Late abstracts

Posters

PL.1

Interaction of heparin-binding alarmin HMGB1 with human mast cell

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HMGB1 (amphoterin) is a 30-kDa transcription factor that is released and secreted to extracellular compartment where its functions as proinflammatory cytokine like alarmin capable to mediate organ damage and lethality. HMGB1 has ability to complex with heparin, LPS, IL-1beta, and DNA that might contribute to its alarmin function. High concentrations of HMGB1 have been detected in serum samples of patients with trauma, septic shock, cancer, and various inflammatory diseases. These observations might reflects two known sources of extracellular HMGB1 that are released from necrotic cells and active secretion from monocytes/macrophages, endothelial cells, neurons, and certain tumor cells. Mast cells are important elements of immune response known to play a role in inflammatory processes. There are very limited data on expression of HMGB1 in mast cells and effect of this alarmin on mast cell function. We investigated expression of HMGB1 and its known receptors (TLR2, TLR4, and RAGE) in human mast cell lines HMC-1 and LAD-2. We found that resting HMC-1 cells secreted low amounts of HMGB1 (less than 4 ng/ml/million cells) and were TLR2/TLR4 negative and RAGE negative. LAD-2 mast cells secreted substantial amounts of HMGB1 (more than 30 ng/ml/million cells) and were TLR4 positive and TLR2/RAGE negative. In HMC-1 mast cells LPS upregulated both TLR2 and TLR4 and also induced high level of RAGE expression. In LAD-2 mast cells LPS upregulated TLR4 and induced TLR2 but failed to induce RAGE expression. In conclusion human mast cell lines spontaneously secrete HMGB1 and express receptors known to recognize this alarmin. LPS is capable to upregulate HMGB1 receptors on mast cell surface. These observations might be important for understanding of mast cells involvement in inflammatory processes where bacterial endotoxins occur concomitantly with HMGB1.

PL.2

COMT Val108/158Met polymorphism and its association with overweight and obesity in Polish population

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Introduction: Obesity is a chronic non-infectious disease of complex etiology. Its causes can be both genetic defects and changes in the functioning of the nervous system. Obesity is defined as excessive accumulation of body fat. The most useful way to assess prevalence of obesity is the body mass index (BMI), which is defined as one's weight in kilograms divided by the square of height in meters. Individuals with BMI ≥ 30 kg/m² are assumed to be obese. One of the genes affecting the pathogenesis of obesity is COMT. It encodes catechol-O-methyltransferase, which plays a key role in the degradation of dopamine, and thereby regulates its concentration in the brain.

It is believed that COMT is involved in behaviors associated with the reward system, including feeding. COMT stability is affected by the Val108/158Met polymorphism. Met variant shows a higher lability at 37°C, which is associated with dysfunction of the dopaminergic transduction. This, in turn, is linked to obesity.

Aim: The goal of the analysis was to examine the correlation between the presence of the Val108/158Met polymorphism in COMT gene and the prevalence of overweight and obesity.

Materials and Methods: The age of the study population ranged from 18 to 84 years (mean 51.92, SD 13.97). From all the 873 recruited individuals peripheral blood samples were taken and the measurements were made to calculate BMI. DNA was isolated from the samples and the COMT gene fragment covering Val108/158Met polymorphic site was amplified using the polymerase chain reaction. The obtained DNA fragments were digested with the restriction enzyme HinfI. Met and Val variants were distinguishable from each other based on electrophoretic band patterns. As a method of statistical evaluation, Kruskal-Wallis test and χ² analysis were used.

Results and Conclusions: Nonparametric analysis of variance revealed correlation between Met/Met COMT genotype and increased BMI, both in the general population and in subpopulations of women. This relationship was not observed in subpopulation of men. χ² analysis indicated that in a subpopulation of women there is a correlation between Met/Met genotype and the prevalence of BMI ≥ 35 kg/m², while in men – BMI ≥ 40 kg/m². The data suggests gender effect on the development of obesity. This can be partially explained by estrogens-COMT interactions, suggested by the literature review. Our findings may constitute a starting point for the development of personalized genetic test allowing evaluation of obesity risk.
New factors involved in regulation of alternative splicing in Arabidopsis thaliana

