
Session 11: Medical Biotechnology in Regenerative Medicine and Drug Research

Lectures

L11.1

Targeting inflammation for treatment of Duchenne muscular dystrophy

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Muscle damage in Duchenne muscular dystrophy (DMD), the incurable diseases caused by the lack of dystrophin, is strongly linked to inflammatory reactions. Accordingly, modulation of inflammation can ameliorate the disease progression.

In our recent studies we have generated the double knock-out (dKO) *mdx* mice lacking additionally either the active heme oxygenase-1 gene (HO-1, *Hmox1*) or miR-378a coding sequence. The HO-1 is the crucial anti-inflammatory protein affecting also muscle stem (satellite) cells differentiation, while miR-378a is involved in regulation of muscle differentiation and cellular metabolism.

Lack of HO-1 aggravates the severity of DMD in a *mdx* mice and *Hmox1*^{-/-}*mdx* dKO animals demonstrate strongly impaired exercise capacity. Interestingly, *mdx* satellite cells (SCs) show disturbed and enhanced differentiation, which is further aggravated by *Hmox1* deficiency. Interestingly, *mdx* SCs expressed lower level of *Hmox1*, and RNA sequencing demonstrated the significant changes in *mdx* SCs transcriptome. Importantly, restoration of *Hmox1* expression normalized the SCs differentiation.

Surprisingly, mice lacking miR-378a were able to run longer distance than their wild-type (WT) in a treadmill test. Lack of miR-378a ameliorated the impairment characteristic for *mdx* mice, as 3-months old dKO animals were stronger than *mdx* mice and ran similar distance to WT. The muscles of dKO animals showed less infiltration of inflammatory cells. RNA-sequencing revealed the fibroblast growth factor 1 (*Fgf1*) among the most downregulated genes in animals lacking miR-378^{-/-} and in dKO mice, what might be of relevance to lower fibrosis observed in dKO than *mdx* mice

In summary, HO-1 and miR-378a can be considered as targets to alleviate muscle injury and DMD severity.

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L11.2

HO-1 as a possible drug target

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Heme oxygenase-1 (HO-1), a cytoprotective, pro-angiogenic and anti-apoptotic enzyme is highly expressed in various cancer types what in turn correlates with the more advanced tumor development as well as poorer survival of patients. Moreover, HO-1 expression is further induced upon chemo-, radio- and photodynamic therapy, what results in decreased effectiveness of the treatment.

On the basis of its cytoprotective features, scientists have pursued the targeting of HO-1 as an attractive target for anticancer treatment. The genetic inhibition of HO-1 by RNA interference (RNAi) or CRISPR/Cas9 approaches provides the possibility to specifically target HO-1; however, the potential therapeutic application of those methods are distant at best. Possibilities of specific pharmacological inhibition of HO-1 are strongly limited as commercially available metalloporphyrins exert many unspecific effects. Therefore, we initiated a drug discovery program aimed at the identification and characterization of novel small-molecule inhibitors of HO-1. Virtual High Throughput Screening (vHTS) followed by structure-based rational design led to the identification of molecules which actively inhibit the catalytic function of HO-1 at submicromolar concentrations. The potent anti-cancer activity of analyzed compounds *in vitro* was demonstrated using broad spectrum of methods, but *in vivo* experiments are required to fully establish potential application of small inhibitors of HO-1 as anti-cancer drugs.

In summary, HO-1 seems to be an attractive cellular pathway for drug discovery and genetic manipulation, however the ideal strategy for HO-1 targeting requires further studies.

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Oral presentations

O11.1

Regulation of hair follicle Stem Cells homeostasis by activation of gene inhibitory network

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Previously, we revealed how the underlying molecular mechanism of Bone Morphogenetic Protein (BMP) signaling governs the homeostasis of hair follicle stem cells (hfSCs) *in vivo*. Here, we focused on testing role of Id2 gene, one of the target genes which we identified to be down-regulated in hfSCs after inhibition of BMP pathway. To test the function of Id2 gene in hfSCs *in vivo* we used Id2 gain of function approach (GOF) by generating transgenic mouse line. Our data demonstrated that Id2 overexpression in hfSCs results in prolonged telogen and a delay in anagen activation, maintaining stem cells quiescence. Further, we performed FACS sorting and RNA-seq analysis of GOF-Id2 hfSCs at first postnatal hair cycle. By comparison with common signature genes in quiescent bulge we demonstrated that approximately 10% of the genes were affected by overexpression of Id2 in hfSCs. Interestingly approximately half of those genes overlapped with common signature genes which we previously published to be affected by BMP pathway, thus working synergistically with that pathway. On the other hand our data also suggest that although Id2 is a direct target and an effector of BMP pathway in hfSCs *in vivo*, second half of those genes work at least partially independent from BMP signaling on gene regulatory network of quiescent bulge.

