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ORIGINAL ARTICLE

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Expression of matrix metalloproteinase-1 mRNA related to eosinophilia and interleukin-5 gene expression in head and neck tumour tissue

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Abstract In situ hybridization (ISH) of the expression of matrix metalloproteinase-1 (MMP-1) in areas of local invasion of head and neck tumours was performed. Expression of MMP-1 mRNA was found in stromal inflammatory cells adjacent to the tumour nests, apparently in eosinophils. MMP-1 mRNA expression was seen only in cases with moderate or greater stromal eosinophilia. MMP-1 mRNA was detected in benign papilloma and verrucous carcinoma. In squamous cell carcinomas, expression of the gene was detected only in well-differentiated cases. Expression of MMP-1 mRNA was not related to the grade of malignancy, and appeared in stromal eosinophils, suggesting to us that it is involved in a remodelling process in the reaction of the host to tumour invasion. ISH examination of the expression of interleukin-5 (IL-5), the activating factor for eosinophils, was also performed. In the tumour, its expression was consistent with the location of eosinophils, as with MMP-1 mRNA, suggesting an autocrine mechanism.

Key words Matrix metalloproteinase · In situ hybridization · Eosinophil · IL-5 · Head and neck tumour

Introduction

The polyfunctional molecules that fill the space between cells in biological tissue are known collectively as the extracellular matrix and comprise of collagen, elastin, proteoglycan, fibronectin, laminin and others. In order for a tumour to spread it must infiltrate the extracellular matrix, which is degraded by proteolytic enzymes. It is believed that matrix metalloproteinases (MMPs), which are active in the neutral environment and contain Zn^{2+} in their active centres, play an important part [20]. MMPs were first reported in 1986, when Goldberg et al. [10] identified the primary structure in interstitial collagenase, matrix metalloproteinase-1 (MMP-1). Several types of MMPs are known today, which can be classified according to their primary structure and substrate specificity as follows: the interstitial collagenase group, whose substrates are the various collagens that form the main component of stroma; the gelatinase group, whose substrate is type IV collagen, the main component of basement membrane; and the stromelysin group, which shows substrate specificity to a wide range of molecules, including fibronectin and laminin; and others. MMP-1 belongs to the collagenase group and degrades collagen types I, II, III and X [29]. Collagen types I and III are found in a wide range of connective tissue.

One of the characteristics of the squamous cell carcinoma, which is the most frequent type of head and neck carcinoma, is its capacity to infiltrate connective tissue [4], and the extent of local invasion is an index of its level of malignancy. We focused on the MMP-1 as a potential index of invasion, and evaluated the expression of MMP-1 using in situ hybridization (ISH) with samples obtained from head and neck tumours. In the course of this study, a relationship between the expression of MMP-1 and eosinophilia was suggested. Thus we evaluated the expression of interleukin-5 (IL-5), which is involved in the differentiation and the proliferation of eosinophils [6], and examined the interrelation between MMP-1 in tumour tissue, eosinophilia and IL-5.

Materials and methods

We took surgical samples of diseased head and neck tissue and cut out about a 5-mm square from the border between normal and diseased tissue as quickly as possible. It was fixed in a 4% buffered paraformaldehyde solution [0.1 M phosphate-buffered saline (PBS, pH 7.4) with 4% paraformaldehyde], embedded in paraffin,

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Table 1 Expression of MMP-1 mRNA, IL-5 mRNA, and tissue eosinophilia. (*SCC* squamous cell carcinoma, eosinophilia; −0–2/hpf (high power field), +3–9/hpf, ++10–29/hpf, +++30≦hpf, expression of mRNA; +positive, −negative)

