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ORIGINAL STUDY

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Clinical significance of serum levels of matrix metalloproteinase 2 (MMP-2) and its tissue inhibitor (TIMP-2) in gastric cancer

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Abstract: Matrix metalloproteinase 2 (MMP-2) is able to degrade type IV collagen, and thus plays a key role in the migration of tumor cells. MMP-2 activity is inhibited by its tissue inhibitor (TIMP-2). The imbalance between MMPs and TIMPs may facilitate progression of cancer cells. The aim of this study was to compare the clinical importance of MMP-2 and TIMP-2 to that of classical tumor markers, namely carcinoembryonic antigen (CEA) and carbohydrate antigen (CA 19-9) in the diagnosis of gastric cancer (GC) by calculating the diagnostic criteria and estimating the levels of MMP-2, TIMP-2, CEA and CA 19-9 in GC patients in relation to clinicopathological features of cancer. We found that serum levels of MMP-2 and TIMP-2 were significantly lower, whereas serum tumor markers were higher, in GC patients than in healthy subjects. Moreover, concentrations of TIMP-2 and CEA correlated with gastric wall infiltration, while CA 19-9 levels correlated with gastric wall infiltration and the presence of nodal metastasis. None of the proteins tested was found to be an independent prognostic factor for GC patients' survival. The percentage of true positive results of TIMP-2 (61%) was higher than those of MMP-2 (54%) and the classical tumor markers CEA (21%) and CA 19-9 (31%). The highest diagnostic sensitivity was observed for the combined use of TIMP-2 with MMP-2 (77%). The results suggest the greater importance of serum MMP-2 and TIMP-2 than of the classical tumor markers CEA and CA 19-9 in the diagnosis of GC. But this issue requires further investigation. (*Folia Histochemica et Cytobiologica 2011; Vol. 49, No. 1, pp. 125–131*)

Key words: gastric cancer, MMP-2, TIMP-2, tumor marker

Introduction

The degradation of extracellular matrix (ECM) is a crucial step in tumor progression, aggressive growth and metastases [1, 2]. The invasion of cancer cells within the basement membrane depends on matrix metalloproteinases (MMPs) and their inhibitors' activities [1, 3].

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Matrix metalloproteinases (MMPs) are a family of extracellular zinc endoproteases capable of degrading all the ECM components [3]. MMPs are produced by tumor cells, so they may be associated with tumor progression including invasion, migration, angiogenesis and metastasis [1, 2, 4, 5]. Among the MMPs, matrix metalloproteinase 2 (MMP-2) and matrix metalloproteinase 9 (MMP-9) play important roles in the migration of malignant cells, because of their ability to degrade type IV collagen [6]. The mechanisms of activation of these enzymes are different.

MMP-9 modulates permeability of the vascular endothelium, whereas MMP-2 promotes cleavage of extracellular matrix proteins and is intensively expressed by tumor and stromal components of cancer [7].

The activity of MMP-2 is regulated at several levels, including transcriptional, post-transcriptional and post-translational levels, as well as via their endogenous inhibitors — TIMPs (tissue inhibitor of matrix metalloproteinase) [8, 9]. Moreover, MMP-2, either in latent or activated form, is able to produce complexes with TIMP-2. It has been found that tissue inhibitor of matrix metalloproteinase 2 (TIMP-2) is more than ten times more effective in inhibiting MMP-2 activity than tissue inhibitor of matrix metalloproteinase 1 (TIMP-1) [8–11]. Several studies have established the role of MMP-2 and TIMP-2 and MMP-2/TIMP-2 complex in the growth and progression of many types of cancer, including colorectal [12, 13], pancreatic [14], lung [15] and gastric cancer [16–18].

Gastric cancer (GC) remains a major cause of mortality and morbidity worldwide [19]. The rapid invasion and metastasis of tumor cells are responsible for poor prognosis [20]. The high expression of MMP-2 and TIMP-2 in GC tissues has been determined in several studies [16, 17, 21, 22]. It has been shown that MMP-2 expression correlates with clinicopathological features of GC, such as tumor stage, depth of tumor invasion and the presence of lymph node and distant metastases, while immunoreactivity of TIMP-2 correlates with tumor differentiation and the presence of distant metastases [16–18, 21]. Moreover, Kubben et al. [22] established the importance of MMP-2 as an independent prognostic factor for the survival of GC patients.

