# Expression of Human Macrophage Metalloelastase (MMP-12) by Tumor Cells in Skin Cancer

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**Matrix metalloproteinases play an essential role in tumor growth and invasion. Different matrix metalloproteinases are often expressed in cancers with distinct patterns. To investigate the role of human macrophage metalloelastase (MMP-12) in epidermal tumors, we studied human macrophage metalloelastase mRNA and protein expression in malignant squamous cell and basal cell carcinomas, and in premalignant Bowen's disease. Human macrophage metalloelastase was detected in 11 of 17 squamous cell carcinomas in epithelial cancer cells, whereas macrophages were positive in 15 of 17 samples. In basal cell carcinomas, human macrophage metalloelastase was more often found in macrophages (seven of 19) than in cancer cells (four of 19). Human macrophage metalloelastase mRNA was also detected in three cell lines derived from squamous cell carcinomas of the head and neck and in transformed HaCaT cells, whereas premalignant tumors and primary keratinocytes were negative for human macrophage metalloelastase mRNA. Western analysis revealed human macrophage metalloelastase protein in squamous cell carcinoma cells. Our results show that human macrophage metalloelastase can be expressed *in vivo* and *in vitro* by transformed epithelial cells and indicate that the level of human macrophage metalloelastase expression correlates with epithelial dedifferentiation and histologic aggressiveness.** ***Key words: carcinogenesis/immunohistochemistry/in situ hybridization. J Invest Dermatol 114:1113–1119, 2000***

Matrix metalloproteinases (MMPs) are a group of proteolytic enzymes that play a crucial role in degradation of the extracellular matrix (Birkedal-Hansen *et al*, 1993; Kähäri and Saarialho-Kere, 1999). Proteolytic activity is required both in normal physiologic processes, such as wound healing and fetal development, as well as in pathologic tissue destruction occurring in chronic wounds, dermal photoageing, and tumor cell invasion and metastasis. MMPs seem to play an important role in all aspects of tumor progression by enhancing tumor-induced angiogenesis, destroying local tissue architecture to allow tumor growth, and breaking down basement membranes in the process of metastatic spread (Chambers and Matrisian, 1997). Different MMPs are often coexpressed in cancers in a cell-type-specific manner, yet in most tumors stromal fibroblasts are the main source of MMPs (see Kähäri and Saarialho-Kere, 1999).

MMPs are divided into five subgroups (collagenases, gelatinases, stromelysins, membrane-type MMPs, and other MMPs) according to their structure and substrate specificity. Human macrophage metalloelastase (HME, MMP-12) is often considered a member of the stromelysin subgroup and it was initially found in alveolar macrophages of cigarette smokers (Shapiro *et al*, 1993). HME is able to degrade elastin and a broad selection of matrix and nonmatrix substrates including type IV collagen, fibronectin, laminin, vitronectin, entactin, heparan, and chondroitin sulfates (Chandler *et al*, 1996; Shipley *et al*, 1996; Gronski *et al*, 1997). The expression of HME *in vivo* has so far been demonstrated only in macrophages, e.g., in intestinal ulcerations (Vaalamo *et al*, 1998), cutaneous granulomas, and macrophage migration (Vaalamo *et al*, 1999). Furthermore, it degrades elastic fibers in atherosclerosis (Halpert *et al*, 1996) and aneurysms (Curci *et al*, 1999), as well as various basement membrane components (Shipley *et al*, 1996; Vaalamo *et al*, 1999). HME has also been found in macrophage-like cells in breast cancer (Heppner *et al*, 1996). Interestingly, granulocyte-macrophage colony stimulating factor (GM-CSF) stimulates tumor infiltrating macrophages to produce metalloelastase, which cleaves plasminogen into angiostatin (Dong *et al*, 1997; Cornelius *et al*, 1998). Thus, HME may prevent tumor growth by inhibiting angiogenesis, whereas other MMPs promote tumor progression.

To investigate the role of HME in epithelial cancers, we studied premalignant and malignant skin tumors using *in situ* hybridization, immunohistochemical, northern blot, and western blot analyses. In this study we show that, in addition to macrophages, malignantly transformed keratinocytes are able to express HME. *In vivo* it is expressed even in basal cell carcinomas (BCCs), slow growing but noninvasive, locally destructing tumors. The greatest number of HME-positive cells were detected in squamous cell carcinomas

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Abbreviations: BCC, basal cell carcinoma; HME, human macrophage metalloelastase; MMP, matrix metalloproteinase.