Case	Tissue	Histology	Differentiation	Sex	Age	TNM stage	Eosinophilia	MMP-1 mRNA	IL-5 mRNA
1	Tongue	SCC	Well	F	72	3 2c 0 4	+++	+	+
2	Tongue	SCC	Well	M	73	2 1 0 3	++	+	+
3	Tongue	SCC	Moderate	M	55	3 0 0 3	+	–	–
4	Tongue	SCC	Poor	M	75	2 0 0 2	+	–	–
5	Floor of mouth	SCC	Well	M	47	2 0 0 2	+++	+	+
6	Floor of mouth	SCC	Moderate	M	45	3 1 0 3	+	–	–
7	Floor of mouth	SCC	Moderate	M	72	1 0 0 1	+	–	–
8	Buccal	SCC	Well	M	70	3 1 0 3	+++	+	+
9	Buccal	SCC	Well	M	63	3 0 0 3	–	–	–
10	Buccal	SCC	Moderate	M	82	3 0 0 3	–	–	–
11	Palate	SCC	Well	M	50	4 3 0 4	+	–	–
12	Oropharynx	SCC	Well	M	73	2 0 0 2	–	–	–
13	Hypopharynx	SCC	Moderate	M	78	4 2c 0 4	+	–	–
14	Maxillary sinus	SCC	Poor	M	53	3 0 0 3	+	–	–
15	Tongue	Verrucous Ca.	–	M	78	3 0 0 3	+++	+	+
16	Tongue	Papilloma	–	M	72	– – – –	++	+	+
17	Turbinate	Inflammation	–	M	44	– – – –	++	–	+
18	Maxillary sinus	Inflammation	–	M	69	– – – –	+++	–	+

and cut into serial sections. The composition of the samples is shown in Table 1. There were a total of 18 cases, made up of 14 cases of squamous cell carcinoma, 1 case each of verrucous carcinoma, and papilloma, and 2 cases of inflammation. Of the squamous cell carcinomas, 7 cases were well differentiated, 5 were moderately differentiated and 2 were poorly differentiated.

For in situ hybridization, the MMP-1 cDNA (1.7-kb) probe was established from the cDNA library of cultured fibroblasts from the human synovium by Saus et al. [28]. The expression of IL-5 was evaluated using human IL-5 oligonucleotide cDNA probe (40 bp, Oncogene Science, N.Y.), which has a base sequence complementary to Exon 1 of human IL-5. These probes were labelled with [α-^{35}S] dCTP (New England Nuclear, Boston, Mass.) with the specific activity of 39.8 TBq/mmol using an Oligolabelling Kit (Pharmacia, Uppsala, Sweden). The specific activity of the probe was set at 2.0×10^7 cpm/μg DNA.

The hybridization procedure used in this study was that of Hayashi et al. [14] with a slight modification. The paraffin sections were deparaffinized in xylene and ethanol. Predigestion was completed with a 50-mM Tris-HCl solution (pH 7.6) containing 5 mM EDTA with proteinase K (Sigma Chemical Co., St. Louis, Mo.) and left to react on the sections in the humid chamber for 10 min. The specimens were washed in PBS containing 2 mg/ml of glycine and PBS, and then re-fixed in 4% paraformaldehyde solution for 20 min. The specimens were washed in PBS containing 2 mg/ml of glycine and then soaked in 0.1 M triethanolamine buffer (pH 8.0). Following this procedure, the specimens were placed in 0.1 M triethanolamine buffer containing 0.25% acetic anhydride and left to react for 10 min at room temperature. The specimens were washed twice in 2 × SSC solution for 5 min each, then dehydrated and dried in air under sterile conditions. A hybridization solution composed of 2 μg/ml labelled cDNA probe, 500 μg/ml yeast tRNA, 80 μg/ml salmon sperm DNA, 50% formamide, 10 mM Tris HCl (pH 7.0), 0.15 M NaCl, 1 mM EDTA (pH 7.0) and 1 × Denhardt's mixture (Sigma) was heated for 3 min at 80°C to denature the probe DNA and then ice-cooled quickly for 5 min. Between 12 μl and 20 μl of this solution was placed on the sections, covered with a cover glass, sealed in a container and reacted in an incubator at 45°C for 16–20 h. The coverglass was then removed in 2 × SSC, and washed in this liquid three times for 10 min at room temperature, then in 0.5 × SSC for 10 min at 45°C, then three times in 0.1 × SSC for 10 min at 45°C, after which the specimens were dehydrated and dried. NTB2 emulsion (Eastman Kodak, Rochester, N.Y.) and 0.6 M ammonium sulfate solution were mixed at 1:1 under safelight, and the mixture was left to stand for 10 min at 43°C to form a uniform solution. The sections were dipped in this solution and ice-cooled for 5 min, placed in a dark box, and then exposed at 4°C for 24–36 h. They were then developed at 18°C for 3 min with D-19 (Kodak) and fixed. After rinsing with water, nuclear staining was performed with haematoxylin, and the specimen was sealed. In each process in this experiment, gene expression-positive specimens were used as positive controls. Surrounding non-tumour regions were used as negative controls.