However, to the best of our knowledge, this is the first study assessing diagnostic criteria for MMP-2 and TIMP-2 in the sera of GC patients in comparison with the classical tumor markers, carcinoembryonic antigen (CEA) and carbohydrate antigen (CA 19-9).

The aim of the current study was to compare the clinical importance of serum MMP-2 and its inhibitor (TIMP-2) with classical tumor markers (CEA and CA 19-9) in the diagnosis of GC by calculating the diagnostic criteria, such as diagnostic sensitivity and specificity (the percentage of true positive and negative results), and positive and negative predictive values for all the proteins tested. In addition, we determined MMP-2, TIMP-2 and classical tumor marker levels in the sera of GC patients in relation to clinicopathological features of cancer, including tumor stage, gastric wall infiltration, and the presence of lymph node and distant metastasis as well as patients' survival.

Material and methods

Patients

The study included 100 patients with GC and 91 healthy subjects. Eighty-nine of the GC patients (27 women and 73 men, aged 27–83 years) underwent surgical tumor resection in the Second General Surgery Department of the Medical University Hospital in Bialystok, while the other 11 patients had non-resectable tumors. The control group comprised 60 women and 31 men, aged 21–65 years. The GC patients were observed over a period of four years. There were no significant differences between female and male subjects in GC patients and control groups. However the differences were statistically significant for age of patients in both analyzed groups. The diagnosis of GC was confirmed by microscopic examination of the tumor samples obtained during gastroscopy and/or surgery. The staging of cancer was based on TNM (tumor-nodulus-metastases) classification, according to the 5th International Union Against Cancer [23]. For statistical analysis, the GC patients were divided into three groups: 27 cancer patients in stage I + II, 31 patients in stage III, and 42 patients in stage IV. They were also sub-divided into three groups depending on gastric wall infiltration (T1 + T2, T3, and T4), two groups depending on nodal involvement (N0, N1 + N2 + N3), and two groups depending on the presence of distant metastases (M0 and M1). Sixty-six patients had intestinal type gastric adenocarcinoma by Lauren classification, whereas 34 patients had diffuse type GC. The study was approved by the Local Ethics Committee and all the patients gave informed consent.

Biochemical analyses

Blood samples from all the patients were drawn before treatment. All the sera were separated within an hour of blood collection and stored at –80°C until assayed.

Serum levels of MMP-2 and TIMP-2 were measured using enzyme-linked immunosorbent assay kits (ELISA) (R&D Systems, Abingdon, UK) according to the manufacturer's instructions. The serum samples were diluted 10-fold before determination for MMP-2, and 50-fold for TIMP-2. The manufacturer of assay kits described the intra-assay coefficient of variation (CV%) for MMP-2 as 5.8% at mean concentration of 18.9 ng/mL, SD = 1.1, and for TIMP-2 as 4.4% at a mean level of 1.23 ng/mL with SD = = 0.054. Serum concentrations of CEA and CA 19-9 were measured by microparticle enzyme immunoassay kits (MEIA) (Abbott, Chicago, IL, USA). The intra-assay CV% for CEA was reported by the manufacturer of the assay kits to be 4.9% at a mean concentration of 2.2 ng/mL, SD = 0.11 and the intra-assay CV% for CA 19-9 — 4.7% at a mean concentration of 38.2 U/mL, SD = 1.80.

The reference cut-off values for classical tumor markers (the 95th percentile), 4.0 ng/mL for CEA and 30.0 U/mL for CA 19-9, were established previously in our department by examining blood sera of healthy volunteers [24]. The cut-off values for serum MMP-2 (188 ng/mL) and TIMP-2 (85 ng/mL) levels correspond to the highest accuracy (minimal false-negative and false-positive results) and were determined using Microsoft Office Excel software. The positive results of MMP-2 and TIMP-2 are below cut-off values.

