

---

## Session 7. Protein Functions in Cellular Signaling

---

### Lectures

#### L7.1

##### Molecular basis of macrophage activation by lipopolysaccharide

Katarzyna Kwiatkowska

Nnenci Institute of Experimental Biology, Department of Cell Biology, Warsaw, Poland

e-mail: Katarzyna Kwiatkowska <k.kwiatkowska@nencki.gov.pl>

Lipopolysaccharide (LPS, endotoxin) is a component of the outer membrane of Gram-negative bacteria. LPS is an archetypical pathogen-associated molecular pattern which activates one of Toll-like receptors (TLR), TLR4, of immune cells. Upon infection it strongly stimulates production of pro-inflammatory mediators by macrophages. Excessive host responses to LPS can lead to a systemic inflammatory condition named septic shock. A relatively high incidence and mortality of the disease drive studies on molecular mechanisms of activation of cells by LPS.

In a typical scenario, activation of TLR4 is a multistep process which begins in blood serum with binding of LPS monomers to LPS-binding protein. The protein transfers LPS to CD14, a protein anchored within sphingolipid- and cholesterol-rich microdomains (rafts) of the plasma membrane. CD14 transfers LPS to TLR4/MD-2 complex which dimerizes and triggers two signaling pathways engaging MyD88 and TRIF adaptor proteins. The TRIF-dependent signaling pathway requires endocytosis of activated TLR4. We study mechanisms which control fast and reliable organization of TLR4 signaling complexes in the plasma membrane addressing questions such as: Are raft lipids shaping macrophage responses to LPS? What is the role of raft accumulation of CD14 for LPS-triggered signaling? Can we identify other raft proteins controlling TLR4 signaling?

#### L7.2

##### Neutrophil extracellular traps (NETs) in autoinflammatory responses

Joanna Cichy

Jagiellonian University, Faculty of Biochemistry, Biophysics & Biotechnology, Department of Immunology, Kraków, Poland  
e-mail: Joanna.Cichy@uj.edu.pl>

Neutrophils also referred to as polymorphonuclear leukocytes (PMNs) form a part of the front line of host defense against pathogens and are key component of tissue infiltrates in autoinflammatory diseases such as systemic lupus erythematosus (SLE) and psoriasis. Activated neutrophils can eliminate microbes by the formation of neutrophil extracellular traps (NETs), extracellular decondensed chromatin networks decorated with microbicidal agents. NETs were first reported to limit the growth and spread of microorganisms through mechanically trapping pathogens and facilitating interaction of anti-microbial agents that are displayed on NETs with bacteria or fungi. More recently, NETs were associated with tissue damage and autoinflammatory diseases. NETs constituents, including self DNA, histones and myeloperoxidase are potential autoantigens. Since in addition to their beneficial role in host defense, NETs may be an underlying component of cell cytotoxicity and autoimmunity, it is likely that specific counter-regulatory mechanisms evolved to constrain NETs formation. Here we will discuss our data on regulatory mechanisms underlying NETs generation and discuss a contribution of NETs to a skin disease-psoriasis.

## L7.3

### NF-kappa B regulation in response to LPS and poly(I:C) stimulation

Maciej Czerkies<sup>1</sup>, Zbigniew Korwek<sup>1</sup>, Wiktor Prus<sup>1</sup>, Sławomir Błoński<sup>1</sup>, Bing Tian<sup>2</sup>, Marek Kimmel<sup>3</sup>, Allan Brasier<sup>2</sup>, Tomasz Lipniacki<sup>1</sup>

<sup>1</sup>Institute of Fundamental Technological Research, Laboratory of Modelling in Biology and Medicine, Warsaw, Poland; <sup>2</sup>University of Texas Medical Branch (UTMB), Department of Internal Medicine, USA; <sup>3</sup>Rice University, Department of Statistics, USA  
e-mail: Tomasz.Lipniacki <tlipnia@ippt.pan.pl>

NF- $\kappa$ B, IRF3 and AP-1 are most potent transcription factors controlling innate immune responses to pathogens. Combining single cell and population techniques with mathematical modeling we analyzed crosstalk of these pathways in mouse embryonic fibroblasts in response to LPS and poly(I:C).

We found that both LPS and poly(I:C) activate mediating kinases IKK $\alpha$ / $\beta$  and TBK1; interestingly, only poly(I:C) stimulation leads to activation of IRF3 (activated by TBK1) and triggering of transcription of IFN $\beta$ , IRF7, and RIG-1 and other interferon regulated genes. LPS stimulation leads to transient or oscillatory (in some cells) responses of NF- $\kappa$ B, in contrast to switch-like responses (preceded by one or two pulses in a fraction of cells) to poly(I:C) stimulation, with fraction of switched-on cells increasing with the stimulation dose. As suggested by the experiment in which cells are costimulated by LPS and IFN $\beta$ , the difference in NF- $\kappa$ B responses is caused by IFN $\beta$  para- and autocrine regulation that leads to activation of Eif2ak2 and suppression of NF- $\kappa$ B inhibitors synthesis. Correspondingly the blockade of IFN $\beta$  receptor causes attenuation of response to poly(I:C) at latter time points. Overall this suggests that autocrine regulation breaks the negative regulation of NF- $\kappa$ B (and IRF3) leading to build up of nuclear NF- $\kappa$ B and IRF3, followed by apoptosis in a fraction of cells (not observed in the case of LPS stimulation). The IRF3 activation is stabilized by positive feedback involving strongly upregulated IRF7 and RIG-1 (which is IRF7 responsive).

We confirmed by mathematical modeling the dynamically divergent responses to LPS (mimicking bacterial infection) and to poly(I:C) (mimicking viral infection). NF- $\kappa$ B is known for exhibiting oscillatory responses to TNF $\alpha$ , which are replaced by switch-like responses in A20-deficient cells. Here, we found that activation of the IRF3 pathway, or IFN $\beta$  stimulation leads to the similar effect on NF- $\kappa$ B signaling, possibly due to inhibition of translation of NF- $\kappa$ B inhibitors. The switch-like behavior, frequently associated with cell fate decisions, is associated with bistability arising here due to the positive feedbacks in IRF3/IRF7 regulation.

## Oral presentations

### 07.1

#### Heme oxygenase-1 deficiency triggers monocyte mobilization and impairs heart function after acute myocardial infarction

Mateusz Tomczyk, Karolina Bukowska-Strakova, Krzysztof Szade, Alicja Jozkowicz, Jozef Dulak, Agnieszka Jazwa

Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland  
e-mail: Agnieszka.Jazwa <agnieszka.jazwa@uj.edu.pl>

Coronary embolism resulting mostly from atherosclerosis, leads to ischemic injury of myocardium, subsequent incorrect heart contractility and heart failure, the main cause of death in industrialized countries. Heme oxygenase-1 (HO-1) is an inducible form of heme-degrading enzyme, which possesses cytoprotective, antioxidative and proangiogenic properties.

A role of HO-1 in acute myocardial infarction (AMI) was investigated in HO-1 knock-out and wild type mice exposed to a permanent ligation of left anterior descending (LAD) coronary artery. Cardiac function was monitored weekly for 3 weeks with high frequency ultrasonography (Vevo2100 platform) and revealed more potent reduction of ejection fraction in HO-1 knock-out mice. Coronary artery occlusion in wild type mice lead to strong upregulation of HO-1 expression, with no changes in expression of other genes related to iron metabolism (HO-2, ferritin). Some genotype-dependent differences were observed in the expression of antioxidant enzymes (superoxide dismutase 1, glutathione peroxidase 1, catalase) and cardiac myocytes-associated miRNAs (miR-29a, miR-199) at 1, 7 and 21 days after AMI. Moreover, we found much higher numbers of classical (Ly6C<sup>++</sup>CD43<sup>+</sup>) and intermediate (Ly6C<sup>++</sup>CD43<sup>++</sup>) monocytes, both basal and induced by AMI, in peripheral blood of HO-1 deprived mice. Contrary in spleen, we observed higher numbers of classical and intermediate monocytes in wild type animals when compared to HO-1 knock-outs. Finally, we noticed that abundance of SKL cells (Sca-1<sup>+</sup>/c-kit<sup>+</sup>/Lin<sup>-</sup>) including hematopoietic stem cells (HSCs, Sca-1<sup>+</sup>/c-kit<sup>+</sup>/Lin<sup>-</sup>/CD48<sup>-</sup>/CD150<sup>+</sup>/CD34<sup>-</sup>) populations in bone marrow of HO-1 deprived mice was larger. No differences were found in case of bone marrow hematopoietic progenitor cells (Sca-1<sup>+</sup>/c-kit<sup>+</sup>/Lin<sup>-</sup>/CD48<sup>-</sup>/CD150<sup>-</sup>/CD34<sup>+</sup>) between both genotypes.

Consequences of AMI seem to be more severe in HO-1 knock-out mice. Lack of HO-1 may influence early and late responses to AMI related to antioxidant genes expression and monocyte/macrophage-dependent cardiac remodeling.

## 07.2

### Silencing of connective tissue growth factor by small interfering RNA prevents fibroblasts to myofibroblast transition in bronchial asthma

Katarzyna Wójcik<sup>1,3</sup>, Marta Michalik<sup>2</sup>, Paulina Koczurkiewicz<sup>3</sup>, Zbigniew Madeja<sup>2</sup>, Marek Sanak<sup>1</sup>

<sup>1</sup>Jagiellonian University Medical College, Department of Medicine, Laboratory of Molecular Biology and Clinical Genetics, Kraków, Poland; <sup>2</sup>Jagiellonian University, Faculty of Biochemistry, Biophysics and Biotechnology, Department of Cell Biology, Kraków, Poland; <sup>3</sup>Jagiellonian University Medical College, Faculty of Pharmacy, Department of Pharmaceutical Biochemistry, Kraków, Poland  
e-mail: Katarzyna Anna Wójcik <katarzynaanna.wojcik@uj.edu.pl>

Bronchial asthma is one of the most common chronic lung diseases. It is inflammatory disorder of the lower airways manifested by reversible constriction of bronchi but also progressing with an irreversible bronchial wall modeling. Despite basic pathomechanism of asthma, characterized by influx of blood cells, many evidences suggest that extracellular matrix thickening and increased number of smooth muscle cells is an early event. Growth factors and cytokines like transforming growth factor type  $\beta$  (TGF- $\beta$ ) and connective tissue growth factor (CTGF) are known to induce fibroblast to myofibroblast transition (FMT) during the course of asthma.

Our previous studies demonstrated that human bronchial fibroblasts (HBFs) cultured from patients with asthma have much higher FMT rate, both spontaneously and at the presence of TGF- $\beta$ . Moreover, we demonstrated that asthmatic HBFs are capable of CTGF synthesis and secretion, especially when stimulated with TGF- $\beta$ .

In the current study we show that CTGF silencing significantly attenuated TGF- $\beta$  induced FMT in HBFs from asthmatics. We used small interfering RNA specific for CTGF transcripts, and noted marked inhibition of FMT in HBFs. This was accompanied by an attenuation of TGF- $\beta$  ability to stimulate this process. In control experiments, we demonstrated that CTGF is able to enhance TGF- $\beta$ -induced FMT in a synergistic manner. These results, especially in the light of our previous reports, suggest that CTGF may contribute to FMT phenomenon as positive feedback stimulus, both by induction and boosting TGF- $\beta$  triggered FMT. Otherwise, CTGF expression seems controlled by the presence of TGF- $\beta$ . Specific mechanism of synergism observed between this cytokines is amplified in the asthmatic HBFs. The advantage of CTGF inhibition over TGF- $\beta$  targeting seems to be a more specific involvement of CTGF into mechanism of remodeling, whereas TGF- $\beta$  signaling is important also for immunoregulation. In conclusion a bronchial fibroblast CTGF biosynthesis in the asthmatic lung amplifies FMT and is a promising target for pharmacological intervention in order to prevent bronchial remodeling in asthma.

## 07.3

### Physiological and pharmacological consequences of 2-methoxyestradiol, a plausible novel hormone

Magdalena Gorska<sup>1</sup>, Alicja Kuban Jankowska<sup>1</sup>, Monika Gorzynik<sup>1</sup>, Michał Aleksander Zmijewski<sup>2</sup>, Maciej Wnuk<sup>3</sup>, Iwona Rzeszutek<sup>3</sup>, Anna Lewinska<sup>3</sup>, Michał Wozniak<sup>1</sup>

<sup>1</sup>Department of Medical Chemistry, Medical University of Gdansk, Gdańsk, Poland; <sup>2</sup>Department of Histology, Medical University of Gdansk, Gdansk, Poland; <sup>3</sup>Department of Genetics, University of Rzeszow, Rzeszow, Poland

e-mail: Michał Wozniak <mwozniak@gumed.edu.pl>; Magdalena Gorska <m.gorska@gumed.edu.pl>

2-methoxyestradiol is one of the principal physiological 17 $\beta$ -estradiol derivatives, believed to be novel and potentially active anticancer agent evaluated in ongoing advanced clinical trials. Preclinical research suggests a wide spectrum of possible anticancer mechanisms of 2-ME action that seem to be directly associated with the inhibition of angiogenesis and induction of apoptosis in tumorous and proliferating cells. According to Sutherland *et al.*, 2-methoxyestradiol inhibits cell proliferation at threshold concentrations of 0.1  $\mu$ M–0.3  $\mu$ M, reaching maximum effect at 10  $\mu$ M–20  $\mu$ M.

The aim of the study was to determine physiological and pharmacological anticancer and genotoxic properties of 2-methoxyestradiol.

We used highly metastatic osteosarcoma 143B cell line. The cells were treated with physiological (1 nM, 10 nM) and pharmacological (1  $\mu$ M, 10  $\mu$ M) concentrations of 2-methoxyestradiol. Induction of cell death were measured by PI/Annexin V staining, respectively. Inhibition of cell cycle, level of reactive nitrogen and nitrogen species and neuronal nitric oxide synthase localization were determined by imaging cytometry. Comet assay was performed in order to determine DNA damage.

Fundamental long-lasting viewpoint on 17 $\beta$ -estradiol metabolites as biologically inactive excretion products has been refuted by various researchers (Yang *et al.*, 2013; Yuan *et al.*, 2013). We evidenced here that 2-methoxyestradiol exerts anticancer properties not only at pharmacological but also physiological concentrations. Due to that fact it could be reported as a plausible novel hormone.

#### References:

- Sutherland TE *et al* (2007) 2-methoxyestradiol — a unique blend of activities generating a new class of anti-tumour/anti-inflammatory agents. *Drug Discovery Today* **12**: 577–584.  
Yang CY *et al* (2013) Osteoblastoma in the region of the hip. *Journal of the Chinese Medical Association* **76**: 115-120.  
Yuan W *et al* (2013) Estrogen Metabolite 2-methoxyestradiol Prevents Hypertension in Deoxycorticosterone Acetate-salt Rats. *Cardiovascular Drugs 160 and Therapy* **27**: 17-22.

#### Acknowledgments

The project was funded by grant No. 2012/07/B/NZ1/00010 from National Science Center resources. M. Wnuk, I. Rzeszutek, A. Lewinska are supported by the Operational Programme Development of Eastern Poland 2007-2013, NoPOPW.01.03.00-18-018/09, the project Centre of Applied Biotechnology and Basic Sciences.

## 07.4

### The role of AnxA2-S100A10 complex in intracellular translocation of TRPV5/6 channels in osteoblast-like cells undergoing mineralization

Anna Cmoch<sup>1</sup>, Patrick Groves<sup>2</sup>, Sławomir Pikula<sup>1</sup>

<sup>1</sup>Nencki Institute of Experimental Biology, Department of Biochemistry, Warsaw, Poland; <sup>2</sup>Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Department of Biological Chemistry, Portugal  
e-mail: Anna.Cmoch <anna.cmoch@gmail.com>

Calcium ions, aside from their role as a second messenger, are fundamental components of the vertebrate bone matrix.  $\text{Ca}^{2+}$  enter cells through the plasma membrane via channels of the transient receptor potential family, vanilloid type (TRPV). Among them TRPV5 and TRPV6 are involved in the efficient uptake and storage of calcium in bone [1]. *Trpv5* (-/-)-deficiency leads to an osteoporosis development [2]. The role of AnxA2-S100A10 complex in regulation of TRPV5/6 channels has been recently broadly investigated. In this study we provide the first evidence for the regulatory mechanism of the AnxA2-S100A10 complexes in the intracellular translocation of TRPV5/6 channels to the plasma membrane of differentiating osteoblasts. To test this we used osteoblast-like cells stimulated for mineralization with 50  $\mu\text{g}/\text{ml}$  ascorbic acid and 7.5 mM  $\beta$ -glycerophosphate. Subcellular fractionation evidenced the increased content of TRPV5/6, AnxA2 and S100A10 in plasma membranes of cells upon treatment with stimulators of mineralization. Then, a direct molecular interaction of AnxA2 and TRPV5/6 channels mediated via S100A10 was confirmed by FRET confocal analysis. Obtained results were complementary to previous observation from our laboratory [3], that AnxA2-S100A10 complexes and TRPV5/6 channels are selectively translocated into matrix vesicles (centres of mineral formation) released from mineralizing Saos-2 cells. Here we demonstrate that knock-down of S100A10 in Saos-2 cells strongly diminishes the distribution of TRPV5/6 channels to plasma membranes and has profound impact on mineralization. Herein, we postulate the role of AnxA2-S100A10 in the modulation of TRPV5/6 function in osteoblast-like cells as a novel regulatory mechanism in the mineralization of osteoblasts. The identification of mechanisms contributing to the bone formation represents a new strategy in screening for therapeutic targets of osteoporosis.

#### References:

1. Vanoevelen J *et al* (2011) *EASEB J* **25**: 3197–3207.
2. Nijenhuis T *et al* (2008) *J Bone Min Res* **23**: 1815–1824.
3. Cmoch A *et al* (2011) *BBRC* **412**: 683–687.

#### Acknowledgements

The work was supported by grant 2012/05/N/NZ3/00330 from the Polish National Science Centre.

## 07.5

### Overexpression of adaptor protein Ruk/CIN85 accompanied by activation of signalling pathways, depending on the PI3-kinase and transcription factor NF- $\kappa$ B, in human breast adenocarcinoma cell line MCF-7

Iryna Horak<sup>1,2</sup>, Ganna Pasichnyk<sup>1</sup>, Olga Povorozniuk<sup>1,3</sup>, Anatoliy Samoilenko<sup>1</sup>, Liudmila Drobot<sup>1</sup>

<sup>1</sup>Palladin Institute of Biochemistry, NASU, Kyiv, Ukraine; <sup>2</sup>Kyiv Mohyla National Academy, Kyiv, Ukraine; <sup>3</sup>Kyiv National Taras Shevchenko University, Kyiv, Ukraine  
e-mail: Iryna.Horak <iryna.horak@gmail.com>

**Introduction:** Molecular basis of cancer is the deviation of certain signaling pathways, including PI3K/Akt/mTOR and NF- $\kappa$ B, which is accompanied by the emergence of resistance to chemotherapy and hormonal drugs, a violation of the regulation of apoptosis and cell differentiation, increased their invasive properties. It was previously shown that MCF-7 cells with overexpression of the adaptor protein Ruk/CIN85 are more resistant to anoikis, have increased transforming potential and resistance to drugs compared to wild type cells. The aim of this study was to investigate the features of functioning of dependent of PI3K and transcription factor NF- $\kappa$ B signaling pathways in the Ruk/CIN85-overexpressing cell line MCF-7.

**Methods:** Activation of kinases mTOR, ERK1/2, Akt, Src, IKK, inhibitor I $\kappa$ B and p65 subunit of transcription factor NF- $\kappa$ B in response to short-term stimulation of MCF-7 cells with overexpression Ruk/CIN85 epidermal growth factor (EGF) was investigated by Western blot analysis. The activity of NF- $\kappa$ B additionally analyzed using the reporter luciferase system.

**Results:** MCF-7 cells with overexpression Ruk/CIN85 was found to be characterized with constitutive activation of signaling pathways PI3K/Akt/mTOR and NF- $\kappa$ B, but not ERK1/2. Using PP2, the nonreceptor kinase Src inhibitor, we demonstrated Src-dependent phosphorylation of kinase Akt. EGF stimulation of cells leads to increase in the phosphorylation of kinases ERK1/2, Akt, mTOR, Src, IKK, inhibitor I $\kappa$ B and p65 subunit of NF- $\kappa$ B transcription factor in Ruk/CIN85-overexpressing cells compared to control. Response of these cells to growth factor stimulation was developed earlier and lasted longer compared with wild type cells. It has been demonstrated that the reporter luciferase activity of NF- $\kappa$ B in wild type cells is significantly lower than in the subline with overexpression of Ruk/CIN85.

**Conclusions:** These data suggest that Ruk/CIN85 involved in the regulation of signaling pathways PI3K/Akt/mTOR and NF- $\kappa$ B in cells of human breast adenocarcinoma cell line MCF-7.

## 07.6

### Changes in level, subcellular localization and posttranslational modifications of CacyBP/SIP phosphatase upon oxidative stress

Agnieszka M. Topolska-Woś<sup>1,2</sup>, Walter J. Chazin<sup>2</sup>, Anna Filipek<sup>1</sup>

<sup>1</sup>Nencki Institute of Experimental Biology, Department of Molecular and Cellular Neurobiology, Warsaw, Poland; <sup>2</sup>Vanderbilt University, Center for Structural Biology, USA  
e-mail: Agnieszka M. Topolska-Woś <a.topolska-wos@nencki.gov.pl>

CacyBP/SIP binds several ligands such as S100 proteins, Siah-1, Skp1, actin, tubulin and tropomyosin (Schneider & Filipek, 2011). It has been suggested that, through the interaction with cytoskeletal proteins, CacyBP/SIP might play a role in various cellular processes, like cell differentiation or cytoskeleton organization. Recently, it has been shown that CacyBP/SIP exhibits phosphatase activity toward ERK1/2 and down-regulates the MAP kinase cascade (Kilanczyk *et al.*, 2009). In this work we investigated the influence of oxidative stress on the CacyBP/SIP phosphatase level, its subcellular localization and posttranslational modifications of in neuroblastoma NB2a cells.

By applying Western blot we have shown that the level of CacyBP/SIP increases in NB2a cells after H<sub>2</sub>O<sub>2</sub> treatment. Other methods, immunofluorescence staining and proximity ligation assay, show that in control NB2a cells CacyBP/SIP is present in the cytoplasm and that dimer is the major form of this protein. Moreover, we have found that upon H<sub>2</sub>O<sub>2</sub> treatment CacyBP/SIP translocates to the nucleus, where it exists mainly as a monomer. As it is shown by 2D electrophoresis, nuclear translocation of CacyBP/SIP is accompanied by changes in posttranslational modifications of this protein. Furthermore, after treatment of NB2a cells with H<sub>2</sub>O<sub>2</sub>, the spots representing highly acidic forms of CacyBP/SIP (pI 3-4) disappear in the cytoplasmic fraction, which makes the pattern of the forms similar to that seen in the nuclear fraction. Interestingly, changes in posttranslational modifications of CacyBP/SIP observed in NB2a cells after oxidative stress are similar to those found in brain of Alzheimer disease subjects (Wasik *et al.*, 2013). As it is known, this pathology is characterized, among others, by an increase in the amount of reactive oxygen species. In conclusion, our results showing changes in CacyBP/SIP level, its subcellular localization and posttranslational modifications after oxidative stress suggest that the protein follows to the nucleus its ligands/substrates, for example ERK1/2, and that reactive oxygen species are involved in this process.

#### Acknowledgements

This work was supported by the European Union through the European Regional Developmental Fund within the scope of the International Ph.D. Studies in Neurobiology (MPD4-502) and by statutory funds from the Nencki Institute of Experimental Biology.

## Posters

### P7.1

#### Comparison of protein disulfide reducing agents – properties of old and new reductants

Justyna Adamczyk<sup>1</sup>, Wojciech Bal<sup>2</sup>, Artur Krężel<sup>1</sup>

<sup>1</sup>Laboratory of Chemical Biology, Faculty of Biotechnology, University of Wrocław, Wrocław, Poland; <sup>2</sup>Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland  
e-mail: Artur Krężel <krezel@biotech.uni.wroc.pl>

Protection of thiol groups from oxidation is extremely important for the maintaining the activity and structure of many proteins. Approximately 20% of human proteins are predicted to contain disulfide bonds between cysteine residues. The most common reductant used in molecular biology and biochemistry are  $\beta$ -mercaptoethanol ( $\beta$ ME) and dithiotreitol (DTT), both possess unpleasant smell. These agents are very basic and their usage is limited to basic pH. Reduction of disulfides in neutral pH is rather slow and usually requires their high excess over the protein. There is still a need for new and more efficient reductants. In recent years TCEP (tris(2-carboxyethyl) phosphine) became very popular. TCEP is advantageous over DTT with respect to the pH range of application. Its pKa value is 7.5, while pKa value of  $\beta$ ME and DTT (thiol) is 9-10 [1]. TCEP compared to these two agents has also the advantages of being odorless, a more powerful reducing agent, more hydrophilic, and more resistant to oxidation in air [2]. In 2012 a new protein disulfide reducing agent appeared on the market – DTBA (dithiobutylamine). DTBA is a dithiol reducing agent that overcomes basicity limitation of DTT. The presence of the primary amine group in DTBA, as opposed to the two hydroxyl groups of DTT, leads to significantly lower pKa values of thiols. Another advantage of the primary amine group is that it allows for easy removal of DTBA from the system by ion-exchange methods [3]. Our study revealed that DTT and DTBA are a very efficient chelating agent for a range of biologically relevant metal ions, while TCEP remains almost non-coordinating reductant. Both reducing dithiols form complexes with transition metal ions (Zn(II), Co(II), Cd(II), Ni(II) and Cu(I)) with low solubility which significantly limit their usage in IMAC. We showed that application of DTT and DTBA to metalloproteins affect their function. Our main goal is to summarize the knowledge of reducing agents properties (both positives and drawbacks) regarding their usage in biochemistry and molecular biology.

