Session 9. Cellular Motility in Pathology

Lectures

L9.1

Myosin VI in autophagy and neurodegeneration

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Myosins of class VI are unique actin based motor proteins that move cargo towards the minus ends of actin filaments. This motor interacts with a wide variety of adaptor proteins, which regulate cargo attachment and thus mediate the very specific intracellular functions of myosin VI in the endocytic and exocytic membrane trafficking pathways. These adaptor proteins may also act as molecular switches to regulate the on/off state of myosin VI.
Our new data shows that myosin VI, in concert with its adaptor proteins optineurin, NDP52, T6BP and Tom1 plays a crucial role in autophagy, a degradative pathway that the cell uses to clear pathogens, damaged organelles and protein aggregates. The ESCRT-0 protein Tom1 is a novel myosin VI adaptor protein in mammalian cells that is involved in targeting of myosin VI to early endosomes. The loss of either myosin VI or Tom1 reduces delivery of endocytic cargo to autophagosomes, thereby preventing autophagosome maturation and autophagosome-lysosome fusion. As a consequence loss of myosin VI function causes a defect in autophagosome clearance and an accumulation of toxic protein aggregates in the cell. This observed dysfunction in autophagy may help to explain the profound astrogliosis phenotype that we observed in the myosin VI KO mouse brain.

L9.2

The molecular basis of inherited cardiomyopathies

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The inherited heart disorder Hypertrophic Cardiomyopathy (HCM) is primarily caused by mutations in genes encoding cardiac contractile proteins, leading it to be labeled a disease of the sarcomere. Mutations in the thick filament proteins beta myosin heavy chain and cardiac myosin binding protein-C predominate, although HCM can also be caused by mutations in thin filament proteins (actin, alpha-tropomyosin, troponin subunits). The different disease of Dilated Cardiomyopathy (DCM) can also be caused by mutations in the same contractile protein genes. A different set of mutations is responsible for DCM, suggesting that distinct changes in contractile protein structure and function can lead to the different diseases. I will summarise the functional effects of the HCM and DCM mutations on cardiac contractility and its regulation, focusing on our work on mutations in troponin subunits and alpha-tropomyosin; this has shown that HCM mutations increase myofilament calcium-sensitivity and DCM mutations have the opposite effect of decreasing this parameter. I will discuss how these changes may alter intracellular calcium handling and cardiac energetics, and how these alterations may trigger the disease states.

L9.3

Role of tropomodulins in neuritogenesis

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Assembly of the actin cytoskeleton is an important part of formation of neurites in developing neurons. Tropomodulin (Tmod), a tropomyosin (TM)-dependent capping protein for the pointed end of the actin filament, is one of the key players in this process. In PC12 cells, a cellular model used to study neuronal differentiation *in vitro*, the overexpression of Tmod isoforms, Tmod1 or Tmod2, showed opposing effects on neurite formation and outgrowth. Tmod1 did not affect neuronal differentiation; while cells expressing Tmod2 showed a significant reduction in the number and the length of neurites. Tmod consists of two structurally and functionally distinct domains, the N-terminal disordered domain, which contains two TM-binding sites and one actin-binding site, and the well-folded C-terminal domain, so called LRR domain. Both of these domains are important for proper function of tropomodulin isoforms in neurones. The integrity of the TM binding sites is critical for the proper function of Tmod. Mutations in one of the tropomyosin-binding sites of Tmod1, which increased its affinity to short g- and d-tropomyosin isoforms and made it similar to that of Tmod2, caused a 2-fold decrease in the length of neurites. Overexpression of Tmod1 with truncated LRR domain closely mimicked the phenotype observed after Tmod2 overexpression, while overexpression of the Tmod2 with truncated LRR domain had no effect. Circular dichroism and limited proteolysis were utilized to assess the Tmod2 structure for comparison to that of Tmod1. Tmod2 had a lower alpha-helical content than Tmod1, indicating that Tmod2 is a less structurally ordered protein. Tmod2 proved to be less stable than Tmod1; it was more susceptible to proteolysis and began to denature when exposed to lower urea concentrations. The dissimilar stability of the two isoforms suggests differences in their tertiary structures, which may account for their functional differences during neuritogenesis.

Oral presentations

O9.1

Reconstitution of EB1-dependent plus-end tracking of kinesin-14 Ncd on tyrosinated and detyrosinated microtubules

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During cell division, the kinesin-14 Ncd accumulates at the plus ends of growing microtubules, although the motor itself is minus-end directed. Previous work suggested that the Ncd localization is mediated by the end-binding protein 1 (EB1). Here, we have reconstituted *in vitro* the microtubule tip-tracking process by Ncd and EB1 using the Ncd tail and full-length EB1 from *D. melanogaster*. Subsequently, we have studied the effect of the tyrosine removal from the a-tubulin C-terminal GluGluTyr/Phe sequence motif on the tracking efficiency. Ncd was GFP-tagged and the microtubule growth was visualized using the TIRF microscopy. The binding of Ncd tail alone to taxol-stabilized detyrosinated and control microtubules was also studied; microtubule detyrosination produced no change in the affinity. However, when dynamic microtubules were used, Ncd in the presence of EB1 tip-tracked the plus-ends of detyrosinated microtubules with the efficiency of about two times higher than that of control microtubules. The efficiency of plus-end tracking by GFP-tagged EB1 without Ncd on detyrosinated microtubules was also about two times higher. We measured the tracking efficiency of human EB1 on detyrosinated microtubules and found that it was only slightly higher in comparison to control microtubules. These findings suggest that the underlying mechanism for the difference in the tracking efficiency of Ncd on control and detyrosinated microtubules originates from different tracking efficiency of EB1. The above results help in understanding the mechanism of interaction of EB1 with the MT plus ends.

