

Posters

P21.1

Gap and tight junctions present within the basal ectoplasmic specialization are primary targets for flutamide action in adult rat testis

Barbara Bilinska¹, Katarzyna Chojnacka¹, Anna Hejmej¹, Marta Zarzycka¹, Wacław Tworzydło², Alicja Kaminska¹, Laura Pardyak¹

¹Institute of Zoology, Department of Endocrinology, Jagiellonian University in Krakow, Kraków, Poland; ²Institute of Zoology, Department of Developmental Biology and Morphology of Invertebrates, Jagiellonian University in Krakow, Kraków, Poland
Barbara Bilinska <barbara.bilinska@uj.edu.pl>

Present study was designed to establish a causal connection between changes in the cell-cell junction protein expression at the blood-testis barrier (BTB) and alterations in the adult rat testis histology following an anti-androgen flutamide exposure. Particular emphasis was placed on the basal ectoplasmic specialization (bES) in the seminiferous epithelium and expression of gap and tight junction proteins, connexin 43 (Cx43) and zonula occludens (ZO-1). Flutamide (50 mg/kg b.w.) was administered to male rats daily from 82 to 88 postnatal day. Testes from 90-day-old control and flutamide-treated rats were used for all analyses. Gonadal morphology was assessed by light and electron microscopy. Gene and protein expressions were analyzed by qPCR, Western blotting and immunohistochemistry, and steroid hormone concentrations determined radioimmunologically. Seminiferous epithelium of both groups of rats displayed normal histology without any morphologically recognizable loss of germ cells. Examination of semithin and ultrathin sections has shown that the length of the bES connecting neighboring Sertoli cells and the number of gap and tight junctions were apparently reduced in flutamide-exposed rats. Moreover, the appearance of unconventional circular ES indicated enhanced internalization and degradation of these junctional complexes. The changes were accompanied by decreased Cx43 and ZO-1 expression ($p < 0.01$) and a loss of linear distribution of the proteins at the region of the BTB. On the other hand, Cx43 expression in the interstitial tissue of flutamide-treated rats increased ($p < 0.01$), which could be associated with Leydig cell hypertrophy. Concomitantly, both intratesticular testosterone and estradiol concentrations were elevated ($p < 0.01$), but testosterone to estradiol ratio decreased significantly ($p < 0.05$) in flutamide-treated rats compared to controls.

In conclusion, short-term treatment with flutamide applied to adult rats exerts its primary effect on the bES, coexisting junctional complexes, and their constituent proteins Cx43 and ZO-1, without any apparent morphological alterations in the seminiferous epithelium. In the interstitial compartment, however, short-term exposure leads to both histological and functional changes of Leydig cells, involving altered steroidogenic activity and increased Cx43 expression. Of note, these observations suggest diverse mechanisms of flutamide action in different cellular targets within the testis of adult rat.

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P21.2

A novel mutation of the *SPTB* gene: a truncated beta-chain responsible for dominant hereditary spherocytosis

Dżamila M. Bogusławska¹, Elżbieta Heger¹, Beata Machnicka¹, Michał Skulski³, Kazimierz Kuliczkowski², Aleksander F. Sikorski³

¹University of Zielona Góra, Faculty of Biological Sciences, Department of Molecular Biology, Poland; ²Wrocław Medical University, Department of Haematology, Blood Neoplasms and Bone Marrow Transplantation, Wrocław, Poland; ³University of Wrocław, Biotechnology Faculty, Department of Cytobiochemistry, Wrocław, Poland
Aleksander F. Sikorski <afsbc@ibmb.uni.wroc.pl>

Hereditary spherocytosis (HS) is the most common inherited haemolytic anaemia among people of Northern European ancestry (1 in 2000 individuals), characterized by increased fragility of erythrocytes and spheroidal red blood cells, which are prematurely trapped in the spleen. Anaemia, jaundice, splenomegaly, reticulocytosis, gallstones and spherocytes on a blood smear are the typical HS manifestations. This anaemia is heterogenous at the clinical and biochemical levels. In the majority of cases HS is dominantly inherited and the mutations are located in the genes encoding the following membrane proteins: ankyrin, α - and β -spectrins, anion exchanger 1 and protein 4.2. Twenty one known, mostly missense and frameshift *SPTB* gene mutations responsible for human cases of HS are located in the sequence encoding the N-terminal and the central part of the β -spectrin.

During our studies on HS cases in the population of Western Poland we found a new mutation in family with HS. RBC protein abnormalities and molecular basis in HS two-generation family origin with clear autosomal dominant inheritance pattern were investigated. Three HS patients with moderate symptoms of the disease were recruited for this study: mother (C10, splenectomized), son (C14) and daughter (C9), age ranged from 27 to 67.

We report on a new mutation, insertion of a G into codon 466 of the *SPTB* gene, leading to a frameshift and a premature stop codon 29 codons downstream in the region encoding the dimerization domain (the first two spectrin repeats) which is close to the N-terminal region of the β -spectrin subunit consisting of actin-binding domain. Frameshift mutations of the β -spectrin gene associated with hereditary spherocytosis have been previously reported and in some cases the mutant mRNA could not be detected or was detected in decreased levels. Most probably instability of mutant mRNA and monoallelic expression of β -spectrin gene results in spectrin deficiency and clinically moderate to serious hereditary spherocytosis in the reported family. This in consequence is a reason for a decreased surface density of the membrane skeleton and disturbed support of the membrane lipid bilayer and hereditary spherocytosis.

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P21.3

Pseudomonas donghuensis P482 antagonism towards plant pathogenic fungus *Rhizoctonia solani* – a link between antifungal and antibacterial activities

Magdalena Jabłońska, Adam Ossowicki, Sylwia Jafra

Intercollegiate Faculty of Biotechnology of University of Gdansk and Medical University of Gdansk, Department of Biotechnology, Gdańsk, Poland

