Session 11. Regulation of Gene Expression

Lectures

L11.1

Looking for regulatory domain boundaries in high-throughput epigenetic data
Bartek Wilczyński
Institute of Informatics, University of Warsaw, Warsaw, Poland
e-mail: Bartek.Wilczynski@impan.pl

Transcription regulation in development is a complex process involving many elements tightly co-operating to achieve highly reproducible expression patterns. One of the key types of interactions are between tissue-specific enhancers and target gene promoters. While it was previously assumed that the majority of such interactions are defined by chromosomal proximity, new results from chromosome interaction studies indicate that the situation is more complex. I will present some results of our efforts to uncover the mechanisms of formation of regulatory domain boundaries: functional elements responsible for limiting the interactions between promoters and enhancers.

L11.2

Sequences regulating gene expression and their application in plant biotechnology
Anna Kulma, Jan Szopa
Faculty of Biotechnology, University of Wroclaw, Wroclaw, Poland
e-mail: Anna Kulma <anku3@wp.pl>

The expression of genes is regulated on many levels, the most common being transcriptional regulation depending on a promoter sequence, transcription factor availability and chromatin structure including DNA methylation. However the post transcriptional regulation in particular non-translated region structure and regulatory small RNAs emerge as a possible targets for biotechnological engineering. Understanding of the structure and function of those elements allows for their use in genetic modifications of plants including synthetic promoter and UTR strategy and modification of pathways through small interfering RNA strategy.

Plant biotechnology relies heavily on the genetic manipulation of crops. Quite often, the gene of interest is expressed in a constitutive fashion, however not all genes can be ectopically transcribed in plants under constitutive promoters such as genes encoding for proteins with a lethal or detrimental phenotype or introducing severe modifications to metabolic or developmental pathways that inhibit plant transformation, regeneration or growth. The alternative to constitutive promoters are inducible and tissue specific promoters. In the course of our work we isolated and characterized several plant promoters with a distinctive expression patterns and successfully used them in a generation of a transgenic plants. This approach allowed us to circumvent problems with a exogenous gene expression such as stunted growth and transgene silencing among others.

One such example is use of a vascular tissue specific promoter for a expression of a polyhydroxybutyrate synthesis genes in flax resulting in composite fibers with PHB bound to cellulose. Another example was use of a tissue specific and inducible promoter to overexpress flavonoid synthesis genes in potato, where expression of those genes under constitutive promoter resulted in a reduced productivity or a transgene silencing.

Even though the direct overexpression or silencing of a gene of interest is a most commonly used strategy the understanding of a regulatory RNA pathways allows for a possibility of a gene regulation in plants through expression of small RNAs and in some cases through homologous genes expression activating DNA methylation probably via miRNA pathways. This combined with a transient introduction of oligonucleotides opens a possibility in a future for a targeted induced methylation resulting in modified crops without transgene insertion.
**L11.3**

**Regulation of barley microRNA444 expression by alternative splicing (AS) under heat stress conditions**

Andrzej Pacak1, Katarzyna Kruszka1, Aleksandra Swida-Barczecka1, Wojciech Karłowski2, Artur Jarmolowski1, Zofia Szweykowska-Kulinska1,2

1Institute of Molecular Biology and Biotechnology, Department of Gene Expression, Adam Mickiewicz University in Poznan, Poznań, Poland; 2Institute of Molecular Biology and Biotechnology, Computational Genomics Laboratory - Bioinformatics Laboratory, Adam Mickiewicz University in Poznan, Poznań, Poland

MicroRNAs are key molecules that regulate gene expression. Mostly 21 nt long, plant microRNAs are produced through the joined action of DCL1 (Dicer-like 1 enzyme) and other miRNA biogenesis proteins. First, primary transcript of MIR gene (pri-microRNA) is processed to form stem-loop structure - pre-microRNA. Then dsRNA duplex consisting of mature microRNA and microRNA* sequences is excised from pre-microRNA stem. MicroRNA as a part of a RNA induced silencing complex (RISC) is involved in specific gene silencing either by mRNA endonucleolytic cleavage or translation repression. Expression of many plant microRNAs is regulated by abiotic stresses. For example, in Arabidopsis mature miR400 is downregulated by heat treatment as a result of specific ratio of two alternatively spliced isoforms of miR400 transcript.

We characterized several barley microRNAs — their gene structures, transcripts and pri-microRNA processing events (Kruszka et al., 2013). There are three genes encoding different species of barley microRNA444 (MIR444.1, MIR444.2 and MIR444.3) all of which contain long introns that separate microRNA from microRNA*. Functional pre-miRNA444 can be formed only after intron removal. MicroRNA 444.1, 444.2, and 444.3 differ from each other by one or two nucleotide substitutions. We recently have tested barley response to different abiotic stresses and found that heat stress induces significant expression of miR444. By Northern blotting and small RNA NGS analyses, we identified MIR444.1 as an induced gene. The transcript of the MIR 444.1 gene undergoes complex alternative splicing events that generate three main RNA isoforms: (i) fully spliced, functional pri-miR444.1 isoform, (ii) alternatively spliced non-functional isoform in which the exon encoding miR444.1* is removed, and (iii) non-functional spliced isoform in which the whole region containing exons encoding miR444.1* and miR444.1 together with the intron separating these exons is spliced out. We found that upon heat stress conditions, the general level of pri-miR444.1 transcript, as well as the level of functional spliced pri-miRNA isoform, was significantly elevated compared to the control conditions. Under stress conditions the level of non-functional isoforms only slightly increased at 3 and 6 hours but was significantly higher after 24 h of stress duration. This resulted in almost two-fold higher level of mature microRNA444.1 at 3 hours of heat and slightly decreased at 24 hours of heat. Thus the interplay between the level of spliced isoforms regulates the level of the mature miR444.1. Interestingly, miR444.1 and its mRNA target have perfect matches. It is due to the fact that the target gene encoding transcription factor belonging to the MADS-box type II gene family is localized at the opposite strand to barley MIR444.1 gene. Accordingly to the elevated level of mature miR444.1 during the heat stress, we observed dramatic decrease of its target mRNA (AK363243).

**References:**

Kruszka K et al (2013) BMC Genomics 14: 34.

