Late Abstracts

LA1

Purification of *Ultraspiracle* from *Drosophila melanogaster* and its basic biochemical tests

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Ultraspiracle (Usp) is a nuclear receptor present in arthropods which participates in control of their molting and metamorphosis. It has been shown that Usp is part of complex engaged in the cross talk between 20E and juvenile hormone (JH) signaling pathways where it interacts with ecdysone receptor (EcR). However Chd64 was also proposed as potential interaction partner for Usp in this cross-talk. Thus, biochemical studies of Usp are important for understanding of its interaction with Chd64. For that reason a new efficient method of Usp purification is presented.
Drosophila Usp was cloned into pQE-80L vector. Transformed bacterial BL21(DE3)pLysS cells were grown in LB medium. The first step of purification includes fractionation with solid (NH4)2SO4. Fractions between 0-35% saturation containing Usp were dissolved and then desalted on a PD10 column. Next, Usp was purified using HisTrap column. Target protein was eluted with a buffer containing 250 mM imidazole. Finally Superdex 200 column was used for separation of remaining contaminations. Functionality of the obtained protein was confirmed by electrophoretic mobility shift assay (EMSA) using ^{32}P-labeled *hsp27* regulatory element.
The previous procedure required heparin column and here it was excluded. Therefore overnight dialysis step can be omitted what reduces loss of Usp and shortens purification making it one-day instead of two-days procedure. EMSA confirmed that obtained protein was functional and it bound to *hsp27* in form of monomer and dimer. Our purification method allows to obtain at least 0.6 mg of Usp protein from 1 L culture. Compared with the previous procedure

LA2

Thymoquinone interactions with tetracycline and gentamicin influence their antibacterial action

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Thymoquinone is a phytochemical compound present in Nigella Sativa seeds essential oil (Piras *et al*., 2013, *Industrial Crops and Products* **46**: 317-323). Thymoquinone has shown various biological activities. The most relevant properties of thymoquinone regarding its biological activity are: anti-inflammatory action (El Gazzar *et al*., 2006, *International Immunopharmacology* **6**: 1135-1142), antioxidant activity (Erkan *et al*., 2008, *Food Chemistry* **110**: 76-82) and antibacterial action against various species of bacteria (Chaieb *et al*., 2011, *BMC Complementary and Alternative Medicine* **11**: 29).
Tetracycline is a very well described antibiotic used against both gram-positive and gram-negative bacteria. Gentamicin is an antibiotic active against most gram-negative bacteria and some gram-positive bacteria. Both antibiotics' activities depend on their interaction with bacterial rybosomes (Chopra & Roberts, 2001, *Microbiology and Molecular Biology Reviews* **65**: 232-260; Tangy *et al*., 1985, *European Journal of Biochemistry* **147**: 381-386).
In this work interactions between thymoquinone and tetracycline, as well as interactions between thymoquinone and gentamicin were investigated. In order to observe these interactions biophysical techniques (UV-VIS spectroscopy and Isothermal Titration Calorimetry) were utilised. The effects of observed interactions on antimicrobial actions of antibiotics and thymoquinone were studied using checkerboard assay for antibiotics synergy testing. In the checkerboard assay strains of *Escherichia coli, Pseudomonas aureginosa, Salmonella enterica* and *Staphylococcus aureus* were used.

LA3

Influence of chlorpyrifos on the level of expression of selected enzymes responsible for the vitamin D_3 synthesis in skin cells

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The aim of the study was to investigate the effect of chlorpyrifos on the expression of cytochrome P450 isoforms (CYP27A1 and CYP27B1),the isoforms involved in the synthesis of vitamin D_3 in the skin cells.

Studies were performed on fibroblast BJ cell line. Cells were pre-incubated with the precursor of vitamin D_3 (7-DHC) and the two concentrations of chlorpyrifos (CHP) (50, 250 μM). The synthesis of vitamin D_3 in BJ fibroblasts was initiated by UVB irradiation with intensity 5 or 20 mJ/cm^2. Control cells were UVB irradiated after preincubation with 7-DHC alone. To investigate the effect of chlorpyrifos on the expression of selected cytochrome P450 isoforms and the protein level, samples were analyzed by Real-Time PCR and Western blot methods. A significance of differences was calculated using the one-way ANOVA with post-hoc Tukey's test, difference were considered statistically significant when the *p*-value was less than <0.05.