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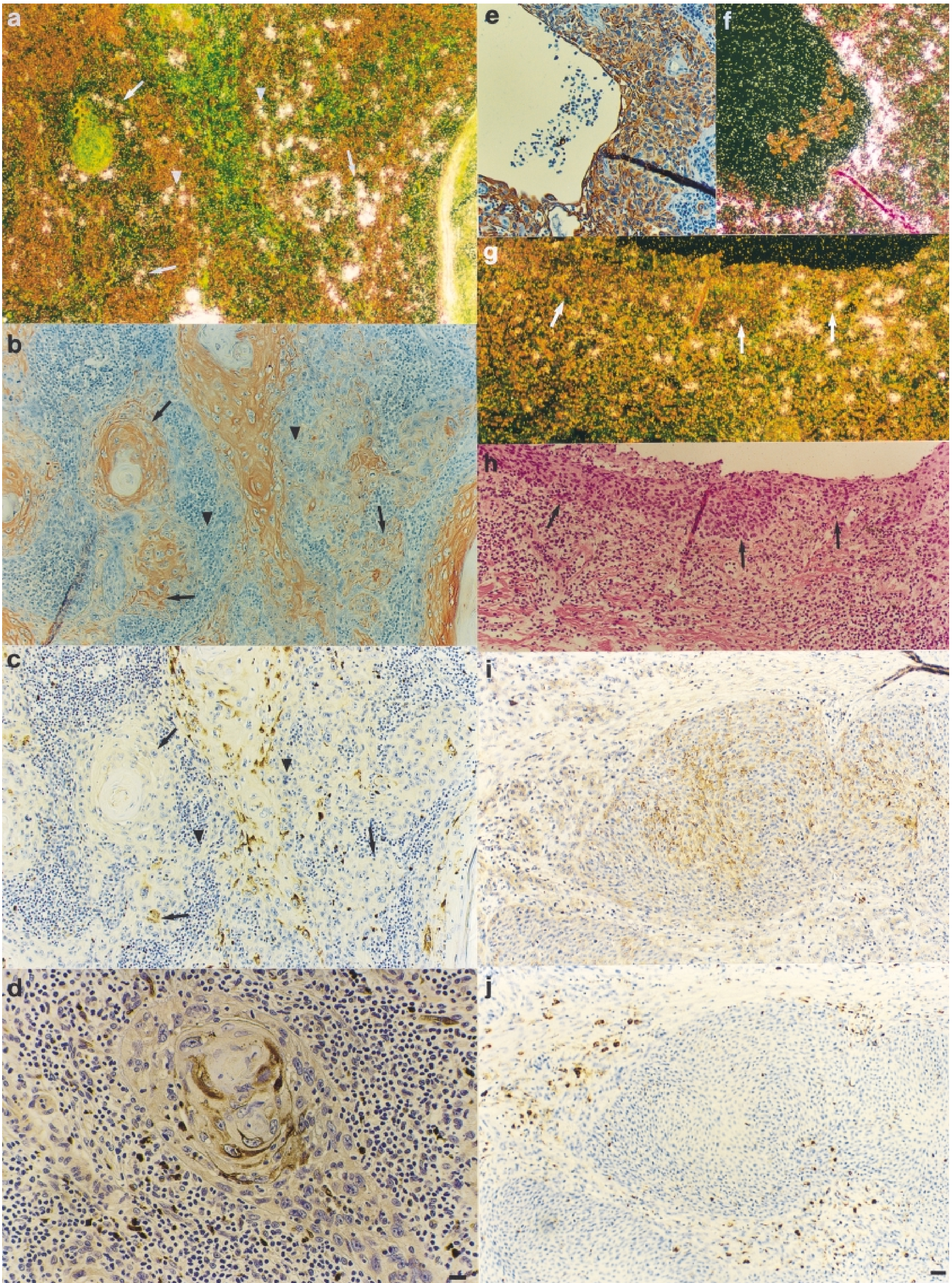
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(SCCs), however, in which HME expression was higher in less differentiated and aggressive tumor subtypes. These results show that HME can be expressed *in vivo* and *in vitro* by transformed epithelial cells and indicate that the level of HME expression correlates with epithelial dedifferentiation and histologic aggressiveness.

## MATERIALS AND METHODS

**Tissue samples** Formalin-fixed, paraffin-embedded specimens were obtained from the Department of Dermatopathology, University of Helsinki. The diagnoses were confirmed by two experienced dermatopathologists. The following histologic specimens were examined: SCCs, grades I (n = 4), II (n = 9), III (n = 5); BCCs (n = 19) [histologic subtypes: sclerosing (n = 11), keratotic (n = 3), adenoid (n = 5)], melanomas (n = 12), and Bowen's disease (n = 5).

***In situ* hybridization** A 650 bp fragment of HME cDNA (corresponding to nucleotides 600–1250) was used to transcribe sense and antisense RNA probes as described previously (Saarialho-Kere *et al*, 1994; Vaalamo *et al*, 1998). All sections were pretreated with 1 μg of proteinase K per ml and washed in 0.1 M triethanolamine containing 0.25% acetic anhydride. Sections were hybridized with $^{35}$S-labeled probes ($4 \times 10^4$ cpm per μl of hybridization buffer) at 50°C for at least 18 h in a humidified chamber. Slides were then washed under stringent conditions, including treatment with RNAse A to remove unhybridized probe (Prosser *et al*, 1989). After 20–35 d of autoradiography, the photographic emulsion was developed, and the slides were stained with hematoxylin and eosin. Samples previously positive for HME (sarcoidosis) (Vaalamo *et al*, 1999) were used as positive controls. The slides were analyzed independently by two investigators (U.S.-K., L.J.).