O11.2

Administration of HO-1/SDF-1 α -overexpressing mesenchymal stromal cells in murine and swine model of myocardial infarction and heart failure – analysis of therapeutic effect

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Objective: Administration of mesenchymal stromal cells (MSC) have been proposed as an approach to regenerate damaged myocardium. The survival of cells upon administration into infarcted tissue, however, is limited. Therefore, the aim of this study was to evaluate the therapeutic potential of mesenchymal cells overexpressing proangiogenic (SDF-1 α) as well as anti-inflammatory [heme oxygenase-1 (HO-1)] factors in murine and swine model of myocardial infarction (MI). **Methods:** In mice (immunocompromised athymic Nude) MI was induced by left anterior descending coronary artery (LAD) ligation upon which 5×10^5 cells (MSC-Luciferase-GFP, MSC-SDF-1-Luciferase-GFP and MSC-HO-1-Luciferase-GFP) were injected in 4 sites of infarct border zone (2.5 μ l each, 10 μ l in total). In pigs, MI was induced by temporary occlusion of LAD with balloon catheter. Subsequently, after 2 weeks, 8×10^6 cells (MSC-Luciferase-GFP and MSC-HO-1-Luciferase-GFP) were administered intracoronary with microcatheter. **Results:** Echocardiographic evaluation of heart function performed 7, 14, 28 and 42 days upon MI induction in mice indicated no therapeutic effect of genetically modified MSC. Similarly, echocardiographic analysis of ejection fraction in pigs four weeks upon cells administration revealed no therapeutic effect of either MSC-Luciferase-GFP and MSC-HO-1-Luciferase-GFP. **Conclusions:** Our study did not confirm therapeutic benefits of genetically modified MSC in both acute and chronic model of myocardial damage.

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O11.3

Alpha ketoglutarate (AKG) induces apoptosis in osteosarcoma cells *in vitro* through the activation of JNK MAP kinase

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AKG is a key metabolite in the Krebs cycle. It has been shown that exogenous AKG exerts anti-tumour activity by reducing the level of the HIF-1 α subunit and inhibiting angiogenesis in hypoxic conditions [1, 2]. Moreover, it inhibited proliferation of colon adenocarcinoma cells *in vitro* in normoxic conditions [3]. The aim of this study was to evaluate the anticancer activity of AKG in human osteosarcoma cell lines, Saos-2 and HOS, and identify the mechanisms involved in this action. Anti-proliferative activity of AKG was estimated by the BrdU assay. An Annexin V-FITC/PI double labelling was used to detect apoptosis. The expression of apoptosis-associated proteins such as caspase-9 and Bax was determined by Western blot analysis, while caspase-3 activity was assessed by flow cytometry technique. The phospho- and total MAP kinases (JNK, ERK1/2) levels were measured by the ELISA method. The results showed that AKG inhibited proliferation of both osteosarcoma cell lines in concentration-dependent manner. It suppressed the activity of ERK 1/2 and enhanced phosphorylation of JNK kinases, increased the Bax protein level, and activated caspases -9, and -3 in osteosarcoma cells. SP600125, the selective inhibitor of JNK, abolished the effect of AKG on induction apoptosis and phosphorylation of JNK. Our results demonstrated that AKG induces intrinsic apoptotic pathway in osteosarcoma cells via JNK activation. Therefore, it is suggested that AKG may be used as an anti-cancer agent in the treatment of osteosarcoma.

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O11.4

3-Bromopyruvic acid: a small molecule with strong anti-tumor potency

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3-Bromopyruvic acid (3-BP), an analog of pyruvate and lactate, can “trick” cancer cells and enter as a Trojan horse. We characterized the kinetics of 3-BP transport into erythrocytes and demonstrated competitive inhibition of its transport by pyruvate. Flavonoids, such as luteolin and quercetin, also inhibited 3-BP transport. We found that 3-BP induces oxidative stress in erythrocytes and human non-invasive MCF-7 and invasive MDA-MB-231 breast cancer cells via depletion of glutathione, inactivation of antioxidant enzymes and provoking transient increase in the production of reactive oxygen and nitrogen species. Our studies showed that glutathione depletion is mainly due to the formation of a 3-BP conjugate (pyruvyl-S-glutathione). 3-BP can sensitize to cytostatic drugs cells showing multidrug resistance dependent on overexpression of ABC transporters since ATP depletion inhibits active export of chemotherapeutics. Cells overexpressing ABCB1 transporter are hypersensitive to 3-BP even in the absence of cytostatic drugs, apparently due to secondary alterations induced by overexpression of this protein. While most studies concentrate on short-term cellular effects of 3-BP, long-term effects of exposure of this compound seem to be of crucial importance for the fate of treated cells. We found that 3-BP induces changes in the expression of a small number of genes, mostly coding for antioxidant proteins, i. a. metallothioneins and low molecular weight thiol homeostasis enzymes.

