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The plasminogen activator and matrix metalloproteinase systems in colorectal cancer: relationship to tumour pathology

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Abstract

The aim of this study was to determine the expression of proteinases and inhibitors from the matrix metalloproteinase (MMP) (MMPs 1, 2, 3, 9, tissue inhibitors of metalloproteinases (TIMPs) 1, 2) and plasminogen activator ((PA) urokinase (uPA), tissue type (tPA), uPAR, plasminogen activator inhibitors (PAIs) 1, 2) systems in colorectal cancer pathology by gelatin zymography, enzyme-linked immunosorbent assays (ELISAs) and quenched fluorescent substrate hydrolysis. The levels of all studied MMPs, uPA, uPAR, TIMP-1 and PAIs were significantly greater in tumour tissues than normal tissues. However, tPA and TIMP-2 were greater in normal colon ($P<0.05$, Mann–Whitney) e.g. PAI-1: tumour, median 14.9 (range 0.2–80.2) ng/mg total protein; normal, 2.1 (0.1–65.0). Tumour levels of several factors, in particular MMP-1 and PAI-1, correlated with pathology, i.e. Dukes' stage, differentiation, lymphatic or vascular invasion and tumour depth. The interactions between proteinase systems in colorectal cancer are complex and the balance between active proteinases and their inhibitors is important for extracellular matrix (ECM) degradation/remodelling at each stage of the metastatic cascade.

Keywords: Matrix metalloproteinases; Plasminogen activators; Tissue inhibitors of metalloproteinases; Plasminogen activator inhibitors; Colorectal cancer

1. Introduction

Extracellular matrix (ECM) degradation by proteinases occurs in both normal and pathological processes. In cancer, ECM degradation occurs at several stages of the metastatic cascade including local invasion, angiogenesis and extravasation. The proteinase systems primarily responsible for ECM degradation *in vivo* are matrix metalloproteinase (MMPs) and plasminogen activator (PA) systems; these proteinases have the combined ability to break down all ECM components.

The matrix metalloproteinase system consists of at least 20 human MMPs that are often divided into 5 groups based on their substrate specificity; gelatinases, collagenases, stromelysins, membrane type MMPs and other less well characterised MMPs [1–3]. Each MMP breaks down several ECM components and therefore normally MMPs are tightly regulated. Levels of regulation include their activation, the presence of specific tissue inhibitors of metalloproteinases (TIMPs) and at the level of gene expression. The members and properties of the MMP system have been extensively reviewed [1–3]. The plasminogen activator system (PAS) consists of two plasminogen activators (PAs), urokinase (uPA) and tissue type (tPA), which as their names suggest, activate plasminogen to the active serine proteinase plasmin. Plasmin can degrade ECM components directly, e.g. fibronectin and proteoglycans or indirectly by activating other proteinases, e.g. MMP-3. The other PAS components are a receptor for uPA (uPAR), which is thought to focus proteolysis *in vivo* and two plasminogen activator inhibitors PAI-1 and PAI-2. The PAS has also been reviewed elsewhere [4,5].

Although many studies have determined the expression and involvement of various proteinases in colorectal and other cancers, the majority have involved small patient numbers [6–9] and/or studied individuals [6,9,10–14], or a small number of proteinases and their inhibitors. [8,9,15–18] No previous study has determined the detailed expression of two proteinase systems

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in colorectal cancer and related expression to tumour pathology. Therefore, the aim of our study was to determine the levels of proteinases and inhibitors from the MMP (MMP-1, 2, 3, 9; TIMP-1,-2) and PA (uPA, tPA, uPAR, PAI-1, PAI-2) proteinase systems in 101 paired colorectal tumour and normal tissue samples. The secondary aim was to determine whether a correlation existed between the tumour levels of each factor and the tumour pathology.

2. Materials and methods

2.1. Patient and tissue samples

101 paired colorectal tumour and normal mucosal tissue samples (taken at a site 10 cm or more from the primary tumour) were collected immediately after surgical resection (April 1998–September 2001). Each sample was finely diced with a scalpel, homogenised (Polytron PT 2,100; Kinematica) in phosphate-buffered saline (PBS) for 5 min and centrifuged for 15 min at 5000 rpm. The homogenate was pipetted off and stored at −80 °C until analysis. The total protein concentration of each tissue sample was determined by the method of Oshini and Barr [19].