#### References:

1. Krężel A *et al* (2001) *J Inorg Biochem* **84**: 77–88.
2. Krężel A *et al* (2003) *Inorg Chem* **42**: 1994–2003.
3. Lukesh JL *et al* (2012) *J Am Chem Soc* **134**: 4057–4059.

#### Acknowledgements

This work was supported by the Ministry of Science and Higher Education (IP2012 018272). Instrumentation were supported by the Polish Foundation for Science (F1/2010/P/2013).

## P7.2

### Modulation of enzymes involved in synthesis of estrogens by methoxy derivatives of resveratrol in non-tumorigenic MCF10A breast epithelial cells

Wanda Baer-Dubowska, Hanna Szaefer, Barbara Licznarska

Poznan University of Medical Sciences, Department of Pharmaceutical Biochemistry, Poznań, Poland

e-mail: Wanda Baer-Dubowska <baerw@ump.edu.pl>

Resveratrol and some of its natural derivatives are considered as promising chemopreventive and/or therapeutic agents interacting in pleiotropic way with carcinogenic process. Because resveratrol has several pharmacokinetic and bioavailability limitations searching for its new synthetic analogues with higher anticancer activity, especially in breast cancer, is reasonable. In postmenopausal women when gonads stop to produce estrogens, active hormones are produced locally. Interference in the local production and metabolism of estrogens, which are considered as major breast cancer risk factor, seems to be a good alternative for chemotherapy and/or chemoprotection of breast carcinoma.

In this study we assessed the modulation of enzymes involved in synthesis of estrogens: sulfatase (STS), sulfotransferase (SULT1E1) and 17 beta-hydroxysteroid dehydrogenase 1 and 2 (17-beta HSD 1 and 2) by resveratrol and its three methoxy derivatives: 3,4,2'-trimethoxy-*trans*-stilbene (B), 3,4,2',4'-tetramethoxy-*trans*-stilbene (D) and 3,4,2',4',6'-pentamethoxy-*trans*-stilbene (I) in breast non-tumorigenic epithelial cell line MCF10A. Cells were treated with the tested compounds for 72 hours and whole cell lysates were isolated. The level of enzymes was determined by Western blot analysis.

MCF10A cells showed low constitutive expression of 17-beta HSD 1, what is consistent with the literature data. Methoxy derivatives and parent compound, resveratrol were able to modulate the level of all enzymes involved in synthesis of estrogens, examined. The most significant changes were found in case of 17-beta HSD 2, which level was significantly increased as result of resveratrol or its methoxy derivatives treatment. 17-beta HSD 2 is the dominant form of this enzyme in normal epithelium of the breast, and allows the epithelial cells to balance the amount of estrone against estradiol. Thus 17-beta HSD 2 induction by resveratrol and its methoxy derivatives may be considered as important mechanism of breast cancer chemoprevention by these compounds.

## P7.3

### A dramatic conformational change is involved in allosteric regulation of muscle FBPase

Jakub Barciszewski<sup>1</sup>, Janusz Wiśniewski<sup>2</sup>, Dariusz Rakus<sup>2</sup>, Andrzej Dżugaj<sup>2</sup>, Mariusz Jaskólski<sup>1,3</sup>

<sup>1</sup>Institute of Bioorganic Chemistry PAS, Center for Biocrystallographic Research, Poznań, Poland; <sup>2</sup>Wrocław University, Department of Animal Molecular Physiology, Wrocław, Poland; <sup>3</sup>Adam Mickiewicz University, Faculty of Chemistry, Department of Crystallography, Poznań, Poland  
e-mail: Jakub Barciszewski <agent007@man.poznan.pl>

Until recently, it has been accepted that in vertebrate muscles the glycolysis process dominates. Lactate, which is the anaerobic product of glucose catabolism, is transferred to the liver where it is converted to glucose, which is finally returned to the muscles. Out of the four regulatory enzymes that control glucose synthesis in human liver, only the fructose-1,6-bisphosphatase isozyme (FBPase) is present in muscles. Due to the absence of glucose-6-phosphatase in muscles, the synthesis of glucose is not possible there, in contrast to glycogen synthesis. Interestingly, the activity of muscle FBPase differs from that of its liver counterpart, as it is 100 times more susceptible to AMP (adenosine monophosphate) inhibition. FBPase is composed of four identical polypeptide chains (Mr 34,000Da/chain) and exists as a dimer of dimers. The enzyme can assume at least two distinct conformations, known as the R (relaxed) and T (tense) states. Without effectors the enzyme exists in the R state. AMP can induce the transition from the active R-state to the inactive T-state. The allosteric site has been identified at the center of the tetrameric molecule where the four subunits converge. Recently, we have shown that FBPase is active in muscles despite the presence of AMP. These findings offer a possibility to better understand the mechanisms of activation and inhibition of this intriguing enzyme. In this work, we have crystallized human muscle FBPase with and without AMP. X-Ray diffraction data were collected and the two structures, of the R and T state, have been solved. Their analysis reveals a dramatic change of the conformation of the N-termini of all subunits upon AMP binding. Furthermore, these changes induce a rotation of 64° of the dimers with respect to each other.

## P7.4

### Identification of palmitoylated proteins involved in TLR4 signaling

Justyna Dembińska, Paula Roszczenko, Kinga Borzęcka, Katarzyna Kwiatkowska

Nencki Institute of Experimental Biology, Warsaw, Poland  
e-mail: Katarzyna Kwiatkowska <k.kwiatkowska@nencki.gov.pl>

Palmitoylation is a post-translation modification of proteins via a covalently bound palmitic acid (C:16) which is catalyzed by palmitoyl acyltransferase. In eukaryotic cells, the acyl chain is transferred onto thiol group of cysteine residues (*S*-palmitoylation) or, less frequently, onto hydroxyl group of serine and threonine residues (*O*-palmitoylation). This reversible modification controls translocation of proteins to plasma membrane microdomains enriched in cholesterol and sphingolipids (rafts), their stability, cellular trafficking of proteins and protein-protein interactions. We found that protein palmitoylation is crucial for pro-inflammatory signaling triggered by Toll-like receptor 4 (TLR4) in cells stimulated with lipopolysaccharide (LPS). An exposure of RAW264 macrophage-like cells to 50–250  $\mu$ M of 2-bromopalmitic acid (BPA), an inhibitor of palmitoyl acyltransferase, reduced in a dose dependent manner the production of pro-inflammatory cytokines, TNF- $\alpha$  and RANTES, stimulated with 100 ng/ml LPS. Therefore, we aimed to identify proteins which are palmitoylated in LPS-stimulated RAW264 cells. Traditional methods to study protein *S*-palmitoylation rely on isotopic labeling of cells, which requires special safety precautions and is time-consuming. We have applied a recently developed non-radioactive technique based on a “click chemistry” in two parallel approaches. In the first one, living RAW264 cells were labeled with  $\omega$ -alkynyl-palmitate analog of palmitic acid (17-ODYA), and after lysis, subjected to a “click-it” reaction with azido-azo-biotin catalyzed by  $\text{Ca}^{2+}$ . Subsequently, labeled proteins were bound to streptavidin beads and eluted with sodium dithionite which cleaved the azido-azo-biotin, and analyzed by immunoblotting. With this technique it was found that LPS induced palmitoylation of several proteins, including Lyn tyrosine kinase of the Src family. Palmitoylation of these proteins was inhibited in the presence of BPA. The second approach of detection of palmitoylated protein in LPS-stimulated cells was based on the fact that the activation of TLR4 by LPS occurs via CD14 located in plasma membrane rafts. Therefore, we decided to perform immunoprecipitation of CD14, preceded by labelling of RAW264 cells with 17-ODYA and followed by “click chemistry” in order to detect palmitoylated proteins which associate with CD14 in LPS-treated cells. Identification of these proteins will involve the mass spectrometry methodology.

## P7.5

### Structural and functional properties of proteins involved in miRNA-mediated gene silencing revealed by hydrogen/deuterium exchange mass spectrometry

Maja K. Cieplak<sup>1</sup>, Krzysztof Tarnowski<sup>2</sup>, Michal Dadlez<sup>2</sup>, Marc R. Fabian<sup>3</sup>, Nahum Sonenberg<sup>4</sup>, Edward Darzynkiewicz<sup>1</sup>, Anna Niedzwiecka<sup>1,5</sup>

<sup>1</sup>Division of Biophysics, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, Warsaw, Poland; <sup>2</sup>Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland; <sup>3</sup>Department of Oncology, Lady Davis Institute for Medical Research, McGill University, Canada; <sup>4</sup>Department of Biochemistry and McGill Cancer Centre, McGill University, Canada; <sup>5</sup>Laboratory of Biological Physics, Institute of Physics, Polish Academy of Sciences, Warsaw, Poland  
e-mail: Anna Niedzwiecka <annan@ifpan.edu.pl>

miRNAs specifically regulate the expression of genes by a network of protein-protein interactions. As they work on the post-transcriptional level, they provide a much faster way to react to changes in the cellular environment and can propagate the received signal by affecting multiple mRNAs containing the same miRNA-binding site.

miRNAs interact with Argonaute proteins, which in turn bind GW182 proteins (Glycine-Tryptophan proteins of 182 kDa). Artificial tethering of GW182 proteins to target mRNAs has shown that GW182 proteins act downstream of Argonaute proteins and are able to silence mRNAs in their absence. GW182 proteins act by recruiting the CCR4-NOT deadenylase complex *via* interactions with the scaffolding CNOT1 subunit [1, 2] of the complex.

The GW182 proteins are predicted to be mostly disordered, excluding the Ubiquitin Binding Domain and the RNA Recognition Motif (RRM). The NMR structure of the RRM of the *Drosophila melanogaster* GW182 protein was published by Eulalio *et al.* in 2009 [3].

Here, we study the structure and CNOT1 binding properties of the silencing domain of TNRC6C, one of the human paralogs of GW182, using hydrogen/deuterium exchange (HDX) coupled with ion mobility mass spectrometry (IMS). HDX-IMS is a powerful method to study solvent accessibility of particular protein regions, which in turn depends on the structural properties of a protein.

#### References:

1. Fabian MR *et al.* (2011) *Nat Struct Mol Biol* **18**: 1211–1217.
2. Braun JE, Huntzinger E, Fauser M, Izaurralde E (2011) *Mol Cell* **44**: 120–133.
3. Eulalio, A. *et al.* (2009) *Nucleic Acids Res* **37**: 2974–2983.

#### Acknowledgements

This work was supported by the European Regional Development Fund through the Foundation for Polish Science International PhD Projects Programme and the Innovative Economy Operational Programme POIG.02.02.00-00-025/09 NanoFun.

## P7.6

### GPER/GPR30 estrogen receptor dysregulation in neoplastic transformation

Adam I. Cygankiewicz<sup>1</sup>, Damian Jacenik<sup>1</sup>, Andrzej Semczuk<sup>2</sup>, Tomasz Rechberger<sup>2</sup>, Radziszław Trzciński<sup>3</sup>, Adam Dziki<sup>3</sup>, Wanda M. Krajewska<sup>1</sup>

<sup>1</sup>Department of Cytochemistry, Faculty of Biology and Environmental Protection, University of Lodz, Łódź, Poland; <sup>2</sup>IInd Chair and Department of Gynecology, Medical University of Lublin, Lublin, Poland; <sup>3</sup>Department of General and Colorectal Surgery, Medical University of Lodz, Łódź, Poland  
e-mail: Adam.Cygankiewicz<adam.c@biol.uni.lodz.pl>

Estrogens are sex hormones, which play central role in modulation of both physiological as well as pathological processes through the human body in both genders. The molecular mechanism of estrogen action on cell has been slowly deciphered in previous decades. However, classical signaling pathway does not explain rapid changes in the metabolism of the cells after exposure to estrogens. In 2005, it was demonstrated that estrogens are natural ligands not only for the canonical receptors ER $\alpha$  and ER $\beta$ , but also for GPER/GPR30 receptor belonging to the family of G protein coupled receptors (GPCRs).

GPER/GPR30 was found to transactivate EGFR receptor and as a result, ERK 1/2 and JNK of the MAP kinase pathway. Two genes i.e., *c-Fos* and *CTGF* were identified as being regulated by GPER/GPR30. Initial reports have described GPER/GPR30 role in neoplastic transformation, blood pressure, bone metabolism and glucose homeostasis. The importance of GPER/GPR30 has been established after publications described that tamoxifen and other classical ER inhibitors are agonists for GPR30. Additionally, estrogen-like compounds like phyto- and xenoestrogens appeared to be the activators of GPER/GPR30, which make this receptor a key factor in cellular metabolism modulation by environmental factors.

The aim of the work was to assess the expression of GPER/GPR30 as compared to classical estrogen ER $\alpha$  and ER $\beta$  as well as EGFR receptors in endometrial and colorectal cancers of different degree of neoplasia and to find out if methylation of promoter regions has impact on the GPER/GPR30 expression.

In endometrial cancer as compared to normal endometrium up-regulation of not only canonical estrogen receptors ER $\alpha$  and ER $\beta$ , but also GPER/GPR30 receptor was shown. It was also noticed that GPER/GPR30 expression increase is associated with tumor grade. Interestingly, similar results, although to lesser extent were noticed in the case of colorectal cancer.

Lower level of methylation of two CpG islands in the promoter region of the GPER/GPR30 receptor gene in endometrial cancer comparing to normal material was found. In the case of colorectal cancer, only one of two identified CPG islands was found to be hypomethylated. It suggests that one of the mechanisms responsible for the up-regulation of GPR30 receptor expression during neoplastic transformation may be the decrease of methylation of CpG islands in its promoter region.

## P7.7

### Identification of palmitoylated proteins involved in TLR4 signaling

Justyna Dembińska, Paula Roszczenko, Kinga Borzęcka, Katarzyna Kwiatkowska

Nencki Institute of Experimental Biology, Warsaw, Poland  
e-mail: Katarzyna.Kwiatkowska<k.kwiatkowska@nencki.gov.pl>

Palmitoylation is a post-translation modification of proteins via a covalently bound palmitic acid (C:16) which is catalyzed by palmitoyl acyltransferase. In eukaryotic cells, the acyl chain is transferred onto thiol group of cysteine residues (*S*-palmitoylation) or, less frequently, onto hydroxyl group of serine and threonine residues (*O*-palmitoylation). This reversible modification controls translocation of proteins to plasma membrane microdomains enriched in cholesterol and sphingolipids (rafts), their stability, cellular trafficking of proteins and protein-protein interactions. We found that protein palmitoylation is crucial for pro-inflammatory signaling triggered by Toll-like receptor 4 (TLR4) in cells stimulated with lipopolysaccharide (LPS). An exposure of RAW264 macrophage-like cells to 50-250  $\mu$ M of 2-bromopalmitic acid (BPA), an inhibitor of palmitoyl acyltransferase, reduced in a dose dependent manner the production of pro-inflammatory cytokines, TNF- $\alpha$  and RANTES, stimulated with 100 ng/ml LPS. Therefore, we aimed to identify proteins which are palmitoylated in LPS-stimulated RAW264 cells. Traditional methods to study protein *S*-palmitoylation rely on isotopic labeling of cells, which requires special safety precautions and is time-consuming. We have applied a recently developed non-radioactive technique based on a "click chemistry" in two parallel approaches. In the first one, living RAW264 cells were labeled with  $\omega$ -alkynyl-palmitate analog of palmitic acid (17-ODYA), and after lysis, subjected to a "click-it" reaction with azido-azo-biotin catalyzed by Ca<sup>2+</sup>. Subsequently, labeled proteins were bound to streptavidin beads and eluted with sodium dithionite which cleaved the azido-azo-biotin, and analyzed by immunoblotting. With this technique it was found that LPS induced palmitoylation of several proteins, including Lyn tyrosine kinase of the Src family. Palmitoylation of these proteins was inhibited in the presence of BPA. The second approach of detection of palmitoylated protein in LPS-stimulated cells was based on the fact that the activation of TLR4 by LPS occurs via CD14 located in plasma membrane rafts. Therefore, we decided to perform immunoprecipitation of CD14, preceded by labelling of RAW264 cells with 17-ODYA and followed by "click chemistry" in order to detect palmitoylated proteins which associate with CD14 in LPS-treated cells. Identification of these proteins will involve the mass spectrometry methodology.



## P7.8

### *Hansenula polymorpha* glucose sensor Gcr1 functions as a putative pH-dependent glucose H<sup>+</sup>-symporter

Iryna O. Denega<sup>1,2</sup>, Olena G. Stasyk<sup>1,2</sup>,  
Natalia O. Sybirna<sup>1</sup>, Oleh V. Stasyk<sup>2</sup>

<sup>1</sup>Ivan Franko National University of Lviv, Department of Biochemistry, Ukraine; <sup>2</sup>Institute of Cell Biology NAS of Ukraine, Department of Cell Signaling, Ukraine  
e-mail: Iryna Denega <deneha.ira@gmail.com>

Several proteins involved in glucose sensing, signaling and transport have been described for methylotrophic yeast *Hansenula polymorpha*. One of them is Gcr1, the close homologue of MstA, a functional high-affinity H<sup>+</sup>-dependent monosaccharide symporter from fungus *Aspergillus niger*. Gcr1 is a peculiar C-tale-less glucose sensor, required for glucose repression and regulation of hexose transport in *H. polymorpha*.

It was previously demonstrated with bakers' yeast *S.cerevisiae* *SrSnf3* and *SrRgt2* glucose non-transporting sensors that substitution of one of the conserved arginine residues by a lysine residue in the hexose sensor's core sequence converts them into constitutively signalling form. We demonstrated that expression of the *GCR1* allele encoding protein with the corresponding R165K substitution in *H. polymorpha ger1Δ* mutant cells did not complement their growth defect on glucose, but additionally impaired growth on fructose-, sucrose-, xylose-, ethanol- and methanol-containing media relative to *ger1Δ* recipient strain. Remarkably, production of Gcr1<sup>R165K</sup> protein also did not restore the normal catabolite repression of AOX synthesis in cells grown on glucose. We analyzed the growth of the strains with overexpression of *GCR1* and *HXT1* on different carbon substrates under presence of glucose antimetabolite 2-deoxy-D-glucose (2-DOG) at different pH values. Strain NCYC 495/*GAP-GCR1* with constitutive expression of *GCR1* was more sensitive to the exogenous 2-DOG relative to the control strains. Such sensitivity was pH dependent, since we observed the difference in growth of the strains at pH 3.8, while at pH 4.0 all strains were equally sensitive to 2-DOG in glycerol-containing medium. Sensitivity of all investigated strains to 2-DOG on pH 4.0 could depend on expression of other genes encoding high-affinity glucose transporters. Therefore, glucose sensor Gcr1 can most probably perform the H<sup>+</sup>-symport function in its native host, since 2-DOG sensitivity was dependent on pH (H<sup>+</sup> concentration). To our knowledge, if confirmed, this is a first example of functional glucose-transporting hexose sensor in yeasts.

## P7.9

### Catabolite degradation of fructose-1,6-bisphosphatase in methylotrophic yeasts

Olena Dmytruk<sup>1</sup>, Nina Bulbotka<sup>1</sup>, Andriy Sibirny<sup>1,2</sup>

<sup>1</sup>Institute of Cell Biology, National Academy of Science of Ukraine, Lviv, Ukraine; <sup>2</sup>Department of Biotechnology and Microbiology, University of Rzeszow, Rzeszow, Poland  
e-mail: Olena Dmytruk <verbaolena@gmail.com>

Fructose-1,6-bisphosphatase (FBP) is a key gluconeogenic enzyme, it catalyses the conversion of fructose-1,6-bisphosphate to fructose-6-phosphate, which later metabolizes to glucose-6-phosphate. FBP is synthesized when cells are grown on non-fermentable carbon sources like ethanol. When yeast cells are subsequently shifted to a glucose-containing medium, FBP is rapidly inactivated and degraded. This process is called catabolite degradation.

In the methylotrophic yeasts, such as *Pichia pastoris* and *Hansenula polymorpha*, FBP also participates in methanol utilization pathway. Therefore the investigation of catabolite degradation of FBP in methylotrophic yeasts has a great scientific interest.

The wild type strains of *P. pastoris* and *H. polymorpha* were analyzed by Western blot analysis. It was shown that FBP is mostly degraded after 5 hours of incubation of cells pre-grown in gluconeogenic substrates in glucose-containing medium. After shifting the cells from methanol or ethanol-containing media on glucose medium, FBP activities of *P. pastoris* and *H. polymorpha* wild-type strains decreased 4.5 and 2.5 times, respectively. FBP degradation of the *P. pastoris* mutants defective in autophagy (*Δmon1*, *Δypt7*, *Δccz1*) was also investigated and compared with the wild type strain.

The recombinant strains of *P. pastoris* with the deletion of *FBP1* gene were constructed and analyzed. *Δfbp* strains did not grow in the medium with non-fermentable carbon sources, such as methanol and ethanol, and revealed impaired growth in glycerol-containing medium. To study the mechanisms of catabolite degradation, recombinant strains with the expression of *FBP1* fused with *GFP* and *FBP1* fused with *Myc* were constructed and analyzed. It was shown that two different ways of FBP degradation can occur in *P. pastoris*, but ubiquitin-proteasome dependent degradation pathway is more preferred.

## P7.10

### Changes in mRNA and protein expression levels of key components of Renin-Angiotensin System and Relaxin Family Peptide System under the influence of Angiotensin II and Relaxin 2 in prostate cancer cells

Kamila Domińska, Agnieszka W. Piastowska-Ciesielska, Karolina Kowalska, Zuzanna E. Matysiak, Tomasz Ochędalski

Department of Comparative Endocrinology, Medical University of Lodz, Łódź, Poland  
e-mail: Kamila Domińska <kamila.dominska@umed.lodz.pl>

Several groups, including ours, have demonstrated that Angiotensin II and Relaxin 2 play a important role in prostate carcinogenesis. It has been shown that these peptides can modulate cell proliferation and apoptosis, invasion and spread of many types of cells. High expression of Relaxin 2 and relaxin receptor RXFP1 as well as Angiotensin II and angiotensin receptor type 1 (AT1) was observed in tumor tissue compared to normal prostate tissue. Studies suggest that receptors expression: RXFP1, AT1 and also these peptide hormones levels in blood can be new early markers of metastatic potential in primary tumors. It is worth noting that interactions between renin-angiotensin system (RAS) and therelaxin family peptide systems (RFPS) have been reported in the organ fibrosis, the cardiovascular system associated with blood-pressure and central nervous system associated with osmoregulation but so far, never in the carcinogenesis of reproductive tissues. In the present study, we investigated the effects of Angiotensin II on some elements of Relaxin Family Peptide System, such as RXFP1 and RXFP2. Furthermore we examined the influence of Relaxin 2 on key components of Renin-Angiotensin System for instance AT1, AT2, ACE. Changes in mRNA and protein expression levels were measured by Real-time RT-PCR and Western blot, respectively. The fluorescent immunocytochemical localization of angiotensin and relaxin receptors in prostate cancer cells was achieved using Alexa Fluor. The changes in local RA and RFP systems were examined in two prostate cancer cell lines (LNCaP and PC3) which are characterized by different invasive potential, as well as sensitivity to androgen. It seems that Rel-2 may influence on function and efficiency of renin-angiotensin system, while Ang II may have an impact on relaxin family peptide systems in prostate cancer cells. The results suggest that deregulation of the locally secreted peptide hormones, such as Rel-2 or Ang II can increase the risk of prostate cancer development and progression.

#### Acknowledgements

This work was financially supported by the National Science Center, research grant: NN403 2081 39.

## P7.11

### Diversity of human metallothionein isoforms — new light on protein stability and cellular functions

Agnieszka Drozd, Artur Krężel

University of Wrocław, Laboratory of Chemical Biology, Wrocław, Poland  
e-mail: Artur Krężel <krezel@biotech.uni.wroc.pl>

Human metallothioneins are encoded by 17 genes located on chromosome 16q13. They consist of 61–68 amino acid residues, with 25–30% cysteine content. MTs exist in four isoforms: MT1, MT2, MT3 and MT4, whereof the MT1 is present in seven sub-isoforms: MT1A, B, E, F, G, H, X. Differences between isoforms arise mainly from changes in the amino acid sequence, despite the sequence homology reaches ca. 90%. MT1 and MT2 are present in every tissue type but MT3 and MT4 are found only in nervous system and squamous epithelium, respectively. Metallothioneins are involved in numerous cellular processes, they are important regulators of metal ion homeostasis and protectors against oxidative damage [1]. Their altered mRNA expression has been correlated with variety of cancers [2]. The understanding of metallothionein functions is hampered due to its high diversity.

