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Expression of matrix metalloproteinase 9 (MMP-9) and tissue inhibitor of metalloproteinases 1 (TIMP-1) by colorectal cancer cells and adjacent stroma cells – Associations with histopathology and patients outcome

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ABSTRACT

Aim: To elucidate cellular features accountable for colorectal cancers' (CRC) capability to invade normal tissue and to metastasize, we investigated the level of the collagenase matrix metalloproteinase 9 (MMP-9) and its physiological inhibitor tissue inhibitor of metalloproteinases 1 (TIMP-1) in cancer cells and supporting stroma cells of CRC.

Methods: Immunoreactivity of MMP-9 and TIMP-1 by carcinoma cells, lymphocytes and fibroblasts in archival specimens of paraffin-embedded primary tumours were retrospectively associated with outcome in 340 consecutive patients completely resected for CRC stages II–IV and subsequently treated with adjuvant 5-fluorouracil.

Results: Expression of MMP-9 by carcinoma cells was demonstrated in 9% of specimens without association to recurrence free survival (RFS) (HR = 1.0; 95% CI: 0.6–1.8; $P = 0.9$) or overall survival (OS) (HR = 0.9; 95% CI: 0.5–1.6; $P = 0.6$). TIMP-1 expression by carcinoma cells, which appeared in 64% of the specimens, was inversely related with RFS (HR = 1.3; 95% CI: 0.9–1.8; $P = 0.08$) and OS (HR = 1.5; 95% CI: 1.1–2.1; $P = 0.02$). Expression of TIMP-1 by fibroblasts at the invasive border was directly related to RFS (HR = 0.7; 95% CI: 0.6–0.9; $P = 0.02$) and OS (HR = 0.7; 95% CI: 0.6–1.0; $P = 0.05$). Expression of MMP-9 by lymphocytes correlated significantly with the degree of peritumoural inflammation ($P = 0.02$) but not with RFS (HR = 0.9; 95% CI: 0.7–1.1; $P = 0.2$) or OS (HR = 0.8; 95% CI: 0.7–1.0; $P = 0.07$).

Conclusion: TIMP-1 in cancer cells is associated with poor prognosis independent of its function as inhibitor of MMP-9. MMP-9 and TIMP-1 are important mediators of the host–cancer cell interaction in the tumour microenvironment with significant influence on the histopathology and on prognosis of CRC.

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1. Introduction

Colorectal cancer is the fourth most common malignant tumour in Western Europe and Northern America affecting 7% of the population and ranks as the second leading cause of cancer-related mortality.[1]

The matrix metalloproteinases are a group of proteolytic enzymes that collectively mediate degradation of fibrillar components of the extracellular matrix during maintenance and remodelling of tissue in physiological and pathological conditions such as growth, involution, inflammation, wound healing and cancer progression.[2–17]Matrix metalloproteinase 9 (MMP-9), also termed gelatinase B or the 92-kDa type IV collagenase, is an extracellular protease that specifically degrades collagen type IV, which is the major structural component of basement membranes.[13] MMP-9 is particularly important in the context of cancer, as it allows cancer cells to infiltrate the adjacent stromal compartment, thereby facilitating a critical early step in the metastatic cascade.[2,4–6,9,11,12,14,16–19] Moreover MMP-9 activates various growth factors and angiogenesis inhibitors like angiostatin with significance to tumour progression indicating a role as a general molecular switch in the microenvironment.[20] Indeed an increased MMP-9 level found in malignant colorectal tumours[2,4–7,9,16–18,21–23] compared to adjacent normal epithelia has been associated with advanced disease stage at the time of diagnosis[6,12,18] and with poor prognosis.[2,4,5,9,11,14,16,17,19]

MMP-9 activity is tightly regulated at various levels including being secreted as an inactive zymogen that requires removal of an amino-terminal domain in order to acquire extracellular enzyme activity.[24] The secreted soluble glycoprotein tissue inhibitor of metalloproteinases 1 (TIMP-1) is the main physiological inhibitor of MMP-9, as it forms high-affinity, non-covalent, but essentially irreversible 1:1 complexes with the active proteinase.[2–4,9,10,25]

The previous observations that high levels of TIMP-1 in the primary tumour[2–4,6,9–11,23,25] and in plasma of colorectal cancer patients[26–28] are correlated with poor outcome[2–4,9,25–27] was unexpected considering the well-established role of TIMP-1 as an inhibitor of MMP-9-mediated matrix degradation during tumour cell invasion. However, as opposed to the invasion controlling effect of TIMP-1 linked to MMP-inhibition, recent studies have demonstrated that TIMP-1 possesses additional functions including enhancing of malignant transformation[29], stimulation of cell growth[30] and inhibition of apoptosis[31], as well as promotion of migration, invasion and angiogenesis[32] indicating a potential tumour-promoting role of TIMP-1 in the early stages of tumourigenesis.[31] These observations suggest that TIMP-1 actually plays dual roles in cancer progression.

Detailed studies on the underlying mechanisms of cancer invasion into the supporting connective tissue are complicated by the fact that supporting stromal cells (e.g. fibroblasts, macrophages, granulocytes and lymphocytes) alter their expression of matrix-degrading enzymes in neoplastic conditions. In response to colorectal cancer cell invasion the stromal cells express matrix-degrading enzymes more frequently than the cancer cells themselves[8,15,33–37], suggesting that matrix proteinases derived from the peritumoural stroma may be significantly more involved in tumour invasion than previously recognised.[8,15,33–37]

The net matrix-degrading activity in a tumour depends on the ratio between MMPs and TIMPs[2–4] and the biological function of these molecules may differ whether they are expressed by cancer cells or by stromal cells.[8,15,33–37] These reflexions argue for a comparative study of the significance of MMP-9 and TIMP-1 expression in relation to prognosis of colorectal cancer, considering the cellular site of expression of the mentioned molecules.