---

**L11.4**

**Chromatin remodeling by SWI/SNF complexes as global regulator of gene expression in eukaryotes**

Tomasz J. Sarnowski1, Andrzej Jerzmanowski1,2

1Institute of Biochemistry and Biophysics Polish Academy of Sciences, Department of Protein Biosynthesis, Warsaw, Poland; 2University of Warsaw, Faculty of Biology, Institute of Experimental Plant Biology, Warsaw, Poland

e-mail: Tomasz Sarnowski <tsarn@ibb.waw.pl>

ATP-dependent chromatin remodeling complexes (CRCs) regulate the structure, activity and organization of chromatin and play critical roles in the maintenance, transmission and expression of eukaryotic genomes. Different types of CRCs are defined by the type of central SNF2-type ATPases and unique composition of other subunits including auxiliary proteins. The SWI/SNF class of ATP-dependent chromatin remodeling complexes, a prototype of which was first described in *Saccharomyces cerevisiae*, is conserved from fungi to mammals. The SNF2 ATPase is associated with a small set of highly conserved “core” subunits including homologues of yeast SWI/SNF5 and SNF5-type proteins, which upon in vitro reconstitution are capable of full SWI/SNF remodeling activity. In multicellular eukaryotes, the lack or aberrant stoichiometry of individual core subunits causes embryolethality or severe defects in development and in animals also leads to carcinogenesis. The studies of human CRCs represent an important area of cancer research. Depending on subunit composition, distinct mammalian SWI/SNF complexes can act as either activators or repressors, and thus may exert opposite effects on cellular activities and serve as the interface for various processes. A particularly well studied role of SWI/SNF CRCs in animals is their involvement as co-activators in the activity of nuclear hormone receptors.

Plant genomes encode homologues of all major core subunits of SWI/SNF complexes. A comparative sequence analysis performed for *Arabidopsis thaliana* revealed our putative SWI/SNF-type SNF2-ATPases, four SWI3-type proteins as well as a single SNF5 subunit. The multiplicity of genes encoding SNF2-type ATPase and SWI3-type subunits in Arabidopsis suggests that plant CRCs contain different combinations of these core components. Genetic analyses confirm that different classes of plant SWI/SNF complexes are involved in regulation of specific processes. The recent studies in ours and other laboratories have demonstrated an important role of SWI/SNF CRCs in transduction of plant hormone signals, including direct interactions with key elements of major hormonal pathways and control of their target genes.
Mechanisms regulating pericentric heterochromatin structure during oogenesis and early mammalian development

Ewa Borsuk

Institute of Zoology, Department of Embryology, University of Warsaw, Warsaw, Poland

e-mail: Ewa Borsuk <borsuk@bial.uw.edu.pl>

In eukaryotic cells two basic types of chromatin can be distinguished, namely euchromatin and heterochromatin. Pericentric constitutive heterochromatin, one of the heterochromatin subtypes, surrounds the centromeric regions of chromosomes. The proper structure of pericentric heterochromatin domains, which is essential for the proper centromere functions, depends mainly on the presence of certain histone variants, non-histone proteins and specific patterns of epigenetic modifications of core histones. In mammalian cells the HP1 protein, a homolog of yeast Swi6, acts as a key regulator of pericentric heterochromatin structure. Two, out of three isoforms of this protein, HP1a and HP1b, are constitutively present in pericentric regions during interphase of somatic cells. Two modifications of histone H3, trimethylation of lysine 9 (H3K9Me3) and phosphorylation of serine 10 (H3S10Ph) are responsible for dynamic changes in localization of HP1 proteins. Although their dynamic in pericentric regions during the cell cycle has been described, the function of HP1 proteins in mammalian cells is not fully understood. The knowledge about the regulation of pericentric heterochromatin structure during gamete formation and early mammalian development is limited. During oogenesis and at the beginning of mouse embryonic development, dramatic changes in pericentric heterochromatin structure and its localization in the nucleus take place. Its organization in the nuclei of zygote, called male and female pronucleus, is distinct from observed in the nuclei of somatic and embryonic cells. Moreover, the protein composition and posttranslational modifications in heterochromatin regions differ significantly between male and female pronucleus. We have investigated the appearance, mechanism of regulation and possible functions of HP1 proteins during oogenesis and first cell cycles of mouse embryo. The phosphorylation of H3S10 as a regulator of HP1a recruitment into heterochromatin regions was of our special interest. We showed that the appearance of HP1a and HP1b in primary oocytes, zygotes and cleaving mouse embryos is unique and distinct from described for somatic cells. From 2-cell stage onward binding of HP1a to heterochromatin regions is regulated by a cytoplasmic clock, regulatory mechanism dependent on the time that passed from the fertilization. The amount of HP1a that can bound to the pericentric heterochromatin is regulated by H3S10 phosphorylation. In the first and second cell cycle H3S10 is phosphorylated in heterochromatin before the end of S phase, which is earlier than in somatic cells. This phosphorylation may constantly protect some subdomains from association with HP1a protein from late S phase until the end of interphase. The presence of regions that contain HP1 proteins and lack H3S10Ph, and regions lacking HP1 proteins and enriched in H3S10Ph may have functional consequences.

The role of MCPIP1 in the inflammatory reaction as an example of complex regulation of gene expression

Jolanta Jura, Łukasz Skalniak, Barbara Lachowicz, Janusz Ligeza, Aleksander Koj

Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland

e-mail: Jolanta Jura <jolanta.jura@uj.edu.pl>

Gene expression regulation is a complex process, tightly controlled at various levels (transcriptional, post-transcriptional, translational and post-translational) enabling cells to cope with intracellular or extracellular stimuli. The inflammatory response is a good model to study the coordinated expression of hundreds of genes. On the systemic level inflammation includes recruitment of blood leukocytes, activation of tissue macrophages, and production of pro-inflammatory mediators such as certain cytokines. On the cellular level there is activation of signaling pathways forwarding signals to key transcription factors, responsible for initiation of expression of specific genes. In this respect the principal role is played by the nuclear factor κB (NF-κB), which is present in the cytoplasm but after degradation of its inhibitor - IκB, is quickly translocated to the nucleus where it controls the expression of genes coding for either pro- or anti-inflammatory mediators. The latter group is best represented by a newly discovered multifunctional protein – MCPIP1 (monocyte chemokine protein-induced protein-1). MCPIP1 is a potent antiinflammatory agent acting on several levels by degradation of mRNA coding for proinflammatory cytokines, by negative regulation of key transcription factors: NF-κB and AP1, and by controlling the level of pre-miRNA. We found that on the transcriptional level expression of gene coding for MCPIP1, ZC3H12a, is controlled by transcription factors NF-κB and ELK1, while regulation on the post-transcriptional level includes degradation by MCPIP1 of its own transcript. The synthesis of MCPIP1 protein is regulated also on the post-translational level by rapid degradation following cell exposure to typical proinflammatory cytokine, such as IL-1beta. Recently It was found that phosphorylation of MCPIP1 at two serine residues (435 and 439) followed by ubiquitination results in its proteasomal degradation. Our studies showed that proteasome regulates MCPIP1 expression not only on the protein level but also on the mRNA level. All available information suggests that control of MCPIP1 expression may be a new therapeutic target in the inflammatory diseases.

