American Journal of Pathology, Vol. 143, No. 6, December 1993

Differential Expression of SKALP/Elafin in Human Epidermal Tumors

Hans A. C. Alkemade,*
Henri O. F. Molhuizen,*
Ivonne M. J. J. van Vlijmen-Willems,*
Urbaan J. G. M. van Haelst,† and
Joost Schalkwijk*

*From the *Department of Dermatology and the †Department of Pathology, Academic Hospital Nijmegen, Nijmegen, The Netherlands*

Recently we described a new epidermal serine proteinase inhibitor, skin-derived antileukoproteinase (SKALP), also known as elafin. SKALP/elafin was found to be absent in normal human epidermis, but can be induced* in vitro *and* in vivo *under hyperproliferative conditions. Here we studied the expression of SKALP/elafin in several types of epidermal tumors (basal cell carcinoma, squamous cell carcinoma, Bowen's disease, actinic keratosis, and keratoacanthoma). Using immunohistochemical staining SKALP/elafin appeared to be differentially expressed in these tumors. Functional measurements of anti-proteinase activity, and Western blotting of tumor extracts confirmed our findings at the histological level. In well differentiated squamous cell carcinoma, SKALP/elafin messenger RNA was demonstrated by non-radioactive* in situ *hybridization. We conclude that SKALP/elafin is a marker for abnormal or disturbed squamous differentiation. A possible role of SKALP/elafin in the control of tumor cell invasion is discussed. (Am J Pathol 1993, 143:1679–1687)

Deregulation of gene expression may cause transcription of oncogenes or loss of action of growth suppressor genes, leading to abnormal growth of tumor cells. However, in addition to disturbed growth control, also invasion and metastasis are features of malignant behavior. Several proteinases such as plasminogen activators, cathepsins, and metalloproteinases, as well as the inhibitors of these enzymes, have been reported to be associated with tumor invasion and metastasis.[1–9] Expression of proteinases is thought to promote the migrational capacity of cells; specific inhibitors of these proteinases counteract this process. Hence, the term metastasis suppressor proteins has been coined for these inhibitors.[10,11]

Recently we described a new serine proteinase inhibitor (skin-derived antileukoproteinase, SKALP), which is expressed in lesional psoriatic epidermis and in epidermis after injury, but is absent in normal epidermis.[12,13] Initially, SKALP was shown to be a low molecular weight, cationic, heat-stable protein that was similar to elafin, an epidermal proteinase inhibitor described by others.[14] We have recently cloned and sequenced the cyclic DNA of SKALP, which proved SKALP and elafin to be identical, and which showed that SKALP/elafin as expressed in cultured epidermal keratinocytes is translated as a 12.3-kd protein.[15] Cleavage of the signal peptide yields a 9.9-kd protein that is the major form found in cultured cells, as was confirmed by purification and N-terminal amino acid sequencing.[15] Elastase-specific inhibitors from bronchial secretions with molecular weights of 10 kd, 5 kd, and 2.5 kd as reported by Hochstrasser, Kramps, and Sallenave, respectively,[16–18] are biological active fragments of the same larger precursor molecule.[19] SKALP/elafin inhibits at least three serine proteinases derived from polymorphonuclear leukocytes (PMN), namely human leukocyte elastase, porcine pancreatic elastase, and human leukocyte proteinase 3.[12,13,20]

Recently we could assign the chromosomal localization of the SKALP gene to chromosome 20, band q12–13.[21] The gene has been given the approved name of Protease Inhibitor, skin derived (SKALP), symbol: PI3, in the Genome Data Base of the HUGO nomenclature committee.

The exact biological function of SKALP/elafin is not known at present, although the substrate specificity for polymorphonuclear cell-derived, elastolytic proteinases suggests that SKALP/elafin is involved in regulation of cutaneous inflammation, or protection

Accepted for publication August 26, 1993.

Address reprint requests to Dr. Joost Schalkwijk, Department of Dermatology, University Hospital Nijmegen, P. O. Box 9101, 6500 HB Nijmegen, The Netherlands.

against polymorphonuclear cell-dependent tissue damage. However, it is very likely that other target enzymes (eg, from the keratinocytes or dermal fibroblasts) exist; hence other functions cannot be excluded.

Because the expression of SKALP/elafin appears not to be associated with the differentiation program of normal epidermis but is found in differentiating cells in the context of the hyperproliferative differentiation program (eg, psoriasis and wound healing), we investigated its expression in a number of epidermal tumors that are characterized by abnormal differentiation. The data clearly show that SKALP/elafin is differentially expressed in these tumors.

Materials and Methods

Chemicals

Methoxysuccinyl-alanyl-alanyl-prolyl-valyl-7-amino-4-methyl coumarin, was obtained from Bachem, Bubendorf, Switzerland. Cetyltrimethylammoniumbromide was obtained from ICI, UK. All reagents for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) including prestained markers were obtained from Bio-Rad Laboratories, Richmond, CA. Polybuffer exchanger 118, a Superdex 75 fast protein liquid chromatography column, a Superdex 75 PC 3.2/30 column, and a SMART chromatography system were obtained from Pharmacia, Uppsala, Sweden. Polyvinylidenedifluoride membrane was from Millipore, Etten-Leur, the Netherlands. Low molecular weight markers used for SDS gels, goat anti-rabbit IgG biotin conjugate, avidin-alkaline phosphatase, 5-bromo-4-chloro-3-indolyl phosphate, and nitroblue tetrazolium were obtained from Sigma Chemicals, St. Louis, MO. Calibration proteins used in chromatography were obtained from Boehringer Mannheim GmbH, Mannheim, Germany. Recombinant elafin was a gift from Dr. Norman Russell, ICI Pharmaceuticals, United Kingdom.