In order to elucidate some of the cellular features accountable for the colorectal cancers' capability to invade normal tissue and to metastasize we studied the immunoreactivity of MMP-9 and TIMP-1 in carcinoma cells and in cells of the supporting stroma and inflammatory cells in archival specimens of paraffin-embedded primary tumours. These results were associated with the histopathological characteristics of the primary tumour and with the outcome of 340 patients, who were completely resected of colorectal cancer stages II–IV and subsequently, consecutively selected for adjuvant 5-fluorouracil treatment.

2. Patients and methods

2.1. Patients and chemotherapy

This study included formalin-fixed paraffin-embedded tissue blocks from 340 patients who were completely resected for colorectal adenocarcinomas stages II–IV and subsequently, consecutively selected for adjuvant chemotherapy at Department of Oncology, Rigshospitalet, University of Copenhagen during the period from February 1996 to December 2003.

The included stage II cancer patients received chemotherapy because of additional risk factors such as bowel perforation, perineural tumour growth or vascular invasion. The stage IV cancer patients mainly had resectable liver metastases. Rectal cancers treated with downstaging-radiation therapy were not included in this study. Adjuvant treatment was given according to the Mayo regimen, including bolus infusion of 5-FU (425 mg/m^2) and isovorin (10 mg/m^2) for 5 d, repeated every 4 weeks, for six courses. Data on clinical and pathological characteristics and on chemotherapy were obtained from surgery, pathology and oncology records. Latest follow-up on survival data was September 2007 (censoring date) using The Danish Register of Cause of Deaths. The study was approved by the local research ethics committee (KF01-201/03, 01-286965).

2.2. Tumour specimens and histopathology

Archival formalin-fixed and paraffin-embedded tumour samples were collected from the pathology archives of the hospitals referring cancer patients to the Department of Oncology at Rigshospitalet.

Amongst the 352 tumour specimens requested, 12 were not accessible either due to surgery in other countries or to loss during relocation of tissue archives. From 340 tumour

specimens, tissue blocks containing the invasive border of the primary tumour were selected, and 4 μm sections were cut and prepared for immunostaining. One section from each block was stained by routine haematoxylin and eosin for histopathological reevaluation including tumour type (WHO 2001), pT-stage (IUCC 5th edition), vascular and perineural invasion, focal necrosis, degree of differentiation, lymphocyte infiltration (inflammation) and tumour cell-budding. Tumour cell-budding was defined as the presence of at least 10 groups of 1–4 tumour cells dissociating from the main tumour at the invasive front observed at a magnification of ×200. Tumour cell-budding was categorised as either absent or present.

2.3. Immunohistochemistry for MMP-9 and TIMP-1

The slides were deparaffinized together with antigen retrieval in Dako PT module at pH 9.0 and at 97 °C for 20 min. using the target retrieval high pH 2 solution and subsequently blocked with peroxidase for 5 min in order to reduce non-specific staining. The sections were incubated for 20 min at room temperature with monoclonal antibodies raised against MMP-9 (clone 15W2 NCL-MMP-9[38], dilution 1:50, Novocastra, UK) or TIMP-1 (clone VT-7 as previously described[39]) and visualised using the Dako EnVision Flex+ technique (DAKO, Glostrup, Denmark) according to the manufacturer's instruction using diaminobenzidine as a chromogen. Negative controls were performed by omitting the primary antibodies and by application of an isotype-matched non-reactive immunoglobulin. Slides were counterstained with Mayer's haematoxylin.

MMP-9 and TIMP-1 immunoreactivity was evaluated semiquantitatively. Staining intensity was assessed for both MMP-9 and TIMP-1, using the scale 0: no staining, 1: faint ambiguous staining, 2: partly staining of cytoplasm and 3: strong staining of cytoplasm.

MMP-9-staining intensity was evaluated separately in cancer cells and lymphocytes at the invasive front, whereas TIMP-1 staining intensity was assessed separately in benign epithelial cells, in neoplastic epithelial cells and in fibroblasts inside the tumour as well as at the invasive border. The proportions of TIMP-1 positive cancer cells were scored as 0 (⩽10%), 1 (11–50%), 2 (51–75%) or 3 (>75%). Tissue specimens were analysed blinded to all other analyses and to the clinical information.

2.4. Statistics

Associations between biomarker distributions and clinicopathological parameters were evaluated using non-parametric Mann–Whitney *U*-test or Kruskal–Wallis test, followed by a Wilcoxon test for trend across ordered groups where appropriate. Survival time was calculated from the time point of the complete resection of the tumour. Efficacy variables were relapse free survival (RFS), defined as time from resection to relapse of primary disease or death, whichever occurred first, and overall survival (OS) defined as time from primary resection to death from any cause. The distributions of RFS and OS were evaluated using the actuarial Kaplan–Meier method. Univariate associations were tested using log-rank statistic. Cox proportional hazard modelling was applied to evaluate the association of outcome with clinicopathological and biomarker characteristics. The interaction between biomarkers expression by carcinoma cells for their association to outcome was evaluated using Cox analysis. Graphical methods were used to ascertain underlying model assumptions as proportional hazards. Two sided $P < 0.05$ was regarded statistically significant. Statistics were performed with Statistica software (Statsoft Inc., Tulsa, OK, USA).

