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Increased elafin expression in cystic, dysplastic and neoplastic oral tissues

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Robinson PA, Markham AF, Schalkwijk J, High AS: Increased elafin expression in cystic, dysplastic and neoplastic oral tissues. J Oral Pathol Med 1996; 25: 135-9. © Munksgaard, 1996.

Expression of human leukocyte elastase inhibitor, elafin, otherwise known as skin-derived antileukoproteinase inhibitor (SKALP), was investigated in normal and abnormal oral tissues using a specific anti-SKALP rabbit antiserum. Weak staining was observed in keratinizing epithelia of normal oral mucosa but not in non-keratinizing mucosa. Increased expression was also observed in the suprabasal layers of dysplastic oral epithelia and in well-differentiated squamous cell carcinoma, but not in basal cell carcinoma. A uniform strong expression was observed in all supra-basal layers of odontogenic keratocyst epithelia, except in regions where inflammatory infiltrate was adjacent to keratocyst epithelia. In contrast, elafin expression in a small number of dentigerous cysts and ameloblastomas was more patchy. The increased levels of elafin in keratocyst epithelia and dysplastic tissue may be a cellular homeostatic response to generate a protective barrier preventing proteolytic degradation of underlying elastic tissue.

Key words: ameloblastoma; basal-cell carcinoma; elafin; keratocyst; squamous-cell carcinoma

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Accepted for publication August 18, 1995.

An understanding of mechanisms controlling levels of proteolytic activity in normal and diseased tissues is being sought by many independent research groups in widely different fields because of their importance in processes such as morphogenesis, angiogenesis, tissue repair and tumour invasion (1, 2). Unbalanced human leukocyte elastase (HLE) proteolytic activity is widely recognised to play an important pathological role in a number of diseases including emphysema, adult respiratory distress syndrome, psoriasis and the bullous dermatoses (3, 4). BRIGGAMAN *et al.* (5) demonstrated that proteases such as HLE, at physiological concentrations, could disrupt the epithelial-connective tissue junction of human skin without having adverse effects on epithelial or connective tissue matrices. HLE function is regulated by endogenous inhibitors such as elafin, β_2 -macroglobulin, antileukoprotease and α_1 -antitrypsin. However, with the exception of elafin, all these protease inhibitors tend to be relatively non-specific in that they are

potent trypsin inhibitors as well as being active against serine elastases.

Elafin, alternatively known as skin-derived antileukoproteinase inhibitor (SKALP), is an epithelial-specific, cationic, elastase inhibitor, of molecular mass 6-kDa in free and 9.9-kDa in immobilized forms (6), that has been identified in cultured keratinocytes (7), the scales of psoriatic lesions and in bronchial secretions (8). It is expressed as preproelafin, which is then processed to the mature elafin molecule found in psoriatic lesions (9). Although an increased level of elafin expression is observed in psoriatic scales compared to normal epidermis, the level of HLE activity was reported to be thirty-one fold greater than that observed in normal skin (3). Hence, increased levels of elafin may be swamped by markedly increased levels of HLE.

The odontogenic keratocyst (10), unlike all other dental cyst types, is peculiar in demonstrating an aggressive, locally destructive growth pattern. Further, during routine histological

processing, separation of epithelium from underlying connective tissue is often observed. The action of proteases, produced from cells located either in epithelia or connective tissue, could underlie this apparent weakness (11). The formation of multiple odontogenic keratocysts in oral tissue is a feature of the naevoid, basal-cell carcinoma syndrome (NBCCS) (12, 13). Normal levels of proteolytic activity (stromelysin-3) have been reported in fibroblasts derived from skin of NBCCS patients (14, 15).