Tumors

Material used for histology was selected from archive biopsy material sent for routine analysis. The specimens had been fixed in buffered 4% formalin for at least 24 hours, and had been embedded in paraffin. Seven basal cell carcinomas, seven squamous cell carcinomas, three Bowen's diseases, seven actinic keratoses, and seven keratoacanthomas were selected. The tissue was cut in 5-μ sections, using a Reichert Jung rotation microtome (Leica, Rijswijk, The Netherlands), and mounted on slides, which were coated with 3-aminopropyltriethoxysilane. Sections were dried 48 hours at 37 C. Some of the sections were used for *in situ* hybridization experiments as well.

A limited amount of fresh tumor material was available for extraction and was used for functional measurements and Western blotting (two basal cell carcinomas, two keratoacanthomas and one squamous cell carcinoma).

Construction of plasmid and synthesis of RNA probes

A 0.42-kb *Pvu*II/*Eco*RI fragment of pGESKA (15), containing almost complete SKALP/elafin complementary DNA was cloned into the plasmid pGEM4 (Promega, Madison, WI). Sense and anti-sense complementary RNA probes were prepared. Briefly, after linearizing the construct with either *Bam*HI or *Kpn*I, the anti-sense and sense cRNAs were transcribed *in vitro* using digoxigenin-labeled UTP, T7, and SP6 RNA polymerase, respectively, according to the manufacturer's instructions (Boehringer). The cRNAs were fragmented with limited alkaline hydrolysis to reduce the size of the synthesized RNAs to approximately 0.2 kb.[22]

In Situ *hybridization*

Nonradioactive *in situ* hybridization was, with some modifications, performed as described by Yokouchi et al.[23] Sections were deparaffinized with xylene, rehydrated using solutions of diminishing ethanol concentrations (99.8 to 50%), and finally rinsed in phosphate-buffered saline (PBS). Sections were then treated with 0.01% Triton X-100 for 1.5 minutes, rinsed with PBS, incubated with pepsin 1 mg/ml in 0.2 N HCl for 15 minutes at 37 C, and again rinsed with PBS. Thereafter postfixation with 4% paraformaldehyde in distilled water was performed. After acetylation with 0.1 M triethylamine, pH 8.0, and 0.25% acetic anhydride for 10 minutes, sections were rinsed with PBS and dehydrated with increasing concentration of ethanol (50 to 99.8%).

The hybridization mixture consisted of 50% formamide, 2X standard sodium citrate (SSC), 10% dextransulfate, 5X Denhard's solution (0.1% bovine serum albumin, 0.1% polyvinylpyrrolidone, 0.1% Ficoll 400), 10 mM dithiothreitol, 1 mg/ml *Escherichia coli* transfer RNA, and 4 μg/ml probe. After overnight hybridization at 37 C, the sections were washed twice for 45 minutes at 37 C in 2X SSC and 1X SSC, respectively, incubated for 20 minutes at

room temperature with 20 μg/ml DNase-free RNase A, (Boehringer) and 50 mM NaCl in Tris EDTA. A second wash step with 1X SSC was carried out for 30 minutes at room temperature.

Hybridization signals were detected by an enzyme-linked immunoassay using a nucleic acid detection kit as suggested by the manufacturer (Boehringer).

Isolation and purification of SKALP/elafin

Skin-derived antileukoproteinase/elafin was isolated both from psoriatic scales and from cultured human keratinocytes as described before.[12,13] In short, psoriatic scales were homogenized in distilled water, yielding a suspension that was boiled and centrifuged, followed by chloroform extraction and centrifugation. After concentration, the preparation was further purified by chromatofocusing (PBE 118 column, triethylamine buffer) and affinity chromatography (porcine pancreatic elastase coupled to cyanogen bromide-activated Sepharose 4B, phosphate-buffered saline (PBS) washing buffer, and 0.1 M acetic acid eluting buffer). Final purification was by gel permeation chromatography on a Superdex-75 fast protein liquid chromatography column. Extracts of cultured keratinocytes were prepared by sonication of the cells in distilled water and subjected to the SMART chromatography system (Superdex 75 PC 3.2/30 column). Anti-elastase activity in obtained fractions was measured; relevant fractions were pooled and vacuum evaporated to dryness, dissolved in nonreducing SDS-sample buffer and used for SDS-PAGE.

Anti-SKALP/elafin serum

Skin-derived antileukoproteinase/elafin purified from psoriatic scales or recombinant elafin was used for immunization procedures as described before.[13] In short, a rabbit was immunized intracutaneously with highly purified SKALP/elafin that was partially cross-linked with glutaraldehyde and emulsified in Freund's complete adjuvant. A booster with the same preparation was given after 2 weeks, and 4 weeks later serum was collected via standard methods. Control (preimmune) serum was drawn before the immunization procedure. The specificity of the antiserum was validated on Western blots and in functional assays which showed 1) that the elastase-inhibiting activity could be absorbed by the antiserum and 2) that the band stained on a Western blot corresponded with a band of anti-elastase activity eluted from the SDS-PAGE gel as shown before.[13] The two antisera yielded identical staining patterns both in immunohistology and Western blotting.

Immunohistology

Before staining, sections were deparaffinized with xylene, rehydrated using solutions of diminishing ethanol concentrations (99.8 to 50%) and finally rinsed in PBS. Thereupon, sections were preincubated with normal swine serum 20% for 15 minutes, and incubated with a polyclonal rabbit antiserum against SKALP/elafin at a dilution of 1/100 in PBS with 1% bovine serum albumin for 60 minutes. After washing in PBS, sections were incubated with peroxidase conjugated swine anti-rabbit immunoglobulin at a dilution of 1/50 in PBS with 1% bovine serum albumin and 5% human AB serum. Subsequently, sections were washed in PBS and Na-acetate buffer and developed with aminoethylcarbazole as the chromogenic substrate (incubation period, 10 minutes). Finally, sections were washed in distilled water, counterstained with hematoxylin and eosin, washed again, dried, and embedded in glycerol-gelatin solution.