**Immunohistochemistry** Immunostaining was performed on sections parallel to those used for *in situ* hybridization by the avidin-biotin-peroxidase complex technique. Diaminobenzidine and aminoethyl-carbazole were used as chromogenic substrates and hematoxylin as counterstain, as described in detail by Saarialho-Kere *et al* (1993). Sections were pretreated with trypsin (10 mg per ml). Antibodies included polyclonal anti-HME (Belaaouaj *et al*, 1995; Curci *et al*, 1999; Vaalamo *et al*, 1999), rabbit antihuman cytokeratin (dilution 1:200, A0575; Dako A/S, Glostrup, Denmark), and CD-68 (KP-1, dilution 1:300, M814; Dako, Carpinteria, CA) for tissue macrophages. Controls were performed with normal mouse immunoglobulin or with rabbit preimmune serum.

**Cell cultures** Keratinocytes were established from adult abdominal or breast skin obtained at laparotomies or mammoplasties for nonmalignant disease, as described previously (Boyce and Ham, 1985). Pieces of the skin were incubated in 0.25% trypsin (solution A, Gibco BRL, Life Technologies, Paisley, Scotland) overnight. Keratinocytes were suspended in keratinocyte growth medium (KGM, Gibco BRL) containing 2% decalcified fetal bovine serum (FBS) and maintained in KGM supplemented with epidermal growth factor (5 ng per ml) and bovine pituitary extract (50 μg per ml) (both from Gibco BRL).

HaCaT cells, transformed human epidermal keratinocytes, were cultivated in Dulbecco's modified Eagle's medium (DMEM, Gibco BRL) containing 10% FBS, 1% penicillin-streptomycin, and 1% sodium puryvate.

Four SCC cell lines examined were established from primary SCCs of tongue (UT-SCC-15) and glottic larynx (UT-SCC-38), as well as from metastases of an SCC of supraglottic larynx (UT-SCC-17) and of a cutaneous SCC (UT-SCC-7) at the time of operation in the Turku University Central Hospital. Cell lines were cultured in DMEM supplemented with 6 mM glutamine, nonessential amino acids, and 10% FBS. The SCC cells were examined in subcultures 5–10 and were homogeneous by visual inspection. For experiments, an equal number of cells was plated on cell culture dishes. The medium was changed the following day, and after an overnight incubation the growth factors and cytokines were added and the incubation was continued for 24 h.

**RNA analysis** Total cellular RNA was isolated from cell cultures using the single-step method (Chomczynski and Sacchi, 1987). Northern blot hybridizations were performed as described previously (Johansson *et al*, 1997a) with cDNAs labeled with [α-$^{32}$P]dCTP using random priming. The 650 bp fragment of HME cDNA utilized for *in situ* hybridizations was also used for northern blot hybridizations. In addition, human MMP-13 cDNA fragments covering the coding region and part of the 3′-untranslated region, altogether 1931 bp (Johansson *et al*, 1997a), a 2.0 kb human cDNA for human MMP-1 (Goldberg *et al*, 1986), and a 1.3 kb rat cDNA for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Fort *et al*, 1985) were used for hybridizations. [$^{32}$P]-cDNA/mRNA hybrids were visualized by autoradiography. Human recombinant tumor necrosis factor α (TNF-α) and transforming growth factor β1 (TGF-β1) were purchased from Sigma (St. Louis, MO). Human recombinant interferon-γ (IFN-γ) was obtained from Promega (Madison, WI).

RNA isolated from HaCaTs and keratinocytes was reverse transcribed to cDNA according to the manufacturers' instructions with Multiscribe Reverse Transcriptase and random hexamers (Perkin Elmer) and used as a template in a polymerase chain reaction (PCR). The PCR consisted of 38 cycles with an initial 10 min denaturing temperature of 95°C followed by 1 min of denaturing, 30 s of annealing and elongation (60°C), and 2 min of final elongation. Primers (Pharmacia Biotech) used for HME amplification were 5′-TGCTGATGACATACGTGGCA-3′ (forward, nucleotides 765–784) and 5′-AGGATTTGGCAAGCGTTGG-3′ (reverse, nucleotides 834–816) to produce a product of 70 bp. To quantitate the starting material, GAPDH primers (TaqMan GAPDH Control Reagents, PE Biosystems, Foster City, CA) were also used in the same reaction. Finally, 15 μl aliquots of the products were run in a 3% low melting point agarose gel in the presence of 5 ng ethidium bromide per ml and visualized under ultraviolet light.