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Global analyses of splicing of precursor messenger RNAs (pre-mRNAs) have revealed that alternative splicing (AS) is highly prevalent in plants. The mechanisms that control splicing and the roles of splice variants generated from a gene has not been yet elucidated. We have previously shown that the nuclear cap-binding protein complex (AtCBC) is involved in alternative splicing in Arabidopsis thaliana. Here we show that AtCBC interacts with SERRATE (AtSE) through binding to the large subunit of CBC, AtCBP80. Moreover, using the RT-PCR alternative splicing panel we have found that AtSE influences alternative splicing, mostly affecting selection of 5' splice site of first introns. The AtSE protein acts in cooperation with AtCBC: many changes observed in the mutant lacking the correct SERRATE activity were common to that observed in the cbc mutant. Interestingly, some changes in the ratios of splicing isoforms were also observed in other mutants of plant miRNA biogenesis pathway, hyl1 and del1-7, suggesting a novel mechanism of alternative splicing regulation in plants. The most intriguing cases were analyzed in terms of finding small RNAs that can influence the level of particular alternatively spliced isoform. Using RNA deep sequencing and global analyses of splicing of precursor messenger RNAs (pre-mRNAs) have revealed that alternative splicing (AS) is highly prevalent in plants. The mechanisms that control splicing and the roles of splice variants generated from a gene has not been yet elucidated. We have previously shown that the nuclear cap-binding protein complex (AtCBC) is involved in alternative splicing in Arabidopsis thaliana. Here we show that AtCBC interacts with SERRATE (AtSE) through binding to the large subunit of CBC, AtCBP80. Moreover, using the RT-PCR alternative splicing panel we have found that AtSE influences alternative splicing, mostly affecting selection of 5' splice site of first introns. The AtSE protein acts in cooperation with AtCBC: many changes observed in the mutant lacking the correct SERRATE activity were common to that observed in the cbc mutant. Interestingly, some changes in the ratios of splicing isoforms were also observed in other mutants of plant miRNA biogenesis pathway, hyl1 and del1-7, suggesting a novel mechanism of alternative splicing regulation in plants.
PL.5

Lovastatin inhibits TGF-β-induced myofibroblasts differentiation of human bronchial fibroblasts derived from patients with asthma

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Asthma is a disease with a complex pathogenesis and differentiated clinical picture with airway inflammation in its background. During multistage remodeling of airways caused by chronic inflammation the differentiation of bronchial fibroblasts to myofibroblasts occurs. This process is mediated by the profibrotic cytokine TGF-β, which leads to gene and protein expression of α-smooth muscle actin (α-SMA) — the major myofibroblasts marker. Statins [3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors] have been described as a principal and the most effective class of drugs to reduce serum cholesterol level. Statins abrogate the conversion of HMG-CoA to mevalonate and thereby inhibit the catabolism of other isoprenoids intermediates of the cholesterol pathway including farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP). These isoprenoids are vital for multiple cellular functions such as covalent attachment, the post-translation and prenylation of numerous proteins, mainly the small GTP-binding proteins. Thus, statins indicate numerous pleiotropic effects such as cell shape, cytoskeleton organization, proliferation and cell signaling. The aim of the study was to determine the effects of lovastatin on the viability, proliferation and — the most important — TGF-β-induced myofibroblasts differentiation of human bronchial fibroblasts (HBF) derived from patients with asthma. The results show that lovastatin exhibits minimal cytotoxicity on HBF and reduces the proliferation activity of the cells in culture. The most important is that lovastatin inhibits the TGF-β-induced myofibroblasts differentiation of HBF and expression of α-SMA on protein level in a dose-dependent manner. The addition of GGPP abolishes the inhibitory effects of lovastatin on TGF-β-induced α-SMA expression but not on differentiation into myofibroblasts, whereas the treatment with FFP has no effect. This antifibrotic activity of lovastatin could play a beneficial role in the treatment of asthma but further studies are required to better understand the mechanism of statins action.

