Session 9: Extracellular Matrix Biochemistry

Lectures

L9.1

Regulation of matrix metalloproteinase MMP-9 expression and function in the brain

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Matrix Metalloproteinases (MMPs) and their endogenous inhibitors (tissue inhibitors of matrix metalloproteinases, TIMPs) have repeatedly been shown to be major players in developmental processes and in a variety of neuronal pathologies involving tumor growth and metastases, excitotoxicity and other kinds of neurodegeneration as well as neuroinflammation. In addition, there is an emerging evidence for pivotal role of MMP/TIMP system in neuronal and synaptic plasticity. Levels of numerous proteins and their respective mRNAs display dynamic changes in parallel to the phenomena of the plasticity as well as modifications of numerous genes and encoded proteins alter the progress and outcome of the plasticity. Clearly, the plasticity relies on external information conveyed predominantly to the neurons by means of neurotransmitters, neurotrophins, cytokines and steroids. Furthermore, plentiful of proteins that are active inside the neurons were shown to be involved in the plasticity. Much less is, however, known about a major brain molecular structure that is surrounding cells and their processes such as synapses, i.e., extracellular matrix (ECM) and associated cell adhesion molecules (CAMs). Hence, extracellular enzymatic activities, including proteolysis that allow for ECM modifications appear to add a new dimension to our understanding of the brain plasticity. The evidence for specific roles of MMP/TIMP system in the aforementioned phenomena is very compelling as derived from multiple lines of evidence obtained both in vivo and in vitro, with various experimental approaches including transgenic and knockout rodents as well as human gene polymorphism and gene expression studies.

L9.2

Osteogenesis imperfecta: understanding the molecular basis of OI and developing new therapeutic approaches using the knock in murine model BrlIV

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The brittle bone disease Osteogenesis Imperfecta (OI) is a bone disorder characterized by skeletal fragility and deformity. The classical forms of OI are caused by mutations in COL1A1 and COL1A2 genes coding respectively for the α1 and α2 chains of type I collagen. The most common mutations are single amino acid substitutions, changing one of the Glycine present at the first position of each of the 338 Gly-X-Y triplets, characteristic of the triple helical region of type I collagen. The other molecular defects responsible for OI are: single base substitutions, affecting the splice sites and causing exon skipping or alternative splicing, small deletion and small insertion. Recently recessive forms of OI have been described due to mutations in genes involved in collagen folding, post translational modifications or regulation of transcription.

Several years ago we generated the BrlIV mouse, which is the first non-lethal knock-in murine model for the classical dominant OI. BrlIV carries a typical Glycine substitution (Gly349Cys) in one col1a1 allele, has the typical dominant transmission of human OI and his phenotype models the moderately severe human type IV. BrlIV shows a moderate/severe or a lethal OI outcome in presence of the same molecular defect, thus this model reproduces the phenotypic variability reported for human patients.

The availability of a mouse model represents a valid tool both to investigate the molecular basis and to develop new therapies for this disease, since it allows the investigation of a large number of animals and it gives the full access to bone tissues, difficult to obtain from human patients due to obvious ethical limitations.

To elucidate the molecular basis of phenotypic variability we investigated mRNA expression by microarray and protein profile by 2-DE and mass spectrometry of calvarial bone of newborn BrlIV with lethal and non lethal outcome. We found in lethal BrlIV an increase of Gadd153/CHOP and a lower expression of the αB-crystallin, the latter was on the contrary elevated in mutant mice with type IV outcome. These data indicated an effect on the phenotypic outcome of the intracellular machinery encountering retention of the mutant collagen. In vitro study using osteoblasts further supported our hypothesis. Osteoblasts from mice with lethal outcome showed slower proliferation and delayed mineralization. We also determined in those cells by RT-PCR an increase in Gadd153, Bim and Cebpβ and a decrease in Cebpβ expression suggesting the activation of an apoptotic pathway.