Sylwia Jafra <sylwia.jafra@biotech.ug.edu.pl>

Pseudomonas spp. is a very ubiquitous and genetically diversified genus which includes plant pathogenic as well as plant beneficial strains. The beneficial *Pseudomonas* species inhabiting the rhizosphere can play important ecological roles because of their ability to protect plants by the production of a set of antimicrobials which significantly inhibit various plant pathogenic microorganisms. Investigations of the genetic mechanisms which determine these antagonistic activities often result in finding the undescribed genes or gene clusters relevant to the interesting features. *Pseudomonas donghuensis* P482, a tomato rhizosphere isolate, is one of the two strains of the newly established species. Despite the absence of any of the known genes encoding for well-characterised secondary metabolites of pseudomonads (except HCN) it exhibits a broad and strong antimicrobial activity towards fungal (e.g. *Rhizoctonia solani*, *Fusarium culmorum*, *Verticillium dahliae* and fungal-like oomycetes *Pythium ultimum*) and bacterial (e.g. *Dickeya solani*, *Pectobacterium carotovorum* subsp. *brasiliense*) plant pathogens. The results of our studies suggested that antifungal effect of P482 shares similar genetic background as its bacterial antagonism as both activities result from a unique combination of genes. Former *in silico* analyses unveiled the new gene cluster crucial for the antibacterial activity of P482 strain (Krzyżanowska *et al.*, 2016). The previously selected insertion mutants of P482 affected in the antibacterial activity towards soft rot bacteria proved to fail also in antifungal activity against *R. solani* in a *dual-culture* assay. Additionally, the involvement of volatiles produced by P482 was confirmed in top-bottom assays with common plant pathogenic fungi. A mutant in one gene of the cluster mentioned above expressed a significant decrease in the ability to inhibit the growth of *R. solani*, *F. culmorum* and *P. ultimum*. We therefore state that the antimicrobial activities of P482 share the same genetic pathway leading not only to the synthesis of the antibacterial factor(s) towards soft rot bacteria but also to the synthesis of antifungal metabolite(s). Studies aiming at the evaluation of the chemical nature of the antimicrobial compounds of *P. donghuensis* P482 are in progress.

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P21.4

Slow- and fast-growing *Mycobacterium* species differentially regulate cytokine expression in human monocyte- and macrophage-like cells

Iwona Karwaciak, Lukasz Pulaski

Institute of Medical Biology of PAS, Laboratory of Transcriptional Regulation, Poland

Iwona Karwaciak <isachrajda@cbm.pan.pl>

Mycobacteria can be categorized into *M. tuberculosis* complex and nontuberculous mycobacteria (MOTT). It can be further classified by growth rate.

Monocytes and macrophages play important roles in host defence against mycobacterial infections. Both cell types not only phagocytose and kill microbes through the production of reactive oxygen intermediates in their respiratory bursts, but also influence the immune response by modulating cytokine production.

Cytokines have an essential role in the modulation of the immune response and, to a large extent, determine the course of a disease. Therefore we investigated the cytokine response induced by species of heat-killed *Mycobacterium*. In this study, we were predominantly interested in the binding of mycobacteria to monocytes and macrophages as a measure of the recognition of mycobacterial ligands by these cell types. As binding must precede ingestion, the studies reported here do not differentiate binding from ingestion.

We sought to characterize in the human like system the similarities and differences between major *Mycobacterium* species differed in their pathogenicity, including *M. tuberculosis*, *M. avium*, *M. smegmatis*, *M. abscessus*, *M. marinum*, *M. goodii*, *M. kansasii*.

To determine the ability of heat-killed Mycobacteria to stimulate cytokine expression in human monocyte- and macrophage-like cells, undifferentiated and differentiated THP-1 and Mono Mac 6 cells were exposed to Mycobacteria. Cell lines were differentiated for 48 h: THP-1 with PMA, Mono Mac 6 with Vitamin D3 and TGF- β 1 and treated with 100 μ g/ml heat-killed Mycobacteria for 6 h.

Heat-killed, fast growing Mycobacteria: *M. abscessus* and *M. smegmatis* induced lower level of TNF- α and IL-1 β than slow growing Mycobacteria: *M. tuberculosis*, *M. avium*, *M. marinum*, *M. goodii*, *M. kansasii* in undifferentiated and differentiated THP-1 cells. In macrophage-like cells we observed more pronounced induction of gene expression for IL-6, particularly in differentiated Mono Mac 6 cells. *M. marinum* exhibited, additionally, high potency to induce gene expression of IL-10 and IL-12B.

We concluded that there are differences in recognized structures between fast and slow growing mycobacteria and this modulate immune cells response and may contribute to disease severity.

P21.5

Expression patterns of aquaporin transcripts in different organs of estivated snail *Helix pomatia* L.

Ewa Kosicka¹, Hanna Kmita², Andrzej Lesicki¹, Joanna R. Pieńkowska¹

¹Adam Mickiewicz University in Poznań, Faculty of Biology, Department of Cell Biology, Poznań, Poland; ²Adam Mickiewicz University in Poznań, Faculty of Biology, Laboratory of Bioenergetics, Poznań, Poland
Ewa Kosicka <ewatom@amu.edu.pl>

Aquaporins facilitate transport of water and small hydrophilic molecules through membranes. The proteins have been characterized in many organisms of all kingdoms of life and are known to share the conserved structure. They are homotetramers, with each monomer containing one water pore. Two separate subfamilies of aquaporins are distinguished, namely orthodox water channels called aquaporins (AQPs) which are only able to transport water, and glycerol-transporting channels called aquaglyceroporins (AQGPs) which beside water also transport glycerol, urea, and other small solutes. Aquaporins play an important physiological role in osmoregulation and control of body hydration. For example, it has been shown that AQPs and AQGPs are critical for dehydration, cold, and freezing tolerance of insects. However, molluscan aquaporins are poorly sampled. This data scarcity is surprising, especially when it comes to different habitats they live in and consequent water management.

Our research consisted in identification of cDNA sequences encoding putative *Helix pomatia* (Roman snail) aquaporins, functional analysis of predicted proteins based on complementation assay in the yeast *Saccharomyces cerevisiae* cells and estimation of the encoding transcript levels in the snail organs during osmotic stress related to an estivation state by real-time PCR reaction. The yeast mutant ($\Delta fps1$) applied for complementation assay is depleted of *FPS1* gene encoding AQGP and possess two nonfunctional AQPs. Consequently, the experimental system allows for detection of transport activity of heterologously expressed proteins. Based on the transport capability analysis concerning water, glycerol and H_2O_2 as well as bioinformatics analysis the identified proteins were divided into AQPs and AQGPs. During estivation, synthesis of majority of proteins is down-regulated and metabolic depression occurs. However, the observation did not applied to the studied aquaporins. Some of them maintained the same expression level as during normal physiological activity whereas others were even upregulated. In addition, the pattern of aquaporin transcript expression was different for tested organs. This suggests that the identified aquaporins contribute differently to protection mechanisms against osmotic stress during the estivation state in *H. pomatia*. Thus, the results may be important for explanation of the mechanisms crucial for survival of terrestrial snails.