We have previously reported a large increase in the concentration of elafin transcripts in odontogenic keratocytes compared to those observed in normal oral (palatal) mucosa (16). The numbers of samples available for RNA extraction and study was, however, limited. We wished, therefore, to further analyse elafin protein in keratocytes to confirm these earlier findings and to establish whether increased transcriptional levels were matched by increased protein expression. We also wished to determine whether this increase was

limited to keratocysts epithelia or included other abnormal oral and perioral tissues. In this study we describe the levels of expression of elafin in a range of archival biopsies of normal and diseased oral tissues, using anti-SKALP antiserum.

Material and methods

Tissue samples

10% formalin-fixed, paraffin-embedded, normal and diseased oral biopsies were obtained from pathology laboratory files. Sections were cut at 5 µm. Specimens are listed in Table 1.

Immunohistochemistry

The characterisation of the polyclonal anti-SKALP rabbit antiserum has been described previously (17). It was used throughout at a dilution of 1/500 in 50 mM tris/HCl buffer, pH 7.6, containing 0.15 M sodium chloride (TBS).

Sections were hydrated by passage through xylene and graded alcohols to water. Endogenous peroxidase activity was blocked by incubating hydrated sections in 1% hydrogen peroxide in methanol for 20 min at 20°C. Non-specific protein binding sites were then

blocked by incubation of sections in a solution of 20% (v/v) normal swine serum in TBS for 15 min at 20°C. Parallel sections were then overlaid with anti-SKALP-specific rabbit antiserum or pre-immune serum in TBS and incubated at 20°C for 1 h. A 20 min wash with TBS was followed by 1 h incubation at 20°C with swine anti-rabbit peroxidase conjugate (P217, Dako Ltd, Bucks, UK, diluted 1:25 in 5% normal swine serum, in TBS). Sections were then washed as above with TBS and developed with 3 mM 3,3'-diaminobenzidine (D-5637 Sigma, Poole, UK) in TBS, containing 3% (v/v) hydrogen peroxide. They were then given a light Mayer's haematoxylin counterstain, dehydrated and mounted.

Sections of odontogenic keratocyst were used as positive controls. Negative controls included the concurrent use of pre-immune rabbit serum on parallel sections. In addition, sections from which primary or secondary antibodies had been excluded were also processed.

In order to indicate relative staining intensities of sections, the staining of each section was assessed visually and scored on a non-parametric 4-point scale:-

negative	0
consistent but weak staining	1
consistent moderate staining	2
consistent heavy staining	3

The uniformity of staining throughout the suprabasal layers of sections was assessed and expressed as (U) uniform, or (P) patchy. Ratings are given as a combination of both intensity and uniformity.

Mucosa was assessed for atypical epithelial features and judged using histological criteria as described by SMITH & PINDBORG (18).

Results

Elafin expression in normal gingiva

No staining was observed in any normal non-keratinizing gingival epithelium. There was some mild, patchy cytoplasmic staining of upper prickle cell layers in normal keratinizing mucosal epithelia, and in epithelia of cyclosporin-enlarged gingival and fibro-epithelial polyps, that related to surface keratinisation. We have shown previously that normal adult skin does not stain for elafin (17).

Elafin expression in cysts

All examples of uninfamed odontogenic keratocyst epithelia demonstrated strong, uniform cytoplasmic staining throughout all epithelial layers (Fig. 1, Table 1). At sites where chronic inflammatory infiltrates impinged on epithelia, no staining was observed (Fig. 1), whilst adjacent epithelium gave strong positive staining. No differences were noted between keratocysts of NBCCS ($n=10$) or non-syndrome origin ($n=10$). No staining was observed in normal tooth follicles or in the majority of dentigerous cyst epithelia ($n=8/10$). However, in two cases a mild, patchy staining pattern was observed in suprabasal positions (Table 1). No clinical or histological differences were observed in these two cysts to account for this.

Elafin expression in dysplastic and neoplastic lesions

An increased expression of elafin was observed in mild and moderate epithelial dysplastic lesions (Table 1). In severe dysplasia the epithelial staining, although strong, was not uniform (Table 1). Staining was uniform in carcinoma-in-situ (Table 1).

