



**Karolinska
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Department of Molecular Medicine and Surgery

NEW PATHOGENIC MECHANISMS IN DIABETIC WOUND HEALING

AKADEMISK AVHANDLING

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ABSTRACT

Diabetic foot ulcers (DFU) represent one of the most feared and invalidating complication of diabetes with high financial pressure for the healthcare system. For the moment, there is no specific therapy available and it has become a priority to develop novel rational therapeutic strategies based on new pathophysiological mechanisms. Our focus was therefore to delineate relevant pathogenic pathways specifically deregulated in diabetes that could contribute to the defective wound healing in diabetes. Cellular proliferation, migration and differentiation, angiogenesis, extracellular matrix deposition, local recruitment of endothelial precursors cells are some of the essential processes activated during wound healing. We decided to focus our investigation on two central signaling pathways (HIF-1 pathway and Notch signaling) that modulates most of the above cellular events.

Hypoxia plays an important role in the development of DFU. We showed that hyperglycemia complexly repressed the function of Hypoxia inducible Factor (HIF), which is the main cellular adaptor to low oxygen tension. The repressive effect of hyperglycemia on HIF-1 alpha was pVHL dependent and affected complexly its transactivation. This was mirrored by suppression of several HIF-1 target genes essential for wound healing. However, by blocking HIF-1 α degradation through chemical interference with HIF hydroxylases (DMOG or DFX), it was possible to reverse the repressive effect of hyperglycemia on HIF and to improve the wound healing process in a diabetic mouse model (the db/db mouse). Moreover, local adenovirus-mediated transfer of two stable HIF constructs demonstrated that stabilization of HIF-1alpha is necessary and sufficient for promoting wound healing in a diabetic environment. Hyperbaric oxygen therapy (HBOT) has been used as therapeutical option for severe foot ulcers, resistant to standard therapy. The detailed mechanisms activated by HBOT are however still unraveled. We showed that HBOT activated HIF-1alpha at several levels with functional consequence on cellular proliferation. Moreover, we could show that local transfer of a stable form of HIF has additive effect to HBOT improving wound healing in the db/db mice.

Notch signaling is a cell-to-cell contact system that consists of several receptors (Notch 1-4) and ligands with a high specific cell-dependent effect. Binding of the ligands to the receptors is followed by proteolytic cleavage of the receptor by a γ -secretase complex, which is followed by activation of the intracellular signaling. Here we show that hyperglycemia activated Notch signaling at several levels both *in vitro* and *in vivo*. The effect of hyperglycemia on Notch signaling is canceled in the presence of γ -secretase inhibitors with positive functional effect both on *in vitro* migration and on *in vitro* angiogenesis assays. Moreover local treatment with γ -secretase inhibitors improved wound healing of db/db mice despite chronic hyperglycemia. The effect is specific for diabetes since neither γ -secretase inhibitors nor immunization with a DNA vaccine against Dll4 influenced the wound healing in non-diabetic animals. Using a loss of function genetic approach (specific siRNA and cre/lox system), we showed that Notch 1 has a central pathogenic role in Notch dependent repression of wound healing in diabetes.

In conclusion, we identified two new pathogenic mechanisms important for impaired wound healing in diabetes. Our findings warrant development of specific therapeutics that address HIF and Notch signaling for normal healing of diabetic wounds.

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