Luna staining [19], which selectively stains eosinophilic granules, was applied to each section. In accordance with the report published by Goldsmith et al. [11], the infiltration was rated by averaging the number of infiltrated eosinophils in three fields of view under a light microscope at a high magnification (×400, high-power field,/hpf) and rated; – (absence, 0–2/hpf), + (mild, 3–9/hpf), ++ (moderate, 10–29/hpf) and +++ (severe, 30 or more/hpf; Table 1).

Each section was observed under a light microscope.

Results

The results of ISH and eosinophilia are shown in Table 1. Expression of MMP-1 mRNA was detected in 6 of the 18 cases. Among squamous cell carcinomas, expression was detected in 4 of 7 well-differentiated cases (Fig. 1), but was not detected in cases of either moderately or poorly differentiated tumour. Expression was also detected in verrucous carcinoma (Fig. 2) and benign papilloma. There was no MMP-1 gene expression in the cases of inflammation. In every case, expression was detected in the stroma adjacent to the tumour nest and was consistent with the location of inflammatory cells. Expression was not detected in tumour cells, nor in any cell in the non-tumour area.

According to evaluation of the eosinophilia levels with Luna staining, 8 cases displayed moderate (++) or severe (+++) infiltration, and its location in the tumour tissue was limited to the stroma adjacent to the tumour nest in every case. Comparison of cells with MMP-1 mRNA expression under ISH and Luna staining in serial sections revealed a characteristic morphology of bilobed

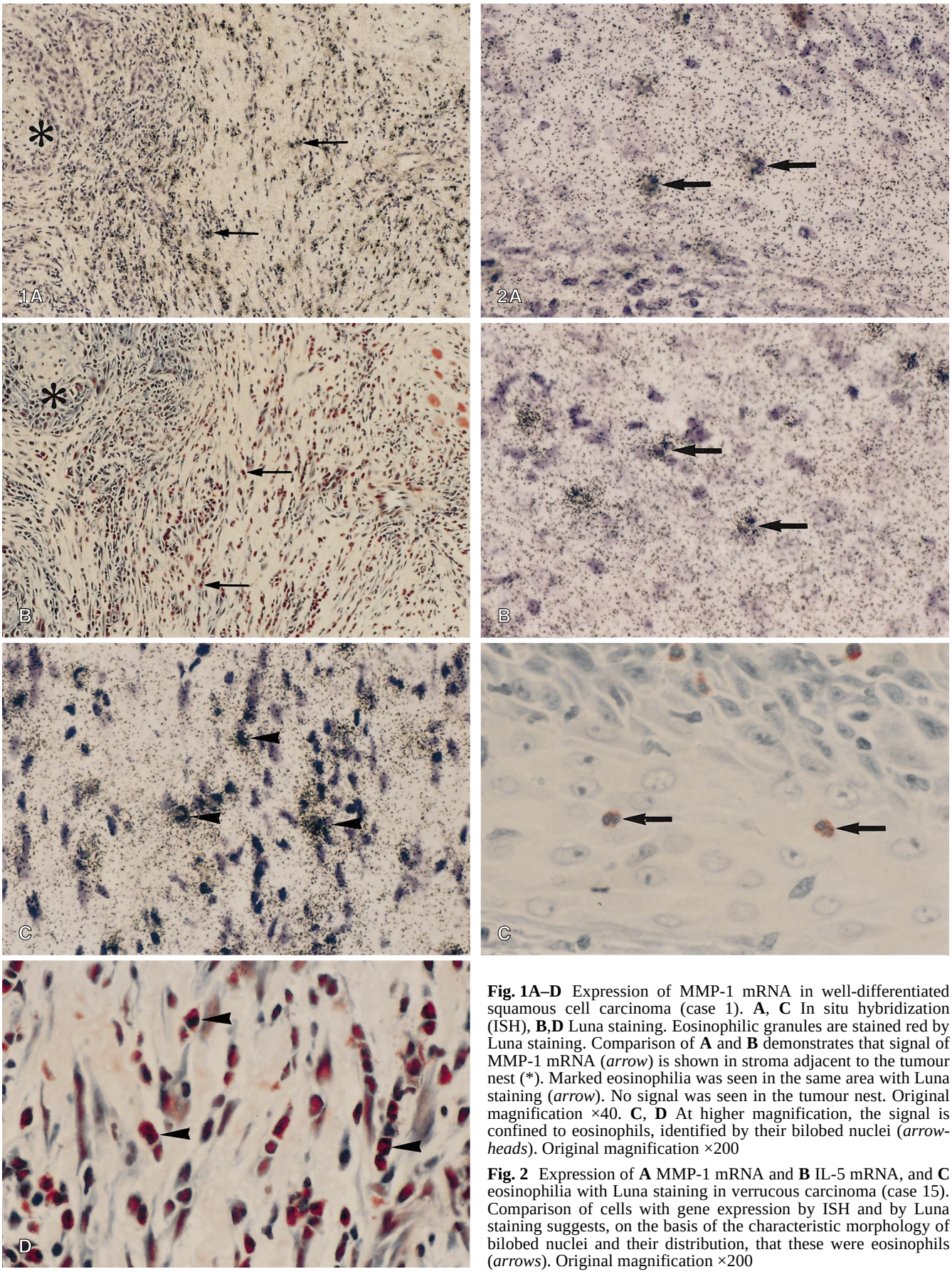
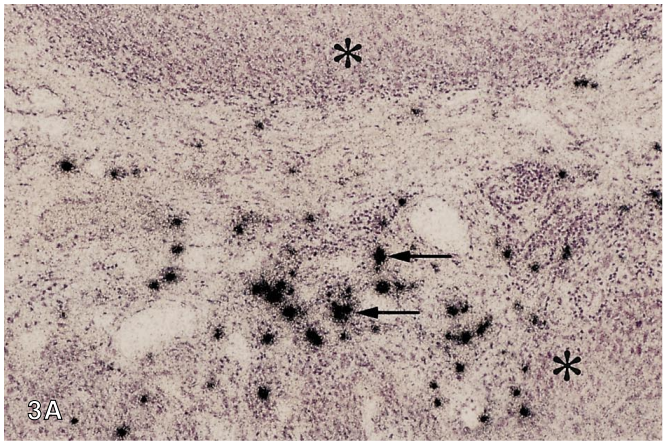