All colorectal tumours were classified according to their pathological staging: Dukes' stage, differentiation, tumour depth and whether the tumour had undergone lymphatic and/or vascular invasion.

2.2. Gelatin zymography

Gelatin zymography was performed on all samples as adapted from the method of Heussen and Dowdle [20]. In brief, tissue homogenates (50 μg protein) were electrophoresed down a 12% (w/v) sodium dodecyl sulphate (SDS) gel containing 0.1% gelatin at 200 V for 45 min (Mini protean plus; Biorad, Hemel Hempstead, UK). Gels were washed and incubated overnight as previously described [20,21]. Control gels were incubated with the MMP inhibitor, ethylenediamine tetraacetic acid (EDTA) (10 mM).

Quantification of the gels was performed using densitometry (GS-700; Biorad) linked to Molecular Analyst software (Biorad). The area and optical density (OD) of each latent and active band were determined for each sample.

2.3. Enzyme-linked immunosorbent assays (ELISAs)

MMP-1, MMP-3, TIMPs-1 and-2 (Biotrack ELISAs, Amersham Pharmacia Biotech, Buckinghamshire, UK) and uPA, tPA, uPAR, PAI-1 and PAI-2 (American Diagnositca, Axis Shield, Aberdeen, UK) protein levels were determined. Duplicate samples were diluted where necessary (1:3 TIMP-1; 1:10 uPA and PAI-1; 1:5 uPAR) and the ELISA performed following the assay protocol.

The microplate reader was set to 450 nm and linked to Revelation software to determine antigen concentrations (Dynatech Laboratories, Billinghurst, UK). Results were expressed as nanograms of antigen per milligram total protein (ng/mg) for all proteinases and inhibitors.

2.4. MMP-2 and MMP-9 activity assays

Total and endogenous MMP-2 and MMP-9 activity were assayed by gelatinase activity assays (Amersham Pharmacia Biotech, Buckinghamshire, UK). The activity assays were performed following the assay protocol [21]. In brief, all standards and samples where total MMP-2 or MMP-9 levels were to be measured were activated by incubating with amino phenyl mercuric acetate (APMA) (0.5 mM for MMP-2 and 1 mM for MMP-9) and samples where endogenous gelatinase activity was to be measured were incubated with assay buffer alone. The concentration (ng/ml) of total and endogenous active MMP-2 and MMP-9 in each colorectal tissue sample was determined using a microplate reader set at 405 nm. Final tissue levels were determined as ng/mg protein.

2.5. Quenched fluorescent substrate hydrolysis

Total MMP activity was determined for each sample by quenched fluorescent substrate hydrolysis (QFSH) [22] using the fluorescent substrate Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2, as we have previously described in Ref. [7]. Results were expressed as pM/min, i.e. the rate of substrate hydrolysis.

2.6. Statistical analysis

For comparisons between expression in colorectal tumour and normal tissue samples, the Mann–Whitney U test for non-parametric data with 95% confidence limits (CLs) was performed. For comparisons between the number of samples expressing latent and active MMP-2 and MMP-9 as determined by gelatin zymography, the χ^2 test was performed. Spearman's correlation coefficient was performed to determine whether a correlation existed between these components and clinical pathological staging (Dukes' stage, tumour depth, differentiation and vascular and lymphatic invasion). Any differences were considered to be statistically significant at the $P<0.05$ level.

3. Results

Of the 101 patients, there were 35 females and 66 males and their median age was 69 years with a range

from 25 to 93 years old (males 70.5 years (34–86 years) and females 68 years (25–93 years)).

The pathology of the excised tumour was five adenomas, 19 Dukes' A tumours, 36 Dukes' B, 38 Dukes' C, and 3 patients had distant metastatic disease ('Dukes' D'). For the 96 malignant tumours; 18 were described as being well differentiated, 63 moderately differentiated and 14 poorly differentiated; 25 tumours had undergone vascular invasion and 41 lymphatic invasion; finally, for tumour depth, there were 6 T_1 tumours, 18 T_2, 59 T_3 and 13 T_4.