O9.2

The role actin rearrangement in methamphetamine-induced disruption of the blood-brain barrier

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Methamphetamine (METH) abuse is one of the fast growing drug problems. METH neurotoxicity has been characterized by dysregulated synaptic reuptake of major monoamine neurotransmitters and the generation of oxidative stress. Neurotoxic and neuroinflammatory effects of METH also include disruption of the blood-brain barrier (BBB) and alterations of tight junction protein expression. The actin cytoskeleton is important for cell shaping, biological cell movements, and cytokinesis. Indeed, the paracellular permeability is maintained in part by the equilibrium between contractile forces generated by the cytoskeleton and adhesive forces produced by cell-cell junctions and cell-matrix contracts. One of the principal actin polymerizing and organizing factors is the actin-related protein 2/3 (Arp2/3) complex. Therefore, the present study focused on the actin cytoskeletal rearrangement as a modulator of METH-induced redistribution of tight junction protein occludin.

Exposure to METH enhanced occludin endocytosis and resulted in a shift of occludin localization from plasma membranes to endosomes. These changes were accompanied by activation of the Arp2/3 and actin polymerization. In addition, METH induced coronin-1b phosphorylation, which diminishes the inhibitory effect of non-phosphorylated coronin-1b on actin nucleation. To further address the role of coronin-1b in METH-induced Arp2/3 complex activity, cells were transfected with coronin-1b-specific or control siRNA followed by exposure to METH. METH significantly increased the binding of WASp to Arp2; however, coronin-1b silencing protected against this effect, suggesting that METH-induced binding between Arp2 and WASp is dependent on the presence of coronin-1b. In addition, blocking actin nucleation with CK-666, a specific inhibitor of the Arp2/3 complex, protected against METH-induced occludin internalization and increased transendothelial monocyte migration. Importantly, treatment with CK-666 attenuated a decrease in occludin levels in brain microvessels of METH-injected mice. The present findings indicate that actin cytoskeletal dynamics is detrimental to METH-induced BBB dysfunction by increasing internalization of occludin.

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O9.3

Does arginine deprivation impairs β-actin arginylation?

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Arginylation is posttranslational modification that is involved in cytoskeleton organisation and seems to play a key role in cardiovascular development (Kashina *et al*., 2006, *Science* **313**: 192–196). In this report, we have found for the first time that lack of arginine reduces β-actin filament formation level, without significant effect on total actin level and causes collapse of the leading edge lamella of human U251MG glioblastoma cells. To test whether the observed changes in β-actin organisation could be due to differences in posttranslational modification pattern, 2D-electrophoresis was performed. Obtained data indicate that depletion of arginine evokes changes in this actin isoform as positively charged β-actin variants were absent in arginine-deprived cells, in comparison with control cells. Moreover, we did not observe significant changes in isoelectric pattern of β-tubulin. While decreased F-actin level and other cellular defects may be mediated by different arginylation-dependent events, the leading edge collapse is most probably due to lack of arginine, and consequently- lack of N-terminally arginylated glutamic acid residue in β-actin isoform. We do not expect γ-actin arginylation as arginylated form is removed by a specific mechanism. In order to further confirm that arginylation of β-actin (known to be reversible) is involved in the observed collapse of the lamellipodium, we re-introduced arginine into the deprived cells. Incubation the cells in re-supplemented media rescued the phenotype as the cells were oval and formed wild lamellae, typical for the control cells.

However, final conclusion has to made by demonstrating by means of mass spec analysis that β-actin is indeed arginylated.

O9.4

The effect of AnxA2 on expansion of human osteosarcoma cells *in vitro*

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Osteosarcoma (OS) is an aggressive primary tumor of the skeleton and is characterized by the rapid development of metastases. Annexin A2 (AnxA2) belongs to the class of proteins participating in bone remodeling, therefore, it is proposed to have an impact on OS phenotype. We have shown that AnxA2 and its binding partner (S100A10) are selectively translocated into matrix vesicles as well as secreted into the extracellular milieu by OS cells undergoing mineralization. These findings prompted us to analyze the role of extracellular AnxA2 on the balance between the expansion and osteogenic potential of OS cells. For this purpose, we examined two human OS cell lines: osteoblast-like Saos-2 cells and osteolytic, highly metastatic 143B cells, grown for 7 days in the presence of human recombinant AnxA2 in the culture media at final concentrations varying from 0.05 μg/ml to 5 μg/ml. As a vehicle, OS cells were treated either with human recombinant AnxA6 or bovine serum albumin at the same concentration range as AnxA2. An *in vitro* control of mineralization was assayed after 7 days of cell culture in the growth medium supplemented with 50 μg/ml ascorbic acid and 7.5 mM β-glycerophosphate (AA/β-GP). Each experimental group was tested for cell expansion (viability, proliferation, cell cycle, migration, invasion) and differentiation (alkaline phosphatase activity, staining of mineral deposits). Herein, we report that supplementation of Saos-2 cells with AnxA2 promoted mineralization process. However, this phenomenon was not accompanied by decreased proliferation rate and prolongation in G2-M phase transition unlike in case of stimulation of cells with AA/β-GP. Moreover, AnxA2 or AA/BGP independent supplementation decreased migration and invasion of OS cells in a time- and dose-dependent manner when compared to the non-treated control. Furthermore, AnxA2 distribution on the cell surface and its active uptake by OS cells was observed by confocal microscopy using FITC-stained human recombinant AnxA2. In conclusion, the obtained results suggest that stimulation of OS cells with exogenous AnxA2 promotes the differentiation and inhibits motility of OS cells. Further studies are required to identify the protein binding partners of AnxA2 and mechanisms involved in the described above processes.