Fig. 1A–D Expression of MMP-1 mRNA in well-differentiated squamous cell carcinoma (case 1). **A**, **C** In situ hybridization (ISH), **B**,**D** Luna staining. Eosinophilic granules are stained red by Luna staining. Comparison of **A** and **B** demonstrates that signal of MMP-1 mRNA (*arrow*) is shown in stroma adjacent to the tumour nest (*). Marked eosinophilia was seen in the same area with Luna staining (*arrow*). No signal was seen in the tumour nest. Original magnification ×40. **C**, **D** At higher magnification, the signal is confined to eosinophils, identified by their bilobed nuclei (*arrowheads*). Original magnification ×200

Fig. 2 Expression of **A** MMP-1 mRNA and **B** IL-5 mRNA, and **C** eosinophilia with Luna staining in verrucous carcinoma (case 15). Comparison of cells with gene expression by ISH and by Luna staining suggests, on the basis of the characteristic morphology of bilobed nuclei and their distribution, that these were eosinophils (*arrows*). Original magnification ×200



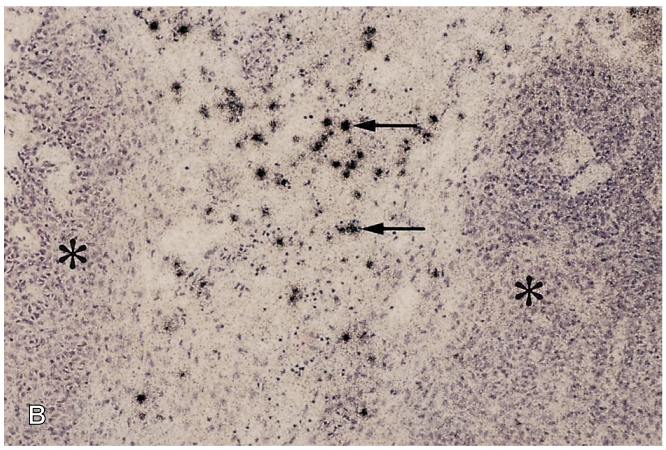
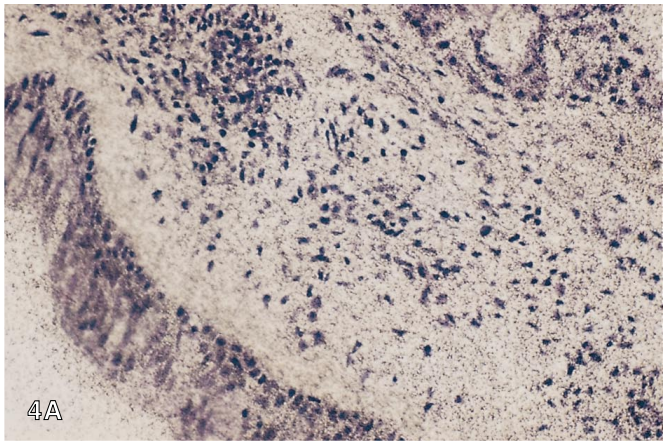
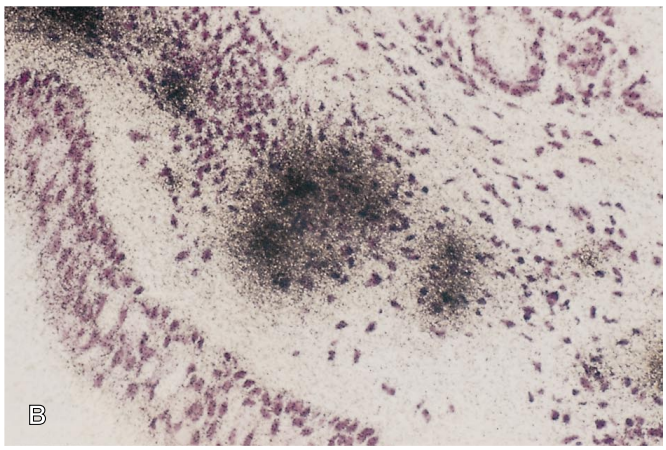