Posters

P11.1

Alpha ketoglutarate promotes differentiation and mineralization of osteoblasts *in vitro*

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Alpha ketoglutarate (AKG), the key intermediate in the Krebs cycle, is a multifunctional endogenous metabolite involved in different metabolic and cellular pathways. In recent years, numerous *in vivo* studies have shown that AKG supplementation may have a beneficial effect on bone tissue. The purpose of our study was to assess the impact of AKG on osteogenesis *in vitro*. The human and mouse pre-osteoblastic cell lines, hFOB 1.19 and MC3T3-E1, were used in our studies. Cell proliferation was measured by BrdU assay. *ALPL*, *COL1A1*, *IBSP*, *SPP1*, *BGLAP*, *Runx2* and *SP7* mRNA expression was analyzed by qRT-PCR. pNPP was used as a substrate to quantify ALP activity in cell cultures using colorimetric assay. The type I collagen level was determined by Western blot analysis. The BSPII, OPN, and OCN levels were measured in cell culture supernatants with the ELISA method. The degree of extracellular matrix mineralization was determined using Alizarin Red S staining. The results showed that AKG did not increase the proliferation of osteoblasts. However, it enhanced the mRNA and protein levels of differentiation markers including ALP, COL1A1, BSPII, OPN, and OCN as well as the levels of mineralization in both cell lines. Moreover, it enhanced the mRNA expression of RUNX2 and Osterix, the key osteogenesis-related genes. We can conclude that AKG might be a promising candidate for bone anabolic drug in the prevention or treatment of osteoporosis.

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P11.2

Small-molecule inhibition of PEX5-PEX14 protein-protein interaction

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The *Trypanosoma* parasites infect humans and domestic mammals, causing significant mortality and huge economic losses. African sleeping sickness (HAT) is an example of life threatening trypanosomiasis. It is caused by *Trypanosoma brucei*. While recent clinical trials provide hope for more efficient cure for HAT other trypanosomiasis require medication that have serious side effects, require long treatment period and often fail to cure parasitemia. That is why new therapeutic strategies are needed.

Trypanosoma protists carry out glucose metabolism in organelle called glycosomes. The biogenesis of glycosomes depends on the PEX5/ PEX14 protein-protein interaction (PPI). It is essential for the transport of glycosomal enzymes (recognized and bound by PEX5 receptor) from the cytoplasm into the organelle. It was presumed that disruption of the PEX5/PEX14 complex formation results in mislocalisation of enzymes, causing metabolic collapse and it kills the parasite.

We have reported a way to selectively kill *T. brucei*. The PEX14 has been identified as an "Achilles' heel" of the parasite suitable for the development of new therapies. We used our nuclear magnetic resonance (NMR) and high-resolution X-ray structural information of PEX5-PEX14 complex, to design small molecules that efficiently disrupt the interaction. These molecules have trypanocidal activities comparable to approved medications and are active *in vivo*. We are currently optimizing our PEX14 inhibitors for better ADME parameters. This is essential for optimal *in vivo* efficacy.

P11.3

Improved production of caffeoylquinic acid derivatives in pRi-transformed plants of *Rhaponticum carthamoides* and biological activity

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Rhaponticum carthamoides (Willd.) Iljin of the family Asteraceae, is an endemic, perennial plant found in the natural habitat in South Siberia, Central Asia and China [1]. *R. carthamoides* roots and rhizomes are valued in traditional Siberian medicine for the treatment of weakness after illness, and are used in supplements as an anabolic and adaptogenic and are thought to alleviate physical weakness and mental weariness [1]. Phytochemical studies indicate that this plant contains various classes of secondary metabolites such as ecdysteroids, phenolic acids with caffeoylquinic acid derivatives, flavonoids, polyacetylenes, sesquiterpene lactones and triterpenoid glycosides [1, 2].

Transformed roots of *R. carthamoides* have previously been obtained by the transformation with *Agrobacterium rhizogenes* [2]. Such transformed roots may represent an efficient route of obtaining valuable plant materials, a relevant alternative to plants cultivation in the field, especially when the raw materials are the roots: this approach avoids the destruction of the whole plant. The aim of this study was to obtain whole transformed *R. carthamoides* plants from shoots regenerated from transformed roots, to perform a chemical analysis of plant materials, to investigate the antioxidant activity of the aqueous methanol extracts from transformed plants on human blood plasma and to assess their cytotoxic activity on the viability of the A2058 human melanoma cell line.