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P21.6

Selected biochemical parameters and blood morphology changes in Polish paralympic rowers after the progressive efficiency test on rowing ergometer during the preparatory stage before Paralympic Games Rio 2016 – a case study

Dorota Kostrzewa-Nowak^{1,4}, Robert Nowak^{2,4}, Jerzy Eider³

¹University of Szczecin, Faculty of Physical Culture and Health Promotion, Department of Modern Movement Forms, Szczecin, Poland; ²University of Szczecin, Faculty of Physical Education and Health Promotion, Department of Biological Bases of Physical Culture, Szczecin, Poland; ³University of Szczecin, Faculty of Physical Culture and Health Promotion, Department of Individual Sports, Szczecin, Poland; ⁴University of Szczecin, Faculty of Physical Culture and Health Promotion, Functional and Structural Human Research Centre, Szczecin, Poland

Dorota Kostrzewa-Nowak <dorota.kostrzewa-nowak@usz.edu.pl>

Long-term intensive physical effort influence athletes' cellular metabolism, can lead to muscle damage, and induce oxidative stress causing metabolic changes on different levels. Adaptive rowing represents an endurance discipline of sport for athletes with physical disabilities. The aim of the study was to evaluate changes in biochemical and haematological parameters of two highly skilled rowers during preparatory stage to Paralympic Games Rio 2016. Polish rowing crew TAmix2x (double scull, man and woman, only use of trunk muscles) qualified to Paralympics Rio 2016 participated in the study. They performed progressive test until exhaustion on rowing ergometer. Before, just after the test, and in the recovery time (17 hours after the test) blood samples were taken by venepuncture. Whole blood was used for morphology and percentages of lymphocyte subsets: total T lymphocytes, helper/inducer T (Th) lymphocytes, suppressor/cytotoxic T (Tc) lymphocytes, B lymphocytes and natural killer (NK) cells determination. Blood serum was used to determine metabolites (glucose, creatinine, urea, uric acid and bilirubin), albumin, total protein, lipid profile, enzymes activities (aminotransferases: aspartate (AST) and alanine (ALT), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), creatine kinase (CK), lactate dehydrogenase (LDH) and amylase) and selected jones. An extreme effort performed in this study caused changes in all studied metabolites. The highest changes were found in both, total and direct bilirubin levels in both participants. No changes in GGT, ALT, CK, amylase activities in women's serum, ALT, LDH, GGT, ALP activity in men's serum were observed. An increase in AST activity after the test but ca. 2-fold decrease during the recovery time in both rowers were found. It was also found that white blood cell levels after the test were almost twice as high as before the effort in both rowers. Interestingly, the percentages of NK cells were higher, and total T lymphocytes were lower after the test but there were higher percentage of Tc and lower percentage of Th lymphocytes subpopulation. No changes in B lymphocytes distribution in both participants were observed. The different changes on physiological, biochemical and immunological levels are related to the adaptive mechanism on physical effort providing to increase in aerobic and anaerobic performance of athletes. Therefore it is crucial to monitor and adapt training individually for each athlete.

P21.7

Testicular protein NWC (c11orf74 homolog) – the search for its role in spermatogenesis

Michał Majkowski¹, Agnieszka Łaskiewicz¹, Łukasz Śnieżewski¹, Sylwia Janik¹, Violetta Kapuśniak², Paweł Kisielow¹, Małgorzata Cebrat¹

¹Hirschfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Department of Tumor Immunology, Laboratory of Molecular and Cellular Immunology, Weigla 12 str., 53-114 Wrocław, Poland; ²Wrocław University of Environmental and Life Sciences, Department of Histology and Embryology, Chair of Biostructure and Physiology, Wrocław, Poland
Agnieszka Łaskiewicz <cebrat@itd.pan.wroc.pl>

NWC gene reveals unique structure and both strong sequence conservation as well as close association with RAG (Recombination Activating Genes) locus in vertebrate evolution, however its function remains unknown. Our previous studies revealed that NWC co-immunoprecipitates with cytoskeletal proteins and most of them are involved in the formation and functioning of primary cilia. We also found NWC ciliary localization in NIH-3T3 cells. In mouse NWC protein at Western Blot detectable level has been found in testis so far. We present here results showing NWC expression in cell populations that form testis functional unit: seminiferous epithelium. To further characterize NWC function NWC-deficient mice were generated. They are viable, have no obvious morphological defects and are fertile in standard laboratory conditions. In order to fertilize an egg sperm cell must undergo a massive exocytosis event termed acrosome reaction (AR) which might be triggered *in vitro* by calcium ionophore. We found that, after AR induction, population of acrosome reacted sperm cells isolated from NWC-deficient mice is reduced when compared to control WT mice. The question if such reduction affects fertilization rate in conditions mimicking sperm competition in female genital track, that occurs in mice natural populations, remains open. Interestingly NWC is not present in sperm cells what indicates its engagement in spermatogenesis process rather than in regulation of acrosome reaction.

P21.8

Effects of R90P and E240K substitutions in tropomyosin Tpm1.1 on the thin filament regulation

Małgorzata Śliwińska, Katarzyna Robaszekiewicz, Joanna Moraczewska

Kazimierz Wielki University in Bydgoszcz, Department of Biochemistry and Cell Biology, Faculty of Natural Sciences, Poland
Joanna Moraczewska <moraczjo@ukw.edu.pl>

Binding of Ca^{2+} to troponin complex (Tn) induces changes in the position of tropomyosin (Tpm) on actin filament (F-actin), which regulate actin-myosin interactions. Direct interactions of Tpm with actin and Tn are involved in the regulation, but the mechanism is still not fully understood [1]. Tpm is a coiled-coil protein, which binds to actin through seven periodic sites containing charged residues located outside the coiled-coil that are suited for electrostatic interactions with actin surface [2]. The C-terminal segment of Tpm is engaged in binding to Tn, but the exact Tpm-Tn interactions are not known.

In the present work two missense mutations in TPM1 gene were introduced. The first substitution – R90P, is located within the third actin-binding period, in the Tpm-actin interface. The second substitution – E240K, is in the region of Tpm-Tn interactions within the sixth actin-binding period, but outside the Tpm-actin interface.

Wild type and mutant Tpm were expressed in BL21 cells, purified and tested using *in vitro* assays. The affinity of Tpm for F-actin was measured using a co-sedimentation assay. R90P and E240K reduced Tpm affinity for F-actin alone 3 and 4-fold, respectively, showing that not only R90, but also E240 are important for interactions of Tpm with actin alone. Binding in the presence of Tn and $\pm\text{Ca}^{2+}$ compensated for the negative effect of the mutation in E240. In contrast, the substitution R90P strongly reduced actin affinity even in the presence of Tn. The effect of mutation on regulation of actin-myosin interactions was assessed by measurements of actin-activated myosin S1 ATPase activity. In contrast to the inhibition of the ATPase in the absence of Ca^{2+} , which was close to normal, the activation of the ATPase by Ca^{2+} in the presence of both Tpm mutants was abolished.