Our previous studies on MT2A showed that metallothionein differs in Zn(II) affinity ( $10^{-8}$ – $10^{-12}$  M) and demonstrated high coordination dynamic of metal clusters [3]. So far, not all metallothionein isoforms were characterized in terms of metal ion binding and their thermodynamic stability. Here, we present the procedure of expression and purification of ten metallothionein isoforms MT1-MT4 with special attention to their apo- and holo-forms preparation. All purified apo-isoforms were characterized in terms of Zn(II) and Cd(II) binding using UV-Vis and CD spectroscopy. Zn(II)-saturated proteins were analyzed in detail in terms of the number of metal ions bound per protein molecule using UV-Vis spectroscopy, fluorimetry and ICP-MS. We found that all metallothionein isoforms bind seven Zn(II) ions per molecule ( $Zn_7T$ ) in a tetrathiolate coordination environment. Our studies uncovered that all metallothionein isoforms demonstrate similar Zn(II) affinities compared to MT2A, varying from nanomolar to low picomolar range. This unusual properties of metallothioneins are responsible for controlling of free Zn(II) ions under cellular conditions.

#### References:

1. Krężel A *et al* (2007) *Arch Biochem Biophys* **463**: 188–200.
2. Mehus AA *et al* (2014) *Mol Cell Proteomics* **4**: 1020–1033.
3. Krężel A, Maret W (2007) *JACS* **129**: 10911–10921.

#### Acknowledgements

This work was supported by the Ministry of Science and Higher Education (IP2012 018272). Instrumentation used in this study was founded by the Polish Foundation for Science (F1/2010/P/2013).

## P7.12

### Ribosomal L12 protein facilitates maturation of ribosomal 60S subunit in yeast cells

Leszek Wawiórka<sup>1</sup>, Barbara Michalec-Wawiórka<sup>1</sup>, Eliza Molestak<sup>1</sup>, Kamil Deryło<sup>1</sup>, Monika Szajwaj<sup>1</sup>, Monika Dźwierzyńska<sup>1</sup>, Przemysław Grela<sup>1</sup>, Vladislava Liudkowska<sup>2</sup>, Joanna Kufel<sup>2</sup>, Marek Tchorzewski<sup>1</sup>

<sup>1</sup>Department of Molecular Biology, Maria Curie-Skłodowska University, Lublin, Poland; <sup>2</sup>Institute of Genetics and Biotechnology, Faculty of Biology, Warsaw University, Warsaw, Poland  
e-mail: Marek Tchorzewski <maro@hektor.umcs.lublin.pl>

Eukaryotic Ribosomal T-site, especially GTPase Associated Centre (GAC), is one of the most intriguing part of translational apparatus. This region is responsible for binding and stimulation of the translational factors (TFs) at every step of the protein synthesis cycle. Molecular activity of GAC is based on the orchestrated interplay between rRNA fragments (highly conserved sarcin-ricin loop) and several protein components including heteropentameric P-stalk structure consisting of three types of P-proteins uL10/P1/P2 and single uL11 protein. The GAC involvement in translational cycle has been extensively studied, however, its direct involvement in other cellular processes extending beyond its canonical functions was also postulated. As it was reported P-proteins, especially uL10, exhibit additional, so called extraribosomal functions in respect to various metabolic pathways. In this communication we show that uL11 activity also goes beyond translation *per se*. We show that uL11 protein is an active element contributing to the ribosomal biogenesis process in yeast cells. Mutant yeast cells, totally depleted of both genomic copies of genes for uL11 protein, exhibit extremely slow growth phenotype, accompanied with the severe defect in ribosomal formation and pre-rRNA processing. The GAC was shown to be a docking platform for the translational GTPases (e.g. eEF-2), so it is tempting to assume that this ribosomal region may also interact with the biogenesis factor Efl1, which shares high degree of homology with translation factor eEF2. Like other translational GTPases, Efl1's activity is strictly dependent on ribosome. The most prominent molecular function of Efl1 refers to Tif6 antiassociation factor release at the very late cytoplasmatic step of pre-60S maturation. Consequently, lack of Efl1 causes accumulation of Tif6 protein on the pre-60S particles, observed as its re-localization from the nucleus into the cytoplasm. The similar phenotype was observed in the uL11 depleted yeast cells which strongly suggest severe perturbations in the cross-talk between ribosome and Efl1. Noteworthy, our data suggest that the function of uL11 seems not to be rigorously limited to the Efl1 stimulation. Instead, uL11 may exert more broad effect, affecting early steps of ribosome biogenesis. Our results extend current understanding of the molecular basis of the eukaryotic ribosome maturation process, bringing new insight into molecular functions of the uL11 protein.

## P7.13

### The role of the LD loop in activation process of the HtrA (DegP) protease from *Escherichia coli*

Donata Figaj<sup>1</sup>, Artur Giełdoń<sup>2</sup>, Marlena Bartczak<sup>1</sup>, Jolanta Jurek<sup>1</sup>, Barbara Lipińska<sup>1</sup>, Joanna Skórko-Glonek<sup>1</sup>

<sup>1</sup>Department of Biochemistry, University of Gdańsk, Gdańsk, Poland; <sup>2</sup>Faculty of Chemistry, University of Gdańsk, Gdańsk, Poland  
e-mail: Donata Figaj <donata.figaj@biol.ug.edu.pl>

Exposition of bacteria to stress agents frequently leads to intracellular protein misfolding and aggregation. Aggregated proteins cannot fulfil their biological function and seriously endanger cell's safety. The occurrence of the improperly folded and aggregated proteins leads to excessive expression of genes involved in cell protection against destructive effect of stressful agents. One of them is *htrA* gene, whose product, the HtrA protein, plays a crucial role in the extracytoplasmic protein quality control.

HtrA (DegP) protein from *Escherichia coli* is a conserved in evolution serine protease, localized in the periplasm. Synthesis of HtrA is increased under heat, oxidative or reducing stress. HtrA plays a dual role in protection of a bacterial cell- it prevents protein aggregation and degrades incorrectly folded proteins, due to its chaperone and proteolytic activity, respectively. Proteolytic activity is regulated in thermal and/or allosteric manner. Under physiological conditions HtrA protein exists as a proteolytically inactive hexamer. During cell exposure to stress agents, when incorrectly folded proteins occur in the periplasmic space, PDZ-1 domain of HtrA recognizes and binds the specific amino acid sequence of a substrate. Binding of a peptide to the PDZ1 domain leads to conformational changes within this domain, which are sensed by the L3 loop. Allosteric signal is passed on the LD loop and next on the L2/L1 loops. As a result the protease active center achieves proper conformation. At the same time hexamer dissociates into trimers, which can form a proteolytically active higher order oligomers. The active HtrA ensures proper bacterial cell protection against stress conditions. Moreover, in the pathogenic *E. coli* strains (UPEC, EPEC) HtrA is implicated in virulence and the *htrA* mutant strains are non-pathogenic.

The aim of our work was to examine the role of the LD loop in the activation process of HtrA. According to the crystal structure (pdb 3ou0) we selected amino residues which seem to play an important role in maintenance of the HtrA proteolytic activity. For this purpose we introduced a set of point mutations in the region coding for the LD loop to obtain the following amino acid substitutions: P170G, F171A, L173A, E175A, E175L, G174S. Almost all of the introduced mutations disturbed the activity in comparison to wild type HtrA. Obtained data confirm the importance of LD loop in the HtrA protease activation. In particular, an LD shape (role of P170 residue) and interactions with the L2 loop (role of F171, L173 residues), or the L3 loop (E175 residue) play crucial roles in the maintenance of the proteolytic activity.

## P7.14

### The lack of Nrf2 transcription factor and heme oxygenase-1 in murine bone marrow macrophages affects osteoclasts differentiation

Urszula Florczyk, Monika Viscardi, Witold Nowak, Alicja Józkwicz, Józef Dulak

Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland  
e-mail: Urszula.Florczyk<urszula.florczyk@uj.edu.pl>

Increased number and activity of osteoclasts (OCLs) are associated with intense bone demineralization in osteoporosis, many types of tumors or inflammatory disorders such as arthritis. Nrf2 transcription factor and heme oxygenase-1 (HO-1), its downstream target, have been suggested to play a role in osteoclastogenesis. Nevertheless, the studies investigating the effect of genetic manipulation of those mediators are lacking. Therefore, we aimed to investigate the effect of Nrf2 and HO-1 deficiency in murine bone marrow macrophages (BMMs) on OCL differentiation.

Bone marrow cells were isolated from tibial and femoral bones of Nrf2- or HO-1-deficient mice (Nrf2<sup>-/-</sup> or HO-1<sup>-/-</sup>, respectively) and appropriate wild type mice and stimulated for 3 days with macrophage colony-stimulating factor (M-CSF) to obtain BMMs. Analysis of surface markers expression (F4/80, Mac1) confirmed macrophage phenotype of cells irrespective of the genotype. For OCL differentiation BMMs were cultured further for 3 days in the presence of M-CSF and receptor activator of nuclear factor κB ligand (RANKL). Mature OCLs were identified at day 6 by tartrate-resistant acid phosphatase (TRAP) histochemical detection.

We showed that Nrf2 deficiency resulted in higher number of OCLs. Reversely, after Nrf2 activation by sulforaphane in RANKL-stimulated Nrf2<sup>+/+</sup> BMMs no TRAP-positive cells corresponding to OCLs were observed, confirming inhibitory effect of Nrf2. In addition, osteoclasts-specific genes, such as Nfat-c1, Calcr, Ctsk, Itgb3, tended to be upregulated in RANKL-stimulated Nrf2<sup>-/-</sup> BMMs in comparison to Nrf2<sup>+/+</sup> BMMs. In contrast, less TRAP+ cells were detected when HO-1 was absent. Accordingly, other osteoclasts-specific genes were all downregulated in RANKL-stimulated HO-1<sup>-/-</sup> BMMs (*vs.* HO-1<sup>+/+</sup> BMMs). In summary, Nrf2 exerts inhibitory effect on osteoclastogenesis, while in our experimental setting HO-1 has opposite effect. It cannot be excluded that in case of the latter the differences may come from different properties of BMMs HO-1<sup>+/+</sup> and HO-1<sup>-/-</sup>, however, such hypothesis requires further investigation.

#### Acknowledgements

Supported by Iuventus Plus grant from the Ministry of Science and Higher Education (0244/IP1/2013/72).

## P7.15

### Role of Amyloid Precursor protein and its processing in the regulation of cellular Store Operated Calcium Entry

Kinga Gazda<sup>1</sup>, Jacek Kuźnicki<sup>1,2</sup>, Tomasz Węgiński<sup>1</sup>

<sup>1</sup>International Institute of Molecular and Cell Biology, Laboratory of Neurodegeneration, Warsaw, Poland; <sup>2</sup>Nencki Institute of Experimental Biology, Warsaw, Poland

e-mail: Kinga.Gazda<kgazda@iimcb.gov.pl>

Alzheimer's disease (AD) is the most common form of dementia. It is characterized by the extraneuronal deposition of amyloid-β-peptide (Aβ) plaques, products of amyloid precursor protein (APP) proteolysis by β-secretase and γ-secretase. Although the β-amyloid-induced toxicity remains the prevailing hypothesis of AD pathogenesis, the research based on it has neither clarified the disease etiology nor brought the long-awaited cure. Altered calcium homeostasis has been recently proposed as one of the early events responsible for disease development, but its exact effect is poorly understood. Endoplasmic reticulum (ER) is the major store of intracellular Ca<sup>2+</sup>. The regulated release of calcium from the ER causes activation of ER-localized calcium sensors STIM1/STIM2 and the subsequent influx of calcium through Orai1 plasma membrane channel. This Store Operated Calcium Entry (SOCE) is crucial for ER-refilling and cell signaling. It has been well demonstrated that familial AD mutations in presenilin-1 (PS1), the enzymatic component of γ-secretase complex, and APP affect intracellular calcium concentration and dynamics, including SOCE. Several mechanisms have been proposed to explain the observed effects of PS1 mutations. However, the role of APP and its FAD-causing variants in calcium homeostasis is controversial. At present, there is no clear and compact explanation how APP influences cellular calcium.

The purpose of this study is to analyze how disturbances of APP processing pathway affect SOCE response. We characterized a human cell line expressing high endogenous levels of APP, α- and γ-secretase components as well as proteins constituting SOCE machinery. We have stably silenced the expression of APP, PS1 and ADAM genes by viral gene transfer of specific shRNA cassettes. We aim to perform quantitative co-localization analysis to characterize dynamic interaction between endogenous Orai1 and STIM1/STIM2 proteins, and to measure the SOCE intensity in cells with impaired APP processing pathway. The results will give insight into mechanisms by which development of AD is connected to cellular calcium disturbances and may help to elucidate the effective disease treatment.

## P7.16

### Searching for the function of human Sgt1A and Sgt1B isoforms

Agnieszka Góral<sup>1</sup>, Małgorzata Sierant<sup>2</sup>,  
Barbara Nawrot<sup>2</sup>, Anna Filipek<sup>1</sup>

<sup>1</sup>Nencki Institute of Experimental Biology PAS, Warsaw, Poland; <sup>2</sup>Centre of Molecular and Macromolecular Studies PAS, Łódź, Poland  
e-mail: Agnieszka Góral <a.goral@nencki.gov.pl>

The Sgt1 protein, originally identified in yeast as a suppressor of G2 allele of Skp1, conservatively functions throughout eukaryotes as a Hsp90 co-chaperone in the kinetochore assembly and proper chromosome alignment (Kitagawa *et al.*, 1999; Davies & Kaplan, 2010). Moreover, in human cells, Sgt1 is required for activation of some NLR proteins during cell response to pathogen attack (da Silva Correia *et al.*, 2007; Mayor *et al.*, 2007). In humans, contrary to the majority of organisms, two isoforms, Sgt1A (37.8 kDa) and Sgt1B (41.0 kDa), are expressed (Niikura and Kitagawa, 2003; Zou *et al.*, 2004). The Sgt1B isoform contains a 33-amino acid insertion (Ile<sup>110</sup>-Gly<sup>142</sup>) instead of the Ser<sup>110</sup> residue characteristic for Sgt1A sequence. It has been shown that RNAi-mediated silencing of both Sgt1 isoforms leads to a mitotic delay and eventually cell death (Steensgaard *et al.*, 2004).

The aim of this work is to establish the role of particular Sgt1 isoforms in human cells, and we mainly focus on analysis of cell proliferation and on identification of protein ligands specific for Sgt1A and Sgt1B. In order to find out whether both isoforms are essential for cell cycle progression, we decided to silence separately each Sgt1 isoform. For that, siRNA molecules were designed and synthesized and then used to transfect HEp-2 (Human Epidermal Carcinoma) cells. At different time points after transfection, the protein level of individual Sgt1 isoforms was analyzed by Western blot. The most effective siRNA sequences were selected and cloned into pPURhU6 vectors in order to establish stably transfected cell lines with diminished level of a given Sgt1 isoform. In turn, to identify protein targets specific for Sgt1A and Sgt1B, HEp-2 cells were transfected with plasmid encoding Sgt1A-3xFLAG or Sgt1B-3xFLAG. Then, co-immunoprecipitation on anti-FLAG agarose was performed and proteins bound to this resin were analyzed by mass spectrometry. The analysis of the cell cycle of HEp-2 cells with diminished level of a given Sgt1 isoform as well as the analysis of the results from mass spectrometry is in progress and will be presented.

#### 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.

## P7.17

### Special features of B-lymphoblastoid cell lines from patients with X-linked lymphoproliferative disease type 1

Inna Gordiienko, Larysa Shlapatska, Larysa Kovalevska, Svetlana Sidorenko

R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology NAS of Ukraine, Laboratory of Signal Transduction Pathways, Ukraine  
e-mail: Inna Gordiienko <hordiienko24@mail.ru>

X-linked lymphoproliferative disease type 1 (XLP1) belongs to genetically determined primary immunodeficiency syndromes and is characterized by mutations in *SH2D1A/DSSH/SAP* gene. *SH2D1A* gene encodes a small 128 aa adaptor protein SH2 domain protein 1A (SH2D1A), also called Duncan's disease SH2-protein (DSHP), or SLAM/CD150-associated protein (SAP). SAP is involved in regulation of development and functions of T lymphocytes, NKT and NK cells. A number of evidence support the idea that SAP is also involved in B cell differentiation and function, so the absence of functional SAP in XLP1 may affect the B cells as well, and contribute to the dramatic increase of B-cell lymphoma development in XLP1 patients. The aim of our study was to analyze receptor-mediated Akt and ERK1/2 activation, and expression of several transcription factors in EBV-transformed B-lymphoblastoid cell lines from XLP1 patients (XLP B-LCLs) in comparison with conventional B-lymphoblastoid cell lines (B-LCLs). Studies were performed on EBV-transformed XLP B-LCLs IARC 739, SC-XLP and RP-XLP; SAP<sup>-</sup> B-LCLs T5-1 and SAP<sup>+</sup> B-LCLs MP-1. Western blot analysis was used for evaluation of Akt and ERK1/2 phosphorylation in response to ligation of CD150, CD40, and IgM cell surface receptors. The expression levels of transcription factors IRF4, IRF8, BCL6, BLIMP1, SPIB, PU.1, and MITF were assessed using quantitative RT-PCR. It was shown that SAP deficiency in XLP B-LCL did not abrogate CD150-mediated Akt and ERK1/2 phosphorylation. CD150 crosslinking on XLP B-LCL IARC 739 led to two waves of ERK1/2 phosphorylation and induced rapid and sustained Akt phosphorylation. IgM ligation on XLP B-LCL IARC 739 mediated very rapid, but transient ERK1/2 activation. However, IgM and CD150 coligation resulted in rapid intense and sustained ERK1/2 phosphorylation. Kinetics of pAkt after CD150 and IgM coligation remained on the level of CD150 alone. Thus, in XLP B-LCL the CD150 signaling with IgM coligation play the dominant role in both Akt and ERK1/2 phosphorylation. SAP<sup>+</sup> and SAP<sup>-</sup> conventional B-LCLs have differences in kinetics and amplitude of receptor-initiated Akt phosphorylation. We found that significantly reduced IRF4, IRF8 and PU.1 expression levels are the key features of XLP B-LCLs. Conventional SAP<sup>+</sup> B-LCL MP-1 demonstrated elevated expression level of MITF, while SPIB was practically undetectable in this cell line. On the other hand, SAP<sup>-</sup> T5-1 and XLP B-LCL IARC 739 cell lines were characterized by highest level of SPIB expression. Taking together, we found that XLP and conventional B-LCL have differences in kinetics and amplitude of receptor-mediated Akt and ERK1/2 phosphorylation. Analysis of transcription factors profile revealed the distinguishing features of XLP B-LCLs with SAP deficiency that may impair B cell differentiation in patients with XLP1.

## P7.18

### Dual effects of 2-methoxyestradiol — anticancer agent and neurotoxin?

Monika Gorzynik<sup>1</sup> Alicja Kuban-Jankowska<sup>1</sup>,  
Michał A. Zmijewski<sup>2</sup>, Agnieszka Dąca<sup>3,4</sup>,  
Michał Wozniak<sup>1</sup>, Magdalena Gorska<sup>1</sup>

<sup>1</sup>Department of Medical Chemistry, Medical University of Gdańsk, Gdańsk, Poland <sup>2</sup>Department of Histology, Medical University of Gdańsk, Gdańsk, Poland <sup>3</sup>Department of Pathophysiology, Medical University of Gdańsk, Gdańsk, Poland <sup>4</sup> Department of Pathology and Experimental Rheumatology, Medical University of Gdańsk, Gdańsk, Poland

e-mail: Magdalena Gorska <m.gorska@gumed.edu.pl>

Osteosarcoma is one of the most malignant bone tumors of childhood and adolescence. So far there is no effective treatment of OS. Constantly looking for new drugs that could effectively change the lives of patients. The existing operating methods are limited due to the high chemoresistance and metastasis of cancer. The research represent that carcinogenicity and angiogenesis can be influenced by 17 $\beta$ -estradiol metabolites [1]. 2-methoxyestradiol (2-ME), one of the natural 17 $\beta$ -estradiol derivatives is a very promising pharmaceuticals fulfilling the role of a potential anti-cancer drug over the still under investigation [2, 3].

Our studies have been conducted on highly metastatic osteosarcoma 143B and rat immortalized hippocampal HT22 cell lines. The cells were treated with pharmacological (1  $\mu$ M, 10  $\mu$ M) concentrations of 2-methoxyestradiol. Neuronal nitric oxide synthase and 3-nitrotyrosine protein level was determined by western blotting. Cell viability, induction of cell death were measured by MTT and PI/Annexin V staining, respectively. 2-ME. Intracellular level of nitric oxide was determined by flow cytometry.

Pathway signaling of neurodegenerative diseases and cancer may be overlapped. Justified by the 2-methoxyestradiol, one of the natural 17 $\beta$ -estradiol derivatives, induces neuronal signaling not only in hippocampal HT22 cells, but also in non-neuronal tissue- osteosarcoma cancer. We observed that 2-methoxyestradiol, in contrast to 17 $\beta$ -estradiol, specifically affects neuronal nitric oxide synthase and augments 3-nitrotyrosine level leading to osteosarcoma and hippocampal cell death. Thus, we present dual face of 2-methoxyestradiol that leads to cancer cell death but on the other side may play the key role as a neurotoxin in pathogenesis of neurodegenerative diseases.

#### References:

1. Yang CY *et al* (2013) Osteoblastoma in the region of the hip. *J Chinese Med Association* **76**: 115–120.
2. Peyrat JF, Brion J-D, Alami M (2012) Synthetic 2-Methoxyestradiol Derivatives: Structure-Activity Relationships. *Curr Med Chem* **19**: 4142–4156.
3. Bruce JY *et al* (2012) A phase II study of 2-methoxyestradiol nanocrystal colloidal dispersion alone and in combination with sunitinib malate in patients with metastatic renal cell carcinoma progressing on sunitinib malate. *Invest New Drugs* **30**: 794–802.

#### Acknowledgments

The project was funded by grant No. 2012/07/B/NZ1/00010 from National Science Center resources.

## P7.19

### Altered S100A6 level in keratinocytes affects EGFR signaling and growth differentiation

Agnieszka Graczyk<sup>1</sup>, Michał Młacki<sup>2</sup>, Tomasz Wilanowski<sup>2</sup>, Wiesława Leśniak<sup>1</sup>

<sup>1</sup>Nencki Institute of Experimental Biology PAS, Department of Molecular and Cellular Neurobiology, Warsaw, Poland; <sup>2</sup>Nencki Institute of Experimental Biology PAS, Department of Cell Biology, Warsaw, Poland

e-mail: Agnieszka Graczyk <a.graczyk@nencki.gov.pl>

S100A6 is a signaling, calcium binding protein most abundant in fibroblasts and epithelial cells including keratinocytes. Its postulated main role is regulation of cell proliferation and, according to some reports, also cell differentiation. Due to the lack of transgenic S100A6 knock-out mouse model its role in tissue differentiation was never examined.

In this study we have established stably transfected S100A6 knockdown (KD), overexpressing (OE) and control (C) HaCaT keratinocytes and utilized them to investigate changes in epidermal growth and differentiation. We induced differentiation of those cells either by increased [Ca<sup>2+</sup>] in 2-dimensional culture or by growing them in an air-lifted 3-dimensional culture and monitored the levels of protein markers of epidermal differentiation using RT-PCR, Western blot and immunofluorescence techniques.

We observed that in 2D culture S100A6 OE keratinocytes had higher amount of keratin 10, the early differentiation marker, in comparison to S100A6 KD and control cells while the synthesis of lorricrin — the late differentiation marker was markedly disturbed. Immunofluorescence analysis of 3D cell cultures revealed that there were minor changes between control and S100A6 KD epidermis. However, epidermis built of S100A6 OE HaCaT cells showed striking differences such as: impaired keratin 10 staining, present only in the uppermost epidermal layers, and no lorricrin staining. We also discovered that the staining of Ki67, the proliferation marker, in S100A6 OE epidermis was not confined to the basal layer but could be detected throughout all epidermal layers. The above characteristics suggested higher growth potential of S100A6 OE epidermis and therefore we decided to assay the activation of EGFR and ERK1/2 kinase pathways. EGF receptor staining showed that, in S100A6 OE epidermis its expression was higher than in control and S100A6 KD epidermis and not restricted to the basal layer. Higher EGFR level in S100A6 OE HaCaT cells was subsequently confirmed at mRNA and protein level.

Our study revealed that overexpression of S100A6 leads to higher cell proliferation as shown by Ki67 staining and higher EGFR level and in this way stimulates epidermal growth but delays differentiation. These results give us an insight into a novel S100A6 signaling pathway that may lead to understanding of S100A6 function in tissue differentiation.