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O9.5

Silencing the expression of genes involved in extracellular matrix remodeling influences migration and invasiveness of cancer cells

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Introduction: Cells of the growing tumor are able to overcome the barrier of extracellular matrix (ECM), which leads to the invasion of surrounding tissue. They also pass through the walls of blood and lymph vessels which enables the formation of metastases [1]. Most of death cases among patients suffering from cancer is not due to local complications caused by the presence of tumor, but is related to the metastatic potential of cells. Motility and invasiveness is associated with the proteolytic activity which provides the degradation of ECM components. Enzymes belonging to a family of extracellular matrix metalloproteinases (MMPs) are secreted both by tumor and microenvironmental cells [2, 3]. Many publications point exceptionally strong correlation between the level of MMP-2 and MMP-9 and tumor invasiveness [4, 5]. Therefore, the inhibition of metalloproteinases may potentially reduce the invasive potential of cancer cells.
The aim of the study: The aim of the study was to evaluate the changes in the motility and invasiveness of T24 cancer cells with modulated expression of *MMP-2* and *MMP-9* genes.
Material and methods: Studies were carried out on cellular model of human urinary bladder cancer. T24 cells (HTB-4) were cultivated under *standard conditions*. Molecular vectors encoding shRNA/MMP-2 or shRNA/MMP-9 were introduced using Lipofectamine®2000 transfection reagent (Invitrogen). The expression of modulated genes was examined at the level of RNA and protein, using Real Time™ PCR and immunoenzymatic method (ELISA, Abcam). Changes in migration of modulated cells were assessed using *in vitro* wound-healing assay. Changes in cell invasiveness were evaluated using Matrigel™ Invasion Chambers (BD Bioscences).
Results: The effectiveness of silencing the *MMP-2* and *MMP-9* expression was successfully confirmed at both the transcriptional and at the protein level. Conducted modulation resulted in a decrease of cell motility. The cells have migrated a shorter distance and slower occupied migration field created in the wound healing assay. There was also a decrease in invasiveness of modulated cells, which was observed as reduced number of cells that was able to pass through the Matrigel™ membrane.
Conclusions: The ability to control the mobility of cancer cells could be a valuable tool in the fight against cancer. Based on our results of *MMPs* expression silencing using RNA interference technique we summit this strategy as an interesting target point for anti-cancer gene therapy.

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O9.6

The synthetic triterpenoids, HIMOXOL and Br-HIMOLID, inhibit migration and invasiveness of breast cancer cells

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Inhibition of cancer cells proliferation by modulation of their adhesion and migration as well as invasion is a key element of the anticancer therapy strategy. The ability of cancer cells to metastasise is the most important factor associated with a reduced therapy efficacy. This phenomenon also concerns breast cancer, one of the most common tumour types in women. The metastatic spread of this cancer is responsible for 90% of human cancer-related deaths.
Triterpenoids represent the group of natural compounds with a proven wide spectrum of antitumor activity expressed by e.g. inhibition of tumour cell promotion and angiogenesis. Oleanolic acid (OA), known for its biological properties, belongs to this group. However, the pharmacological activity of this natural compound is associated with different side effects and a non-selective mechanism of action. Thus, designing and synthesizing new modified triterpenoids constitutes an important challenge. Consequently, this might lead to a significantly increased effectiveness of their activity in cancer treatment.
The aim of this study was to evaluate the ability of two selected oleanolic acid derivatives to modulate migration and invasion processes in breast cells *in vitro*. MCF-12A (ER+) normal breast cells, MCF7 (ER+) and MDA-MB-231 (ER-) breast cancer cells were treated by oleanolic acid, methyl 3-hydroxyimino-11-oxoolean-12-en-28-oate (HIMOXOL) and 12α-bromo-3-hydroxyimonoolean-28→13-olide (Br-HIMOLID) in different concentrations (up to 50 μM). The semisynthetic OA derivatives were synthesized at the Organic Chemistry Department, Poznan University of Medical Sciences. After incubation for 24 h, cell viability was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Migration of all cell lines was studied by migration "scratch assay". Moreover, invasion potential of breast cells was measured by BD BioCoat Tumor Invasion System and western blot (WB) analysis of focal adhesion proteins, which included focal-adhesion kinase (FAK), a receptor protein — integrin β1 and adaptor molecule — paxillin.
We found that OA, HIMOXOL and Br-HIMOLID treatment in non-cytotoxic concentrations did not inhibit the migration of normal breast cells, MCF-12A. However, studied compounds retarded the migration and invasion of breast cancer cells in a dose-dependent manner. It was revealed that FAK, integrin-β 1 and paxillin are the proteins which are responsible for regulation of migration and invasion of treated breast cancer cells.
Conclusion. The synthetic derivatives of oleanolic acid inhibit migration and invasion of breast cancer cells but do not affect normal breast cells *in vitro*.