Statistical analysis

The levels of MMP-2 and classical tumor markers CEA and CA 19-9 did not follow a normal distribution based on preliminary statistical analysis (χ^2 test). Consequently, nonparametric statistical analyses were used. The Mann–Whitney U-test was employed to compare the two groups, while differences between more than two groups were compared using ANOVA on ranks (Kruskal–Wallis tests). The *post hoc* Dwass-Steel-Critchlow-Fligner test was conducted to assess which groups were different, if significant differences were found. Data are presented as the median and range. The differences were considered statistically significant when $p < 0.05$. The Kaplan–Meier method was used for the calculation of the survival curves. The log-rank test for univariate analyses of survival and the Cox proportional hazards model for multivariate analyses were employed. Moreover, we calculated diagnostic criteria, such as percentage of true positive and negative results, positive and negative predicted values for MMP-2, TIMP-2 and tumor markers. Statistical analyses were carried out using the STATISTICA 5.1 PL program (StatSoft Inc., Tulsa, OK, USA). Diagnostic criteria were calculated using Med-Calc statistical software (Med-Calc Software, Mariakerke, Belgium) and Microsoft Office Excel.

Results

The median and range of MMP-2 and TIMP-2 levels and classical tumor markers (CEA and CA 19-9) in GC and healthy subjects (the control group) are presented in Tables 1 and 2. The levels of MMP-2 and TIMP-2 were significantly lower, whereas CEA and CA 19-9 were higher, in GC patients compared to healthy subjects.

According to the tumor stage, based on TNM classification, the MMP-2 levels were lower in stages I + + II and IV compared to stage III, while concentrations of TIMP-2 were lower in stages III and IV than in stages I + II, although these differences were not statistically significant (Table 1). The levels of CEA and CA 19-9 increased with more advanced GC stage and were highest in stage IV; however, the differences were found to be significant only for CA 19-9 ($p = 0.049$) (Table 2).

Serum levels of all the proteins tested varied according to the clinicopathological features of GC, such as gastric wall infiltration, the presence of lymph node and distant metastasis. Statistically significant differences were found between the serum levels of TIMP-2 ($p = 0.002$), CEA ($p = 0.018$), CA 19-9

Table 1. Serum levels of MMP-2 and TIMP-2 in GC patients in relation to clinicopathological features of tumor

		n	MMP-2 [ng/mL]				TIMP-2 [ng/mL]			
			Median	Range		p	Median	Range		p
Group tested	Gastric cancer	100	184	118	500	0.006*	79	53	149	< 0.001*
	Healthy controls	91	205	118	384		94	54	162	
Tumor stage	I + II	27	178	132	500	NS	88	62	149	NS
	III	31	195	134	394		78	63	123	
	IV	42	184	118	499		79	53	106	
Gastric wall infiltration	T1 + T2	24	182	136	500	NS	90	63	149	0.002*
	T3	40	179	120	387		76[A]	53	117	
	T4	36	189	118	499		85	58	123	
Nodal involvement	N0	25	175	132	495	NS	83	62	149	NS
	N1 + N2 + N3	75	187	118	500		78	53	133	
Distant metastases	M0	65	185	132	500	NS	79	62	149	NS
	M1	35	183	118	499		80	53	106	

*Statistically significant when $p < 0.05$; NS — not statistically significant; [A] — statistically significant when compared to T1 + T2 group in Dwass–Steel–Critchlow–Fligner *post hoc* test

Table 2. Serum levels of classic tumor markers in GC patients in relation to clinicopathological features of tumor

		n	CEA [ng/mL]				CA 19-9 [U/mL]			
			Median	Range		p	Median	Range		p
Group tested	Gastric cancer	100	1.4	0.1	620.0	< 0.001*	6.9	0.0	50000.0	< 0.001*
	Healthy controls	91	0.8	0.1	11.4		0.8	0.0	53.0	
Tumor stage	I + II	27	1.2	0.3	6.9	NS	3.4[A]	0.0	431.0	0.049*
	III	31	1.2	0.3	120.3		7.0	0.0	2169.0	
	IV	42	2.0	0.1	620.0		16.0	0.0	50000.0	
Gastric wall infiltration	Tl + T2	24	1.1	0.3	11.9	0.018*	3.6	0.0	339.0	0.034*
	T3	40	1.2	0.1	120.3		8.7	0.0	10880.0	
	T4	36	2.1[B]	0.6	620.0		16.0[B]	0.0	50000.0	
Nodal involvement	N0	25	1.4	0.1	6.9	NS	2.0	0.0	431.0	0.001*
	N1 + N2 + N3	75	1.7	0.3	620.0		11.7	0.0	50000.0	
Distant metastases	M0	65	1.3	0.3	120.3	NS	5.8	0.0	4005.0	NS
	M1	35	1.9	0.1	620.0		8.8	0.0	50000.0	