Acknowledgements:

This study was supported by the National Science Centre; Grant number 2011/03/N/NZ1/01193 awarded to Lukasz Skalniak.

Session 11. Regulation of Gene Expression
O11.1

Protein synthesis alterations in response to perturbation in assembly/stability of mitoribosomes in Arabidopsis

Małgorzata Kwaśniak, Aleksandra Adamowicz, Hanna Jańska
University of Wrocław, Department of Biotechnology, Laboratory of Molecular Cell Biology, Wrocław, Poland
e-mail: Małgorzata Kwaśniak <gosiakwasniak@op.pl>

Mitoribosomes, like oxidative phosphorylation (OXPHOS) complexes have a dual genetic origin. In Arabidopsis, all of the mitochondrially-encoded ribosomal subunits are well known, but the full repertoire of nuclear-encoded mitoribosomal proteins remains to be established. One of the known components of the small subunits of Arabidopsis mitoribosomes encoded in the nucleus is the S10 protein. We found that silencing of RPS10 gene encoding S10 protein affects mitoribosome biogenesis. This silencing provokes a compensatory response increasing the abundance of the components of both small and large subunits of mitoribosomes. However, the up-regulation is imbalanced since the S10-deficient small subunits are unstable, resulting in an excess of large subunits. In turn, the perturbation in assembly/stability of mitoribosomes in rps10 mutant seems likely to affect mitochondrial translation. The analysis of polynomial fractions revealed that the efficiency of translation of various classes of mitochondrial mRNAs is differently affected. The majority of mitochondrially-encoded transcripts of OXPHOS subunits are less actively translated, whereas mitochondrially-encoded transcripts of most ribosomal proteins are preferentially synthesized. By contrast, the translational efficiency of nuclear-encoded mRNAs for mitoribosomes and OXPHOS complexes seems to be less affected. To provide an independent evidence for altered mitochondrial translation in rps10 mutant we conducted in organello protein synthesis. Our result clearly indicate that the pattern of proteins synthesized in rps10 mitochondria differs from that in wild type. As we expected from the affected association of mRNAs with polysomes, several of OXPHOS proteins were poorly translated in rps10 mitochondria, while some of ribosomal proteins barely synthesized in wild type mitochondria were very strong translated in rps10. These results confirm that mitochondrial translation is misregulated in the rps10 mutant with enhanced synthesis of ribosomal proteins and repression of OXPHOS proteins. We postulate that mitoribosomes are not simply translation machines but also function as regulatory elements that differently affect or filter the translation of particular mRNAs.

References:

O11.2

Influence of CacyBP/SIP on CREB activity in neuronal type of cells

Sara Rosińska1, Ewa Kilańczyk2, Michał Hetman2, Wiesława Leśniak1, Anna Filipek1
1Nencki Institute of Experimental Biology PAS, Warsaw, Poland; 2Kentucky Spinal Cord Injury Research Center, University of Louisville, KY40202, USA
e-mail: Sara Rosińska <srosinska@nencki.gov.pl>

The CacyBP/SIP protein was discovered as a calcyclin (S100A6) binding protein (1) and later as a Siah-1 interacting protein (2). CacyBP/SIP is present in different cells and tissues with the highest level in brain, where it was suggested to play a role in cytoskeleton reorganization (3,4). Recently, it has been shown that CacyBP/SIP has phosphatase activity towards ERK1/2 kinase and, in consequence, inhibits the activity of the Elk-1 transcription factor (5). In this work we examined the influence of CacyBP/SIP on transcription factors engaged in different aspects of neuronal activity such as SRF, CREB, AP-1, STAT-3, NFκB and NFAT. In the current studies we focused on CREB (cAMP response element-binding) we chose mouse neuroblastoma NB2a cells and rat cortical neurons and applied two methods, dual luciferase reporter assay and Western blot analysis. The influence of CacyBP/SIP on CREB activity was analyzed after CacyBP/SIP overexpression or silencing.

We found that the transcriptional activity of CREB after CacyBP/SIP overexpression was inhibited in NB2a cells and in rat cortical neurons by about 22,7% and 43.8%, respectively. In cells with diminished level of CacyBP/SIP the activity of CREB in NB2a cells and cortical neurons was increased up to 360% and 190%, respectively. Furthermore, CacyBP/SIP overexpression correlated with a diminished level of phosphorylated CREB. Altogether, our results suggest the influence of CacyBP/SIP on CREB activity in a neuronal type of cells.

Acknowledgements:
This work was supported by grant from the National Science Center (M 425) and by statutory funds from the Nencki Institute of Experimental Biology.

References:
Transfer RNAs (tRNAs) are not only a fundamental component of the translation machinery but also play roles in modulating key cellular processes like proliferation and stress responses. There are many pathways involving tRNAs action. Recent studies have described a novel aspect of stress responses in eukaryotic cells wherein cytosolic tRNAs are separated into half molecules by cleavage in the anticodon loop. The mechanism and significance of such tRNA cleavage remains unknown.

tRNA fragments have been uncovered by deep sequencing projects in all three domains of life. Recently we have reported for the first time differential processing of tRNA molecules in positions other than the anticodon loop and the association of small RNAs derived from tRNAs (tsRNAs) with *Saccharomyces cerevisiae* ribosomes.