3.1. Proteinase and inhibitor levels in colorectal tissues

3.1.1. MMP system

3.1.1.1. Gelatin zymography. Following gelatin zymography, four main lysis bands corresponding to the gelatinases were observed; 92 kDa latent MMP-9, 84 kDa active MMP-9, 72 kDa latent MMP-2 and 66 kDa active MMP-2. The proportion of tissue samples expressing each form of gelatinase is illustrated in Fig. 1 and the actual observed amounts are summarised in Table 1. In brief, latent MMP-9 and latent MMP-2 was observed in 99% of the tumour samples (100/101) and 92% of the normal tissue samples (91/99). Active MMP-9 and active MMP-2 were expressed by a significantly greater proportion of colorectal tumours than normal tissue samples (active MMP-9; 89% tumours (90/101) and 16% normal (16/99); active MMP-2: 97% tumours (98/101) and 56% normal (55/99); $P<0.05$, χ^2). The band sizes for both the latent and active forms of MMP-2 and MMP-9 were significantly greater in the tumour tissues than the normal colon (Table 1a).

3.1.1.2. Gelatinase activity assays. Total MMP-9 levels (latent plus active) and endogenous active MMP-9 levels were significantly greater in colorectal tumours

Table 1
(a) Latent and active gelatinase levels in colorectal tumour and normal tissue samples following gelatin zymography; (b) total and endogenous active gelatinase levels in colorectal tumour and normal tissue samples as determined by gelatinase activity assays[a]

	Tumour	Normal
(a)		
Latent MMP-9	11.8 (0–35.9)*	8.7 (0–23.1)
Active MMP-9	8.0 (0–30.3)*	0 (0–8.5)
Active/latent MMP-9	0.8 (0–1.8)*	0 (0–1.1)
Latent MMP-2	4.7 (0–12.7)*	3.8 (0–12.9)
Active MMP-2	4.9 (0–14.4)*	0.9 (0–5.6)
Active/latent MMP-2	0.9 (0–3.2)*	0.3 (0–1.4)
(b)		
Total MMP-9	17.4 (0.3–217.3)*	2.8 (0.2–35.3)
Endogenous MMP-9	0.4 (0–22.6)*	0.2 (0–5.7)
Total MMP-2	5.3 (0.2–63.2)	4.8 (0.8–55.1)
Endogenous MMP-2	0.2 (0–6.3)	0.06 (0–5.7)

*$P<0.05$ Mann–Whitney U. OD, optical density; MMP, matrix metalloproteinase.

[a] Levels are expressed as medians with the range in parentheses and values are in arbitrary units (OD of band *x* area).

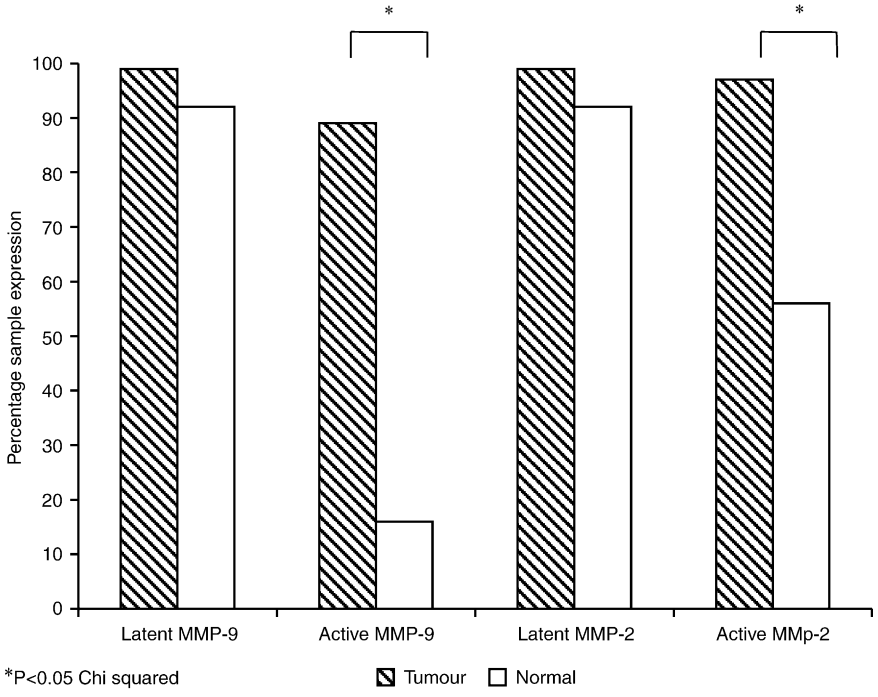

Fig. 1. Bar chart illustrating the differences in the percentage of tumour and normal tissue samples expressing the latent and active forms of the gelatinases, matrix metalloproteinase (MMP)-2 and MMP-9 as determined by gelatin zymography. A significantly greater proportion of tumour samples expressed the active form of both gelatinases than in normal colorectal tissue ($P<0.05$ χ^2).