Since OI is a genetic disorder no definitive cure is at the present available, but a lesson from nature tells us that mosaic carriers of collagen mutations, some of the parents of OI patients, have very mild or absent clinical outcome.
Cellular therapy is the treatment that mimics the mosaic situation, thus could be an effective therapy for OI. Thus we developed in utero stem cell transplantation to treat the BrtlIV mice using as donor cells the whole bone marrow from GFP transgenic mice. Engraftment in bone from 2 months old transplanted mice was confirmed by inverted microscopy analysis at the sacrifice and laser confocal microscopy of cryosections. MicroCT analysis demonstrated that femora of treated Brtl mice had significant improvement in geometric parameters (p < 0.05) versus untreated Brtl mice, and mechanical properties attained WT values. Biochemical analysis of bone composition finally demonstrated that donor cells synthesized up to 20% of all type I collagen in the host bone and IR analysis revealed a more homogeneous mineralization in the bone regions surrounding green cells.

In conclusion, our results suggest that the engrafted cells form bone with higher efficiency than the endogenous ones, pointing out that in utero transplantation is a promising approach for the treatment of genetic bone diseases and anyway, regardless of potential applications to disease treatment, the autosomal dominant mouse model for IUT presented here also offers an opportunity for better understanding the molecular mechanisms of OI.

**Oral Presentations**

**O9.1**

**Utilization of fluorogenic peptides for determination of neutrophil serine proteinase activity**

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This study is focused on selection, characterization and utilization the FRET peptides in activity assay of neutrophil serine proteinases (NSP). This group of enzymes is expressed in neutrophile and released upon its activation. In humans it is consisted of three proteinases: cathepsin G, neutrophil elastases and proteinase 3.

The physiological role of these proteases is manifested in several processes such cytokines and chemokines processing, platelet activation, degradation of extracellular matrix proteins, activation of protease receptor 2 PAR-2 and displaying antimicrobial action. Recently a novel mechanism has been described on how neutrophils release granule proteinases and chromatin that form together an extracellular trap to attract and kill bacteria. Despite its common/mutual function NSP some enzyme display unique roles in the human organism. Cathepsin G is involved in blood pressure regulation by processing angiotensin I into angiotensin II. Activity of human neutrophil elastase is associated with some inflammatory lung disorders. Proteinase 3 is the main antigen in Wegener's granulomatosis, a chronic inflammatory disorder of unknown etiology. It is characterized clinically by respiratory tract and renal disease, in which there is a high prevalence of antineutrophil cytoplasmic antibody (ANCA) mostly against proteinase 3 in patient serum.

The aim of this study was a selection (using combinatorial chemistry methods) the FRET displaying peptides that will be efficiently and selectively hydrolyzed by the selected neutrophil protease. Obtained compounds will be characterized in terms of their chemical and kinetic properties. Furthermore the substrates with the highest specificity and selectivity will be used for determination of proteolytical of single protease activity in human serum. The outcome of such approach could be the diagnostic test for detection of neutrophil serine proteinases in body fluids.

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Eleutherococcus spp. as a potential inhibitors of matrix metalloproteinase

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MMPs are a special group of proteases degrading of extracellular matrix (ECM). Their natural substrates are insoluble proteins, complex mixtures of proteins and associate macromolecules, structural components of extracellular matrices. There are 28 matrix metalloproteinases. MMPs are divided into six families: the collagenase family, the gelatinase family, the stromelysin family, the matrilysin family, the membrand-type family and the unclassified family. They play a critical role in a variety of physiological and pathological conditions such as organogenesis, tumor metastasis, wound repair, angiogenesis, and arthritis.

The activity of metalloproteinases is regulated at transcriptional, translation levels and by endogenous inhibitors such as tissue inhibitors of metalloproteinases.

Currently known are four natural tissue inhibitors of metalloproteases: TIMP-1, TIMP-2, TIMP-3 and TIMP-4. The main function of TIMPs is participation in controlling the local activities of MMPs in tissues.

Many researchers from all over the world search for chemical substances as potential inhibitors of MMPs. Their natural source are plants, e.g.; Macrocystis pyrifera (L.), Camellia sinensis (L.) and Eucommia ulmoides (Oliv.).

The aim of the present study was to search for new potential inhibitors of MMPs within the plants from Eleutherococcus genus (E. senticosus, E. setchuenensis, E. henryi, E. gracilistylus, E. divaricatus, E. sessiliflorus). These species, especially E. senticosus possesses a wide biologically activity (anticancer, antiviral, antibacterial, and immunomodulatory).

In our study we used MMP-1, MMP-2, MMP-3, MMP-4 and azocoll as a substrate. Ethanol and chloroform extracts from the roots of these plants were used as potential inhibitors of MMPs. The activity of MMPs was assayed by spectrophotometric and electrophoretic method.