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Posters

P9.1

Cooperation between Angiotensin II and Relaxin in the progress of spread of the prostate cancer cell lines

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Interactions between renin-angiotensin system (RAS) and peptides from the relaxin-like family (RLF) were reported in cardiovascular system and the central nervous system. Angiotensin II and Relaxin have both chronotropic and inotropic properties however, their actions are contradictory to each other. On the other hand, it has been shown that Relaxin may act by the renin-angiotensin system to induce the release of vasopressin and oxytocin. Dysregulation of certain peptide hormones, such as Angiotensin II and Relaxin, have been linked to cancer initiation and progression. Therefore we decided to evaluate biological relationship between RAS and RLF in prostate carcinogenesis. The two prostate cancer cell lines used in the study were characterized by a different invasive potential *in vitro*, as well as different sensitivity to androgen (LNCaP and PC3).The Wound Healing Test and a Transwell Migration Chamber with 8 μm pore size were used as methods to assess the migration ability before and after peptides treatment. Cell migration is a process which is essential during cell invasion and metastasis. Furthermore we examined the influence of combined peptide hormones on ability of prostate cancer cells to adhere to laminin, collagen I, collagen IV and fibronectin. Migration/invasion of cancer cells is critically regulated by physical adhesion of cells to extracellular matrix (ECM). Cell proliferation and viability were defined by using BrdU Assay and Alamar Blue Assay, respectively. Survival and the number of cell divisions play an important role in both the growth and development of cancer. The obtained results confirm the influence of the above mentioned peptides on prostate cancer cells, regarding to the invasion and migration, cell adhesion, cell viability and proliferation. Nevertheless, the individual, stimulatory potence of Ang II and Rel 2 has rather not been translated into the synergistic or cumulative effects in the case of both peptides. Effects of combination of Ang II and Rel 2, were the most frequently intermediate or lower than the ones which were accomplished by separate incubation experiments. The obtained results suggested that the investigated systems: RAS and RLF, have impact on spread of prostate cancer at least a partial overlapping of the signal pathways.

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P9.2

Analysis of cell growth and morphology in three-dimensional culture — effect of silk scaffold porosity

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Introduction: Two-dimensional, cell culture has proven to be inadequate to study complex cell-cell interactions and cell — extracellular matrix interactions. 3D cultures based on scaffolds provide microenvironment more similar to that *in vivo*. Thanks to its biocompatibility, excellent mechanical properties and slow biodegradability, silkworm silk is a perfect biomaterial for cell studies and *in vivo* experiments. In this work silk protein was shaped into a porous scaffolds which were used to study cell attachment and proliferation in three dimensional (3D) environment.
Aim of the study: Analysis of proliferation of cells of different origin on the 3D silk scaffolds with different pore sizes.
Materials and Methods: Breast cancer cells (EMT6) and fibroblasts (NIH 3T3) were genetically modified by lentiviral vectors to express fluorescent proteins. Aqueous *B. mori* silkworm silk solution was used to produce scaffolds by two methods: salt leaching and lyophilization. Cells were cultured on the scaffolds (3D) with pore sizes: 100–250 um, 250–500 um, 500–750 um and lyophilized. The proliferation rate of cultured cells was compared using Alamar Blue reagent. Cells were cultured separate as well as in coculture. The morphology of cell cultures was observed using confocal laser microscopy and scanning electron microscopy.
Results: Proliferation rate of cancer cells was higher than fibroblasts in all cultures. In coculture fibroblast were outgrown by cancer cells. Scaffolds made by salt leaching method were better penetrated by cells and overall better suited for cell culture than lyophilized ones.
Conclusion: Silkworm silk scaffolds made by salt leaching method have proven to be good matrices for cell culture. The heterologous 3D cancer model composed of cells like epithelial, fibroblast and cancer will provide the microenvironment closely related to one observed *in vivo*.

P9.3

Nuclear translocation of myosin VI upon stimulation of neurosecretory PC12 cells: a possible role of MVI in gene expression