than normal colon (Table 1b; $P<0.05$ Mann–Whitney). 86% of the tumour samples demonstrated measurable levels of endogenous active MMP-9 compared with 77% of the normal tissue samples.

Total and endogenous MMP-2 levels were also greater in tumour than normal colorectal tissues. However, the differences were not significant (Table 1b). 85% of the tumour samples expressed measurable levels of endogenous active MMP-2 compared with 92% of the normal colorectal samples.

3.1.1.3. Other MMPs. Total MMP activity, MMP-1 and MMP-3 levels were all significantly greater in the colorectal tumour samples than in the normal mucosa (Table 2; $P<0.05$ Mann–Whitney).

3.1.1.4. TIMP-1 and TIMP-2. TIMP-1 levels were significantly greater in tumour than normal tissue samples. However, TIMP-2 levels were significantly greater in normal colon (Table 2; $P<0.05$ Mann–Whitney).

3.2. PAS components

uPA, uPAR, PAI-1 and PAI-2 were all demonstrated to be at significantly greater levels in the colorectal tumour tissues. However, tPA levels were significantly greater in the normal colon (Table 3; $P<0.05$ Mann–Whitney).

3.2.1. Correlations between proteinase and inhibitor levels

There were several significant correlations ($P<0.05$ Spearman's correlation) between the levels of proteinases and inhibitors both within and between the two proteinase systems, MMP and PAS, and these are summarised in Table 4.

In brief, we have demonstrated correlations, in colorectal tumour and normal tissues, between (a) total MMP activity and individual MMP levels, (b) gelatinase levels between techniques (zymography and activity assays), (c) proteinases and inhibitors within a proteinase system and (d) proteinases and inhibitors between the two systems.

3.3. MMP and PA systems and colorectal cancer pathology Dukes' stage

A significant positive correlation was observed between total MMP-9 (activity assay), latent and active MMP-9, active MMP-9 and active MMP-2 (zymography), MMP-1, uPA, uPAR and PAI-1 tumour levels and the Dukes' stage of colorectal tumours (Fig. 2). In addition, the normal tissue levels of PAI-1 and PAI-2 demonstrated a significant positive correlation whilst the ratio/proportion of endogenous active MMP-2 and MMP-9 compared with the total levels (activity assays) demonstrated a significant negative correlation with Dukes' stage.

The tumour/normal tissue levels of total MMP-9 and MMP-1 also significantly correlated with the Dukes' stage.

3.4. Differentiation

Although there were several trends in increasing tumour levels of several proteinases with the tumour differentiation the only factors demonstrating a significant positive correlation were PAI-1 levels in normal colorectal tissue and the TIMP-2 tumour:normal ratio.

3.5. Tumour depth

The tumour levels of MMP-1 and both tissue levels of PAI-1 significantly correlated with the tumour depth.

3.6. Vascular and lymphatic invasion

Tumour levels of total MMP-9, active MMP-9, MMP-1, uPA and PAI-1, normal tissue PAI-1 levels and tumour:normal ratio for latent MMP-2 (zymography) and MMP-1 demonstrated significant positive correlations with whether the tumour had undergone

Table 2
MMP (1 and 3) and TIMP (1 and 2) levels in colorectal tumour and normal tissue samples as determined by ELISA (ng/mg protein) and total MMP activity (pM/min) as determined by QFSH[a]

	Tumour	Normal
MMP-1	10.6 (0.1–86.9)*	0.2 (0–15.2)
MMP-3	2.9 (0.1–69.1)*	0.9 (0–57.0)
TIMP-1	37.0 (1.5–351.1)*	11.4 (1.1–107.0)
TIMP-2	2.2 (0–21.9)	3.0 (0–23.5)*
Total MMP activity	8,725 (793–174,400)*	5,710 (328–354,650)

*$P<0.05$ Mann–Whitney U. TIMP, tissue inhibitors of metalloproteinases; ELISA, enzyme-linked immunosorbent assays; QFSH, quenched fluorescent substrate hydrolysis.