3. Results

3.1. Clinicopathology and MMP-9 and TIMP-1 immunoreactivity

Tumour tissue MMP-9 or TIMP-1 immunoreactivity was assessed in 96% (325/340) of the collected tumour specimens. Staining failed in the remaining archival samples due to scarcity of cancer tissue.

3.1.1. Carcinoma cells

The distributions of MMP-9 and TIMP-1 immunoreactivity in carcinoma cells according to clinical and pathological characteristics are displayed in Table 1. Immunoreactivity of MMP-9 in carcinoma cells appeared in 9% (29/319) of specimens and was especially marked at peripheral tumour borders in deeply invading nests, whereas only a faint positive reaction was observed in superficial tumour areas (Fig. 1). Immunoreactivity of MMP-9 in carcinoma cells did not significantly covariate with clinical or histopathological characteristics (Table 1).

Immunoreactivity of TIMP-1 was generally strong and diffuses in the cytoplasma of carcinoma cells in 64% (203/318) of the specimens (Fig. 2; Table 1). Significantly more colon carcinomas than rectal carcinomas contained cancer cells having immunoreactivity of TIMP-1 ($P = 0.04$), whereas this had no association with other clinicopathological parameters (Table 1).

3.1.2. Normal epithelium

The normal colorectal epithelium essentially lacked immunoreactivity of MMP-9, whereas the supranuclear cytoplasmic region stained positive for TIMP-1 in 2% (4/246) of the specimens.

3.1.3. Stroma cells

Inflammatory cells, including plasma cells, a portion of the lymphocytes and macrophages at the invasive tumour border and occasionally in the lamina propria appeared to express MMP-9. In the majority of tumours (71%, 231/316) peritumoural infiltrating lymphocytes had varying intensity of MMP-9 immunostaining, which correlated directly to the degree of inflammation ($P = 0.02$). Tumours with perineural invasion had significantly weaker MMP-9 immunostaining by lymphocytes ($P = 0.01$). Immunoreactivity of TIMP-1 was demonstrated in stromal cells including myofibroblasts, endothelial cells and smooth muscle cells, which was especially distinct around the nests of carcinoma cells but was faint or negative in non-malignant tissues remote from sites of invasion. Fifty percentage (157/316) of the specimens had TIMP-1 positive fibroblasts located centrally in the tumour intermingled with the neoplastic cells, whereas 85% (263/311) contained TIMP-1

Table 1 – Matrix metalloproteinase 9 (MMP-9) and tissue inhibitor of metalloproteinases 1 (TIMP-1) expression in colorectal carcinoma cells and peritumoural lymphocytes (MMP-9) and fibroblasts (TIMP-1) according to clinicopathological characteristics. Significant *P*-values in bold.

	n = 325%	Carcinoma				Peritumoural			
		MMP-9 (0, 1–2%)	*P*	TIMP-1 (0–1, 2–3%)	*P*	MMP-9 (0–1, 2–3%)	*P*	TIMP-1 (0–1, 2–3%)	*P*
Proportions by score 0, 1, 2, 3%	–	–	–	37, 26, 26, 11	–	–	–	–	–
Gender									
Male	50	(92, 8)	0.6	(58, 42)	0.6	(66, 34)	0.2	(49, 51)	0.9
Female	50	(90, 10)		(56, 44)		(64, 36)		(49, 51)	
Age									
<70	79	(90, 10)	0.1	(58, 42)	0.4	(65, 35)	0.3	(50, 50)	0.2
⩾70	21	(96, 4)		(52, 48)		(63, 37)		(46, 54)	
Tumour site									
Colon	81	(91, 9)	0.9	(54, 46)	**0.04**	(63, 37)	0.2	(50, 50)	0.9
Rectum	19	(90, 10)		(69, 31)		(72, 28)		(46, 54)	
Disease stage									
II	11	(91, 9)	0.9	(49, 51)	0.3	(70, 30)	0.5	(53, 47)	0.9
III	78	(91, 9)		(57, 43)		(64, 36)		(49, 51)	
IV	11	(92, 8)		(66, 34)		(69, 31)		(43, 57)	
Tumour stage									
T2	4	(77, 23)	0.2	(62, 38)	0.1	(62, 38)	0.9	(33, 67)	0.3
T3	59	(92, 8)		(62, 38)		(64, 36)		(46, 54)	
T4	37	(91, 9)		(49, 51)		(65, 35)		(55, 45)	
Differentiation (WHO)									
Well	31	(95, 5)	0.2	(58, 42)	0.8	(63, 37)	0.4	(52, 48)	0.5
Intermediate	42	(90, 10)		(58, 42)		(66, 34)		(43, 57)	
Poor	27	(87, 13)		(53, 47)		(66, 34)		(54, 46)	
Perineural invasion									
Yes	19	(93, 7)	0.7	(64, 36)	0.3	(77, 23)	**0.01**	(64, 36)	**0.04**
No	51	(92, 8)		(57, 43)		(61, 39)		(47, 53)	
Not assessed	30	–		–		–		–	
Vascular invasion									
V0	52	(93, 7)	0.5	(58, 42)	0.8	(65, 35)	0.9	(50, 50)	0.9
V1	22	(90, 10)		(59, 41)		(63, 37)		(51, 49)	
Vx	26	–		–		–		–	
Intestinal perforation									
Yes	9	(90, 10)	0.9	(55, 45)	0.9	(76, 24)	0.3	(52, 48)	0.6
No	91	(91, 9)		(57, 43)		(64, 36)		(49, 51)	
Bowel obstruction									
Yes	15	(91, 9)	0.9	(53, 47)	0.6	(72, 28)	0.08	(52, 48)	0.2
No	85	(91, 9)		(57, 43)		(64, 36)		(48, 52)	
Necrosis									
Yes	37	(90, 10)	0.3	(57, 43)	0.9	(62, 38)	0.2	(44, 56)	0.06
No	63	(93, 7)		(57, 43)		(68, 32)		(52, 48)	
Budding									
Yes	25	(93, 7)	0.8	(53, 47)	0.3	(71, 29)	0.7	(43, 57)	**0.01**
No	75	(92, 8)		(59, 41)		(65, 35)		(51, 49)	
Inflammation									
Strong	37	(91, 9)	0.9	(52, 48)	0.1	(61, 39)	**0.02**	(50, 50)	0.3
Weak	63	(92, 8)		(60, 40)		(69, 31)		(49, 51)	