In general, there was increased elafin

Table 1. *Expression of Elafin/SKALP in normal and diseased tissue.* Sections of tissues were screened as described in Material and methods. Results are expressed as the numbers of cases investigated, comparative staining intensities observed and uniformity of expression throughout suprabasal layers, expressed as U – uniform or P – patchy

Tissue	Number	Staining	Uniformity
Normal gingival epithelium ¹	6	0/1	U
Normal tooth follicle	5	0	–
Cysts			
Odontogenic keratocyst (NBCCS)	10	3	U
Keratocyst (non-NBCCS)	10	3	U
Dentigerous cyst ²	10	0/1	P
Residual cyst	10	0	–
Radicular cyst	10	0	–
Dysplasias			
Mild epithelial dysplasia	10	2/3	U
Moderate epithelial dysplasia	10	1/2	U
Severe epithelial dysplasia	10	2/3	P
Neoplasms			
Carcinoma-in-situ	10	2	U
Well-differentiated SCC ³	10	0/1	P
Poorly-differentiated SCC	10	0/1	P
Basal cell carcinoma ⁴	10	0	–
Ameloblastoma ⁵	5	0	–
Other epithelia			
Cyclosporin-enlarged gingiva	5	1	P
Fibro-epithelial polyp	5	0/1	P

Notes.

¹ Grade 1 staining of prickle layer, only in keratinizing epithelia.

² Staining only noted in 2 of 10 cases.

³ SCC – Squamous cell carcinoma; staining was seen centrally in keratinized areas.

⁴ BCCs – Basal cell carcinoma; staining was observed only in the granular layer of the surface epithelium directly over BCCs.

⁵ Ameloblastomas were only positive in the centre of acanthomatous (keratinizing) areas.