*Statistically significant when $p < 0.05$; NS — not statistically significant; [A] — statistically significant when compared to stage IV in Dwass–Steel–Critchlow–Fligner *post hoc* test; [B] — statistically significant when compared to Tl + T2 group in Dwass–Steel–Critchlow–Fligner *post hoc* test

(p = 0.034) and gastric wall infiltration (T factor), as well as between CA 19-9 (p = 0.001) concentrations and nodal involvement (N factor). Moreover, TIMP-2 levels were significantly lower in the T3 subgroup compared to patients in the Tl + T2 group (Table 1), while CEA and CA 19-9 were significantly higher in the T4 subgroup in compared to the Tl + T2 subgroup (Table 2).

The Kaplan–Meier method was used to assess the relationship between survival of GC patients and proteins tested levels. Univariate log-rank analysis showed that tumor size (p = 0.004), the presence of nodal involvement (p = 0.003) and distant metastases (p = 0.001), as well as the levels of CEA (p = = 0.007) and CA 19-9 (p = 0.008), were significant factors affecting overall survival. However, multivariate regression analysis using Cox's proportional hazards model failed to establish the significance of the classical tumor markers as independent prognostic factors for the survival of GC patients.

The percentage of true positive results (diagnostic sensitivity) of proteins tested is presented in Figure 1. The diagnostic sensitivity of TIMP-2 (61%) was higher than that of MMP-2 (54%) and double that of classical tumor markers CEA and CA 19-9 (respectively: 21% and 31%). The highest percentage of true positive results was observed for combined use of TIMP-2 with MMP-2 (77%) compared to the diagnostic sensitivity of tumor markers (Figure 1). The percentage of true negative results (diagnostic specificity) for TIMP-2 (73%) and MMP-2 (68%) levels was lower than those for classical tumor markers, CEA (98%), and CA 19-9 (97%), similarly for positive predictive value. However, negative predictive value was higher for TIMP-2 (63%) and MMP-2 (57%) compared to classical tumor markers. The highest negative predictive value was observed for combined use of TIMP-2 with MMP-2 or CA 19-9 (68%).

Discussion

Matrix metalloproteinase-2 is a zinc-dependent protease released from a cell as a zymogen. This enzyme requires extracellular post-translational cleavage to gain biological activity [9, 11, 25]. MMP-2 is found in various cells, including normal myocytes and vascular cells, and therefore may play a role in cardiac remodeling, heart tube formation and angiogenesis [26, 27]. Some clinical investigations have indicated that MMP-2 is an important protease implicated in the proteolytic regulation of various intracellular proteins in myocardial oxidative stress injury [9, 26]. This enzyme is also produced by tumor cells and may facilitate the migration of malignant cells because of its ability to degrade type IV collagen of extracellular matrices and basal membranes [6, 21]. MMP-2 activity is inhibited by its tissue inhibitors, including TIMP-2 [8, 21]. The imbalance between MMPs and their inhibitors may facilitate progression of cancer cells [5, 8]. It has been shown that increased MMP-2 and TIMP-2 expression plays a key role in invasion and metastases of several types of cancer, such as colorectal [12, 13], pancreatic [14] and gastric cancer [16–18, 28].

The objective of the current study was to investigate the differences between diagnostic criteria, in-