Fig. 3 Expression of IL-5 mRNA in **A** papilloma (case 16) and **B** well-differentiated squamous cell carcinoma (case 8). IL-5 mRNA signal is seen in stromal cells (*arrows*) adjacent to the tumor nest (*). Original magnification ×33





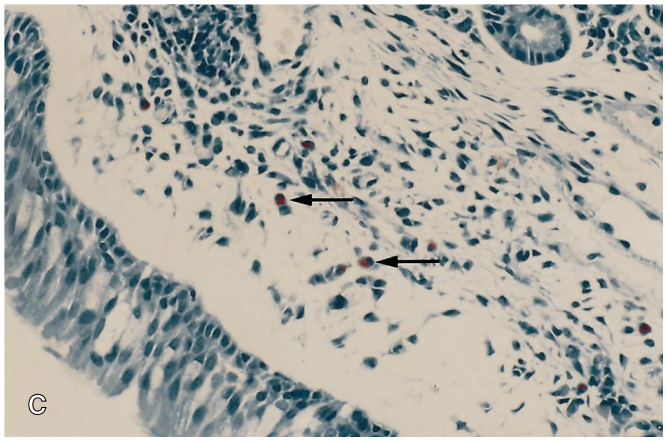


Fig. 4 Expression of **A** MMP-1 mRNA and **B** IL-5 mRNA with ISH and **C** eosinophilia with Luna staining in inflammation (inferior turbinate, case 17). IL-5 mRNA signal is found only where eosinophils are located (*arrows*). Original magnification ×80

nucleus and a characteristic distribution that led us to believe these cells were eosinophils (Figs. 1, 2). Expression was all seen in 6 cases with either moderate or severe eosinophilia, and no MMP-1 gene expression was found in the cases where eosinophilia was mild or absent.

Scanning for the expression of IL-5 mRNA showed expression in every case in which there was expression of MMP-1 mRNA, and its location was limited to the stroma adjacent to the tumour nest in every case (Fig. 3). Expression was not detected in any cell in the non-tumour regions. In comparison with Luna staining, gene-expressed cells were identified as eosinophils, just as with MMP-1 mRNA (Fig. 2). Expression of IL-5 mRNA was not found in tumour cases from which expression of MMP-1 was absent, but in cases with inflammation, IL-5 mRNA was found alone, consistent with the location of eosinophils (Fig. 4).

Discussion

Since Liotta et al. [18] reported that the metastatic potential of malignant tissue correlated with degradation activity of collagen type IV, there have been many inquiries into the relationship between expression of MMPs and tumours. However, there is still no consensus about the relationship between cells with expression and the level of malignancy of a cancer. There are few reports on MMP-1, and little is known about its relationship with metastatic activity or its pathological significance [13, 26].

Different institutions and methodologies have produced inconsistency in the reporting of MMP expression. Immunohistochemical staining is reported to have revealed expression of MMP-2 in the cancer cells of oral and oesophageal squamous cell carcinoma [16, 30] and thyroid carcinoma [5], and ISH examinations are reported to have detected expression of MMP-2 and MMP-9 in cancer cells of hypopharyngeal squamous cell carcinoma [21] in proportion to the level of malignancy.

