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ORIGINAL ARTICLE

Immunoexpression of metalloproteinase 14 and tissue inhibitor of metalloproteinase 2 in colorectal carcinomas and lymph node metastases

Francisco Nélson Nóbrega Furtado · João Paulo Aguiar Sampaio · João Tarcisio Alves Maia-Filho · Renato Braga Vieira · Roberto César Pereira Lima-Júnior · Ronaldo Albuquerque Ribeiro · Paulo Roberto Carvalho Almeida

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Abstract Changes in metalloproteinase (MMP) and tissue inhibitor of metalloproteinases (TIMP) have been associated with tumor progression in colorectal cancer. However, the role of MMP-14 and TIMP-2 has yet to be determined. We investigated the differential expression of MMP-14 and TIMP-2 in colorectal carcinomas of the left and the right colon, as well as in mononuclear cells in primary tumors and their lymph node metastases. We performed an immunohistochemistry analysis of tumor samples obtained from 50 cases of colorectal cancer. We found that MMP-14 staining was positive in 100 % of cases, in contrast to normal mucosa (86 % positivity, P= 0.0451). Additionally, neoplastic cells showed a higher frequency of TIMP-2-positive staining (70 % versus 14 % of normal mucosa, P=0.0004). Furthermore, MMP-14 expression in primary tumor-associated mononuclear cells was higher in cases without lymph node metastases (N0) in comparison to more advanced carcinomas (N1–N3) (P=0.0353). MMP-14 and TIMP-2 expression was observed in neoplastic cells in primary tumors, with a higher frequency of increased expression of MMP-14 (82 %) than increased expression of TIMP-2 (22 %, P<0.0001). The expression of MMP-14 and TIMP-2 was evaluated in each cell type and at each site, and the frequency of TIMP-2 expression in colonic lesions and in the lymph nodes was significantly higher than in tumor-infiltrating mononuclear cells (P=0.0003 and P=0.0406, respectively). Expression of MMP-14 and TIMP-2 in primary colorectal carcinomas and in their lymph node metastases suggests the involvement of these proteins in local invasion and tumor progression.

Keywords Metalloproteinases · Tissue inhibitor of metalloproteinases · Colorectal carcinomas · Macrophages

F. N. N. Furtado · J. P. A. Sampaio · J. T. A. Maia-Filho · R. B. Vieira · P. R. C. Almeida (✉)
Department of Pathology and Forensic Medicine, Faculty of Medicine, Federal University of Ceará, Fortaleza, Ceará, Brazil
e-mail: paulo.almeida@ufc.br

P. R. C. Almeida
e-mail: fnnf1233@yahoo.com.br

R. C. P. Lima-Júnior · R. A. Ribeiro
Department of Physiology and Pharmacology, Faculty of Medicine, Federal University of Ceará, Fortaleza, Ceará, Brazil

R. A. Ribeiro
Department of Clinical Oncology, Haroldo Juaçaba Hospital, Cancer Institute of Ceará, Fortaleza, Ceará, Brazil

Introduction

Colorectal cancer (CRC) is the third most common cancer and the fourth leading cause of cancer death worldwide (Kanazawa et al. 2010). The majority of CRC cases show no identifiable inherited genetic mutations. The most accepted pathogenic context for this malignancy is the adenoma-carcinoma sequence (Mclean et al. 2011). There are two pathways involved in carcinogenesis of the colon and the rectum, which are also related to sporadic cancers: (1) the adenomatous polyposis coli (APC)/β-catenin pathway, also known as the "chromosome instability pathway," in which changes occur in the *APC*, *TP53*, and *KRAS* genes (Felin et al. 2008), is related to intestinal carcinogenesis of the left colon and to the familial adenomatous polyposis (FAP) condition (Mclean et al. 2011), and (2) the "microsatellite instability pathway" with mutations

in DNA repair genes such as *MSH2* and *MLH1* (Felin et al. 2008), which is associated with right colonic carcinogenesis, as exemplified by the hereditary non-polyposis colorectal cancer syndrome, also known as Lynch syndrome and also present in sporadic tumors (Sugai et al. 2006; Benedix et al. 2010).

In recent years, attention has been increasingly given to the tumor microenvironment in the genesis of neoplasias and the tumor stroma, including the extracellular matrix and nontumor cells, such as fibroblasts and macrophages (Mbeunkui and Johann 2009; Joyce and Pollard 2009; Hadler-Olsen et al. 2011; Zagouri et al. 2011).

The matrix metalloproteinases (MMPs) are a family of approximately 25 zinc-dependent endopeptidases capable of degrading almost all molecular components of the extracellular matrix (Altadill et al. 2012). Changes in MMPs have been associated with tumor progression and are also associated with poor clinical prognosis (Felin et al. 2008; Kanazawa et al. 2010). The MT1-MMP, also called MMP-14, was the first membrane-associated metalloproteinase to be identified and is considered to trigger the activation of several secreted (nonmembranous) MMPs, including pro-MMP-2 and pro-MMP-13 (LaFleur et al. 2001). MMP-14 expression has been involved within the process of tumor invasion in cancers of the stomach, pancreas, colon, and rectum (Nabeshima et al. 2002).