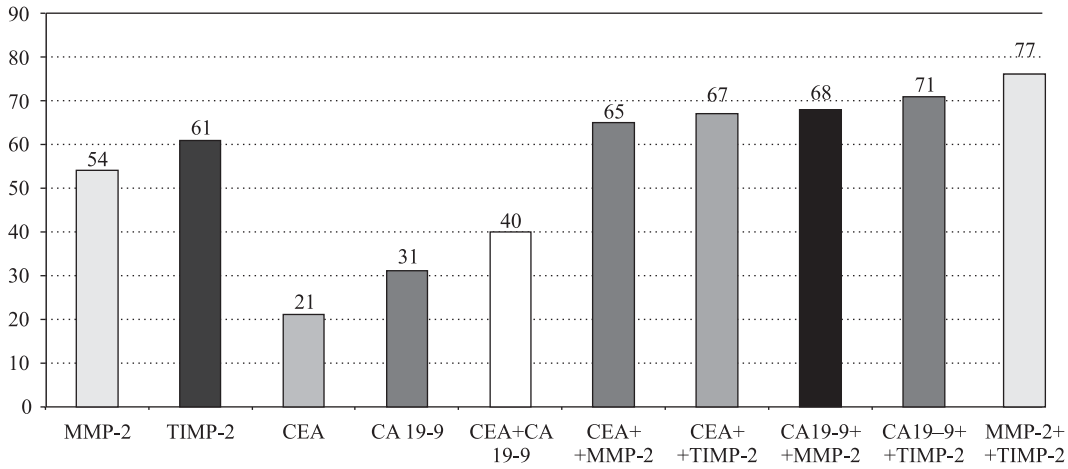

Figure 1. The percentage of true positive results of MMP-2, TIMP-2 and classic tumor markers in GC patients

cluding diagnostic sensitivity and specificity (the percentage of true positive and negative results), positive and negative predictive values for MMP-2, TIMP-2 and classical tumor markers (CEA and CA 19-9) as well as serum levels of all the proteins tested in relation to clinicopathological features of the tumor as well as GC patients' survival.

We found the serum levels of MMP-2 and TIMP-2 were significantly lower in the GC patients group than in healthy subjects. Our results agree with the findings of Waas et al. [29], who revealed that the levels of precursor form of MMP-2 (proMMP-2) were significantly lower in the plasma of patients with colorectal cancer (CRC) than in healthy controls. Contradictory results were found in the study of Endo et al. [30], who indicated that serum as well as plasma levels of proMMP-2 in GC patients were significantly higher compared to healthy individuals. Moreover, Kubben et al. [22] demonstrated that MMP-2 expression was significantly increased in GC tissue compared to normal gastric mucosa. We found the serum concentrations of classical tumor markers were significantly higher in GC patients compared to the control group. This was similar to our previous study, where we showed that the CEA levels were significantly higher in GC patients than in healthy subjects [31]. Our current results indicate that the concentrations of MMP-2 were lower in stage IV compared to stage III, whereas TIMP-2 levels were lower in more advanced tumor stages than in early stages (I + II). Similar results were obtained by Alakus et al., who suggested that aggressive forms of GC are associated with low TIMP-2 expression [21].

Serum concentrations of MMP-2, TIMP-2 and classical tumor markers varied according to clinicopathological features of GC, such as gastric wall infiltration, the presence of lymph nodes and distant metastases. Serum TIMP-2 levels were significantly lower in the T3 subgroup compared to patients in the Tl + T2 group. However, we did not find statistically significant differences between the serum levels of MMP-2 and gastric wall infiltration (T factor). Contradictory results were shown by Shim et al. [1], who failed to establish any significant differences between expression of TIMP-2 and clinicopathological parameters of GC.

Similarly as in our findings, Endo et al. [30] were unable to demonstrate a significant association between serum proMMP-2 concentrations and gastric wall infiltration. However, several studies have indicated that expression of MMP-2 is positively correlated with depth of tumor invasion, lymphatic node and distant metastases, while the intensity of TIMP-2 staining is positively correlated with the presence of distant metastasis [16–18, 21].

Our current study assessed the association between the survival of GC patients and the serum classical tumor markers concentrations using the Kaplan–Meier method, although we did not detect any tendency that would point to MMP-2 and TIMP-2 as prognostic factors for GC patients' survival. Our observations are in line with the findings of Alakus et al. [21], who indicated that high expression of MMP-2 in GC tissue was associated with poor prognosis, but not as an independent prognostic factor, while TIMP-2 showed no correlation with the GC patients' survival, in a similar way to CEA levels in our previous study [31]. However, Kubben et al. [22] proved the significance of enhanced tumor MMP-2 levels as an independent prognostic factor of the survival of GC patients as well as plasma TIMP-1 concentrations, which was confirmed in our previous study [31].

The percentage of true positive results of TIMP-2 (61%) was higher than those of MMP-2 (54%) and had twice the diagnostic sensitivity of classical tumor markers. The highest frequency of true positive results was observed for combined use of TIMP-2 with MMP-2 (77%). The percentage of true positive results established in the present paper was slightly lower than those obtained in our previous data, where we calculated the diagnostic sensitivity of other MMPs and their inhibitors in GC patients, including MMP-9 (60%) and TIMP-1 (78%) [31].