A statistically significant and dose-dependent decrease of the CYP27B1 isoform expression was observed at the mRNA level. Also decrease of CYP27A1 isoform at the mRNA level was observed after 5 mJ/cm^2 UVB irradiation, however a slight increase of its mRNA level was observed after the higher intensity of UVB irradiation. These results were confirmed at the protein level by Western blot analysis.

Our research showed that exposure to chlorpyrifos interfered with the regulation of the expression of key cytochrome P450 isoforms, which are involved in the synthesis of vitamin D_3 in the skin cells.

Key words: chlorpyrifos, cytochrome P450, fibroblast, vitamin D3

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LA4

Quantitative analysis of Drp1 oligomeric states in the living cell

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Dynamin related protein 1 (Drp1) is involved in the process of mitochondrial fission, which is very important in maintaining cell homeostasis, as well as in apoptosis, cell division and mitophagy. Upon fission signals in the cell, Drp1 intermediate assemblies form oligomeric ring-like structure which wraps around mitochondria and then constricts thanks to the energy gained from GTP hydrolysis. This constriction results in mitochondrial fragmentation. Precise state of Drp1 protein population in the cytosol is not yet known. So far we do not exactly know if this protein stays in cytosol in dimeric, tetrameric or any other state. With the use of Fluorescence Correlation Spectroscopy (FCS) and length scale dependent viscosity model we can quantify the diffusion coefficient of Drp1 and the extent of its aggregation. This allows us to estimate the size of Drp1 oligomer in the cytosol. Our results suggest that Drp1 likely exists in the cytosol in oligomeric complexes of more than two Drp1 proteins. What is more, CRISPR-Cas9 gene editing technique allowed us to establish cell line with GFP sequence attached to Drp1 gene, what then let us to visualize Drp1 cellular distribution and dynamics at endogenous expression level. The distribution of Drp1 oligomers forming on the mitochondrial network can be estimated using FCS calibrated concentration maps toghether with the information about the size of the dominant diffusing species. Moreover, observations from Spinning Disc microscope give us supplementary data concerning dynamics of Drp1 in the cytosol and on mitochondria. These observations showed that Drp1 forms stable large oligomers which can move along mitochondria, but the Drp1 ring formation does not necessarily lead to mitochondrial fission.

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LA5

The enigmatic involvement of p66Shc signaling pathway in MCF-7 and MDA-MB-231 human breast cancer cells

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Background: p66Shc protein as a member of ShcA family may play a dual role in the cell. On the one hand it has been implicated in the inhibition of proliferation as well as apoptogenic signals in response to a variety of different factors, acting as a negative regulator of proliferation. On the other hand, p66Shc protein undergoing phosphorylation at Ser36 residue can have a substantial impact on mitochondrial metabolism through regulation of cellular response to the oxidative stress. These facts make p66Shc pathway a potential target concerning the cancer proliferation, tumor progression or metabolic reprogramming. Hence, the main aim of our project is focused on studies of p66Shc signaling pathway and its impact on bioenergetics parameters in human breast cancer cell lines and corresponding controls.
Materials and methods: To achieve our goal two human breast cancer cell lines (MDA-MB-231 and MCF-7) and corresponding normal control cell lines (AG11137, MCF-10A) have been used. The level of Shc isoforms (p46, p52 and p66) as well as the status of antioxidant defense system have been estimated with the use of Western Blot technique. Mitochondrial bioenergetic parameters and ROS production have been determined with the use of respective fluorescent probes and measured using Infinite M200 Tecan microplate reader.
Results: Investigation of the profile of p66Shc signaling pathway, reactive oxygen species level as well as antioxidant defence system, has revealed differences between human breast cancer cell lines and corresponding controls.
Conclusions: Our data indicate that p66shc protein may play the specific role depending on the cancer type. However, it remains still mysterious in which mechanism p66Shc exerts its impact on tumor cells and requires further investigations.