Another group of molecules, the tissue inhibitors of metalloproteinases (TIMPs), modulates the function of MMPs by regulating their activity. Four TIMPs (TIMP-1, TIMP-2, TIMP-3, and TIMP-4) have been identified, and they share many similarities and overlapping specificities, while their biochemical properties and the patterns of expression exhibit distinct characteristics (Gomez et al. 1997; Baker et al. 2002; Murphy and Nagase 2008; Hadler-Olsen et al. 2011; Ra and Parks 2007). However, the function of TIMP-2 remains unknown. Several studies have reported either an inhibitory or an activating action on MMP-2 function (Schwandner et al. 2007; Park et al. 2011; Nabeshima et al. 2002; Webster and Crowe 2006)]. Additionally, the expression of TIMP-2 is positively associated with tumor recurrence and poor prognosis (Nabeshima et al. 2002; Webster and Crowe 2006)].

We also studied the simultaneous expression of MMP-14 and TIMP-2 in CRC and their role in the development of right and left primary colorectal carcinomas, especially in their respective lymph node metastasis.

Material and methods

Case selection

Our study was carried out using 50 CRC cases. Tumor samples were obtained from the files of the Department of Pathology and Forensic Medicine, Federal University of Ceará. Sections of colonic mucosa with histologically standard surgical margin were taken, apart from the tumor, in 16 cases. The collected material was fixed in 10 % formalin, embedded in paraffin, sectioned into 3-μm-thick slices, and then stained with hematoxylin-eosin. Exclusion criteria included poorly fixed samples, cases with insufficient material or with extensive tumor necrotic areas, patients who underwent chemotherapy, and cases that did not meet the criteria for histological classification as a carcinoma. This study was approved by the Research Ethics Committee (Protocol number 126.12.10.).

Tissue microarray (TMA-Tissue microarray)

We performed a TMA method according to a previous report (Gurgel et al. 2012) to remove the cylinder samples from the donor blocks. The recipient blocks were also prepared as in a previous study (Sampaio et al. 2014). After tissue core transference into the receiver block, hot paraffin (62 ° C) was added to improve adherence between the tissue cores and the recipient block. The recipient blocks were incubated in a stove at 60 °C for 15 min and allowed to reach room temperature. Routine histopathological and immunohistochemistry were then performed.

Immunohistochemistry

Sections were deparaffinized and rehydrated. Treatment with a solution of 3 % H2O2 in methanol for 10 min was used to block endogenous peroxidase. Antigen unmasking was achieved by a 20-min incubation in a Tris/EDTA retrieval solution (Target Retrieval Solution pH 9.0, 3-in-1; Ref: S2375, DAKO Co., São Paulo, Brazil) at 98 °C. Ultra V block (TA-125-UB; LabVision) was utilized for 10 min to inhibit unspecified ground reactions. The slides were incubated with diluted (1:10) mouse monoclonal antibodies anti-MMP-14 (mouse anti-human sc-80210, Santa Cruz Biotechnology) and anti- TIMP-2 (mouse anti-human sc-21735, Santa Cruz Biotechnology) at 25 °C for 12 h. A mouse monoclonal anti-human CD68 (KP1 clone, DAKO Co., São Paulo, Brazil) antibody was also utilized at 1:800 for 1.5 h. The slides were processed in an automated marking module (Ventana Benchmark XT/Roche™). Negative controls, for which no primary antibody was applied, were included. Following primary antibody incubation, a secondary donkey anti-mouse IgG biotinylated antibody (sc-2098, Santa Cruz Biotechnology) was applied at 1:100. Next, sections were incubated in a streptavidin-coupled peroxidase complex (TS-125-HR; LabVision) for 15 min. An automated immunostainer (Ventana Benchmark XT/Roche™) was utilized to process the reactions, and a biotin-free-UltraView Universal diaminobenzidine Detection Kit (DAKO Co., São Paulo, Brazil) was used as the chromogen. The sections were counterstained with hematoxylin, dehydrated, diaphanized, mounted, and analyzed. Kidney and lung were used for positive controls for MMP-14 (Bonfil et al. 2007) and TIMP-2 (Dong et al. 2005).

Table 1 MMP-14 and TIMP-2 immunoexpression in epithelial and mononuclear stromal cells in both normal and tumor tissues of colorectal carcinoma samples

Stained tissue	Scores	Tumor marker				Statistics	
		MMP-14		TIMP-2		P^1	P^2
		Normal	Tumor	Normal	Tumor		
Epithelial cells	0	2	0	12	15		
	1	5	9	2	24		
						0.0314*	0.1027
	2	4	12	0	11		
	3	3	29	0	0		
	Total	14	50	14	50		
Mononuclear cells	0	1	1	6	6		
	1	2	7	8	11		
						0.7034	<0.0001*
	2	5	15	0	16		
	3	6	24	0	10		
	Total	14	47	14	43		

P^1 =MMP-14 or P^2 =TIMP-2 high expression (2 or 3 score) versus low expression (0 or 1 score)

Score analysis

The following scores were based on a previous report (Buskens et al. 2003), taking into account the intensity of the staining and the percentage of stained tumor or stromal cells in each sample, as follows: 0=absence of immunoreactivity or sparse labeled cells (<5 %), 1=discrete staining in more than 50 % of tumor/mononuclear inflammatory cells or less than 50 % of cells moderately stained, 2=moderate staining in most (>50 %) tumor/mononuclear inflammatory cells or less than 50 % of cells strongly stained, and 3=strong staining in more than 50 % of tumor or inflammatory mononuclear cells (confirmed by CD68 expression). Scores 0 and 1 were grouped as "low expression," while scores 2 and 3 corresponded to "high expression" samples.