Control staining was performed as indicated above, with polyclonal rabbit anti-SKALP/elafin serum substituted by preimmune serum of the same animal.

SDS-PAGE and Western blotting

Three types of tumors were studied (basal cell carcinoma, squamous cell carcinoma, and keratoacanthoma). Tumor tissue (average weight approximately 110 mg) was homogenized in distilled water with the help of a glass-glass grinder, whereafter the homogenate was centrifuged for 10 minutes at 12,000 revolutions per minute. Anti-elastase activity of the supernatant was assayed as described before.[12,24] The clear supernatant was vacuum evaporated to dryness, and subsequently dissolved in 500 μl nonreducing SDS-sample buffer; 5 μl of this preparation was subjected to SDS-PAGE. As a reference, samples of recombinant elafin, psoriatic scale extract, and partially purified SKALP/elafin of cultured keratinocytes were used in the same run of electrophoresis. Proteins were separated on a 16% polyacrylamide gel, using tricine as a trailing ion instead of tris(hydroxymethyl)aminomethane-glycine.[25] Gels were blotted on polyvinylidenedifluoride membranes, and immunological detection of

proteins was performed using biotinylated goat anti-rabbit immunoglobulin G, avidin-conjugated alkaline phosphatase, 5-bromo-4-chloro-3-indolyl phosphate, and nitroblue tetrazolium. Both a polyclonal rabbit antiserum raised against highly purified SKALP/elafin from psoriatic scales and a rabbit antiserum against recombinant elafin were used.

Microphotography

Microphotographs were taken with a Leitz photomicroscope and a Zeiss Axiophot, Wetziar and Oberkochen/Württemberg, Germany, respectively, using black-and-white negative film Agfapan APX 25 from Agfa-Gevaert AG, Leverkusen, Germany.

Results

Immunohistology

In basal cell carcinoma, all tumor cells with a typical basaloid phenotype were negative with respect to SKALP/elafin expression. Cells of the granular layer in epidermis overlying the tumor were positive in some cases. The epidermis of distant normal skin did not show any SKALP/elafin expression at all (not shown).

In squamous cell carcinoma, SKALP/elafin expression varied both between tumors and within the individual tumors. However, most tumors and tumor cell nests that had a clear squamous phenotype were strongly positive as shown in Figure 1. In all specimens basal cells were negative. SKALP/elafin expression was seen from the suprabasal cells upward, with the strongest staining in the cells just underneath the layer of the cornified envelopes, namely, in the stratum granulosum and the most superficial layers of the stratum spinosum.

In Bowen's disease we found that the tumor cells showed a weak cytoplasmic staining, which was less pronounced than in the well differentiated squamous cell carcinomas (not shown).

All biopsies from actinic keratoses were positive, with a variation both in intensity of SKALP/elafin expression and in the number of cell layers that were stained (two to eight layers, basal cells were negative; an example of an actinic keratosis with only one to two positive layers of keratinocytes is shown in Figure 2.

Keratoacanthomas were strongly positive for SKALP/elafin as shown in Figure 3. The staining pattern was comparable with that of the other tumors, ie, the suprabasal layers were stained, and staining was most pronounced in the cell layer just below cornified envelope formation.

Staining of the stratum corneum in all positive tumors was inconsistent, varying both in intensity and distribution pattern from strong to nearly absent, and from continuous to patchy.

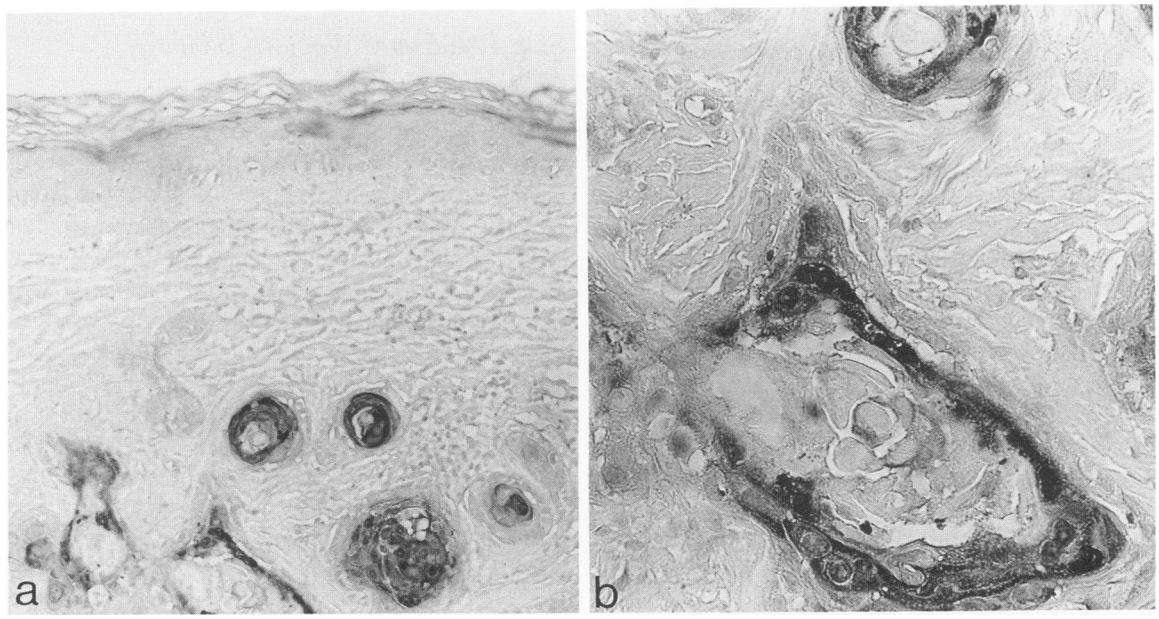

Figure 1. *Immunohistology of squamous cell carcinoma, staining was performed with anti-SKALP/elafin antibody.* (a) *Cell nests with a clear squamous phenotype are strongly positive. Overlying epidermis is negative. Magnification: ×125.* (b) *Detail. SKALP/elafin staining is most distinct in cells with a granular cell morphology. Magnification: ×313.*

