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 defence, 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.

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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.

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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.

ment of a wide range of skin diseases such as acne, psoriasis, photoaged skin and ichthyosis. In addition, retinoids have been used with success in the prevention and treatment of skin malignancies. The human epidermis contains a significant amount of vitamin A, and several enzymes are responsible for its metabolism toward either storage or conversion to more active metabolites. Retinol (ROH) can be converted into retinoic acid (RA), the biological most active metabolite. Another major epidermal metabolite is 3,4-didehydroretinol (ddROH), which is formed from ROH in epidermis and in cultured keratinocytes and melanocytes. The concentration of ddROH in human epidermis and keratinocytes in culture makes up about 30% of the total retinoid content. ddROH can be metabolized into the corresponding acid ddRA, but the concentration is extremely low and its formation difficult to study. No specific role for ddROH or ddRA has been demonstrated in human epidermis.

Two protein families are important for the retinoid metabolism of the skin cells. First of these are the cellular retinol-binding protein (CRBP I) and the cellular retinoic acid-binding proteins (CRABP I and II) located in the cytosol. These proteins protect, transport or direct the substrates to specific enzymes for further conversion or metabolic inactivation. The second protein family is the nuclear retinoid receptor family, which mediates the effects of retinoids. This group of proteins consists of the retinoic acid receptors (RARα, β, and γ) and the retinoid X receptors (RXRα, β, and γ). These receptors belong to the steroid/thyroid hormone superfamily of ligand-modulated transcriptional regulators. The RARs are activated by several isoforms of natural RA, while RXRs are activated only by 9-cis RA. RARs and RXRs bind to DNA as homodimers, but they function optimally as RAR:RXR heterodimers. These complexes bind to specific oligonucleotide sequences designated as RA response elements, located in the promoter region of many genes involved in the regulation of cell growth and differentiation. In addition, retinoid receptors may suppress the expression of other genes by inhibiting the action of other transcription factors, e.g. activator protein-1 (AP-1). Because retinoid receptors and AP-1 proteins use the same limited amounts of co-factors for transcription, the two signalling pathways compete with one another.

In this thesis we have investigated the retinoid metabolism and the concentration of retinoid-binding proteins and retinoid receptors in human keratinocytes and melanocytes in vitro. The results were compared to similar studies done in human malignant epithelial cells (HeLa) and malignant melanoma cells. Keratinocytes and melanocytes contained high concentrations of ROH, ddROH, while HeLa- and melanoma cells contained lower levels. Keratinocytes contained the highest level of the retinoid-binding proteins CRBP I and CRABP II compared to melanocytes, HeLa and melanoma cells. High CRABP II levels showed a correlation with the ability to accumulate ddROH. In melanocytes, CRABP I was highly expressed, but in melanoma cells CRABP II dominated. The difference between melanocytes and melanoma cells in receptor levels was most pronounced for RARβ, which was highly expressed in melanoma cells. Such dissimilarities between benign and malignant melanocytes might play a role in differentiation and growth regulation.

To study the retinoid metabolism, radioactive ROH was used. The conversion of [^{3}H]ROH to [^{3}H]ddROH was examined in various cell types. Keratinocytes and HeLa cells have the highest capacity to produce [^{3}H]ddROH, accounting for 10% and 30% of the cellular radioactivity after 24 hours. Melanocytes generated 4% [^{3}H]ddROH of the total cell-associated tracer activity, and melanoma cells only 1%. The uptake of [^{3}H]ROH was significantly higher in melanocytes than in keratinocytes. Both keratinocytes and melanocytes converted [^{3}H]ROH to [^{3}H]ddROH and [^{3}H]RA.

Dietary β-carotene has been considered to play a critical role in the natural defence against cancer. For this reason, we investigated β-carotene uptake and metabolism in human keratinocytes and melanocytes in vitro. Both keratinocytes and melanocytes have the ability to accumulate β-carotene. When cells were incubated identically with β-carotene for five days, the uptake was much higher in melanocytes than in keratinocytes. During β-carotene incubation, a significant increase in cellular ROH was found in both keratinocytes and melanocytes compared to control cells. To verify whether β-carotene was converted to ROH, radioactive β-carotene was used. We were able to

En gång om dagen – gärna till kvällen!