The intensity of MMP-14 and TIMP-2 immunoexpression was analyzed by an experienced pathologist (PRCA) who was blinded to the case identities.

Statistical analysis

The scores were compared using Fisher's exact test using GraphPad Prism 5.0 software. A P value of <0.05 was considered statistically significant.

Results

Table 1 shows the level of MMP-14 and TIMP-2 immunoexpression in samples of primary colorectal carcinomas and normal colonic mucosa. MMP-14 expression was higher in tumor samples, both in neoplastic cells and mononuclear cells. In regard to neoplastic cells, MMP-14 staining was positive in 100 % of the cases (50/50), in contrast to 86 % positivity in normal mucosa (12/14, P= 0.0451).

The high MMP-14 expression (scores 2 and 3) was observed mainly in tumor epithelial cells (41/50=82 % versus 7/14=50 % of normal mucosa, P=0.0314, Table 1). In mononuclear cells, MMP-14 expression was not significantly different when compared to the normal mucosa (Table 1).

Additionally, neoplastic cells had a relatively higher frequency of TIMP-2-positive staining (35/50=70 % versus 2/14=14 % of normal mucosa, P=0.0004). Furthermore, 22 % of tumor samples (11/50) demonstrated a high frequency of TIMP-2 expression, which was not seen in normal epithelium (P=0.1027, Table 1). Positive scores in mononuclear cells were present in the tumor in 37/43 cases (86 %) and present in normal colonic mucosa in only 8/14 cases (57 %) (P=0.0528). Moreover, frequently, mononuclear cells were moderately to intensely stained within the tumor but not in normal mucosa (26/43=60 % versus 0/14=0 % in the normal mucosa, P<0.0001, Table 1).

We investigated the possible association between the expression of MMP-14 and TIMP-2 in neoplastic and mononuclear cells with several clinicopathologic variables, including sex, age, anatomic location of the tumor, tumor size, angiolymphatic invasion, perineural infiltration, and TNM (T and N) score system in 50 cases of colorectal carcinoma; 36 of which were in the left and 14 in the right colon (Table 2). However, no correlation was found (P>0.05). In contrast, while analyzing the mononuclear

cells, we verified that MMP-14 expression in primary tumor-associated mononuclear cells was higher (scores 2 and 3) in cases without lymph node metastases (N0) when compared to more advanced carcinomas (N1–N3) (Table 2, high versus low expression, $P=0.0353$). There was no correlation between the TIMP-2 expression in mononuclear cells and other clinicopathological variables.

As shown in Table 2, MMP-14 immunoexpression in tumor cells was positive both in primary tumors (50/50=100 %) and in metastatic cells (7/8=88 %). No significant difference was observed between the two anatomical sites or primary tumors in the right colon or the left. The comparison between high and low expression also did not show a significant difference (high expression: 41/50=82 % primary tumor and 6/8=75 % in the metastasis, $P=0.6391$, Table 2). The immunoreactivity for MMP-14 in mononuclear cells was also predominantly positive, both in primary tumors (46/47=98 %) and in metastatic (7/8=88 %) tumors in the right and left colon, with no significant difference. We also did not find a significant difference between high- and low-expressing cells (high: 39/47=83 % colon and 6/8=75 % lymph nodes, $P=0.6273$, Table 2).

Table 2 MMP-14 expression in mononuclear cells according to clinical-pathological variables

Clinical-pathological variables	MMP-14 immunoexpression (mononuclear cells)						
	Scores						
	n	0	1	2	3	n.d.	*P*
Gender							
Male	29	0	4	11	13	1	0.6972
Female	21	1	3	4	11	2	
Age							
<50	11	0	0	3	8	0	
≥50	38	1	7	12	15	3	0.1691
n.d.	1	0	0	0	1	0	
Tumor size							
<5 cm	12	0	3	2	7	0	
≥5 cm	37	1	4	12	17	3	0.4124
n.d.	1	0	0	1	0	0	
Angiolymphatic invasion							
Absent	27	0	5	8	12	2	
Present	21	1	1	7	11	1	0.4367
n.d.	2	0	1	0	1	0	
Perineural infiltration							
Absent	9	0	2	4	3	0	
Present	7	1	1	0	5	0	1.0000
n.d.	34	0	4	11	16	3	
Local invasion (T)							
T1	2	0	0	0	2	0	
T2–T4	46	1	7	14	21	3	1.0000
n.d.	2	0	0	1	1	0	
Lymph node metastasis (N)							
N0	26	0	1	9	14	2	0.0353*
N1–N3	21	1	5	6	8	1	
n.d.	3	0	1	0	2	0	
Total	50	1	7	15	24	3	

Exact Fisher's test (0 or 1 scores versus 2 or 3 scores)

n.d. nondetermined

TIMP-2 immunostained neoplastic cells were found both in primary (35/50=70 %) carcinomas and in their respective lymph nodes (8/8=100 %), although the difference was not statistically significant (Table 3). The immunoreactivity for TIMP-2 in mononuclear cells was also predominantly positive in primary tumors (37/43=86 %) and present in all cases of metastasis (8/8=100 %). High expression was observed in 60 % (26/43) and 75 % (6/8) in the primary carcinoma and lymph nodes, respectively. These differences were not statistically significant (Tables 3 and 4).