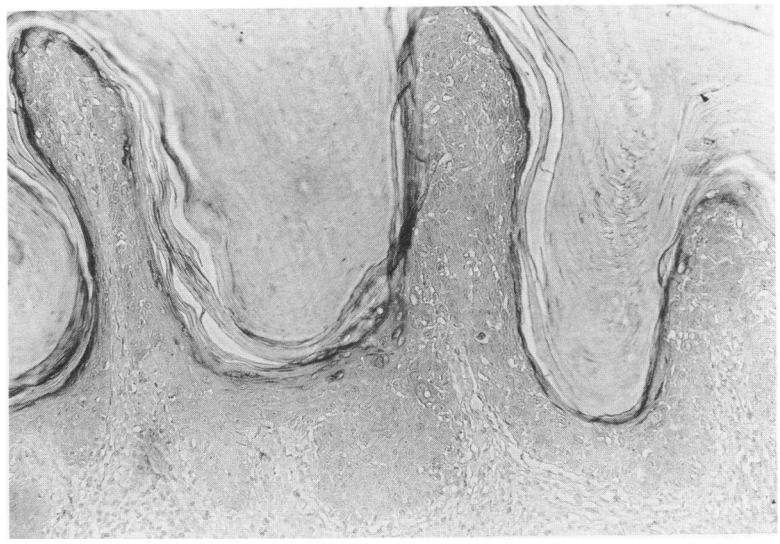

Figure 2. *Immunohistology of actinic keratosis. Variation in intensity of SKALP/elafin staining and in the number of stained cell layers as well, was observed. Here SKALP/elafin staining is limited to one or two layers of granular cells, just beneath ridges of thickened stratum corneum. Magnification: ×125.*

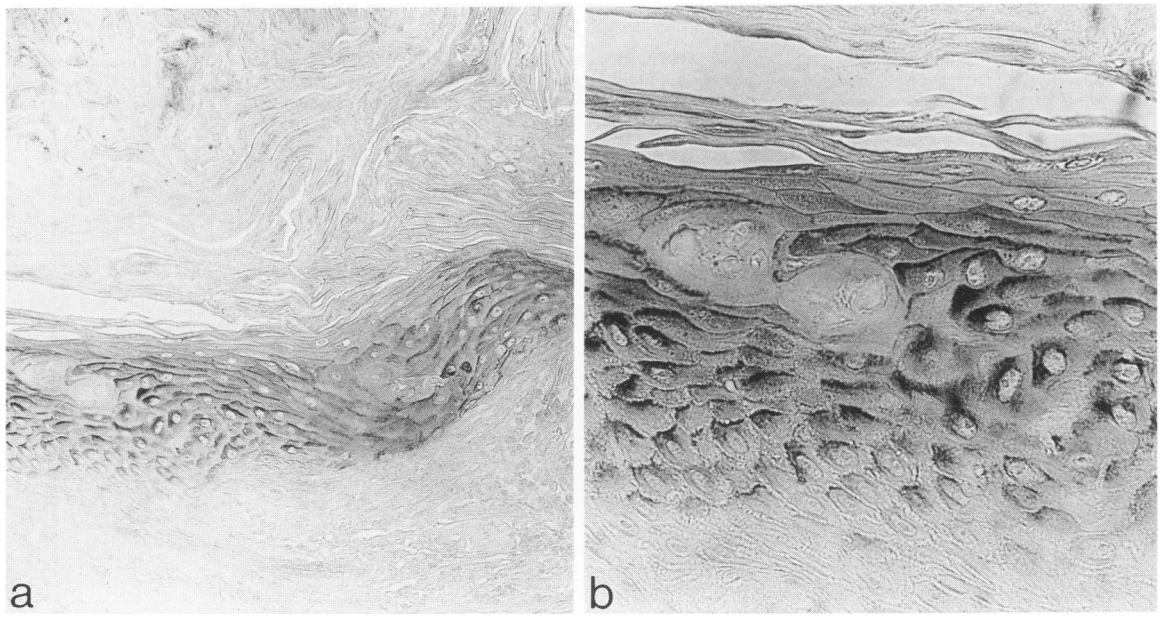

Figure 3. *Immunohistology of keratoacanthoma.* (a) *Strong SKALP/elafin staining is seen in multiple layers of well differentiated suprabasal cells. Magnification: ×125.* (b) *Detail. Note the polarized distribution of SKALP/elafin-staining pattern close to the upper cell membrane, in the direction of increasing differentiation, possibly because of cross-linking to the cornified envelope by transglutaminase. Magnification: ×313.*

In situ *hybridization*

Using cRNA probes, sections of a well differentiated squamous cell carcinoma, which showed high expression of SKALP/elafin using immunohistochemistry, were studied for the presence of SKALP/elafin messenger RNA. In general, mRNA expression colocalized with expression of SKALP/elafin at the protein level as shown on serial sections (Figure 4). Sense cRNA probes, used as controls, were completely negative.