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Myosin VI (MVI) is a unique, actin-based motor protein that moves along actin filaments in the opposite direction of all other myosins. It is implicated in processes associated with the actin-cytoskeleton such as for example: endocytosis, cell migration, cytokinesis and possibly in secretion. Our previous studies demonstrated that in neurosecretory PC12 cells MVI localized both in the cytoplasm and nucleus. Moreover, we showed that nuclear localization of MVI was even more pronounced upon stimulation with 59 mM KCl, especially after 5 min of treatment. Stimulation-dependent MVI translocation was accompanied by its co-localization with several nuclear proteins involved in transcription and nascent transcript maturation, namely active form of RNA polymerase II, transcription factor Sp1, PML bodies, SC35-containing nuclear speckles and hnRNP U. Also, MVI colocalized with transcriptionally active sites as measured by BrUTP incorporation. Interestingly, we also observed that after stimulation total amount of MVI in the cell was increased, with the highest increase after 5 min. At the same time, the levels of PML, SC35 and hnRNP U proteins were increased as well. Contrary to that, in PC12 cells with MVI knockdown, characterized among others by the proliferation decrease, a significant reduction of PML level was observed. Also, our mass spec analyses of the eluate fraction of MVI-based pull-down assay identified numerous nuclear proteins, including hnRNP U and several proteins involved in transcription and post-transcriptional processes.
These data indicate the existence of functional interactions between MVI and transcription machinery. Thus it is plausible that in neurosecretory cells MVI may play a role in transcription, and consequently in gene expression.

P9.4

Myosin VI involvement in myoblast migration and adhesion as well as in maintenance of Golgi apparatus and endoplasmic reticulum organization

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Myosin VI (MVI) is the only known myosin walking towards the minus end of actin filament. Acting as a cargo transporter and/or an anchor, MVI is involved in numerous processes associated with actin cytoskeleton such as cell spreading and migration, endocytosis and intracellular trafficking as well as gene transcription. It seems to have a very important role in striated muscle functioning as a point mutation in human *MYO6* is associated with hypertrophic cardiomyopathy. However, the function of MVI in muscle tissue remains unknown. Our studies showed that in striated muscles MVI could be involved in postsynaptic trafficking as well as in maintenance of and/or transport within the sarcoplasmic reticulum, and possibly in gene transcription (Karolczak J *et al.*, 2013, *Histochem Cell Biol* **139**: 873–85). Recently we found that MVI knockdown (MVI-KD) in C2C12 murine myoblasts caused significant changes in cell morphology and cytoskeleton organization. MVI-KD cells were oval-shaped and migrated slower than control cells. Also, their adhesive properties were changed as they were more adhered to the glass surface. This was accompanied by a significant reduction in the size but not the number of adhesive structures. Also, immunostainings for TGN130 and calreticulin, the markers of Golgi apparatus and endoplasmic reticulum (ER), respectively, revealed that MVI deficiency affected organization of those membranous compartments. They were more compact, and less ramified, especially in the case of ER. Thus our observations indicate that MVI is involved in myoblast migration and adhesion, as well as in maintenance of its Golgi apparatus and ER morphology. Also, they justify a question as of possible involvement of this molecular motor in myoblast differentiation.

P9.5

Effect of the low oxygen concentration on the migration and clonogenicity of colon cancer cells preselected by anticancer agents — therapeutic implications

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Hypoxia inside tumor promotes maintenance of cancer stem/progenitor cell phenotype. The cancer cell invasiveness and the cancer cell resistance to therapy increases in the hypoxic conditions. Sunitinib as a small-molecule, multi-targeted receptor tyrosine kinase (RTK) inhibitor could be used as a therapeutic agent against cells with stem/progenitor cell phenotype.

The aim of our study was to assess the impact of hypoxia on invasiveness and clonogenic potential of colorectal cancer cells exposed to sunitinib or 5-fluorouracil (5-FU). Cultures of human colon cancer cell line HT-29 derived from a moderately differentiated tumor and of human cell line HCT-116 derived from a poorly differentiated, highly metastatic colon cancer, were carried out at 5% O_2 concentration or at the standard oxygen conditions used for *in vitro* cultures. In the monolayer wound healing assay, migration of HCT-116 cells cultured in the standard oxygen conditions was markedly reduced by sunitinib. Migration of HCT-116 cells cultured in the hypoxic conditions was not affected by sunitinib. In the monolayer wound healing assay, migration of HT-29 cells was equally reduced by sunitinib in the standard and in the hypoxic conditions. The clonogenicity of colon cancer cells preselected with 5-FU was higher if the preselected cells were subsequently cultured in hypoxic conditions in comparison to the clonogenicity of the cells cultured in the standard oxygen pressure conditions. The yield of HCT-116 cell clones formed in the hypoxic conditions was 1.9 times higher, and the yield of HT-29 cell clones formed in the hypoxic conditions was 3.8 times higher than the yield of clones formed in the respective cultures set in the standard oxygen conditions. By flow cytometry, the expression of colon cancer stem cell markers CD133 and CXCR4 on HT-29 cells cultured for 4 days at 5% O_2 concentration did not substantially differ from the expression of these markers on HT-29 cells cultured in the standard oxygen conditions.

Study results demonstrate that the low partial pressure of oxygen can promote the recovery potential of cancer cells exposed to anticancer agents, such as classical cytostatic 5-FU and multi-targeted receptor tyrosine kinase (RTK) inhibitor sunitinib. Therefore anti-cancer agents designed to target stem/progenitor cancer cells should be preferentially tested in the hypoxic culture conditions.