The transformed roots of *R. carthamoides* were able to spontaneously regenerate green buds and shoots. Bud and shoot regeneration was observed in more than 90% of the cultures grown in Schenk and Hildebrandt (SH) or Gamborg (B5) liquid medium with full micro- and macronutrient concentrations, under light conditions. The regeneration of shoots from transformed roots may be improved by supplementation of Murashige and Skoog (MS) agar medium with indole-3-acetic acid (IAA) and benzyladenine (BA). The shoots were multiplied on MS medium supplemented with 0.1 mg×L⁻¹ IAA and 0.2 mg×L⁻¹ BA and then rooted successfully. The transformation of the plants derived from transformed roots was confirmed by PCR analysis and Southern hybridization. The three-month-old transformed plants (TR-plants) were able to biosynthesize caffeoylquinic acid derivatives such as chlorogenic acid, 3,5- or 4,5-di-O-caffeoylquinic acids. The transformed plants demonstrated higher secondary metabolite levels and greater biomass accumulation than the plants obtained directly from seeds (SD-plants) of the same age and grown under the same conditions as transformed plants. It was found that extracts from TR-plants inhibited the growth of A2058 human melanoma cells depending on the tested concentration and bestowed antioxidant properties on human blood plasma.

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P11.4

Growth of *Leonurus sibiricus* L. roots with over-expression of AtPAP1 transcriptional factor in closed bioreactor, production of bioactive phenolic compounds and evaluation of their biological activity

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Leonurus sibiricus L. belongs to the Lamiaceae family. It is an aromatic, herbaceous plant, which can be annual, bisannual or perennial. Most significantly from a pharmacological perspective, the species has been reported to have a wide range of anti-inflammatory, antioxidant, anti-diabetes, anti-bronchitis and anti-cancer effects (1, 2). Biological manipulation showed that, fast-growing transgenic root cultures of *Leonurus sibiricus* with the AtPAP1 transcriptional factor may be used as a base for potential large-scale phenolic acids production. The present study investigates the effect of different volumes of medium (300 mL, 1 L, 3 L, 5L flasks) and a 5 L bioreactor on biomass increase and phenolic acids production by transgenic roots of *L. sibiricus*. Of these cultures, those from the 5 L bioreactor demonstrated the greatest increase in dry weight (20.83 g/L) and highest yields of phenolic acids (chlorogenic acid 448 mg/L and caffeic acid 302 mg/L). Additionally, they also displayed a cytotoxic effect on melanoma cells across the range of tested concentrations, as well as antioxidant activity on human blood plasma. This approach may serve as an alternative to conventional field crops for enabling large-scale production of the active constituents of AtPAP1 root extract, which would be of great value for pharmaceutical production.

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P11.5

Peptide-based biopolymers PolyRGD to be used for pro-regenerative purposes

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The RGD motif (Arg-Gly-Asp) has been selected for poly-epitope/poly-signal protein construction. This motif is common for cell-secreted extracellular matrix molecules (ECM). Activity of the RGD sequence exhibits mainly during the extracellular signal transduction and cell proliferation. The PolyRGD proteins can find applications in the regeneration of cells, tissues and organs. Placed in a wound, they can act directly or gradually release the biologically active peptides, due to their autodegradation or activity of the extracellular matrix proteases.

First, monomeric peptide units for construction of poly-peptide protein structures were selected and the PolyRGD proteins were designed. Then, a series of DNA vectors was constructed, enabling amplification of the selected epitopes and expression of the recombinant genes in *Escherichia coli*. The PolyRGD proteins were obtained using: (i) bioinformatic methods for the design of the genes, (ii) chemical synthesis and molecular cloning of the designed, monomeric DNAs units and (iii) the DNA units' amplification. For this purpose, a patented technology was used (PL 228341). The technology involves a directional, vector-enzymatic amplification of the designed DNA segment, with maintenance of the correct open reading frame for a given insert.

The obtained recombinant genes were successfully expressed in *E. coli*. Procedures for efficient isolation and purification of the bioactive PolyRGD protein variants were developed.

P11.6

Identification of a novel protein interaction with the EKLF transcription factor (Erythroid Krüppel-like factor)

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Erythroid Krüppel-like factor (EKLF/KLF1) is a transcription factor that plays a critical role in activation of genes essential for the red blood cell development. It is achieved by EKLF's ability to directly integrate basal transcription and chromatin-modifying machineries. EKLF interacts with chromatin remodeling complex SWI/SNF, the HIRA chaperon protein and components of the transcription machinery: p62 a subunit of TFIID and TAF9. Post-translational modifications of EKLF provide alternate protein interaction platforms that lead to varied downstream effects in erythroid differentiation.

