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Distinct pattern of matrix metalloproteinase 9 and tissue inhibitor of metalloproteinase 1 mRNA expression in human colorectal cancer and liver metastases

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Summary The matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) are perceived as essential for tumour invasion and metastasis. In the present study, we compare the topographical pattern of MMP-9 and TIMP-1 expression in colorectal cancer and liver metastases by *in situ* hybridisation. TIMP-1 mRNA was detected in all 26 colorectal cancers examined, while only 18 out of 26 (69.2%) were positive for MMP-9. Both MMP-9 and TIMP-1 mRNA were observed in all ten liver metastases but were absent in three adenomas and in all normal colonic mucosa and liver. There was no association between MMP-9 or TIMP-1 mRNA expression and degree of differentiation or size of tumours. MMP-9 and TIMP-1 mRNA were similarly observed in the peritumour stroma cells rather than in tumour cells themselves. MMP-9 mRNA-positive cells were round and identified as macrophages by immunostaining with an anti-macrophage antibody (KP1), while TIMP-1 mRNA was detected in spindle-shaped stromal cells. In liver metastases, MMP-9 localised within peritumour stroma or at the interface between the tumour stroma and normal liver, whereas TIMP-1 mRNA was located throughout the malignant tumour stroma. Our data demonstrate a distinct pattern of MMP-9 and TIMP-1 mRNA expression in colorectal cancer and liver metastases suggesting distinct cellular origins as well as separate patterns of regulation.

Keywords: *in situ* hybridisation; matrix metalloproteinase; tissue inhibitor of metalloproteinase; type IV collagenase

Matrix metalloproteinases (MMPs) are a family of metal-dependent endopeptidases with proteolytic activities for various components of the extracellular matrix (Liotta and Stetler-Stevenson, 1991; Ennis and Matrisian, 1994). The MMP family comprises at least nine members (Woessner, 1994). All family members are secreted in a latent form that becomes active upon cleavage of a 10 000 dalton peptide (Ennis and Matrisian, 1994). Since type IV collagen is a major component of basement membranes, the 72 kDa (MMP-2) and 92 kDa (MMP-9) type IV collagenases are of particular interest.

Over the last decade, much attention has focused on the role of MMPs in tumour invasion and metastases (Liotta and Stetler-Stevenson, 1991; Murphy *et al.*, 1989). Both *in vitro* and *in vivo* data have indicated that MMPs participate, via accelerated breakdown of extracellular matrices, in tumour invasion and metastases (Liotta *et al.*, 1980; Nakajima *et al.*, 1987; Ura *et al.*, 1989). Furthermore, increased production of MMP has been associated with increased invasive and metastatic potential in several human malignant tumours (Levy *et al.*, 1991; Urbanski *et al.*, 1992; Boag and Young, 1993; Brown *et al.*, 1993). Although, MMP-9 is secreted by a wide range of cells, including macrophages, neutrophils, capillary endothelial cells and trophoblasts, from both normal and malignant tissue (Hibbs *et al.*, 1985, 1987; Herron *et al.*, 1988; Werb and Alexander, 1993) in primary colorectal cancer, it appears that MMP-9 mRNA may be macrophage in origin (Pyke *et al.*, 1993).

MMP activity, *in vivo*, is thought to be regulated in part by natural tissue inhibitor proteins such as tissue inhibitors of metalloproteinases (TIMP) (Liotta and Stetler-Stevenson, 1991; Ennis and Matrisian, 1994). Thus far, three TIMP genes (TIMP-1, TIMP-2 and TIMP-3) have been identified (Murphy *et al.*, 1981; Stetler-Stevenson *et al.*, 1989; Pavloff *et al.*, 1992). TIMP-1 is a glycoprotein with an apparent molecular size of 28.5 kDa and is produced by a variety of human tissues and many human tumour cell lines (Murphy *et al.*, 1981; Stricklin and Welgus, 1983; Dean *et al.*, 1992; Sato *et al.*, 1992). TIMP-1 inhibits MMPs by forming a 1:1 complex with activated MMP-1, MMP-3 and MMP-9 (Liotta and Stetler-Stevenson, 1991; Ennis and Matrisian., 1994). Although several studies have shown an inverse correlation between TIMP-1 levels and the metastatic potential of murine and human tumour cells (Schultz *et al.*, 1988; Khokha *et al.*, 1989; Alvarez *et al.*, 1990), we and others have demonstrated a direct correlation between TIMP-1 expression and clinical aggressiveness of colorectal cancer (Guillem *et al.*, 1990; Lu *et al.*, 1991; Zeng *et al.*, 1995). Recently, using Northern blot analysis of whole-tissue homogenates, we demonstrated a parallel overexpression of TIMP-1 and MMP-9 mRNA in colorectal cancer and liver metastases, suggesting co-regulation of these two genes (Zeng *et al.*, 1993). However, because of tissue cellular heterogeneity, the exact cellular origin of TIMP-1 and MMP-9 mRNA in colorectal cancer and liver metastases homogenates remained unclear. In the present study, *in situ* hybridisation analysis was used to investigate the cellular distribution of TIMP-1 and MMP-9 mRNA expression in human colorectal cancer and liver metastases.