Functional measurements and Western blotting

Using a sensitive microassay (12,24), both squamous cell carcinoma and keratoacanthoma showed inhibitory activity against human leukocyte elastase, whereas basal cell carcinoma was negative. Squamous cell carcinoma contained an anti-elastase activity of 2.3 U/mg tissue, anti-elastase activity of keratoacanthoma was 167 U/mg tissue, and in

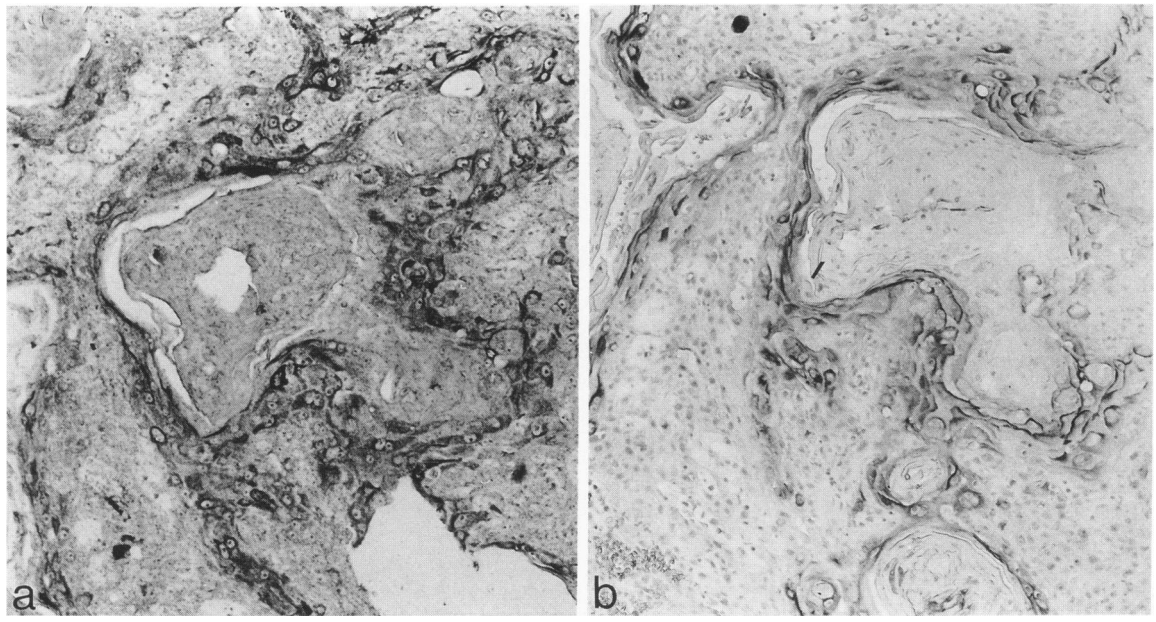

Figure 4. In situ *hybridization and immunohistology on serial sections of a well differentiated squamous cell carcinoma. Using antisense cRNA probes, SKALP/elafin messenger RNA is shown* (a), *and immunostaining demonstrates presence of SKALP/elafin protein* (b). *Note that the expression at the mRNA level roughly coincides with the expression at the protein level; eg, see the region indicated by the arrows. Magnification:* ×100.

basal cell carcinoma no inhibitory activity was measured. Western blotting showed SKALP/elafin expression both in squamous cell carcinoma and in keratoacanthoma, but not in basal cell carcinoma (Figure 5). Interestingly, there were clear differences in staining patterns between the tumors. Keratoacanthoma showed one clear band with an apparent molecular weight of 13.6 kd. In contrast, squamous cell carcinoma showed four different bands with apparent molecular weights of 15.7 kd, 15.0 kd, 13.8 kd, and 12.4 kd. Basal carcinoma was negative. SKALP/elafin from cultured keratinocytes (apparent molecular weight about 16 kd), recombinant elafin (apparent approximate molecular weight 11 kd), and psoriatic scale extract were used as positive controls and as a reference range for the various forms of SKALP/elafin that are known to exist.[12,13]

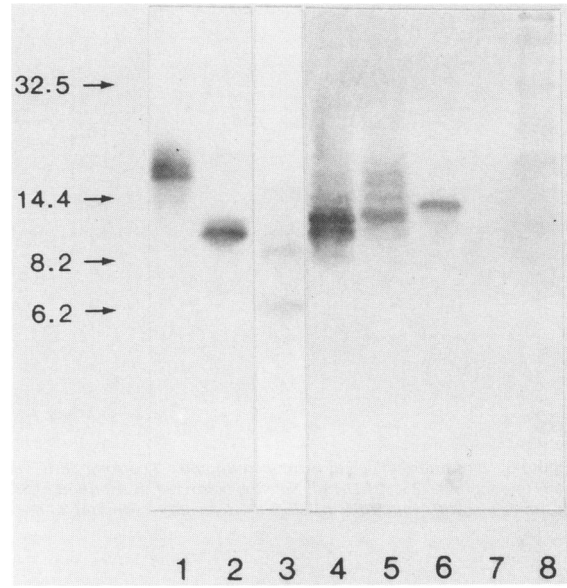

Figure 5. *Western blot of human skin tumor extracts stained with a polyclonal anti-SKALP/elafin serum. In lane five distinct bands were stained in a squamous cell carcinoma extract. In* lane 6, *only a single band is seen in an extract from keratoacanthoma.* Lane 7 *shows the absence of SKALP/elafin staining in a basal cell carcinoma extract. Partially purified SKALP/elafin of cultured keratinocytes, recombinant elafin, and psoriatic scale extract have been used as reference samples, and staining is shown in* lanes 1, 2, *and* 4, *respectively. Molecular weight markers (kilodaltons) are shown in* lanes 3 *and* 8.

