Session 26: Biochemistry of the Connective Tissue

Lectures

L26.1

Matrix metalloproteinase, MMP-9 in the brain: Beyond the connective tissue

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Matrix metalloproteinase 9, MMP-9 is an extracellularly operating enzyme that has been demonstrated as important regulatory molecule in control of synaptic plasticity, learning and memory. We have shown that either genetic or pharmacological inhibition of MMP-9 impairs synaptic plasticity in the brain, as well as certain forms of learning and memory formation. MMP-9 is locally translated and released from the excitatory synapses in response to neuronal activity. Extrasynaptic MMP-9 is required for growth and maturation of the dendritic spines to accumulate and immobilize AMPA receptors, making the excitatory synapses more efficacious. Our studies on animal models have implicated MMP-9 in such neuropsychiatric conditions, as e.g., epileptogenesis, autism spectrum disorders, development of addiction, and depression. We have also reported that in humans MMP-9 appears to contribute to epilepsy, alcohol addiction, Fragile X Syndrome, schizophrenia and bipolar disorder. In aggregate, all those conditions may be considered as relying on alterations of dendritic spines/excitatory synapses and thus understanding the role played by MMP-9 in the synaptic plasticity may allow to elucidate the underpinnings of major neuropsychiatric disorders.

L26.2

Tolloids, function-structure relations and related disorders resulting from extra-cellular matrix pathology

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Mammalian tolloids are the evolutionarily conserved family of zinc-dependent metalloendopeptidases, also known as BMP1/TLD/Tolloid-like. The enzymes are present across the animal world, including in humans. The first discovered tolloid of the family in mammals was BMP1, also known as procollagen C-endopeptidase, which engages in the formation of the extracellular matrix (ECM). BMP1 and the longer alternatively spliced variant of the BMP1 gene product, TLD, demonstrate high homology to a protein named drosophila tolloid-related (tlrd) in fruit flies. The drosophila tlrd is involved in embryonal signaling and development, leading to the designation of the dorsal-ventral body axis. Another protein, tolloid-related protein 1 (TLR1), is a key factor in transformation of the fly larvae. Currently, four tolloid proteins have been detected in mammals. Two of them, BMP1 and TLD1, are products of the alternative splicing of the BMP1 gene product. The two other proteins, (mammalian tolloid-like 1 [TLL1] and mammalian tolloid-like 2 [TLL2]) are encoded by separate genes.
Mutations in the gene encoding BMP1/TLD1 have been linked to familial severe form of brittle bone disease (*osteogenesis imperfecta – OI)*. In the *TLL1* gene three mutations were found in individuals with atrial septal disorder (ASD6). There is no doubt its role in heart development is related to its ability to cleave chordin, therefore its role should be considered in the context of substrate cleavage, which may regulate the genesis and proliferation of cells forming the endocardium via the leading signaling involving BMP2 and BMP4. The TLL2 role has be yet clarified.
The roles of tolloids in human health and pathologies will be detailed discussed in this presentation.

Oral presentations

O26.1

Collagen metabolism within cardiac fibroblast is modified by physical properties of cell environment

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The study is aimed at verification of hypothesis claiming that collagen metabolism within cardiac fibroblast (CF) is regulated by hardness of the cell environment. Moreover, we examined whether this regulation is dependent on α2β1 integrin. The study is aimed at verification of hypothesis claiming that collagen metabolism within cardiac fibroblast (CF) is regulated by hardness of the cell environment. Moreover, we examined whether this regulation is dependent on α2β1 integrin.The research were conducted on stable CF cell line, cultured on polyacrylamide gels with different hardness (soft gel 15kNt/m^2, hard gel 28kNt/m^2). Decrease in hardness of CF environment caused elevation of both α2 integrin gene expression and the protein density on cell membrane. These changes were accompanied by augmentation of collagen levels on soft gel as well as increase in gene expression of type I procollagen α1 chain. Furthermore, we observed that lower hardness of the surface is connected with increase in levels of MMP-1 and with decrease in levels of TIMP-3 and TIMP-4. Levels of Src kinase were lower on soft gels. Addition of TC-I 15 to cells on both soft and hard gels result in augmentation of intracellular collagen deposition. Data obtained in this experiment suggest that metabolic activity of CF was regulated by hardness of the cells environment. The tested physical modification of the cell environment modify expression of α2 integrin subunits and α2β1 integrin receptor. These effects were accompanied by modification of intracellular signaling and changes of collagen metabolism. Finally the results showed inhibitory influence of α2β1 integrin on intracellular collagen deposition in CF.