Materials and methods

Tissue samples

All surgical samples used in this study were randomly obtained from the operating room immediately after resection. Eighteen primary colorectal cancers and corresponding normal mucosa and seven colorectal cancer liver metastases and matched normal liver were examined by Northern blot analysis. In addition, 26 primary colorectal cancers, three benign adenomas and ten liver metastases as well as corresponding normal adjacent tissue were examined by *in situ* hybridisation. Table I summarises the clinicopathological variables of these 26 primary colorectal cancers. The Surgical Pathological Laboratory of the Memorial Sloan-

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Table I Clinicopathological characteristics of 26 colorectal cancers and *in situ* MMP-9 and TIMP-1 hybridisation status

Case	Age	Sex	Location[a]	Diff[b]	Size (cm) (Max)	Dukes' stage	*In Situ* hybridisation TIMP-1	MMP-9
1	80	F	RE	M	6.5	C	+	+
2	65	F	RS	M	4.0	A	+	−
3	66	F	R	M	4.0	C	+	−
4	78	M	RS	M	4.8	D	+	+
5	71	M	RE	M	3.5	A	+	−
6	54	M	RE	P	7.0	D	+	+
7	81	F	S	P	3.2	C	+	+
8	55	F	R	M	6.0	B	+	+
9	64	F	S	M	2.5	B	+	−
10	63	M	R	M	5.0	C	+	+
11	72	F	RE	M	10.0	D	+	+
12	67	M	S	M	4.5	D	+	+
13	64	M	R	M	3.0	A	+	−
14	32	F	RC	M	4.5	D	+	+
15	77	M	R	M	3.2	B	+	−
16	47	M	S	M	6.0	D	+	+
17	75	M	RE	M	6.5	B	+	+
18	78	M	RE	M	7.5	C	+	+
19	74	F	R	M	3.6	A	+	+
20	62	M	R	P	10.0	D	+	+
21	62	F	R	M	9.0	D	+	+
22	86	M	R	M	6.0	B	+	+
23	50	M	L	M	3.0	D	+	+
24	69	F	L	M	3.7	C	+	−
25	71	F	R	M	4.5	A	+	−
26	74	F	RC	M	7.0	C	+	+

[a]R, right colon; L, left colon; S, sigmoid colon; RS, rectum–sigmoid colon; RE, rectum.
[b]Diff refers to tumour differentiation: M, moderate; P, poor.

Kettering Cancer Center (MSKCC) performed routine histopathological examination on the resected specimens using haematoxylin and eosin staining.

Tissue processing

Northern blot analysis Specimens obtained from the tumour edge, avoiding a necrotic centre, were quick-frozen in liquid nitrogen and stored at −80°C. Samples were handled and stored under strict RNAse-free conditions. Samples of normal mucosa were obtained from the surgical resection margin by sharply dissecting the mucosa off the muscularis.

In situ *analysis* Specimens were fixed in 4% paraformaldehyde overnight at 4°C, sequentially dehydrated with 50%, 70%, 85%, 95% and 100% ethanol and embedded in paraffin. To prevent RNA degradation, reagents were treated with diethyl pyrocarbonate (0.1%).

Preparation of RNA probes

Sense and antisense ^{35}S-labelled RNA probes were prepared from human TIMP-1 and MMP-9 cDNAs cloned in Bluescript KS (Stratagene). The TIMP-1 sense probe was generated by T3 polymerase following digestion with *Kpn*L, while the antisense probe was generated by T7 polymerase after *Eco*RI template digestion. For MMP-9 RNA probes, sense and antisense plasmid vectors were linearised with *Xba*L and *Eco*RI respectively. Transcribed RNA was labelled with [α-^{35}S]UTP (1200 Ci mmol^{-1}, Dupont, NEW). DNA template was removed by incubating with RNAse-free DNAse. Ten micrograms of tRNA was added and samples were extracted with phenol–chloroform. RNA probes were hydrolysed with sodium carbonate buffer, pH 10.2, for 60 min at 60°C, neutralised and ethanol precipitated. Probes were redissolved in 10 mM DTT at a final concentration of 1.5 ng μl^{-1} and stored at −20°C.

In situ *hybridisation*

Prehybridisation Paraffin sections 5–10 μm thick were dried at 42°C overnight, deparaffinised in xylene and then redehydrated by passage through graded ethanol. Slides were incubated with proteinase K (100 μg ml^{-1}) at room temperature for 7.5 min and then serially dipped in PBS, washed in 2 mg ml^{-1} glycine (w/v) and finally washed in freshly prepared triethanolamine buffer containing 0.25% acetic anhydride for 10 min.