Our laboratory is interested in elucidation of molecular mechanism of genetic distortions resulting from the presence of dominant mutations in EKLF. We investigate whether the functional differences between WT and mutated EKLF follow from formation of slightly different protein complexes e.g. with transcriptional machinery.

In order to analyze this, we established stable K562 cell lines with doxycycline inducible expression of Flag-EKLF variants. Protein complexes were isolated by immunoprecipitation with the ANTI-FLAG M2 magnetic beads and identified by mass spectrometry. After applying the Mascot program and differential comparison with negative controls approximately 100 EKLF specific partners were selected. A new EKLF's interaction with USP7 (Ubiquitin carboxyl-terminal hydrolase 7) was empirically confirmed.

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P11.7

Cytotoxic potential analysis of biomaterials modified with proteolytic enzyme inhibitors

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The formation of infections within the prosthesis implanted into the human body is a serious problem for modern medicine. Currently, many new solutions are sought in the field of biomaterial engineering, thanks to which it is possible to increase the aseptic potential of various types of biomedical materials and to give them antimicrobial properties. One of the innovative solutions is the use of proteolytic enzyme inhibitors (synthetic inhibitor AEBSF and α_1 -antitrypsin) as agents preventing the adhesion of pathogenic microorganisms (such as *E. coli*, *P. aeruginosa*, *S. aureus* or *C. albicans*) to the surface of prostheses and inhibiting the formation of biofilm produced by these microorganisms. During the development of new biomaterials, their possible cytotoxicity to the tissues of the human body should be tested.

Our study investigates the influence of vascular prostheses modified with two proteolytic enzyme inhibitors on the growth of human skin fibroblasts (HSF). The LDH (lactate dehydrogenase) cytotoxicity analysis of the modified biomaterials revealed that the Uni-Graft prosthesis modified with AEBSF inhibitor and α_1 -antitrypsin does not show cytotoxic effect on human epithelial cells (HSF). The results of the LDH test were also confirmed by microscopic photographs on which no abnormalities in the morphological structure of HSF cells were observed.

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P11.8

Functionalization of silk with metal binding peptides to produce iron oxide/silk composite spheres for theranostics purpose

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Spider silk is a biocompatible and biodegradable material with broad biomedical applications. It can be processed into different morphological forms including micro- and nanospheres. Moreover, it can be genetically engineered to obtain material of modified properties. Previously we characterized the EMS2/IONPs spheres that were composed of bioengineered silk EMS2 and iron oxide nanoparticles (IONPs). The drug loading studies exhibited more than two-fold higher loading efficiency of doxorubicin to spheres EMS2/IONPs than to EMS2. To further increase the affinity of silk to IONPs, we functionalized silk with metal binding peptides Fe1 and Fe2.

The study aimed to develop iron oxide/silk composite material for the theranostic application.

The functionalized (MS1Fe1)₂, (MS1Fe2)₂ and MS-1Fe1MS1Fe2 and control sMS1 silks were produced. The functionalizations did not impede the self-assembly of silk, and spheres were obtained. The SEM/EDS analysis of the composite silk/IONPs spheres indicated that the functionalized silks bound more IONPs comparing with the control silks (sMS1 and EMS2). Moreover, the Fe1 peptide bounded more IONPs than the Fe2 peptide. The binding of IONPs of negative charge resulted in the formation of spheres with more separated and more spherical morphology than spheres with positive IONPs. The spheres with a higher IONPs content may result in higher loading efficiency of doxorubicin, which may be beneficial for medical applications.

P11.9

How to create drug delivery systems based on supramolecular compounds, proteins and carbon nanotubes?

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Construction of high capacity drug-delivery systems presenting tissue specificity that allows to avoid side effects is nowadays one of the most important research topics. Congo red (CR) type supramolecular compounds (SC) were previously shown to interact with some proteins, including albumin (BSA) and antigen-bound antibodies (Ag-Ab), and can also bind to and disperse carbon nanotubes. The presented results concern the studies on the complexes between SC with anticancer drugs: doxorubicin (DOX) or imatinib (IM) and the interaction of these complexes with antibodies (Ab), albumin and carbon nanotubes (CNT).

Hypothesis: SC-drug complexes interact with CNT and with proteins and can potentially deliver drugs to areas affected by pathological processes.

- SC associate in water solutions creating ribbon-like multimolecular assemblies
- organic molecules (such as DOX and IM) can intercalate into SC assemblies
- the structure and protein-binding properties of CR-DOX significantly differs from CR

Goals: Description of the properties of complexes formed by SC-DOX and SC-IM with Ag-Ab, BSA, CNT and CNT-linked proteins.