Figures 1 and 2 are representatives of the MMP-14 (Fig. 1) and TIMP-2 (Fig. 2) expression in both mononuclear and tumors cells in primary tumors as well as in lymph node metastasis. We found that MMP-14 is highly expressed in mononuclear cells when compared with tumor cells in the primary tumor (Fig. 1c). However, lymph node metastases were weakly stained for MMP-14 (Fig. 1d). Kidney was adopted as a positive control (Fig. 1b), which shows the tubular structures highly reactive to MMP-14 staining. TIMP-2 was intensely expressed in mononuclear cells in primary tumors (Fig. 2c) as well as in lymph node metastasis (Fig. 2d), but the tumor cells showed a weak immunostaining (Fig. 2c). Figure 2b represents the positive control for TIMP-2 (lung). General background, when no primary antibody was used, showed an absence of staining (Figs. 1a and 2a).

The major component of mononuclear cells observed in the primary tumor (Fig. 3a) represents macrophages because most of these cells were positively stained for CD68 (Fig. 3b).

Figure 4 summarizes the findings shown in Figs. 1, 2, and 3. MMP-14 and TIMP-2 expression was observed in neoplastic cells in primary tumors, with high expression of MMP-14 more frequently observed (score 2 and 3 41/50=82 %) than high expression of TIMP-2 (11/50=22 %, $P<0.0001$, Fig. 4a). Accordingly, highly stained tumor cells in the lymph nodes were observed (6/8=75 %), in contrast to the lower frequency of high expression of TIMP-2-positive tumor cells in that anatomical site (1/8=13 %, $P=0.0406$, Fig. 4b). The MMP-14 and TIMP-2 expression levels were compared in mononuclear cells in the tumor stroma of primary carcinomas. A higher expression level of MMP-14 was observed relative to TIMP-2 expression (39/47=83 % versus 26/43=60 %, $P=0.0202$, Fig. 4c). However, there was no difference in

Table 3 MMP-14 expression in tumor and mononuclear cells according to tumor site and position into the colon in colorectal cancer samples

Cell type	Tumor site	Tumor position into the colon	MMP-14 immunoexpression (scores)					Statistics
			n	0	1	2	3	*P*
Tumor cells	Primary tumor	Right	14	0	2	5	6	
								1.0000[a]
		Left	36	0	7	6	23	
		Total	50	0	9	12	29	
								0.6391[b]
	Lymph node	Right	–	–	–	–	–	
								–
		Left	8	1	1	4	2	
		Total	8	1	1	4	2	
Mononuclear cells	Primary tumor	Right	13	0	2	5	6	
								1.0000[a]
		Left	34	1	5	10	18	
		Total	47	1	7	15	24	
								0.6273[b]
	Lymph node	Right	–	–	–	–	–	
								–
		Left	8	1	1	2	4	
		Total	8	1	1	2	4	

P=MMP-14 high expression (2 or 3 score) versus low expression (0 or 1 score); P^{a} =right colon versus left colon; P^{b} =primary versus metastatic tumor

the lymph nodes (high expression in 75 % of cases for both MMP-14 and TIMP-2, *P*=1.0000).

Additionally, while comparing the expression of MMP-14 and TIMP-2 in each cell type and at each site, we observed

Table 4 TIMP-2 expression in tumor and mononuclear cells according to tumor site and position into the colon in colorectal cancer samples

Cell type	Tumor site	Tumor position into the colon	MMP-14 immunoexpression (scores)					Statistics
			n	0	1	2	3	*P*
Tumor cells	Primary tumor	Right	14	4	6	4	0	
								0.4578[a]
		Left	36	11	18	7	0	
		Total	50	15	24	11	0	
								1.0000[b]
	Lymph node	Right	–	–	–	–	–	
								–
		Left	8	0	7	1	0	
		Total	8	0	7	1	0	
Mononuclear cells	Primary tumor	Right	13	1	4	7	1	
								1.0000[a]
		Left	30	5	7	9	9	
		Total	43	6	11	16	10	
								0.6936[b]
	Lymph node	Right	–	–	–	–	–	
								–
		Left	8	0	2	4	2	
		Total	8	0	2	4	2	

P=MMP-14 high expression (2 or 3 score) versus low expression (0 or 1 score); P^{a} =right colon versus left colon; P^{b} =primary versus metastatic tumor