Hybridisation The labelled probes were diluted in hybridisation buffer containing 50% formamide, 10% dextran sulphate, 0.1 M DTT and Denhardt's. ^{35}S-labelled RNA probe was added to each slide in a 25 μl volume of hybridisation mixture. The slides were covered with coverslip and incubated at 57°C overnight in a 50% formamide, 2 × SSC humidified chamber.

Post-hybridisation After hybridisation, coverslips were gently removed with 4 × SSC and slides washed at room temperature in 4 × SSC for 1 h and then in 2 × SSC, 50% formamide, 10 mM DTT solution for 40 min at 68°C. Slides were then treated with 20 μg ml^{-1} RNAse A in 20 mM Tris, pH 7.5, 0.5 M sodium chloride, 1 mM EDTA at 37°C for 30 min followed by washing in the same buffer without RNAse A for 30 min. The final washes were in 2 × SSC, 50% formamide, 1 mM DTT for 40 min at 68°C, 2 × SSC for 5 min at room temperature and 0.1 × SSC for 15 min at 50°C. Slides were dehydrated through graded ethanol and air dried.

Autoradiography Autoradiographic detection of the hybrids was carried out by dipping in Kodak NTB-2 emulsion (Eastman Kodak, Rochester, NY, USA) at 42°C under safe light and dried at room temperature for at least 2 h. Slides were placed in a light tight box at 4°C for 1–2 weeks. After exposure, slides were subsequently developed in Kodak D-19 developer for 4 min at 15°C, washed in water and fixed in Kodak fixer. Tissue sections were counterstained with haematoxylin and eosin (H&E) and mounted. Silver grains were visualised by dark-field microscopy (Nikon).

Northern blot hybridisation

RNA was extracted by the guanidium isocyanide/caesium chloride method as previously described (Zeng *et al.*, 1994*a*). Ten micrograms of total RNA was electrophoresed on 1.0% agarose–formaldehyde gel and blotted onto a Duralon-UV

membrane (Stratagene). TIMP-1 and MMP-9 DNA probes were radiolabelled with [^{32}P]dCTP by the random primer technique (Feinberg and Vogelstein, 1983). A 28S oligo probe was used as an internal control (Barbu and Dautry, 1989) for equal RNA loading and ethidium bromide staining of gels to confirm equal RNA transfer. Hybridised RNA was quantitated by LKB XL laser densitometry (Pharmacia LKB Biotechnology, Uppsala, Sweden). The results were expressed as a ratio of the average fold increase of transcript in tumours to that in the paired normal tissues. The difference in standardised TIMP-1 and MMP-9 between tumour and paired normal tissue was assessed by the paired *t*-test. Significant differences were analysed by Student's *t*-test.

Immunohistochemistry

Tissue sections adjacent to those used for *in situ* hybridisation were used for immunohistochemistry. Tissue macrophages were identified by peroxidase–antiperoxidase immunostaining using a monoclonal anti-macrophage antibody KP-1 (CD-68). This antibody is known to react specifically with tissue macrophages (Pulford *et al.*, 1989). The immunohistochemical staining technique was performed as previously described (Zeng *et al.*, 1994*b*).

Results

Expression of MMP-9 and TIMP-1 in colorectal cancer and liver metastases

Northern blot analysis of both MMP-9 and TIMP-1 in 18 primary colorectal cancers and matched adjacent normal mucosa as well as seven colorectal cancer liver metastases and matched normal liver revealed a parallel overexpression of MMP-9 and TIMP-1 mRNA in both human colorectal cancer and liver metastases (Figure 1). The mean tumour/normal (T/N) fold increases of TIMP-1 and MMP-9 in primary cancer were 9.1 ± 1.9 ($P<0.01$) and 12.9 ± 5.0 ($P<0.01$), while in liver metastases they were 11.1 ± 3.6 ($P<0.05$) and 3.47 ± 1.2 ($P<0.05$) respectively.

In situ *hybridisation of MMP-9 and TIMP-1 mRNA in primary colorectal cancer*

As seen in Figure 2, primary colorectal cancers show detectable signals for MMP-9 and TIMP-1 mRNA. All 26 colorectal cancers were positive for TIMP-1 expression, while only 18 out of 26 (69.2%) colorectal cancers were positive for MMP-9 (Table I). Intensity of staining varied between different cases and in different areas of the same tumour. In three adenomas and in corresponding normal mucosa, insignificant (background) quantities of MMP-9 and TIMP-1 mRNA were detected and the control sense probes for MMP-9 and TIMP-1 showed only background autographic signals (figures not shown). Since silver grain signals occurred only with the antisense probe and none were detected with the sense probe, the hybridisation was felt to be specific.