#### Acknowledgements

Financial support: Grant no. 2012/05/N/NZ3/00905 to AG; EFS Scholarships for PhD students to AG and MM.

## P7.20

### Design of substrates of human 20S proteasome caspase-like subunit using combinatorial chemistry approach

Natalia Gruba, Magdalena Wysocka, Adam Lesner, Dawid Debowski, Krzysztof Rolka

Institute of Biochemistry, University of Gdansk, Gdańsk, Poland  
e-mail: Natalia Gruba <natalia.gruba@ug.edu.pl>

The degradation of proteins has become one of the most commonly studied processes in nowadays' biology. Degradation of proteins in eukaryotic cells is a rapid and irreversible process that keeps the concentration of intracellular proteins to a level that allows for precise regulation of cellular functions [1]. A proteolytic enzyme, which has great importance in the cells regulatory is a proteasome that is responsible for the removal of damaged, modified and misfolded proteins or proteins that have become expendable [2]. As proteasome is involved in many fundamental biological processes, like the cell cycle, gene expression, immune responses or carcinogenesis, even the smallest disruption in its activity may cause very serious physiological consequences [3, 4].

In this project we decided to investigate systematically the substrate specificity of 20S proteasome in P nonprime and P prime (based on the Schechter-Berger notation) positions.

Here we report the selection of internal quenched substrates for caspase-like specificity applying combinatorial chemistry approach. The library used, consist of peptides that on N- and C- termini have accordingly donor and acceptor fluorophore of electron excitation. The general formula of the peptide library for "non-prime" positions is presented below:

ABZ-X<sub>4</sub>-X<sub>3</sub>-X<sub>2</sub>-X<sub>1</sub>-ANB-NH<sub>2</sub>  
ANB-NH<sub>2</sub> – amide of 5-amino-2-nitrobenzoic acid  
ABZ – 5-aminobenzoic acid  
X<sub>1</sub> – Asp, Glu;  
X<sub>2</sub>, X<sub>3</sub>, X<sub>4</sub> – coded amino acids except for Cys.

In the next step of our research the peptide resulting from above described studies was incorporated into internal quenched library with general formula: ABZ-Ile-Leu-Met-Asp-X<sub>1</sub>'-X<sub>2</sub>'-X<sub>3</sub>'-Tyr(3-NO<sub>2</sub>)-NH<sub>2</sub> where in position X<sub>1</sub>', X<sub>2</sub>', X<sub>3</sub>' set of proteinogenic amino acid except Cys. The libraries of fluorescent substrates were synthesized manually on solid resin support using "split and mix" method, Fmoc/*t*Bu procedure. Deconvolution against human 20S proteasome was carried out using iterative solution method.

#### References:

1. Mizushima T *et al* (2004) Viley-VCH: Weinheim.
2. Elofsson M *et al* (1999) *Chem Biol* **6**: 811–822.
3. Borissenko L *et al* (2007) *Biol Chem* **388**: 947–955.
4. Groll M *et al* (2005) *ChemBioChem* **6**: 222–256.

#### Acknowledgements

This work was supported by the project contributing to the development of young scientist and PhD students under grant No 538-8710-B324-14.

## P7.21

### The role of central carbon metabolism in the control of DNA replication in eukaryotic cells

Aleksandra Konieczna, Aneta Szczepańska, Karolina Sawiuk, Robert Łyżeń, Grzegorz Węgrzyn

University of Gdańsk, Department of Molecular Biology, Gdańsk, Poland  
e-mail: Aleksandra Konieczna <a.konieczna@biol.ug.edu.pl>

Replication of genetic material is a basic biological process, essential for each organism. Processes occurring during the synthesis of DNA are the subject of many works and have been relatively well studied. However, the control mechanism of the regulation of this process remains poorly understood. Disturbances in the process of DNA replication result in the accumulation of harmful mutations, often leading to appearance of genetic diseases, and development of cancer. Therefore, it seems important to investigate the mechanisms that regulate this fundamental biological process.

The aim of this study is to understand the global regulatory processes that control DNA replication in eukaryotic organisms, as well as to answer the question whether these mechanisms are universal. This work focus primarily on glycolysis conjunction with DNA replication. The expression of selected isoforms of genes of the glycolysis pathway was reduced at the level of up to 95 % by using siRNA. Then the cell cycle was analyzed with particular regard to the transition of the cells into the S phase. Moreover, the analysis of viability and cell number was carried out. The most pronounced difference in the transition of cells treated with siRNA into the S phase was observed in the case of the gene encoding glyceraldehyde 3-phosphate dehydrogenase (GAPDH), it amounted to about 50%. Furthermore, silencing of the genes encoding the hexokinase (HK), phosphofructokinase (PFK) and enolase (ENO) resulted in decreased number of cells in the S phase by 30% and 15%, respectively. Surprisingly, silencing the expression of the gene encoding the enzyme GPI resulted in enhancement of the replication phase.

These results facilitate understanding of the correlation between metabolic processes and DNA replication. This can be practically used in studies on carcinogenesis and its prevention, as well as biotechnological use of cell cultures.

## P7.22

### Role of AMPK and HGF in epithelial to mesenchymal transition

Paweł Konieczny, Tomasz Adamus, Marcin Majka

Department of Transplantation, Jagiellonian University Medical College, Kraków, Poland

e-mail: Paweł Konieczny <p.konieczny@uj.edu.pl>

AMP-activated kinase (AMPK) is a metabolic sensor that helps maintain cellular energy homeostasis. It is also involved in tumorigenesis and metastatic progression, however its role in these processes remains unclear. Evidence that AMPK cellular level is adjusted in response to HGF induced signaling is missing. However, it seems that AMPK level and state of its activation might influence the epithelial to mesenchymal transition (EMT) process and led to tumor progression and development of metastasis.

The aim of this study was to investigate changes in expression and activation of AMPK as a result of HGF stimulation. We used three cervical carcinoma cell lines according to their different clinical stage and invasiveness. C-4I, isolated from primary tumor mass, represented an early stage of cervical cancer. HTB-35 originated also from tumor *in situ*, however, cells acquired mesenchymal characteristic, indicating the implementation of EMT. The third cell line was HTB-34, derived from metastatic site in lymph node, regained their epithelial phenotype indicating the implementation of mesenchymal to epithelial transition program. Thus, such cell lines represented comprehensive model of tumorigenesis and metastatic progression of cervical carcinoma. qRT-PCR analysis were performed to examine the level of AMPK catalytic subunits ( $\alpha 1$  and  $\alpha 2$ ) after stimulation with HGF. Simultaneously, Western Blotting was used to investigate total AMPK level and its phosphorylation status.

Our analysis showed immense differences in AMPK transcript and protein levels between examined cell lines. C-4I cell line revealed the highest AMPK mRNA and protein levels, followed by HTB34 with moderate expression and HTB 35 with lowest expression among cells lines. HGF stimulation resulted in decreased expression of AMPK in C4I cell line. In other cell lines HGF did not induce any changes in AMPK mRNA or protein levels.

Our results allow to trace the process of metabolic deregulation of cancer cell in metastatic progression. Moreover, we tried to examine the connection between EMT promoting factors like HGF and tumor cell metabolism at different clinical stages.

## P7.23

### HtrA protease from *Escherichia coli* and its redox state: implication for substrate degradation/affinity and the activation process

Tomasz Koper<sup>1</sup>, Katarzyna Węgrzyn<sup>2</sup>, Igor Konieczny<sup>2</sup>, Bogdan Banecki<sup>2</sup>, Adam Lesner<sup>3</sup>, Barbara Lipińska<sup>1</sup>, Joanna Skórko-Glonek<sup>1</sup>

<sup>1</sup>Department of Biochemistry, Faculty of Biology, University of Gdańsk, Gdańsk, Poland; <sup>2</sup>Intercollegiate Faculty of Biotechnology UG-MUG, Gdańsk, Poland; <sup>3</sup>Faculty of Chemistry, University of Gdańsk, Gdańsk, Poland

e-mail: Tomasz Koper <tomasz.koper@biol.ug.edu.pl>

HtrA protease from the bacterium *Escherichia coli* is a crucial factor of the periplasmic protein quality control system. HtrA recognizes and degrades irreversibly damaged proteins that emerge under various stress conditions such as heat shock, oxidative stress, reducing stress, non-physiological pH, etc.

HtrA exists in various oligomeric forms. In the inactive/resting state it forms hexamers. Upon the activation process triggered by the presence of misfolded/unfolded substrates it re-assembles into higher-order oligomers consisting mainly of 12- and 24-mers.

The structure of the hexamer is stabilized by the LA loops which interact with the regulatory loops L1 and L2 of the opposite subunit. The LA-L1-L2 loops cover the active site. In the active conformation the LA-L1-L2 contacts are no longer present and the active site becomes available for the substrate. This process is accompanied by hexamer dissociation into trimers and simultaneous assembly into higher order oligomers.

The proteolytic activity of HtrA shows strong temperature-dependence and at temperatures below 30°C is hardly detectable *in vitro*. Our previous studies showed that the redox state of Cys57 and Cys69 of the LA loop may influence the proteolytic activity of HtrA at low temperatures (20-25°C): (1) HtrA deprived of the S-S bond more efficiently degraded reduced alkaline phosphatase *in vivo* and (2) the cysteineless LA loops were more exposed to the solvent and had more flexible structure, that probably facilitated activation process or substrate binding.

The aim of our work was to further examine the role of the disulphide bridge in the activation process of HtrA. For this purpose we checked for the kinetics of proteolysis, affinity to substrate and oligomeric state of HtrA variants containing or deprived of the disulphide bridge in the presence of the model substrates/ligands.

The HtrA deprived of the S-S bond: (i) digested a model substrate more efficiently at 20 and 37°C, (ii) exhibited higher affinity to immobilized substrate, (iii) required significantly lower concentration of model ligand to assemble into higher-order oligomers, (iv) showed altered oligomer equilibrium in the absence of substrate, in comparison to HtrA with the preserved S-S bond.



## P7.24

### Cx43 up-regulation contributes to TGF- $\beta_1$ -induced activation of Smad-dependent signaling during fibroblast-to-myofibroblast transition of primary human bronchial fibroblasts

Milena Kosińska,<sup>1</sup> Izabela Borek,<sup>1</sup> Damian Ryszawy,<sup>1</sup> Katarzyna Anna Wójcik,<sup>1,2</sup> Katarzyna Piwowarczyk,<sup>1</sup> Marta Michalik<sup>1</sup>, Jarosław Czyż<sup>1</sup>

<sup>1</sup>Jagiellonian University, Faculty of Biochemistry, Biophysics and Biotechnology, Department of Cell Biology, Kraków, Poland; <sup>2</sup>Jagiellonian University Medical School, Department of Medicine, Kraków, Poland

e-mail: Milena Kosińska <milu.kosinska@uj.edu.pl>

Fibroblast-to-myofibroblast transition (FMT) is TGF $\beta_1$ -dependent process which plays a pivotal role in the induction of airway wall remodeling during bronchial asthma [1]. It is regulated by the activation of Smad signaling in human bronchial fibroblasts (HBF) and accompanied by up-regulation of  $\alpha$ -SMA expression - a marker of myofibroblasts [2]. The involvement of Cx43, a protein which constitutes gap junctional channels, in the regulation of FMT in cardiac tissue has recently been reported [3]. It prompted us to estimate the role of Cx43 in TGF $\beta_1$ -induced FMT undergone by primary HBFs derived from asthmatic patients (AS HBFs). Prolonged exposure of AS HBFs to TGF $\beta_1$  efficiently induced FMT, which was accompanied by up-regulation of  $\alpha$ -SMA and Cx43 levels as demonstrated by immunofluorescence, immunoblotting and FACS analyses. These effects were correlated with the induction of Smad signaling, illustrated by nuclear translocation and accumulation of p-Smad2 in TGF $\beta_1$ -treated AS HBFs. Inhibition of gap junctional intercellular coupling by 18- $\alpha$ -glycyrrhetic acid considerably reduced the efficiency of FMT. However, it had no effect on TGF $\beta_1$ -induced nuclear translocation of Smad2 and Cx43 expression level in AS HBFs. On the other hand, transient inhibition of Cx43 expression by siRNA attenuated the  $\alpha$ -SMA transcript quantity, FMT and Smad signaling activity in these cells. Our observations indicate overlapping gap junctional channel-dependent/independent effects of Cx43 on the efficiency of FMT during airway wall remodeling. Gap junctional coupling may participate in FMT of AS HBFs through mediating by-stander effects in a Smad2-independent manner. On the other hand, Cx43 affects Smad activity in these cells in a channel-independent fashion.

#### References:

1. Makinde T Murphy RF Agrawal DK (2007) *Immunol Cell Biol* **85**: 348–356.
2. Evans *et al* (2003) *Exp Cell Res* **282**: 90–100.
3. Asazuma-Nakamura Y *et al* (2009) *Exp Cell Res* **315**: 1190–1199.

## P7.25

### Yeast YIL096C binds and methylates 25S rRNA

Jan Kutner<sup>1</sup>, Andrzej Dziembowski<sup>2,3</sup>, Maga Rowicka<sup>4,5,6</sup>, Krzysztof Ginalski<sup>1</sup>

<sup>1</sup>Centre of New Technologies, Laboratory of Bioinformatics and Systems Biology, University of Warsaw, Warsaw, Poland, <sup>2</sup>Institute of Genetics and Biotechnology, Faculty of Biology, University of Warsaw, Warsaw, Poland, <sup>3</sup>Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland, <sup>4</sup>Department of Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston, Texas, United States of America, <sup>5</sup>Institute for Translational Sciences, University of Texas Medical Branch, Galveston, Texas, United States of America, <sup>6</sup>Sealy Center for Molecular Medicine, University of Texas Medical Branch, Galveston, Texas, United States of America

e-mail: Jan Kutner <j.kutner@cent.uw.edu.pl>

The ribosomal RNA contains 109 specific modifications that affect translation and ribosome biogenesis. Only few of them correspond to base methylations catalyzed by methyltransferases (MTases). In eukaryotes, most of the rRNA modifications are maintained by enzymes that require guide snoRNA. In this work we identify a specific substrate for *Saccharomyces cerevisiae* YIL096C (BMT5) [1, 2], a snoRNA-independent MTase, using *in vitro* isotope dependent assays. We show that YIL096C binds and methylates yeast 25S rRNA and possesses substrate specificity to oligoRNA containing already known methylation site m<sup>3</sup>U2634. This is highly consistent with the recent study by independent group showing that BMT5 is a new m<sup>3</sup>U2634 MTase [3]. Our results contribute to better understanding of rRNA base methylation process, ribosome biogenesis and function. Presented approach should be helpful in further studies on substrate specificity of rRNA base MTases.

#### References:

1. Włodarski T *et al* (2011) *PLoS One* **6**: e23168.
2. Szczepińska T *et al* (2014) *PLoS Comput Biol* **10**: e1003514.
3. Sharma S *et al* (2014) *Nucleic Acids Res* **42**: 3246–3260.

## P7.26

### TROSPA — an intrinsically disordered protein involved in the tick colonization by *Borrelia*

Dominik Lewandowski<sup>1</sup>, Anna Urbanowicz<sup>1</sup>, Kamil Szpotkowski<sup>1</sup>, Karol Kamel<sup>1</sup>, Mariusz Jaskólski<sup>1,2</sup>, Marek Figlerowicz<sup>1,3</sup>

<sup>1</sup>Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland; <sup>2</sup>Faculty of Chemistry, Adam Mickiewicz University, Poznań, Poland; <sup>3</sup>Institute of Computing Science, Poznań University of Technology, Poznań, Poland  
e-mail: Anna Urbanowicz <aniau@ibch.poznan.pl>

Borreliosis (Lyme disease) caused by bacteria belonging to *Borrelia* genus is one of the most prevalent tick-borne diseases in North America and Europe. In the latter, *Borrelia* spirochetes are transmitted by *Ixodes ricinus* (Castor bean tick). Earlier it has been demonstrated that a protein produced in tick's epithelial cells called TROSPA is essential for the bacteria to colonize the vector. Bacterial surface protein OspA binds TROSPA and this way enables *Borrelia* to attach to tick gut.

Despite intensive studies conducted during the last decade, the structure of TROSPA and its basic functions remain unknown. In order to learn more about this protein we produced a recombinant TROSPA\_NΔ44 deletion mutant. It lacked transmembrane domain but still was capable of binding OspA at the similar level to the wild type protein. The structure of the highly purified TROSPA\_NΔ44 mutant in a solution was examined by using a number of techniques, such as small-angle X-ray scattering (SAXS), dynamic light scattering (DLS), size-exclusion chromatography (SEC) or circular dichroism spectroscopy (CD). The results coming from our analyses clearly demonstrated that TROSPA shows the features characteristic for intrinsically disordered proteins (IDP). In addition, based on the data collected so far, we generated the computational model of three dimensional structure of the TROSPA\_NΔ44 mutant.

#### Acknowledgements

This work was partially supported by the Polish Government through grant N N302 041536.

## P7.27

### Regulation of Nrf2-dependent pathway in normal and tumor cells of the digestive system by ITC 2-oxoheptyl

Katarzyna Lubelska<sup>1,2</sup>, Katarzyna Wiktorska<sup>1</sup>, Lidia Śliwka<sup>1,2</sup>, Małgorzata Milczarek<sup>1,3</sup>, Zdzisław Chilmonczyk<sup>1</sup>

<sup>1</sup>National Medicines Institute, Department of Cell Biology, Warsaw, Poland, <sup>2</sup>Medical University of Warsaw, Faculty of Pharmacy with the Laboratory Medicine Division, Warsaw, Poland, <sup>3</sup>University of Lublin, Faculty of Pharmacy with Medical Analytics Division, Lublin, Poland  
e-mail: Katarzyna Wiktorska <k.wiktorska@nil.gov.pl>

The nuclear factor (erythroid-derived 2)-like 2) Nrf2 plays a key role in regulation of detoxification enzyme NAD(P)H dehydrogenase (quinone) (NQO1) [1]. Increasing in NQO1 activity contributes in normal cells to the maintenance of cellular homeostasis, whereas in cancer cells it is unwanted phenomena, as it desensitizes cells to endogenous or exogenous mechanisms of their elimination [2].

The isothiocyanates (ITC) are compounds which induce NQO1 activity through Nrf2 in the cells of a gastrointestinal tract *in vitro* [3]. Due to the deregulation of signaling pathways in cancer cells it is not known whether the Nrf2 induction in normal and cancer cells by the ITC influences in a similar way individual molecular targets which could result in increasing activity or lack thereof.

ITC 2-oxoheptyl was selected for the study. It is a selective compound with potential activity on the normal and cancer cells. The tests were performed on CRL1790 normal cells and HT29 colon cancer cells. The effects of ITC 2-oxoheptyl on translocation of Nrf2 from the cytoplasm to the nucleus in both cell lines was examined and compared. Then, changes in the expression level of the gene encoding NQO1 and changes in the level of NQO1 protein were examined. The final step was to compare the level of induction of NQO1 activity in both types of cell lines. NQO1 protein levels and the location of the transcription factor Nrf2 was examined by immunocytochemistry. Examination of the gene encoding the expression of NQO1 was performed using the method of quantitative real-time PCR (qPCR). NQO1 enzyme activity was tested biochemically.

It was found that ITC 2-oxoheptyl induced Nrf2 translocation from the cytoplasm to the nucleus in both cell lines, leading to an increase in the expression of the gene encoding NQO1. However, changes in the cellular localization of Nrf2 and increased expression of the gene encoding NQO1 resulted in the increase in the level and activity of NQO1 protein only in normal cells. In the tumor cells, instead of induction of translocation and increase in expression of the gene encoding NQO1, reduced protein levels and NQO1 activity were observed.

The results indicate a possible influence of ITC 2-oxoheptyl subsequent post-translational modifications, which may play an essential role in the final level of NQO1 activity in tumor cells HT29.

#### References:

Dinkova-Kostova AT *et al* (2010) *Arch Biochem Biophys* **501**: 116–123.  
Lau A, Villeneuve NF *et al* (2008) *Pharmacol Res* **58**: 262–270.  
Morimitsu Y *et al* (2002) *J Biol Chem* **277**: 3456–3463.

#### Acknowledgements

The research was co-financed by the European Union within the European Social Fund.

## P7.28

### The chiral Schiff base as the activators of adenylate kinase

A. Ludwiczak<sup>1</sup>, E. Błocka<sup>2</sup>, A. Wojtczak<sup>1</sup>, M. Komoszyński<sup>3</sup>

<sup>1</sup>Faculty of Chemistry, Nicolaus Copernicus University, Department of Crystallochemistry and Biocrystallography, Poland; <sup>2</sup>Faculty of Chemistry, Nicolaus Copernicus University, Department of Organic Chemistry, Poland; <sup>3</sup>Faculty of Biology and Environmental Protection, Department of Biochemistry, Poland  
e-mail: Agnieszka.Ludwiczak <cagnieszka.b.1987@o2.pl>

Adenylate kinase (AK, EC 2.7.4.3) are the ATP: AMP phosphotransferases catalyzing the reaction of bidirectional transfer of phosphate residues from adenosine triphosphate (ATP) to adenosine monophosphate (AMP). The substrates of the enzymatic reaction are two molecules of adenosine diphosphate (ADP). The presence of ADP in the blood is necessary for initiation of platelet aggregation and maintain the process of repairing damaged tissue. However, uncontrolled and growing the ADP concentration increases platelet aggregation and thrombus formation. On the other hand, increase in the concentration of ATP initiates the inflammatory processes and formation of an atherosclerotic plaque. In our opinion, the reduction of non-physiological concentrations of ADP and ATP in the blood by the activation of adenylate kinases is more favorable than block platelet purinergic receptors. Understanding the molecular basis for regulation of the activity of these kinases may be a starting point for effective treatment of cardiovascular diseases, including heart attacks.

A completely new group of regulators of kinases belonging to the Schiff bases was obtained and tested. The synthesis of these compounds, including derivatives of norephedrine was conducted for catalytic applications [1,2]. These compounds have structural similarity to the derivatives of suramin or anthraquinone-2-sulfonic acid- inhibitors of nucleotidase. Additionally, they are characterized by a similar distribution of heteroatoms to the ATP molecule, which led us to research their effect on the activity of adenylate kinase from *Aquifex aeolicus* and *Bacillus stearothermophilus*. The tested compounds showed a 40-60% activation of kinase. Both tested regulators was crystallized and their crystal structures was determined[3].

#### References:

1. Błocka E, Jaworska M, Kozakiewicz A, Welniak M, Wojtczak A (2010) *Tetrahedron: Asymmetry* **21**: 571–577.
2. Hayashi M, Miyamoto Y, Inoue T, Oguni N (1993) *J Org Chem* **58**: 1515–1522.
3. Iocka E, Bosiak MJ, Welniak M, Ludwiczak A, Wojtczak A (2014) *Tetrahedron: Asymmetry* **25**: 554–562.

## P7.29

### Novel mTOR isoform and tumorigenesis

Oksana Malanchuk, Sergiy Palchevskyy, Oleg Garifulin

The Institute of Molecular Biology and Genetics of NAS of Ukraine, Department of Cell Signalling, Ukraine  
e-mail: Oksana.Malanchuk <malanchook@ukr.net>

**Background:** The mammalian target of rapamycin (mTOR) plays a striking role in signal transduction and regulates cell growth, metabolism, proliferation and survival in response to nutrients and growth factors. Deregulation of mTOR signaling pathway has been associated with numerous pathologies, including many human cancers.

There is only one gene encoding mTOR. Therefore the diversity of the mTOR-mediated signaling is conferred by two functionally and structurally distinct multiprotein complexes (mTORC1 and mTORC2) with the well known mTOR (mTOR $\alpha$ ) in their core. Previously in our Laboratory for a first time mTOR splicing isoform (mTOR $\beta$ ) was identified. This isoform differs from well known mTOR $\alpha$  lacking HEAT and partially FAT domains but carrying intact Kinase domain. It has been shown that mTOR $\beta$  is an active protein kinase that mediates downstream signaling through complexing with Raptor (mTORC1) and Rictor (mTORC2) proteins and, significantly, is a potential oncogene.

Recently, we have identified a novel mTOR isoform – mTOR $\delta$ . Importantly, this isoform has another principle of isoform formation than mTOR $\beta$  (unpublished data). Deletion in kinase domain resulted in frame shift within 3' - region and finally translation of unique protein sequence which is absent in mTOR $\alpha$ . So, current research proposal is focused on clarifying the involvement of mTOR $\delta$  in tumorigenesis. Characterization of oncogenic and signaling activities of mTOR $\delta$  and its expression in cancer will offer new perspective target for cancer therapy.

**Results:** Early using RT-PCR of various cell lines we have identified a novel splicing isoform of mTOR kinase – mTOR $\delta$ . The aim of this study was to characterize the functional role of mTOR $\delta$  in tumorigenesis. So, initially, we have generated polyclonal antibody against mTOR $\delta$  and specific antibodies against Raptor and Rictor which are applicable in various immunoassays. Then we have shown by Western-blot analysis that mTOR $\delta$  expressed in different cell lines *in vivo*. Next, HEK293 stable cell line overexpressing mTOR $\delta$  has been generated. To examine the oncogenic potential of mTOR $\delta$  the various assays have been done: MTT assay to analyze the cellular activity and proliferation; Soft Agar Assay to measure cell anchorage-independent proliferation potential; Scratch Migration Assay to study the migration capacity of cells. In result, HEK293 stable cell line overexpressing mTOR $\delta$  demonstrates a low proliferative potential and not considerable migration capacity in compare to control HEK293 cell.