[a] Levels are expressed as medians with the ranges in parentheses.

Table 3
Tissue levels of PAS components in colorectal tumours and normal mucosa following ELISA[a]

	Tumour	Normal
uPA	1.6 (0–6.7)*	0.1 (0–2.6)
tPA	3.4 (0.5–35.2)	6.8 (1.0–37.5)*
uPAR	2.5 (0.2–72.9)*	0.6 (0–7.0)
PAI-1	14.9 (0.2–80.2)*	2.1 (0.1–65.0)
PAI-2	1.3 (0- 25.3)*	0.6 (0–7.0)

*$P<0.05$ Mann–Whitney U. PAS, plasminogen activator system; uPA, urokinase plasminogen activator; tPA, tissue type plasminogen activator; PAI, plasminogen activator inhibitor.

[a] Levels are expressed as median levels with the range in parentheses (ng/mg protein).

Table 4
Illustrating the significant correlations (positive and **negative**) between the levels of proteinases and inhibitors in colorectal tumour and normal tissues

Factor	Tumour	Normal
RSH	T9, **T2**, A9, MMP-3, TIMP-1, uPA, **PAI-2**	**MMP-1**
Total MMP-9	RSH, E9, T2, L9, A9, L2, A2, MMP-1, TIMP-2, uPA, uPAR, PAI-1	E9, TIMP-1
End MMP-9	T9, T2, E2, MMP-1, PAI-1	T9, T2, E2, **uPA, PAI-1, PAI-2**
Total MMP-2	**RSH,** T9, E9, E2, L9, A9, L2, A2, MMP-1, TIMP-2, uPAR, PAI-1, PAI-2	E9, E2, L2, MMP-1, TIMP-2, uPAR,
End MMP-2	E9, T2, MMP-1, uPAR, PAI-1, PAI-2	E9, T2, L9, MMP-1, **MMP-3, uPA**
Latent MMP-9	T9, T2, A9, L2, A2, uPA	L2, A2, uPA, PAI-1
Active MMP-9	RSH, T9, T2, L9, L2, A2, MMP-1, uPA, uPAR, PAI-1	A2, uPA, PAI-1
Latent MMP-2	T9, T2, L9, A9, A2, MMP-3, uPA, uPAR, PAI-1	T2, L9, A2, uPA, uPAR, PAI-1 PAI-2
Active MMP-2	T9, T2, L9, A9, L2, MMP-1, uPA, uPAR, PA-1	L9, A9, L2, uPA, PAI-1, PAI-2
MMP-1	T9, E9, T2, E2, A9, A2, MMP-3, uPA, uPAR, PAI-1	**RSH,** T2, E2, uPAR
MMP-3	RSH, L2, MMP-1, TIMP-1, uPA	**E2**, TIMP-1, TIMP-2, uPA, uPAR
TIMP-1	RSH, MMP-3, uPA, **PAI-2**	T9, MMP-3, TIMP-2, uPA
TIMP-2	T9, T2	T2, MMP-3, TIMP-1, uPAR
uPA	RSH, T9, A9, L2, A2, MMP-1, MMP-3, TIMP-1, uPAR, PAI-1	E9, E2, L9, A9, L2, A2, MMP-3, TIMP-1, uPAR, PAI-1, PAI-2
tPA	–	PAI-2
uPAR	T9, T2, E2, L9, A9, L2, A2, MMP-1, uPA, PAI-1, PAI-2	T2, L2, MMP-1, MMP-3, TIMP-2, uPA, PAI-1, PAI-2
PAI-1	T9, E9, T2, E2, A9 L2, A2, MMP-1, uPA, uPAR	E9, L9, A9, A2, L2, uPA, uPAR, PAI-2
PAI-2	**RSH,** T2, E2, **TIMP-1**, uPAR	E9, L2, A2, tPA, uPA, uPAR, PAI-1

RSH, rate of substrate hydrolysis. Gelatinase activity assays: T9, total MMP-9; E9, endogenous active MMP-9; T2, total MMP-2; E2, endogenous active MMP-2. Gelatin zymography: L9, latent MMP-9; A9, active MMP-9; L2, latent MMP-2; A2, active MMP-2.

lymphatic invasion, the levels of all these factors were greater in samples that had undergone lymphatic invasion than those that had not (Fig. 3a).