positive stromal cells along the invasive border. Perineural invasion was significantly associated with weak TIMP-1 immunostaining by peritumoural fibroblasts ($P = 0.04$), whereas tumour-budding was significantly associated with strong immunostaining of TIMP-1 by peritumoural fibroblasts ($P = 0.01$). Tumour-budding was not significantly associated with disease stage ($P = 0.2$), T stage ($P = 0.4$) or gross tumour size ($P = 0.1$).

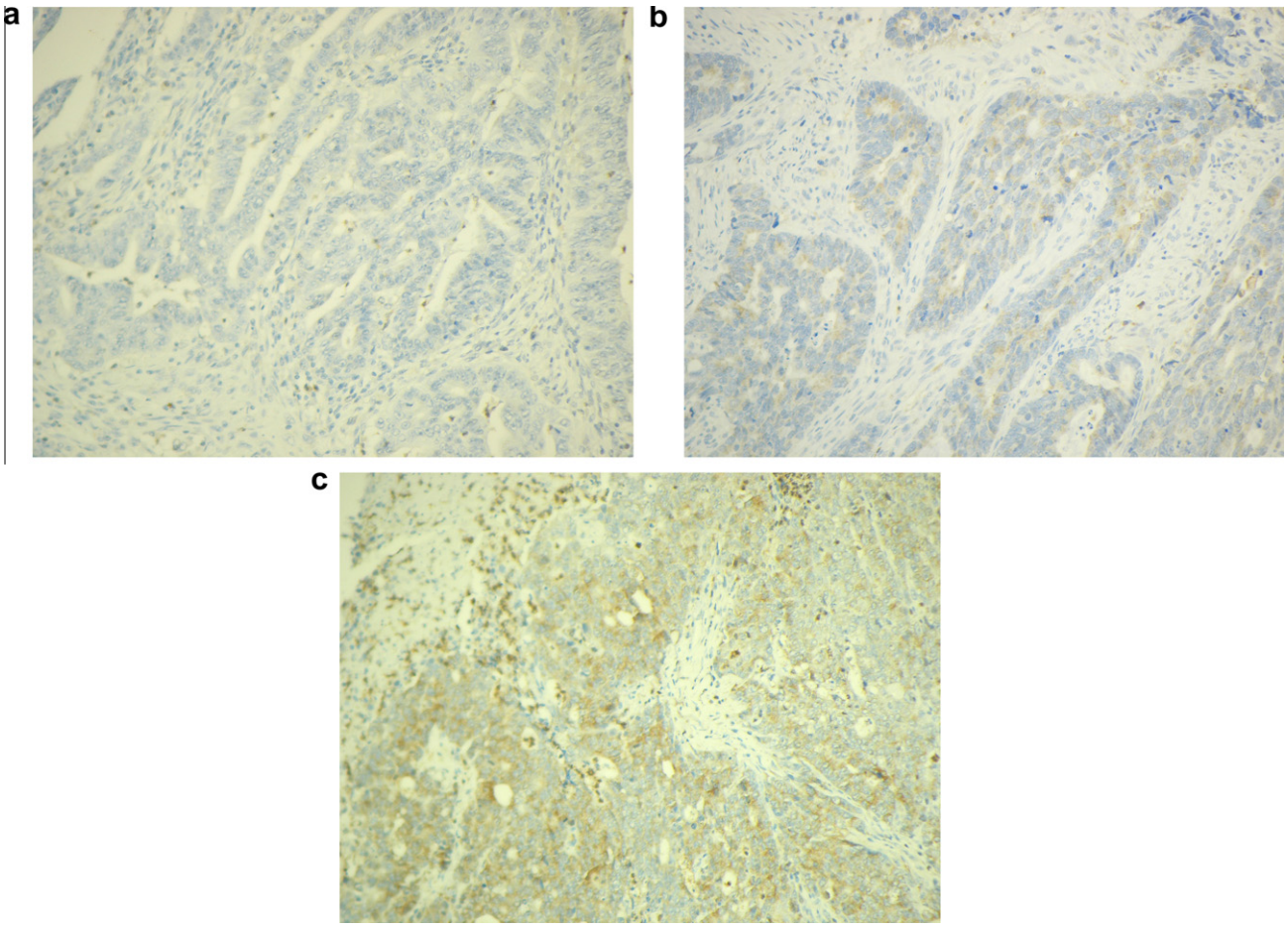

Fig. 1 – Photomicrographs of matrix metalloproteinase 9 (MMP-9)-staining on colorectal carcinomas. Staining was done with clone NCL-MMP-9 and assessed semiquantitatively using the scale: (a) score 0 = no staining; (b) score 1 = faint ambiguous staining; and (c) score 2 = partly staining of cytoplasma. Original magnification ×200.