Chloroformic extract from E. gracilistylus gave a high inhibitory effect on MMP-1 (74.1 U), MMP-3 (62.9 U) activity, and E. divaricatus on MMP-9 (117.3 U).
O9.4

NOTCH signaling pathway activation during early in vitro differentiation of liver-derived mesenchymal stem cells to osteoblasts

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The NOTCH signaling plays important roles in cell proliferation and differentiation. In this study changes in expression and distribution of NOTCH 1, 2, and 3, and Delta1, among cytoplasm and nuclei, in rat liver-derived mesenchymal adherent stem cells, differentiating into osteoblasts were investigated. Expression of the Notch receptors and Delta1 was detected following reverse transcription and by primer specific nested PCR and verified at protein levels by immunoprecipitation and Western blot analysis. Distribution of the proteins in cells was analyzed following staining with antibodies specific to intracellular domains of the receptors. The mineralization of differentiating cell cultures was detected using AlizarinS assay. The significant changes in the number of cells expressing NOTCH1, 2, and Delta1 and with their activation, except for NOTCH3, was detected following 24-hours of culture under osteogenic conditions. Although, the number of cells expressing NOTCH3 remained at the same time almost unchanged, the cell number with activated NOTCH3 was elevated. The number of cells positive for NOTCH3 was higher than other NOTCHs after 48 hours of differentiation, but only small fraction of the cells did show presence of the receptor's cytoplasmic domain in the nuclei. The culture mineralization started on the day four, and at that time all analyzed receptors were present at levels corresponding to control numbers, except for Delta1. The three analyzed receptors and one ligand seem to play important roles in regulation of osteogenesis at early stage of stem cell differentiation. They also are differently activated when the mineralization begins at day 4 of osteogenesis.

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P9.1

The activity of esterases in ontogenesis of honey bee (Apis mellifera carnica)

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Honey bee as a pollinator plays a significant role in both the environment and human economy. Recently they have become the object of widely researches because of unexplained phenomena of Colony Collapse Disorder (CCD). Assays were conducted at 12 developmental stages of the honeybee. In the extracts of them the carboxyesterases activity was determined in relation to nitrophenyl (pNP) derivatives of four acids: acetate (C2), butyrate (C4), caprate (C10) and palmitate (C16). The activities of acidic and alkaline phosphatases were also studied. Carboxyesterases activity was found to decrease with increasing length of the substrate. It was the highest for acetate acid ester, and lower by half for C4, and by 70% for C10. The C16-ester was not hydrolyzed at all. The activity of esterases varied in the development of honeybees, e.g. for C2 esterase its value ranged from 0.111 to 0.953 U/mg. The activity profiles for all studied substrates were similar in honeybee development. Enzymes were most active in two first and two final stages. In the capped brood the enzymes activity decreased up to 10 times. It was the lowest in prepupea. Similar profiles of the activity changes were for carboxyesterases and phosphatases. The activity of alkaline phosphatase was always higher than acidic phosphatase during development. The results of this work are in opposition to the generally accepted opinion that lipid metabolism plays the important role in the initial period of metamorphosis of honey bees.
P9.2

Steroid-induced autophagy in the development and remodelling of bovine mammary gland

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Autophagy is a process responsible for degradation and recycling of long-lived proteins and organelles by lysosomes. During starvation period autophagy is regarded as a temporary survival mechanism providing an alternative energy source. This situation is observed in bovine mammary gland during involution, which is typically overlapped by next pregnancy in dairy cows. In the dry period mammary epithelial cells (MECs) undergo stress connected with milk stasis and deprivation of nutrients and biologically active compounds, extensively used by simultaneously developing foetus. At this time also high levels of steroid hormones are observed in the gland. Using an in vitro model of involution based on the culture of bovine mammary epithelial cell line BME-UV1 in a medium with low concentration of foetal bovine serum (0.5% FBS) we have investigated the effect of 17β-estradiol and progesterone on autophagy. Immunofluorescence, LysoTracker, stable expression of GFP-LC3, and immunoblotting techniques were used as methods for analysis of lysosomes activation, and expression of MAP1LC3 - the marker of autophagy. Additionally a three-dimensional (3D) culture of BME-UV1 cells on laminin-rich extracellular matrix (ECM) — Matrigel was used to investigate the role of the selected hormones in the development of acinar structures, that mimic the round, polarized, hollow alveoli of the mammary gland.