Research methods: electrophoresis, gel filtration, cell cultures, fluorescence microscopy, DLS.

Results: Using BSA increase capacity for binding drugs while the application of Ab enables targeted drug delivery. The presented system offers a wide range of biomedical applications including drugs delivery to cancer cells. As carriers of drugs, these complexes present an interesting alternative to the currently used systems.

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P11.10

Tetragametic chimeras in DNA testing, diagnostics, and therapy

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Tetragametic chimeras are created by the fusion of two zygotes, typically at the very early stage of dizygotic pregnancy. The result is an organism containing two cell populations, carrying distinct DNA sets and distributed randomly within the body. For a long time medicine did not realize their existence and its consequences. Secondary cell population can occur within the pigmented tissues and manifest itself by color difference, allowing easy identification - but if it localizes within the internal organs, it becomes barely detectable. There is still no certain information about the rate of the occurrence of the tetragametic chimerism. As during the zygote fusion cells segregate randomly, there is no way to predict which tissues can have different DNA set than the rest of the individual. Up to now all known cases of tetragametic chimerism were diagnosed during unrelated medical procedures such as transplantation or parentage testing. To increase chances of identifying a chimera, the samples for DNA test would have to be taken from multiple tissues, including internal organs, which would be very invasive and costly procedure. Moreover, such analysis could be insufficient to confirm a disease of genetic background in patients, as the cause of the disease would remain unknown because the genetic change occurred in the different cell population. It could also potentially obstruct the treatment due to problems with selective drug delivery.

P11.11

Endocytosis, intracellular trafficking and degradation behavior of functionalized silk particles for targeted drug delivery

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One of strategies for cancer treatment is to design a nanodelivery system, directing active agents specifically to the site of tumor, therefore avoiding potential side effects. The bioengineered spider silk is a material with a great potential in biomedical application. Drug-encapsulated biodegradable spheres made of functionalized silk have considerable potential for use as delivery systems. Inclusion of targeting ligands displayed at the surface of a carrier, with specific affinity towards a particular receptor, can further enhance the accumulation and uptake of these nanoparticles at the site of action.

The objective was to investigate the mechanism involved in the internalization pathway into target cells, intracellular trafficking and biodegradation of the obtained spheres in live cells.

The hybrid constructs were obtained by adding a Her2-binding peptide (H2.1) to MS1 and MS2 bioengineered silks based on the MaSp1 and MaSp2 proteins from *N. clavipes*, respectively. The H2.1MS1 and H2.1MS2 proteins were blended at a weight ratio of 8:2. Stable silk particles were formed by mixing a soluble protein with potassium phosphate using a micromixing technique. Obtained spheres were characterized in terms of size and morphology using an SEM. The uptake of spheres by human Her2-positive breast cancer cells SKBR3 in the presence of endocytosis inhibitors was assessed using flow cytometry. The subcellular distribution of silk particles was investigated using CLSM by evaluating the signal colocalization with organelle-specific tracker. CLSM imaging was performed in order to analyze the degradation kinetics of silk particles by employing different lysosomal and exosomal inhibitors. Functionalized spheres were actively internalized by Her2-positive cells. Silk particles facilitated the entry into cells both through the clathrin- and caveolae-dependent pathway of endocytosis. Silk spheres upon entering the cells localized in lysosomes. Applying various lysosomal inhibitors substantially extended the fluorescence signal from silk particles indicating the intracellular degradation took place in lysosomes. Furthermore, the prolonged presence of silk particles was not observed when using inhibitors of exosomal release, which further proved that the lysosomal function is essential for silk-based carriers elimination.

We showed for the first time, that silk spheres could be processed into cells by the endocytosis pathway and their degradation occurred in lysosomes. The degradation of the carrier is of a great importance to develop drug delivery system.

P11.12

Genistein-mediated lysosomal degradation of main pathogenic factors of Alzheimer's disease as a novel therapeutic strategy

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Alzheimer's disease (AD) is one of the most severe neurological diseases, and the number of patients is growing rapidly. Unfortunately, no effective treatment is currently available despite many therapeutic strategies. The most recent studies indicated that the main pathogenic factor of AD – β -amyloid can be degraded by lysosomes, thus its accelerated lysosomal degradation became a promising strategy for treatment of AD. However, search for the safe activator of lysosomes is still ongoing. Our previous studies indicated that genistein, one of isoflavones, can stimulate biogenesis of lysosomes and activity of acid hydrolases by positive regulation of transcription factor EB gene expression. Thus, the aim of this study was to determine therapeutic effects of genistein on AD. The streptozotocin rat model of this disease was used. Behavioral studies showed that genistein corrected locomotor activity of AD rats (actometers, open field), alleviated stress (elevated plus maze) and improved memory (Morris water maze), making them indistinguishable from healthy rats. Biochemical assays indicated that genistein decreased levels of both β -amyloid and hyperphosphorylated tau protein in the brain to the level of these proteins in healthy rats (Western blotting, immunohistochemistry) while cell culture experiments showed that this effect required lysosomes. In summary, genistein as a safe compound which can be used in long-term treatment has a great chance to become a cure for AD.