**Conclusions:** In contrast to mTOR $\beta$  isoform, mTOR $\delta$  can inhibit cell proliferation. To elucidate the mechanism by which cell proliferation may be inhibited by mTOR $\delta$  now we are exploring its effect on the cell cycle progression by flow cytometric analysis. Besides we plan to investigate both the localization of mTOR $\delta$  in cell and its co-localization with Rictor or Raptor proteins.

**P7.30****Transport of the opioid peptides across Caco-2 monolayer**

Michał Matysiewicz, Beata Jarmołowska, Marta Bukała, Natalia Smulska, Ewa Fiedorowicz, Anna Cieślińska, Elżbieta Kostyra

Medical Biochemistry Peptides and Proteins Research Team; Faculty of Biology & Biotechnology; University of Warmia and Mazury in Olsztyn, Olsztyn, Poland  
e-mail: Elzbieta.Kostyra <elzbieta.kostyra@uwm.edu.pl>

Milk is the source of  $\beta$ -casomorphins (BCMs) – biologically active peptides with opioid activity – which are suspected to play various roles in the human body. The local influence of exogenous opioid peptides on gastrointestinal functions has been widely reported. After passing the gut barrier,  $\beta$ -casomorphins may affect the functions of immunological system, as well as dopaminergic, serotonergic and GABA-ergic systems in brain, regulate the opioid receptor development and elicit behavioral effects. However, possibilities and mechanisms of the intestinal transport of  $\beta$ -casomorphins in human body in vivo have not been accurately described so far. However, it is known that in the gastrointestinal tract as well as in the blood,  $\beta$ -casomorphins can be degraded by the dipeptidyl peptidase IV (DPPIV; EC 3.4.14.5), a highly specified aminopeptidase that cleaves dipeptides (X-Pro) from N-terminus fragments of peptide. The aim of this research was to determine the permeability coefficients of the  $\mu$ -opioid agonists human  $\beta$ -casomorphin-5 and -7 (BCM5, BCM7) in time intervals, and to compare them with the permeability coefficient of  $\mu$ -opioid antagonist peptide, human lactoferroxin A (LCF A) – using Caco-2 monolayer. In order to determine the pathway of investigated peptide transport across Caco-2 monolayer, two directions of the transport (apical to basolateral and basolateral to apical) have been studied. All investigated peptides were transported across the human intestinal cell line Caco-2 and the curves of cumulative amount of transported peptides in time were linear in each case. In addition, the hydrolysis of  $\beta$ -casomorphins during 60 min of experiment by dipeptidyl peptidase IV was observed.

Our studies have shown that the transport of opioid peptides across epithelial cells may be more efficient in the case of lower DPPIV activity. These data have also confirmed that DPPIV is the main factor limiting the half-life of opioid peptides in the intestine. Intestinal transport of  $\mu$ -opioid receptor agonist and antagonist peptide has the potential to regulate the access of those peptides to their target sites in the body. It prompts the need to investigate the transport abilities of opioid peptides, especially in the cases of diseases such as allergy, mucosal immunity, celiac disease, autism and schizophrenia.

**P7.31****Calix[4]arene C-90 as selective inhibitor of plasma membrane calcium pump and new myometrium contraction agent**

Luliia Mazur<sup>1</sup>, Tetyana Veklich<sup>1</sup>, Oleksandr Shkrabak<sup>1</sup>, Olga Tsymbalyuk<sup>2</sup>

<sup>1</sup>Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine, Department of Muscle Biochemistry, Ukraine; <sup>2</sup>Taras Shevchenko National University of Kyiv, Institute of Higher Technology, Ukraine

e-mail: Luliia.Mazur <yuliy.vorona@gmail.com>

Plasma membrane  $Mg^{2+}$ ,ATP-dependent  $Ca^{2+}$ -pump eject  $Ca^{2+}$  from cytoplasm to extracellular space and control local  $Ca^{2+}$ -signal in intracellular membrane microdomain. Nonetheless, prior to our research there are no low-molecular, selective and affine inhibitors. The calix[4]arene C-90 (5,11,17,23-tetra(trifluoro)methyl(phenyl-sulfonylimino)-methylamino-25,26,27,28-tetrapropoxycalix[4]arene) is shown to efficiently and selectively inhibit the ATP hydrolyse activity of  $Ca^{2+}$ , $Mg^{2+}$ -ATPase in the myometrium cell plasma membrane fraction. Calix[4]arene C-90 was synthesized in Institute of organic chemistry NAS of Ukraine by prof. V.I. Kalchenko and characterized by methods of infrared spectroscopy and nuclear magnetic resonance.

Activity of  $Ca^{2+}$ , $Mg^{2+}$ -ATPase was determined on swine myometrium plasma membrane fraction by measurement of  $P_i$  formed as a result of ATP hydrolysis. The inhibition coefficient  $I_{0.5}$  values was  $20,2 \pm 0,5$  and calyx[4]arene C-90 (100  $\mu M$ ) decrease  $Ca^{2+}$ , $Mg^{2+}$ -ATPase velocity maximum on 75% comparatively to control. The inhibitory effect of calix[4]arene C-90 was selective comparatively to other ATPases localized in the plasma membrane:  $Na^+$ , $K^+$ -ATPase and “basal”  $Mg^{2+}$ -ATPase. The inhibitory effect of calix[4]arene C-90 on the  $Ca^{2+}$ , $Mg^{2+}$ -ATPase activity was associated with the cooperative action of four trifluoromethyl-phenyl sulfonylimine (sulfonylamidine) groups spatially oriented on the upper rim of the calix[4]arene macrocycle (the calix[4]arene “bowl”).

Due to results of confocal microscopy study calix[4]arene C-90 increased calcium concentration in cytoplasm of myometrium smooth muscle cells in rest on  $45 \pm 9$  % comparatively to control, however, during 1,5-2 minutes the calcium concentration was turning to output level. It is mean that other calcium-control system (as  $Na^+$ , $Ca^{2+}$ -exchanger, mitochondria) became more actively involved in calcium extrusion if plasma membrane  $Ca^{2+}$ -pump is inhibited. Additionally the method of laser correlation spectroscopy was used to determine effective hydrodynamic diameter of smooth muscle cells and inference about cell contractility was made. Calix[4]arene C-90 (50  $\mu M$ ) caused decrease of effective hydrodynamic diameter on 26% like as uterotonic oxytocin (100 nM). Thus, calyx[4]arene C-90 provoke smooth muscle cells contraction. Also calix[4]arene C-90 (10  $\mu M$ ) decrease relaxation velocity maximum of uterine smooth muscle normalized on contraction amplitude on 20% comparatively to control measured in spontaneous muscle activity.

The experimental findings seem to be important for studies of regulation of calcium homeostasis in smooth muscle cells and also for investigation of the participation of the plasma membrane  $Ca^{2+}$ -pump in control of electro- and pharmacomechanical coupling in myocytes.

## P7.32

### New piroxicam derivatives with antitumor activity and their effect on multidrug-resistant colon cancer cells

Kamila Środa-Pomianek<sup>1</sup>, Berenika Szczęśniak-Sięga<sup>2</sup>, Wiesław Malinka<sup>2</sup>, Jadwiga Maniewska<sup>2</sup>, Krystyna Michalak<sup>1</sup>

<sup>1</sup>Department of Biophysics, Wrocław Medical University, Wrocław, Poland; <sup>2</sup>Department of Chemistry of Drugs, Wrocław Medical University, Wrocław, Poland  
e-mail: Krystyna.Michalak <krystyna.michalak@umed.wroc.pl>

Data on potential antitumor activity of commonly used non-oncological drugs, such as non-steroidal anti-inflammatory drugs (NSAIDs) often appear in literature, recently. Many studies have revealed that NSAIDs may decrease the risk of cancer incidence, including breast, colon, lung, and stomach cancer. The challenge is to obtain new, more active compounds possessing anticancer and/or multidrug reversing activity. Piroxicam is a NSAID that belongs to the oxamic class of drugs. It is used as an analgesic in case of post-operative pain or in rheumatoid and osteoarthritis. NSAIDs are inhibitors of cyclooxygenase-2 (COX-2), the enzyme which is expressed in the most solid tumors.

The research goal was to design and synthesize compounds that would be able both to inhibit COX-2 activity and modulate multidrug resistance (MDR). The question was which structural changes should be introduced into chemical structure of piroxicam to obtain derivatives with maximal anticancer activity. Two of the newly synthesized derivatives exhibit the ability to inhibit the growth of colon cancer cells LoVo and LoVo/Dx, sensitive and resistant to doxorubicin, respectively. The expression level of the multidrug resistance proteins and some proteins associated with apoptosis, and their corresponding genes (mRNA determination) have been analyzed by immunodetection and PCR, respectively. Analysis at the level of protein has shown that new piroxicam derivatives decrease the expression of multidrug transporter ABCG2 (BCRP), as well as an anti-apoptotic Bcl-2 protein and change expression of pro-apoptotic Bax protein. It was observed that both oxamic compounds were also able to reduce the level of COX-2 expression. The studies with use of confocal microscopy have confirmed the influence of new derivatives on cytoskeleton structure in studied cancer cell lines. In conclusion, the newly synthesized piroxicam derivatives show promising properties as potential anticancer agents in case of colon cancer.

## P7.33

### Induction of particulate guanylyl cyclase type A in monocytes

Małgorzata Mitkiewicz, Jakub Siednienko, Joanna Zyzak, Marianna Kuropatwa, Ewa Kurowska, Janusz Matuszyk

Laboratory of Signaling Proteins, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland  
e-mail: Malgorzata.Mitkiewicz <ciuman@iitd.pan.wroc.pl>

Cyclic GMP (cGMP) is a common secondary messenger involved in many cellular processes. Synthesis of this nucleotide is catalyzed by guanylyl cyclases (GCs), which exist as cytosolic (sGC) or membrane-bound (particulate, pGC) proteins. The data show that in monocytes mainly sGC is present and the NO/sGC/cGMP pathway in regulation of immune cells response is well known [1, 2]. However, the role of particulate guanylyl cyclase A (GC-A) in immune cells remains unknown. Our observations and other reports [3, 4] indicate that active GC-A appears during culture of monocytes isolated from peripheral blood mononuclear cells (PBMCs). It is supposed that such change in the phenotype of cells may influence their function in certain diseases, i.a. in cirrhosis, hypertension and heart failure. In the plasma of patients was observed even 30-fold increase in concentrations of natriuretic peptides (ANP and BNP), the activators of GC-A. Due to the small amount of studies on the molecular mechanism of GC-A expression, this process remains unclear. In this light the main aim of our studies was to examine what external factors influence the expression of GC-A in monocytes and how this process is regulated. As a model we used the monocytic cell line THP-1. It was found that among many extracellular factors only phorbol ester (PMA) and all-trans retinoic acid (much more weakly) affect induction of GC-A in THP-1. Using real-time PCR, Western blotting and ELISA we found that in PMA-stimulated cells active form of GC-A is produced. Subsequently it was shown that both protein kinase C (PKC) and mitogen extracellular kinases (MEK1/2) play an important role in the activation of GC-A encoding gene in response to PMA. The implementation of specific inhibitors of these kinases resulted in significant decrease of GC-A expression and activity in PMA-differentiated cells. The participation of PKC in GC-A induction was also shown in the cultured PBMCs. Preliminary data indicate that Sp1 transcription factor may be involved in the regulation of GC-A gene expression in THP-1 cells and PBMCs.

#### References:

1. Mitkiewicz M *et al* (2011) *Immunobiology* **216**: 423–430.
2. Siednienko J *et al* (2011) *Cytokines* **54**: 282–288.
3. Bender AT *et al* (2004) *Cellular Signalling* **16**: 365–374.
4. Morita R *et al* (2003) *J Immunol* **170**: 5869–5875.

#### Acknowledgements:

This project was supported by National Science Centre, Poland (Project SONATA No. UMO-2011/01/D/NZ3/04249).

## P7.34

### Subcellular distribution of the immunophilin FKBP39 in living cells

Marek Orłowski<sup>1</sup>, Katarzyna Popławska<sup>1</sup>, Joanna Pieprzyk<sup>1</sup>, Aleksandra Szczygiał-Sommer<sup>1</sup>, Mirosław Zarębski<sup>2</sup>, Jurek Dobrucki<sup>2</sup>, Andrzej Ożyhar<sup>1</sup>

<sup>1</sup>Department of Biochemistry, Faculty of Chemistry, Wrocław University of Technology, Wrocław, Poland; <sup>2</sup>Division of Cell Biophysics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland  
e-mail: Marek.Orlowski <marek.orlowski@pwr.edu.pl>

The FK506-binding proteins (FKBPs), a distinct class of immunophilins, interact with immunosuppressants and catalyze the *cis-trans* conversion of prolyl bonds in proteins using their peptidyl-prolyl isomerase (PPIase) activity during protein folding events. Immunophilins are intracellular receptors of the immunosuppressants FK506 and rapamycin that block antigenic responses and/or cell cycle progression. These proteins are also a unique group of chaperons found in a wide variety of known organisms. FKBPs are composed of variable numbers and types of domains, which enable them to perform many important cellular functions in addition to protein folding. The peptidyl-prolyl *cis-trans* isomerase FK506-binding protein of 39 kDa (FKBP39) from *Drosophila melanogaster* is an inhibitor of autophagy in larval fat body and the protein is probably involved in ecdysone and juvenile hormone signal transduction as a transcriptional modulator of gene expression. Thus, the aim of this work was to study the subcellular distribution of FKBP39 fused to a yellow fluorescent protein (YFP) in living cells. The experiments were carried out in mammalian COS-7 and HEK293 cells to facilitate the investigation of the subcellular trafficking of YFP-FKBP39 in the absence of its endogenous expression. The data revealed that the distribution of YFP-FKBP39 was exclusively nuclear and nucleolar in both cell types analyzed. In order to determine the nuclear localization signal (NLS), a series of FKBP39 mutants tagged with YFP were prepared and examined in cells. In a series of deletion mutants, a segment (residues 180–250) displayed constitutive nuclear import suggesting the presence of a novel complex NLS signal in a basic domain in the middle of FKBP39. Detailed mutagenesis studies revealed that residues K188 and K191 are crucial for nuclear targeting of FKBP39. The results show that the immunophilin FKBP39 possess an active NLS in close proximity to a putative helix-turn-helix motif responsible for binding of DNA.

#### Acknowledgements

This work was supported by the National Science Centre grant (2012/05/B/NZ1/00659) and partly by a statutory activity subsidy from the Polish Ministry of Science and Higher Education for the Faculty of Chemistry of Wrocław University of Technology.

## P7.35

### Cytochrome *c* interaction with tRNA, the new properties of the known molecules

Roza Pawłowska, Dominika Jedrzejczyk, Magdalena Janicka, Anna Graczyk, Arkadiusz Chworos

Department of Bioorganic Chemistry, Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Łódź, Poland  
e-mail: Roza.Pawłowska <rozapech@cbmm.lodz.pl>

Transfer RNAs (tRNAs) are the key molecules involved in protein biosynthesis, responsible for transport of specific amino acids to the ribosome during translation. Some recent data suggest, that tRNAs not only play role as key elements in protein synthesis machinery, but also this class of small RNAs is involved in regulation of programmed cell death [1] and gene expression regulation in response to stress conditions [2].

The apoptosis regulation by tRNA is conducted by direct binding of tRNA molecule to cytochrome *c* and thereby interfering with the apoptosome formation [1]. This novel, surprising discovery sheds a new light on possible role of increasing expression of tRNA in cancer cells and opens possibility to study tRNA in this regard, as potential model molecules for therapeutic agents design.

Since Mei and Yong discovery in 2010 [1], new findings show quantitatively how cytochrome *c* binds to tRNA [3], however the mechanism of this interaction is still unclear. Another unexplored problem is the affinity of individual tRNAs to cytochrome *c*.

Cytochrome *c* is a well explored protein primarily responsible for the electron transport in mitochondrial respiratory system located in an inter-membrane of mitochondria and their release into the cytoplasm is one of the earliest steps of induction of intrinsic apoptosis pathway [4].

In current studies, we have designed and synthesized, specific RNA molecules imitating mitochondrial tRNA<sup>Gly</sup>, tRNA<sup>Ala</sup>, tRNA<sup>His</sup> and tRNA<sup>Phe</sup> and test their ability to bind to cytochrome *c* and possible influence on the apoptosis. The cytochrome *c*/tRNA complexes formation was investigated using electrophoretic methods (based on the mobility shift measurement) and spectroscopy techniques. Obtained results show, that all tested tRNA molecules form complexes with cytochrome *c* and thereby may influence on induction of apoptosis *in vitro*. Simultaneously there are some differences observed between individual tRNAs.

#### References:

1. Mei Y, Yong J *et al* (2010) *Molecular Cell* **37**: 668–678.
2. Nawrot B *et al* (2011) *Cellular and Molecular Life Sciences*.
3. Suryanarayana *et al* (2012) *Mol Biol Rep* **39**: 9187–9191.
4. Yong-Ling P *et al* (2008) *Nature* **9**: 532–542.

#### Acknowledgements

Presented research was financially supported by the National Science Centre (NCN) in Poland, grant number 2011/01/B/NZ3/02090 and a statutory fund.

## P7.36

### BAL cellular analysis in the lung diseases diagnosis using new intramolecular quenched substrates for human PR3 and HNE

Jadwiga Popow-Stellmaszyk<sup>1</sup>, Beata Popławska-Wiśniewska<sup>2</sup>, Magdalena Wysocka<sup>1</sup>, Joanna Chorostowska-Wynimko<sup>2</sup>, Krzysztof Rolka<sup>1</sup>, Adam Lesner<sup>1</sup>

<sup>1</sup>Department of Biochemistry, Faculty of Chemistry, University of Gdańsk, Gdańsk, Poland; <sup>2</sup>Department of Genetics and Clinical Immunology, National Institute of Tuberculosis and Lung Diseases, Warsaw, Poland

e-mail: Jadwiga Popow-Stellmaszyk <jadzia.popow@gmail.com>

Bronchoalveolar lavage (BAL) still occupies a very important position in the diagnosis of primary lung diseases of various origins: infectious, interstitial, and neoplastic pulmonary pathology occurring in the course of other diseases [1]. Of these, interstitial lung diseases caused by human neutrophil elastase (HNE) and proteinase 3 (PR3), is a hallmark of numerous pulmonary diseases [2, 3]. Optimal treatment of respiratory infections requires the establishment of a pathogenic agent. Obtained during this procedure data (composition of cells, enzymes and mediators) allowed at a large extent explain a number of pathophysiological pulmonary diseases. However, the limitation of this method and lack of standardization cause the difficulty in comparing the results. Thus, other quick, sensitive and uncomplicated diagnostic features are still needed.

This work is an attempt to design and obtain new, selective intramolecular quenched substrates of HNE and PR3 using combinatorial chemistry approach (split and mix method and Fmoc chemistry) on TentaGel S RAM resin as a solid support. The iterative deconvolution in solution of such libraries (parallel against both enzymes) allowed us to obtain two substrates ABZ-Met-Pro-Val-Ala-Trp-Glu-Tyr(3-NO<sub>2</sub>)-NH<sub>2</sub> for HNE and ABZ-Tyr-Tyr-Asn-Glu-Pro-Tyr(3-NO<sub>2</sub>)-NH<sub>2</sub> for PR3 hydrolyzed in the presence of only one of the desired enzymes. The last step was to apply these highly sensitive substrates, perform measurement of concentration of these enzymes in BALF samples and see if it is possible to discriminate between different lung disorders.

#### References:

1. Chcialowski A *et al* (2011) *Pneumonol Alergol Pol* **79**: 2, 75.
2. Prok J-A *et al* (2005) *AJP* **167**: 651.
3. Drent M *et al* (1993) *Eur Respir J* **6**: 803.

#### Acknowledgments

This work was supported by National Council of Science (NCN) under grant number DEC-2012/07/N/ST5/00178.

## P7.37

### ISG'ylation increases stability of hundreds proteins including STAT1 that prevents premature termination of immune responses in LPS stimulated microglia

Piotr Przanowski<sup>1</sup>, Stefan Loska<sup>1</sup>, Dominik Cysewski<sup>2</sup>, Michal Dabrowski<sup>1</sup>, Bozena Kaminska<sup>1</sup>

<sup>1</sup>Laboratory of Molecular Neurobiology, Nencki Institute of Experimental Biology, Warsaw, Poland; <sup>2</sup>Laboratory of RNA Biology and Functional Genomics, Institute of Biochemistry and Biophysics, Warsaw, Poland

e-mail: Piotr Przanowski <piotr.przanowski@gmail.com>

The initiation, progression and termination of brain inflammation requires changes in gene expression, posttranslational protein modifications and regulation of protein degradation. Microglia are brain resident macrophages which become activated in most neurological diseases. Inflammatory activation could be mimicked in primary microglial cultures by treatment with lipopolysaccharide (LPS). We demonstrated the increase of Uba7 expression – an E1 enzyme that is crucial for ISG'ylation – posttranslational modification similar to ubiquitination in inflammatory microglia. ISG'ylation is an important part of anti-viral response and inflammatory processes, but unlike ubiquitination, ISG'ylation do not lead to protein degradation and its function remains unclear. We found the increased level of ISG'ylation after LPS stimulation. Silencing of UBA7 expression in immortalized BV2 microglial cells led to decrease in a steady state level of hundreds proteins as demonstrated by mass spectrometry. Many of these proteins have been described as ISG'ylated proteins in earlier studies. One of such proteins is transcription factor STAT1, which is also a main activator of UBA7 expression. We demonstrated that in microglia stimulated by LPS STAT1 is ISG'ylated and the levels of both total and phospho-STAT1 decrease after UBA7 silencing. This is accompanied by premature termination of the immune response, as shown by the reduction of *iNos* and *Ccl5* expression. Those observations suggest that ISG'ylation increases stability of many proteins, including STAT1, that prevents premature termination of immune responses.

## P7.38

**Bacteriocins produced by lactic acid bacteria**

Anna Pyra<sup>1</sup>, Natalia Jazienicka<sup>2</sup>, Krzysztof J. Pawlik<sup>3</sup>, Ewa Brzozowska<sup>3</sup>

<sup>1</sup>University of Wrocław, Faculty of Chemistry, Department of Crystallography, Wrocław, Poland; <sup>2</sup>Wrocław University of Environmental and Life Sciences, Faculty of Food Science, Department of Biotechnology and Food Microbiology, Wrocław, Poland; <sup>3</sup>Polish Academy of Sciences, Institute of Immunology and Experimental Therapy, Wrocław, Poland  
e-mail: Anna Pyra <anna.pyra@chem.uni.wroc.pl>

Bacteriocins are ribosomally synthesized antibacterial molecules lethal to bacteria other than the producing strain (Galvez *et al.*, 2007, Joerger 2003). Purified bacteriocins could be used as preservatives or for reduction or elimination of certain pathogens. Bacteriocins produced by lactic acid bacteria (LAB) gained interest because they are produced by bacteria largely considered beneficial to human health and food production. Bacteriocins produced by LAB have relatively broad spectrum of antimicrobial activity and they are not active and not toxic to eukaryotic cells (Klaenhammer 1993). They are usually plasmid-encoded which allows genetic manipulation. The mechanism of action is not well known for bacteriocins but the most of the low molecular weight bacteriocins appear to interact with the bacterial membrane. They display activity or their activity increases after complexation with other bacteriocins. The final goal of our research is determination of the three-dimensional structures of low-molecular weight bacteriocins produced by LAB. It helps to know the mode of action of these small proteins. The genes of selected bacteriocins were cloned into several types of expression vectors and try to overexpressed using different strains of bacterial cells. These genes were modified by cutting of the short nucleotide sequences corresponding to signal-peptides in protein.

For our experiments, we chose bacteriocins completely unknown in terms of 3D structure. It is worth to note that there is no X-ray structure of bacteriocin produced by LAB in Protein Data Bank (PDB). There are only 23 bacteriocin's NMR structures synthesized by LAB. In those NMR structures the N-terminal region is usually followed by a well-defined central amphiphilic  $\alpha$ -helix followed by less well defined  $\alpha$ -helix in the C-terminal.

Here we present the results of cloning and overexpression of selected bacteriocins produced by *Lactobacillus sp.*

**References:**

- Galvez A *et al* (2007) *Int J Food Microbiol* **120**: 51–70.  
Joerger RD (2003) *Poult. Sci.* **82**: 640–647.  
Klaenhammer TR (1993) *FEMS Microbiol Rev.* **12**: 39–85.