17β-estradiol and progesterone induced autophagy in BME-UV1 cells, which was manifested by an increased level of the 16kDa active form of MAP1LC3 protein - LC3-II (found in the membrane of autophagosomes), and increased LysoTracker Green fluorescence, related with the organelles with low pH, such as lysosomes and autophagosomes. The experiments on Matrigel showed that steroids play an important role in the development of mammary glands' alveoli. The 3D culture of BME-UV1 cells showed that the bovine MECs, cultured on ECM components, form acini structures with hollow lumen within 16 day. In the presence of 17β-estradiol and progesterone the growth arrest of the cells was observed earlier (between day 6 and 9 of culture). Additionally an intensive autophagy was noted, manifested by high levels of GFP-LC3-related, punctuated fluorescence localised mainly in the centre of the developing acini, and high expression of LC3-II. Simultaneously a process of apoptosis, responsible for the formation of hollow lumens, was also observed.

P9.3

IGF-I stimulates the expression of fibronectin, integrins α5, β1 and disintegrin metalloprotease ADAM12 in mouse C2C12 myogenic cells

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The are functional interactions between the extracellular matrix (ECM) molecules and the elements of insulin-like growth factor (IGF)-I system, that regulate the progression of cell and tissue growth. The purpose of the present study was to examine the potential modifications of the expression of ECM components in mouse C2C12 myogenic cells subjected to 5-day differentiation in the presence of IGF-I. The cellular content of specific ECM proteins, fibronectin and laminin, integrin α3, z5, β1 subunits, and disintegrin metalloprotease ADAM12 was assessed by immunoblotting and immunofluorescence method. Fibronectin and laminin were expressed both in proliferating and in differentiating C2C12 myogenic cells. IGF-I (30 nmol/l) strongly stimulated the expression of fibronectin on the 5th day of differentiation. The level of laminin was not affected by the presence of IGF-I. The expression of integrin α3, a subunit forming the specific receptor of fibronectin, was markedly augmented by the treatment with IGF-I, the effect being particularly evident on the 5th day of myogenesis. The protein level of integrin α3, forming one of the adhesion receptors of laminin, was hardly detectable in control cultures and increased in myogenic cells exposed to growth factor during differentiation. IGF-I augmented the cellular content of the integrin β1 both on the 3rd and on the 5th day of differentiation. The supplementation with IGF-I resulted in the increase in the cellular content of full length 100 kDa form of ADAM12, and this effect was evident in the whole observation period. The expression of 75 kDa form of ADAM12 (probably “secreted” form) was also elevated by IGF-I, however this effect was detectable on the 5th day of myogenesis. The cellular content of 50 kDa form of ADAM12, which probably represents the protein without metalloprotease domain, was not affected significantly by the addition of growth factors. IGF-I did not modify the level of actin, however it markedly activated the expression of myosin heavy chain, indicating the stimulation of myotube formation and growth. In conclusion, IGF-I caused the increase in extracellular matrix content and, probably, its function in C2C12 myogenic cells, which was manifested by enhanced expression of fibronectin, integrin α3, z5, and β1 as well as a disintegrin metalloprotease ADAM12. The stimulatory effect on ECM could comprise a part of cellular mechanisms involved in IGF-I-dependent myogenesis.
P9.4
Alterations in plasma glycosaminoglycan profiles in rheumatoid arthritis patients

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Qualitative and quantitative analyses of glycosaminoglycans (GAGs) in plasma obtained from rheumatoid arthritis (RA) patients were carried out to provide a clearer understanding of the role of these macromolecules in the disease pathogenesis. Plasma samples were obtained from 39 healthy volunteers and 50 patients with RA. All patients fulfilled the American College of Rheumatology criteria for RA diagnosis. Disease activity was determined using the 28 joints diseased activity score (DAS 28). The subjects participating in this study were divided into the following three groups: patients with high, moderate and low disease activity. Glycosaminoglycans were isolated from plasma samples. Total GAGs were quantified using a hexuronic acid assay and subjected to electrophoretic fractionation. Plasma levels of keratan sulfate (KS) and hyaluronic acid (HA) were measured using immunoassay kits. Total plasma glycosaminoglycans concentrations in RA patients and in healthy subjects did not differ significantly. The electrophoretic analysis allowed to identify chondroitin sulfates (CS), dermatan sulfates (DS), heparan sulfates/heparin (HS/H) in plasma of healthy subjects and patients with RA. Chondroitin sulfates were the predominant type of GAGs in plasma of all the investigated subject groups. Enhanced level of CS, DS and HS/H in all rheumatoid arthritis patient groups were accompanied by the increased structural heterogeneity of these compounds as compared to controls. CS and DS concentrations in RA patients and in healthy subject were almost similar. HS/H and HA plasma levels were significantly higher in the patients with high and moderate disease activity than in controls. KS plasma levels were significantly increased in all rheumatoid arthritis patient groups as compared to controls. The study shows that rheumatoid arthritis lead to the remodeling of the extracellular matrix, reflected by the qualitative and quantitative changes in plasma glycosaminoglycans. The mentioned plasma changes of glycosaminoglycans in rheumatoid arthritis depend on disease activity and may contribute to the systemic alterations of the properties of the extracellular matrix tissue.