**Assay of HME production** Equal aliquots of the conditioned media of cells were fractionated by 7.5% sodium dodecyl sulfate polyacrylamide gel

**Table I. Results of *in situ* hybridization and immunohistochemistry for HME in SCC and BCC[a]**

| Diagnosis | Number of samples | Signal strength for HME | |
|---|---|---|---|
| BCC sclerosing | 5 | − | |
| | 3 | + | mf |
| | 3 | + | c. |
| BCC keratotic | 3 | + | mf |
| BCC adenoid | 4 | − | |
| | 1 | + | c./mf |
| SCC grade I | 2 | + | mf |
| | 1 | + | c. |
| | 1 | ++ | c./mf |
| SCC grade II | 1 | − | |
| | 3 | + | mf |
| | 2 | + | c./mf |
| | 2 | ++ | c./mf |
| | 1 | +++ | c./mf |
| SCC grade III | 1 | + | c./mf |
| | 3 | +++ | c./mf |

[a]Immuno and *in situ* hybridization signal for HME was evaluated using bright and dark field microscopy. Signal strength was assessed as follows: −, no detectable specific signal; +, specific signal in few cells; ++, specific signal in moderate number of cells; +++, specific signal in high number of cells. Cells expressing HME were identified as cancer cells (c.) or macrophages (mf).

**Figure 1. Expression of HME mRNA and protein in SCCs.** (*A*) *In situ* hybridization dark-field for HME mRNA in a grade II SCC. (*B*) Staining for cytokeratin in a serial section. (*C*) A near-by section stained with polyclonal HME antibodies. *Arrows* depict corresponding spots in cancer, *arrowheads* in the stroma. (*D*) Higher magnification from the same tumor with HME-immunopositive cells in epithelial areas and in the stroma. (*E*) Immunostaining for cytokeratin in a grade III SCC. (*F*) *In situ* hybridization dark-field of the same tumor depicting HME mRNA positive area inside the tumor. (*G*) HME mRNA in stromal cells of a previously ulcerated grade I SCC. (*H*) The corresponding bright-field image. *Arrows* mark corresponding spots. (*I*) Immunostaining for HME in a grade II SCC showing HME in tumor cells. (*J*) Immunostaining for CD-68 in a serial section. Counterstaining was performed with hematoxylin and eosin (*A*, *F-H*) or with hematoxylin in (*B-E*, *I*, *J*). *Scale bars*: (*A-C*, *E-J*) 50 μm; (*D*) 25 μm.

electrophoresis and transferred to Hybond enhanced chemiluminescence (ECL) filter (Amersham); the amount of HME was determined by western blot analysis using monoclonal antibody against human recombinant MMP-12 (R&D Systems, Minneapolis, MN) in a concentration of 1 μg per ml followed by detection of specifically bound primary antibodies with peroxidase-conjugated secondary antibodies visualized by ECL (Amersham).