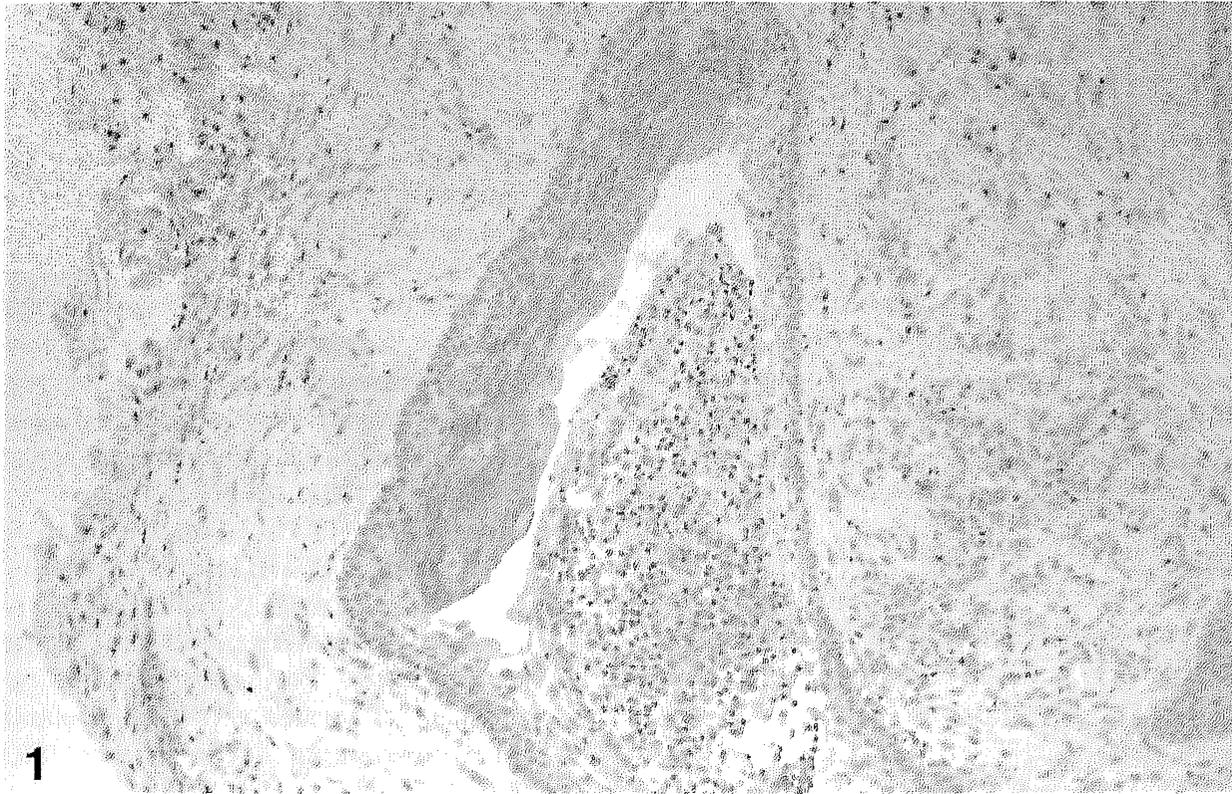


Fig. 1. Odontogenic keratocyst. Uninflamed cyst lining (top left of picture): there is strong, even, perinuclear, cytoplasmic supra-basal epithelial staining with elafin. Centre picture (lower and upper right) shows keratocyst epithelium associated with an inflammatory cell response (midright) and a loss of elafin reactivity, compared to uninflamed epithelium (top left) (peroxidase label $\times 250$ approx).