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PL.6

GADD45α in IL-1β-mediated beta cell death

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Beta cells make up 65–80% of the cells in so called islets of Langerhans and secrete insulin in a response to elevated glucose concentration in blood. During the course of Type 1 Diabetes tremendous reduction of β cell mass has been reported. Beta cell death is caused by the autoimmune assault, partially by the exposure to soluble mediators of the inflammatory state, mainly IL-1β (Cnop M et al., 2005, Diabetes 54 Suppl 2: 97–107). Recently, a protective role of growth arrest and DNA damage-inducible (GADD) 45β protein against IL-1β-induced apoptosis of the insulin producing INS-1E cell line was reported (Larsen CM et al., 2006, Diabetologia 49: 980–989). This effect was linked to the inhibition of JNK activation observed in cells overexpressing GADD45β. GADD45β belongs to the family of GADD45 proteins, consisting of three highly conserved isoforms. GADD45 proteins play important roles in regulation of cell cycle arrest, DNA repair, survival and apoptosis. In our study we characterize the influence of the second member of GADD45 family, GADD45α protein, on beta cell death rate following the exposure of cells to IL-1β. We show that GADD45α at the close to physiological expression level is deleterious for insulin-producing INS-1E cells, which seems to be a common phenomenon for numerous cell types. However, our study shows also that high levels of GADD45α expression may provide survival advantage for beta cells upon exposure to IL-1β. The effect of GADD45α overexpression on INS-1E cells is mediated by modulation of activity of JNK, p38, ERK and NF-κB. Conversely, expression of GADD45α in stimulated cells is tightly regulated by JNK and NF-κB in both nitric oxide-dependent and independent manner. Altogether, our findings suggest that GADD45α protein is engaged in a complex mechanism regulating beta cell fate, and depending on its expression level may contribute to or protect against IL-1β-mediated beta cell death.

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Downregulation of MET receptor influences rhabdomyosarcoma cell differentiation

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Introduction: MET receptor, encoded by MET proto-oncogene, belongs to the family of growth factor receptors with intrinsic tyrosine kinase activity. It has been shown that deregulation of MET activity is a key event underlying tumor metastasis and MET overexpression and hyperactivation have been reported to correlate with metastatic ability of tumor cells. Physiologically, Met is rapidly downregulated at the onset of myogenic differentiation. Rhabdomyosarcoma (RMS) seems to be a good candidate for differentiation therapy because RMS cells are blocked on their way to terminal muscle differentiation. The precise molecular mechanism responsible for the disruption of myogenesis, characteristic for RMS tumors, is not fully understood.

Maturation protocol: RH30 cells were maintained in DMEM, supplemented with horse serum (2%) and 100 nM TPA. Cells were cultured in this medium for 4, 8 and 10 days and subsequently used in appropriate experiments.

Methods: Lentiviral vectors construction, production and in vitro transduction, RNA extraction and reverse transcription, Quantitative real time RT-PCR analysis, Chemotaxis assay, Western blot, FACS analysis, Murine models - NOD-SCID mice.

Results: Differentiation process caused downregulation of MyoD to undetectable level and increased expression of Myogenin. We also observed MET receptor downregulation after differentiation process. Cells subjected to differentiation showed strong defect in ability to migrate and lower expression of CXCR4.

Conclusion: In this study, for the first time, we have shown that differentiation of RMS cells is connected to the decrease expression and signaling of MET receptor. These findings might have a significant clinical implication for the treatment of RMS cells because they suggest that induction of differentiation of RMS cells by e.g. blocking MET receptor might have influence on the aggressiveness/metastatic potential of these tumors.

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Analysis of porcine piwi genes and piRNA expression during gametogenesis

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The Argonaute proteins are the main components of RISC complex which plays a crucial role in the mechanism of RNA interference. This protein family can be divided into two subfamilies, Ago and Piwi. Ago proteins are ubiquitously expressed and preferentially bind to miRNA and siRNA. In contrast to Ago proteins, expression of Piwi proteins is restricted to germline cells where they associate with a new class of small RNAs – piRNAs. Piwi/piRNA complexes are presumably involved in silencing of mobile genetic elements ensuring genomic stability of germ cells. Using a bioinformatics approach, we identified three porcine piwi genes (Piwi1, Piwi2 and Piwi4) which encode Siwi, Sili and Siwi2 proteins of 861, 985 and 853 amino acids, respectively. Porcine piwi genes were cloned and sequenced. The determined identities of porcine and human Piwi proteins were 96% for Siwi and human ortholog Hiwi, 86% for Sili and Hili and 81% for Siwi2 and Hiwi2, respectively.