In order to find similarities of yeast tsRNAs to other known or predicted tRNA-derived RNAs, we have conducted a systematic computational analysis of all tRNA processing by means of high-throughput reads mapping. We have used tRNA sequences from cDNA libraries generated from *Homo sapiens*, *Mus musculus*, *Saccharomyces cerevisiae*, *Haloferax volcanii*, and *Drosophila melanogaster*. We've observed that the anticodon loop with the 3' part of tRNA is the only evolutionary conserved processing region. However, in some organisms we have also observed shorter fragments appeared to be the results of processing events in other regions like DHU stem-loop. Moreover, we present experimental evidences that yeast tsRNAs are able to inhibit protein biosynthesis in model eukaryotic species.
P11.3

The influence of valproic acid and hydroxyurea on expression level of histone H3/a in human connective cell cultures
Anna Kulczycka, Arkadiusz Orzel, Zofia Dzierżewicz
Department of Biopharmacy, School of Pharmacy with the Division of Laboratory Medicine, Medical University of Silesia in Katowice, Katowice, Poland
e-mail: Zofia Dzierżewicz <zofiad@gmail.com>

Development of tissue engineering is associated with the use specific spatial (3D) cultures to generate the tissue structures in vitro. Proliferative activity assessment in these types of cell culture is limited mainly by the diffusion rate of test reagents (commercial markers) into a spatial environment. The expression of replication-dependent subtypes of histone H3 seems to be a precise and useful tool for the assessment of the exact proliferative status of cells growing in 3D cultures. The mRNA level of H3/a subtype is cell cycle-dependent. The highest expression ratio was detected in cells passing through the S phase. In this study, we evaluated the accuracy of histone H3/a expression assessment by the use of real-time RT-PCR technique as a proliferation marker in human chondrocytes and fibroblasts. For this purpose, histone H3/a level was determined in the cells having various proliferative activity: growing exponentially and growth-arrested with valproic acid (VPA) and hydroxyurea (HU). Cell proliferation is a result of cell cycle progression including DNA replication and following mitotic division. The normal passage of cells through the division cycle can be inhibited by addition of specific agents to the culture environment, like valproic acid and hydroxyurea. Valproic acid is a representative of histone deacetylase inhibitors (HDACIs). It inhibits transition from G1 to S phase. After incubation with VPA, the massive accumulation of cells is detected in G1 phase. In turn, hydroxyurea treatment results in inhibition of ribonucleotide reductase causing alternations in deoxyribonucleotide synthesis. The DNA replication is blocked and cells accumulate on the border of G1 and S phase. The aim of the study was to evaluate an impact of the two cell cycle inhibitors (VPA and HU) on histone H3/a mRNA levels in cultured human chondrocytes and fibroblasts.

The experiment was performed on CCD-11Lu human fibroblast cell line and normal human articular chondrocytes (NHAC-kn). After 24-hr incubation with VPA and HU (both at 2 and 10 mM concentrations), RNA was isolated and expression ratios of histone H3/a were determined using real-time RT-PCR. The control RNA was isolated from untreated chondrocyte and fibroblast cell cultures respectively.

Acknowledgements:
The work was supported by KNW-1-002/K/3/0.

P11.4

Silencing of human Sgt1 isoforms
Agnieszka Góral1, Małgorzata Sierant2, Barbara Nawrot2, Anna Filipek1
1Nencki Institute of Experimental Biology PAS, Warsaw, Poland; 2Centre of Molecular and Macromolecular Studies PAS, Łódź, Poland
e-mail: Agnieszka Góral <agoral@nencki.gov.pl>

The Sgt1 protein was identified in yeast as a component of the kinetochore complex and SCF ubiquitin ligase, but later it was found also in plant and mammalian cells (Kigawa et al., 1999; Spiechowicz, Filipek, 2005). In most of the studied organisms, including mouse and rat, there is only one Sgt1 isoform. Interestingly, due to alternative splicing, two isoforms, named Sgt1A (37.8 kDa) and Sgt1B (41.0 kDa) exists in humans (Niikura, Kitagawa, 2003). The difference between these two splice variants includes a 33-amino acid insertion within Sgt1A sequence, that is not found in Sgt1B. Moreover, there is a specific Ser110 residue present in Sgt1A, but not in Sgt1B isoform.

It has been shown that Sgt1, a co-chaperone of Hsp90, is a critical factor for human kinetochore assembly and for cell response to pathogen attack by activation of NLR proteins (Davies, Kaplan, 2010; da Silva Correia et al., 2007). RNA interference-mediated silencing of both Sgt1 isoforms causes cell arrest, due to activation of the spindle checkpoint, and an increase in the amount of tetraploid G1 cells. Lack of Sgt1 is lethal for the cells (Steensgaard et al., 2004).

To answer the question whether both human Sgt1 variants are essential for cell viability and proliferation, we designed specific siRNA molecules in order to silence separately the Sgt1A or Sgt1B isoform. To obtain effective siRNA molecules, we have tested several sequences for specific silencing of both isoforms (as a control). For that, HEp-2 cells were transfected with siRNAs at the final concentration of 200 nM using LipoFectamine 2000. Then, cell lysates were prepared 24, 48 and 72 hours after transfection and Western blot analysis was performed. These studies allowed us to identify siRNA sequences which caused 50-84% decrease in the level of Sgt1A, Sgt1B or both isoforms. At present we search for the function of the particular Sgt1 splice variants in HEp-2 cells.

Acknowledgements:
This work was supported by statutory funds from the Nencki Institute of Experimental Biology, Warsaw and from Centre of Molecular and Macromolecular Studies, Łódź.
P11.5

The possible molecular mechanisms of azole resistance in *Candida albicans* strains

Karolina Gołąbek1, Joanna K. Strzelczyk1, Anna Ślemp-Migiel2, Aleksander Owczarek3, Andrzej Wiczkowski1

1Medical University of Silesia, Chair and Department of General Biology, Poland; 2Laboratory of Microbiology in Hospital in Nowy Targ, Poland; 3Medical University of Silesia, Division of Statistics, Poland

e-mail: Karolina Gołąbek <karolina.golabek@med.sum.edu.pl>

Azoles are the most common class of drugs for yeast infections. The possible molecular mechanism of azole resistance is associated with overexpression of CDR1, CDR2, MDR1 genes (genes encoding active transporters of drugs), and overexpression of ERG11 gene (the gene encoding lanosterol 14a-demethylase).

The aim of the study was the assessment of the expression of CDR1, CDR2, MDR1, and ERG11 genes in *Candida albicans* strains sensitive and resistant to azole drugs and analyze nucleotide substitutions in the *Candida albicans* ERG11 gene.

120 *C. albicans* strains were isolated from clinical samples (sputum, pus, urine, blood and bronchial aspirates). RNA isolates were used for cDNA synthesis by a reverse transcription reaction. Received cDNA was used to determine the genes expression level by quantitative real-time PCR.

The ERG11 genes of isolates of *Candida albicans* strains were amplified in four overlapping regions of the gene and sequenced. The MSSCP (Multitemperature Single Strand Conformation Polymorphism) method was performed due to the selection of samples presenting genetic differences in the *Candida albicans* ERG11 gene fragments.

Analysis by quantitative real-time PCR allowed to demonstrate differences in gene expression of CDR2, MDR1, and ERG11 in strains of *C. albicans* resistant and susceptible to azoles. Based on the sequencing results we managed to detect 19 substitutions of nucleotides in the *Candida albicans* ERG11 gene fragments for sequence analysis.