A significant positive correlation was also observed with tumour PAI-2 levels and normal PAI-1 levels with vascular invasion (Fig. 3b). However a significant negative correlation was observed with tumour PAI-1 levels and both tumour and normal tissue levels of endogenous MMP-9 and the ratio of endogenous MMP-9 compared to total MMP-9 with vascular invasion.

4. Discussion

Several previous studies have determined the presence of individual proteinases/proteinase systems in colorectal and other human cancers, but only two studies have determined the simultaneous expression of components of both the MMP and PA proteinase systems in colorectal cancer. Saito and colleagues determined immunohistochemical staining of uPAR and MMP-9, and Inuzuka determined uPA, MMP-3 and MMP-9 expression. [17,23] Therefore to our knowledge, this is the first study to determine the detailed involvement of proteinases and inhibitors from both the MMP and PA systems in human colorectal cancer, correlating the levels of each factor with the other measured factors and with the tumour histopathology.

Similar to previous studies on proteinase expression in human colorectal cancer we have demonstrated a significant up-regulation of proteinases MMP-1,-2,-3,-9, uPA and uPAR, and inhibitors TIMP-1, PAI-1 and PAI-2 in colorectal tumour tissue compared to normal colon. [7,9,13,21,24–26] Normal mucosal tissue levels of tPA [27] and TIMP-2 were greater than those observed in tumour tissue. However this is the first study to determine the tissue levels of these factors simultaneously.

Colorectal tumours like other human tumours are heterogeneous in nature and this may in part account for the observed variations in proteinase levels between samples. However the important finding is that tumour tissue demonstrated greater proteinase levels than normal colorectal tissue, implying that tumours with high proteinase levels are capable of causing proteolytic degradation of the surrounding ECM and this may affect the tumours' potential for invasion and metastasis.

ECM degradation by proteinases in both normal and pathological processes is tightly regulated, primarily by specific proteinase inhibitors. However in cancer these proteinases are not 'normally' regulated. Complex