Discussion

In this study we have demonstrated that the recently discovered serine proteinase inhibitor SKALP/elafin is differentially expressed in human epidermal tumors. On immunohistology, the cytoplasmatic staining was mostly limited to the upper differentiating layers of the tumors. Basal layers were completely negative, which is in accordance with previous findings in psoriatic epidermis and in a model for wound healing.[26,27] Basal cell carcinomas were negative as well. *In situ* hybridization of well differentiated squamous cell carcinoma showed presence of messenger RNA in the same regions where SKALP/elafin protein is demonstrated by immunostaining.

Previous studies showed that SKALP/elafin exists in multiple forms.[12–14] In material from psoriatic patients a consistent pattern of two major bands emerges. As shown in Figure 5, in squamous cell carcinoma and keratoacanthoma different forms of SKALP/elafin are found. Because only a limited number of fresh tumors was available to us, it is not clear whether these patterns are specific for a type of tumor. The antisera used in this study were polyclonal rabbit antisera against recombinant elafin or SKALP/elafin purified from psoriatic scales. These sera yielded identical staining patterns on Western blots and in immunohistology. Both sera were raised against SKALP/elafin fragments that contained the anti-proteinase activity, which is located in the C-terminal half of the mature SKALP/elafin molecule. Because the different forms of SKALP/elafin are generated by various N-terminal deletions, all these cleavage products are recognized by these antisera. As can be seen in Figure 5, the apparent molecular weights of SKALP/elafin obtained on SDS-PAGE significantly deviate from the calculated molecular weights (as mentioned above). This is probably caused by the strong cationic nature of the molecule (isoelectric point = 9.6) which causes a slower migration in this electrophoresis system.

At present there are no conclusive data on the biological significance of SKALP/elafin in human skin. However, previous studies suggest that the expression of SKALP/elafin *in vivo* is linked to inflammatory processes. We have shown that SKALP/elafin-activity is found in epidermis from inflammatory skin diseases and that it is absent in normal skin and in non-inflammatory skin diseases.[28] Recently we have found that SKALP/elafin is secreted in the urine of psoriatic patients and that the levels found roughly correlate with the severity of the disease.[29] In addition we have found that SKALP/elafin can be induced in normal human skin as a result of standardized injury by tape stripping.[12,27] These findings show that SKALP/elafin is induced in human skin under inflammatory conditions, which suggests (but not proves) that SKALP/elafin could act as a negative feedback on the inflammatory response. Whether this would be at the level of interference with migration of polymorphonuclear cells/monocytes or as a protective mechanism against tissue damage remains to be investigated.

Apart from this teleological interpretation, SKALP/elafin expression can also be regarded as part of the regenerative/hyperproliferative differentiation program of human epidermis as seen in psoriasis and wound healing. The normal differentiation program includes the expression of keratin 1 and 10, the absence of keratin 6 and 16 in the suprabasal layers, and expression of differentiation-related proteins (eg, filaggrin, involucrin, transglutaminase) exclusively in the stratum granulosum.[30] The differentiation program of the regenerative/hyperproliferative type is characterized by the induction of keratin 6 and 16, down-regulation of keratin 10, premature expression of differentiation-related proteins and, as we have shown, the induction of SKALP/elafin. Within the context of this differentiation program, SKALP/elafin could function as an inhibitor of proteinases from inflammatory cells or could, speculatively, be directed to keratinocyte-derived proteinases to control other, hitherto unknown processes. Evidence that SKALP/elafin expression is coupled to the regenerative/hyperproliferative phenotype is further supported by the notion that cultured keratinocytes, which are also negative for keratins 1/10 and positive for keratins 6/16, produce large amounts of SKALP/elafin.[12,15]

In this study we demonstrate that SKALP/elafin expression is high in tumors with a clear squamous phenotype, and is absent in poorly differentiated squamous cell carcinomas and in basal cell carcinomas. Tumor cells display disturbed differentiation programs that are distinct from the differentiation programs mentioned above. Speculatively, loss of expression of anti-proteinase activity could promote tumor cell migration, invasive growth, or induce detachment of tumor cells. This could be caused either by proteinase activity from the tumor cells or by proteinases derived from neighboring cells (stroma, inflammatory cells). The finding in the present study that SKALP/elafin expression is low or absent in the tumors that are able to invade the dermal connective tissue is in line with this hypothesis.

In general, little is known with respect to the role of other proteinases and proteinase inhibitors in normal human skin. It has been suggested that cathepsin D is involved in transglutaminase processing.[31] Both urokinase-type plasminogen activator and its inhibitor, plasminogen activator inhibitor were demonstrated in human epidermis,[32–34] and are supposed to be involved in keratinocyte migration.[35,36] Expression levels of proteinases and proteinase inhibitors have been reported to correlate with tumor proliferation, tissue invasion, and tissue destruction. The resulting tissue destruction would be caused by a local imbalance between proteinases and their inhibitors.[7,37–42] Recently the presence of urokinase-type plasminogen activator in squamous cell carcinoma and the absence in basal cell carci-

noma was described, with the interesting finding that those squamous cell carcinomas that were lacking urokinase-type plasminogen activator were histologically well differentiated tumors.[34,43]

Because not a single factor but cascades and combinations of different events are important in carcinogenesis, it is interesting to speculate on a possible role of SKALP/elafin in this process. SKALP/elafin may interfere with elastolytic activity that tumor cells need to penetrate the dermal tissue either directly by inhibiting elastase[44,45] or indirectly through interference with an elastase-dependent pathway of plasminogen activation.[46] In addition, SKALP/elafin might inhibit unknown proteinases and interfere with proteolysis in an elastase-independent way. Therefore, within the spectrum of keratoacanthoma, actinic keratoses, and squamous cell carcinomas with varying degrees of differentiation, a progressive loss of SKALP/elafin expression could facilitate invasive growth. To date, a correlation between tumor aggressiveness and the expression of lytic enzymes such as heparanases, plasminogen activators, cathepsins, and metalloproteinases has been described (for review see references 7 and 47). Furthermore, it has been shown that inhibitors of metalloproteinases or inhibitors of serineproteinases can block tumor cell invasion.[1,48,49] Most likely a panel of different enzymes and inhibitors will determine the migrational behavior of tumor cells, and SKALP/elafin might be part of this panel.