LA6

Distribution and trafficking of mitochondria in fibroblasts derived from patients diagnosed with sporadic form of Alzheimer's and Parkinson's diseases

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Proper trafficking of mitochondria is crucial for maintenance of vital functions of the cell. Accordingly, its dysregulation can contribute to changes in bioenergetics profile of the cell and can lead to cell death. Wide events connected to mitochondrial transport defects were noticed in the most neurodegenerative diseases. We observed differences in mitochondrial movement speed in fibroblasts derived from patients with Alzheimer's (AD) and Parkinson's (PD) diseases in reference to the controls. Predominantly the velocity was lower, especially in the case of large size of mitochondria. Forasmuch mitochondrial transport seems to be determined by their size, we assessed mitochondrial network integrity and estimated the quantity and area of mitochondria separated from the network. Our results indicated slightly changes for AD and PD group in comparison to the controls. Owing to the fact, that mitochondrial trafficking depends on mitochondrial state, we investigated the "age" of mitochondria using Mitotimer vector as a tool in our study. We observed a diminished mitochondrial turnover in AD and PD cells.
To summarise, our results revealed, that mitochondrial transport is slightly different in fibroblasts derived from patients with sporadic Alzheimer's and Parkinson's diseases in comparison to the healthy control cells.

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LA7

Mitochondrial stress in sporadic and familial form of ALS

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Amyotrophic lateral sclerosis (ALS) is one of the most common neurodegenerative diseases leading to progressive and selective loss of both upper and lower motor neurons. Sporadically occurring disease (sALS) affects the majority of patients (90%) while the rest of cases is inherited and referred to as familial ALS (fALS). Usually the clinical and pathological features of sporadic and familial forms of ALS are indistinguishable, suggesting share similar pathogenic mechanism. Defects in mitochondrial energy production, mitochondrial dynamics and trafficking are early symptoms in ALS development. Understanding the relationship between mitochondrial malfunctions and progress of ALS may provide a useful tool for early diagnosis and potential pharmacological targets for treatment of the disease.
We characterized mitochondrial network and physiology in primary fibroblasts derived from patients diagnosed with familial and sporadic form of ALS showing numerous differences in the mitochondrial functioning in patients' cells in comparison to control cells.
These all data indicate that chronic mitochondrial stress is present in sALS and fALS cells. The changes in organization of mitochondrial distribution in ALS indicate that distribution and structure of mitochondrial network may be involved in the activity of mitochondrial retrograde signaling. Principal Component Analysis (PCA) performed on obtained data set gave a clear separation of investigated cell lines into 3 distinct groups: sALS, fALS and control.
Profound analysis of the mitochondrial functioning may be a useful (albeit time-consuming and expensive) method of ALS diagnosis, especially at an early stage of the disease, before appear symptoms of neurodegeneration. It is possible that mitochondria could be a therapeutic target in the treatment of this type of disorder.
Although our results suggest, that mitochondrial stress is present in investigated ALS fibroblasts model, the question if these changes are primary or secondary event in ALS pathophysiology, still needs answer.

LA8

Measurements of intracellular mobility of proteins associated with glycogen

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Glycogen, a large glucose polymer serving as a reservoir of energy can be seen as a molecular hub integrating signals from proteins involved in its synthesis.
Due to microscopic observations of transfected primary fibroblasts we have determined that glycogen synthase (GS) (83,7kDa) forms cytoplasmic granule-like deposits, most probably corresponding to glycogen. By contrast, another enzyme, glycogen branching enzyme (GBE1) (80 kDa) is uniformly distributed in the cell. Mutations of the Gbe1 gene lead to abnormal structure of glycogen causing glycogen storage disease type IV (P1 cell line derived from the patient with GSDIV, which corresponds to abnormal glycogen;). We assume that poor branching of glycogen molecule can result in impaired binding of GBE1 to the glucose polymer.
Results of the measurements of diffusion using Fluorescence Correlation Spectroscopy (FCS) for different probes (e.g.. GBE1 and GS tagged with GFP) indicate distinct mobilities of studied proteins. This could tell us about the oligomeric state of proteins as well as their interactions, like affinity of GS and GBE1 to this large glucose polymer. Moreover, the size of the glycogen could also be estimated based on the obtained results.