P11.6

Hypoxia downregulates HSP genes expression in human keratinocytes

Anna Habryka1,2, Agnieszka Gogler-Pigłowska1, Bernadeta Pyclik1, Mariusz Kryj2, Zdzisław Krawczyk1,2, Dorota Ścieglińska1

1M. Skłodowska-Curie Memorial Cancer Center and Institute of Oncology Gliwice Branch, Center for Translational Research and Molecular Biology of Cancer, Poland; 2Silesian University of Technology, Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology, Poland

e-mail: Anna Habryka <annahabryka@interia.pl>

The epidermis is the most superficial layer of skin, composed mostly of keratinocytes. It was established that keratinocytes environment is hypoxic due to a lack of vessel system in epidermis. This phenomenon is reflected by stabilization of HIF1α in the basal layer, which is most hypoxic part of epidermis. Many studies show that various HSP genes (*HSPB1, HSPA1, HSPA2, HSPC, HSPH1*) are expressed in human epidermis. Functional data shows that HSPs are required for protection against UV-induced skin damages. HSPs also contribute to skin pathological processes like wound healing, psoriasis and cancer. The view that hypoxia induces HSPs expression is well established, however there are no data showing that hypoxia activates heat shock response in keratinocytes.

Here we aimed to investigate HSPs expression in keratinocytes under low oxygen tension (hypoxia) and treatment with hypoxia-mimicking agent (cobalt chloride). We found that HSPs expression was downregulated in normal human keratinocytes (NHEK), immortalized keratinocytes (HaCaT) and in epidermal cancer cells (A431) under hypoxia (1% O₂). While treatment with CoCl₂, which stabilizes HIF1α and also generates reactive oxygen species (ROS), did not affect HSPs level in normal keratinocytes. We noticed an ... that changes of ROS level can influence HSP genes expression. This idea was confirmed by hypoxia/reoxygenation experiment and hydrogen peroxide treatment.

We can conclude that hypoxia does not activate HSPs expression in keratinocytes, nevertheless HSPs are an important element of keratinocytes response to oxidative stress.
P11.7

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

Joanna Jabłońska1, Piotr Wasąg1, Anna Goc1, Robert Lenartowski2

1Nicolaus Copernicus University, Department of Genetics, Poland; 2Nicolaus Copernicus University, Isotope and Instrumental Analysis Laboratory, Poland

Tyrosine hydroxylase (TH) [EC. 1.4.16.2] catalyzes the first and rate-limiting step in catecholamine biosynthesis. TH expression is regulated in a tissue-specific manner and occurs only in the catecholaminergic cells of the nervous system and in the adrenal medulla. It has been documented that TH undergoes multilevel regulation, including both short-term modulation of enzyme activity and long-term regulation of gene expression. Several regulatory elements recognized by trans-acting factors are located in the TH promoter, its coding sequence, and downstream of the TH gene. Nuclear mapping studies that connect nuclear function and structure have indicated that higher-order chromatin organization has an impact on the transcriptional status of many genes. Binding chromatin loops to the nuclear matrix (NM) through specific DNA sequences, called scaffold/matrix attachment regions (S/MARs), constitutes another transcriptional regulatory mechanism. S/MARs act in cis to anchor the chromatin to the NM structure in a cell-type- and cell-cycle-specific manner. However, the distribution of S/MARs does not appear to have any defined pattern, and S/MAR location can change in response to the NM protein composition. The nucleoprotein complexes formed by S/MAR and NM proteins protect DNA sequences from nucleolytic digestion. To assess attachment of the TH gene to the NM, we examined DNase I sensitivity of the 30 kb genomic fragment containing the bovine TH gene. Nuclear matrices were isolated from two different bovine tissues with differing TH transcriptional status: the adrenal medulla, in which TH is transcriptionally active, and the liver, in which the TH gene is inactive. NM samples were digested with DNase I, and the DNA fraction bound to the NM was used as a template in PCR reactions. Five pairs of primers for the 18 kb TH promoter, two pairs for the TH gene, and two pairs hybridizing downstream of the coding sequence were used to amplify DNA. The regions -18484/-18048, -7703/-7081, +3173/+3614, +4554/+5106 were DNase I sensitive in the chromatin isolated from the adrenal medulla. By contrast, chromatin obtained from bovine liver was less sensitive to digestion and the regions from +3173 to +3614 and from +4554 to +5106 bp were released from the NM.

P11.8

Studies on the regulation of CacyBP/SIP gene expression

Beata Kądziołka, Wiesława Leśniak, Anna Filipek

Laboratory of Calcium Binding Protein, Nencki Institute of Experimental Biology PAS, Warsaw, Poland

CacyBP/SIP was discovered as a calcyclin (S100A6) binding protein (1) and later as the Siah-1 interacting protein (2). At present, other targets of CacyBP/SIP are known. Among them are Skp1, tubulin, actin, tropomyosin and ERK1/2 kinase (3). As to the interaction with ERK1/2, it has been revealed that CacyBP/SIP dephosphorylates this kinase (4) and, in consequence, decreases the activity of Elk-1 transcription factor which is involved in the regulation of cell proliferation and differentiation pathways. In this work we examined the regulation of CacyBP/SIP gene expression. For that we analyzed its promoter sequence (1.6 kb downstream from the Transcription Start Site, TSS) using the MatInspector program. We found the binding sites, among others, for transcription factors such as NFAT (Nuclear Factor of Activated T cells) and CREB (cAMP response element-binding), which may link CacyBP/SIP expression with the activity of other kinases and phosphatases, a binding site for E2F transcription factors that promote proliferation or cell death and a binding site for DREAM (downstream regulatory element antagonist modulator) which might transmit the effect of intracellular calcium concentration on CacyBP/SIP gene expression. Our preliminary results show that these transcription factors change the activity of CacyBP/SIP gene promoter as well as evoke changes in CacyBP/SIP mRNA and protein level in the cell. Performing the analyses simultaneously in human neuroblastoma SH-SY5Y and colorectal cancer cell HCT116 cells, we observed that, depending on the cell line, different factors can be engaged in regulation of CacyBP/SIP expression.

Acknowledgements:

This work was supported by grant from the National Science Center (G 595) and by statutory funds from the Nencki Institute of Experimental Biology.

References:

P11.9

Do the different levels of microRNA overexpression affect cell phenotype?

