Investigation of Internalin B 321 CD on immortalized dermal keratinocytes and dermal organotypic co-cultures

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Introduction

• Internalin B (InB) is an invasion protein of Listeria monocytogenes, which facilitates its uptake into the host cells via activation of the receptor tyrosine kinase c-Met. It was proposed that receptor dimerization, which is essential for activation, is mediated through an InB dimer. The variant InB 321 CD [1] (crystal dimer), i.e. a dimerized fragment of Internalin B, was designed to stabilize the InB dimer in solution and to study the effects on c-Met activation.

• In human skin the c-Met receptor is mainly expressed on keratinocytes where it leads among other effects to proliferation after being stimulated by its physiological ligand hepatocyte growth factor (HGF). The receptor plays an important role in human dermal wound healing.

• The present project aims at the influence of dimeric as well as monomeric InB 321 on re-epithelialization of dermis with keratinocytes. Subsequent to studying the potency of InB 321 CD on the HaCaT cell line (human dermal immortalized keratinocytes), the effects of InB 321 CD on bioengineered skin of a keratinocyte/dermal fibroblast co-culture as a human skin equivalent were studied to test whether the morphology of the bioengineered skin as a model of intact skin was affected.

Experimental setup

Cell culture

• Human dermal fibroblasts from the foreskin of newborns were cultured in DMEM supplemented with 10 % FCS, 2 mM L-glutamine and antibiotics. They were used from passage three to twelve.

• Organotypic co-cultures consisting of both cell lines called artificial skin constructs (mASC) were manufactured according to Weber [2]: Type 1 collagen was extracted in acetic acid, neutralized with NaOH, dialyzed and lyophilized. In order to build up the dermal equivalent in which the dermal fibroblasts could be incorporated, the collagen gel was then cast into a transwell (Corning) at a volume of 1 ml/mASC. One week later without serum, 76,000 HaCaT cells were seeded onto each contracted dermis and covered with medium. After one hour further medium was added at the air-liquid interface (ALI) and the medium was changed to MSBM 2 %. The mASCs were ready to use after 14 days in ALI.

Proliferation assay via MT

HaCaT cells were seeded with a density of 60,000 cells/well in a 12-well plate and cultured for three days. After washing with PBS, the medium was exchanged to serum free DMEM. One day later, the keratinocytes were incubated for another day with different concentrations of InB 321 CD versus equimolar concentrations of monomeric InB 321. 30 nM HGF and medium. Afterwards, an MTT assay was carried out [2]. The HaCaT monolayers were treated with an aqueous dilution of MTT (0.5 %), two hours later it was removed and the dye was dissolved in dimethyl sulfoxide. Afterwards, the blue colored liquid was measured twice at 570 nm with a multiplate reader (KGA, Biotek). Furthermore, the number of cells of the untreated control group, cultivated with or without serum, was counted at a Coulter Counter Z2 (Beckman).

The mASCs were investigated in analogy to HaCaT cells, the change to serum free MSBM was accomplished one day before incubating with 1 nM dimer, 2 nM monomer or 30 nM HGF for 2 days.

Histological analysis

The effects of InB 321 were visualised histologically in non-wounded skin constructs. Those mASCs were incubated for three days with monomer, dimer or medium, respectively. Afterwards, the constructs were dehydrated with a series of increasing ethanol concentrations, infiltrated and embedded in EMA (Technovit 7100, Heraeus Kulzer). Sections of 6 µm were prepared of the hardened constructs with a microtome (RM2835G). Subsequently, the hematoxylin-eosin staining was performed.

Conclusion

• InB 321 CD showed a proliferative effect on HaCaT monolayer, i.e. immortalized dermal keratinocytes, which are supposed to express the c-Met receptor [3]. Hepatocyte growth factor (HGF) as a physiological agonist of the c-Met receptor also stimulates the mitosis of the HaCaT cell line.

• In contrast to that, the organotypic co-cultures did not seem to be influenced after being stimulated with InB 321 CD or monomer, only a tendency could be observed. This might be due to the fact that dermal fibroblasts serve as a natural source of HGF [4], consequently a proliferative effect of c-Met receptor agonists, i.e. InB 321 CD, could not be evoked.

• In consideration of the histological analysis, no morphological alteration of mASC could be detected. That is very important, because the c-Met receptor is also involved in invasive events.

References


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