In conclusion, for the regulation of contraction not only the correct Tpm-actin, but also Tpm-Tn interactions are required. Single substitutions in functionally important sites of Tpm have a global effects on the function of tropomyosin molecule.

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P21.9

Regulatory mechanisms of the two related plasmid-borne restriction-modification systems of an Arctic *Psychrobacter* sp. strain

Robert Lasek, Dariusz Bartosik

University of Warsaw, Faculty of Biology, Institute of Microbiology, Department of Bacterial Genetics, Warsaw, Poland
Robert Lasek <lasek@biol.uw.edu.pl>

Restriction-modification (R-M) systems are commonly considered as a specific defence mechanism of prokaryotic cells against exogenous DNA. The classic model of their action assumes that the protection of the host's DNA against the nucleolytic activity of restriction endonucleases (REases) is conferred by methyltransferases (MTases) which methylate cytosines or adenines in specific sequences. The foreign DNA which lacks this kind of modification is degraded by REases. R-M systems are ubiquitous in bacterial genomes and present a decisive impact on horizontal gene transfer events – the main driving force of the prokaryotic evolution.

Given their biological role, the genetic modules of this kind have to accommodate a number of functions: (i) the maintenance of the restriction activity against the exogenous genetic material invading the cell, (ii) the inhibition of the potential "autotoxic" behaviour of REases, and (iii) the maintenance of an adequate level of the host's DNA methylation. For this reason they tend to be controlled by fine-tuned regulatory mechanisms (Mruk & Kobayashi, 2014). Here we present the preliminary results of the project aimed at the elucidation of the regulatory mechanisms of the two closely related type II R-M systems identified in plasmid pP62BP1 of an Arctic strain *Psychrobacter* sp. DAB_AL62B (Lasek *et al.*, 2012). *In vivo* and *in silico* analyses that we have performed suggest that the activity of the systems is under control of a complex regulatory network. It encompasses (i) the function of MTases encoded therein (MTase-DNA interactions in regulatory sequences and cytosine methylation in CCNGG sequences, influencing the activity of the component gene promoters) as well as (ii) the effect of a potential regulatory RNA transcribed from P_{REV} promoters which are located within the genes encoding REases.

A regulatory mechanism of such a level of complexity has not been described for any other R-M system to date. Taking into consideration a high level of mutual similarity between the pP62BP1-encoded R-M systems, the studied case constitutes a unique experimental set-up for the further investigation of possible regulatory cross-talk in control mechanisms of gene expression of both modules.

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P21.10

Epigenetic mechanisms involved in expression regulation of Epidermal Differentiation Complex (EDC) genes

Wiesława Leśniak, Barbara Sobiak

Nencki Institute of Experimental Biology, Department of Molecular and Cellular Neurobiology, Warsaw, Poland
Wiesława Leśniak <w.lesniak@nencki.gov.pl>

Epidermal differentiation is a process during which keratinocytes acquire morphological and molecular features that enable them to form a tight physical barrier that protects the organism from environmental insults. In particular, in differentiating keratinocytes, there is a huge increase in expression of specialized epidermal proteins, many of them encoded by genes located in the Epidermal Differentiation Complex (EDC) on human chromosome 1. So far, there is almost no information on epigenetic mechanisms that facilitate activation of EDC gene expression during epidermal differentiation. In this work we have examined changes in DNA methylation and histone modifications in selected gene promoters and putative regulatory regions within EDC during differentiation of primary human keratinocytes and keratinocyte-derived non-carcinogenic cell line, HaCaT. Our results show that there are no major changes in DNA methylation pattern in the analyzed regions, with only single cytosine residues undergoing demethylation upon differentiation. Analysis of histone modifications within promoters of late cornified envelope (LCE) genes, which form a subcluster within EDC, by chromatin immunoprecipitation (ChIP), revealed an altered histone modification pattern. The most pronounced change was demethylation of lysine 9 in histone 3 (H3K9), which probably contributed to activation of LCE1 genes transcription during epidermal differentiation. Thus, we plan to examine the effect of overexpression of histone modifying enzymes (e.g. H3K9 methyltransferase) on the expression of LCE1 and other genes located in EDC in differentiating keratinocytes.

P21.11

Telomerase inhibitor TMPyP4 reduces adhesion and lifespan of human breast cancer cells and alters doxorubicin-mediated toxicity

Natalia Lipińska¹, Aleksandra Romaniuk¹, Mariusz Kaczmarek², Błażej Rubiś¹

¹Department of Clinical Chemistry and Molecular Diagnostics, Poznan University of Medical Sciences, 49 Przybyszewskiego St., 60-355 Poznań, Poland; ²Department of Immunology, Chair of Clinical Immunology, Poznan University of Medical Sciences, Rokietnicka 5D, 60-806 Poznań, Poland

Natalia Lipińska <nat.lipinska@gmail.com>

Human telomeric sequence was one of the first discovered and characterized sequences forming G-quadruplex structure. Association of these structures with oncogenic and tumor suppressor proteins suggests its important role in cancer development and treatment. Since cationic porphyrin TMPyP4 is known as a G-quadruplex stabilizer and telomerase inhibitor the aim of the study was to analyze the mechanisms of anticancer activity of this compound in human breast cancer MCF7 and MDA-MB-231 cells.

The cytotoxic effect of TMPyP4 alone and in combination with doxorubicin was measured by MTT and colony formation assays. We found that TMPyP4 potentiated the cytotoxic effect of DOX especially at higher concentrations (i.e. > 50 µM), and when applied alone, it was significantly less toxic in non-cancer MCF12A cells. TMPyP4 caused cell cycle arrest in G0/G1 phase or G2/M phase depending on a cell line and led to changes in corresponding cyclins and cyclin-dependent kinases levels. Interestingly, TMPyP4 attenuated the effect of G2/M arrest triggered by doxorubicin. We revealed that TMPyP4 inhibited both telomerase expression and activity, that suggested two different mechanisms of action – due to direct blocking of G-quadruplex structures at telomeres or through regulation of hTERT gene expression. Moreover, neither TMPyP4 nor 0.1 µM doxorubicin led to senescence of cancer cells. Scratch and adhesion assays showed that telomerase inhibition was accompanied by decreased adhesion and migration properties of breast cancer cells. TMPyP4 at concentration 50 µM caused a significant decrease of FAK and paxillin proteins which are crucial for the adhesion complex formation.

Our novel findings indicate that TMPyP4, an effective telomerase inhibitor, alters adhesion and migration of breast cancer cells, suggesting a telomere-unrelated mechanism of toxicity. Thus, co-treatment of cancer cells with TMPyP4 and doxorubicin might constitute a promising approach in breast cancer cells fighting.