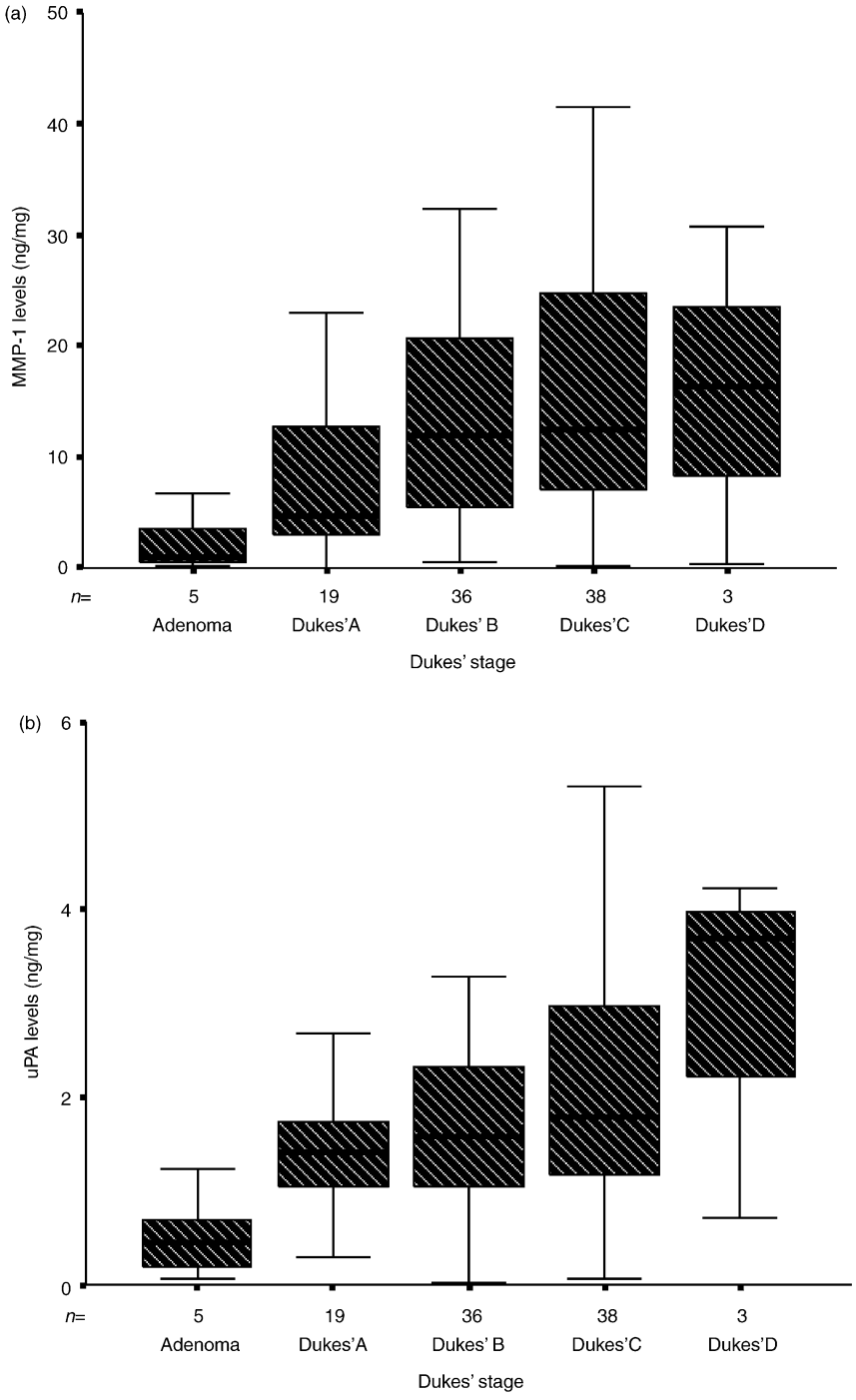

Fig. 2. Box plots illustrating the significant positive correlation between (a) matrix metalloproteinase (MMP)-1 and (b) urokinase (uPA) levels (ng/mg protein) with the Dukes' stage of the tumour as determined by enzyme-linked immunosorbent assays (ELISA) ($P < 0.05$ Spearman's correlation coefficient). The horizontal bold line within the box corresponds to the median value and the whiskers to the interquartile ranges.

interactions occur between proteinases and inhibitors and between proteinase systems, maybe representing a proteinase cascade, and this study has demonstrated many significant correlations between proteinases and inhibitors both within and between the MMP and PA systems (summarised in Table 4). However, it is the balance between the levels of active proteinases and their inhibitors that is important in determining whether ECM degradation/remodelling will occur at each stage of the metastatic cascade.

The technique of QFSH determines total MMP activity within a sample and there was a significant positive correlation between the tumour levels of several MMPs (MMP-2,-3 and-9) with the observed total MMP activity in tumour tissue, as would be expected. A positive correlation was also observed with uPA, the major