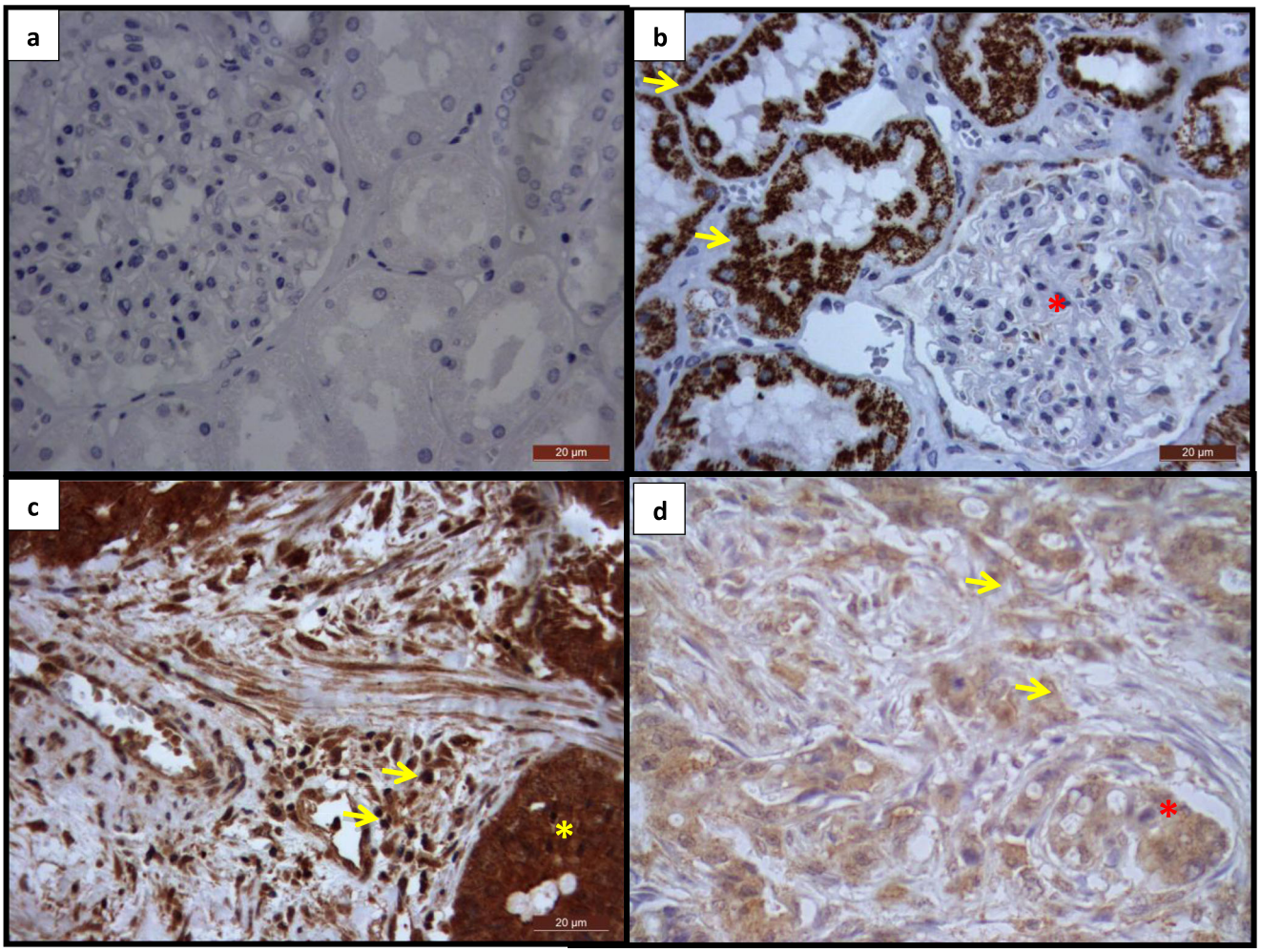

Fig. 1 Primary tumor and mononuclear cells express MMP-14. **a** Negative control in which no primary antibody was used. Kidney—×400. **b** Intense staining in renal tubules (*yellow arrows*). Glomerulus showed no MMP-14 expression (*black arrow*, internal negative control), Kidney—×400. **c** High MMP-14 expression (score=3) in mononuclear cells is more evident (*yellow arrows*) than in primary tumor neoplastic cells (*)—×400. **d** The expression of MMP-14 in mononuclear cells in the lymph node was identical to that of neoplastic cells (*)—×400

that the frequency of TIMP-2 expression in colonic lesions was significantly higher than in tumor-infiltrating mononuclear cells (26/43=60 % versus 11/50=22 %, respectively, P=0.0003, Figs. 4a and c). The same observation was made in the lymph nodes (6/8=75 % versus 1/8=13 %, P=0.0406). There was no difference in MMP-14 immunoreactivity in neoplastic and mononuclear cells in either primary tumors or in metastases; it was highly expressed in most of the cases, in both cell types and in both anatomical sites (Fig. 4a–d).

Discussion

In the present study, we observed that MMP-14 and TIMP-2 are highly expressed in colorectal carcinoma and mononuclear cells, which may contribute to tumor invasion and metastasis. Additionally, we found no difference in the expression of these markers according to tumor localization in the right or left colon.

The comparison of MMP-14 and TIMP-2 expression revealed a higher expression level (higher scores) in epithelial and stromal mononuclear cells of tumor samples compared to that in normal mucosa. In some cases, these differences were statistically significant; epithelial tumor cells stained for MMP-14, and tumor epithelial cells and mononuclear cells of the tumor microenvironment stained positively for TIMP-2. The expression of TIMP-2 in both cell types has been previously described in colorectal cancer and other sites (Kikuchi et al. 2000; Têtu et al. 2006), primarily in the stromal cells (Kikuchi et al. 2000; Trudel et al. 2008).