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P21.12

Studying the gene regulatory network of heart development in *Danio rerio* using genomics approach

Katarzyna Nieścierowicz, Cecilia Winata

International Institute of Molecular and Cell Biology in Warsaw, Laboratory of Zebrafish Developmental Genomics, Warsaw, Poland
Katarzyna Nieścierowicz <kniescierowicz@iimcb.gov.pl>

The development of the heart is a dynamic and intricate process involving multiple interactions at the molecular, cellular and tissue level. Genetic or environmental perturbations to such a highly complex developmental program can lead to congenital heart defects (CHD). At the heart of the gene regulatory network of cardiogenesis are transcription factors (TFs), which initiate the transcription of cardiac genes, triggering a cascade of genetic regulation of heart development. Mutations in several cardiac TFs have been identified in patients and families with some of the most common forms of cardiac morphological and functional abnormalities. Despite the remarkable progress of our knowledge about regulators of heart development, there are still some missing information about their downstream regulatory networks and how they interact. We have initiated a study to elucidate the molecular mechanism of several transcription factors (Hand2, Gata5, Nkx2-5, Tbx5) involved in key phases of the vertebrate heart development. The zebrafish is an ideal model to study heart development as it compensates for the early embryonic lethality of heart development mutants in other model organisms. We will present our ongoing work to elucidate the genetic regulatory network of heart development and discuss its possible contribution to a better understanding of the mechanism of CHD.

P21.13

Distribution of white blood cells' subpopulations among junior football players during the final round of Central Junior League competitions

Robert Nowak^{1,3}, Dorota Kostrzewa-Nowak^{2,3}, Rafał Buryta^{1,3}

¹University of Szczecin, Faculty of Physical Education and Health Promotion, Department of Biological Bases of Physical Culture, Szczecin, Poland; ²University of Szczecin, Faculty of Physical Culture and Health Promotion, Department of Modern Movement Forms, Szczecin, Poland; ³University of Szczecin, Faculty of Physical Culture and Health Promotion, Functional and Structural Human Research Centre, Szczecin, Poland

Robert Nowak <robert.nowak@usz.edu.pl>

According to literature data, chronic high-intensity exercise could stimulate T cells resulting in immunosuppression. It might be manifested by an increased incidence of infections, mainly of upper respiratory system among athletes. The aim of this study was to monitor changes in percentages of lymphocyte subsets: T lymphocytes (CD3⁺), helper/inducer T lymphocytes (Th; CD3⁺/CD4⁺), suppressor/cytotoxic T lymphocytes (Tc; CD3⁺/CD8⁺), B lymphocytes (CD3⁻/CD19⁺), and natural killer (NK) cells (CD3⁻/CD16⁺/CD56⁺) in capillary blood of junior football players during final round of Central Junior League competition. Ten participants median aged 18 years old (range 17–19 years) were recruited from Pogoń Szczecin S.A. football club. Capillary blood collection system for haematological analysis with ethylenediaminetetraacetic acid (EDTA) anticoagulant was used to collect blood samples. Blood samples were collected 24 hours after the football match during 8 consecutive weeks of final round of Central Junior League competition. White blood cells (WBC) phenotyping was performed by flow cytometry analysis using BD Multitest™ IMK kit (BD Biosciences) to determine the percentages of T lymphocyte subsets in erythrocyte-lysed blood. Local Ethics Committee approval No. 13/KB/V/2014 and informed consent of the participants (and their parents, where appropriate) was received before the beginning of the testing in accordance to Declaration of Helsinki. Significant decrease in percentage of NK cells, and no changes in percentage of B lymphocytes were observed during the study. Significant changes in T lymphocytes percentages exhibiting decreasing tendency were also found. It was observed that the most probable cause of this phenomenon was the significant decrease in percentage of Tc lymphocytes since there were no changes in Th lymphocytes subpopulation. The results of the study seem to evidence the important influence of exercise bouts on the changes in WBC subpopulations. Therefore, WBC phenotype monitoring might be an important part of sport diagnostics.

P21.14

Modulation of cofilin severing activity by tropomyosin isoforms

Zofia Ostrowska, Joanna Moraczewska

Department of Biochemistry and Cell Biology, Faculty of Natural Sciences, Kazimierz Wielki University in Bydgoszcz, Poland
Zofia Ostrowska <zofostr@ukw.edu.pl>

In the non-muscle cells the rapid remodeling of the actin cytoskeleton is fundamental to the proper functioning of these cells and is under control of many actin-binding proteins, including tropomyosin (Tpm) and ADF/cofilins. Tpm is a protein which binds along actin filaments (F-actin), stabilizes the filaments and controls actin interactions with other binding partners. ADF/cofilins accelerate filaments depolymerization through severing and sequestering of actin monomers. Tpm1.6, Tpm1.8, and Tpm3.2 are Tpm isoforms expressed in many non-muscle cells. Tpm3.4 is a brain-specific isoform. The goal of this project was to verify the hypothesis that tropomyosin isoforms differentially regulate F-actin depolymerization by cofilin 1. Severing and depolymerization of rhodamine-labelled F-actin by cofilin-1 was observed with the use of fluorescence microscopy. Saturation of F-actin with Tpm1.6 and Tpm1.8 strongly protected the filaments from fragmentation and depolymerization by cofilin-1. This correlated with reduced affinity of cofilin-1 for F-actin in the presence of Tpm1.6/8. In contrast, in the presence of Tpm3.2 and Tpm3.4 both activities of cofilin-1 were accelerated, which at least partially could be explained by the fact that Tpm3.2/4 did not change cofilin-1 affinity for F-actin.

In order to find the mechanism used by Tpm3.2 and Tpm3.4 to enhance the cofilin's activities, we resorted to the ability of cofilin to twist actin subunits, which disrupts actin-phalloidin binding site. In the presence of each Tpm the filaments were stable and the release of rhodamine-labelled phalloidin was not accelerated. Addition of cofilin-1 to F-actin alone decreased the fluorescence showing that cofilin-1 induced a conformational change, which released rhodamine-phalloidin. Tpm1.6 and Tpm1.8 strongly reduced the cofilin-induced rate of the rhodamine-phalloidin fluorescence decrease. In contrast the rate was accelerated by Tpm3.2 and Tpm3.4.

We concluded that Tpm1.6 and Tpm1.8 are cofilin-1 competitors, but Tpm3.2 and Tpm3.4 act as collaborators. Because all Tpm stabilize untwisted conformation of F-actin, the mechanism of regulation does not involve conformational changes in F-actin synergistically induced by cofilin-1 and the isoforms Tpm3.2/4. To understand the mechanism underlying this synergy, further structural studies are required.