Acknowledgments

We thank C. Diepenbroek for technical assistance.

References

1. Reich R, Thompson E, Iwamoto Y, Martin GR, Deason JR, Fuller GC, Miskin R: Effects of inhibitors of plasminogen activator, serine proteinases and collagenase IV on the invasion of basement membranes by metastatic cells. Cancer Res 1988, 48:3307–3312
2. Ossowski L, Reich E: Antibodies to plasminogen activator inhibit human tumor metastasis. Cell 1983, 35: 611–619
3. Liotta LA, Tryggvason K, Garbisa S, Hart I, Foltz CM: Metastatic potential correlates with enzymatic degradation of basement membrane collagen. Nature 1980, 284:67–68
4. Basset P, Bellocq JP, Wolf C, Stoll I, Hutin P, Linacker JM, Podhajcer OL, Chenard MP, Rio MC, Chambon P: A novel metalloproteinase gene specifically expressed in stromal cells of breast carcinomas. Nature 1990, 348:699–704
5. Alidina R, Kikuchi M, Kashima M, Epstein JH, Fukuyama K: Cysteine protease and its inhibitor in experimentally produced squamous cell carcinomas in hairless mouse skin. Exp Mol Pathol 1988, 49:118–127
6. Bauer EA, Gordon JM, Reddick ME, Eisen AZ: Quantitation and immunocytochemical localization of human skin collagenase in basal cell carcinoma. J Invest Dermatol 1977, 69:363–367
7. Liotta LA, Steeg PS, Stetler-Stevenson W: Cancer metastasis and angiogenesis: an imbalance of positive and negative regulation. Cell 1991, 64:327–336
8. Tsuboi R, Yamaguchi T, Kurita Y, Nakao H, Ogawa H, Ishihara K: Comparison of proteinase activities in squamous cell carcinoma basal cell epithelioma and seborrheic keratosis. J Invest Dermatol 1988, 90:869–872
9. Tsushima H, Hopsu Havu VK: Cysteine proteinase inhibitors in human squamous cell carcinoma. Acta Histochem 1989, 85:23–28
10. Liotta LA: Introductionary overview. Cancer Metastasis Rev 1990, 9:285–287
11. Liotta LA, Stetler-Stevenson W, Steeg PS: Metastasis suppressor genes. Important Adv Oncol 1991, 85–100
12. Schalkwijk J, Chang A, Janssen P, de Jongh GJ, Mier PD: Skin-derived antileucoproteinases (SKALPs): characterization of two new elastase inhibitors from psoriatic epidermis. Br J Dermatol 1990, 122:631–641
13. Schalkwijk J, de Roo C, de Jongh GJ: Skin-derived antileukoproteinase (SKALP) an elastase inhibitor from human keratinocytes. Purification and biochemical properties. Biochim Biophys Acta 1991, 1096:148–154
14. Wiedow O, Schröder J, Gregory H, Young JA, Christophers E: Elafin: an elastase specific inhibitor of human skin. Purification characterization and complete amino acid sequence. J Biol Chem 1990, 265:14791–14795
15. Molhuizen HOF, Alkemade HAC, Zeeuwen PLJM, de Jongh GJ, Wieringa B, Schalkwijk J: SKALP/Elafin: an elastase inhibitor from cultured human keratinocytes. Purification, cDNA sequence and evidence for transglutaminase crosslinking. J Biol Chem 1993, 268: 12028–12032
16. Hochstrasser K, Albrecht GJ, Schönberger OL, Rasche B, Lempart K: An elastase-specific inhibitor from human bronchial mucus. Isolation and characterization. Hoppe-Seyler's Z Physiol Chem 1981, 362:1369–1375
17. Kramps JA, Klasen EC: Characterization of a low molecular weight anti-elastase isolated from human bronchial secretion. Exp Lung Res 1985, 9:151–165
18. Sallenave J-M, Ryle AP: Purification and characterization of elastase-specific inhibitor. Sequence homology with mucus proteinase inhibitor. Biol Chem Hoppe-Seyler 1991, 372:13–21
19. Sallenave J-M, Marsden MD, Ryle AP: Isolation of elafin and elastase-specific inhibitor (ESI) from bronchial secretions. Evidence of sequence homology and