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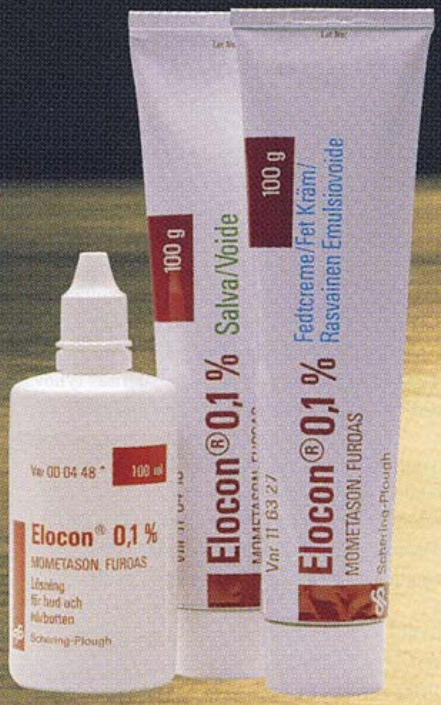

Med Elocon® (mometason furoat grupp III-steroid) räcker det med endast en behandling en gång per dygn för att uppnå god effekt. Det är enkelt och bevämt för patienten. Det är också ekonomiskt.

Elocon® dämpar och lindrar snabbt symtomen vid eksem och psoriasis. Elocon® finns som salva, fet kräm och lösning. Lösningen är ett effektivt och bekvämt alternativ för behandling av hårbotten vid seborroiskt eksem och psoriasis. Oavsett beredningsform och symtom räcker det med att använda Elocon® en gång per dygn. Om Elocon® appliceras till kvällen ger detta praktiska fördelar.

Kliniska studier visar ofta att behandling med Elocon® en gång per dygn ger bättre effekt än andra grupp III-steroider givna två gånger per dygn. [1] [2] [3] [4]

ELOCON® ×1

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demonstrate that [^{14}C]β-carotene was converted to [^{14}C]ROH in both these cell types. This suggests that the local storage of β-carotene in the skin might serve as an alternative supply for vitamin A.

Over the last decades, the frequency of skin cancer has been increasing worldwide among the fair-skinned population. The non-melanoma skin cancers, such as basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), are the most common. SCC is thought to be the result of the total cumulative exposure of ultraviolet (UV) radiation, while intermittent sun exposure may play a critical role for BCC development. Sunburns at a young age are considered to be associated with malignant melanoma, which is more frequent on body areas that are intermittently exposed to the sun. Despite the compelling evidence that UV irradiation causes skin cancer, more knowledge is needed concerning the mechanisms involved, for instance how UV radiation affects cellular signalling via retinoids and other hormone-like natural compounds. The influence of UV irradiation on the cellular vitamin A metabolism in general and on the RAR expression in particular was investigated in cultured keratinocytes and melanocytes. We found that a combination of a moderate dose of UVA and UVB immediately decreased the level of ROH by 50% in both keratinocytes and melanocytes, compared to control cells. After 1–2 days the level returned to starting levels. The concentration of ddROH decreased by about 20% as a result of irradiation and was normalized again within 1–2 days. These results are in line with the UV induced reduction seen in human epidermis, but the regeneration seen in vivo was more gradual. One obvious way to normalize vitamin A levels after UV exposure might be to increase the cellular uptake of ROH, while an alternative mechanism would be to suppress the metabolism from ROH to more polar metabolites. To try to establish the mechanism behind this recovery of the retinoid levels, the cells were exposed to radioactive ROH. Our experiments hold no support for increased cellular uptake of ROH after UV. Instead the metabolism of ROH was decreased and the biologically most active metabolite RA was rescued. In fact our data showed that the level of RA was increased by 160% both in keratinocytes and in melanocytes compared to control cells. Our findings emphasize the importance of a well-controlled RA production and degradation. A failure to direct this process during and after UV exposure may promote carcinogenesis.