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P21.15

***N*-homocysteinylation impairs collagen cross-linking in cystathionine β -synthase-deficient mice: a novel mechanism of connective tissue abnormalities**

Joanna Perla-Kajan¹, Marta Rusek², Agata Malinowska³, Ewa Sitkiewicz³, Olga Utyro⁴, Hieronim Jakubowski^{1,2,4}

¹Poznan University of Life Sciences, Department of Biochemistry and Biotechnology, Poznań, Poland; ²Rutgers-New Jersey Medical School, International Center for Public Health, Department of Microbiology, Biochemistry and Molecular Genetics, USA; ³Institute of Biochemistry and Biophysics, Poland; ⁴Institute of Bioorganic Chemistry, Poznań Poland

Joanna Perla-Kajan <asiape1@gmail.com>

Cystathionine β -synthase (CBS) deficiency, a genetic disorder in homocysteine (Hcy) metabolism in humans, elevates plasma Hcy-thiolactone and leads to connective tissue abnormalities affecting cardiovascular and skeletal systems. However, the underlying mechanism of these abnormalities is not understood. Hcy-thiolactone has the ability to form isopeptide bonds with protein lysine residues, which generates *N*-homocysteinylation of protein. Because lysine residues are involved in collagen cross-linking, *N*-homocysteinylation of these lysines should impair cross-linking. Using a Tg-I278T *Cbs*^{-/-} mouse model of hyperhomocysteinemia (HHcy) that recapitulates connective tissue abnormalities observed in CBS-deficient patients, we show that *N*-Hcy-collagen was elevated in the heart, bone, and tail of *Cbs*^{-/-} mice, while pyridinoline cross-links were significantly reduced. Plasma deoxypyridinoline crosslink and cross-linked carboxyterminal telopeptide of type I collagen were also significantly reduced in *Cbs*^{-/-} mice. Lysine oxidase expression and activity were not affected by the *Cbs*^{-/-} genotype. We also show that collagen carries *S*-linked Hcy bound to the thiol of *N*-linked Hcy. *In vitro* experiments show that Hcy-thiolactone modifies lysine residues in collagen type I α -1 chain. Residue K160, located in the non-helical *N*-telopeptide region and involved in pyridinoline cross-link formation, was also *N*-homocysteinylation *in vivo*. Taken together, our findings show that *N*-homocysteinylation of collagen in *Cbs*^{-/-} mice impairs its cross-linking. These findings explain at least in part connective tissue abnormalities observed in HHcy.

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P21.16

ModeLang – a new approach to biologists-friendly modelling

Tomasz Prejzandanc, Szymon Wąsik, Jacek Błażewicz

Institute of Computing Science, Poznan University of Technology, Poznań, Poland

Tomasz Prejzandanc <t.prejzandanc@gmail.com>

The main way of modelling – systems of differential equations – are requiring a lot of time for adopting models for specific purpose and, which is the case, require to go into deep mathematical definitions. One of the answers is multi-agent systems modelling. ModeLang is the new approach to modelling which allows definition in the form of controlled natural language. User is able to note interactions in the subset of natural language, e.g., English. Afterwards the data is being processed by the software to simulator, which is allowing users to retrieve results afterwards. Modelling using ModeLang is helpful approach for specialists in various fields of science including mostly tested bioinformatics, but also, e.g., economy.

P21.17**The modulation of radiation effects in normal and cancer cells via antioxidant vitamins and ferulic acid**Maria Konopacka¹, Jacek Rogoliński¹, Krzysztof Śłosarek²

¹Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Center for Translational Research and Molecular Biology of Cancer, Poland; ²Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Department of Radiotherapy and Brachytherapy Treatment Planning, Poland

Jacek Rogoliński <rogolinski@io.gliwice.pl>

Radiotherapy is the major form of treatment for many human cancer. During the course of treatment, the ionizing radiation produce many biological effects not only in cancer but also in normal cells. This study concerns the radio-protection of normal versus cancer cells, *via* antioxidants.

We tested the modulating effects of vitamin C, vitamin E and ferulic acid on clastogenic activity of ionizing radiation in human lung cancer and normal bronchial epithelial cell lines. The study was used a water phantom to model radiotherapy of lung tumour in humans, with cancer cell located in a beam axis and receiving 5 Gy dose, and normal cells located outside of the radiation field and receiving 0,2 Gy of scattered radiation. Formation of micronuclei were evaluated using micronucleus test.

Results indicate the discrepancy between the distribution of physical dose at different depths of the water phantom and biological effects. It is of special meaning in case of irradiation at bigger depths or placed outside the field during the exposure.

Treatment with either ferulic acid or vitamin E reduced micronuclei frequency in normal cells irradiated outside the beam, while at the same time increased micronuclei frequency in directly irradiated cancer ones. The effect of vitamin C was concentration-dependent and did not vary between normal and cancer cells. The use of vitamin E and ferulic acid may augment the efficacy of radiation therapy by enhancing the response of cancer cells to the radiation and simultaneously these substances can protect normal cells exposed to low dose of scattered radiation outside the radiation field during radiotherapy.

Our results suggest that healthy cells exposed outside the radiation field during radiotherapy can be damaged to a greater extent than can be predicted from the dosimetric curve of absorbed dose. The protection of normal cells against these distant effects appears to be an important element of radiotherapy.

P21.18**Profiling the dynamics of mitochondrial co-translational import during Zebrafish development**Sreedevi Sugunan¹, Ania Sokol¹, Piotr Chroscicki¹, Michal Bazala¹, Agnieszka Chacinska¹, Cecilia Winata^{1,2}

¹International Institute for Cellular and Molecular Biology, Warsaw, Poland; ²Max-Planck Institute for Heart and Lung Research, Germany
Sreedevi Sugunan <kittysree77@gmail.com>

The mitochondrion is an essential organelle in eukaryotic cells, which mainly functions to generate chemical energy for the cell. To perform its function, mitochondria require more than 1000 proteins that are encoded mostly by the nuclear genome, synthesized in the cytosol and subsequently imported into various sub-organellar compartments. The mechanisms underlying post-translational import, which is the main mode of protein import into mitochondria, are well established. Recent genome wide analysis in yeast has revealed that many nuclear encoded mRNAs are associated with the mitochondrial outer membrane, giving rise to the hypothesis that they are translated locally by the ribosomes attached to the mitochondrial surface and concurrently imported into mitochondria. Yet, direct support for such co-translational import is still scarce. Moreover, the proteins that may be involved in such a process are largely unknown. Furthermore, this process is completely unexplored in vertebrates. In order to uncover the repertoire of nuclear encoded mRNAs, products of which undergo the co-translational import, we perform translational profiling of mRNAs associated with mitochondrial surface isolated from 5 dpf zebrafish larvae. The purity of mitochondrial preparation and the presence of polysomes are confirmed using western blot against mitochondria specific proteins and ribosomal proteins respectively. To further validate our approach, mRNAs are isolated from MARS, microsomal and cytosolic fractions and analyzed by qPCR of specific genes which were reported to undergo co-translational import. Subsequently, we performed sequencing of RNA from these three fractions.

