 variant with a more acidic pI was expressed in livers of $Blmh^{-/-}$ mice (Suszynska-Zajczyk J *et al*., 2014, *Amino Acids*). To elucidate a mechanism underlying this change in Glod4 expression we carried out RT-PCR assays using primers specific for Glod4 mRNA isoforms 1, 2, and 3. We detected only isoform 1 Glod4 mRNA in the livers of $Blmh^{-/-}$ and $Blmh^{+/+}$ mice. Glod4 isoform 1 mRNA was also expressed in heart, kidneys, spleen, lungs, muscles, bones, brain, and eyes of $Blmh^{+/+}$ and in kidneys, brain, and spleen of $Blmh^{-/-}$ mice. Isoform 3 of Glod4 mRNA was detected in kidneys, brain, and eyes of $Blmh^{+/+}$ mice and in kidneys of $Blmh^{-/-}$ animals. Isoform 2 was not detected in any of the tissues analyzed. Glod4 isoform 1 mRNA was in significantly more abundant than isoform 3 mRNA. Inactivation of the *Blmh* gene reduced the expression of isoform 3 mRNA in the kidney.
Taken together, our results indicate that the *Blmh* locus affects the expression of Glod4 and that the low pI Glod4 protein variant arises in livers of $Blmh^{-/-}$ by a post-transcriptional or post-translational mechanism.

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P6.41

A novel resveratrol derivatives modulating expression of CYP19 and CYP1B1 in non-tumorigenic MCF10A breast epithelial cells

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Estrogens play critical role in breast cancer development. Both receptors mediated and non-receptor mediated pathways are involved in this process. The latter activity is related to mutations caused by certain estrogen metabolites. Resveratrol (RES) has gained much interest because of its potential chemopreventive activity against human cancer including breast cancer. Unfortunately, RES is unstable in water solutions, possesses relatively low bioavailability, and possible phytoestrogenic activity. These facts led to searching new synthetic RES derivatives with potential chemopreventive and/or chemotherapeutic activity in breast cancer. Our and many others studies showed that most promising analogues of RES are metoxy derivatives.
The aim of the present study was the assessment of the ability of newly synthesized RES derivatives to interfere with molecular mechanisms related to breast cancer progression: estrogen synthesis (CYP19) and initiation (CYP1B1) in breast non-tumorigenic epithelial cell line MCF10A. Expression analysis was performed by real-time PCR and Western blot.
The results demonstrated the capability to modulate the expression of CYP19 and CYP1B1 by RES and its three derivatives: 3,4,2'-trimetoxy-*trans*-stilbene (A), 3,4,2',4'-tetrametoxy-*trans*-stilbene (B), and 3,4,2',4',6'-pentametoxy-*trans*-stilbene (C). The most promising in breast cancer prevention seemed to be the compound C, which in the concentrations of 1 μM and 5 μM decreased *CYP19* mRNA, and in the concentration of 5 μM reduced also *CYP1B1* mRNA level. The compound B, in the doses of 1 μM and 5 μM, significantly diminished the expression of *CYP1B1*, but in the 5 μM increased CYP19 protein level. The compound A in the concentration of 0.5 μM increased the mRNA of *CYP1B1*. The parent compound RES, in the concentration of 5 μM reduced significantly the CYP1B1 mRNA and protein, but increased both mRNA and protein level of CYP19.
Since down-regulation of *CYP19* may result in the reduction of active estrogens level and the decrease of *CYP1B1* expression of their carcinogenic metabolites (e.g. 4-hydroxyestradiol) in breast epithelial cells, the new synthetic derivatives of RES could be considered as potential preventive agents against breast cancer development.

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Polymorphisms in base excision repair genes and Alzheimer disease risk

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The progression of Alzheimer's disease (AD) is associated with a phenomenon of oxidative DNA damage accumulation resulting from progressive decrease in efficiency of repair system for these damages such as Base Excision Repair (BER). There is an evidence suggest that the presence of fundamental polymorphisms of genes involved in repair of oxidative damage is associated with a reduced DNA repair activity. Therefore a deficiency in BER was proposed to be an important factor in etiology of AD.
In our study, we evaluated the occurrence of frequent polymorphisms in MUTYH and XRCC1 genes as a risk factor for AD.
Objective: In this study we checked the correlation between genotypes and haplotypes of polymorphisms the p.Gln335His (rs3219489) of MUTYH and p.Arg194Trp (rs1799782) of XRCC1 genes and the risk of Alzheimer Disease in 110 AD patients and 120 healthy controls. For polymorphism genotyping, we used the TaqMan SNP Genotyping Assays method. Correlation between both genes genotypes and the risk of AD occurrence was evaluated by calculating odds ratio (OR) with 95% confidence interval (CI). It was done by using unconditional multiple logistic regression.
Results: The AA genotype of p.Arg194Trp polymorphism in *XRCC1* gene significantly increased the risk of AD (OR 3.71, 95% CI 1.91-7.20) while heterozygous AG genotype decreased the risk (OR 0.110, 95% CI 0.043-0.283). The presence of A allele in the AA was associated with increased risk of AD (OR 1.598, 95% CI 1.048-2.438). We didn't find any statistical correlation between the occurrence of polymorphism p.Arg194Trp of MUTYH gene and the risk of AD incidence.
Conclusion: The p.Arg194Trp polymorphism of the *XRCC1* gene may play a role in the pathogenesis of Alzheimer's disease.

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