On Keratin Mutations in Epidermolytic Hyperkeratosis and the Regulation of Keratin Expression by Retinoids

Marie Virtanen
Department of Medical Sciences, Dermatology and Venereology, Uppsala University, University Hospital, SE-751 85 Uppsala, Sweden.
E-mail: marie.virtanen@medsci.uu.se

Dr Marie Virtanen defended her thesis on May 30th, 2001, at the University Hospital, Uppsala. Faculty Opponent was Prof. Torbjörn Egelrud, Dept of Dermatology, University of Umeå. Prof. Anders Vahlquist (*right*) and Assoc. Prof. Hans Törmä (*left*) acted as supervisors.

Epidermolytic hyperkeratosis (EHK), or bullous ichthyosis, is a rare inherited disease of the skin caused by dominant-negative mutations in keratin 1 (K1) or 10 (K10) expressed in suprabasal epidermis. Keratins are the major structural proteins in epidermis, forming heterodimers during polymerisation. Mutations in one of the keratins may cause instability of the intermediate filaments and hence keratinocyte fragility. In about 50% of the cases, there are sporadic mutations, i.e. there is no previous family history of EHK. The disease is evident at birth with generalised erythema, scaling, erosions and blisters, i.e. findings that can be difficult to distinguish from a severe form of epidermolysis bullosa or certain bacterial infections. Within a few weeks, the erythema and blistering diminish and a thick verrucous scaling appears instead, particularly over flexural regions. The blisters mainly cause problems during the first couple of years, but even in adulthood blisters can cause problems, especially in connection with skin trauma, heat or bacterial infections. Some of the patients also have an associated palmoplantar keratoderma.

No curative treatment is available, but some patients benefit from retinoid therapy. More knowledge is needed about the genotype/phenotype correlation in EHK and the mechanism of action of retinoids, including regulation of the keratin expression.

Fifteen patients were identified in Sweden and Norway, 13 with a generalised disease and 2 with localised lesions indicating genetic mosaicism. In eight of these patients novel mutations in K1 or K10 protein were identified. The discovered mutations were of many different types, such as point mutations, splice site mutations, deletion, and deletion-insertion mutations. An association was found between mutations in K1 and the appearance of palmoplantar keratoderma.

Topical and/or systemic treatment with retinoids was given for 4 weeks in five patients with K1 mutation and in seven patients with K10 mutation. Only patients with K10 mutation seemed to benefit from the treatment, while other patients even got worse on retinoids, with increased irritation and blistering. In four patients from each group, the mRNA expression level for epidermal keratins K1, K2e, K4 and K10 were examined by quantitative PCR, before and after retinoid treatment. Retinoids caused a pronounced down-regulation of K2e and up-regulation of K4 but no change in the expression of mutated keratins K1 and K10.

The effect of retinoids on keratin expression was further investigated in normal healthy skin and in keratinocytes grown in a reconstructed skin model. After 2 days application of 0.025% retinoic acid to normal skin under occlusion, the induction of K4 mRNA expression reached a maximum of 10,000 times above baseline. CRABP II (cellular retinoic acid binding protein II), used as a marker of bioacting retinoids, was only up-regu-

lated 15-fold. Conversely, the mRNA expression of K2e was down-regulated more than 1000-fold.

By using keratinocytes grown in a reconstructed skin model, the retinoid regulation of K2e and K4 expression was further investigated. Retinoids with various affinities for the nuclear receptors RAR and RXR were added to the culture and the keratin mRNA expression was monitored for several days. The most potent retinoids were found to be RARα agonists, the effects of which could be inhibited by addition of a panRAR antagonist.

In conclusion, several novel keratin mutations have been shown to cause epidermolytic hyperkeratosis, and a few examples of a pertinent genotype/phenotype correlation have been found. Treatment with retinoids seems more useful in patients carrying a K10 mutation than in those carrying a K1 mutation, possibly because the former are less vulnerable to the pronounced down-regulation of K2e also seen in normal skin. Keratin 4 is a sensitive marker for retinoid activity in the skin, both on the mRNA and protein level, compared to CRABP II. This up-regulation of K4 and the down-regulation of K2e seems to be mediated through RARa, a nuclear receptor expressed in the keratinocytes. This opens up the possibility of designing new drugs which will hopefully be more effective in treating, for example, bullous ichthyosis due to K2e mutations (Siemens type).

List of original publications

The thesis is based upon the following papers:

I. Virtanen M, Kaye Smith S, Gedde-Dahl Jr T, Vahlquist A, and Bowden PE. Novel spontaneous keratin mutations (KRT1 and KRT10) causing epidermolytic hyperkeratosis in Scandinavia. Manuscript.

II. Virtanen M, Gedde-Dahl Jr T, Mörk N-J, Leigh I, Bowden PE, Vahlquist A. Phenotypic/genotypic correlations in patients with epidermolytic hyperkeratosis and the effects of retinoid therapy. Acta Derm Venereol 2001; 81: 163–170

III. Virtanen M, Törmä H, Vahlquist A. Keratin 4 up-regulation by retinoic acid *in vivo*: A sensitive marker for retinoid bioactivity in human epidermis. J Invest Dermatol 2000; 114: 487–493.

IV. Virtanen M, Törmä, H, Rollman O, Sirsjö A, Vahlquist A. Keratins 2e and 4 in reconstituted human skin are reciprocally regulated by retinoids, acting via the nuclear receptor RARa. Manuscript

Adolescent Sexuality and Sexual Abuse – A Swedish Perspective

Karin Edgardh
Department of Venhälsan, Södersjukhuset, Karolinska Institute, Stockholm
karin.edgardh@ulleval.no

Dr Edgardh is specialized in dermatovenereology and gynaecology. At present, Dr Edgardh holds a position at the Department of Venereology, Olafia Clinic, Ullevål University Hospital, Oslo.

In the late 1980s, teenage abortions and genital chlamydial infections were increasing adolescent health problems in Sweden, indicating unsafe sex practices among young people. The emergence of HIV highlighted the need for research on adolescent sexual health issues. The national cross-sectional questionnaire-based survey, SAM 73–90, was conducted in 1990 among 1,943 high school students and 210 school drop-outs born in 1973. The response rate was high, 92% and 44%, respectively. Consensual sexual experience was varied. Coital experience was reported by 54% of the boys and 64% the girls. Factors associated with coital experience were early puberty, not living with both parents, vocational study program or school non-attendance, and risk-taking behaviour with regard to smoking, alcohol and drugs. Non-coital sexual experience included cunnilingus and fellatio. Early starters, with the first heterosexual intercourse before age 15, reported risky sexual behaviour with multiple partners, casual sex and varied sexual practices as part of a generalized adolescent risk-taking behaviour. Consequently, early starters were, compared to later starters, at increased risk for unwanted pregnancy and sexually transmitted infections. School drop-outs constituted a group at risk.

Child sexual abuse was reported by 11.2% of female and 3.1% of male students, and by 28% of female and 4% of male non-schoolers. Alcohol and drug abuse, along with suicidal ideation, was reported significantly more often by abused youths of both gen-