The long term goal of our work is to explore the transitions in the mitochondrial proteome that take place during the development. Towards this direction, we are creating a mutant fish for proximity specific ribosome profiling By CREISPR/Cas9 genome editing. Our work will contribute to new insights into the dynamics of mitochondrial biogenesis during vertebrate development.

P21.19

The role of lysine specific demethylase 1 in shaping of the inflammatory reaction of endothelial cells

Martyna Wojtala¹, Dorota Rybaczek², Aneta Balcerczyk¹

¹University of Lodz, Department of Molecular Biophysics, Łódź, Poland

²University of Lodz, Department of Cytophysiology, Łódź, Poland

Martyna_Wojtala <mwojtala89@biol.uni.lodz.pl>

Epigenetic modifications such as methylation of cytosine in DNA, histone post-translational modifications are a key mechanism of gene expression regulation, essential for normal development. Among histone modifications methylation and its effect on gene expression are extensively studied since it was found that methylation marks are not static but dynamically regulated by histone methyltransferases and histone demethylases.

In the presented studies we shed light on the role of the lysine-specific demethylase 1 (LSD1), responsible for demethylation of mono- and di-methylated histone H3 lysine 4 (H3K4me1/me2) through a FAD-dependent oxidative reaction, in the inflammatory response in endothelial cells. As a model human microvascular endothelial cells (HMEC-1) were used, and the activation of cells was induced by lipopolysaccharide (LPS; 100 ng/ml). To define the role of LSD1 in the response of HMEC-1 to the inflammatory stimuli pharmacological as well as transcriptional inhibition of the enzyme activity was performed, using 2-PCPA, and specific shRNA sequence, respectively.

Looking at the cytokines and chemokines level, we did not find global changes in the release or expression of the proinflammatory molecules after inhibition of LSD1 with 2-PCPA, but preincubation of cells with the LSD1 inhibitor (100 μ M) followed by exposure of cells to LPS (100 μ g/ml), significantly attenuated the inflammatory response of HMEC-1 (decreased expression of CCL-2, IL-6, IL-8 and ICAM-1, was observed in compare to the cells treated by the LPS only). Anti-inflammatory profile of HMEC-1 revealed after pharmacological inhibition of LSD1 activity was confirmed in the experiments on HMEC-1 LSD1 KDs. The silencing of LSD1 we reached oscillated at 0.21–0.29 in compare to the control (HMEC-1 nonTarget). Apart of the decreased expression of inflammatory mediators in HMEC-1 LSD1 KDs stimulated by 4 h with LPS, we also found that silencing of LSD1 affects the p65 cytosol-nucleus shuttling, a subunit of NF κ B transcriptional factor, involved in the response to inflammatory reaction LPS-induced.

In conclusion, performed analysis strongly suggest an engagement of the LSD1 in shaping of the inflammatory response of human microvascular endothelial cells *via* NF κ -B dependent manner.

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P21.20

Expression and localization of myosin VI in mouse testes

Przemysław Zakrzewski¹, Robert Lenartowski¹, Maria J. Rędownicz², Marta Lenartowska¹

¹Nicolaus Copernicus University in Torun, Faculty of Biology and

Environmental Protection, Toruń, Poland; ²Nencki Institute of

Experimental Biology, Department of Biochemistry, Warsaw, Poland

Przemyslaw_Zakrzewski <przezak@doktorant.umk.pl>

Myosin VI (MVI) is only the one molecular motor traveling towards the minus end of actin filaments. This protein has three main domains: N-terminal motor domain (head) with ATP- and actin-binding motifs; neck (lever arm) which has regulatory function; and tail which binds cargo and/or adaptor proteins. Due to presence of two inserts in the tail, small (SI) and large (LI), four alternatively spliced forms of MVI are formed in animal cells: isoforms with SI or LI, with both inserts or without any of them. The presence/lack of these inserts can determine specific localizations and functions of MVI in different cell types [1].

MVI function has been documented in various cellular processes, such as endo- and exocytosis, stabilization of Golgi apparatus, morphology of hair cells in Corti organ, cell adhesion and migration, cytokinesis, nuclear processes, autophagy, myogenesis, and iris development [2]. It was also demonstrated that lack of MVI in *Drosophila* testes leads to male infertility [3]. Thus, MVI role seems to be crucial in spermatogenesis of invertebrates. Here we report that this protein is also involved in sperms development in mammals. Using RT-PCR and immunoblotting we showed that two splicing variants of MVI, with SI and with no inserts, were highly expressed in mouse testes. Moreover, our immunocytochemical studies confirmed that both MVI and actin were particularly involved in the late phase of spermatogenesis. Using immunogold technique and electron microscopy we revealed MVI localization in maturing acrosome as well as in the acroplaxome – a highly specialized actin structure that anchors the acrosome to the spermatid nucleus. Furthermore, we confirmed the presence of MVI in a temporary structure called the “manchette” which assists in sperm’s tail elongation. Taken together, our results reported here for the first time suggest an important role of MVI in spermiogenesis of mammals.

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P21.21

On the interplay between phosphatidic acid and cholesterol in membrane recruitment of proteins

Jolanta Zegarlińska, Magda Chmielewska, Aleksander F. Sikorski, Aleksander Czogalla

Institute of Biotechnology, Department of Cytobiochemistry, Wrocław, Poland

Jolanta_Zegarlińska <aleksander.czogalla@uwr.edu.pl>

For a long time lipid membranes were seen only as building blocks and solvents for a proteins. Moreover, lipid-protein interactions were often ignored and thought to be irrelevant for protein function. However, most recently lipids are more and more appreciated for their role as modulators and initiators of physiological processes.

Phosphatidic acid (PA), the simplest diacyl-glycerophospholipid, is present in small quantities in cell membranes. Recent studies revealed its important role in the cell as a signaling lipid. There are two major pathways of synthesis of PA in mammalian cells. Phospholipase D produces mainly lipids with the 16:0 and 18:1 acyl chains (POPA), while diacylglycerol kinase – lipids with 18:0 and 20:4 (SAPA) acyl chains.