There have been successive reports of expression of MMPs detected in stromal cells within tumour tissue. Polette et al. [27] have reported that MMP-1 and MMP-10 (stromelysin 2) in head and neck squamous cell carcinoma is expressed principally in the fibroblasts in the stroma and have observed that the production and the activation of MMPs occur as an interaction between tumour cells and stromal cells. Gray et al. [12] have found MMP-1 mRNA in stromal fibroblasts of oral squamous cell carcinoma and cite the following possible mechanisms: an increase in tensile forces generated by the continued expansion of a growing tumour mass, factors released by the local inflammatory response, and factors released by tumour cells themselves. Gray et al. [13] have also observed gene expression of MMP-1 in eosinophils, fibroblasts and endothelial cells in their examination of colonic adenocarcinomas and observed that the the level of expression seems to be correlated with the degree of inflammatory infiltration and to show no obvious correlation with the depth of tumour invasion or tumour stage. Ohtani et al. [25] have observed expression of MMP-2 mRNA abundantly in the “reactive fibrous zone” surrounding the invasive margin of cancer. Because its expression is similar to that of non-neoplastic fibrous granulation tissue and MMP-2 appears during the healing process of wounds, it has been postulated that MMP-2 has a role as a turnover enzyme for the extracellular matrix. Ohtani et al. pointed out the possibility that it might be involved in the remodelling process induced by cancer cell invasion. Okada et al. [23] observed that membrane-type metalloproteinase (MT-MMP) transcripts were detected only in stromal cells of carcinomas as wound healing occurred and postulated that MT-MMP contributed to the tissue remodelling process associated with the formation of both stroma and granulation tissue.

In this study, the expression of MMP-1 mRNA was seen in verrucous carcinoma and benign papilloma. In squamous cell carcinoma it was observed only in the well-differentiated tumour tissue. In every case expression was seen in the inflammatory cells adjacent to the tumour. Verrucous carcinoma is known to have a heavy inflammatory reaction in the stroma adjacent to the tumour [2], and this is where MMP-1 mRNA was found. Thus, as with MMP-2 and MT-MMP, the pattern of expression hints at the possibility that the enzyme is involved in the remodelling process, as a part of the reaction of the host against the tumour invasion.

From examination of the morphology of the cells showing expression and the Luna staining of the serial sections, we conclude that the cells observed were eosinophils. In earlier reports, the expression of MMP-1 mRNA in the stromal cells was reported to be in fibroblasts and macrophages in many cases [12, 22, 26, 27]. There are, however, some reports that they are found in inflammatory cells [26] and in the interstitial eosinophils of colon cancer [13]. There are also reports that MMP-9 were found in the stromal eosinophils of squamous cell carcinoma and basal cell carcinoma of the skin [31, 32]. There is a real possibility that in ISH examination using radioisotopes, emulsion particles may interfere with cell identification. But the use of serial sections and Luna staining and performance of comparative studies may be expected that to allow clarification of the relationship between MMPs and eosinophils in the future.

Eosinophilic infiltration is common in areas of inflammation, cancers and wound healing. In particular, eosinophilia of tumour tissue (tumour-associated tissue eosinophilia; TATE) was recognized 100 years ago [34]. The presence of collagenase activity in eosinophils was first reported by Bassett et al. [1] in 1976 when they discovered it in the eosinophils of rats. Hibbs et al. [15] reported that eosinophils in the abdominal cavity of guinea pigs contained metalloprotein which selectively degraded collagen types I and III. In 1984, Davis et al. [7] reported that human eosinophils also contain collagenase, and that they degrade collagen types I and III at approximately the same ratio as eosinophils in guinea pigs. It can be concluded that eosinophils also produce MMP-1 in head and neck tumours.

It is conceivable that various cytokines are factors inregulation of the production of MMPs in tumour tissues. IL-1 [17], TNF-α [24], and TGF-α [9] have previously been reported as factors facilitating the production of MMP-1. In this study, however, since MMP-1 is being produced in eosinophils, we focused on IL-5, which is responsible for their maturation and activation, and examined the expression of IL-5 mRNA. The expression found was consistent with the location of eosinophils, just as with MMP-1 mRNA, suggesting an autocrine mechanism in which the eosinophil itself facilitates its own activity. IL-5 mRNA production by eosinophils in atopic dermatitis [33], bronchial asthma [3], and coeliac disease [8] has already been reported. The expression of IL-5 mRNA is also conceivable in TATE, but these are the first reported cases of IL-5 mRNA expression in TATE of head and neck tumours. Expression of IL-5 mRNA was also detected in inflammation, but expression of MMP-1 mRNA was not detected, which suggests a difference in the mechanism of the activated eosinophils and warrants further study.