## P7.39

**The crucial role of sphingosine kinases in regulation of dopaminergic cell survival exposed to alfa-synuclein oligomers. Implication in Parkinson's disease**

Joanna Pyszko, Anna Wilkaniec, Agata Adamczyk, Joanna B. Strosznajder

Mossakowski Medical Research Centre, Polish Academy of Sciences, Department of Cellular Signalling, Warsaw, Poland  
e-mail: Joanna Pyszko <asiapyszko@o2.pl>

Alpha-synuclein (ASN) is a presynaptic terminal protein, involved in the regulation of neuronal plasticity. In oligomeric form ASN promotes oxidative stress and leads to neuronal cell death in Parkinson's disease (PD). Our previous data indicated that oxidative stress activates ASN release from brain synaptoneuroosomes. The aim of the current study was to examine the effect of extracellular ASN in the native and mutated forms (E46K, A53T, A30P) on gene expression and activity of sphingosine kinase 1 (Sphk1), a key enzyme regulating the balance of the sphingolipids mediators and cell survival/death signaling. This study was carried out using dopaminergic cells: PC12 and human SH-SY5Y. The immunochemical, spectrofluorometrical and Real Time PCR methods were applied. Our data indicated that extracellular native and mutated ASN (0.5  $\mu$ M) in monomeric/oligomeric forms down-regulated Sphk1 gene expression/protein level and significantly reduced its activity. ASN and its mutated forms decreased dopaminergic cell survival in a concentration dependent manner. Subsequently, pharmacological Sphks inhibition, by using SKI II (5  $\mu$ M) evoked enhancement of ASN secretion, activation of gene expression for pro-apoptotic Bcl-2 proteins: Bax and BH3-only protein Hrk, cytochrome c release from mitochondria and caspase-dependent degradation of poly(ADP-ribose) polymerase (PARP-1). Reduced Sphk1 gene expression and activity was observed in our previous study, using 1-methyl-4-phenylpyridinium PD model. In this stress exogenous S1P (1  $\mu$ M) exerted neuroprotective effect, which was dependent on the S1P1 receptor stimulation. Summarizing, extracellular ASN in oligomeric form decrease gene expression and activity of Sphk1. On the other hand, Sphks inhibition leads to enhancement of ASN secretion and to caspase-dependent apoptotic neuronal death. Therefore, our results show the close relationship between extracellular ASN and Sphks inhibition and indicate Sphk1 as a molecular switch of neuronal cell survival and death. S1P and agonists of S1P receptor offer the promising strategy against ASN toxicity and should be considered in PD therapy.

**Acknowledgements**

Supported by NCN grant 5870/B/PO1/2011/40 and by The European Union under The European Social Fund.



## P7.40

### Redistribution of CD14 in LPS-stimulated macrophages

Agnieszka Płóciennikowska, Mykola I. Zdioruk, Gabriela Traczyk, Andrzej Sobota, Katarzyna Kwiatkowska

Nencki Institute of Experimental Biology, Warsaw, Poland  
e-mail: Agnieszka.Płóciennikowska <a.plociennikowska@nencki.gov.pl>

CD14 is an abundant cell surface glycoprotein of macrophages and other immune cells. It possesses a glycosylphosphatidylinositol (GPI) linker which anchors CD14 within cholesterol- and sphingolipid-rich microdomains of the plasma membrane. CD14 serves as an acceptor of lipopolysaccharide (LPS), a major constituent of the outer membrane of Gram-negative bacteria. CD14 transfers LPS to a complex of MD-2 protein and toll-like receptor 4 (TLR4). Activated TLR4 triggers signaling pathways which engage MyD88 or TRIF adaptor proteins and lead to expression of pro-inflammatory cytokines. To reveal mechanisms which govern an involvement of CD14 in TLR4 signaling, we followed dynamics of CD14 in LPS-stimulated macrophages. Laser scanning cytometry analysis showed that stimulation of cells with LPS led to a moderate elevation of the amounts of CD14 on the surface of J774 cells. LPS is likely to up-regulate trafficking of CD14 toward the plasma membrane from Golgi apparatus, as indicated by confocal microscopy analysis. In agreement, biochemical analysis revealed that CD14 was enriched in raft fractions at the onset of cell stimulation with LPS. To get insight into CD14 distribution in the plane of the plasma membrane, large sheets of the membrane were obtained by mechanical cleavage of cells and subjected for immunogold labeling and ultrastructural analysis. Prior to LPS stimulation, CD14 was dispersed in the plane of the plasma membrane and occupied its invaginations where caveolin 1, but not clathrin, was found. Stimulation of cells with 100 ng/ml LPS induced transient clustering of CD14 in the plasma membrane reflected by a shift of CD14-attributed gold labels from singlets to aggregates. CD14 clustering was fast and after 5 min of LPS action the amount of singular gold particles marking CD14 was reduced by 42 % and more than 17% of CD14 was accumulated in clusters of at least 10 particles. CD14 clusters co-localized with PI(4,5)P<sub>2</sub>, a plasma membrane lipid recruiting adaptor proteins crucial for TLR4 signaling. Specificity of LPS action was confirmed using antibody which inhibited binding of LPS to CD14-clustering of CD14 was abolished in these conditions. No clustering of GM1, raft-enriched ganglioside, was induced by LPS. Taken together, our studies indicate that clustering of CD14 is an initial step during stimulation of cells with LPS and CD14-enriched regions of the plasma membrane are involved in an initiation of signaling cascades of TLR4.

## P7.41

### Do LTA, UEA, MAA and PNA lectins influence binding of *Helicobacter pylori* to gastric mucins?

Iwona Radziejewska<sup>1</sup>, Małgorzata Borzym-Kluczyk<sup>2</sup>, Katarzyna Leszczyńska<sup>3</sup>

<sup>1</sup>Medical University of Białystok, Department of Medical Chemistry, Białystok, Poland; <sup>2</sup>Medical University of Białystok, Department of Pharmaceutical Biochemistry, Białystok, Poland; <sup>3</sup>Medical University of Białystok, Department of Microbiology, Białystok, Poland  
e-mail: iwona.radziejewska <iwona@umb.edu.pl>

*Helicobacter pylori* colonizes the gastric mucosa of about half of the world's population and is responsible for gastroduodenal diseases such as chronic gastritis, gastric and duodenal ulcers and also gastric cancers. It seems to be clear that both bacterial virulence factors and host susceptibility features play a role in the development of infection. *H. pylori* colonizes the gastric mucosa by adhering to the mucous epithelial cells and the mucous layer lining the epithelium. The best defined bacterial adhesins are the blood group binding adhesin (BabA) with affinity to Lewis b and H type 1 antigens and sialic acid binding adhesin (SabA) that binds sialyl dimeric Lewis x. Human Lewis antigens represent terminal modifications on mucins. Lewis b structure on MUC 5AC mucin seems to be the best proved receptor for *H. pylori* adhesins. Recently, the involvement of MUC 1 mucin in the interaction with the bacterium has been proposed [1, 2]. Lewis blood antigens are also expressed on the O-specific chains of the lipopolysaccharides (LPS) of *H. pylori*. This phenomenon can be understood as a kind of molecular mimicry between bacteria and host. This, in turn, may result in immune tolerance against antigens of the pathogen and can facilitate colonization. Moreover, it is known that *H. pylori* strains are able to adapt their outer membrane expression profile according to alterations in host environment, including changes in mucosal glycosylation patterns, by switching on and off specific gene expression [3]. The main aim of our study was to assess the effect of addition of four lectins on binding of *H. pylori* to gastric mucins. The lectins used in our experiments have high specificity to exact carbohydrate structures (that can be present on both mucins and *H. pylori* lipopolysaccharides. LTA (from *Lotus tetragonolobus* seeds) and UEA (from *Ulex europaeus* seeds) bind  $\alpha$ Fuc, MAA (from *Maackia amurensis* seeds) binds Neu5Ac $\alpha$ 3Gal $\beta$ 4GalNAc and PNA (from *Arachis hypogaea* peanuts) binds Gal $\beta$ GalNAc. The study was carried on gastric juices taken from patients after eradication treatment and on *babA2* genopositive and *babA2* genonegative *H. pylori* strains. To assess the binding of bacterium to gastric mucins ELISA test was used. We assumed that lectins used in the study could "block" potential carbohydrate receptors (on mucins) for bacterial adhesins. However, such inhibition was not observed in all examined samples.

#### References:

- Linden SK *et al* (2009) *PLoS Pathog* **5**: e1000617.  
Guang W *et al* (2010) *J Biol Chem* **285**: 20547–20557.  
Sheu SM *et al* (2007) *Infect Immun* **75**: 2661–2667.

## P7.42

### Personalized therapy of chronic lymphocytic leukemia

Małgorzata Rogalińska<sup>1</sup>, Paweł Góralski<sup>2</sup>, Jerzy Z. Błoński<sup>3</sup>, Jan Barciszewski<sup>4</sup>, Karolina Tarnowska<sup>1</sup>, Marta Wawro<sup>1</sup>, Katarzyna Woźniak<sup>5</sup>, Aneta Kocewa-Chyła<sup>6</sup>, Henryk Piekarski<sup>2</sup>, Tadeusz Robak<sup>3</sup>, Zofia M. Kilińska<sup>1</sup>

<sup>1</sup>University of Lodz, Faculty of Biology and Environmental Protection, Department of Cytobiochemistry, Łódź, Poland; <sup>2</sup>University of Lodz, Faculty of Chemistry, Department of Physical Chemistry, Łódź, Poland; <sup>3</sup>Medical University of Lodz, Department of Hematology, Łódź, Poland; <sup>4</sup>Polish Academy of Science, Institute of Bioorganic Chemistry, Poznań, Poland; <sup>5</sup>University of Lodz, Faculty of Biology and Environmental Protection, Department of Molecular Genetics Łódź, Poland; <sup>6</sup>University of Lodz, Faculty of Biology and Environmental Protection, Department of Thermobiology, Łódź, Poland  
e-mail: Małgorzata Rogalińska <gosiar@wp.pl>

B-chronic lymphocytic leukemia (CLL) is a common type of leukemia in Europe and North America with growing incidence of disease for younger people.

The general goal of therapies directed towards lymphoproliferative disorders, like CLL, is elimination of leukemic cells by apoptosis induction. Due to unpredictable clinical picture and personal patient's differences in anti-cancer treatment sensitivity, establishing optimal therapy for this type of leukemia sometimes reflects difficulties. Therefore, a special importance in case of patient's resistance to therapy is to search drug administration with potency to eliminate *in vitro* leukemic cells from patient's peripheral blood. The comparative analysis of CLL cells incubated with anticancer agents (purine analogs combine with alkylating agent mafosfamide or monoclonal antibody – rituximab) by cytometric analysis (cell viability, apoptosis level), thermal profiles by differential scanning calorimetry, and protein expression related to apoptosis (PARP and Mcl-1), could be useful in the most effective drug treatment choice or even monitoring patient's treatment *in vivo*.

Because of personal differences between patients in disease dynamics and the response to drugs registered to cure of this type of leukemia, it seems to be important to personalize therapy by choosing potentially effective type of treatment with ability to induce apoptosis before its *in vivo* administration to reduce ineffective patient's response to anticancer therapy.

## P7.43

### Mechanism of inhibition of human platelet aggregation by Lys-plasminogen

Yana M. Roka-Moiia, Dmytro D. Zhernossekov

Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine, Department of Enzyme Chemistry and Biochemistry, Ukraine  
e-mail: Yana Roka-Moya <yanulia@bk.ru>

Plasminogen/plasmin system takes part not only in fibrinolysis but also in regulation of functional state of different cells. Platelet membrane can provide a surface for assembly of plasminogen and its activators. On the surface of certain blood cells Glu-plasminogen is transformed into Lys-form, which possesses open conformation and can be more easily activated with the plasmin formation. We showed that exogenous Lys-plasminogen but not its native Glu-form inhibits platelet aggregation stimulated by ADP, thrombin and collagen. The aim of this work is to define the role of certain structural domains of plasminogen molecule in the observed inhibitory effect. One of the possible mechanisms of plasminogen interaction with the surface receptors is the binding of carboxyl-terminal lysines by lysine-binding sites (LBS) of plasminogen molecule. In our experiments lysine analogue, 6-aminohexanoic acid abolishes above mentioned inhibitory effect. Serine protease inhibitor, aprotinin (5.5 IU/ml) does not make any influence on the inhibitory effect of Lys-plasminogen during thrombin-induced platelet aggregation. The used concentration of aprotinin has no effect on platelet aggregation. Preincubation of washed platelets with K1-3, K4 and K5 taken into equimolar concentration with Lys-plasminogen completely abolishes inhibitory effect of the last one. The plasminogen kringle K5 added to the reaction mixture (washed platelets and 1.2  $\mu$ M Lys-plasminogen) even at concentration 0.12  $\mu$ M recovers platelet aggregation till 80%, whereas K1-3 and K4 at this concentration reach only near 60% as compared as control aggregation level. Plasminogen kringles K1-3, K4 and their combination have no influence on aggregation of washed platelets. There was no change in platelet aggregation in case of mini-plasminogen (K5 plus serine protease domain), where catalytic activity was inhibited by aprotinin or p-nitrophenyl-p'-guanidine benzoate. The observed effect of plasminogen kringles on the inhibition of platelet aggregation by Lys-plasminogen let us suggest that LBS of plasminogen kringles provide the plasminogen binding to the adhesive proteins of platelet surface (e.g. thrombospondin, vitronectin and fibrinogen). These proteins are secreted from  $\alpha$ -granules and remain bound to platelet surface. The binding of plasminogen kringles to these proteins may lead to the disturbance of protein-protein interaction which is the necessary condition for efficient platelet aggregation.

## P7.44

### The Membrane-Type Matrix Metalloproteinases MT3-MMP and MT5-MMP in human umbilical cord blood

Lech Romanowicz, Zofia Galewska, Tomasz Gogiel, Krzysztof Sobolewski

Medical University of Białystok, Department of Medical Biochemistry, Białystok, Poland  
e-mail: Lech.Romanowicz <lech.romanowicz@umb.edu.pl>

The vascular system of mother and placenta plays an important role in the intrauterine development of the fetus. Preeclampsia is a conditions characterized by systemic vascular endothelial dysfunction. Metalloproteinases (MMPs) are enzymes engaged in degradation of collagen and protein cores of proteoglycans, including those which bind peptide growth factors. The Membrane-Type Matrix Metalloproteinases (MT-MMPs) are extracellular matrix-degrading endopeptidases. Thus they exhibit a broad spectrum of substrate degradation including activation of some secreted MMPs such as MMP-2 (gelatinase A). MMP-2 is the main collagenolytic enzyme of both umbilical artery and vein. The activation of proMMP-2 is mediated on the cell surface by MT1, 2, 3 and 5-MMP. We decided to evaluate the presence, content and activity of MT3-MMP and MT5-MMP in the umbilical cord blood of preeclamptic patients. Since some MMPs may be released from blood cells during blood clotting it was decided to compare these proteinases in both the umbilical cord plasma and serum of healthy newborns and those delivered by mothers with preeclampsia. We used Western Immunoblot method and immunoenzymatic assay (ELISA) for detection of both metalloproteinases.

Umbilical cord blood plasma and serum of control and preeclamptic newborns contained MT3-MMP and MT5-MMP. Both enzymes existed in form of complexes with other extracellular matrix components and/or their tissue inhibitors in control and preeclamptic subjects. Free latent form of both MT-MMPs were observed after the action of reducing agent. Only trace of free active enzymes existed in control material. Furthermore, we found a huge increase in the amount of MT3-MMP in preeclamptic umbilical cord blood. MT5-MMP content increased also but in a lesser degree. Earlier studies showed no significant changes in MMP-2 active form presence in preeclamptic umbilical cord blood compared to control subjects. Simultaneous decrease in MT1-MMP observed earlier with an increase in content of both investigated MT-MMPs suggested the switch of main activating agent of MMP-2 between those membrane type metalloproteinases in preeclampsia.

## P7.45

### Does CacyBP/SIP influence the level of p-CREB via MAP kinase cascade?

Sara Rosińska, Wiesława Leśniak, Anna Filipek

Nencki Institute of Experimental Biology PAS, Warsaw, Poland  
e-mail: Sara.Rosinska <s.rosinska@nencki.gov.pl>

CacyBP/SIP has been shown to dephosphorylate ERK1/2 kinase which, in consequence, resulted in lower activity of the Elk-1 transcription factor (Kilianczyk E *et al.*, 2011, *Biochem Biophys Res Commun*). Since CacyBP/SIP is highly expressed in brain (Schneider & Filipek, 2011, *Amino Acids*) in the present work we examined the influence of CacyBP/SIP overexpression on transcription factors engaged in different aspects of neuronal activity such as SRF, CREB, AP-1, STAT-3, NFκB and NFAT. We used mouse neuroblastoma NB2a cells and applied two methods, dual luciferase reporter assay and Western blot analysis. Since preliminary data showed that CacyBP/SIP overexpression had a strongest effect on CREB (cAMP response element-binding) protein activity, we focused on analyzing this transcription factor in NB2a cells under normal conditions and after induction of cells with KCl, agent which increases the level of intracellular Ca<sup>2+</sup> ions and the level of phosphorylated form of CREB (p-CREB).

Using Western blot method we found that the level of p-CREB after CacyBP/SIP overexpression in NB2a cells was inhibited by about 50% and 75% in control and KCl treated cells, respectively. Moreover, the luciferase reporter assay revealed that the activity of CRE sequence was decreased by 15% and 33% in control and KCl treated cells, respectively.

Since CacyBP/SIP is considered to be a member of mitogen kinase phosphatase family (MKP), we analyzed its influence on the level of phosphorylated ERK1/2 (p-ERK1/2) and phosphorylated p38 (p-p38) kinases. The results showed that activity both kinases decreased after CacyBP/SIP overexpression. The activity of ERK1/2 was diminished by about 55% and 45% in control and KCl induced cells, respectively, while the activity of p38 was lower by about 75% and 56%, respectively. Therefore, our results indicate that CacyBP/SIP has an effect on CREB activity in neuroblastoma NB2a cells and suggest that the observed effects are due to down-regulation of the MAP kinase cascade.

#### Acknowledgements

This work was supported by grant from the National Science Center (NZ3/00425) and by statutory funds from the Nencki Institute of Experimental Biology.

## P7.46

### Identification of palmitoylated proteins involved in TLR4 signaling

Justyna Dembińska, Paula Roszczenko, Kinga Borzęcka, Katarzyna Kwiatkowska

Nencki Institute of Experimental Biology, Warsaw, Poland  
e-mail: Katarzyna Kwiatkowska <k.kwiatkowska@nencki.gov.pl>

Palmitoylation is a post-translation modification of proteins via a covalently bound palmitic acid (C:16) which is catalyzed by palmitoyl acyltransferase. In eukaryotic cells, the acyl chain is transferred onto thiol group of cysteine residues (*S*-palmitoylation) or, less frequently, onto hydroxyl group of serine and threonine residues (*O*-palmitoylation). This reversible modification controls translocation of proteins to plasma membrane microdomains enriched in cholesterol and sphingolipids (rafts), their stability, cellular trafficking of proteins and protein-protein interactions. We found that protein palmitoylation is crucial for pro-inflammatory signaling triggered by Toll-like receptor 4 (TLR4) in cells stimulated with lipopolysaccharide (LPS). An exposure of RAW264 macrophage-like cells to 50-250  $\mu$ M of 2-bromopalmitic acid (BPA), an inhibitor of palmitoyl acyltransferase, reduced in a dose dependent manner the production of pro-inflammatory cytokines, TNF- $\alpha$  and RANTES, stimulated with 100 ng/ml LPS. Therefore, we aimed to identify proteins which are palmitoylated in LPS-stimulated RAW264 cells. Traditional methods to study protein *S*-palmitoylation rely on isotopic labeling of cells, which requires special safety precautions and is time-consuming. We have applied a recently developed non-radioactive technique based on a “click chemistry” in two parallel approaches. In the first one, living RAW264 cells were labeled with  $\omega$ -alkynyl-palmitate analog of palmitic acid (17-ODYA), and after lysis, subjected to a “click-it” reaction with azido-azo-biotin catalyzed by  $\text{Ca}^{2+}$ . Subsequently, labeled proteins were bound to streptavidin beads and eluted with sodium dithionite which cleaved the azido-azo-biotin, and analyzed by immunoblotting. With this technique it was found that LPS induced palmitoylation of several proteins, including Lyn tyrosine kinase of the Src family. Palmitoylation of these proteins was inhibited in the presence of BPA. The second approach of detection of palmitoylated protein in LPS-stimulated cells was based on the fact that the activation of TLR4 by LPS occurs via CD14 located in plasma membrane rafts. Therefore, we decided to perform immunoprecipitation of CD14, preceded by labelling of RAW264 cells with 17-ODYA and followed by “click chemistry” in order to detect palmitoylated proteins which associate with CD14 in LPS-treated cells. Identification of these proteins will involve the mass spectrometry methodology.

## P7.47

### The role of tissue-nonspecific alkaline phosphatase and phospholipase D in mineralization

Monika Roszkowska<sup>1</sup>, Agnieszka Strzelecka-Kiliszek<sup>1</sup>, David Magne<sup>2</sup>, Sławomir Pikula<sup>1</sup>

<sup>1</sup>Nencki Institute of Experimental Biology, Department of Biochemistry, Warsaw, Poland; <sup>2</sup>Université Claude Bernard Lyon 1, Institut de Chimie et Biochimie Moléculaires et Supramoléculaires UMR CNRS 5246, Villeurbanne, France  
e-mail: Monika Roszkowska <m.roszkowska@nencki.gov.pl>

Although it is currently accepted that tissue-nonspecific alkaline phosphatase (TNAP) is responsible for the initiation of mineralization [1], the function of phospholipase D (PLD) in this process is still unclear. PLD is able to release phosphatidic acid which may promote budding of matrix vesicles at the early stages of mineralization as well as subsequent release of hydroxyapatite (HA) to the extracellular matrix (ECM) [2].

Physiological mineralization is a process by which HA is deposited in the ECM of bone tissue, whereas pathological mineralization occurs in soft tissues, especially in blood vessel walls. Vascular calcification, the most dangerous type of pathological mineralization, is a common phenomenon in aging. However, excessive calcification of blood vessels may also occur at earlier stages of life and have a dramatic impact on function of cardiovascular system. It is likely that mechanisms of physiological and pathological mineralization are similar. Numerous data suggest that vascular smooth muscle cells (VSMCs) are able to “trans-differentiate” into mineralization-competent cells, to express TNAP and finally, to mineralize ECM [3]. Pathological mineralization accompanies common diseases such as atherosclerosis, diabetes type 2, chronic kidney disease and bone tissue cancers.

The objective of this research was to compare mineralization properties of two mineralizing cell lines: human fetal osteoblasts (hFOB 1.19) and human bone osteosarcoma cells (Saos-2). Cells were stimulated for mineralization by osteogenic factors, ascorbic acid and  $\beta$ -glycerophosphate. The amount of released HA was measured in stimulated cells compared to resting cells by Alizarin Red Staining. The level of TNAP activity was determined by ALP ELISA Assay. In addition, a common inhibitor of TNAP – levamisole - was applied. Furthermore, the role of phospholipase D (PLD) was examined in these cell lines. The activity of PLD was determined by use of Amplex Red PLD Assay Kit. The two inhibitors of PLD were applied - ethanol and 1-butanol. Our findings demonstrated that the activities of both enzymes, TNAP and PLD, are at a lower level in normal cells compared to tumor cells.

#### References:

- Hessle L *et al* (2002) *Proc Natl Acad Sci USA* **99**: 9445–9449.
- Mebarek S *et al* (2013) *Int J Mol Sci* **14**: 5036–5129.
- Shao J S *et al* (2006) *Arterioscler Thromb Vasc Biol* **26**: 1423–1430.

#### Acknowledgements

The work was supported by a grant N401 140639 from Polish Ministry of Science and Higher Education.

## P7.48

### The effectiveness of chemotherapy and electrochemotherapy in human ovarian cell lines (OvBH-1 and SKOV-3) *in vitro*

Jolanta Saczko<sup>1</sup>, Justyna Piłat<sup>1</sup>, Julia Bar<sup>2</sup>, Anna Choromańska<sup>1</sup>, Nina Rembiałkowska<sup>1</sup>, Aleksandra Kuzan<sup>1</sup>, Iwona Kamińska<sup>2</sup>, Małgorzata Kotulska<sup>3</sup>, Julita Kulbacka<sup>1</sup>

<sup>1</sup>Department of Medical Biochemistry, Wrocław Medical University, Wrocław, Poland; <sup>2</sup>Department of Pathomorphology and Clinical Cytology, Medical University, Wrocław, Poland; <sup>3</sup>Institute of Biomedical Engineering and Instrumentation, Wrocław University of Technology, Wrocław, Poland  
e-mail: Jolanta Saczko <jolasak@gmail.com>

Ovarian cancer is one of the common and most lethal cause of gynecological tumor. Currently the aggressive surgery and standard chemotherapy are widely applied to improve the survival rate in patients with this cancer. The prognosis remains poor due to ovarian cancers are diagnosed in advanced stages. Thus there is a need for new therapeutic methods. Electrochemotherapy (ECT) is one of the new technique based on combination of electroporation (EP) and chemotherapy.