Cholesterol, one of the major ingredient of mammalian membranes, plays a key role in regulation of membrane fluidity, permeability, and organization of microdomains. Recent findings show that cholesterol can also modulate lipids' headgroups conformation, which may influence protein binding to lipid bilayer.

We study the specific recognition of PA by peripheral membrane proteins, such as syndapin, and the role of cholesterol in these interactions. One of method used for this study was immuno-electrochemiluminescence (EIA). Saturation binding curves of the protein to lipid vesicles (LUV) composed of (POPC) and (POPA) in a molar ratio of 99/1, 95/5 and 90/10 was obtained. Syndapin bound very weakly to these vesicles, but the situation changed when cholesterol was added. It produced a significant increase in binding syndapin to liposomes, but only in the case of high concentration of POPA (10 mol%). This result was also confirmed by flotation of liposomes in a density gradient. Procedure was repeated with vesicles containing SAPA. In this case, we observed a weak bounding to vesicles SAPA/POPC, and an effect on on the cholesterol-syndapin bounding was much stronger for the liposomes containing SAPA (noticeable already at 5 mol%) compared to liposomes POPA. These observations are confirmed by the designated dissociation constants Kd. In general, we demonstrated a significant effect of cholesterol on the presentation of phosphatidic acid on the membrane surface, which is reflected in changes in the affinity of syndapin to PA.

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P21.22

Comparison of pentraxin 3 and four serum tests in the identification of significant fibrosis in chronic hepatitis C virus (HCV) infection

Jolanta Zuwała-Jagiello¹, Joanna Gorka-Dynysiewicz¹, Monika Pazgan-Simon², Krzysztof Simom²

¹Department of Pharmaceutical Biochemistry, Wrocław Medical University, Wrocław, Poland; ²Division of Infectious Disease and Hepatology, Faculty of Dentistry, Wrocław Medical University, Wrocław, Poland

Jolanta_Zuwała-Jagiello <jolanta.zuwała-jagiello@umed.wroc.pl>

Background and Aims: Long pentraxin 3 (PTX-3) is a soluble pattern recognition receptor produced by phagocytes and nonimmune cells at sites of inflammation or liver injury. The aim of the present study was: (1) to investigate whether PTX-3 serum levels correlate with liver inflammation and fibrosis in patients with HCV; (2) to evaluate the potential of PTX-3 serum levels combined with the use of serum test (APRI, FIB-4, Forns, King score) as a non-invasive method for the diagnosis of fibrosis stage in patients with HCV.

Methods: We performed a prospective case-control study including 35 patients with HCV genotype 1 and 20 healthy subjects matched by age and sex. The HCV group was divided into two groups (non-significant, F0–F2 and significant fibrosis, F3–F4) selected according to liver fibrosis staging (Metavir). The liver function and the following markers PTX-3, sCD163, IL-6, and TNF- α were measured in all patients. APRI, FIB-4, Forns and King scores were calculated based on the values used for Metavir.

Results: Serum levels of PTX-3 were higher in patients with liver fibrosis (Metavir score $F \geq 2$) in comparison to patients without fibrosis. Furthermore PTX-3 was markedly increased in patients with inflammatory activity in liver biopsy (Metavir score ≥ 3 vs. 3; 6.07 vs. 2.12 ng/mL, $P=0.01$). We found a strong relation between the stage of fibrosis and PTX-3 levels and sCD163 (respectively; $r=0.41$, $P=0.05$; $r=0.56$, $P=0.001$). However, the differences weren't significant in IL-6 and TNF- α levels in the significant vs. non-significant fibrosis. An overall significant correlation was found between PTX-3 and the noninvasive diagnostic tests as follows: FIB-4 ($r=0.51$), Forns ($r=0.48$), King ($r=0.46$) and APRI ($r=0.45$), all with $P=0.001$. In identifying significant fibrosis, all 3 tests performed best, with AUC respectively for Forns, FIB-4, and King of 0.90, 0.89 and 0.87, all with statistical significance ($P=0.001$). APRI performed slightly better than PTX-3 in differentiating significant fibrosis from low grades of fibrosis [AUC 0.76 and 0.64, respectively].

Conclusions: This is the first study to demonstrate a marked elevation of PTX-3 in chronic HCV infection and the relation with the fibrosis stage and inflammatory activity grade. PTX-3 is a parameter that has satisfactory sensitivity (74%) and specificity (58%) and it can be useful to reduce the frequency with which biopsies need to be carried out to monitor the evolution of chronic hepatitis C and the right moment for treatment indication.

P21.23

Adhesion dependent signaling leads to RhoA pathway inhibition compensation

Natalia Nowak¹, Wanda Kłopocka², Paweł Pomorski³

¹Laboratory of Imaging Tissue Structure and Function, Neurobiology Center, Nencki Institute of Experimental Biology of Polish Academy of Sciences, Warsaw, Poland; ²Department of Biochemistry and Cell Biology, Faculty of Biology and Environmental Sciences, Cardinal Stefan Wyszyński University in Warsaw, Warsaw, Poland; ³Laboratory of Molecular Basis of Cell Motility, Department of Biochemistry, Nencki Institute of Experimental Biology of Polish Academy of Sciences, Warsaw, Poland

Paweł Pomorski: <pp@nencki.gov.pl>

We have previously showed that in glioma C6 cells UTP-induced P2Y₂ nucleotide receptor activation leads to the recovery from RhoA pathway blockade. RhoA inhibition results in the rounding of the cell body and decreased cell motility. After stimulation with UTP, both cell anatomy and motile properties are restored. In the present work we propose the multiple action of the P2Y₂ receptor as the mechanism of aforementioned phenomenon. First of all, P2Y₂ acts as the G α_q coupled GPCR receptor, activating calcium signal and promoting actomyosin contraction. Activation of this pathway is confirmed by myosin light chain phosphorylation following receptor activation. On the other hand, increased phosphorylation of the cofilin suggests the activity of the Rac1 pathway, mediated by the G α_o G protein subunit. It is suggested that G α_o activation by P2Y₂ receptors requires the integrin-dependent adhesion mechanism and indeed calcium free medium or growing of the cells in suspension inhibit this pathway. Using proximity ligation assay we visualized the distribution of integrin-P2Y₂ coupling. The control and recovered cells exhibited statistically significant increase of the receptor-integrin complex number as compared to RhoA-inhibited cells. We also observed stronger polarization of control and recovered cells where numerous integrin-P2Y₂ coupling events occurred on the cell periphery and less in the cell centre. Therefore, we suggest that not only balance of pathway activation but also distribution of signaling events may be responsible for shape and motility of cells.