## Abstract

Recovery from injury, known also as wound healing, is a precisely regulated process in which four phases occur. The proper healing starts from clot formation, inflammatory cell infiltration, re-epithelization, and finally, matrix remodeling. As a result of a delay in wound healing chronic inflammation and ulcers may happen. Skin is one the most frequently injured organ involving a variety of cells playing distinct roles such as keratinocytes, fibroblasts, macrophages, and endothelial cells, however, in the epidermal layers, keratinocytes comprise about 95% of the cell mass. They serve as the first line cells which encounter pathogenic bacteria, viruses, UV radiation, and allergens leading to the generation of pro-inflammatory cytokines and skin inflammation progression. The machinery of the healing process involves also growth factor receptors, metalloproteinases (MMPs), inflammatory mediators, and enzymes that closely cooperate with the cells to restore the functionality of the injured tissue. Recently PEPD was found to be a ligand of the EGFR. As activation of EGFR signaling promotes cell proliferation, growth, differentiation, and migration, the question was raised whether prolidase may be a stimulating factor for wound healing *in vitro*.

This study aimed to investigate the proliferative capacity of prolidase in models of experimental wound healing under conditions of interleukin (IL)-1β-induced inflammation and mechanical damage in HaCaT keratinocytes.

Immortalized human HaCaT keratinocytes were treated with prolidase (porcine or recombinant human) and cell viability, vitality, proliferation, and migration were assessed. Western immunoblotting and immunocytochemical staining coupled to a confocal microscope were employed to evaluate the protein expression. Determination of collagen biosynthesis and prolidase activity was assayed with radiometric and colorimetric methods, respectively. The liquid chromatography coupled with mass spectrometry was applied for the measurement of proline concentration. MMP activity was evaluated with gelatin zymography assay while cell cycle analysis was analyzed with image cytometry.

The study revealed that PEPD, under scratched conditions, induced cell proliferation and migration via activation of EGFR-downstream signaling in which the PI3K/Akt/mTOR pathway played an essential function. The protein markers of epithelial-to-mesenchymal transition were upregulated and supported the observation of enhanced cell motility. The expression of $\beta_1$-integrin and IGF-1 receptors and their downstream kinases were upregulated and it was accompanied by higher proline concentration and collagen biosynthesis. While under inflammatory conditions, PEPD required the presence of IL-1β to augment keratinocyte

proliferation through activation of EGFR and its downstream signaling proteins (Akt, ERK1/2, and STAT3). Migrating cells expressed the EMT protein markers such as downregulated E-cadherin and upregulated N-cadherin. Extracellular matrix remodeling occurring in the inflammatory phase was reflected by the activation of MMP-9. It may result from activation of NF-κB via IKK-mediated IκBα degradation. Interestingly, mutated PEPD (rhPEPD-G448R, rhPEPD-231delY, and rhPEPD-E412K) were able to activate EGFR-mediated keratinocyte proliferation.

Extracellular prolidase acting through EGFR induces growth, migration, and collagen biosynthesis and ECM remodeling in HaCaT keratinocytes under the conditions of experimental wound healing. PI3K/Akt/mTOR, ERK1/2, and STAT3 pathways are involved in the proliferation and migration of keratinocytes. Prolidase activity is not required for EGFR activation. Enzymatically active and inactive prolidase may modulate EGFR signaling with different intensities. The study suggests that not only PEPD activity but also an extracellular function of PEPD may be involved in the mechanism underlying prolidase deficiency. Prolidase may serve as a stimulating factor in injured cells accompanied by inflammation and represent a therapeutic approach to treating skin wounds.