

Wissenschaftliche Posterausstellung: Poster 12

# Effects of INLB321-CD on VEGFA Gene Up-Regulation in Immortalized Human Keratinocytes and on a Model of Wounded Skin

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Internalin B (InlB) is an invasion protein of *Listeria* which facilitates its uptake into host cells by activating the receptor tyrosine kinase MET. It was proposed that activation via receptor dimerization is mediated by an InlB dimer. The dimerized fragment of Internalin B, InlB321-CD<sup>1</sup> (crystal dimer), was designed to stabilize the InlB dimer in solution. In binding studies and in in vitro scatter assays<sup>1</sup>, InlB321-CD revealed to be a stronger agonist than monomeric InlB321 and Internalin B.

In human skin, mainly epithelial cells express the MET receptor whose activation leads to proliferation, migration and vascularization. Its endogenous agonist hepatocyte growth factor (HGF/SF), which is secreted by e.g. dermal fibroblasts, plays an important role in the regeneration of the epidermis of the skin. That is why in preliminary studies, InlB321-CD was investigated with focus on its mitogenic and motogenic properties<sup>2,3</sup> on human epidermal immortalized cellline (HaCaT).

The present study aims at InlB321-CD's influence on the up-regulation of the vascular endothelial growth factor (VEGFa) at RNA level, because HGF/SF stimulates vascularization via secretion of VEGF4. In vivo, particularly in chronic wounds, HGF/SF is degraded by proteases causing retarded vascularization.

Furthermore, InlB321-CD's effect on a mechanically wounded and differentiated epidermis model was analyzed in terms of its wound healing properties.

## Methods:

A confluent HaCaT monolayer was serum-starved (24 h), then incubation with serum-free medium, 0.5 nM HGF, 0.5 nM InlB321-CD and 1 nM InlB321 took place for 6 h. Total RNA was extracted with Trizol<sup>®</sup> according to the manufacturer's guidelines. RNA concentration was quantified with an UV spectrometer. Prior to performing PCR with a pair of gene specific primers for VEGFa, first strand DNA synthesis was carried out. The PCR products were separated with an agarose gel electrophoresis stained with ethidium bromide and detected under UV light (260 nm).



HaCaT cells were cultivated on a polycarbonate membrane (3µm pore size) which was set on a dermis consisting of living fibroblasts incorporated in a collagen matrix<sup>5</sup>. After 3 weeks of co-culture, 3 days of serum-starvation was conducted. Then the membrane with the differentiated epidermis was removed. Subsequent to perforating the epidermis with a punch, cultivation on a dermis with either dead or living fibroblasts was conducted for further 5 days in serum-free medium with or without 0.5 nM InlB321-CD supplementation. An MTT assay was used to test for viability of the epidermis.

#### Results:

The VEGF gene expression at RNA level after incubation with 0.5 nM InlB321-CD was slightly higher compared with medium control, and comparable to 0.5 nM HGF/SF, which served as positive control. However, the equimolar dose of monomeric InlB321 did not increase VEGF gene on mRNA level.

Subsequent to incubation with 0.5 nM InlB321-CD, the epithelial model of wounded skin cultured on a dead dermis showed a higher relative cell viability compared to that treated with plain medium. In contrast to that, the differentiated keratinocytes co-cultured on a living dermis did not benefit the same way from 0.5 nM InlB321-CD supplementation versus medium. This might be due to fibroblasts' secretion of growth factors, i.e. HGF/SF that endogenously stimulates the mitogenic process in keratinocytes.

In conclusion, for proof of wound healing potential of InlB321-CD treatment of a wounded 3D skin model, the endogenous HGF/SF secretion of fibroblasts from the dermal layer underneath the epidermal layer has to be suppressed.

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