Tomasz Kolenda¹,³, Anna Teresiak-Mariczak¹, Weronika Przybyła¹, Marta Kruszyna², Anna Kowalik², Weronika Jackowiak², Renata Bliźniak¹, Katarzyna M. Lamperska¹,³
¹Cancer Genetics Laboratory, Greater Poland Cancer Centre, Poznań, Poland; ²Medical Physics Department, Greater Poland Cancer Centre, Poznań, Poland; ³Postgraduate School of Molecular Medicine, Medical University of Warsaw, Warsaw, Poland

E-mail: Tomasz Kolenda <kolenda.tomek@gmail.com>

Introduction: microRNAs are proposed to be a good candidates as biomarkers for personalization of medicine nowadays. The main problem of miRbiomarkers is how to define their “normal” and pathological expression levels. We still don’t know if two different overexpression levels have the same cellular effect or cause different cell behavior. Are the microRNAs overexpression levels of suppressor important? In this study we focused on influence of different let-7d and miR-18a overexpression levels on cell behavior. let-7d and miR-18a have significant function in the biology of cancer. let-7d acts as tumor suppressor of C-MYC and miR-18a acts as suppressor of K-RAS.

Purpose: Investigation whether different suppressor microRNA overexpression levels have influence on cell behavior.

Methods: Overexpression of let-7 and let-7d-miR-18a in FaDu cell line has been achieved using lentiviral system. Cell line models with different range of examined miRNAs overexpression have been created basing on clonogenic selection method. Overexpression of miRNAs has been investigated by TaqMan microRNA assay (Applied Biosystem) and verified by western blot. The proliferation ratio of cell models has been described by MTT assay. Cell models have been irradiated using dose of 2 Gy and clonogenic assays were performed. FaDu-GFP and non-modified FaDu cell models were used as controls.

Results: We received FaDu-7d and FaDu-7d-18a models with different overexpression range from 5- to 40 times higher than in non-modified cells. The cell models have different proliferation ratio depending on expression levels of let-7d and miR18a. The clonogenic assay showed different proliferation ability and radiosensitivity of FaDu-7d and FaDu-7d-18a. Let-7d shows double nature according to its expression level in cell.

Conclusions: We observed different cellular behavior depending on microRNAs levels. Let-7d may act as tumor suppressor when overexpression is 10- and 21 times higher than in non-modified cell line (control cell line) but it may be also oncogenic factor and makes cells more resistant to irradiation.

P11.10

RNA-chaperone Hfq has an essential role in bacterial nano-cellulose (BNC) biosynthesis control in *Gluconacetobacter xylinus*

Katarzyna A. Kubiak, Agata Jackiewicz, Marta Kurzawa, Marzena Jędrzejczak-Krzepkowska, Stanisław Bielecki

Institute of Technical Biochemistry, Lodz University of Technology, Łódź, Poland

E-mail: Katarzyna Kubiak <katarzyna.kubiak@p.lodz.pl>

*Gluconacetobacter xylinus* is the most efficient bacterial nanocellulose (BNC) producer, used for its production on an industrial scale. Due to its exceptional structure BNC is regarded as a very attractive material especially intensively tested for applications in regenerative medicine. Nevertheless, up to date molecular mechanisms of this polysaccharide biosynthesis regulation in BNC model producer have been not described in detail. Recently, in numerous biofilm-producing bacterial species the gene expression regulation guided by short RNA (sRNA) was proved to play an important role in extracellular polysaccharides biosynthesis and secretion.

Our team’s preliminary studies of *Ga. xylinus* E25 strain genome have revealed the presence of a gene encoding for a protein with high sequence and structural (based on modeling) similarity to Hfq. It is well known RNA chaperon, belonging to evolutionary ancient Sm-like protein family (its representatives are commonly present in both eukaryotic and prokaryotic cells), which is essential for proper function of *sRNA* regulators. Furthermore, we demonstrated that identified *hfq* gene expression is stimulated in the presence of stress inducing factor (ethanol) in culture medium. In order to verify, if mechanisms involving Hfq may play any role in regulation of cellulose biosynthesis process in a direct way and/or influence the intensity of biofilm formation indirectly the construction of *hfq*-disruption mutants of E25 and ATCC 53582 strains was done (by homologous recombination induction). Gene disruption was confirmed by PCR and phenotype of the *hfq-* *Ga. xylinus* mutants was established. Moreover, the E25 strain transformed with pBBR-bla-hfq vector (possessing additional copy of its own *hfq* gene) was obtained and its BNC productivity was tested.
Nuclear matrix (NM) is defined as an insoluble and salt-resistant nuclear scaffold. It was originally isolated from interphase cells after chromatin digestion and extraction of soluble proteins with detergent or high-ionic-strength buffers. In general, NM is composed of non-histone proteins, which form a fibrogranular nuclear framework, and a small percentage of nucleic acids and phospholipids. Protein NM composition is dynamic and varies in a cell-type-specific manner. It has been shown that some NM proteins interact with DNA sequences called scaffold/matrix attachment regions (S/MARs). S/MAR sequences anchor chromatin loops to the NM in spatial-, temporal-, cell- and tissue-specific ways. Any changes in NM protein composition result in the redistribution of chromatin anchoring sites and, consequently, impose a new gene activity profile. Thus, NM can have an impact on tissue-specific gene expression by affecting chromatin remodeling. Expression of the tyrosine hydroxylase (TH) gene is regulated in a developmental-stage- and tissue-specific manner and is controlled by homeostatic stimuli. TH gene expression is activated by trans-acting factors that recognize regulatory elements located at the 5′ promoter region, within the coding region, and downstream of the coding region. Our previous studies indicated that the bovine TH gene can attach to the NM isolated from bovine liver and bovine adrenal medulla. The constitutive S/MAR was located within the +725/+1678 bp distal fragment of the first intron of TH, but its exact position was unclear. To map the minimal region of the TH first intron responsible for anchoring to the NM, we assessed short fragments in an in vitro binding assay. This method allows to identify DNA regions that are specifically bound by the NM proteins and consequently map the S/MAR. Using molecular probes encompassing fragments of the bovine TH gene (+1015/+1843 bp and +1011/+1438 bp), we were able to determine that +1011/+1438 is sufficient for binding to NM proteins. Moreover, the +1011/+1438 TH intron fragment has a higher affinity for NM proteins isolated from bovine adrenal medulla than for NM proteins from bovine liver.
**P11.13**