It is possible that other MMPs and tissue inhibitors of metalloproteinases (TIMPs), as well as the various cytokines, are intricately involved in the response to head and neck tumours. It is hoped that by clarifying the details of this network, the whole picture of the metabolism of the extracellular matrix of the head and neck tumour invasion will be revealed.

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References

1. Bassett EG, Baker JR, Baker PA, Myers DB (1976) Comparison of collagenase activity in eosinophil and neutrophil fractions from rat peritoneal exudates. Aust J Exp Biol Med Sci 54:459–465
2. Batsakis JG (1979) Squamous cell carcinoma of oral cavity and oropharynx. In: Batsakis JG (ed) Tumors of the head and neck, 2nd edn. Williams & Wilkins, Baltimore, pp 144–176
3. Broide DH, Paine MM, Firestein GS (1992) Eosinophils express interleukin 5 and granulocyte macrophage-colony-stimulating factor mRNA at sites of allergic inflammation in asthmatics. J Clin Invest 90:1414–1424
4. Burman JF, Carter RL (1985) Lysis of type-I collagen by squamous carcinomas of the head and neck. Int J Cancer 36:109–116
5. Campo E, Merino MJ, Liotta L, Neumann R, Stetler-Stevenson W (1992) Distribution of the 72-kd type IV collagenase in nonneoplastic and neoplastic thyroid tissue. Hum Pathol 23: 1395–1401
6. Clutterbuck EJ, Hirst EMA, Sanderson CJ (1989) Human interleukin-5 (IL-5) regulates the production of eosinophils in human bone marrow cultures: comparison and interaction with IL-1, IL-3, IL-6, and GMCSF. Blood 73:1504–1512
7. Davis WB, Fells GA, Sun XH, Gadek JE, Venet A, Crystal RG (1984) Eosinophil-mediated injury to lung parenchymal cells and interstitial matrix: A possible role for eosinophils in chronic inflammatory disorders of the lower respiratory tract. J Clin Invest 74:269–278
8. Desreumaux P, Janin A, Colombel JF, Prin L, Plumas J, Emilie D, Torpier G, Capron A, Capron M (1992) Interleukin 5 messenger RNA expression by eosinophils in the intestinal mucosa of patients with coeliac disease. J Exp Med 175:293–296
9. Ganser GL, Stricklin GP, Matrisian LM (1991) EGF and TGF α influence in vitro lung development by the induction of matrix-degrading metalloproteinases. Int J Dev Biol 35:453–461
10. Goldberg GI, Wilhelm SM, Kronberger A, Bauer EA, Grant GA, Eisen AZ (1986) Human fibroblast collagenase: complete primary structure and homology to an oncogene transformation-induced rat protein. J Biol Chem 261:6600–6605
11. Goldsmith MM, Belchis DA, Cresson DH, Merritt WD III, Askin FB (1992) The importance of the eosinophil in head and neck cancer. Otolaryngol Head Neck Surg 106:27–33
12. Gray ST, Wilkins RJ, Yun K (1992) Interstitial collagenase gene expression in oral squamous cell carcinoma. Am J Pathol 141:301–306
13. Gray ST, Yun K, Motoori T, Kuys YM (1993) Interstitial collagenase gene expression in colonic neoplasia. Am J Pathol 143:663–671
14. Hayashi M, Ninomiya Y, Parsons J, Hayashi K, Olsen BR, Trelstad RL (1986) Differential localization of mRNAs of collagen types I and II in chick fibroblasts, chondrocytes, and corneal cells by in situ hybridization using cDNA probes. J Cell Biol 102:2302–2309
15. Hibbs MS, Mainardi CL, Kang AH (1982) Type-specific collagen degradation by eosinophils. Biochem J 207:621–624
16. Kusukawa J, Sasaguri Y, Shima I, Kameyama T, Morimatsu M (1993) Expression of matrix metalloproteinase-2 related to lymph node metastasis of oral squamous cell carcinoma: A clinicopathologic study. Am J Clin Pathol 99:18–23
17. Lefebvre V, Peeters-Joris C, Vaes G (1990) Modulation by interleukin 1 and tumor necrosis factor α of production of collagenase, tissue inhibitor of metalloproteinases and collagen types in differentiated and dedifferentiated articular chondrocytes. Biochim Biophys Acta 1052:366–378