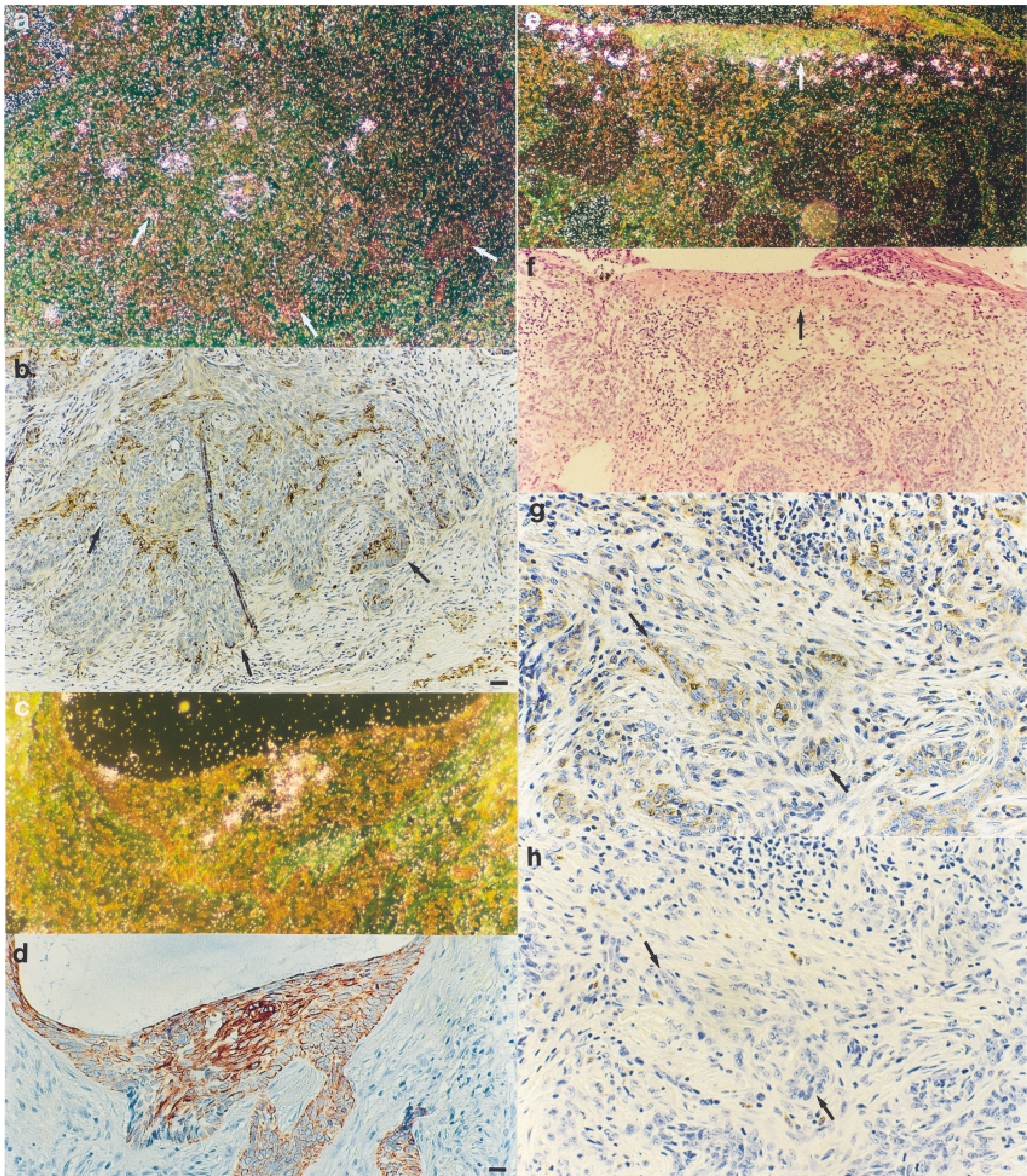


**Figure 2. Expression of HME in BCC.** (*A*) A fibrosing BCC hybridized with the HME antisense probe. (*B*) Staining for HME protein in a serial section. *Arrows* depict corresponding spots. (*C*) A BCC island with HME mRNA positive cells. (*D*) Staining for cytokeratin in a serial section. (*E*) Signal for HME mRNA at the surface of an ulcerated fibrosing BCC. (*F*) Corresponding bright-field. *Arrows* depict corresponding spots. (*G*) Immunostaining for HME in another sample of fibrosing BCC. (*H*) Staining for CD-68 in a serial section. *Arrows* mark corresponding spots. Counterstaining with hematoxylin and eosin (*A*, *C*, *E*, *F*) or with hematoxylin (*B*, *D*, *G*, *H*). *Scale bars*: (*A*, *B*, *E*, *F*) 50 μm, (*C*, *D*, *G*, *H*) 25 μm.