Applying the reverse transcription PCR analysis, we observed a tissue specific expression of these three Piwi mRNAs restricted exclusively to gonads. Additionally, using the real-time PCR technique, we examined expression of siwi, sili and siwi2 genes in ovaries, oocytes and testes derived from sexually mature and immature animals. We identified expression of three piwi genes in porcine oocytes in which twice higher expression of Sili transcript than Siwi and Siwi2 was detected. In contrast to oocytes, in both groups of oocytes Siwi transcript was the most abundant and inconsiderable expression of Siwi2 was observed. Additionally, the expression of piRNA in both groups of the studied ovaries was similar.

In testes of adult animals we observed the highest expression of Siwi and much lower expression of Sili. On the other hand, in gonads of a 1-day-old piglet, Siwi expression was about 2-fold reduced while the level of Sili transcript was higher when compared to mature males. Although expression of Siwi2 transcript was very low in adult testes, its level was 34-fold elevated in the gonads of 1-day-old piglet. Interestingly, a subset of piRNA was only expressed in adult males.
PL.9

Autologous muscle-derived stem cell injection for female stress urinary incontinence: a 1-year follow-up of Polish investigation

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Purpose: We evaluated the effects of muscle-derived stem cells therapy among women with stress urinary incontinence (SUI).

Material and methods: Muscle-derived stem cells were isolated from upper arm muscle biopsy from eighteen women with SUI. They were cultivated and after 6 weeks injected trans urethral into the urethral rhabdosphincter each woman. Endoscopic guidance procedure is a single-dose injection of a cell count in range of 0.9 up to 15 × 10⁶ MDSC circumferential at 9, 12 and 3 o’clock under local anesthesia.

Results: Initial results of SUI treatment with adult muscle-derived stem cells suggest that perspectives of this method are encouraging.

Conclusions: Stem cell therapy is promising to become minimally invasive method for reconstruction of the urethral rhabdosphincter muscles.

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PL.10

Laser microdissection

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Molecular analysis are mostly carried out in heterogeneous biological material, and results obtained in this way illustrate the situations that exist in different cell types, also in cells that are not the object of our interest. An important need of many research projects is the availability of high-quality, pure and well defined cell - for example normal and cancer cells. These challenges can be overcome via the use of laser microdissection which allows separation of single cells or defined groups of cells from complex tissues almost unchanged, both morphologically and biochemically. Laser microdissection is technology that provides the scientific community with a rapid method to isolate a homogeneous population of cells from heterogeneous tissue specimens, thus providing investigators with the ability to analyze DNA, RNA, and protein accurately from pure populations of cells.
The physicochemical properties of T4 phage

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Phage has many potential applications in human medicine, veterinary science, agriculture and food technology. Knowledge of the physicochemical properties of bacteriophages is crucial both to understand their properties and to develop bacteriophage’s technologies. The paper presents the results of bacteriophage aggregation determined by dynamic light scattering and atomic force microscopy.

On the basis of size measurements it was found that critical level of ionic strength below which the process of size increase starts is about 0.03 M. pH of the medium (slightly alkalic) is also important. The AFM imaging indicates that increase in particle sizes can be associated with the process of aggregation of particles emerging in the alkaline environment of lower ionic strength.

Presented results proved that physicochemical properties of T4 bacteriophages depend on medium composition, in particular on the ionic strength and pH. The observed aggregation process was possible due to the change of the nature of interparticle interactions starting to be attractive in solutions of diminished electrolyte content.

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Production of 1-butanol from cassava starch by Clostridium acetobutylicum

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Bio-solvents, such as acetone, butanol and ethanol are typical bulk chemicals whose industrial importance could expand. After ethanol; also butanol could supersede under certain conditions the traditional liquid fuels. Among biofuels, 1-butanol specifically has more suitable physicochemical properties, even better in contrast to ethanol and similar to gasoline and during combustion butanol is less harmful to the environment. These biosolvents are produced by bacterial acetone–butanol–ethanol fermentation. The aim of our studies were to optimize for industrial process the 1-butanol formation with respect to cassava roots application as the main raw material like starch substrate. As optimization parameters were chosen butanol concentration in the cultivation broth, butanol productivity and yield. The medium composition from the point of view of the content of other nutrients was optimized as well. Batch fermentation was carried out in Erlenmeyer flasks under anaerobic conditions at 37°C. The bacterium Clostridium acetobutylicum DSM 1731 manifesting high amylolytic activity was used as a production strain. It yielded the butanol titer of 12.3 g/l and total solvent concentration of 16.6 g/l.