The aim of our investigation was the evaluation of the electrochemotherapy effectiveness in comparison to standard chemotherapy with bleomycin in two ovarian cancer cell lines. Two human ovarian cells lines were used: OvBH-1 — human clear ovarian carcinoma with silent mutation of P53 gene and resistant to chemo- and radiotherapy and SKOV-3 line — human ovarian carcinoma cells resistant to diphtheria toxin, cisplatin and adriamycin. In EP and ECT experiments different voltage values (from 0 to 1300 V/cm<sup>2</sup>), 8 pulse duration of 100µs, intervals between pulses 1s were used. The chemotoxicity of applied treatments was determined by MTT assay. The expression of heat shock protein — HSP27 was examined by immunocytochemistry and by western blot method.

The cytotoxicity with different concentrations of bleomycin was not significant decrease in both cell lines. The highest decrease was observed after EP with bleomycin after 72h of incubation for 1200 (V/cm<sup>2</sup>). The intensity of expression of small heat shock proteins HSP27 increased after ECT in both treated cell lines. The results indicated that electroporation effectively supports chemotherapy with bleomycin on human ovarian cells *in vitro*.

**Key words:** ovarian cancer, bleomycin, electroporation, HSP27.

## P7.49

### Effect of heme oxygenase-1 on C/EBPδ and C/EBPβ in myoblasts and rhabdomyosarcoma cell lines

Marta Seczyńska, Maciej Cieśla, Mateusz Jeż, Magdalena Kusior, Magdalena Kozakowska, Paulina Marona, Józef Dulak, Alicja Józkowicz

Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland  
e-mail: Marta Seczyńska <marta.seczyńska@uj.edu.pl>

Myogenic, adipogenic and osteogenic precursors originate from primitive mesenchymal stroma cells (MSCs). Muscle derived tumors, rhabdomyosarcoma (RMS), can exhibit traces of osteogenic and adipogenic differentiation in place of proper myogenesis. A group of transcription activators regulating these processes are CCAAT enhancer binding proteins (C/EBPs), with C/EBPβ which may attenuate myogenesis, and C/EBPδ which may promote it. Recently we have reported that heme oxygenase-1 (HO-1), the cytoprotective enzyme which degrades heme to produce biliverdin, iron ions and carbon monoxide (CO), inhibits myoblast differentiation, and is elevated in RMS with less favorable outcome. Here we analyzed the effect of HO-1 on C/EBP expression and activity.

All studies were performed in two *in vitro* models: in C2C12 murine myoblasts and in six RMS cell lines with ranging clinical aggressiveness. Some cell lines were genetically modified using retroviral or lentiviral vectors to overexpress the enzymatically active or inactive form of HO-1.

According to our findings, the C2C12 myoblasts with increased level of HO-1 expression display the decrease in nuclear C/EBPδ isoform as demonstrated by western blotting, and decrease in binding of C/EBPδ to MyoD promoter, as shown by chromatin immunoprecipitation. This phenomenon can be mimicked by incubation of cells with carbon monoxide releasing molecule (CORM). Importantly, overexpression of C/EBPδ reverses the inhibitory effect of HO-1 on myoblast differentiation, resulting in upregulation of MyoD and its downstream targets such as myogenin or myosin, and finally in formation of polynucleated myotubes (Kozakowska *et al.*, 2012, *Antioxid Redox Signal*).

In RMS we were not able to show differential expression of C/EBPδ between cell lines of different aggressiveness. Instead, we found that C/EBPβ isoform is upregulated in the malignant RMS cell lines. Moreover, forced overexpression of enzymatically active HO-1 in RMS led to upregulation of proliferative C/EBPβ. This was accompanied by the decrease in levels of myosin, marker of myogenic differentiation. In contrast, forced overexpression of enzymatically inactive HO-1 (H25A HO-1) was ineffective. To sum up, HO-1 regulates transcriptional activity of C/EBPδ in myoblast cell line. In RMS the more important seems to be the effect of HO-1 on C/EBPβ isoform.

## P7.50

### Biophysical properties of hippocalcin signaling in rat hippocampal neurons

Y. Y. Sheremet, V. P. Cherkas, A. V. Dovgan,  
N. I. Kononenko, T. M. Tsugorka, P. V. Belan

Bogomoletz Institute of Physiology, Department of General Physiology of the Nervous System, Ukraine  
e-mail: Yevhenii Sheremet <sheremetik@yandex.ru>

Hippocalcin is a neuronal  $\text{Ca}^{2+}$  sensor protein that signals in hippocampal neurons *via* translocation from the cytosol to the plasma membrane. In spite of importance of this signalling for many different cellular functions precise biophysical mechanisms of hippocalcin signalling are still unknown. In this study Lux-FRET approach was used to measure spatio-temporal pattern of hippocalcin insertion to the plasma membrane during translocation. We found some specific sites on the dendritic plasma membrane, sized from diffractionally limited to several microns, where the local hippocalcin insertion to the plasma membrane was higher than in neighbouring sites. We checked whether translocation at these specific sites are associated with regions of higher intracellular free calcium concentration ( $[\text{Ca}^{2+}]_i$ ). Creating spatially uniform  $[\text{Ca}^{2+}]_i$  transients in dendritic segments, we showed that hippocalcin translocation was significantly different in neighbouring sites having the same (in terms of kinetics and amplitude) patterns of  $[\text{Ca}^{2+}]_i$  changes. Producing long-lasting elevations of  $[\text{Ca}^{2+}]_i$  by activation of different  $\text{Ca}^{2+}$  mobilizing mechanisms, we also demonstrated that hippocalcin translocation was observed in the same set of sites independently of  $\text{Ca}^{2+}$  sources. These results indicate that  $[\text{Ca}^{2+}]_i$  is not the only determinant of hippocalcin translocation and that local differences in the plasma membrane affinity for hippocalcin are an important biophysical mechanism of hippocalcin signaling. Furthermore we have also developed original approaches for quantitative separate and simultaneous measurement of hippocalcin concentration in cytosolic and membrane cellular fractions of single living hippocampal neurons. Based on these approaches and simulation of  $\text{Ca}^{2+}$  and hippocalcin diffusion in the dendrites and spines of hippocampal neurons we have shown that hippocalcin concentration in dendritic membranes can be many times locally increased during intrinsic patterns of neuronal activity. Using FRAP-studies, we have obtained diffusion coefficients of hippocalcin in the cytosol and in the plasma membrane of dendrites, the kinetic constant of hippocalcin insertion into the plasma membrane and other parameters. These data allowed us to construct a quantitative biophysical model of hippocalcin cellular signaling in hippocampal neurons. We conclude that hippocalcin may serve as a site specific messenger with a high dynamic range allowing precise modulation of its targets.

## P7.51

### Bovine $\beta$ -casomorphin-5 levels determined in serum and urine of autistic children (autism spectrum disorders)

Natalia Smulska<sup>1</sup>, Michał Matysiewicz<sup>1</sup>,  
Marta Bukalo<sup>1</sup>, Barbara Chwała<sup>2</sup>, Małgorzata  
Moszyńska-Dumara<sup>2</sup>, Elżbieta Kostyra<sup>1</sup>

<sup>1</sup>University of Warmia and Mazury, Faculty of Biochemistry, Department of Biology & Biotechnology, Olsztyn, Poland; <sup>2</sup>Regional Children's Specialized Hospital, Olsztyn, Poland  
e-mail: Natalia Smulska <natalia.smulska@uwm.edu.pl>

Autism Spectrum Disorders (ASDs) are defined as a group of developmental disabilities characterised by impairment in social interactions and communication, and repetitive, stereotypical patterns of behaviour. ASDs, according to The Autism and Developmental Disabilities Monitoring (ADDM) Network, affect about 1 in 88 children in US (data for 2008).

The opioid-excess hypothesis of ASDs suggests that this dysfunction is the consequence of the incomplete breakdown and excessive absorption of peptides with opioid activity causing disruption to neuroregulatory and biochemical processes. Currently, scientist's attention is drawn to the similarity between the action of the opiates and symptoms of the ASDs. It has been proven that children who suffer from autism have increased the levels of opioid peptides, which are occurred in the central nervous system in humans. It was found that appearance of  $\beta$ -casomorphin-5 in the diet is leads to development of many diseases and psychological disorders. In significant number of people who suffer from autism is application of milk-free and gluten-free diet lessens clinical symptoms of ASDs.

According to one of theories disruptions in opioid system function may be possible cause of so called autistic behaviour. Elevated levels of both endogenous and exogenous ( $\beta$ -casomorphins; BCMs) opioid peptides were reported in sera of ASDs patients.

All experiments were approved by the Bioethics Committee. For the research we used the serum and urine of the children with diagnosed ASD. The samples were taken from Regional Children's Specialized Hospital in Olsztyn. The aim of this study was to determine the content of  $\beta$ -casomorphin-5 in the serum and urine of children, who suffer from autism.

Samples of serum and urine were tested for the presence of  $\beta$ -casomorphin-5 with ELISA test. We observed elevated levels of  $\beta$ -casomorphin-5 in serum in children with diagnosed ASD. We also demonstrated presence of  $\beta$ -casomorphin-5 in urine of children with ASDs. Nevertheless, opiate dependence and autism disorders in children should be confirmed by further studies.

## P7.52

### The role of apolipoprotein III in insect immune response to $\alpha$ -(1,3)-glucan

Sylwia Stączek<sup>1</sup>, Agnieszka Zdybicka-Barabas<sup>1</sup>, Aneta Sowa-Jasilek<sup>1</sup>, Adrian Wiater<sup>2</sup>, Małgorzata Pleszczyńska<sup>2</sup>, Jerzy Wydrych<sup>3</sup>, Paweł Mak<sup>4</sup>, Małgorzata Cytryńska<sup>1</sup>

<sup>1</sup>Department of Immunobiology, <sup>2</sup>Department of Industrial Microbiology, <sup>3</sup>Department of Comparative Anatomy and Anthropology, Faculty of Biology and Biotechnology, Maria Curie-Skłodowska University in Lublin, Lublin, Poland; <sup>4</sup>Department of Analytical Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University in Krakow, Kraków, Poland  
e-mail: Sylwia Stączek <s.staczek@poczta.umcs.lublin.pl>

Apolipoprotein III (apoLp-III), an important component of insect hemolymph, is considered an insect homologue of human apolipoprotein E. This multifunctional protein, involved in lipid transport and immune response in insects plays i.a. a role of a pattern recognition receptor. The binding of pathogen associated molecular patterns (e.g.  $\beta$ -glucan, LPS, lipoteichoic acid) is followed by cellular and humoral response activation, where apoLp-III has documented contribution. ApoLp-III acts also as a signalling molecule transmitting the information about the infection to the hemocytes. This protein may change the localization in the hemocytes in response to the penetration of pathogens into the body of an insect. Despite the comprehensive knowledge of apoLp-III participation in immune response to different pathogens and PAMPs, there is no information about apoLp-III involvement in immune response to  $\alpha$ -(1,3)-glucan. From our studies, it is known that  $\alpha$ -(1,3)-glucan, an important component of fungal cell wall, is recognized by insect immune system and triggers different types of immune reactions. We investigated apoLp-III participation in insect immune response to  $\alpha$ -(1,3)-glucan using the greater wax moth *Galleria mellonella* as a model organism. The objectives of our research were as follows: (i) testing ability of apoLp-III to binding  $\alpha$ -(1,3)-glucan, (ii) checking the influence of  $\alpha$ -(1,3)-glucan injection to insect hemocoel on apoLp-III level in hemolymph, (iii) determination of changes of apoLp-III localization in the hemocytes in response to  $\alpha$ -(1,3)-glucan. *In vitro* incubation of *G. mellonella* hemolymph with  $\alpha$ -(1,3)-glucan isolated from *Aspergillus niger* revealed binding of apoLp-III to  $\alpha$ -(1,3)-glucan molecules. This observation indicated that apoLp-III acts as PRR recognizing fungal  $\alpha$ -(1,3)-glucan. The changes in the level of apoLp-III after immunization of *G. mellonella* larvae with  $\alpha$ -(1,3)-glucan were examined using SDS-PAGE electrophoresis, as well as immunoblotting with anti-apoLp-III antibodies. The level of apoLp-III in the hemolymph of the  $\alpha$ -glucan-challenged larvae changed depending on the dose of  $\alpha$ -(1,3)-glucan and on the time after the immunization.

For apoLp-III immunolocalization, the adherent hemocytes obtained at a different time after immunization of the larvae with  $\alpha$ -(1,3)-glucan were incubated with anti-*G. mellonella*-apoLp-III antibodies and secondary FITC-labelled antibodies. The hemocytes were then imaged in a laser scanning confocal microscope. The observed changes in the localization of apoLp-III in the hemocytes after challenge of *G. mellonella* with  $\alpha$ -(1,3)-glucan suggested apoLp-III involvement, as a signalling molecule, in activation of immune reactions.

All of these results indicate that apoLp-III actively participates in insect immune response to fungal infections through recognizing and signalling the presence of  $\alpha$ -(1,3)-glucan.

#### Acknowledgements

The work was supported by grant 2013/09/N/NZ6/00838 (UMO-2013/09/N/NZ6/00838) from the National Science Center, Poland.

## P7.53

### Nucleic acid chaperone activity of retroviral proteins

Katarzyna Pachulska-Wieczorek<sup>1</sup>, Agnieszka K. Stefaniak<sup>1</sup>, Katarzyna J. Purzycka<sup>1</sup>, Leszek Błaszczuk<sup>2</sup>, Marcin Biesiada<sup>2</sup>, Ryszard W. Adamiak<sup>1,2</sup>

<sup>1</sup>Institute of Bioorganic Chemistry Polish Academy of Sciences, Department of Structural Chemistry and Biology of Nucleic Acids, Group of RNA Structure and Function, Poznań, Poland; <sup>2</sup>Poznań University of Technology, Institute of Computing Science, Poznań, Poland  
e-mail: Agnieszka Stefaniak <astef@ibch.poznan.pl>

Proteins with nucleic acid chaperone activity facilitate nucleic acids folding by destabilizing misfolded, kinetically trapped structures and enabling the formation of the thermodynamically most favored form. The HIV nucleocapsid domain of Gag and mature nucleocapsid protein (NC) act as a nucleic acid chaperone and are involved in critical steps of HIV replication, such as primer tRNA annealing, reverse transcription, vRNA dimerization and packaging, virion assembly and proviral integration into host DNA. Also Ty1 retrotransposon Gag protein lacking canonical NC domain is able to chaperone reverse transcription and dimerization. In addition, the transactivator of HIV transcription – Tat regulatory protein, may influence the remodelling and annealing of nucleic acids during viral splicing and reverse transcription. However it remains unclear if Tat protein displays nucleic acids chaperone activity.

We have comparatively investigated the *in vitro* nucleic acid chaperone properties of diverse retroviral and retrotransposon proteins: NC, Gag, Tat. We determined the ability of those proteins to chaperone nucleic acid aggregation, annealing and strand exchange in duplex structures. Two different experimental models were used to assay the *in vitro* chaperone activity of proteins. The first model mimics the annealing of the (-)ss DNA strand and acceptor RNA at the viral 3' UTR during the first strand transfer of reverse transcription. The second corresponds to the placement of tRNA<sup>Lys3</sup> on primer binding site (PBS) of HIV-2 RNA.

#### Acknowledgements

This study was supported by grants from the National Science Center [2011/01/D/NZ1/03478] and Ministry of Science and Higher Education [IP2012049272].

**P7.54****Distinct role of human annexin A6 isoforms in vesicle dynamics during mineralization**Lukasz Bozycki<sup>1</sup>, Agnieszka Strzelecka-Kiliszek<sup>1</sup>, Rene Buchet<sup>2</sup>, Sławomir Pikula<sup>1</sup><sup>1</sup>Nencki Institute of Experimental Biology PAS, Department of Biochemistry, Warsaw, Poland; <sup>2</sup>University Lyon 1, ICBMS CNRS UMR 5246, Villeurbanne, France  
e-mail: Agnieszka Strzelecka-Kiliszek <a.strzelecka-kiliszek@nencki.gov.pl>

Bone is a dynamic form of connective tissue composed of cells (osteoblasts, osteocytes and osteoclasts), extracellular matrix, providing tensile strength, and hydroxyapatite contributing to the mechanical resistance. Mineralization-competent cells release matrix vesicles (MVs) in which the initial steps of mineralization occur. It has been reported that the release of MVs is correlated with changes in cellular actin distribution and tissue-nonspecific alkaline phosphatase (TNAP) activity. In addition, annexins, a family of calcium- and membrane-binding proteins, were suggested to be involved in the Ca<sup>2+</sup> homeostasis of mineralizing cells and the Ca<sup>2+</sup> influx into MVs. Due to an alternative splicing, human AnxA6 exists in two isoforms, AnxA6-1 and AnxA6-2, the latter of which carries the 524-VAAEIL-529 deletion. The specific role of these two isoforms in mineralization remains unclear.

Two human cell lines, osteoblastic hFOB1.19 and osteosarcoma Saos-2, spontaneously releasing MVs, were used to determine the participation of AnxA6 isoforms in mineralization. Transfection of both types of cells with EGFP-AnxA6 isoform cDNA was performed and the mineralization process, stimulated with ascorbic acid and β-glycerophosphate, was monitored. TNAP activity measured by pNPP ELISA assay was 7-fold higher in osteosarcoma than in osteoblastic cells. Calcium nodule detection by Alizarin Red-S staining showed distinct influence of AnxA6 isoforms on cellular morphology and mineral formation. Spectrophotometric determination of Ca<sup>2+</sup> and Pi concentrations confirmed that AnxA6 isoforms were responsible for different chemical composition of the produced minerals. Immunohistochemical analysis of AnxA6 distribution in stimulated cells revealed an enrichment of both AnxA6 isoforms in plasma membrane of Saos-2 cells, whereas in hFOB1.19 cells these isoforms had different localizations: perinuclear for AnxA6-1 and cytoplasmic for AnxA6-2. F-actin, as determined by fluorescent microscopy, accumulated in focal contacts or under the membrane of mineral containing MVs and its rearrangement was necessary for MV biogenesis and mineral formation. Cytochalasin D and levamisole inhibited the mineralization process in AnxA6-1- as compared to AnxA6-2-overexpressing cells. In conclusion, we assume that different targeting of AnxA6 isoforms to specific cellular compartments, points to their distinct role in vesicle dynamics during mineralization.

**Acknowledgements**

This work was supported by a grant N N401 140639 from Polish Ministry of Science and Higher Education.

**P7.55****Mechanism of interplay between human ribosomal P1-P2 dimer and RTA**Przemysław Grela<sup>1,2</sup>, Xiao-Ping Li<sup>1</sup>, Barbara Michalec-Wawiórka<sup>2</sup>, Leszek Wawiórka<sup>2</sup>, Monika Szajwaj<sup>2</sup>, Eliza Molestak<sup>2</sup>, Kamil Deryło<sup>2</sup>, Monika Dźwierzyńska<sup>2</sup>, Nilgun E. Tumer<sup>1</sup>, Marek Tchorzewski<sup>2</sup><sup>1</sup>Department of Plant Biology and Pathology, School of Environmental and Biological Sciences, Rutgers University, New Brunswick, New Jersey, USA, <sup>2</sup>Department of Molecular Biology, Maria Curie-Skłodowska University, Lublin, Poland  
e-mail: Marek Tchorzewski <maro@hektor.umcs.lublin.pl>

The pentameric architecture of eukaryotic stalk represents a universally conserved entity across the entire domain where two copies of ribosomal P1-P2 protein dimers are regarded as its main functional elements. All P-proteins possess universally conserved C-terminal fragment directly involved in recruitment of external factors to the ribosome. To investigate the role of multiplication of highly conserved motif present at the C-terminal domain of all stalk P proteins, ricin A chain (RTA) which binds to the stalk to depurinate the sarcin/ricin loop (SRL), was used as a molecular probe. Using several structural forms of human P1/P2 protein dimers and their truncated variants we examined the interplay among individual C-terminal fragments of both proteins within the form of dimer and RTA. P1-P2 dimers bearing only one C-terminal domain on P1 protein were shown to have significantly higher level of interaction with RTA comparing to P1-P2 dimer where C-terminal polypeptide from P1 protein was deleted. Our results for the first time demonstrate, although the C-terminal polypeptide is absolutely required for proper binding of RTA to stalk proteins, the internal structural configuration of the intact P1-P2 hetero-dimer protein complex represents crucial component in the web of interactions with external factors like RTA molecule.



**P7.56****Correlation between central carbon metabolism and DNA replication in eukaryotic cells**

Karolina Sawiuk\*, Aneta Szczepańska\*, Aleksandra Konieczna, Robert Łyżeń, Grzegorz Węgrzyn

University of Gdansk, Department Biology, Molecular Biology, Poland

\*both authors contributed equally

e-mail: Aneta Szczepańska <aneta.szczepanska@biol.ug.edu.pl>

Central carbon metabolism and DNA replication are crucial biochemical processes occurring in cells of all eukaryotic organisms. Nevertheless, a very limited number of studies have been carried out to explain the cell growth regulation in relation to genetic material replication. We aimed to test a possible link between central carbon metabolism and DNA replication regulation in human fibroblasts. Expression of selected genes, coding for enzymes involved in central carbon metabolism, were silenced by using siRNA, and kinetics of DNA replication was monitored in such cells relative to controls. We found significant differences in the time of entering the S phase by control cells and fibroblasts in which expression of the gene coding for either isocitrate dehydrogenase 2 or fumarase hydratase was silenced by specific siRNA. Namely, impairment of expression of the isocitrate dehydrogenase 2 stimulated the cell cycle, while inhibition of fumarase hydratase production had an inhibitory effect. Moreover, silencing the gene coding for STAT3, which influenced expression of genes coding for p53 and p27 proteins, caused significant perturbations in DNA replication regulation. These results suggest that changes in central carbon metabolism, and in transcription of some genes, significantly influence regulation of DNA replication in human fibroblasts.

**P7.57****Nucleolar localization of phosphoglycerate mutase as a cell proliferation marker**

J. Wiśniewski<sup>1</sup>, A. Gizak<sup>1</sup>, M. Grenda<sup>1</sup>, P. Mamczur<sup>1</sup>, J. Silberring<sup>2</sup>, D. Rakus<sup>1</sup>

<sup>1</sup>Department of Animal Molecular Physiology, University of Wrocław, Wrocław, Poland; <sup>2</sup>Department of Biochemistry and Neurobiology, AGH University of Science and Technology, Kraków, Poland  
e-mail: Janusz Wiśniewski <jwis@biol.uni.wroc.pl>

Over the last twenty years several lines of evidence has accumulated that that evolutionary primeval enzymes of carbohydrate metabolism may localize not only in cell cytoplasm but also in other cellular structures such as mitochondria, nuclei or nucleoli. It has been also demonstrated that in these compartments, their function is not restricted to the catalytic activity but they play a role in the regulation of cell proliferation and differentiation. One of those enzymes is phosphoglycerate mutase (PGAM) – a glycolytic enzyme catalyzing the transformation of 3-phosphoglycerate into 2-phosphoglycerate. Proteomic studies has demonstrated its presence in nucleoli, however its role in these compartments have not been established yet. Results presented here shed light on the role of PGAM in nucleoli and show that the enzyme may be engaged in ribosomal RNA transcription.

During our research we found that PGAM localized in nucleoli of proliferating cancer, the KLN-205, cells. Withdrawal of serum from the culture medium, which stops cell proliferation, caused removal of PGAM from the nucleoli. The re-supplementation of culture medium with serum or addition of insulin and/or IGF-1 resulted in the returning of PGAM to nucleoli. This nucleolar import was blocked by inhibition of phosphatidylinositol kinase – a protein engaged in transduction of signal in the insulin/IGF-1 pathway. This demonstrates that nucleolar localization of PGAM is connected with high metabolic/proliferatory activity of the cell.

The results of immunocytochemical experiments showing nucleolar localization of PGAM were confirmed by MS analysis of proteins isolated from nucleoli of proliferating cells. On the other hand, the MS experiments did not demonstrate the presence of PGAM in nucleoli of non-dividing cells.

The results presented here show a relationship between the nucleolar localization of PGAM and rRNA synthesis and the capability of cells to divide and grow.