The correlation between MMP-9 and TIMP-1 mRNA expression in 26 colorectal cancer specimens and the relationship to clinicopathological variables is summarised in Table I. MMP-9 and TIMP-1 mRNA expression did not correlate with either the degree of tumour differentiation or the size of the tumours. Figure 3 shows MMP-9 mRNA expression in relation to Dukes' stage. MMP-9 mRNA expression progressively increased with advancing stages (Dukes' A to 'Dukes' D').

In situ *hybridisation of MMP-9 and TIMP-1 mRNA in colorectal cancer liver metastases*

In situ hybridisation analysis revealed a remarkably specific signal for MMP-9 (Figure 4) and TIMP-1 (Figure 5) mRNA transcripts in all ten liver metastases from colorectal cancer. No expression was detected in adjacent normal liver and hybridisations with MMP-9 and TIMP-1 sense probes were also negative (data not shown).

Distinct localisation of MMP-9 and TIMP-1 expression

Although both MMP-9 and TIMP-1 mRNA signals were strongest within stromal cells of tumours, the spatial distributions of MMP-9 and TIMP-1 mRNA were distinctly different. In order to compare directly the spatial expression of MMP-9 and TIMP-1, we hybridised serial sections of the same samples. As seen in serial sections (Figure 2), MMP-9 mRNA hybridisation resulted in a more focused signal, strongest within a small population of stromal cells encircling the tumour epithelial cells (Figure 2b and c), whereas TIMP-1 mRNA hybridisation produced a diffuse scattering signal (Figure 2e and f).

The general expression patterns of MMP-9 and TIMP-1 mRNA in liver metastases were similar to those in primary colorectal cancers. Comparison of MMP-9 and TIMP-1 mRNA expression in liver metastases indicates that, although hybridisation signals for MMP-9 and TIMP-1 transcripts were clearly localised in tumour stroma, MMP-9 and TIMP-1 mRNA have distinctly different patterns of distribution. As seen in Figure 4a and b, large numbers of MMP-9-positive cells were localised at the interface between the liver metastases lesion (LM) and surrounding normal liver parenchyma (NL). However, TIMP-1 mRNA was found throughout malignant tumour stroma (Figure 5a and b).

Higher magnifications of the area bordered by arrows in Figure 2b and e are shown in Figure 2c and f. The MMP-9 mRNA signals were seen in a small population of stromal cells encircling the tumour epithelial cells (Figure 2c), whereas TIMP-1-labelled cells are clearly spindle-shaped stromal cells (Figure 2f). When seen in liver metastases under higher magnification, MMP-9 mRNA-positive stroma cells (Figure 4b, arrow marked) have a circular pattern (Figure 4c), while TIMP-1-positive cells (Figure 5b, arrow marked) are spindle like in shape (Figure 5c).

To identify the cells expressing MMP-9 mRNA three colorectal cancer and two liver metastases samples were examined by immunohistochemical staining with the monoclonal antibody KP1 (CD68), known to be macrophage specific (Pulford *et al.*, 1989). Figure 6 shows that cells

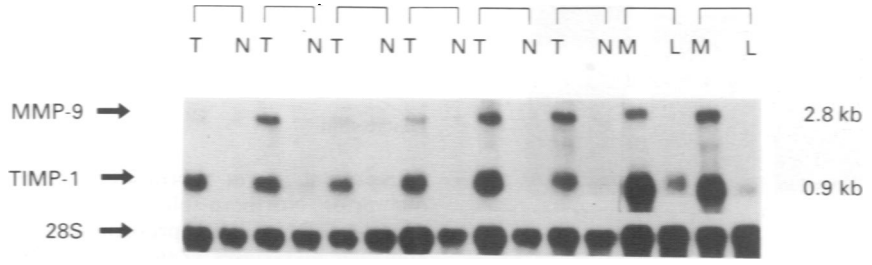

Figure 1 MMP-9 and TIMP-1 expression in colorectal cancer and liver metastases from colorectal cancer. MMP-9 and TIMP-1 cDNA probe co-hybridisation was performed from tumour and adjacent normal tissue (T, tumour; N, normal mucosa; M, liver metastases; L, normal liver tissue). Blots were subsequently stripped and reprobed with the 28S probe.