**Construction of pSuper.GFP/neo Mdr1 short hairpin RNA expression vectors and its application in LoVo/Dx cells**

Anna Palko-Łabuz, Kamila Środa-Pomianek, Daniela Mosiądz, Krystyna Michalak

Wrocław Medical University, Department of Biophysics, Wrocław, Poland

e-mail: Anna Palko-Łabuz <anna.palko-labuz@wp.pl>

The development of multidrug resistance (MDR) in cancer cells remains the major obstacle of effective chemotherapy. The resistance to a wide spectrum of drugs can be a consequence of pharmacological treatment as well as primary drug resistance. The MDR phenotype has most frequently been correlated to the presence of ATP-binding cassette (ABC) transporters, responsible for active extrusion of toxins and xenobiotics. P-glycoprotein (P-gp) encoded by the human MDR1 gene has been extensively studied ABC transporter because of its strong impact on limiting cellular uptake of substances from blood into the brain as well as from intestinal loop into epithelial cells. The protein has been also widely expressed in different kinds of cancer cells, resistant to colchicine, vinblastine, doxorubicin, etc (Bansal et al., 2009, *J Pharm Sci* 12:46–78). Inhibition of the drug efflux activity of P-gp has been studied intensively and the experimental therapeutic strategies are under investigation. RNA interference (RNAi) is a naturally occurring mechanism but also a relatively new technology in therapeutic gene silencing (Pichler et al., 2005, *Clin Cancer Res* 11:4487–4494).

By applying of RNA interference mechanism to resistant cancer cells it is possible to inhibit gene expression and in that way to reduce the drug efflux, mediated by MDR transporters, like P-glycoprotein. In our studies, two different short hairpin RNAs (shRNAs) against MDR1 gene were designed and introduced into pSuper.GFP/neo plasmid, respectively. They have been transfected into P-gp — overexpressing LoVo/Dx cells. To determine an influence of doxorubicin on the growth of cancer cells with anti — Mdr1 shRNA expressing vectors incorporated, the SRB assay was applied. The results were compared with data obtained before transfection. Inhibition of gene expression by RNA interference offers a new approach to combat the drug resistance in cancer.

**P11.14**

**Developing the expression cassette for the gene therapy of muscular dystrophies**

Katarzyna Piekarowicz, Ryszard Rzepecki

Laboratory of Nuclear Proteins, Faculty of Biotechnology, University of Wrocław, Poland

e-mail: Katarzyna Piekarowicz <kasiap@ibmb.uni.wroc.pl>

The expression cassette is a key element of a vector for the gene therapy, that is a possible cure for muscular dystrophies. It should contain control elements responsible for obtaining a stable, specific and high-level expression of a therapeutic gene needed for a correction of a genetic defect.

In order to develop the expression cassette, the hybrid promoter was designed. It consist of controlling elements such as enhancers, core promoter and intron with small intronic enhancer. They originate from murine, muscle specific genes of *desmin* and *muscle creatine kinase*, that were chosen and modified basing on their expression profiles, previous research data and in silico analysis.

The hybrid promoter activity was controlled via the expression of a reporter gene — secretory luciferase *Lucia*. The expression of this protein can be quantitatively measured by a luminescence. Our data shows, that developed promoter provides much higher level of the expression in C2C12 cell line that promoter of muscle specific gene *desmin*, that was already used in a clinical trial of the muscular dystrophy gene therapy treatment. The influence of the particular components of the hybrid promoter on the expression level in muscular and non-muscular cell lines was also investigated.
Comparison of expression levels of microRNA after irradiation of FaDu cell line with wild-type and silenced p53 activity

Weronika Przybyła¹, Tomasz Kolenda¹, Anna Teresiak-Manczak¹, Marta Kruszyna², Anna Kowalik³, Weronika Jackowiak³, Anna Przybyła³, Katarzyna M. Lamperska³

¹Cancer Genetics Laboratory, Greater Poland Cancer Centre, Poznań, Poland; ²Radiobiology Laboratory, Greater Poland Cancer Centre, Poznań, Poland; ³Medical Physics Department, Greater Poland Cancer Centre, Poznań, Poland; ⁴Postgraduate School of Molecular Medicine, Medical University of Warsaw, Warsaw, Poland; ⁵Chair of Medical Biotechnology, University of Medical Science, Poznań, Poland; ⁶Head of Laboratory, Medical University of Warsaw, Warsaw, Poland; ⁷Chair of Medical Biotechnology, University of Medical Science, Poznań, Poland

e-mail: Weronika Przybyła <weronika.przybyla@gmail.com>

text:

Introduction: Head and neck squamous cell carcinomas are one of the worst prognosis cancers with increasing number of cases. Because of its chemoresistant character, radiotherapy is one of the main methods of the complementary treatment after surgery. Interestingly, it turned out there are significant relationships between miRNAs expression and radiotherapy. miRNAs are involved in post-transcriptional regulation of genes which regulate many cell cycle processes and participate in cancerogenesis. Changes in expression levels of miRNA determine changes in profiles of target genes expression. One of them is gene p53 which coding protein p53. This „guardian of the genome“ plays very important role in cells response to irradiation. The fact that p53 can suppress the expression of cell proliferation and apoptosis is very important regarding to radiotherapy treatment and expression levels of miRNAs.

Aim: Analysis of influence of ionizing radiation on changes in miRNA expression in FaDu cell line with wild-type and silenced p53 activity.

Methods: Changes of expression level in miRNA were analysed after irradiation of FaDu cell line and FaDu — sip53 dose of 2Gy in water phantom using Clinac 2300. Then RNA from FaDu (wild-type) cell line was isolated in different point of time after irradiation (2, 4, 8, 24 and 48 hours) and expression levels were studied by qRT-PCR method.

FaDu cell line with silenced p53 gene was achieved using sip53 vector. Next, RNA from FaDu-sip53 will be isolated and expression levels of miRNA will be compared between these two lines.

Results: Significant changes of expression levels of miRNAs in FaDu (wild-type) cell lines were observed. All of examined miRNAs were overexpressed showing similar trends in changes. The biggest differences in dynamic of expression showed miRNA-100 (2-ΔΔCT 0.16–1.39). The lowest one goes to miRNA-205 (2-ΔΔCT 0.84–1.52).

Discussion: The expression of miRNA is a dynamic process. According to cancerogenesis, creation of miRNA expression profiles will allow to get to know cell response to ionizing irradiation and in the future it will allow to make genetic biomarkers and personalisation of therapy.