References:

1. Kalwarczyk T, Ziębacz N, Bielejewska A, Zaboklicka E, Koynov K, Szymański J, Wilk A, Patkowski A, Gapiński J, Butt H, Holyst R (2011) Comparative analysis of viscosity of complex liquids and cytoplasm of mammalian cells at the nanoscale. *Nano Lett* **11**: 2157–2163.
2. Roach PJ, Depaoli-Roach AA, Hurley TD, Tagliabracci VS (2012) Glycogen and its metabolism: some new developments and old themes. *Biochem J* **441**: 763–787.

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LA9

Detecting of the presence of advanced glycation end products AGE 1 and AGE3 in the serum blood of diabetic patients by the slot dot-blot method

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The glycation is a non-enzymatic process which occurs under physiological conditions and determines aging or organism. Glycation is increased by a variety of metabolic disorders, i.e. diabetes, atherosclerosis, Alzheimer's disease, vasculitis. Glycation reaction takes place between the basic groups of proteins, lipids, DNA, and reducing sugars or low molecular weight aldehydes. A lot of different products can be formed as a result of these reactions, which due to its durability, are treated as potential diagnostic markers of metabolic changes. However, due to the low level of AGEs in serum or tissues, the AGE determination is difficult. Developed by our team a method of slot dot-blot has proven to be an effective tool for detection of AGE-1 (BSA-glucose) and AGE-3 (BSA-methylglyoxal) in blood serum. We have detected these products in serum of 300 patients with diabetes. anti-AGE1, anti-AGE3 (Cosmo Bio, Japan) were used for the experiments. The presence of AGE1 and AGE3 was demonstrated practically in the serum of samples of the same patient suggesting that the same metabolic state comes to the formation of both AGE1 and AGE3. Subsequently, the correlation between the level of AGEs and the following parameters: BMI, CRP, HGB, HbA1c, glucose, protein, albuminuria, total cholesterol, LDL, HDL, TG, creatinine, GFR, uric acid, insulin, C-peptide, were checked. However, there any correlation between these parameters and the level of AGE has been established. Maybe these products are new biomarkers, but carrying out further studies is necessary.

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LA10

Spectral properties and secondary structure of C1q-like domain of otolin-1 – influence of calcium ions

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Biomineralization is a biologically controlled process, in which composite materials with unique properties are synthesized. Otoliths and otoconia are biominerals from inner ear of fish and land vertebrates, respectively, which allow to sense linear accelerations. Their inorganic part consists of calcium carbonate, most often aragonite in case of fish otoliths or calcite in case of human otoconia. Organic matrix of otoliths and otoconia contains proteins and proteoglycans. Proteins from otoliths and otoconia can be classified in two groups: scaffold proteins and proteins regulating biomineralization process. Otolin-1 is a collagen like protein from C1q superfamily, which belongs to the first group. It is strongly conserved during evolution, which suggests that it plays a crucial role in biomineralization of otoliths and otoconia. C1q-like domain may be especially important for biomineralization, because it may contribute to oligomerization of otolin-1, bind calcium ions and interact with other proteins from otoliths and otoconia.
In this study, results of investigation of secondary structure and spectral properties of C1q-like domain of otolin-1 from human and zebrafish are shown. Sedimentation velocity analytical ultracentrifugation allowed to observe oligomerization of investigated proteins. Only in presence of calcium ions, both variants formed stable trimers. Circular dichroism and intrinsic fluorescence had shown that structure of C1q-like domain is sensitive to concentration of calcium ions. CD measurements allowed to observe calcium ion concentration dependent induction of β-structures with simultaneous decrease of disordered structure content. Calcium ions also strongly increased thermal stability of C1q-like domain of otolin-1. Significant differences were observed between human and zebrafish variants.
Our results highlight that calcium ions are crucial for trimerization and stabilization of otolin-1, and therefore for formation of stable scaffold for biomineralization of otoliths and otoconia.

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