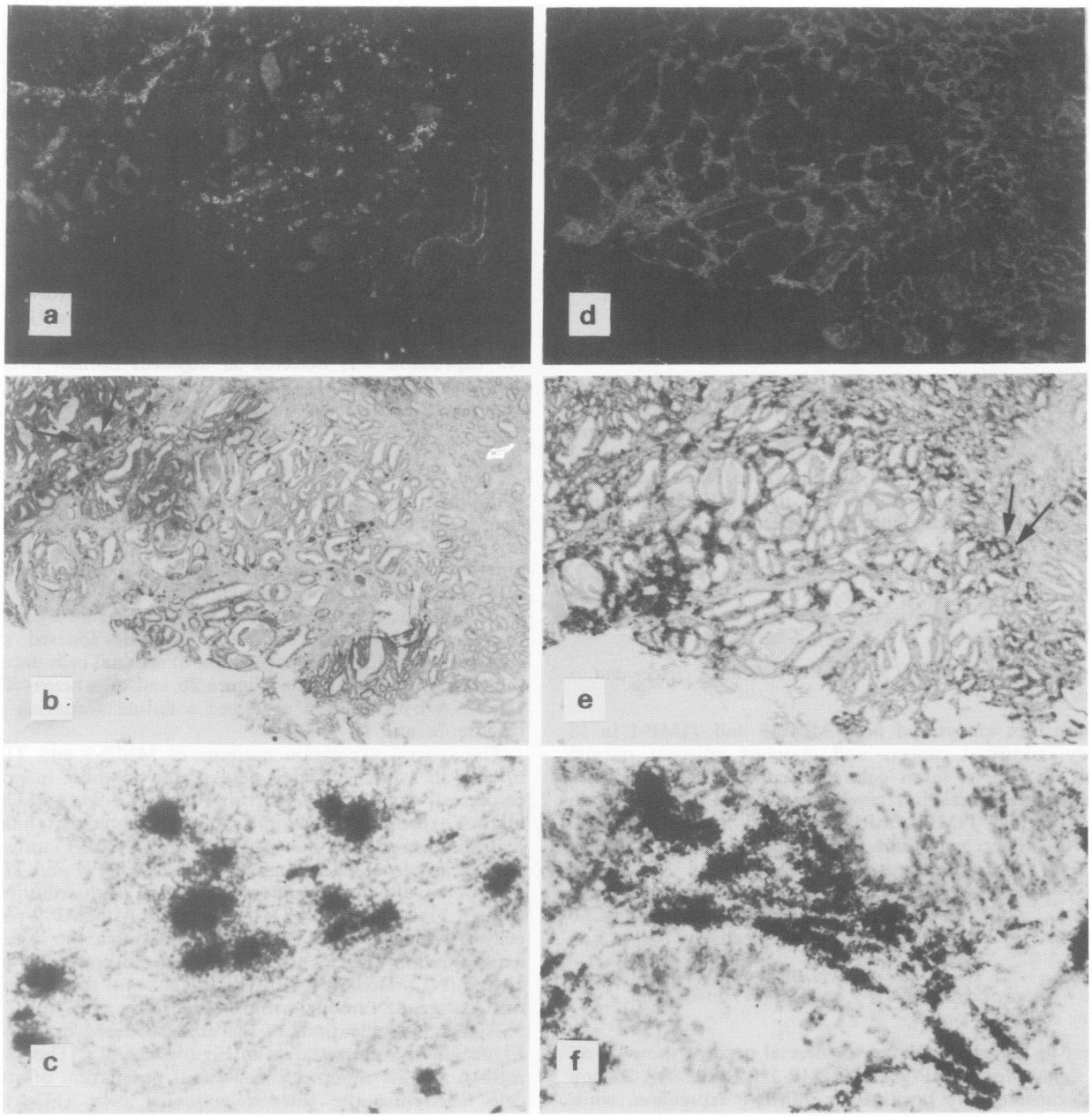

Figure 2 Microphotographs showing *in situ* hybridisation for TIMP-1 and MMP-9 in serial sections of primary human colon cancer. Bright-field and corresponding dark-field microphotography of MMP-9 mRNA (**a**, **b** and **c**) and TIMP-1 mRNA (**d**, **e** and **f**) detected by *in situ* hybridisation with ^{35}S-labelled antisense RNA probe. (**a** and **d**) Low-power dark-field view of colon cancer. (**b**, and **e**) Light-field view of the same areas of **a** and **d**. Both TIMP-1 and MMP-9 mRNA are located in malignant tumour stroma; no signal is observed in malignant epithelium. Higher magnifications of the area bordered by arrows in **b** and **e** are shown in **c** and **f**. MMP-9 mRNA was found in a circular pattern of stromal cells (**c**), while TIMP-1 mRNA localised primarily in fibroblast-like stromal cells (**f**).

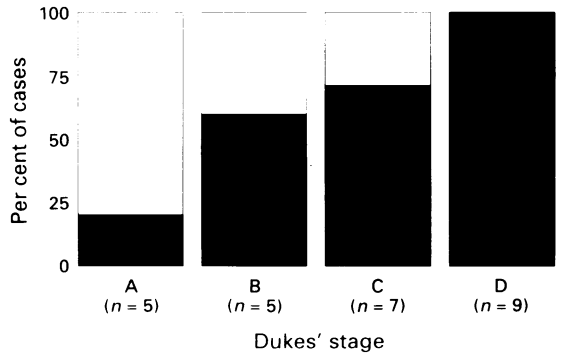

Figure 3 MMP-9 mRNA expression (■, positive; □, negative) in primary human colorectal cancer in relation to Dukes' stage.

positive for MMP-9 mRNA expression in liver metastases have a circular morphology (Figure 6a) and were identified as macrophages by immunostaining of adjacent sections with anti-macrophage antibody (Figure 6b). As noted, not all macrophages were positive for MMP-9 mRNA expression.