3.2. *Outcome associated with MMP-9 and TIMP-1 expression*

During follow-up (median 6.1 years; range 4.1–11.3 years) 121 patients (36%) had documented recurrent disease and 153 patients (45%) had deceased. Median overall survival was 9.5 years, and the 5 years survival rate was 62%.

Univariate analysis revealed that MMP-9 immunoreactivity in cancer cells had no association to RFS (HR = 1.0; 95% CI: 0.6–1.8; $P = 0.9$) or OS (HR = 0.9; 95% CI: 0.5–1.6; $P = 0.6$) (Fig. 2). In contrast, TIMP-1 immunoreactivity in cancer cells was inversely correlated with RFS (HR = 1.3; 95% CI: 0.9–1.8; $P = 0.08$) and OS (HR = 1.5; 95% CI: 1.1–2.1; $P = 0.02$) (Fig. 3). The proportions of carcinoma cells having TIMP-1 immunoreactivity was not significantly associated with RFS ($P = 0.3$) or OS ($P = 0.1$).

TIMP-1 immunoreactivity in fibroblast at the invasive tumour border was directly correlated to both RFS (HR = 0.7; 95% CI: 0.6–0.9; $P = 0.02$) and OS (HR = 0.7; 95% CI: 0.6–1.0; $P = 0.05$). In contrast, TIMP-1 immunoreactivity in fibroblasts inside the tumour had no association with RFS ($P = 0.8$). Immunoreactivity of MMP-9 in lymphocytes had no significant correlation with RFS (HR = 0.9; 95% CI: 0.7–1.1; $P = 0.2$) but borderline significant association with OS (HR = 0.8; 95% CI: 0.7–1.0; $P = 0.07$).

In multivariate analysis (Fig. 4) MMP-9 immunoreactivity in cancer cells was not significantly associated with RFS ($P = 0.8$) or OS ($P = 0.5$), whereas TIMP-1 immunoreactivity in cancer cells was inversely related to RFS ($P = 0.05$) and OS ($P = 0.01$) adjusted for the prognostic significance of disease stage ($P = 0.0001$), perineural invasion ($P = 0.001$), ileus ($P = 0.0001$) and inflammation ($P = 0.05$). The interaction between MMP-9 and TIMP-1 expression by carcinoma cells was not significantly associated with RFS ($P = 0.9$) or OS ($P = 0.09$).

TIMP-1 immunoreactivity in fibroblasts inside tumours was inversely correlated with RFS ($P = 0.1$) and OS ($P = 0.07$), whereas the similar expression in fibroblasts at the invasive border was directly correlated with RFS ($P = 0.06$) and OS ($P = 0.1$). Immunoreactivity of MMP-9 in peritumoural lymphocytes was directly correlated with RFS ($P = 0.3$) and OS ($P = 0.06$).

4. Discussion

MMP-9 and TIMP-1 have diverse roles in cancer progression depending on their site of expression in carcinoma cells and peritumoural stromal cells. This study shows that strong expression of TIMP-1 by carcinoma cells, independent of the expression of MMP-9, is associated with poor outcome of