Overexpression of miR-155 enhances cell growth by targeting the TBRG1 gene in B-cell lymphoma

Izabella Słęzak-Prochazka¹,², Joost Kluiver¹, Debora de Jong¹, Katarzyna Smigielska-Czepe³l, Melanie Winkle¹, Bea Rutgers¹, Bart-Jan Kroesen¹, Anke van den Berg¹

¹University Medical Center Groningen, Department of Pathology and Medical Biology, Groningen, The Netherlands; ²Czeschowa University of Technology, Department of Public Health, Częstochowa, Poland

e-mail: Izabella Słęzak-Prochazka <izabella.slezak@gmail.com>

MicroRNAs (miRNA) are single-stranded noncoding RNA molecules that negatively regulate gene expression by binding to 3’ untranslated region (3’UTR) of target miRNAs. The functional mature miRNAs are part of the RNA-induced silencing complex (RISC), which also includes one of the Argonaute (Ago) proteins. In silico predictions reveals many putative miRNA targets, however until now only a limited number of these targets have been proven experimentally. MiR-155 is an important regulator of B-cell development and immune response. Derepression of miR-155 leads to hematologic malignancies. High miR-155 levels were observed in several types of lymphoma, including Hodgkin lymphoma. In contrast, miR-155 levels were very low in Burkitt lymphoma. To determine function of miR-155, we identified miR-155 target genes in Hodgkin and Burkitt lymphoma.

We showed that retroviral overexpressed miR-155 in in two Burkitt lymphoma cell lines, ST486 and Ramos enhanced growth of ST486 cells but not Ramos cells in a GFP competition assay. To identify miR-155 target genes in ST486 and Ramos cells, we performed Ribonucleoprotein ImmunoPrecipitation-gene Chip (RIP-Chip) using antibodies against wild-type human Ago2 in miR-155-transduced or EV-transduced cells. Upon miR-155 overexpression, 54 genes were more than 2 fold enriched in the targetome of ST486 cells and considered as miR-155 target genes. Only 18 genes were identified in Ramos cells with an overall lower fold enrichment compared to ST486 cells. From the 54 miR-155 target genes in ST486 cells, 32% of genes were predicted by TargetScan and 77% contained 6mer miR-155-binding site in the 3’UTR. We validated 5 most enriched genes DET1, TBRG1, TRIM32, HOMEZ, PSIP1 and a known miR-155 target JARID2 in luciferase assay in ST486 cells. We also showed that DET1, TBRG1, TRIM32, HOMEZ and JARID2 are targeted by endogenous miR-155 in KM-H2 Hodgkin lymphoma cells, since they were depleted in KM-H2 targetome upon inhibition of miR-155 with retroviral miRNA sponge construct. To determine which of the identified miR-155 target genes phenocopy the effect of miR-155 induction in ST486 cells, we inhibited the six selected genes and showed that inhibition of TBRG1 enhanced growth of ST486 cells.

In conclusion, we identify novel miR-155 targets in Burkitt and Hodgkin lymphoma and show that miR-155 overexpression does not always result in increased miR-155 activity. We also show that induction of miR-155 enhances growth of ST486 Burkitt lymphoma cells and this phenotype involves inhibition of the TBRG1 gene.
Genistein, which belongs to isoflavones, is a natural vegetable chemical substance with proven wide therapeutic and preventive action. It is known for its antioxidant properties and the ability to interact with estrogen receptors. The beneficial effect of a diet rich in flavonoids was demonstrated many times—it includes primarily the ability to prevent cancer—just at the stage of initiation and progression of the disease. Here is pointing to the abilities to reduction of tumor cell proliferation, stimulation of apoptosis, effect on angiogenesis, course of inflammatory processes and immune system response. In addition, genistein has the ability to regulate the transcriptional activity of matrix metalloproteinases (MMPs), which are considered to be one of the main proteolytic enzymes for maintaining the correct composition and organization of the extracellular matrix (ECM). Excessive activity of most MMPs leads to an imbalance between synthesis and degradation of ECM components and, consequently, to the infiltration of the tissues surrounding the tumor and distant metastasis formation.

The aim of this study was evaluation of the genes expression involved in apoptosis BAX, BCL-2 and TP53 in breast cancer cells T47-D treated with genistein. At the same time, under the same conditions, tested cells were analyzed for changes in gene expression of selected MMPs (MMP-1, MMP-2, MMP-3, MMP-13, MMP-14, MMP-15) and their inhibitors (TIMP-1, TIMP-2, TIMP-3). This studies used the human breast cancer cell line T-47D, purchased from the American Type Culture Collection (ATCC). The cultures were treated with a concentration of genistein 50 µM. The expression of genes involved in apoptosis, and genes selected MMPs and TIMPs was evaluated using RT-QPCR technique.

Under the conditions of the carried out experiment, showed that in breast cancer cells line T-47D incubated with genistein decreases of anti-apoptotic BCL-2 gene expression and increases of pro-apoptotic BAX gene expression. Increase of BAX/BCL-2 ratio can point to run in tested cells apoptosis pathway. Analysis of MMPs showed an increased expression of genes MMP-1 and MMP-14 and a decrease in gene expression of MMP-2, MMP-3, MMP-13, MMP-15, TIMP-1, TIMP-2 and TIMP-3.
BNIP3 is a member of the Bel-2 family of proteins which regulate programmed cell death. Many cancers show the altered expression of BNIP3, which can be correlated with resistance to anticancer drugs. Cosse et al. have shown that forced BNIP3 expression, depending on oxygen conditions had an impact on the cells’ sensitivity to etoposide. The aim of this study was to investigate the role of BNIP3 gene expression in the context of cancer cells sensitivity to etoposide. We used the DLD-1 (human colon cancer cell line) which do not express BNIP3 due to promoter methylation. In this regard BNIP3 gene transcription can be restored by methyltransferase inhibitor — 5-aza-2′-deoxycytidine (5-aza-dC). To study sensitivity of DLD-1 cells to etoposide we used MTS assay followed by propidium iodide apoptosis assay.

We observed the same pattern of the dose-dependent decrease in viability of the cells treated with etoposide in normoxia as well as in hypoxia. Additionally, preconditioning of DLD-1 with 5-aza-dC, resulting with restoration of BNIP3 expression, leads to increased sensitivity to the etoposide treatment. To determine the direct influence of BNIP3 on cells’ sensitivity to etoposide assessment of apoptosis was done. Etoposide induced apoptosis of DLD-1 cells regardless to BNIP3 expression. To study sensitivity of DLD-1 cells to etoposide we used MTS assay followed by propidium iodide apoptosis assay.

The negligible effect of BNIP3 expression on the sensitivity of DLD-1 cells to etoposide

Magdalena Puniach, Edyta Wysokińska, Alicja Pawlak, Anna Śliwak, Ewa Ziolo, Wojciech Kalas, Leon Strządała

Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland

e-mail: Edyta Wysokińska <edyta.wysokinska@iitd.pan.wroc.pl>