Discussion

Our present *in situ* hybridisation results are consistent with our prior Northern blot demonstration of MMP-9 and TIMP-1 mRNA overexpression in primary colorectal cancer and liver metastases (Zeng *et al.*, 1993). Our striking novel observation is the distinct spatial expression of MMP-9 and TIMP-1 mRNA within pericancer stroma in both primary colorectal cancer and liver metastases. Our findings provide

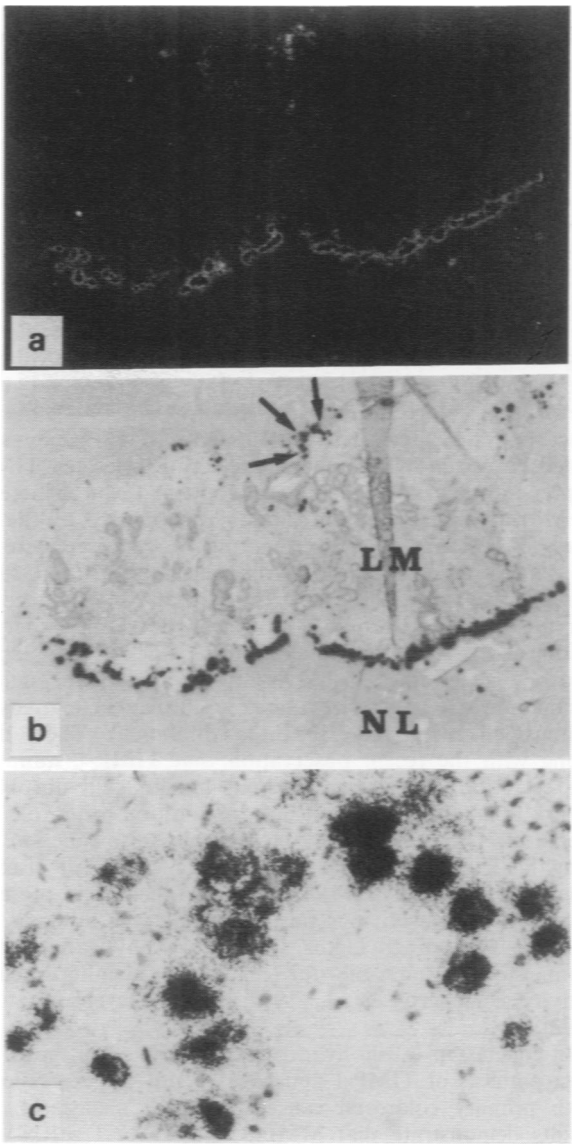

Figure 4 MMP-9 mRNA expression in liver metastases from colorectal cancer. Sections were hybridised with a ^{35}S-labelled antisense RNA probe specific for MMP-9 mRNA. Dark-field (**a**) and bright-field (**b**) microphotographs demonstrate MMP-9 mRNA expression limited to the interface between liver metastases lesion (LM) and normal liver (NL). Higher magnification of the area bordered by arrows in **b** suggests that MMP-9 mRNA-positive stroma cells have a circular morphology (**c**).