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P9.6

IFN-γ and IL-10 at a HIV-infected children with acute rhinosinusitis

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We investigated 25 HIV-infected with acute rhinosinusitis (ARS) children at the age from to 3 till 14 years old. The HIV diagnosis was based on revealing of specific antibodies in standard serological tests. Level cytokines (IFN-γ, IL-10) in the peripheral blood was studied a method of the immune enzyme analysis with use of test systems by firms "Vector-Best" (Russia). Parameters of the immune status studied twice: before and 1 month after treatment.
Spectrum studying cytokines at a HIV-infected of children with ARS has shown that at them presence of significant differences between values of the basic group with control group was marked. So, for example, if at healthy children level IFN-γ made 23.70±5.38 pg/ml, at a HIV-infected of children with ARS the similar parameter was in 3/5 times above and there was at level 82.84±21.17 pg/ml. So, high level IFN-γ at a HIV-infected of children with ARS testified to expressiveness of degree of inflammatory reaction. Level IL-10 in group at a HIV-infected of children with ARS approximately in 8 times higher than those values of the control group. It is known that IL-10 it is described as the factor stimulating B-lymphocytes as it causes proliferation B-cells. The main producers IL-10 are Th2 cells. IL-10 inhibits functions of macrophages and secretion by them IL-1, FNO and IL-6, having thus anti-inflammatory an effect. IL-10 causes proliferation and a differentiation B - and T-lymphocytes, influences development hematopoietic cells, on macrophages, natural killers, basophiles, being the functional antagonist cytokines, produced Th1 cells. IL-10 promotes development of allergic reactions, possesses the expressed anti-inflammatory action.
The comparative analysis has shown that the parity IFN-γ/IL-10 (proinflammatory/anti-inflammatory cytokines or Th1/Th2) at healthy children equaled 2.2. In the presence of the expressed inflammatory process, that is at children of the basic group, this indicator made 0.96. The expressed disbalance in functioning of the core regulator cytokines which was expressed by acute lifting of level anti-inflammatory cytokines and suppression proinflammatory cytokines, acute inflammatory conditions being the basic regulators is revealed.

P9.7

Relationships between kinematic motion parameters and metabolic efficiency of cryopreserved bull spermatozoa

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Freezing-thawing process of semen causes structural and functional changes in spermatozoa, resulting in their reduced metabolic efficiency and fertilizing ability (Buhr *et al.*, 1994)). The main metabolic processes of spermatozoa are oxidative phosphorylation and glycolysis, which provide energy for the sperm functional activity. Such energy is provided in the form of ATP, which is required for sperm movement (Dziekońska, Strzeżek, 2011).
The aim of this study was to determine the relationships between some selected kinematic parameters of motility and the metabolic efficiency of bull spermatozoa following cryopreservation. Cryopreserved semen was obtained from 200 bulls at the Artificial Insemination (SHiUZ Bydgoszcz). The sperm motion kinematic parameters were analyzed using a computer-assisted sperm analysis (CASA) system. The metabolic efficiency of sperm cells was represented by the measurements of ATP content and the assessments of the sperm mitochondrial function, using the JC-1 fluorochrome.
The percentages of total motile and linear motile frozen-thawed spermatozoa were 61.31±7.69 (mean±S.D.) and 21.76±7.27, respectively. It was found that the proportions of frozen-thawed spermatozoa with circular movements were 39.34±3.18%, whereas those with locally motile were 31.40±6.20%. In frozen-thawed sperm samples, ATP content was 15.63±3.27nmol/10^8 spermatozoa, whereas the percentage of spermatozoa with functional mitochondria was 71.45±2.01.
Statistical analysis of the results showed positive correlations between the analyzed sperm parameters after cryopreservation. The percentage of total motile spermatozoa was correlated with sperm ATP content ($r=0.97$, $p\leq0.01$) and mitochondrial function ($r=0.24$, $p\leq0.01$).
The results of this study reaffirm the importance of the functional mitochondria in the maintenance of an efficient motility apparatus of cryopreserved bull spermatozoa.

Acknowledgements:

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P9.8

Monomeric beta actin dominates in nuclear actin pool

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Actin is multifunctional protein existing in all eukaryotic cells. In vertebrates, six actin isoforms are known: two of which are present in striated muscle (alpha skeletal actin and alpha cardiac actin), alpha and gamma smooth muscle actin and two cytoplasmic isoforms beta and gamma actin. Cytoplasmic beta and gamma actin differ from each other by only four amino-acids. Actin appears in the cytoplasm and in the nucleus. During last decade a lot of attention was dedicated to nuclear actin organization and function. Actin participates in nuclear structure organization, chromatin remodeling, transcription and signal transduction, but there is still lack of information on nuclear actin organization and the state of its polymerization- measured by filamentous to monomeric (F:G) actin ratio. Our goal was to recognize beta and gamma actin level and to determine the state of actin polymerization in cancer cells nuclei.
Experiments were performed on human melanoma A375 cell line. The results were compared with those obtained for normal human dermal fibroblasts (NHDF). Filamentous actin - when detected with phalloidin conjugated with Alexa Fluor®568, was not seen in the nucleus of investigated cells. Actin was shown in the nucleus of A375 and NHDF cells, using the immunocytochemistry and monoclonal antibodies, directed against total actin and beta and gamma isoforms. We demonstrated that not only beta but also gamma actin is present in the nucleus. Quantitative actin analysis showed however, that in the A375 human melanoma cells nucleoplasm, the beta actin is about 30% higher than gamma actin level. Our results, based on the method of measurements of actin by its inhibitory activity towards DNase I (from bovine pancreas), showed that in human melanoma A375 cells nuclear actin is mainly monomeric.
The presence and diverse level of actin isoforms in the cell nuclei raises the possibility that they can play different roles in nuclear processes. In contrast to cytoplasm, majority of nuclear actin is monomeric, which may suggest that actin in the nucleus could function in different manner than in cytoplasm.