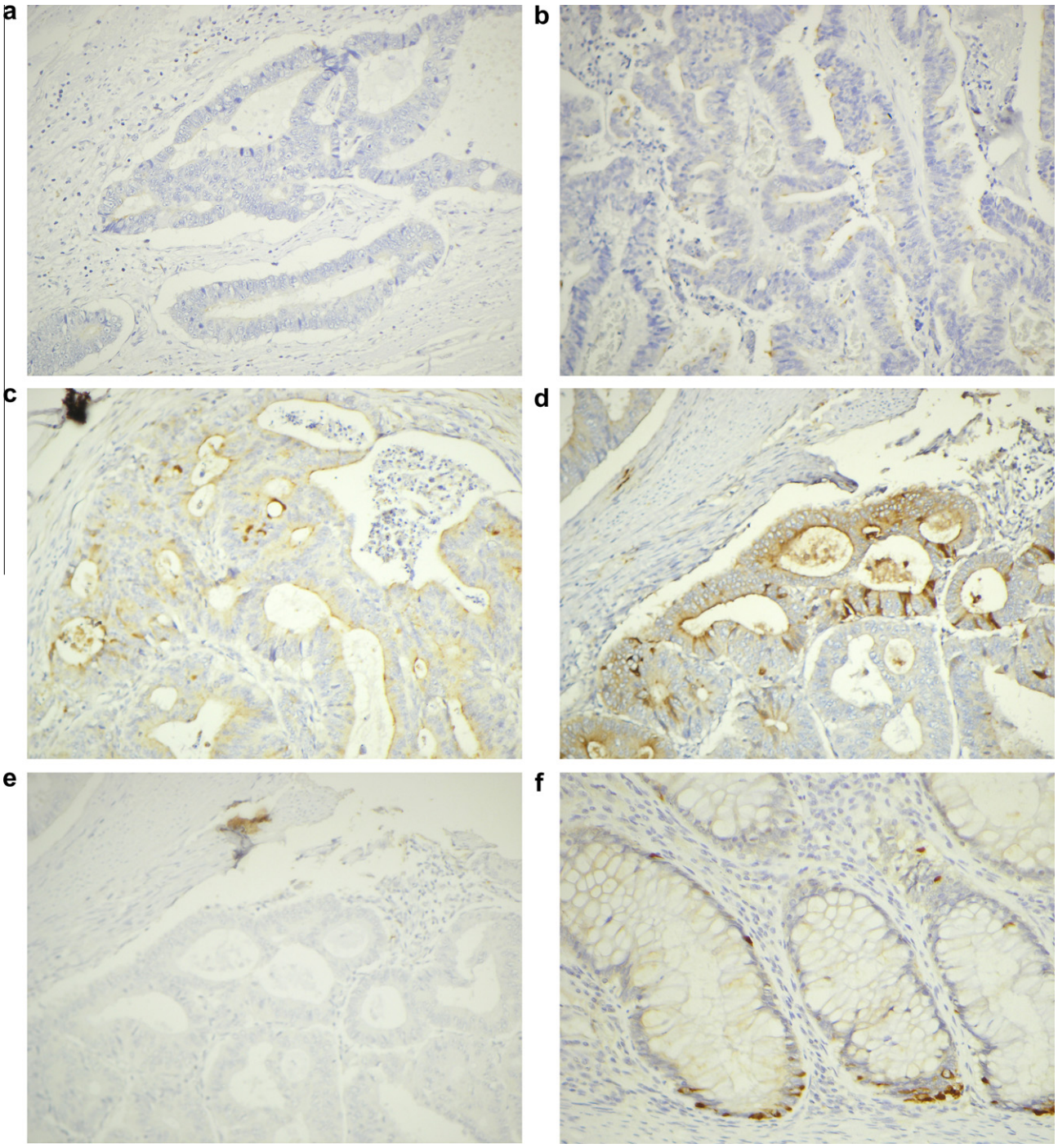

Fig. 2 – Photomicrographs of tissue inhibitor of metalloproteinases 1 (TIMP-1)-staining on colorectal carcinomas. Staining was performed using clone VT-7. (a) 0 = no staining, (b) 1 = faint staining, (c) 2 = partly cytoplasmatic staining and (d) 3 = strong cytoplasmatic staining. Note the localised areas of strongly stained malignant epithelial cells. (e) Shows staining with a non-sense protein (negative control). (f) A selection of the normal (i.e. non-neoplastic) colon mucosa is included demonstrating strong staining of single epithelial cells in the crypt basis, presumed to be neuroendocrine cells due to their position between the absorptive epithelial cells and the basement membrane. Original magnification: (a–e) ×200, (f) ×400.

CRC patients. This finding is consistent with previous reports describing that the levels of TIMP-1 mRNA[9,10], zymogen[2–4,25] and activity[6] in cancer cells or TIMP-1 protein plasma level[26–28] are significantly related to the tumour stage at diagnosis[6,10,23,26–28,40] and to the patient prognosis.[2–4,9,25–27]

The relationship between increased levels of TIMP-1 and poor outcome is contrary to the idea of TIMP-1 acting as an inhibitor of MMPs, which are thought to assist in tumour cell invasion. However, distinct from its role in modulating the MMP-9 activity, TIMP-1 may also facilitate cancer cell