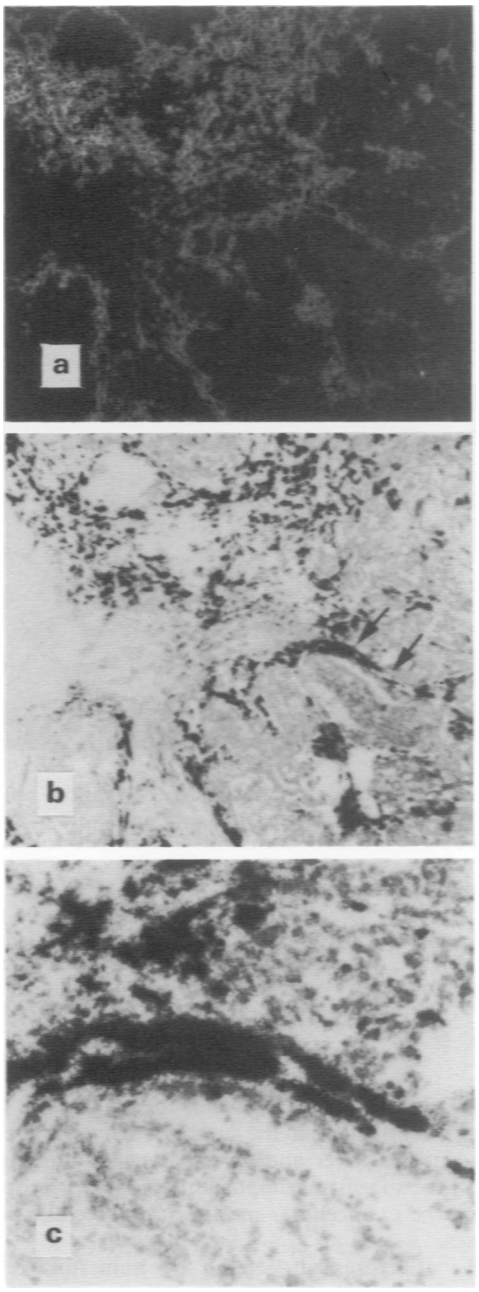

Figure 5 TIMP-1 mRNA expression in liver metastases from colorectal cancer. Sections were hybridised with a ^{35}S-labelled antisense RNA probe specific for TIMP-1 mRNA. Dark-field (**a**) and bright-field (**b**) microphotographs demonstrate TIMP-1 signals principally in malignant tumour stroma. No signal is observed in malignant epithelium. Higher magnification of the area bordered by arrows in **b** reveals TIMP-1 mRNA to be located in fibroblastlike stromal cells (**c**).

the evidence that MMP-9 and TIMP-1 mRNA are predominantly stromal in origin in both primary colorectal cancer and liver metastases. To our knowledge, this is the first report demonstrating a distinct spatial expression of MMP-9 and TIMP-1 mRNA in liver metastases from colorectal cancers.

MMP-9 is produced *in vitro* by a variety of cell types, including fibroblasts, endothelial cells, keratinocytes, macrophages and chondrocytes (Werb and Alexander, 1993). The synthesis of MMP-9 has been induced by SV-40 transformation of human fibroblasts (Wilhelm *et al.*, 1989). Under normal circumstances this enzyme is a major secretion product of tissue macrophages (Wilhelm *et al.*, 1989) and has been found in the granules of polymorphonuclear leucocytes and in cultured keratinocytes (Wilhelm *et al.*,1989). In human granuloma annulare and necrobiosis lipoidica diabeticorum, MMP-9 is expressed in eosinophils (Saarialho-Kere *et al.*, 1993). Our observation that MMP-9 mRNA may be produced by peritumour macrophages is consistent with a study (Pyke *et al.*, 1993) in which MMP-9 was found in stroma cells of colorectal cancers. Similar to colon cancers, *in situ* hybridisation studies have shown MMP-9 mRNA to be localised in the stroma of breast (Davies *et al.*, 1993*a*) and bladder cancers (Davies *et al.*, 1993*b*). In human skin cancer, six of nine squamous cell carcinomas expressed MMP-9 mRNA (Pyke *et al.*, 1992). However, none of the basal cell carcinomas, a rarely metastatic tumour, expressed MMP-9 mRNA (Pyke *et al.*, 1992).

The role for several metalloproteinases in colorectal tumorigenesis is supported by Newell *et al.* (1994) who demonstrated matrilysin (MMP-7) expression in both benign and malignant colorectal tumour. However, the expression of stromelysin-1 (MMP-3), stromelysin-3 (MMP-11) and MMP-2 was noted only in colorectal cancer. These findings suggest that MMP-7 expression is an early event and that MMP-3, MMP-11 and MMP-2 expression is