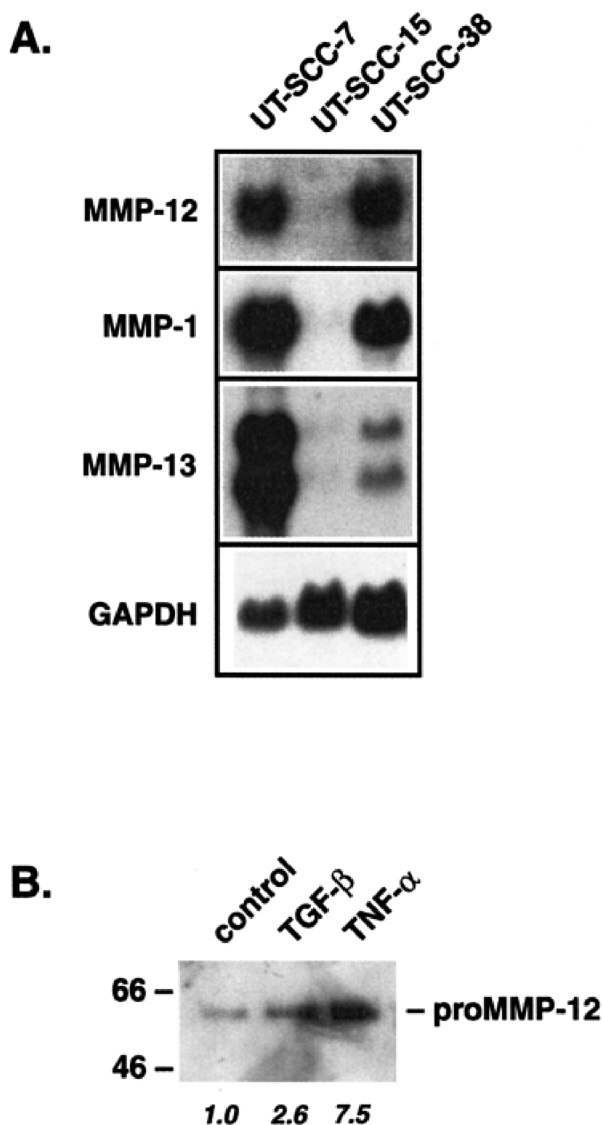


**Figure 3.** (*A*) Expression of MMP-12 mRNA in cell lines from SCCs of the head and neck. SCC cell lines were cultured in DMEM supplemented with 6 mM glutamine, nonessential amino acids, and 10% FBS. Total RNA was extracted and 20 μg aliquots were analyzed for expression of HME (MMP-12), collagenase-3 (MMP-13), collagenase-1 (MMP-1), and GAPDH mRNA by northern blot hybridizations. UT-SCC-7 cell line, established from metastasis of cutaneous SCC; UT-SCC-15 cell line, from primary SCC of tongue; UT-SCC-38 cell line, from primary SCC of glottic larynx. (*B*) UT-SCC-7 cells were treated with TGF-β (5 ng per ml) or TNF-α (20 ng per ml) for 24 h and the production of proMMP-12 was determined by western blot analysis of the aliquots of the conditioned media corresponding to equal number of cells. The levels of proMMP-12 quantitated by densitometric scanning are shown below the western blot relative to levels in untreated control cells (1.0). Migration positions of molecular weight markers (in kDa) are shown on the left.

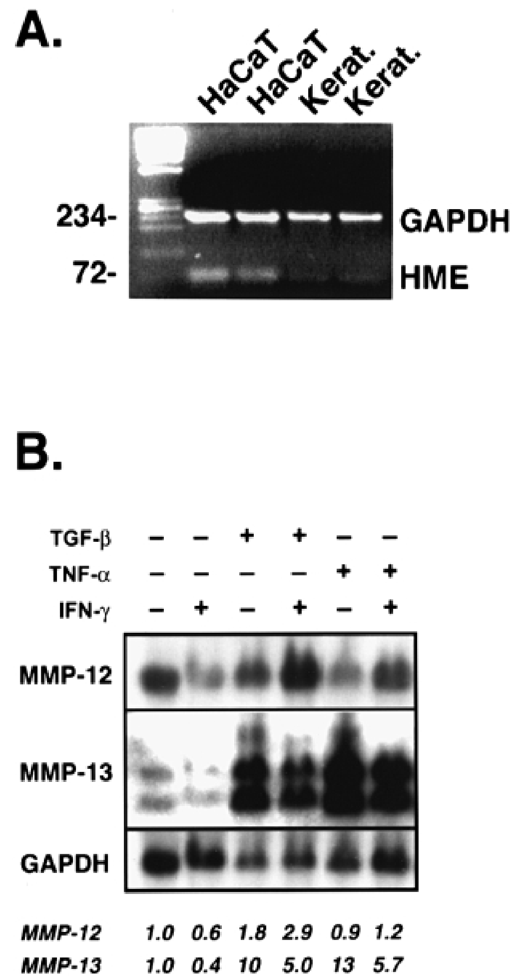


**Figure 4.** (*A*) Expression of HME in transformed (HaCaTs) but not in normal keratinocytes by reverse transcriptase PCR. HaCaT cells were cultured in DMEM containing 10% FBS, 1% penicillin-streptomycin, and 1% sodium puryvate. Keratinocytes were cultured in KGM supplemented with epidermal growth factor (5 ng per ml) and bovine pituitary extract (50 μg per ml). Total RNA isolated was reverse transcribed to cDNA and reverse transcriptase PCR was performed as described in *Materials and Methods*. Results from two different experiments are shown. (*B*) HaCaT keratinocytes were incubated without (–) or with (+) IFN-γ (100 U per ml) for 1 h prior to adding TGF-β1 (5 ng per ml) or TNF-α (20 ng per ml), as indicated. After 24 h incubation cells were harvested and 20 μg aliquots of total RNA were analyzed for MMP-12, MMP-13, and GAPDH mRNA levels by northern blot hybridizations. The levels of MMP-12 and MMP-13 mRNAs were normalized to GAPDH mRNA levels and are shown below the northern blots, relative to the levels in untreated control cells (1.0).

## RESULTS

**HME is abundantly expressed in SCC** Expression of HME mRNA was detected in 16 of 17 SCCs studied (**Table I**). The signal was found in epithelial cancer cells in 11 of 17 tumors (**Fig 1*A*, *F***). This localization was confirmed using cytokeratin and CD-68 immunohistochemistry (**Fig 1*B*, *E***). In 15 of 17 samples HME mRNA was also expressed by stromal macrophage-like cells (**Fig 1*A*, *F–H***). Samples of grade I SCC (highly differentiated tumors) displayed considerably fewer positive cells than grade II and III tumors (**Table I**). In ulcerated SCCs, a signal for HME mRNA was detected in macrophage-like cells just beneath the wound bed.