P9.5
Role of extracellular matrix proteins in functional differentiation of bovine mammary epithelial cells

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Mammary alveoli are the basic functional unit of mammary gland, which develop during gestation and produce and secrete milk during lactation. Fully differentiated alveoli are comprised of a single layer of polarized mammary epithelial cells (MECs) surrounded by myoepithelial cells and extracellular matrix (ECM). In the in vitro studies MECs cultured on plastic display a typical non-polarised, cobblestone morphology. However, when plated on Matrigel, which is a commercially available ECM, they form three dimensional (3D), functional alveoli-like structures - mammospheres. The aim of this study was to compare the differences in the functional differentiation of bovine mammary epithelial cell line BME-UV1 cultured in monolayer and 3D mammospheres. We investigated the ability of BME-UV1 cells to produce, and release β-casein, which is one of the basic milk proteins synthesised and secreted by MECs. Monolayer culture was maintained on plastic until reaching confluence. Cells plated on Matrigel formed 3D acinar structures within 16 days of culture. In both cases cells were grown with, or without lactogenic hormone - prolactin (PRL). The level of β-casein was determined in cell extracts using immunoblotting technique, and in the medium, by HPLC. Our results have shown that ECM increases the synthesis of β-casein. Cells cultured in the 3D system expressed higher levels of β-casein in comparison with MECs grown as monolayer. Additionally a higher amount of this protein was detected in the media collected from the 3D culture. Prolactin significantly increased the synthesis of β-casein in BME-UV1 cells grown on plastic, however it did not show this effect on MECs cultured on Matrigel. In case of the β-casein’s secretion PRL showed a positive effect in both examined cell culture systems, causing a significant increase of the milk protein’s concentration in the media. The obtained results indicate that the functional differentiation of bovine MECs is primarily driven by the ECM components.

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P9.6

High ambient glucose causes the elevation of fibronectin and the inhibition of disintegrin metalloprotease ADAM12 levels in mouse C2C12 myogenic cells

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An important cross-talk between the extracellular matrix (ECM) and the insulin signaling has already been reported. High glucose and high insulin, associated with several insulin resistant states are able to impair metabolic effects of insulin and insulin-dependent protein kinases activation in myotubes. The purpose of the present study was to examine the potential modifications of the expression of ECM components in mouse C2C12 myogenic cells subjected to 5-day differentiation in the presence of high glucose alone or in combination with insulin. The cellular content of specific ECM proteins, fibronectin and laminin, integrin α3, α5, β1 subunits, and disintegrin metalloprotease ADAM12 was assessed by immunoblotting and immunofluorescence method. High ambient glucose (15 mmol/l) markedly elevated the level of fibronectin in C2C12 myogenic cells, but it did not affect the cellular content of laminin, which appeared at a very low level in differentiating cells. The expression of either of integrin subunits chosen for a study i.e. the integrin α5 (a component of the adhesion receptor for fibronectin), the integrin α3 (a component of one of adhesion receptors for laminin), and the integrin β1 (forming functional heterodimers with integrin α2 subunits) were not modified by high glucose alone or used with high insulin. High glucose, however, caused a decrease in cellular content of full length 100 kDa form of ADAM12, both on the 3rd and on the 5th day of differentiation, the effect being prevented by the presence of high insulin. The expression of 75 kDa form of ADAM12 was not affected by experimental treatments, however, high glucose alone or in combination with high insulin decreased the level of 50 kDa form of ADAM12 (probably the form able to bind to integrins), both on the 3rd and on the 5th day of myogenesis. High glucose used alone or accompanied by high insulin did not modify the cellular content of actin. The myosin heavy chain protein level was augmented in myogenic cells treated with high glucose during 5 days, pointing to the stimulation of myotube growth in this condition. In conclusion, high ambient glucose caused an increase in fibronectin levels without modification of cellular content of integrins. The simultaneous inhibition of ADAM12 expression could result in the disturbances in extracellular matrix remodelling and function, in particular, in ECM accumulation which can, in turn, contribute to the impairment of the insulin signaling.