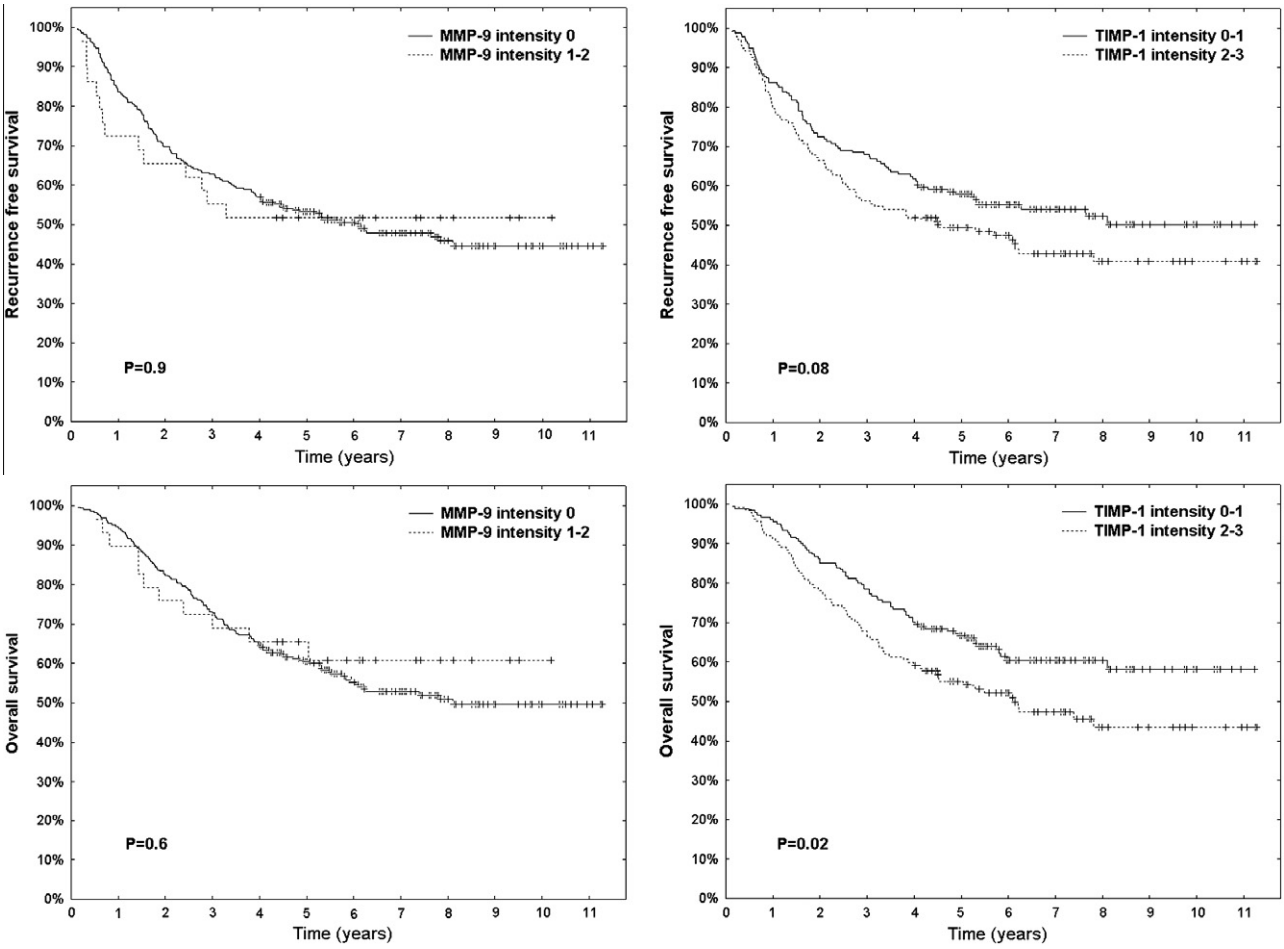

Fig. 3 – Recurrence free survival (upper) and overall survival (lower) associated with MMP-9 (left panel) and TIMP-1 (right panel) immunoquantitation in carcinoma cells after complete resection of colorectal cancer stages II–IV and adjuvant chemotherapy. Censored data (+).

generation[29] and progression by interaction with potent regulatory molecules.[30,32] Hence, TIMP-1 has profound effects on various biological processes that lead to tumour progression including those of stimulating cancer cell growth[30], invasion and angiogenesis[32] and inhibition of apoptosis.[31]

Our finding that cancer cells have infrequent and low expression of MMP-9 suggests that this proteinase is of minor significance to tumour progression in later stages (II–IV) of colorectal cancer. Moreover MMP-2 having similar specific activity towards degradation of the basal membrane may substitute for MMP-9 regarding this function.[4] Conversely, in recent reports considerable higher rates in the range of 44–70% of tumour specimens had cancer cells expressing MMP-9, the level of which was inversely related to disease-free survival.[12,14,41,42] Whilst the employment of different antibodies and technical procedures potentially may explain for such discrepancies between studies the fact that most specimens in the current study had MMP-9 immunostaining in stromal cells contradicted low sensitivity of the immunoreactivity in general. Both of the two antibodies used for immunohistochemistry have previously been validated for their performance on paraffin-embedded tumour tissue.[38,39]

Varying distribution of clinicopathological characteristics between study cohorts may offer some explanation to the different profiles of MMP-9 expression being reported.[41] Hence the biomarker levels were significantly related to the localisation of the tumour as more intense MMP-9 expression was reported in lesions of the right colon as compared to left colon and rectum[41], whereas the expression of TIMP-1 in the current study was significantly stronger in the colon as compared to the rectum. Such distinct patterns of expression associated with the tumour site might reflect some basic differences in the tumour biology between the proximal and distal CRCs.[1,25]

The employment of the immunohistochemical technique to assess matrix proteinases in carcinomas may have shortcomings. Although the antibodies used in this study recognised both the proMMP-9 and the activated enzyme, the immunoreactivity of MMP-9 was mainly confined intracellularly, indicating that it reflects the cytoplasmic pool of proenzyme. The disproportionately greater increase of proenzyme compared to active MMP-9 which has been noticed during carcinogenesis may interfere with the indirect estimate of changes of the extracellular proteolytic activity of MMP-9.[7]

Using other methods, previous studies have reported an increased level of MMP-9 mRNA[9,16,21], protein[7,17,18,23] and enzyme activity[6,7,22,23] in colorectal tumour tissue compared to