## P7.58

### The effect of the exogenous cholesterol on fibroblast-to-myofibroblast transition in bronchial fibroblasts derived from asthmatic patients

Dawid Wnuk<sup>1</sup>, Milena Kosińska<sup>1</sup>, Monika Rak<sup>1</sup>, Katarzyna A. Wójcik<sup>1,2</sup>, Zbigniew Madeja<sup>1</sup>, Jarosław Czyż<sup>1</sup>, Marta Michalik<sup>1</sup>

<sup>1</sup>Jagiellonian University, Faculty of Biophysics, Biochemistry and Biotechnology, Department of Cell Biology, Kraków, Poland;

<sup>2</sup>Jagiellonian University Medical School, Department of Medicine, Kraków, Poland

e-mail: Dawid Wnuk <dwunuk.dwnuk@gmail.com>

The development of bronchial asthma is determined by two processes: chronic inflammation and bronchial wall remodeling in the respiratory tract resulting from local overproduction of cytokines and growth factors, mainly TGF- $\beta_1$ . Prolonged exposure of human bronchial fibroblasts (HBFs) to TGF- $\beta_1$  leads to the surplus of extracellular matrix (ECM) components and phenotypic shifts towards myofibroblast phenotype resulting in subepithelial fibrosis [1]. Multiple studies have demonstrated the increased propensity of “asthmatic” bronchial fibroblasts to myofibroblastic transition (FMT), in comparison to fibroblasts derived from non-asthmatic patients [2]. However, the mechanisms underlying these phenotypic shifts still remain unclear. Previously, we have demonstrated that inhibitors of cholesterol biosynthesis pathway attenuate TGF- $\beta_1$ -induced FMT in “asthmatic” HBFs in a manner dependent on intracellular cholesterol level [3]. The intracellular cholesterol levels depend on the activity of cholesterol biosynthesis and LDL-endocytosis. Consequently, we examined the effect of the exogenous cholesterol on the intensity of TGF- $\beta_1$ -induced FMT in “asthmatic” HBFs. Our data show the correlation between the increased magnitude of TGF- $\beta_1$ -induced phenotypic shifts undergone by “asthmatic” HBFs and reduced levels of extracellular lipids. Moreover, TGF- $\beta_1$ -induced increase of  $\alpha$ -SMA expression in HBFs cultured in the absence of lipids is independent of the canonical TGF- $\beta$ /Smad signaling. It may depend on the non-canonical TGF- $\beta$  pathways which involve lipid raft-dependent ERK or Akt signaling.

#### References:

1. Makinde *et al* (2007) *Immunol Cell Biol* **85**: 348–356.
2. Michalik *et al* (2009) *Med Sci Monit* **15**: 194–201.
3. Michalik *et al* (2013) *Europ J Pharmacol* **704**: 23–32.

## P7.59

### Expression of VEGFs and its receptors in abdominal aortic aneurysm

Malgorzata Wolanska<sup>1</sup>, Emilia Bankowska-Guszczyn<sup>1</sup>, Krzysztof Sobolewski<sup>1</sup>, Radoslaw Kowalewski<sup>2</sup>

<sup>1</sup>Medical University of Białystok, Department of Medical Biochemistry, Białystok, Poland; <sup>2</sup>Medical University of Białystok, Department of Vascular Surgery and Transplantology, Białystok, Poland  
e-mail: Malgorzata Wolanska <ma.wolanska@interia.pl>

**Background:** The development of abdominal aortic aneurysm (AAA) is thought to be related to an imbalance of VEGF and its receptors. This study aims to evaluate the expression of VEGF family members and their receptors in AAA wall, AAA mural thrombus and normal aorta wall.

**Materials and methods:** AAA specimens (mural thrombus — luminal layer, mural thrombus — abluminal layer, AAA wall) were collected from 24 patients undergoing elective open AAA repair. Abdominal aortas from 12 organ donors served as controls. The expression of VEGF (VEGF-A, VEGF-B, VEGF-C and VEGF-D) and VEGF receptors (VEGFR-1, VEGFR-2 and VEGFR-3) was evaluated using Western blot.

**Results:** Increased expression of VEGF-B, VEGF-C and VEGF-D was found in the AAA wall compared to normal aorta. No significant difference in VEGF-A expression was demonstrated between aortic wall samples. The expression of all investigated VEGFs was increased in the mural thrombus of AAA compared to normal aorta. Furthermore, the expression of VEGF-C and VEGF-D was significantly increased in the abluminal layer of the mural thrombus. Increased VEGFR-1 expression was found in the mural thrombus, particularly in the abluminal layer. All aneurysm samples expressed higher levels of VEGFR-2 and VEGFR-3.

**Conclusions:** Our study demonstrates that the abluminal layer in AAA mural thrombus contains high levels of VEGFs and VEGF receptors, suggesting that this layer may play a significant role in AAA disease pathogenesis. Increased expressions of VEGF and their receptors in AAA mural thrombus may impact different pathways involved in AAA etiology.

## P7.60

### The oxidative stress-associated Src activator and c-Src tyrosine kinase-a novel targets in the mechanism of photodynamic therapy

Marta Woźniak, Kamila Duś-Szachniewicz,  
Barbara Ziółkowska, Piotr Ziółkowski

Department of Pathology, Wrocław Medical University, Wrocław, Poland  
e-mail: Marta Woźniak <marta1wozniak@wp.pl>

Photodynamic therapy is an effective method in the diagnosis and treatment of various tumors, but still the detailed molecular mechanisms and pathways are not well known. In response to the cytotoxic influence of PDT, cells activate and inhibit different proteins that prevent them from reactive oxygen species (ROS). It has been reported the oxidative stress-associated Src activator/ *Homo sapiens* chromosome 9 open reading frame 10 protein (Ossa/ C9orf10) protects cancer cells from an oxidative stress-induced apoptosis by Src family kinases activation. The main aim of the present study was investigating whether the PDT leads to Ossa expression and if this may be connected with co-expression of c-Src tyrosine kinase. Additionally, apoptosis and necrosis following an oxidative stress caused by PDT on cancer cells were analyzed. For these purpose, Ossa and c-Src proteins were examined in MCF-7 human breast cancer cells after 5-aminilevulinic acid-mediated photodynamic therapy. The effects of PDT on the expression of the above proteins were detected in different time points (0, 7, 18, 24 hours after irradiation) using immunohistochemistry and Western blot analysis. Our results showed a high expression of Ossa at early time interval after PDT, which was accompanied by a low expression of c-Src tyrosine kinase. This relation between expression of two above proteins indicate that Ossa could protect cancer cells from PDT through activation of c-Src in response to an oxidative stress. In conclusion, analyzed proteins may be targets to achieve better efficiency of PDT treatment.

## P7.61

### Role of mRNA binding proteins in BRCA1 mRNA translation in leukemia cells

Magdalena Wolczyk<sup>1\*</sup>, Paulina Podszywalow-Bartnicka<sup>1\*</sup>,  
Tomasz Skorski<sup>2</sup>, Margaret Nieborowska-Skorska<sup>2</sup>,  
Krzysztof Skowronek<sup>1,3</sup>, Katarzyna Piwocka<sup>1</sup>

<sup>1</sup>Nencki Institute of Experimental Biology PAS, Warsaw, Poland; <sup>2</sup>Temple University, Philadelphia, United States; <sup>3</sup>The International Institute of Molecular and Cell Biology, Warsaw, Poland

\*join the first authorship

e-mail: Katarzyna Piwocka <k.piwocka@nencki.gov.pl>

The BCR-ABL1 oncoprotein plays a major role in the development and progression of chronic myeloid leukemia (CML). To study the role of BCR-ABL1, we employed mouse progenitor cell line expressing high level of BCR-ABL1 corresponding to drug-resistant cells from blast crisis of the disease. We previously demonstrated that CML progression correlates with increased aneuploidy resulting from affected mitosis. This was caused by BRCA1 downregulation leading to decreased expression of protein members of spindle assemble checkpoint [1]. BRCA1 tumor suppressor regulates crucial cellular processes involved in DNA damage repair and cell cycle control. In this study we investigated the mechanisms responsible for BCR-ABL1-mediated BRCA1 downregulation. Herein we found that downregulation of BRCA1 protein is associated with enhanced half-life and increased levels of BRCA1 mRNA in a BCR-ABL1 transformed cell line and in CML primary cells from patients. Lowered luciferase synthesis level under BRCA1 3'UTR control indicated that BRCA1 mRNA translation is affected in BCR-ABL1 expressing cells. Silencing and overexpression studies suggested that mRNA binding proteins regulate BRCA1 mRNA translation and stability. Studies based on site directed mutagenesis revealed that phosphorylation pattern plays a key role in BRCA1 mRNA translation in BCR-ABL1 expressing cells. Currently we use BRCA1 Stellaris RNA FISH probes and SmartFare RNA probes to visualize BRCA1 mRNA together with mRNA binding proteins. Altogether, we showed a novel mechanism affecting BRCA1-dependent signaling in CML, in which BCR-ABL1 expressing cells modulate translation of BRCA1 mRNA, leading to protein downregulation. This may ultimately contribute to genomic instability and provide justification for targeting PARP1 and/or RAD52 to induce synthetic lethality in CML.

#### Reference:

Wolanin K *et al* (2010) *Mol Cancer Ther* 9: 1328–1338.

#### Acknowledgements

This work was supported by grants: IP2011 043071 from the Polish Ministry of Science and Higher Education and 2011/01/B/NZ3/02145 from the National Science Centre.

## P7.62

### The new model of mRNA degradation based on the recent advances in the DcpS enzyme specificity towards m<sup>7</sup>GDP

Anna Wypijewska del Nogal<sup>1</sup>, Marius D. Surleac<sup>2</sup>, Joanna Kowalska<sup>1</sup>, Maciej Lukaszewicz<sup>1</sup>, Janusz Stepinski<sup>1</sup>, Richard E. Davis<sup>4</sup>, Martin Bisaillon<sup>5</sup>, Jacek Jemielity<sup>1,3</sup>, Edward Darzynkiewicz<sup>1,3</sup>, Adina L. Milac<sup>2</sup>, Elzbieta Bojarska<sup>1,3</sup>

<sup>1</sup>University of Warsaw, Division of Biophysics, Warsaw, Poland; <sup>2</sup>Institute of Biochemistry of the Romanian Academy, Department of Bioinformatics and Structural Biochemistry, Romania; <sup>3</sup>University of Warsaw, Centre of New Technologies, Warsaw, Poland; <sup>4</sup>University of Colorado, Department of Biochemistry and Molecular Genetics, United States; <sup>5</sup>University of Sherbrooke, Department of Biochemistry, Canada  
e-mail: Anna Wypijewska del Nogal <wypijewska.del.nogal@gmail.com>

Decapping Scavenger (DcpS) enzyme was reported to hydrolyze the free 5' mRNA cap (m<sup>7</sup>GpppN) in the 3'→5' mRNA decay pathway, following mRNA degradation by exosome exonuclease. The turnover of m<sup>7</sup>GpppN by DcpS generates 7-methylguanosine monophosphate (m<sup>7</sup>GMP) and nucleoside diphosphate (NDP). DcpS was also shown to play a role in the 5'→3' mRNA decay pathway, where it facilitates the Xrn1 exonuclease activity. However, in this case, the cap hydrolysis is performed by another pyrophosphatase, Dcp2, yielding 7-methylguanosine diphosphate (m<sup>7</sup>GDP) and monophosphate mRNA.

Here, using HPLC and fluorescence titration assays, we show that DcpS is not able to hydrolyze m<sup>7</sup>GDP, although it binds m<sup>7</sup>GDP with a high association constant. This finding is of biological significance. Therefore, we proved it on DcpS enzymes from different species: humans, model organisms (*C. elegans* and *S. cerevisiae*) and a parasitic nematode organism (*A. suum*). Moreover, we found that m<sup>7</sup>GDP is a competitive inhibitor of DcpS enzymes. Computational approaches such as molecular modeling and docking of m<sup>7</sup>GDP, m<sup>7</sup>GpppG and several other cap analogs were used to explain m<sup>7</sup>GDP resistance to the DcpS-mediated hydrolysis and to identify molecular determinants for its efficient binding. Furthermore, we show that DcpS enzymes are able to hydrolyze 7-methylguanosine triphosphate (m<sup>7</sup>GTP) *in vitro*, releasing m<sup>7</sup>GMP and a diphosphate. This suggests that DcpS might possibly be involved in m<sup>7</sup>GDP turnover, provided m<sup>7</sup>GDP is initially converted to m<sup>7</sup>GTP.

Overall, we postulate that DcpS specificity towards m<sup>7</sup>GDP may play a regulatory role in both the 3'→5' and 5'→3' mRNA decay pathways, affecting activity of other cap-binding proteins, such as Dcp2 or eukaryotic translation initiation factor, eIF4E. This is important for proper understanding of DcpS function in norm and diseases such as spinal muscular atrophy or cancer. The new model of mRNA degradation will be presented, highlighting that DcpS enzyme is a regulator of the mRNA decay pathways at the level of m<sup>7</sup>GDP metabolism.

## P7.63

### Silencing of parafusin inhibits the secretory pathway

Elzbieta Wyroba<sup>1</sup>, Li Liu<sup>2</sup>, Birgit H. Satir<sup>2</sup>

<sup>1</sup>Nencki Institute of Experimental Biology, Warsaw, Poland; <sup>2</sup>Anatomy and Structural Biology Department, Albert Einstein College of Medicine, New York, USA  
e-mail: Elzbieta Wyroba <e.wyroba@nencki.gov.pl>

Parafusin (PFUS) is an important signaling component for regulatory control of the secretory pathway and the new protein — cloned by us in 1994 (Subramanian SV *et al.*, 1994, *Proc Natl Acad Sci USA* **91**: 9832–9836) — in the evolutionary conserved phosphoglucomutase (PGM) superfamily. Though it is 50.7% identical to rabbit muscle PGM has four insertions and two deletions and negligible enzymatic activity. PFUS attaches to secretory vesicles docked underneath cell surface and undergoes phosphogluco- sation/dephosphorylation during exocytosis. Subsequently it relocates to the newly formed secretory vesicles to enable a next Ca<sup>2+</sup>-triggered exocytic event. These cyclic post-translational modifications are essential for unique PFUS function (Satir BH *et al.*, 1989, *Proc Natl Acad Sci USA* **86**: 930–932; Andersen AP *et al.*, 1994, *Biophys Res Commun* **200**: 1353–1358; Levin S *et al.*, 1999, *Protein Eng* **12**: 737–746) and are reflected in the results obtained after 2D electrophoresis of this protein upon immunoprecipitation with pan-PGM antibody. Six spots at Mr 63 kDa with pI's from 6.8 to 6.3 were detected whereas immunoblot analysis with a PFUS specific peptide antibody (to insertion 2 not present in PGM) revealed only two spots of pI 6.7 and 6.5, respectively.

To elucidate the details of PFUS intracellular targeting three different gene constructs were created to perform gene silencing by feeding in model eukaryote *Paramecium* that produced different phenotypes. The effect of RNAi was followed by Western blot analysis and quantification of PFUS versus PGM combined with double-labeling experiments in the confocal microscopy using two specific antibodies: against PFUS and the content of the dense core secretory vesicles (DCSVs). RNAi experiments proved that knockdown of PFUS inhibits dense core secretory vesicle synthesis with minor or no effect on other PGM isoforms. Thus a proper incorporation of PFUS into the scaffold is indispensable for maintenance and maturation of the DCSVs and subsequently for Ca<sup>2+</sup> regulated exocytosis (Liu L *et al.*, 2011, *Eur J Cell Biol* **90**: 844–853) and isolated DCSVs contain glycosylated PFUS in their scaffold (Liu L *et al.*, 2009, *Eur J Cell Biol* **88**: 301–313). PFUS or its orthologs are present in many eukaryotic cells (Wyroba E & Satir BH, 2000, *Biochem Cell Biol* **78**: 683–690; Wyroba E *et al.*, 1995, *Eur J Cell Biol* **68**: 419–426) and its *Toxoplasma* counterpart was also identified (Matthiesen SH *et al.*, 2001, *Eur J Cell Biol* **80**: 775–783; Matthiesen SH *et al.*, 2003, *Cell Microbiol* **5**: 613–624). Based on the PFUS/PGM sequence analysis against archea and eubacteria sequences we postulate that PFUS appeared early in evolution acquiring a new function apart from the glycolytic one represented in PGM superfamily of proteins.

## P7.64

### High-mannose type N-glycosylation of $\beta$ integrins in human leukocytes is influenced by rapamycin

Marta Ząbczyńska<sup>1</sup>, Ewa Pocheć<sup>1</sup>,  
Katarzyna Bocian<sup>2</sup>, Anna Lityńska<sup>1</sup>

<sup>1</sup>Institute of Zoology, Department of Glycoconjugate Biochemistry, Jagiellonian University, Kraków, Poland; <sup>2</sup>Institute of Zoology, Department of Immunology, Faculty of Biology, University of Warsaw, Warsaw, Poland  
e-mail: Marta Ząbczyńska <marta.zabczynska@uj.edu.pl>

The interactions mediated by N-glycans are crucial in immune cells. Most surface glycoproteins exist in the fully processed form, it means that they contain mainly the complex type N-glycans. Although more and more research have shown that the mannose-rich oligosaccharides remaining at low levels in mammalian cell, also play an important role in their physiology. Our previous results shown that immunosuppressive drugs, rapamycin (RAPA) and cyclosporine A (CsA) increase the high-mannose glycosylation in some proteins in allogeneic stimulated mixed leukocyte reaction (MLR) (Pocheć *et al.*, 2012). The purpose of our present study was to identify RAPA affected proteins using mass spectrometry (MS). We focused on one of the band (102 kDa) from three with increased GNA-positive glycosylation in MLR culture. MS identified proteins for this band belong to  $\beta$  integrin family;  $\beta 3$  subunit (score: 6137) and  $\beta 1$  integrin precursor subunit (score: 636). To confirm these results we used endoglycosidase H which removes mannose-rich oligosaccharides from proteins. The results demonstrated the high-mannose N-glycans are presented on the  $\beta 3$  subunits in allogeneic stimulated and non-stimulated leukocytes, but only in MLR cells we observed two forms of this integrin. What is interesting, only the mature protein is abundant in mannose-rich oligosaccharides, while the precursor variant does not show the presence of this type of glycans. The precursor and mature forms we found also in  $\beta 1$  integrin, but unlike to  $\beta 3$  subunit, their expression is not dependent on allogeneic stimulation. Endoglycosidase digestion showed that the mature  $\beta 1$  subunit did not expressed high-mannose glycans in contrast to premature precursor glycoprotein, which is strongly high-mannose glycosylated.

To the best of our knowledge, this is the first work that shows the effect of immunosuppressive drugs on leukocyte glycosylation. High-mannose structures are expressed on mature variant of  $\beta 3$  and precursor of  $\beta 1$  integrins. Taking into account the significant role of glycans in immune system there is no doubt that RAPA-dependent alternations in high-mannose glycosylation of  $\beta$  integrins have the physiologic relevance in immunosuppressive state.

#### References:

Pocheć E, Bocian K, Popek M, Lityńska, A (2012) *FEBS J* **279**: 249.

#### Acknowledgments

This work was supported by grants: K/ZDS/004193 (JU), IP2010003370 (UW) and 'Bratniak' Foundation of the JU Students and Graduates.

## P7.65

### *Cact-2* and *dorsal-2* mRNA expression in the development of *Apis mellifera*

Ewa A. Zaobidna<sup>1</sup>, Krystyna Żółtowska<sup>1</sup>,  
Elżbieta Łopieńska-Biernat<sup>1</sup>

University of Warmia and Mazury, Department Biology and Biotechnology, Poland  
e-mail: Ewa Zaobidna <ewazaobidna@gmail.com>

Honeybees (*Apis mellifera*) are important ecosystem element as pollinators. They are also producers of several products valuable for humans. The global population of *A. mellifera* is decreasing rapidly. Numerous factors affect this phenomenon: viral, bacterial, fungal and parasitic diseases, chemical-like pesticides (van Engelsdorp & Meixner, 2010). The immunological response of insects involves the Toll pathway. Insect Toll and the Toll-like receptors (TLRs) are transmembrane signal transducing proteins that play critical roles immunity. Following conformational changes of the activated receptor, several intracellular proteins are recruited to form a receptor complex. Activation of this complex leads to the degradation of the NF kappa B inhibitor (I $\kappa$ B) Cactus and subsequent nuclear translocation of the NF- $\kappa$ B transcription factor Dorsal (Valanne *et al.*, 2011). The genes responsible for the synthesis of these receptors are *cact-2* (GB13520) and *dorsal-2* (GB18032).

The aim of the study was analysis of expression of genes: *cact-2* and *dorsal-2*, during development of *A. mellifera*.

The material included 5 larval stages of *A. mellifera*: larvae (L8), pre-pupae (PP), two stages of pupae (P3, P4) and newly emerged imago. RNA was isolated from tissues with kit total miniRNA (A&A Biotechnology, Poland). cDNA synthesis and amplification was done with TransScriba Kit and StartWarm 2xPCR Master Mix (A&A Biotechnology). Quantitative real-time PCR was performed using SYBR-Green PCR-MIX Taq™ (A&A Biotechnology) according to the manufacturer's instructions. The mean value  $\pm$  SD was used for analysis of relative transcript's levels for each time point using the  $\Delta\Delta$ Ct method. The data were analyzed and normalized relative to *rp49* transcript levels by  $\Delta\Delta$  analysis software (7500v2.0). All samples were tested in triplicate with lightcycler (Applied Biosystem, FAST7500). We demonstrated the first time, the presence of expression of mRNA *cact-2* and *dorsal-2* during developmental stages of *A. mellifera*. Up-regulated expression of *cact-2* and *dorsal-2* genes was observed in development in all studied stages. The pattern of *cact-2* and *dorsal-2* genes expression demonstrates the increase of immune activity during development of *A. mellifera*. The highest expression of these genes was detected in imago stage (*cact-2*: 83-times; *dorsal-2*: 13,3-times).

#### References:

Valanne S *et al* (2011) *J Immunol* **186**: 649–656.

Van Engelsdorp D, Meixner MD (2010) *J Invertebrate Pathol* **103**: 80–95.

**P7.66****Effect of TGF $\beta$ 1 signaling pathway inhibition on skeletal muscle myoblasts**

Paulina Kasprzycka, Marta Gawor, Katarzyna Ilach,  
Maria A. Ciemerych, Małgorzata Zimowska

University of Warsaw, Faculty of Biology, Department of Cytology,  
Warsaw, Poland

e-mail: Małgorzata Zimowska <mzimowska@biol.uw.edu.pl>

Matrix metalloproteinases (MMPs) are enzymes involved in many physiological and pathological processes. Their proteolytic activity is essential during the activation and deactivation of signaling molecules. These enzymes affect tissue architecture through the proteolysis of extracellular matrix components, also in repair of damaged skeletal muscles. The crucial role in the regeneration of muscle tissue repair is played by two of metalloproteinases: MMP-9 and MMP-2. Their activity is regulated by TGF $\beta$ 1 and increased during skeletal muscle regeneration. However, the interactions of these factors are not specified. Our recent studies suggest that MMPs plays a role not only in the extracellular matrix transformation, but also in the proliferation and differentiation regulation of myoblasts (cells responsible for the reconstruction of damaged muscle fibers). TGF $\beta$ 1 represses activity of myogenic regulatory factors, expression of genes encoding proteins specific to the skeletal muscle, and thus blocks differentiation of these cells. In our project we inhibited TGF $\beta$ 1 signaling pathway by reducing receptor 1 transforming growth factor  $\beta$  (TGF $\beta$ R1) biological activity. Next, we identified the MMP-9 and MMP-2 activity and also interactions between MMPs and TGF $\beta$ 1 in myoblasts isolated from Soleus muscles. In our study we also tested how the inhibition of TGF $\beta$ R1 impacts on the regulation of differentiation in skeletal muscle myoblasts.

**P7.67****Effect of TGF $\beta$ 1 signaling pathway inhibition of MMP-9 and MMP-2 activity during myoblasts differentiation**

Paulina Kasprzycka, Marta Gawor, Katarzyna Ilach,  
Maria A. Ciemerych, Małgorzata Zimowska

University of Warsaw, Faculty of Biology, Department of Cytology,  
Warsaw, Poland

e-mail: Małgorzata Zimowska <mzimowska@biol.uw.edu.pl>

Matrix metalloproteinases (MMPs) are enzymes involved in many physiological and pathological processes. Their proteolytic activity is essential during the activation and deactivation of signaling molecules. These enzymes affect tissue architecture through the proteolysis of extracellular matrix components, also in repair of damaged skeletal muscles. The crucial role in the regeneration of muscle tissue repair is played by two of metalloproteinases: MMP-9 and MMP-2. MMPs plays a role not only in the extracellular matrix transformation, but also in the proliferation and differentiation regulation of myoblasts (cells responsible for the reconstruction of damaged muscle fibers). MMP-9 and MMP-2 activity is regulated by transforming growth factor beta 1 (TGF $\beta$ 1) and increased during skeletal muscle regeneration.

TGF $\beta$ 1 is a member of the TGF- $\beta$  superfamily, which exerts wide range of biologic effects. This cytokine is involved in cell proliferation, differentiation, extracellular matrix production, and cell migration. In skeletal muscle TGF $\beta$ 1 promotes fibrosis during regeneration, represses expression of genes encoding proteins specific to the skeletal muscle, and thus blocks differentiation of myoblasts. However, the interactions of MMP-9, MMP-2 and TGF $\beta$ 1 in myoblasts are not specified.

We inhibited TGF $\beta$ 1 signaling pathway by reducing biological activity of receptor 1 transforming growth factor  $\beta$  (TGF $\beta$ R1). Next, we identified the MMP-9 and MMP-2 activity and also interactions between MMPs and TGF $\beta$ R1 in myoblasts isolated from Soleus muscles. In our study we also tested how the inhibition of TGF $\beta$ R1 impacts on the regulation of differentiation in skeletal muscle myoblasts.