

Dissertations

The Human Antimicrobial Peptide hCAP18 in Epithelial Defence

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Gene-encoded antimicrobial peptides serve a protective role in host defence. They are multifunctional effector molecules in innate immunity (non-adaptive immune system), with the capacity to kill a broad spectrum of microorganisms. In contrast to the highly specific adaptive immunity, the innate immune system provides a rapid and non-specific response and thereby contributes to the first line of defence. Cathelicidins constitute a family of antibacterial peptides. hCAP18, the precursor of the antimicrobial peptide called LL-37, is the only cathelicidin in humans while there are several in other species.

In our initial work, we analyzed wound fluid from wounds with different aetiology in terms of antibacterial and biochemical factors. We showed that wound fluid can be analyzed reproducibly and can be biochemically characterized. Separation and identification of such fluids yielded peptide components, likely to reflect necrosis and to function as antibacterial and plausible wound healing factors.

We have studied the role of hCAP18 in normal wound healing and chronic non-healing ulcers. We used skin bi-



Margareta Frohm defended her thesis on December 7, 2001, at the Department of Dermato-Venereology, Karolinska Institutet, Stockholm. Faculty Opponent was Professor Alan Ezekowitz, Harvard Medical School, Boston, MA, USA. Professor Mona Bäckdahl, Department of Dermatology, Karolinska Institutet, acted as Supervisor.

opsies from healthy volunteers and patients to determine the level and expression pattern of hCAP18. Our results demonstrate the presence of hCAP18 in both types of wounds. Maximal levels of hCAP18 were attained at 12 h-2days post-wounding in surgical wounds. Total hCAP18 protein levels in the chronic ulcers were less than 20% of maximum levels detected in the surgical wounds. Processed active LL-37 peptide was demonstrated in normal wound healing but was barely detectable in the chronic ulcers.

In addition to providing a mechanical barrier between the body and the environment, epithelia function as an

immunological organ. When we investigated the expression of hCAP18 in different inflammatory skin disorders we found an up-regulation of the hCAP18 gene in the keratinocytes of different inflammatory dermatoses, such as lesional psoriasis, nickel allergy and atopic dermatitis, but not in normal quiescent epidermis.

Further investigation of hCAP18 in squamous epithelia demonstrated that hCAP18 was constantly expressed at both RNA and protein levels in epithelia of tongue, oesophagus, cervix and vagina. Expression of IL-6 co-localized with hCAP18 in these tissues. The hCAP18 gene contains promoter elements that are potentially

regulated by IL-6 and our findings suggest a potential local mechanism for the up-regulation of hCAP18 at the epithelial surfaces.

To assess the potential role of hCAP18 in tumour host defence, we have investigated its expression in human breast cancer of varying types and malignancy. We found that hCAP18 was strongly up-regulated in the tumour cells compared to the low constitutive expression found in normal benign mammary epithelia. Furthermore the highest levels of hCAP18 protein were detected in the most malignant tumours and immuno-blotting revealed a presence of processed active LL-37 only in these tumours. Thus our results do not support a protective role for hCAP18 in tumour host de-

fence, but rather suggest that hCAP18 may provide a survival advantage for the tumour.

In summary our studies reveal a role for the innate immunity effector hCAP18 in epithelial defence.

List of original publications:

- I. Frohm M, Gunne H, Agerberth B, Bergman A-C, Bergman T, Boman A, Lidén S, Jörnvall H, Boman H.G. Biochemical and antibacterial analysis of human wound fluid. *Eur J Biochem* 1996;237: 86 -92.
- II. Heilborn J, Frohm Nilsson M, Kratz G, Sørensen O, Weber G, Ståhle-Bäckdahl M. Human cathelicidin antimicrobial protein, hCAP18, is induced in skin wounding with high levels and release of active C-terminal peptide, LL-37, in

physiological healing but not in chronic non-healing ulcers (Manuscript)

- III. Frohm M, Agerberth B, Ahangari G, Ståhle-Bäckdahl M, Lidén S, Wigzell H, Gudmundsson, G. The expression of the gene coding for the antibacterial peptide LL-37 is induced in human keratinocytes during inflammatory disorders. *J Biol Chem* 1997;272: 15258 - 15263.
- IV. Frohm Nilsson M, Sandstedt B, Sørensen O, Weber G, Borregaard N, Ståhle-Bäckdahl M. The human cationic antimicrobial protein (hCAP18), a peptide antibiotic, is widely expressed in human squamous epithelia and colocalizes with interleukin -6. *Infect. Immun.* 1999;67: 2561-2566.
- V. Frohm Nilsson M, Sørensen O, Sandstedt B, Heilborn J, Tham E, Borregaard N, Weber G, Ståhle-Bäckdahl M. The innate immunity effector protein hCAP18 is over-expressed in tumor cells of human breast carcinoma. (Manuscript)

Vitamin A and β -carotene Metabolism and Effects of UV Irradiation on Human Keratinocytes and Melanocytes

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Vitamin A (retinol) and its derivatives play an important role in the maintenance of normal epithelial growth and differentiation. Both natural and synthetic retinoids are used in the treat-



Eva Andersson defended her thesis on April 12th, 2002, at the University Hospital, Linköping. *From left to right.* Professor Anders Vahlquist, Division of Dermatology, Uppsala, Eva Andersson, Professor Inger Rosdahl and Professor Jörgen Serup, Division of Dermatology, Linköping.