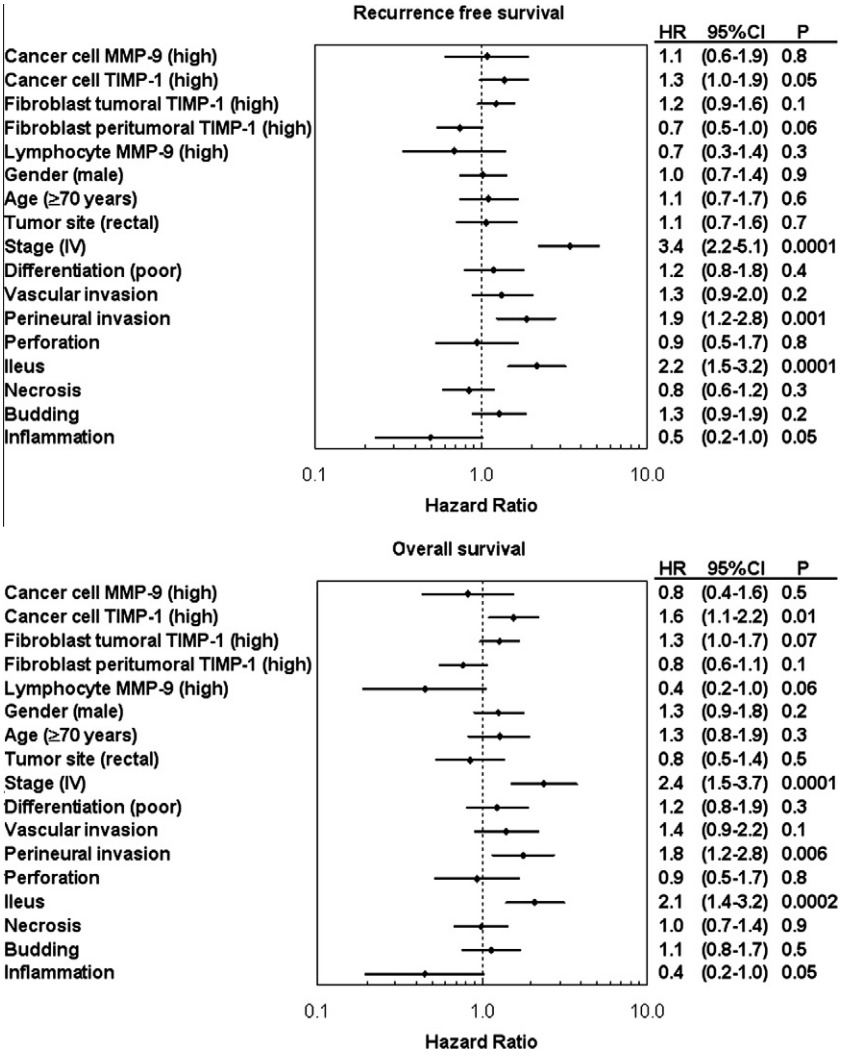

Fig. 4 – Forest plots displaying multivariate Cox analysis of recurrence free survival and overall survival associated with clinicopathological characteristics and with MMP-9 and TIMP-1 immunoreactivity in carcinoma and stromal cells.

the adjacent normal epithelium. A direct relationship between the levels of MMP-9 mRNA[9,11,16], protein[6], enzyme activity[17,19] or plasma level[18], and advanced disease stage at diagnosis[6,12,18] and poor prognosis[5,9,11,14,16,19] suggested a central role of MMP-9 for progression of colorectal cancer. However, as the significance of MMP-9 to tumour progression may differ according to the site of expression, such data must be interpreted cautiously as they make no account of, from which cell types the MMP-9 expression was derived.

The prognosis of CRC in relation to expression of TIMP-1 and MMP-9 by cancer cells has been studied amongst patients receiving uniform treatment consisting of either surgery alone[25,41] or in combination with adjuvant chemotherapy.[3,25,42] In contrast, direct comparison of survival of patients receiving chemotherapy with those not receiving chemotherapy has not been performed in order to investigate whether the levels of TIMP-1 or MMP-9 in carcinoma cells may predict a different efficacy of chemotherapy.

The association between histopathological features and outcome of CRC and the adjacent stromal cells' expression of MMP-9 and TIMP-1 indicated their potential roles as important mediators of a host versus tumour reaction. Hence, the correlation between lymphocytic MMP-9 expression and the degree of inflammation suggests that matrix-degrading proteinases may facilitate transmigration of lymphocytes, neutrophils and macrophages through blood vessels and extracellular matrix[43] to initiate a peritumoural host immune reaction leading to a better outcome.[1,8,15,33–35] Also the pronounced TIMP-1 expression by fibroblasts located at the border of the invading carcinoma nests and its association with a more favourable clinical outcome suggest a host response aiming at inhibiting tumour invasion. The founding mechanisms, however, remain unclarified considering the diverse functions of TIMP-1[29–32] and the ambiguous relationship with perineural invasion and tumour-budding that provided no clear-cut association with either infiltrating or expanding tumour growth characteristics.

A previous report found no significant correlation between TIMP-1 extracted from colorectal cancer tissue and its plasma level indicating that there is no simple relationship between the steady-state level of TIMP-1 in carcinoma and stromal cells and its turnover rate by secretion to the extracellular matrix and subsequent release into the blood circulation.[44] However given the consistent inverse correlation between

the plasma level of TIMP-1 and the prognosis of colorectal cancer[26–28] it is suggested that plasma TIMP-1 mainly reflect the content in carcinoma cells, whereas the contribution derived from peritumoural stroma cells is of minor significance.

In conclusion, this expression study suggests that MMP-9 and TIMP-1 are important mediators of host and cancer interaction through their diverse effects on tumour progression depending on expression by cancer cells and stromal cells in the microenvironment of colorectal adenocarcinomas. This recognition is essential for their application as prognostic markers and for development of rational anti-cancer therapies based on specific targeting of the matrix proteinase system.

Conflict of interest statement

The authors claim no conflicts of interest.

Authors' contributions

SAJ and JBS conceived and planned the study. SAJ collected and integrated the clinical data. BV collected the tumour specimens from the departments of pathology. NB and AB provided antibodies raised against TIMP-1 and conducted the TIMP-1 immunostaining. BV conducted the immunohistochemical scoring and interpretations. SAJ performed data analyses, and made the curve plots and the statistics. SAJ and JBS interpreted the results. SAJ wrote the manuscript draft. All authors critically read through and contributed to the revision of the manuscript draft. All authors approved the final manuscript.

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