P9.7

The trefoil factor 2 (TFF2) protein — a hard man with a chink in its armour

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The trefoil factor family proteins are found in all mucous layers and exert important activities in maintenance of their homeostasis by eg. mucin stabilization or taking part in restitution. There are three subfamilies: TFF1, TFF2 and TFF3. The TFF2 protein contains two P-domains which are supposed to bind oligosaccharide residues of mucins and creates cross-linked structure increasing the viscosity of mucous. The TFF proteins are assumed to be partially responsible for metastasis.

The aim of this study was to investigate the human TFF2 protein stability by: protease digestion assay, mass spectrometry (MS), circular dichroism (CD) and nuclear magnetic resonance (NMR). The human TFF2 protein was expressed in Pichia pastoris and purified on Ni-Sepharose and by HPLC.

We have found that human TFF2 produced in P. pastoris undergoes N-glycosylation. According to the NetGlyce software the 15-th Asn residue of matured protein was pointed to be responsible for glycosylation. This suggestion was confirmed by MS analysis of the protein treated with Endo H glycosylase - the LSPHNR peptide was identified as containing the N-acyethylhexosamine moiety. Therefore we mutated the triplet of tff2 cDNA to the one encoding Ala residue in order to avoid N-glycosylation. As it was shown in Western-blot analysis of TFF2 and TFF2 N15A proteins, the second one exists only in one not-glycosylated form, comparing to the TFF2.

The HPLC analysis linked with MS measurements revealed that the oligosaccharide moiety is not uniform and it preferably contains 10 hexose residues, being in line with P. pastoris glycosylation properties. Additionally, the TFF2 proteins consist of 7 disulphide bridges which can be reduced with DTT and blocked with iodoacetamide. If digested with trypsin, the TFF2 does not show any changes up to 24 h of investigation and the amount of TFF2 N15A seems to be slightly decreased. On the other hand, the digestion carried out in the presence of DTT leads to almost complete disappearance of TFF2 after the first hour. The analysis of CD and NMR spectra showed that these proteins does not change their structure up to 80°C, but with reduced and blocked Cys residues they are deprived of the CD minimum characteristic for the helix secondary structure.

Our studies reveal that the human TFF2 protein is extremely resistant to elevated temperatures and trypsin digestion. The main reason for this lies in the high number of disulphide bridges rather than in the N-glycosylation.
P9.8

The influence of apitherapeutic agent on glycosaminoglycan expression during experimental burn healing

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We compared the therapeutic efficacy of the silver sulfadiazine (SSD) and apitherapeutic agent (Propol T) in the treatment of minor skin burns. The evaluation of therapeutic efficacy of the mentioned agents was based upon the GAGs analysis. Glycosaminoglycans (GAGs) isolated from normal skin and burn wounds inflicted in domestic pigs, were quantified using a hexuronic acid assay followed by the electrophoretic fractionation and densitometric analyses. The accumulation of GAGs in burned wounds was significantly rised after Propol T application. The electrophoretic analysis allowed to identify chondroitin sulfates (CS), dermatan sulfates (DS), heparan sulfates/heparin (HS/H) and hyaluronic acid (HA) in all samples. DS were found initially to increase and at the end of the experiment to decrease after apitherapeutic agent treatment. When SSD was applied the DS content was growing until the end of the experiment. An enhancement in CS amount during the burn healing was observed, particularly visible after apitherapeutic implementation. Short-term elevation in HS/H amount in wounds was manifested in the case of apitherapeutic agent and apitherapeutic vehicle applying. An increase after Propol T treatment in hyaluronan content in wounds followed by the fall and subsequent stability in HA amount was found. The enhanced GAGs expression in wounds after apitherapeutic agent treatment stimulates the repair processes. Found after biochemical investigations, beneficial effects of Propol T treatment on minor burns, make to possible the implementation of the apitherapeutic agent in topical burn management.