P9.9

Tropomyosin mutations related to human congenital myopathies change tropomyosin interactions with troponin

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Myopathies are congenital muscle diseases caused by mutations in genes encoding various muscle proteins, including tropomyosin (TM). TM is a two-chain coiled coil protein, which together with troponin complex (Tn) regulates actin filaments in striated muscle in Ca^{2+}-dependent manner. Our previous study has shown that different myopathy-related mutations in TM reduce activation of acto-myosin ATPase activity and gliding velocity of actin filaments over myosin heads [1].
In the present work we verified the hypothesis that the effects of mutations in TM on Ca-dependent regulation of acto-myosin interactions are correlated with structural changes in TM, which affect TM-Tn interactions. To verify this hypothesis we selected three TM mutants - TM-Leu100Met, TM-Arg168His, and TM-Arg245Gly, which differ in the magnitude of the functional deffect. The three mutants carry single amino acid replacements in the region which does not bind directly Tn (Leu100Met), interacts with the core region of Tn complex (Arg168His) and interacts with TnT subunit.
Wild type and mutant TMs were prepared by protein expression in bacterial cells. Structural changes in the Tn-binding region of TM were assessed by analysis of pyrene excimer fluorescence. Pyrene was attached to Cys190 located in register in the central, Tn-binding region of both TM chains. Labeled TM was bound to actin filament and titrated with Tn. Mutations in TM caused changes in excimer fluorescence. In the presence of saturating concentrations of Tn differences in the fluorescence between wild type and mutant TMs were in the following order: TM-Arg168His>TM-Arg245Gly>TM-Leu100Met. These results correlated well with the Tn-dependent regulation of acto-myosin interactions as measured by actomyosin ATPase initial velocity. In the presence of the mutant TMs the maximal activation of the actomyosin ATPase required higher concentrations of Tn then in the presence of wild type TM. The largest effect was observed in the presence of TM-Arg168His. This suggests that this mutation significantly lowered TM's affinity for Tn. We conclude that conformational changes near the Tn-binding region of TM determines the severity of disfunction of the Tn-dependent regulation of actin-myosin interactions.

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P9.10

Salsolinol — the effect on the glutamate-induced cell apoptosis pathway

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1,2,3,4-Tetrahydroisoquinolines are endogenous substances present in a low concentration in the human and rat brain. As was focused previously, the action of various members of this group ranges from neurotoxicity to neuroprotection (Antkiewicz-Michaluk *et al*., 2006, *J Neurochem* **97**: 846–856). The endogenous neurotoxin, 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (salsolinol), has been considered as a potential neurotoxin in the etiology of Parkinson's disease (Antkiewicz-Michaluk *et al*., 2000, *Neuroscience* **96**: 59–64). The synthesis of salsolinol as the concentration product of dopamine and acetaldehyde, the first metabolite of ethanol, suggested also its involvement in alcohol addiction. In the present study we investigated the effect of salsolinol on the glutamate-mediated apoptosis pathway. We evaluated the effect of salsolinol in different concentrations (50, 100 and 500 μM) on glutamate-induced apoptotic and neurotoxic parameters, such as loss of mitochondrial potential, activation of caspase-3, and lactate dehydrogenase (LDH) release in primary culture of rat hippocampal cells. Biochemical data were complemented with cellular analyses, including Hoechst 33342 and calcein AM staining, to visualize apoptotic DNA-fragmentation and to assess cell survival, respectively. The assessment of all investigated parameters was performed in the rat hippocampal cells. Our studies have shown that salsolinol has biphasic effects, at lower concentrations demonstrating the neuroprotective activity whereas in the higher caused neurotoxic effect. Salsolinol in the concentrations 50-100μM significantly inhibited the pro-apoptotic and neurotoxic effects caused by glutamate treatment as well as diminished the number of bright fragmented nuclei with condensed chromatin and increased cell survival in Hoechst 33342 and calcein AM staining in hippocampal cultures. Additionaly, salsolinol (50 μM) inhibited of glutamate-induced loss of membrane mitochondrial potential. Only the highest dose of salsolinol (500 μM) enhanced the excitotoxicity caused by glutamate. **Conclusions**: Presented molecular studies exclude possibility that salsolinol under physiological conditions could be an endogenous factor involved in neurodegenerative processes, conversely, it may be a neuroprotective agent. This findings may have important implications for the development of new strategies to treat or prevent neural degeneration.

P9.11

Entangled in the signal network: how calcium and adhesion modulates Rho-dependent signaling?

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Migration is one of the crucial features of cells in a living organism, responsible for embryogenesis, regeneration, immune defense, as well as such undesirable phenomena, as spreading of cancer cells. Two major conditions must be fulfilled for cell migration: polarization and adhesion. These are spatially and temporally regulated by signaling pathways related to RhoA and Rac1 proteins — the key regulators of actin cytoskeleton dynamics. The RhoA/ROCK signaling pathway plays an important role in contractile force generation, the assembly of stress fibers, development of focal adhesions and cell tail retraction. In the same time, Rac1/PAK pathway regulates actin polymerization at the leading edge and formation of the adhesion complexes in the lamellipodium.