P11.13

Degradation of glycosaminoglycans through autophagy stimulation in mucopolysaccharidosis type III

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Mucopolysaccharidoses (MPS) belong to lysosomal storage diseases which are caused by lack or decreased activity of enzymes involved in the degradation of unbranched chains of polysaccharides called glycosaminoglycans (GAGs). Normally, GAGs degradation takes place in lysosomes due to the activity of several enzymes that cooperate to remove individual mono-sugars or chemical groups from GAG chains. Defect of one of the enzymes prevents the activity of the others because of sequential way of degradation. Therefore, GAGs degradation stops at some point, and undegraded molecules accumulate in the lysosomes.

Our previous studies shown, that one of the currently tested therapeutics – genistein – can lower the level of GAGs storage in cells by increasing the biogenesis of lysosomes, thus we argue that in the autophagy process accumulated GAGs can be degraded as well. Autophagy, induced by various natural compounds, is considered one of the most promising therapeutic strategies in many diseases related to accumulation of various molecules. Our approach rely on testing various compounds, known to induce autophagy through different pathways. We found that genistein, trehalose, curcumin, capsaicin, resveratrol, and calcitriol differentially affected levels of GAGs in MPS III cells. Further investigation revealed additive affect after combine genistein with different compounds. These results are considered as possible use of the tested compounds in potential therapy for MPS III.

P11.14

Structural investigations on the components synergy of a new proposed regenerative treatment for periodontal lesions

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In the current clinical activity, it is used a paste containing tetracycline, metronidazole and white vaseline for periodontal wounds treatment, but the beneficial effect of this treatment is not complete satisfactory. Therefore, we propose a new topic treatment material, coupling the antimicrobial effects of antibiotics with the specific proliferation and differentiation of osteoblasts activity of melatonin and hyaluronic acid while inhibiting the osteoclasts activity. The new material is proposed for directly usage in the periodontal pockets. During last years, both melatonin and hyaluronic acid were studied due to their beneficial effects in medical area. It has been proved that melatonin interferes in the osteoclasts function thus inhibiting bone resorption, due to its antioxidant properties. The hyaluronic acid showed anti-inflammatory and anti-bacterial effects in the treatment of periodontal diseases, presenting specific tissue healing properties. The present work aims to develop a derivative UV spectrophotometric method for simultaneous estimation of melatonin, hyaluronic acid and antibiotic in combined dosage form. Absorbance of all components within the new treatment were measured at specific λ_{\max} . It has been proved that all components in treatment mixture preserve their specific activity.

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P11.15

3-Bromopyruvic acid affects the mobility and redox balance of metastatic cells

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Cancer treatment becomes very difficult when tumor cells metastasize into distant body organs. Therefore, prevention of cancer cell metastasis is an effective strategy for successful management of human cancers. An alkylating antiglycolytic compound, 3-bromopyruvic acid (3-BP) is a promising anticancer agent, able to act also on multidrug-resistant cells *in vitro*. We have studied the effect of 3-BP on the survival, mobility, ATP content and glutathione content of human prostate metastatic carcinoma DU-145 cells. The cells were sensitive to 3-BP action, IC_{50} estimated with Neutral Red after 24 h being $25.8 \pm 1.1 \mu\text{M}$. The cell mobility was strongly affected by 3-BP, total length of cell displacement being decreased from 100.9 ± 7.3 down to $78.6 \pm 6.7 \mu\text{m}$ during 6 h, and the rate of cell movement being reduced from 0.28 ± 0.02 to $0.22 \pm 0.02 \mu\text{m}/\text{min}$, 24 h after treatment with $10 \mu\text{M}$ 3-BP ($P < 0.05$). The inhibition of cell movement can be attributed to ATP depletion, which decreased to $9.9 \pm 5.6\%$ of the control value at $30 \mu\text{M}$ 3-BP ($P < 0.05$). 3-BP brought about changes in the level of glutathione, increasing glutathione content at low concentrations and decreasing it at higher concentrations ($30\text{--}50 \mu\text{M}$), thus altering the redox equilibrium of the cells. The high sensitivity of metastatic DU-145 cells to the action of 3-BP and inhibition of their motility by low concentrations of this compound suggest that 3-BP may prove useful in the therapy of metastatic tumors.