HME protein expression was detected in all SCCs examined (n = 14). Immunosignal was seen in both macrophages and cancer cells (**Fig 1*C*, *D*, *I***) in the same region as mRNA-positive cells. Immunostaining for CD-68 was performed on serial sections in 19 SCCs or BCCs altogether to exclude the possibility that HME-expressing cells in the middle of cancer nests would be macrophages. The staining demonstrated that some of the HME-positive cells were macrophages, particularly those lying near the borders of tumor islands. HME-positive cells were also detected, however, inside cancer islands devoid of macrophages (**Fig 1*I*, *J***).

**HME expression in BCC** HME mRNA was expressed also in BCCs in 10 of 19 samples studied (**Table I**), but the expression was focal and not as abundant as that of HME in SCCs (**Fig 2*A***). HME was generally expressed by macrophage-like cells, but in four of 19

samples it was also detected in cytokeratin-positive cancer cells within the tumor islands (**Fig 2*C*, *D***). HME protein was expressed in the same area as HME mRNA either in cancer cells (**Fig 2*B*, *G***) or macrophages. As in SCCs, HME was expressed in the wound bed of ulcerated BCCs by macrophages (**Fig 2*E*, *F***), based on the microscopic appearance of HME-positive cells, their presence in cytokeratin-negative areas, and the lack of data on HME production by fibroblasts or endothelial cells.

Tumors representing Bowen's disease were negative for HME except for one sample, in which there was a weak signal in occasional macrophages (data not shown). No HME expression was found in Clark grade II-IV melanomas (not shown). Normal skin has previously been shown to be negative for HME mRNA and protein (Saarialho-Kere *et al*, 1999; Vaalamo *et al*, 1999), and was also included as a negative control in the present *in situ* and immuno studies with similar results. No signal was detected using the sense HME cRNA probe.

**Expression of HME by keratinocyte and SCC cell lines** To confirm the ability of SCC cells to express HME, we used northern blot analysis to determine the expression of HME (MMP-12) mRNA in four cell lines established from SCCs of the head and neck. As shown in **Fig 3(*A*)**, marked expression of MMP-12 mRNA was detected in UT-SCC-7 cells, established from metastasis of a cutaneous SCC, and in UT-SCC-38 cells, established from primary SCC of glottic larynx. Both of these cell lines also expressed high basal levels of MMP-1 and MMP-13 mRNA (**Fig 3*A***). In addition, MMP-12 mRNA was detected in cell line UT-SCC-17, established from metastasis of an SCC of supraglottic larynx (not shown). No expression of MMP-12 mRNA was detected in UT-SCC-15, a primary SCC tongue cell line, which also expressed very low basal levels of MMP-1 and MMP-13 mRNA (**Fig 3*A***).

We also assayed the amount of MMP-12 in the aliquots of conditioned media corresponding to equal number of cells by western blot analysis using a monoclonal anti-MMP-12 antibody. As shown in **Fig 3(*B*)**, UT-SCC-7 cells produced immunoreactive proMMP-12 with an estimated molecular weight of 58 kDa, and its production was enhanced 2.6-fold and 7.5-fold by a 24 h treatment with TGF-β and TNF-α, respectively (**Fig 3*B***).

As HME expression has not been detected in normal or wounded keratinocytes *in vivo* (Saarialho-Kere *et al*, 1999; Vaalamo *et al*, 1999), we examined the expression of HME *in vitro* in normal keratinocytes and in the transformed nontumorigenic human epidermal keratinocyte cell line, HaCaT. Interestingly, a specific product was amplified with reverse transcriptase PCR using HaCaT cell RNA as template, whereas no HME mRNA could be detected in normal keratinocytes (**Fig 4*A***). Marked expression of HME mRNA was also detected in HaCaT keratinocytes using northern blot hybridizations, and its expression was slightly (1.8-fold) enhanced by TGF-β, but not by TNF-α, both of which markedly enhanced the expression of MMP-13 mRNA in the same cells (**Fig 4*B***). We have recently shown that the expression of MMP-13 and MMP-1 in SCC cells is potently inhibited by IFN-γ (Ala-aho *et al*, 2000). In HaCaT cells the basal expression of HME mRNA was suppressed by 40% with IFN-γ (100 U per ml) (**Fig 4*B***). IFN-γ slightly augmented the expression of HME mRNA in combination with TGF-β and TNF-α, however (**Fig 4*B***). In the same cells, IFN-γ potently inhibited the basal MMP-13 expression, as well as the upregulation of MMP-13 mRNA levels by TGF-β and TNF-α (**Fig 4*B***), as we have noted in SCC cells (Ala-aho *et al*, 2000). Interestingly, no expression of HME mRNA was detected in normal epidermal keratinocytes treated with TNF-α, TGF-β, or IL-1 using northern blot analysis (not shown).