In accordance with our findings, Asano et al. (2008) found that cancerous tissues typically have higher levels of expression of MMPs compared to normal mucosa. A study by Schwandner et al. (2007) showed that MMP-14, expressed

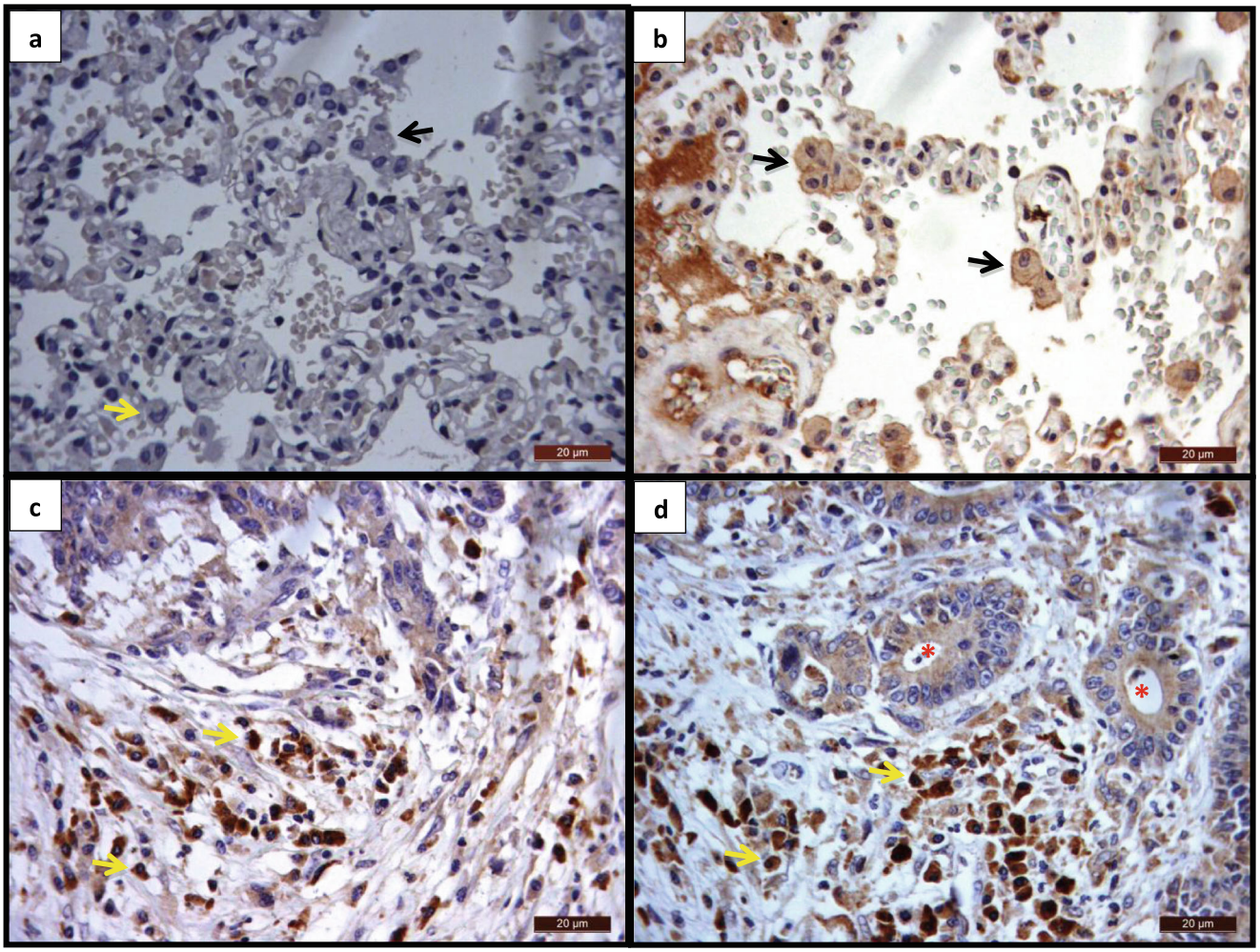

Fig. 2 TIMP-2 is expressed in primary tumor-associated mononuclear cells and in its lymph node metastasis. **a** Absence of primary antibody. Mononuclear cells (macrophages) adjacent to the alveolar septa with no immunolabeling (*arrows*). Lung—×400. **b** Moderately stained mononuclear cells (macrophages) adjacent to the alveolar septa (*black arrows*). Lung—×400. **c** Intense staining (score=3) in mononuclear cells (*yellow arrows*). Tumor cells lightly stained at the top of the figure—×400. **d** The expression of TIMP-2 in mononuclear cells (*yellow arrows*) in the lymph node was higher than that observed in neoplastic cells (*)—×400