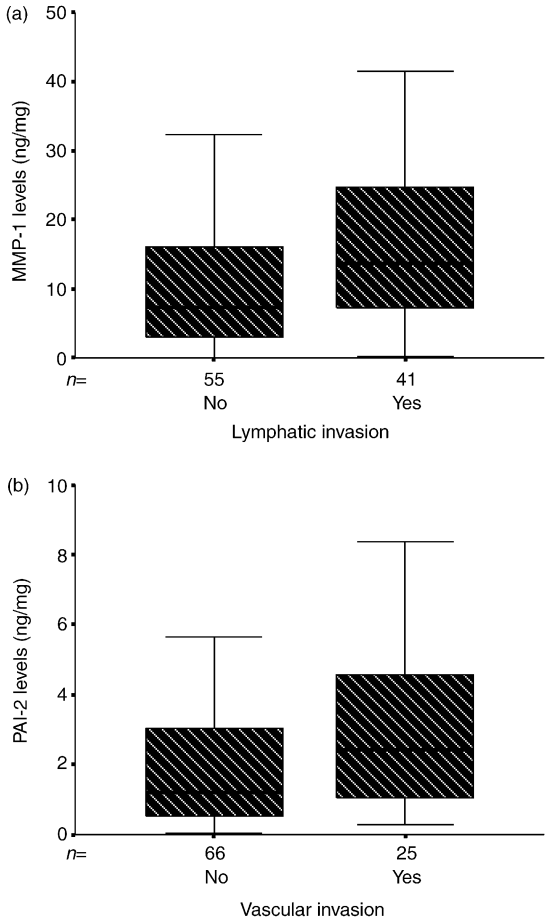

Fig. 3. Box plots illustrating the significant positive correlation between (a) matrix metalloproteinase (MMP)-1 and lymphatic invasion and (b) PAI-2 and vascular invasion in colorectal tumour tissue ($P < 0.05$, Spearman's correlation coefficient). The horizontal bold line within the box corresponds to the median value and the whiskers to the interquartile ranges.

PA expressed in tumour tissue. uPA activates plasminogen to plasmin, which is known to activate several MMPs including MMP-3. There are however other MMPs important in human colorectal cancer progression e.g. MMP-7 whose presence would also contribute to cleaving the fluorescent substrate which were not studied. In addition, there was also a significant correlation between the rate of substrate hydrolysis and the proteinase inhibitors TIMP-1 (positive) and PAI-2 (negative).

Two techniques were used to determine gelatinase levels in colorectal tissue, the semi-quantitative technique of gelatin zymography and the quantitative gelatinase activity assays. A significant positive correlation was observed in gelatinase levels between these techniques in both colorectal tumour and normal tissue samples.

This study has demonstrated a significant positive correlation between the tumour tissue levels of several proteinases and inhibitors with the tumour pathology including the Dukes' stage, tumour differentiation, tumour depth and whether the tumour had undergone lymphatic and/or vascular invasion at the time of resection (Fig. 3). The main factors consistently correlating to tumour pathology were MMP-1, uPA and PAI-1 in tumour tissue and PAI-1 in normal colon. There was also a strong positive correlation in colorectal tumour tissues between the levels of these factors, MMP-1, uPA, uPAR and PAI-1 (Table 4). Previously, several MMP system components have correlated with the pathology, for example, MT-MMP levels with vascular invasion, [6,15] MMP-7 with nodal and distant metastasis, [11] MMP-1 with tumour depth [14] and vascular invasion [10] and TIMP-1 with vascular and lymphatic invasion. [28] Similarly, for PAS components, uPA and uPAR levels have been shown to correlate with distant metastases [29]. However, the majority of studies have either not found a correlation or have not correlated tumour proteinase or inhibitor expression with tumour pathology. Several groups have demonstrated a correlation between proteinase and inhibitor levels with outcome, for example uPA and uPAR levels with distant metastases [29].

In addition, other groups have demonstrated a significant correlation between proteinase and inhibitor levels with outcome, i.e. disease-free and overall survival. In particular with components of the PAS, PAI-1, [30] uPA, [13,30] and uPAR [12,26] have all been shown to correlate with survival.

The possible reasons for discrepancies in proteinase and inhibitor expression between studies (tissue expression and correlation to tumour pathology) are the sample size of the study populations, the methods for tissue disaggregation, the actual laboratory techniques employed and the enzyme specificities within the same techniques.

The clinical relevance of these findings is difficult to extrapolate. It is possible that aggressive cancers could be identified more accurately by recognition of these proteinase systems and give us a better lead as to the use of anticancer therapies such as radiotherapy. Better understanding of the pathophysiology might also be integrated into the response to radiotherapy or chemotherapy. Distant control of proteinases and their inhibitors seems a distant possibility.

In summary, there are several proteinase systems known to be involved in colorectal tumour invasion and metastasis and this study has concentrated on two of these systems, the MMP and the PAS. Differential levels of both MMP and PA system components were observed in and between colorectal tumour and normal tissues. A significant correlation was observed between the tissue levels of various proteinases and inhibitors and it would seem that there are strong interactions between these two systems which may reflect a complex proteinase cascade. However, it is the balance in the

levels of active proteinases and inhibitors in these and other systems that is important in controlling ECM degradation and remodelling in colorectal cancer invasion and metastasis.

In addition, a significant positive correlation was observed between tumour levels of proteinases and inhibitors and colorectal cancer pathology, in particular MMP-1 and PAI-1, and with patient follow-up, these results may be of prognostic value.

It is therefore important to identify proteinase and inhibitor profiles from different proteinase systems to determine their overall involvement in colorectal cancer progression.

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