## DISCUSSION

This study demonstrates HME expression in squamous and basal cell skin cancers. We show that HME mRNA is expressed both *in vivo* and *in vitro* not only by macrophages, but by transformed keratinocytes. Our findings are further substantiated by the fact that premalignant Bowen's disease and actinic keratosis samples (Saarialho-Kere *et al*, 1999) were devoid of keratinocyte-derived HME. Furthermore, although several MMPs have been detected in melanomas (Airola *et al*, 1999), they lacked signal for HME mRNA. In our previous studies on several benign skin disorders as well as on normally healing or chronic wounds, we have never detected HME mRNA in keratinocytes *in vivo* (Vaalamo *et al*, 1999). This observation is further supported by the findings in this study showing no expression of HME mRNA in cultured normal epidermal keratinocytes.

MMPs are believed to have an important role at different stages of tumor development and invasion. Various MMPs might form a network, in which each MMP has a distinct role and their activation would happen in a cascade-like manner (see Kähäri and Saarialho-Kere, 1999). For example in SCC, tumor cells, stromal fibroblasts, and inflammatory cells all express a distinct set of MMPs, which can at least in part complement the proteolytic capacity of each other. HME mRNA expression has previously been found in breast cancer (Heppner *et al*, 1996). Cells producing HME were identified as cytokeratin-negative macrophage-like cells, based on the earlier reported restricted expression of HME in macrophages (Shapiro *et al*, 1993; Belaaouaj *et al*, 1995).

The only study demonstrating HME in cancer cells and not in macrophages *in vivo* is that of Rivas *et al* (1998), in which HME was primarily expressed by epithelial hepatocellular carcinoma cells. Furthermore, using semiquantitative reverse transcriptase PCR Giambernardi *et al* (1998) reported strong expression of HME in immortalized mammary epithelial cell line (MCF-10F) and also low levels in two other cancerous cell lines (melanoma and astrocytoma). Our observations clearly show that HME is expressed by SCC cells in culture, providing further evidence that malignant epithelial cells can express HME. Furthermore, the expression was detected in transformed keratinocytes (HaCaT cells) but not in normal epidermal keratinocytes, indicating that HME expression is specific for transformed epithelial cells.

Among various other MMPs, HME has been suggested to be antiangiogenic, as it generates angiostatin from plasminogen (Dong *et al*, 1997; Cornelius *et al*, 1998). Angiostatin inhibits endothelial cell proliferation and metastatic tumor cell growth, and it could be hypothesized that more invasive tumors express less HME. Indeed, those hepatocellular carcinoma patients whose tumors did not express HME mRNA had less favorable prognosis than those whose tumors expressed high amounts of HME (Rivas *et al*, 1998). In contrast to these results, both HME protein and mRNA were clearly expressed more in less differentiated grade II and grade III SCC tumors than in grade I tumors. Analogously, clinically more aggressive, fibrosing BCCs expressed HME mRNA more often than the keratotic or adenoid BCCs. *In vitro* angiostatin can be generated from plasminogen by several other enzymes (Patterson and Sang, 1997), of which at least matrilysin (MMP-7) and stromelysin-1 (MMP-3) are expressed in BCCs and SCCs (Karelina *et al*, 1994; Airola *et al*, 1997).

In general, MMPs expressed by cancer cells can also be produced by non-neoplastic cells (see Kähäri and Saarialho-Kere, 1999). Collagenase-3 (MMP-13), matrilysin (MMP-7), and MT1-MMP (MMP-14) expression have previously been detected in malignantly transformed keratinocytes in SCCs, but not in normal keratinocytes or in premalignant lesions of the skin (Karelina *et al*, 1994; Airola *et al*, 1997; Johansson *et al*, 1997b, 1999). Thus, their expression serves as a marker for the transformation and invasion capacity of SCC cells. In the same way, the amount of HME-expressing malignant keratinocytes could correlate with invasiveness of SCC. HME expressed by different cells could have different functions in cancer. The surrounding stromal compartment of malignant tumors undergoes extensive tissue remodeling and, if they do not have a part in host defense, as suggested for stromal MT1-MMP (see Seiki, 1999), HME-producing macrophages may contribute to this process.

Besides degradation of matrix components and angiostatin generation, metalloelastase seems to have other functions in tissues. During cutaneous wound healing murine metalloelastase is abundantly expressed by macrophages, first just below the scab and, after reepithelialization, in deeper areas of dermis, specifically around blood vessels (Madlener *et al*, 1998). Based on this finding, Madlener *et al* (1998) suggested that murine metalloelastase may have a function in capillary regression. Whenever our tumor samples were ulcerated, we also detected signal for HME in macrophages at the tumor surface.

HME was detected in BCC, although its expression was more frequent and abundant in aggressive SCCs, suggesting a role for HME in epithelial dedifferentiation and invasiveness. Studies are under way using clinical material of other types of SCCs to investigate their patterns of HME expression and possible correlations to metastasis and patient prognosis. In conclusion, our data show that, in addition to macrophages, HME expression can be induced in transformed keratinocytes.

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