DESCRIPTION OF THE GENERAL PUBLIC

Chronic inflammation and disturbances in wound healing are underlying mechanism of several health problems, including civilization diseases. The inflammatory process can be induced by physical, chemical and biological factors. The chronic inflammation contributes to atherosclerosis, rheumatoid arthritis, asthma, cancer and several other diseases. The process is accompanied by deregulation of cell proliferation, differentiation and biosynthesis of extracellular matrix (ECM) components, mainly collagen. Although, the mechanism of inflammation is well recognized, the complexity of molecular regulation of this process is not completely understood. We considered bi-functional activity of prolidase (intracellular enzyme and extracellular ligand of EGFR) as an important protein in the regulation of regenerative processes in experimental model of inflammation in the skin cells.

Prolidase is intracellular exopeptidase, which splits imidodipeptides with proline or hydroxy-proline. The enzyme plays an important role in the recycling of proline from imidodipeptides (mostly derived from degradation products of collagen) for re-synthesis of collagen and other proline-containing proteins. However, the specific ligand for β1-integrin receptors is collagen type I. The stimulation of β1-integrin receptor causes an increase in collagen biosynthesis. The effect of stimulation of the signaling pathway of this receptor is induction of transcription factors that stimulate expression of genes for many proteins involved in the regulation of cell proliferation, differentiation and metabolism. The linkage of prolidase activity and the process of collagen biosynthesis has been demonstrated in several models of cultured cells and animals, such as the process of wound healing, aging, tissue fibrosis and osteogenesis imperfecta. The regulation of these processes was found at the level of signaling by insulin-like growth factor (IGF-I), the most potent stimulator of collagen biosynthesis. The mechanism of prolidase-dependent regulation of collagen biosynthesis was found at both transcriptional and post-transcriptional level. Prolidase-dependent transcriptional regulation of collagen biosynthesis is regulated at the level of NF-kB, known inhibitor of type I collagen gene expression. Modulation of integrin-dependent signaling by stimulatory or inhibitory ligands or by nitric oxide donors affected prolidase at the level of the enzyme phosphorylation. Prolidase was also found as a ligand of epidermal growth factor receptor (EGFR) that stimulates growth-promoting signaling. This dual action of prolidase suggest its pivotal role in promotion of cell growth and tissue healing. However, this function of prolidase has not been explored yet.

Therefore, the aim of the project is to explain molecular mechanism of prolidase action in regenerative processes in cultured human skin cells. The significance of biological effects of prolidase on skin cells will be studied in respect to potential use in wound healing as well as in prolidase deficiency. The results of these studies may contribute to establish a novel model of regenerative pharmacotherapy.

The purpose of the project is to establish the role of extracellular prolidase in wound healing. Therefore, the impact of the obtained recombinant prolidase and its mutated variants on skin cells (keratinocytes and fibroblasts) under conditions of inflammation and wound healing will be evaluated. The effect of prolidase influence on collagen biosynthesis, cell proliferation, cell cycle and proteins of signaling pathways induced by prolidase-dependent stimulation of the EGFR will be evaluated by RT-qPCR, Western immunoblot, wound healing assay and immunocytochemistry using confocal microscopy and flow cytometry. In the final stage of the research the metabolomic analysis of intracellular amino acids and their metabolites will be evaluated in prolidase-treated skin cells in conditions simulating tissue damage. The results of these studies may contribute to establish a novel experimental model of regenerative pharmacotherapy using human recombinant prolidase.