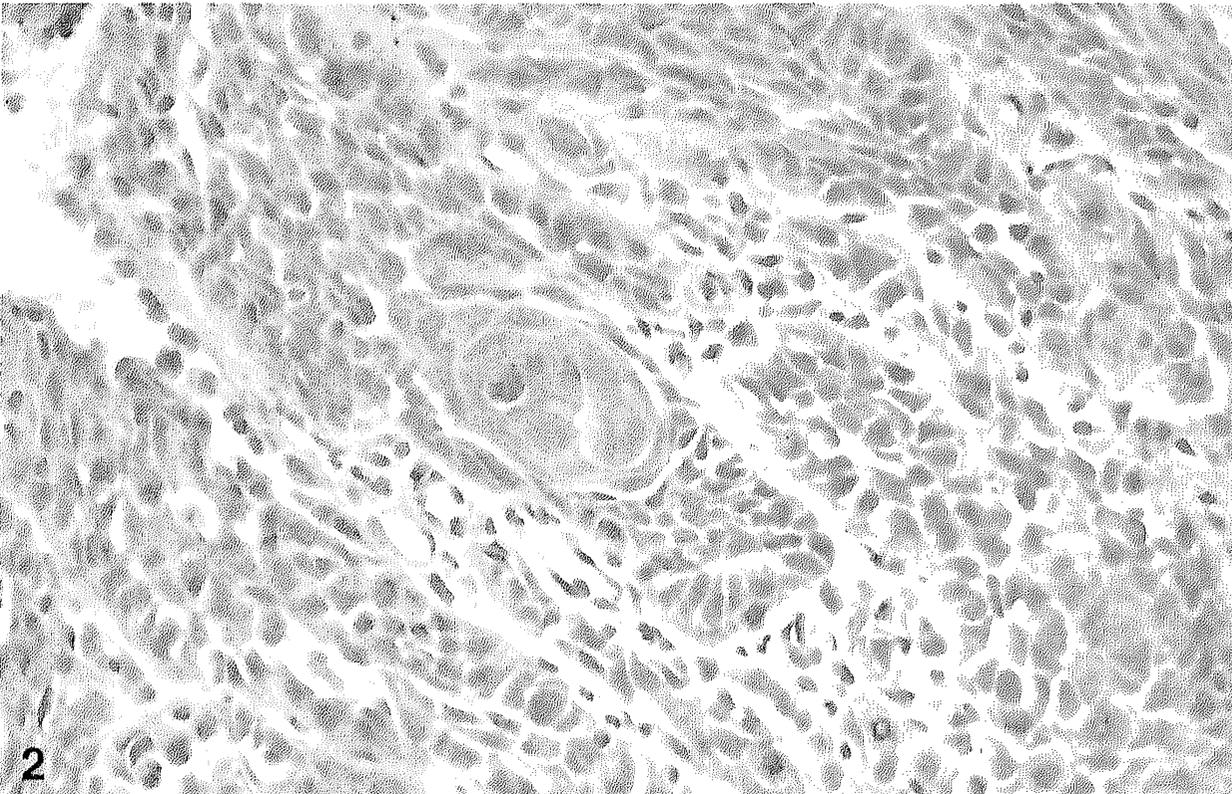


Fig. 2. Squamous cell carcinoma. There is patchy light-to-moderate cytoplasmic staining centrally in the more differentiated islands of carcinoma (top centre) in this overall well-differentiated SCC (peroxidase label $\times 400$ approx).