We studied the ability for compensation of these two signaling pathways in glioma C6 cells by blocking each of them. Under both experimental conditions stimulation of $P2Y_2$ receptors with UTP enabled cells recovery to control like morphology and migration pattern. We examined the differences between cell migration parameters (average velocity, walk persistence, directionality) and adhesion areas in control cells, those with blocked RhoA/ROCK or Rac/PAK pathway (by Y-27632 and NCS inhibitor respectively) and in cells shortly incubated in calcium-free medium. We showed that NCS and calcium-free environment prevent cell recovery from ROCK inhibition after UTP stimulation [1]. Under these experimental conditions interaction of $\alpha_v\beta_5$ integrins with $P2Y_2$Rs is decreased, as microscopy and biochemical studies showed, inhibiting cofilin phosphorylation *via* Rac1/PAK signaling pathway [2]. The role of $\alpha_v\beta_5$ integrins in migration of glioma C6 cells is discussed.

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P9.12

Metabolic and bactericidal effects of "Tauzink" and a derivative of 5-nitrotiazola

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We have tested the new composition consisting of the amino acid taurine and zinc sulphate — "Tauzink" and new compound N-(5-nitrotiazol-2-yl)-N'-(4-aminobenzenesulfonyl) glutarildiamida with taurine which are in preclinical studies.
Rats against toxicity induced by intragastric administration of carbon tetrachloride (CCl4) at a dose of 2 ml/kg of a 30% oil solution for 4 days carried tauzink intragastric administration at a dose of 400 mg/kg body weight. The control group received only CCl4.
Under the influence of "tauzink" in the blood plasma showed normalization of relations nonessential/essential amino acids, phenylalanine hydroxylation rate on higher total number of proteinogenic amino acids that increase the level of glutamic and aspartic acid, tryptophan and alanine, while reducing the concentrations of glutamine, arginine, and lysine. The protective effect observed: the increased ratio of glutamate/glutamine and the number of sulfur-containing amino acids in the blood plasma. The liver increased methionine content recorded, citrulline, cysteic acid, threonine, lysine, proline, and alanine, while reducing the concentration of glutamine, histidine, phenylalanine. At the same time observed a two-fold increase in the content of β-aminobutyric acid on the background of almost two-fold reduction of β-alanine. Morphologically in hepatocytes, while the composition is administered with CCl4 "tauzink" there was a less pronounced degree of fatty degeneration. This reduces the number of cells with large lipid droplets and the number of large drops. Fatty cysts were not found. Most of the hepatocytes were with undisturbed or minimally disturbed ultrastructure.
The method of serial dilutions of the test compounds was studied in agar antimicrobial activity. When combined with taurine (at concentrations of 100, 250 or 500 ug/ml) with a 5-nitrotiazola (at a concentration of 100 ug/ml) a marked antibacterial activity in 4 out of 32 tested strains: B. 11778 cereus, B. subtilis 6633, S. intermedius 1009, S. epidermidis 42a.
Thus, "tauzink" with CCl4 intoxication, provides positive dynamics pool of free amino acids and their derivatives in blood plasma and liver of rats. Taurine combination with a 5-nitrotiazola has antimicrobial action.

P9.13

Changes of fetal pancreas ultrastructure after maternal lead intoxication

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Lead is a well-known industrial toxic agent, spread widely in urban areas and responsible common antioxidant balance in organism and numerous organ malfunction (Lyn, 2006, *Alt Med Rev* **11–2**: 114–127). Moreover, it was shown, that the pregnant women and their offspring are more sensitive to lead exposition, due to its toxicokinetics and placental permeability (Carpenter, 1974, *Env Health Perspect* 129–131). The aim of the experiment here presented is to evaluate the effects of lead acetate administration during the early pregnancy on the fetal pancreas morphology.
Materials and methods: In this study 2 groups ("Lead" and "Intact") of female rats were used. The "Lead" group consisted of 8 animals, exposed to a 1.5 mg/L lead acetate solution in drinking water 20 days before and 10 days after fertilization. Rats from both groups were sacrificed by decapitation on the 20-th day of pregnancy. Pancreas was taken from decapitated rat embryo, and fixed in osmium tetraoxide 1% solution. 40 nm thin sections were stained in uranyl acetate and lead citrate. Sections of exocrine part of pancreas were studied in transmission electron microscope at magnification 4 000–30 000 times.
Results: After lead administration there were observed some changes in the nuclear ultrastructure in rat pancreatic cells. The localization and shape of heterochromatin granules was changed towards the consolidation. In the lead-administered acinar cells we observed highly condensed chromatin, in the shape of lumps, localized all over the nuclear area, while in the "intact" group the heterochromatin was less expressed, and localized mainly near the nuclear membrane as a narrow strip. Knowing that the chromatin in the form of condensed heterochromatin is less active in processes of transcription, we conclude, that the acinar cells after the lead administration have less active nuclei, therefore synthetic function of fetal pancreatic tissue is decreased.