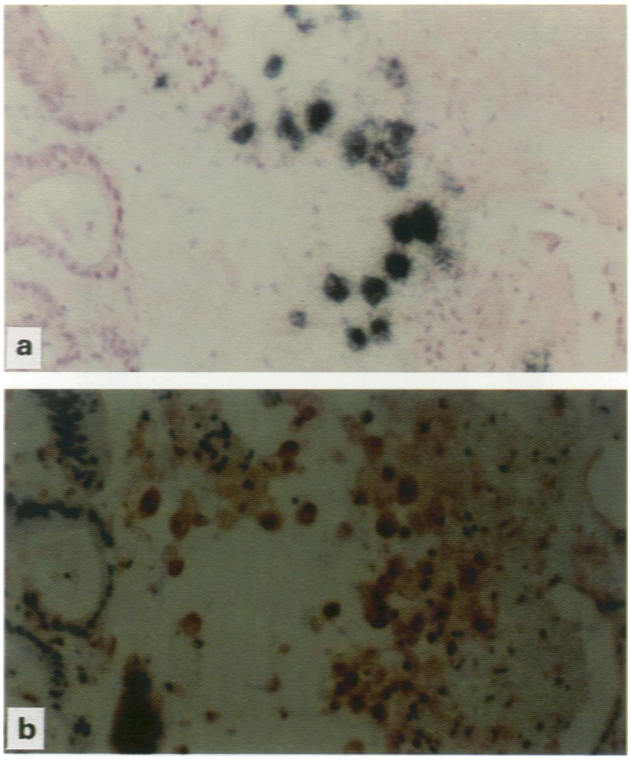

Figure 6 Identification of MMP-9 mRNA-positive cells. Serial sections of a liver metastases were hybridised with a ^{35}S-labelled antisense RNA probe specific for MMP-9 mRNA or stained with a macrophage-specific monoclonal antibody KP1 (CD68). (**a**) *In situ* hybridisation. Cells positive for MMP-9 mRNA have a macrophage-like morphology. (**b**) Immunohistochemical staining. MMP-9 mRNA-positive cells were identified as macrophages by immunostaining of adjacent sections. As noted, not all macrophages are positive for MMP-9 mRNA expression.

primarily a late event in colorectal tumorigenesis. The focal expression of MMP-9 at the interface between liver metastases and normal liver and its apparent macrophage origin reported here suggest an important role for macrophages in degrading the extracellular matrix of colorectal cancer liver metastases.

TIMP-1 is expressed *in vitro* by numerous cell types, including fibroblasts (Stricklin and Welgus, 1983), chondrocytes (Gavrilovic *et al.*, 1987) and endothelial (Herron *et al.*, 1988), and vascular smooth muscle cells (DeClerck, 1988). Analysis of human colorectal cancer specimen homogenates have demonstrated an overexpression of both TIMP-1 mRNA and protein when compared with normal colonic mucosa (Guillem *et al.*, 1990; Lu *et al.*, 1991; Zeng *et al.*, 1995). In this study, we have found that, in human colorectal cancer, TIMP-1 mRNA is located in fibroblast-like stromal cells. A previous study on human granuloma annulare and necrobiosis lipoidica diabeticorum demonstrated TIMP-1 mRNA expression in spindle-shaped cells (Saarialho-Kere *et al.*, 1993), suggesting that fibroblasts may serve as a source for TIMP-1. Our *in situ* hybridisation data are consistent with a study by Hewitt *et al.* (1991) which demonstrated increased protein expression of collagenase and TIMP-1 in colorectal cancers compared with adenomas and normal mucosa. The strongest immunohistochemical collagenase and TIMP signals were noted close to islands of neoplastic cells. Furthermore, in this study, the staining intensity in the invasive edge increased for collagenase but decreased for TIMP (Hewitt *et al.*, 1991).

Parallel overexpression of TIMP-1 and MMP-9 mRNA noted in primary colorectal cancer and liver metastases homogenates (Zeng *et al.*, 1993) suggests possible co-regulation of these two important genes *in vivo*. Although the expression of several MMPs (MMP-2,-3,-7,-9 and -11) is elevated in colorectal cancer (Newell *et al.*, 1994), the distinct pattern of cellular TIMP-1 and MMP-9 expression noted by *in situ* hybridisation suggests that elevated TIMP-1 expression may not be simply a response to local increases in MMP-9 expression. This notion is supported by the fact that TIMP-1 has growth-promoting properties (Gasson *et al.*, 1985; Avalos *et al.*, 1988; Bertaux *et al.*, 1991). Hayakawa *et al.* (1992) have found that TIMP-1 accounts for a significant portion of the growth factor activity of serum and is capable of stimulating a wide range of human and bovine cell lines, including those derived from tumours (human breast adenocarcinoma, erythroleukaemia, myelogenous leukaemia and Burkitt's lymphoma). These data, along with the recent observation that TIMP-1 stimulates the secretion of collagenase from human skin fibroblasts (Clark *et al.*, 1994), suggest that, in addition to its role as a metalloproteinase inhibitor, TIMP-1 may also function as a growth factor in the pathogenesis of a variety of diseases. The mechanism of TIMPs' multiple function is presently unknown. However, it is postulated that the domains responsible for growth factor activity are physically distinct from those responsible for regulating metalloproteinase activity (Docherty *et al.*, 1992; Stetler-Stevenson *et al.*, 1992).

In conclusion *in situ* hybridisation analysis has shown distinct patterns of TIMP-1 and MMP-9 mRNA expression in both primary colorectal cancer and liver metastases. Our results demonstrate that MMP-9 and TIMP-1 mRNA are produced not by colorectal cancer cells themselves, but rather by surrounding macrophages and fibroblast-like cells respectively. In addition, the distinct, non-juxtaposed pattern of MMP-9 and TIMP-1 expression suggests that TIMP-1 may not be produced simply in response to local elevations of MMP-9 and suggests that *in vivo* these genes are independently regulated. Further studies are needed to define the colorectal cancer–stroma cell interactions involved in the regulation of MMPs and TIMP productions.

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