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O26.2

Effect of the extracellular collagen type I on cardiac fibroblasts

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Extracellular collagen bound with integrins exerts regulatory influence on cell metabolism and is hypothesized to change extracellular matrix synthesis and accumulation within a tissue. The study is aimed at evaluation of extracellular collagen (type I) effect on cardiac fibroblast function. The experiments were carried out on immortalized cardiac fibroblasts cultured on surfaces coated with collagen (10 μg/cm^2). The results were compared with controls (cultures without collagen). Genes expression were measured by qPCR. Collagen and glycosaminoglycan (GAG) contents were assessed with Woessner and Farndale methods respectively. Integrin density was evaluated by flow cytometry. Cell proliferation and Src kinase content were investigated with BrdU and ELISA tests.
The density of α2 integrin on plasmolemma was lowered by collagen. Density of both α11 and β1 integrins was elevated by extracellular collagen. These effects were linked with increased level of Src kinase within fibroblasts cultured on collagen coated wells. High level of collagen augmented proliferation of fibroblasts but decreased GAG deposition within the culture. Intracellular collagen accumulation was not affected by tested conditions.
Type I extracellular collagen modifies density of integrins on the surface of the fibroblasts and influence on GAG deposition and fibroblast proliferation. These effects are linked with decreased activity of Src kinase.

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O26.3

Effect of histamine on collagen content in cardiac myofibroblasts culture

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Collagen content in a tissue is regulated by histamine. The described effect is dependent on activation of H3 receptors in myofibroblast culture isolated from scar of myocardial infarction or H1 receptor in myofibroblast from granulation tissue of the superficial wounds.
The aim of the present study is to describe the effect of histamine on collagen deposition by cardiac myofibroblasts culture as well as identification of the receptor involved in this process.
The experiments were carried out on cells isolated from intact heart of a Wistar rat. The cells were identified by flow cytometry. The following antigens were detected: a-smooth muscle actin, vimentin and desmin. The effect of histamine, pirydylethylamine dihydrochloride (H1 agonist), amthamine dihydrobromide (H2 agonist), imetit (H3 agonist) and 4-methylhistamine (H4 agonist) on collagen content within the cell culture were investigated. Collagen content was measured by Woessner method.
The spindle-shaped cells were isolated. The present results foud that obtained cells are a-smooth muscle actin-positive, desmin-positive and vimentin-positive. Moreover collagen content was increased within the culture by histamine and all applied agonists.
The results showed that myofibroblasts were isolated from intact heart and that histamine may increase fibrosis even in naïve cells isolated from intact heart. All histamine receptors are involved in regulation of collagen content within myofibroblast culture.

Posters

P26.1

Exendin-4 improves blood and tissue biochemical markers of inflammation and wound healing in Zucker diabetic rats

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Using Zucker diabetic rats injected daily with exendin-4, an antidiabetic drug, or 0.9 % saline and implanted subcutaneously with polyethylene mesh stimulating the growth of granulation tissue fibroblasts for 3 weeks, we examined the changes in the metabolic (glucose, triglycerides), inflammatory (CRP, C-reactive protein), and wound healing markers, i.e., MMP-9 (matrix metalloproteinase-9 which inhibits both wound repair and fibroblast activity), and TIMP-1 (tissue inhibitor of matrix metalloproteinase-1 which accelerates tissue repair) in the blood serum and wound fibroblast cultures obtained from these animals. Exendin-4 treatment had no significant effect on blood glucose and triglyceride concentrations but decreased significantly both the blood and fibroblast culture medium CRP levels thus reducing the expression of an important pro-inflammatory agent. Also, the drug increased markedly TIMP-1 concentration in the serum leaving, however, the MMP-9/TIMP-1 ratio unaffected. On the contrary, the culture medium MMP-9/TIMP-1 ratio was significantly reduced due to a marked decrease in the MMP-9, and an increase in the TIMP-1 release by cultured fibroblasts. These changes were accompanied with improved cell viability. To conclude, exendin-4 treatment noticeably improved the blood and tissue biochemical environment of diabetic rats to promote wound healing.

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P26.2

The effects of peroxisome proliferator-activated receptor ligands in the TGF-β_1-induced myofibroblastic transition of bronchial fibroblasts derived from asthmatics

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Bronchial fibroblast activation to myofibroblastic transition (FMT) play a key role in the subepithelial fibrosis progression and asthmatic airway remodeling. Recent reports suggest the involvement of peroxisome proliferator-activated receptors (PPARs) ligands in the attenuation of fibrosis in several tissues. We examined the effects of the PPARs agonists and antagonists in the asthma-related FMT.

Profibrotic activation of primary bronchial fibroblasts derived from asthmatic patients was induced by TGF-β_1. The effect of PPARα, β/δ and γ agonists (fenofibrate, GW501516 and rosiglitazone, respectively) and/or antagonists (GW6471, GSK0660, GW9662, respectively) treatment on the FMT efficiency was verify by immunofluorescent staining, western blotting and qPCR analyses. FMT efficiency measured by the percentage of myofibroblasts, α-SMA and Cx43 levels were strongly suppressed by the antagonists of PPARα and PPARγ in contrast to their agonists. In turn, pharmaceutical activation or inhibition of PPARβ/δ had insignificant effects on this process.

In conclusion, antagonists of PPARα and PPARγ suppress TGF-β_1-induced FMT in bronchial fibroblasts from asthmatic patients, but the mechanisms of its action requires further research.

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