We have also examined the mRNA and protein expression of RARα, RARβ, RARγ and RXRα before and after exposure of UVB or sham treatment. Both the mRNA and protein levels dropped dramatically the first 8 h after irradiation. In irradiated melanocytes, the mRNA levels of the four receptors were close to normal after 16 h and the protein levels returned to starting levels during the following 2–3 days studied. In contrast, in keratinocytes only RARα mRNA and protein levels returned to starting levels, while the other receptors examined were consistently lower in irradiated cells compared to sham treated cells. It is known that UV exposure of the skin results in the stimulation of growth factor and cytokine receptors, which leads to the activation of protein kinase signal transduction cascades. This results in an increased expression of c-Fos and c-Jun, which bind to the AP-1 response element, which in turn upregulates genes important for proliferation. When AP-1 is in excess, retinoid receptor activity is repressed, contributing to a perturbed vitamin A signalling. In our study the retinoid receptor levels were decreased during one to two days after UV irradiation in melanocytes and depressed for a longer time in keratinocytes. This, together with decreased levels of ROH and RA, would favour the AP-1 pathway after UV irradiation.

It appears that the retinoid-signalling pathway is more vulnerable to UVR in keratinocytes than in melanocytes, which have a greater ability to restore their retinoid receptor levels after irradiation. Likewise melanocytes have a higher capacity than keratinocytes for restoring the vitamin A and RA levels after UV exposure and a higher capacity than keratinocytes for accumulating β-carotene, which might serve as a quencher of free radicals and excited oxygen molecules. The high capacity for accumulating β-carotene and preserved retinoid function might have a pivotal function in the melanocytic defence against harmful effects of UVR.

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I. Andersson E, Björklind C, Törmä H, Vahlquist A. The metabolism of vitamin A to 3,4-didehydroretinol can be demonstrated in human keratinocytes, melanoma cells and HeLa cells, and is correlated to cellular retinoid-binding protein expression. Biochim Biophys Acta 1994; 1224: 349-359.

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On Dynamics and Gene Expression of Growth Factor Receptors in Human Cultured Skin Cells, Mainly after UVB Irradiation.

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Dr Margareta Lirvall defended her thesis on May 18, 2001, at the University Hospital, Linköping. Faculty Opponent was Ass Prof Gunnar Kratz, Department of Plastic Surgery, Karolinska Hospital, Stockholm. Chairman: Prof Åke Wasteson, Division of Cell Biology, University of Linköping. Board of examination: Prof Olle Larkö, Dept of Dermatology, Sahlgrenska Hospital, Göteborg, Prof Tommy Sundqvist, Dept of Microbiology, and Prof Hans-Jurg Monstein, Dept of Molecular Biology, both University of Linköping.

Dr Margareta Lirvall, specialized in dermato-venereology, presently specializing in general surgical pathology with a focus on dermatopathology at the Department of Pathology, University Hospital, Linköping.

Cells in a tissue carry out genetically based programmes for proliferation and differentiation upon induction by signals received from other cells or tissues. Most of these environmental signals are growth factors that are polypeptides. Growth factors represent a network of intercellular signalling molecules responsible for the development of a tissue or an individual and for the maintenance of integrity. The signals are received by receptors, which trigger a cascade of activities in the cytoplasm that will modulate the activity of nuclear transcription factors, thus regulating gene expression.

Communication between cells involves interactions of a signalling molecule with a receptor at the surface of the cell. Typically the receptor is embedded in the membrane and it is hypothesized that the binding of the signalling molecule causes a change in the state of aggregation of the receptor which, in turn, initiates a biochemical signal within the cell.

Growth factor receptors provide an essential link between diffusible growth factors and their intracellular