P9.9

Differences of gene expression profiles in differentiating to osteoblasts rat bone marrow mesenchymal stem cells cultured in three-dimensional structures versus monolayer cultures

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Extensive bone loss due to an accident, other damage or surgical removal of large fragments of bone during orthopedic surgery require filling of the gap with bone-like material. Thus, in this work we tested new material based on biodegradable polymer for its suitability for obtaining bone equivalent using bone marrow mesenchymal stem cells (BMSC) from rats. Expression profiles of genes commonly linked to osteogenesis were determined for early stages of differentiation to osteoblasts in structured polymer (3D) both uncoated and coated with procollagen type I, in comparison to monolayer cultures. After 24 and 48 hours the cells were harvested and gene expression profile was analyzed using SABiosciences SuperArray kit for osteogenesis. Changes were observed in expression of 24 genes between both, the cells differentiating in the 3D versus monolayer and between the two type of culture environments with no differentiation. The differences in expression were for the following genes: similar to Bone morphogenetic protein receptor, type II, alpha 2 chain of procollagen type I, alpha 1 chains of procollagen, types IV, V and VIII, alpha 3 chain of procollagen type IV, fibronectin 1, insulin-like growth factor 1 receptor, integrin alpha 1, integrin beta 1, matrix Gla protein, matrix metallopeptidase 13, platelet derived growth factor, alpha, scavenger receptor class B, member 1, Serine (or cysteine) proteinase inhibitor, clade H, member 1, Smads 4 and 7, osteonectin, osteopontin, transforming growth factor, beta 3, Vcam 1, Vegf B and C, and Twist. Thus, the culture of cells under conditions mimicking natural tissue, initiates different genetic programs in cells grown in 3D when compared to the monolayer. The differentiating conditions turn on different genetic programs in cells cultured in the 3D than in cells cultured in tissue culture plate.

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IFN-γ but not TNF-α inhibits the expression of fibronectin and integrin-β1 in differentiating mouse C2C12 myogenic cells

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The importance of the extracellular matrix (ECM) molecules as a part of the myogenesis signaling mechanisms has already been demonstrated. Proinflammatory cytokines play an essential role in muscle repair, on the other hand, however, their excess can impair the regenerative processes in skeletal muscle tissue. The purpose of the present study was to examine the potential modifications of the expression of ECM components in mouse C2C12 myogenic cells subjected to 5-day differentiation in the presence of TNF-α and IFN-γ. The cellular content of specific ECM proteins, fibronectin and laminin, integrin α3, α5, β1 subunits, and a disintegrin metalloprotease ADAM12 was assessed by immunoblotting and immunofluorescence method. Both fibronectin and laminin were expressed in C2C12 myogenic cells differentiating in control conditions and in the presence of TNF-α (1 ng/ml). In cell cultures treated with IFN-γ (1 ng/ml), the level of fibronectin detected by immunoblotting was markedly decreased during 5-day myogenesis. The inhibitory action of IFN-γ on laminin expression appeared on the 5th day of differentiation. The level of integrin α5, a subunit of the fibronectin receptor, was not modified by either cytokine. The expression of integrin α3, a subunit forming one of the laminin receptors, was detectable on the 5th day of differentiation and it did not depend on the cell treatment. The cellular content of integrin β1 was markedly higher in differentiating than in proliferating cells and it was decreased on the 5th day of myogenesis in cells exposed to IFN-γ. The 100 kDa (full length) form of ADAM12 was highly expressed in proliferating myoblasts and it decreased dramatically in cells subjected to myogenesis. In differentiating cells, the shorter 75 kDa form of ADAM12 appeared and it was markedly elevated on the 5th day of exposition to IFN-γ. The shortest 50 kDa form of ADAM12 (probably the protein without metalloprotease domain), was declined in cells treated with cytokines on the 5th day of differentiation. The actin level was not modified by experimental treatment, however both cytokines inhibited the growth of myotubes on the 5th day, assessed by myosin heavy chain protein level. In conclusion, IFN-γ exerted a stronger than TNF-α modulatory effect on the expression of ECM components in C2C12 myogenic cells. The alterations of ECM components could, at least partially, explain an inhibitory action of cytokines on myogenesis.