P11.16

Nrf2 influences satellite cells and muscle regeneration but does not aggravate the progression of Duchenne muscular dystrophy

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The nuclear factor (erythroid-derived-2)-like 2 (Nrf2) is a transcription factor known as a master regulator of the inflammatory response. Our aim is to assess the Nrf2 influence on myoblast and muscle satellite cells (mSCs) differentiation and its role in the progression of Duchenne muscular dystrophy (DMD).

In order to evaluate the role of Nrf2 in muscle satellite cells differentiation, mSCs were isolated from Nrf2+/+ and Nrf2-/- mice and *in vitro* differentiation was performed in an environment of different oxygen concentration. Satellite cells lacking Nrf2 demonstrated more robust differentiation in hypoxic condition. Interestingly, their growth and viability were strongly impaired in normoxia in comparison to wild type cells.

To investigate the role of Nrf2 in the progression of the Duchenne muscular dystrophy double knockouts were generated. Firstly, mice were subjected to an exhaustion treadmill test to assess muscle functionality. Animals' performance demonstrated that lack of Nrf2 does not affect exercises capacity. This observation was validated by histological and FACS analysis showing a similar level of muscle damage, as evidenced by the degree in inflammatory cells infiltration and fibrosis in dystrophin-deficient mice (*mdx*) and double knockouts.

In summary, lack of Nrf2 activity in mSCs of *mdx* mice enhanced the mSCs differentiation in the hypoxia condition. However, this does not aggravate the progression of Duchenne muscular dystrophy.

P11.17**The influence of polyphenolic phytochemicals on the redox homeostasis of cells**

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The enhancement of endogenous antioxidant defense system through dietary supplementation appears to be a reasonable approach to reduce oxidative stress. However, to pinpoint the actual role of dietary antioxidants in maintaining cellular redox homeostasis, the relation between their physicochemical, electrochemical and biological properties must be established. The aim of our study was to clarify these relationships for selected polyphenoles as well as to reveal molecular implications resulting from exposure to the tested compounds for oxidative stress response system in HT29 cell line.

Reduction potentials of phenolic compounds were determined by potentiometric titration. The biological tests embraced determinations of cytotoxicity and antioxidant activity in cell culture assessed by CAA assay. Genomic studies concerning modulation of expression of genes involved in oxidative stress response employed RT-PCR array-based technologies.

Our comparative approach to establish the correlation between chemical properties and biological potential of polyphenols suggests their dose-dependent role in maintenance of redox homeostasis. This influence was accompanied with modulated expression of genes involved in oxidative stress response. The set of regulated genes was dependent not only on structure of investigated compounds, but also on their standard reduction potentials. The final conclusion is that dose dependent effects of dietary antioxidants and biological functions potentially modulated by them may become deregulated upon exposure to their excessive doses.

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P11.18**CRISPR/Cas9 editing of heme oxygenase-1 affects cardiomyocytes differentiated from human induced pluripotent stem cells**

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Background: Human induced pluripotent stem cells (hiPSC) are an invaluable platform for generation of human cardiomyocytes for disease modeling, drug screening and possibly also for regenerative medicine. Importantly, efficient and reproducible protocols for differentiation of hiPSC into cardiomyocytes (CMs) have been described, however, hiPSCs-CMs demonstrate fetal phenotype, thus further studies investigating factors involved in their maturation are needed.

Heme oxygenase-1 (HO-1) is a cytoprotective enzyme degrading heme into CO, Fe²⁺, and biliverdin. Via products of its enzymatic activity HO-1 influences plethora of physiological processes including stem cell differentiation.

Aim: Aim of this study was to investigate the role of HO-1 in differentiation of hiPSC into CMs.

Methods: hiPSCs were differentiated into CMs using small molecular inhibitors modulating Wnt pathway. CRISPR/CAS9 technique was utilized to inactivate HMOX1. Lack of HO-1 protein was functionally confirmed by Western blot technique.

Results: Differentiation of hiPSC to CMs was performed with very high efficiency (up to 98.8%, basing on flow cytometry analysis of cardio-troponin T). Importantly, patch-clamp technique revealed shortened depolarization phase in hiPSC-CMs lacking HO-1 whereas qRT-PCR analysis confirmed lower expression of ion channels in CMs devoid of HO-1.

Conclusion: Observed characteristics indicate that hiPSC-CMs lacking HO-1 are less mature than their control counterparts suggesting an important role of HO-1 in process of CMs maturation.