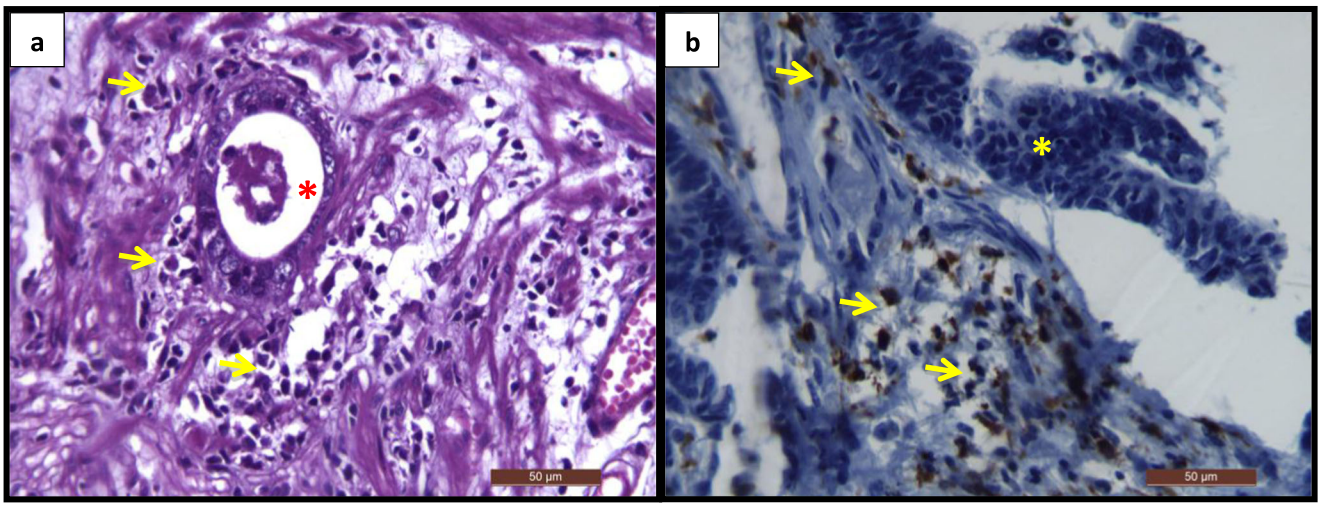

Fig. 3 Mononuclear cells in tumor stroma. **a** Mononuclear cells in the stroma (*arrows*) arranged around tumor cells (*)—×400. **b** CD68-positive mononuclear cells (macrophages, *arrows*). Absence of staining observed in adjacent tumor cells (*)—×400. H&E staining

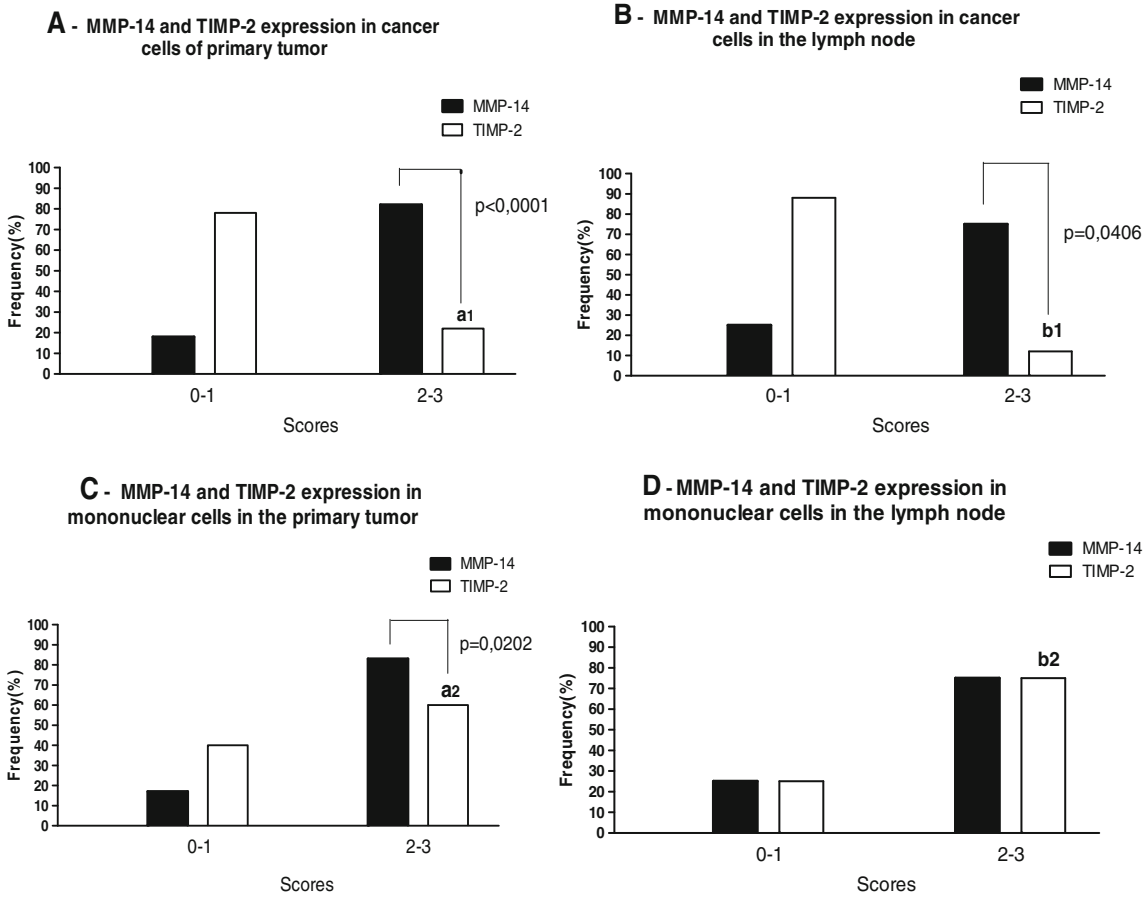

Fig. 4 Summary of scores for MMP-14 and TIMP-2 immunostaining in neoplastic malignant cells and mononuclear cells in the tumor stroma, in primary colorectal carcinoma and lymph node metastases. **a** In the neoplastic cells of primary tumors, high expression of MMP-14 and low expression of TIMP-2 predominate ($P<0.0001$). **b** In the neoplastic cells of the lymph nodes, there are similar findings, also significantly different from other cell types ($P=0.0406$). **c** A higher expression of MMP-14 was present in mononuclear cells compared to TIMP-2 staining ($P=0.0202$), but there was no difference in the expression in the lymph nodes (**d**)

in the cytoplasm of tumor cells (positive in approximately half of the cases), was not expressed in the stroma or in the normal tissues

In regard to TIMP-2, our results are controversial. We found a higher expression level of TIMP-2 in primary colorectal carcinomas than in normal colonic mucosa, which is in accordance to the results reported by Groblewska et al (2014). However, Asano et al. (2008) reported that the expression of TIMP-1, TIMP-2, TIMP-3, and TIMP-4 in cancer tissues has nearly equivalent levels as in normal colonic mucosa. Meanwhile, Baker et al. (2002) and Kim et al (2006) found that tissue levels of TIMP-2 in normal mucosa were higher than those observed in tumor tissue.