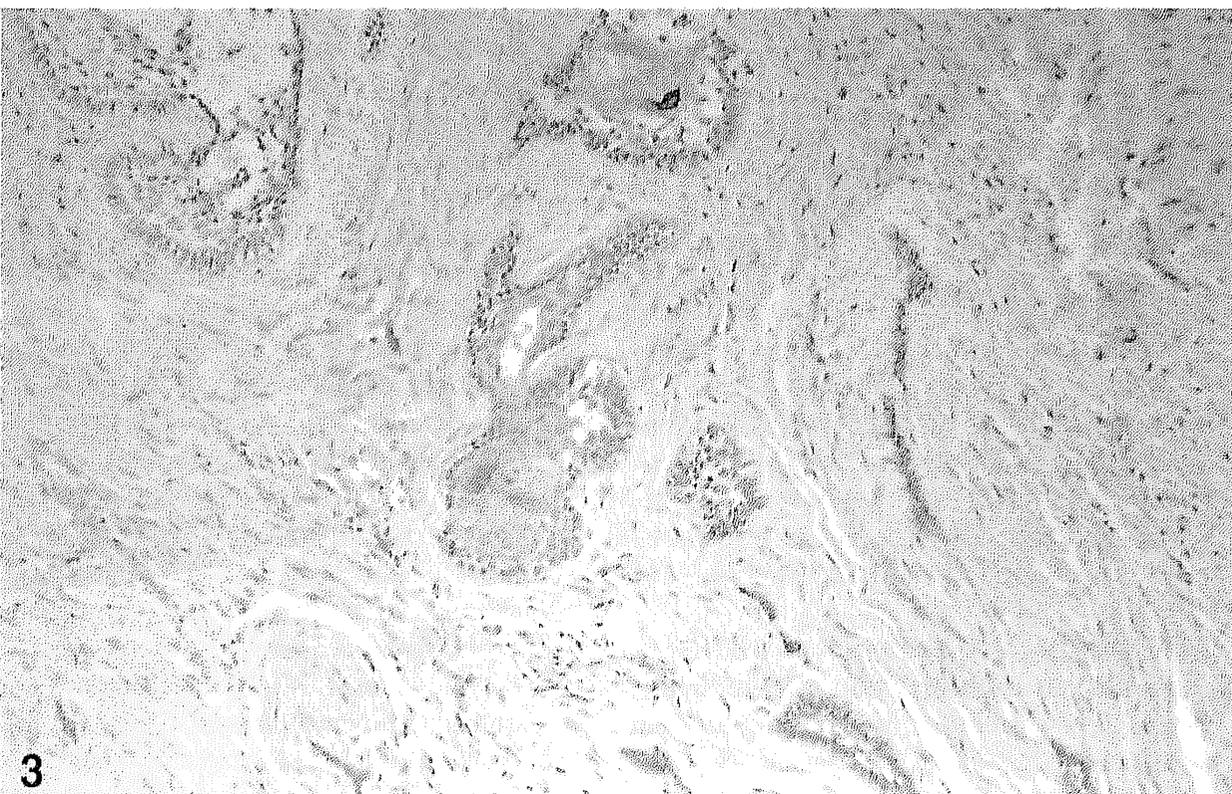


Fig. 3. Ameloblastoma. Patchy dense staining is evident centrally within some acanthomatous (keratinizing) islands of ameloblastoma (lower centre) (peroxidase label $\times 250$ approx).

expression in neoplastic mucosal tissue compared to normal. There was uniform staining in the centre of well-differentiated epithelial islands (Fig. 2), but this became less intense and patchy with increasing loss of differentiation. Positive staining was observed in epithelia overlying BCC islands, whilst the islands of BCC were negative.

No staining was observed in the majority of follicular and plexiform ameloblastomas; however, moderate, uniform, staining was observed centrally in islands of acanthomatous ameloblastomas (Fig. 3).

Discussion

An absence or weak expression of elafin was observed in epithelia of normal oral mucosa using a specific anti-SKALP rabbit antiserum. In contrast, a uniform expression was observed in all layers of keratocyst epithelia. These results indicate that increased levels of transcription of elafin in keratocyst epithelia observed previously are also reflected by increased levels of elafin protein (10). At sites of inflammation beneath keratocyst epithelia, staining was lost. Loss of epitope could result from either an alteration in conformation due to binding or proteolytic degradation of the elafin-protease complex.

Most dentigerous cyst walls and all normal tooth follicles gave a negative staining pattern. However, a patchy expression pattern was observed in some (2/10), despite their similar histological appearance to negatively stained sections. The reasons for the different staining pattern in these sections is not known but may suggest a subtle difference in environmental factors regulating the growth and maintenance of architecture of these cells. Similarly, light, positive staining was observed in the normal skin epithelia overlying BCC islands, although its histological appearance was no different from that of adjacent normal epithelia. This suggests that factors are being secreted from cells of the BCCs that are capable of diffusing to cause molecular changes in overlying epithelial cells, without overt alteration of their morphology. The nature of these factors is not known. *In vitro*, elafin expression was induced after injury in normal epidermis (2). Furthermore, these workers also observed that the transcription of elafin is inducible in epithelial cells *in vitro*. The expression was found to be dependent

on the system used. Keratinocytes cultured on a feeder layer of 3T3 fibroblasts (19) produce elafin. In contrast, those grown in keratinocyte growth medium do not, unless supplemented with 5% fetal calf sera (2). This induction was not due to the increased concentration of calcium in the media. Further evidence from experiments utilising keratinocytes in suspension culture demonstrated that the induction of elafin was not simply due to an induction of terminal differentiation.

Increased levels of elafin expression were also observed in the suprabasal layers of dysplastic oral epithelia. SCC staining appeared to be related to tumour differentiation. It was uniform in well-differentiated tumours but reduced and patchy in poorly-differentiated SCCs. The presence of increased levels of elafin in dysplastic tissue may serve to generate a barrier to protect tissue from proteolytic attack. NARA *et al.* (20) have suggested that the repeating sequence located in the prosegment of elafin, which they named cementoin, could serve as a transglutaminase substrate to anchor it to extracellular matrix proteins, thereby protecting underlying elastic tissues from proteolytic attack.

We have previously reported increased expression of a tumour-associated antigen (gp38) in epithelia of odontogenic keratocysts compared to other cyst types (21) and dysplastic epithelia (22, 23). These expression patterns are similar to those observed with the anti-SKALP antiserum. The use of these antibodies may assist not only for diagnostic purposes to distinguish between cyst types but also be useful in investigations into the development of these lesions.

Acknowledgement – We thank MRS. S. KEAT for her technical assistance.

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