immunological cross-reactivity. Biol Chem Hoppe-Seyler 1992, 373:27–33
20. Wiedow O, Lundemann J, Utecht B: Elafin is a potent inhibitor of proteinase 3. Biochem Biophys Res Commun 1991, 174:6–10
21. Molhuizen HOF, Zeeuwen PLJM, Olde Weghuis D, Geurts van Kessel A, Schalkwijk J: Assignment of the human gene encoding the epidermal serine proteinase inhibitor SKALP (PI3) to chromosome 20, region q12-q13. Cytogenet Cell Genet 1993, in press
22. Cox KH, DeLeon DV, Angerer LM, Angerer RC: Detection of mRNAs in sea urchin embryos by in situ hybridization using asymmetric RNA probes. Dev Biol 1984, 101:485–502
23. Yokouchi Y, Ohsugi K, Sasaki H, Kuroiwa A: Chicken homeobox gene Msx-1: structure, expression in limb buds and effect of retinoic acid. Development 1991, 113:431–444
24. Lammers AM, van de Kerkhof PCM, Schalkwijk J, Mier PD: Elastase a marker for neutrophils in skin infiltrates. Br J Dermatol 1986, 115:181–186
25. Schägger H, von Jagow G: Tricine-sodium dodecyl sulphate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. Anal Biochem 1987, 166:368–379
26. Schalkwijk J, van Vlijmen-Willems I, Alkemade JAC, de Jongh GJ: Immunohistochemical localization of SKALP/elafin in psoriatic epidermis. J Invest Dermatol 1993, 100:390–393
27. Alkemade H, van Vlijmen-Willems I, de Jongh G, Schalkwijk J: Skin-derived antileukoproteinase: cellular localization in psoriatic and tape-stripped skin. J Invest Dermatol 1992, 98:532–532 (abstr)
28. Chang A, Schalkwijk J, Happle R, van de Kerkhof PCM: Elastase-inhibiting activity in scaling skin disorders. Acta Derm Venereol 1990, 70:147–151
29. Alkemade H, van de Kerkhof P, Schalkwijk J: Demonstration of skin-derived antileukoproteinase (SKALP) in urine of psoriatic patients. J Invest Dermatol 1992, 99: 3–7
30. Watt FM: Terminal differentiation of epidermal keratinocytes. Curr Opin Cell Biol 1989, 1:1107–1115
31. Negi M, Matsui T, Ogawa H: Mechanism of regulation of human epidermal transglutaminase. J Invest Dermatol 1981, 77:389–392
32. Grøndahl J, Ralfkiaer E, Nielsen LS, Kristensen P, Frenz G, Danø K: Immunohistochemical localization of urokinase- and tissue-type plasminogen activators in psoriatic skin. J Invest Dermatol 1987, 88:28–32
33. Jensen PJ, Baird J, Morioka S, Lessin S, Lazarus GS: Epidermal plasminogen activator is abnormal in cutaneous lesions. J Invest Dermatol 1988, 90:777–782
34. Sappino A, Belin D, Huarte J, Hirschel Scholz S, Saurat J, Vassalli JD: Differential protease expression by cutaneous squamous and basal cell carcinomas. J Clin Invest 1991, 88:1073–1079
35. Morioka S, Lazarus GS, Baird J, Jensen P: Migrating keratinocytes express urokinase-type plasminogen activator. J Invest Dermatol 1987, 88:418–423
36. Grønddahl-Hansen J, Lund LR, Ralfkiaer E, Ottevanger V, Danø K: Urokinase- and tissue-type plasminogen activator in keratinocytes during wound reepithelialization in vivo. J Invest Dermatol 1988, 90: 790–795
37. Wegener Childers J, Hernandez AD, Kim JH, Stricklin GP: Immunolocalization of collagenase inhibitor in normal skin and basal cell carcinoma. J Am Acad Dermatol 1987, 17:1025–1032
38. Van Roy F, Mareel M: Tumour invasion: effects of cell adhesion and motility. Trends Cell Biol 1992, 2:163–169
39. Blasi F, Vassalli JD, Danø K: Urokinase-type plasminogen activator: proenzyme receptor and inhibitors. J Cell Biol 1987, 104:801–804
40. Ponton A, Coulombe B, Skup D: Decreased expression of tissue inhibitor of metalloproteinases in metastatic tumor cells leading to increased levels of collagenase activity. Cancer Res 1991, 51:2138–2143
41. Quax PHA, van Leeuwen RTJ, Verspaget HW, Verheijen JH: Protein and mRNA levels of plasminogen activators and inhibitors analyzed in 22 human tumor cell lines. Cancer Res 1990, 50:1488–1494
42. Quax PHA, van Muijen GNP, Weening Verhoeff EJD, Lund LR, Danø K, Ruiter DJ, Verheijen JH: Metastatic behaviour of human melanoma cell lines in nude mice correlates with urokinase-type plasminogen activator its type-1 inhibitor and urokinase mediated matrix degradation. J Cell Biol 1991, 115:191–199
43. Miller SJ, Jensen J, Dzubow LM, Lazarus GS: Urokinase plasminogen activator is immunocytochemically detectable in squamous cell but not in basal cell carcinomas. J Invest Dermatol 1992, 98:351–358
44. Lowe FC, Isaacs JT: Biochemical methods for predicting metastatic ability of prostatic cancer using the Dunning R-3327 rat prostatic adenocarcinoma system as a model. Cancer Res 1984, 44:744–752
45. Zeydel M, Nakagawa S, Biempica L, Takahashi S: Collagenase and elastase production by mouse mammary adenocarcinoma primary cultures and cloned cells. Cancer Res 1986, 46:6438–6445
46. Machovich R, Owen WG: An elastase-dependent pathway of plasminogen activation. Biochemistry 1989, 28:4517–4522
47. Nakajima M, Chop AM: Tumor invasion and extracellular matrix degradative enzymes: regulation of activity by organ factors. Cancer Biol 1991, 2:115–127
48. Mignatti P, Robbins E, Rifkin DB: Tumor invasion through the human amniotic membrane: requirement for a proteinase cascade. Cell 1986, 47:487–498
49. DeClerck YA, Perez N, Shimada H, Boone TC, Langley KE, Taylor SM: Inhibition of invasion and metastasis in cells transfected with an inhibitor of metalloproteinases. Cancer Res 1992, 52:701–708