The role of TIMP-2 in regulating MMP-14 function remains under debate. TIMP-2 forms a ternary complex with MMP-14 and MMP-2 and is a potent inhibitor of both. However, some studies have ascertained that high levels of TIMP-2 positively correlate with poor prognosis in cancer patients (Bernardo and Fridman 2003; Strongin 2010). Our findings showed that the expression of the two biomarkers was much higher in tumor samples compared to normal colonic mucosa, both in cancer cells and in mononuclear cells, suggesting their positive correlation with tumor progression.

The possible association between immunostaining for biomarkers and clinicopathological variables was also investigated. We found that mononuclear cells showed a high expression of MMP-14 in the primary tumor, which was more relevant in cases without lymph node metastasis. In neoplastic cells, no relationship was found between the expression of MMP-14 and TIMP-2 with other clinicopathological variables. Similarly, some authors showed no correlation between the expression of various MMPs, including MMP-14, and some of these variables in colorectal cancer (Schwandner, et al. 2007) and other cancers (Meneses-García et al. 2008). However, a more frequent expression of MMP-14 in invasive carcinomas and in cases of vascular invasion has been described (Kikuchi et al. 2000). Kikuchi and coworkers found that the rate of detection of TIMP-2 in the cytoplasm of tumor cells increased with the degree of invasion and that TIMP-2 in

stromal cells was found more frequently in tumor invasion areas and in lymph node metastases (Kikuchi et al. 2000). Additionally, a significant association between the detection of MMP-14 and TIMP-2 was described (Kikuchi et al. 2000).

Pellikainen et al. (2004) described a similar result in breast carcinomas. Whereas MMP-14 initiates the activation of other metalloproteinases (Bernardo and Fridman 2003; Trudel et al. 2008), these findings are unexpected and could suggest that MMP-14 participates mainly in the local invasion of colorectal carcinomas (and some breast carcinomas, as reported), rather than in their spread to lymph nodes.

In our study, a relatively large number of cases presented a high level of MMP-14-positive staining in the two cell types and in both the left and right anatomical sites of the colon. Têtu and colleagues utilized mRNA in situ hybridization of paraffin-embedded material of breast cancers and found MMP-14 mRNA in reactive stromal cells, while TIMP-2 mRNA was expressed in both stromal and cancer cells (Têtu et al 2006). Hong et al (2011) found that MMP-2 was more frequently expressed in malignant epithelial than in stromal cells. Another recent study showed a significantly higher expression of MMP-1, MMP-2, and MMP-3 in the stromal cells compared to neoplastic colorectal carcinoma cells (Kahlert et al. 2014). MMP-14, which is considered the primary activator of several MMPs, is generally expressed in various cancers, along with a decreased expression of tissue inhibitors of MMPs, such as TIMP-2 (Ornstein and Cohn 2002; Têtu et al. 2006; Sato and Takino 2010; Al-Raawi et al. 2011).

We found no difference in the expression of MMP-14 and TIMP-2 in colorectal carcinomas of the right and left colon. Therefore, we suggest that these biomarkers could have similar contributions to carcinogenesis of the right and left colon. To the best of our knowledge, this is the first report regarding the frequency of MMP-14 and TIMP-2 expression in relation to the laterality of lesions in human colorectal carcinomas. The only report concerning other MMPs was published by Hong et al. (2011), who described a higher presence MMP-2 in stromal cells of the left colon (including the rectum) than in the right, while MMP-7 expression was primarily observed in tumor cells of the right colon.

Our findings did not show differences between primary and metastatic lesions, when considering the same immunomarker. There are only a few studies that have evaluated immunoexpression in primary cancer lesions and the respective lymph node metastases. García et al. (2010) described a higher expression of MMP-14 in lymph node metastases than in primary breast carcinomas in both tumor and stromal cells, with significant differences. They also found a higher expression of TIMP-2 in primary lesions in both cell types, but without significant differences.

Interestingly, MMP-14 expression was increased in both cell types in primary and metastatic tumors, along with a clearly more intense expression of TIMP-2 in mononuclear cells compared to neoplastic cells, in both the colon and lymph node sites. These findings suggest the importance of the tumor microenvironment in cancer progression. Furthermore, a dramatically higher level of MMP-14 expression compared to TIMP-2 was found in both anatomical sites, primarily in neoplastic cells. This last finding reinforces the self-sufficiency of neoplastic cells to stimulate the microenvironment by themselves. It is possible that MMP-14 orchestrates the mechanisms of invasion.

Conclusions

The differential expression of MMP-14 and TIMP-2 in colorectal carcinomas, in their lymph node metastases and in stromal mononuclear cells, suggests the involvement of these genes in local invasion and tumor progression. Furthermore, the similar frequency and intensity of MMP-14 and TIMP-2 immunoreactivity in colorectal carcinomas in both the right and left anatomical sites rule out the differential involvement of these enzymes in the development of intestinal cancers in regard to laterality.

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