18. Liotta LA, Tryggvason K, Garbisa S, Hart I, Foltz CM, Shafie S (1980) Metastatic potential correlates with enzymatic degradation of basement membrane collagen. Nature 284:67–68
19. Luna LG (1968) Manual of histologic staining methods of the armed forces insitute of pathology, 3rd edn, pp111-112, McGraw-Hill, New York
20. Matrisian LM (1990) Metalloproteinases and their inhibitors in matrix remodeling. Trends Genet 6:121–125
21. Miyajima Y, Nakano R, Morimatsu M (1995) Analysis of expression of matrix metalloproteinases-2 and -9 in hypopharyngeal squamous cell carcinoma by in situ hybridization. Ann Otol Rhinol Laryngol 104:678–684
22. Muller D, Wolf C, Abecassis J, Millon R, Engelmann A, Bronner G, Rouyer N, Rio MC, Eber M, Methlin G, Chambon P, Basset P (1993) Increased stromelysin 3 gene expression is associated with increased local invasiveness in head and neck squamous cell carcinomas. Cancer Res 53:165–169
23. Okada A, Bellocq JP, Rouyer N, Chenard MP, Rio MC, Chambon P, Basset P (1995) Membrane-type matrix metalloproteinase (MT-MMP) gene is expressed in stromal cells of human colon, breast, and head and neck carcinomas. Proc Natl Acad Sci USA 92:2730–2734
24. Okada Y, Tsuchiya H, Shimizu H, Tomita K, Nakanishi I, Sato H, Seiki M, Yamashita K, Hayakawa T (1990) Induction and stimulation of 92-kDa gelatinase/type IV collagenase production in osteosarcoma and fibrosarcoma cell lines by tumor necrosis factor α. Biochem Biophys Res Commun 171:610–617
25. Ohtani H, Nagai T, Nagura H (1995) Similarities of in situ mRNA expression between gelatinase A (MMP-2) and type I procollagen in human gastrointestinal carcinoma: comparison with granulation tissue reaction. Jpn J Cancer Res 86:833–839
26. Otani Y, Okazaki I, Arai M, Kameyama K, Wada N, Maruyama K, Yoshino K, Kitajima M, Hosoda Y, Tsuchiya M (1994) Gene expression of interstitial collagenase (matrix metalloproteinase 1) in gastrointestinal tract cancers. J Gastroenterol 29:391–397
27. Polette M, Clavel C, Muller D, Abecassis J, Binninger I, Birembaut P (1991) Detection of mRNAs encoding collagenase I and stromelysin 2 in carcinomas of the head and neck by in situ hybridization. Invasion Metastasis 11:76–83
28. Saus J, Quinones S, Otani Y, Nagase H, Harris ED Jr, Kurkinen M (1988) The complete primary structure of human matrix metalloproteinase-3. J Biol Chem 263:6742–6745
29. Schmid TM, Mayne R, Jeffrey JJ, Linsenmayer TF (1986) Type X collagen contains two cleavage sites for a vertebrate collagenase. J Biol Chem 261:4184–4189
30. Shima I, Sasaguri Y, Kusukawa J, Yamana H, Fujita H, Kakegawa T, Morimatsu M (1992) Production of matrix metalloproteinase-2 and metalloproteinase-3 related to malignant behavior of esophageal cacinoma: a clinicopathologic study. Cancer 70:2747–2753
31. Ståhle-Bäckdahl M, Parks WC (1993) 92-kd gelatinase is actively expressed by eosinophils and stored by neutrophils in squamous cell carcinoma. Am J Pathol 142:995–1000
32. Ståhle-Bäckdahl M, Sudbeck BD, Eizen AZ, Welgus HG, Parks WC (1992) Expression of 92-kDa type IV collagenase mRNA by eosinophils associated with basal cell carcinoma. J Invest Dermatol 99: 497–503
33. Tanaka Y, Delaporte E, Dubucquoi S, Gounni AS, Porchet E, Capron A, Capron M (1994) Interleukin-5 messenger RNA and immunoreactive protein expression by activated eosinophils in lesional atopic dermatitis skin. J Invest Dermatol 103:589–592
34. Thompson AC, Bradley PJ, Griffin NR (1994) Tumor-associated tissue eosinophilia and long-term prognosis for carcinoma of the larynx. Am J Surg 168:469–471