However, the frequency of true positive results of MMP-2 and TIMP-2 assessed in the current paper was higher than those of other biomarkers useful in GC diagnosis — hematopoietic growth factors (HGFs), such as granulocyte-colony stimulating factor (G-CSF) (36%), granulocyte-macrophage colony stimulating factor (GM-CSF) (29%), stem cell factor (SCF) (19%) and macrophage colony stimulating factor (M-CSF) (10%) [32]. The percentage of true negative results obtained in the present data for TIMP-2 (73%) and MMP-2 (68%) levels was lower than those for classical tumor markers, CEA (98%) and CA 19-9 (97%) as well as for M-CSF (95%), SCF (94%), G-CSF (92%), and GM-CSF (82%) assessed in our previous paper [32].

In conclusion, to the best of our knowledge, the present study is the first to assess serum levels of MMP-2 and its tissue inhibitor (TIMP-2) compared to classical tumor markers in relation to clinicopathological features of GC, including tumor stage, gastric wall infiltration, the presence of lymph node and distant metastasis as well as patients' survival. Moreover, the current study is the first to compare the diagnostic criteria of MMP-2 and TIMP-2 with classical tumor markers in this cancer. We found that serum levels of MMP-2 and TIMP-2 were significantly lower, but concentrations of classical tumor markers were higher, in GC patients than in healthy subjects. Additionally, serum TIMP-2, CEA and CA 19-9 correlated with gastric wall infiltration. None of the proteins tested was found to be an independent prognostic factor for GC patients' survival. The percentage of true positive results (diagnostic sensitivity) of TIMP-2 (61%) and MMP-2 (54%) were higher than those for the tumor markers CEA (21%) and CA 19-9 (31%).

These findings suggest that serum MMP-2 and TIMP-2 are more useful than classical tumor markers in the diagnosis of gastric cancer. However, our paper is one of the first studies of the serum levels of MMP-2 and TIMP-2 in GC patients, and therefore further investigation seems to be necessary.

References

1. Shim K, Jung S, Joo Y, Yoo K. Clinical significance of tissue levels of matrix metalloproteinases and tissue inhibitors of metalloproteinases in gastric cancer. *J Gastroenterol*. 2007;42:120–128.
2. Schwartz G. Invasion and metastases in gastric cancer: in vitro and in vivo models with clinical correlations. *Semin Oncol*. 1996;23:316–324.
3. Wagenaar-Miller R, Gorden L, Matrisian L. Matrix metalloproteinases in colorectal cancer: is it worth talking about? *Cancer Metastasis Rev*. 2004;23:119–135.
4. Bonomi P. Matrix metalloproteinases and matrix metalloproteinase inhibitors in lung cancer. *Semin Oncol*. 2002;29:78–86.
5. Nabeshima K, Inoue T, Shimao Y, Sameshima T. Matrix metalloproteinases in tumor invasion: role for cell migration. *Pathol Int*. 2002;52:255–264.
6. Kabashima A, Maehara Y, Kakeji Y, Baba H, Koga T, Sugimachi K. Clinicopathological features and overexpression of matrix metalloproteinases in intramucosal gastric carcinoma with lymph node metastasis. *Clin Cancer Res*. 2000;6:3581–3584.
7. Galis Z, Muszynski M, Sukhova G et al. Cytokine-stimulated human vascular smooth muscle cells synthesize a complement of enzymes required for extracellular matrix digestion. *Circ Res.* 1994;75:181–189.
8. Kähäri VM, Saarialho-Kere U. Matrix metalloproteinases and their inhibitors in tumour growth and invasion. *Ann Med*. 1999;31:34–45.
9. Kandasamy A, Chow A, Ali M, Schulz R. Matrix metalloproteinase-2 and myocardial oxidative stress injury: beyond the matrix. *Cardiovasc Res*. 2010;85:413–423.
10. Howard E, Bullen E, Banda M. Preferential inhibition of 72- and 92-kDa gelatinases by tissue inhibitor of metalloproteinases-2. *J Biol Chem*. 1991;266:13070–13075.
11. Jezierska A, Motyl T. Matrix metalloproteinase-2 involvement in breast cancer progression: a mini-review. *Med Sci Monit*. 2009;15:RA32–40.
12. Shin J, Jung S, Kim S et al. Expression of MMP-2, HIF-1alpha and VEGF in colon adenoma and colon cancer. *Korean J Gastroenterol*. 2007;50:9–18.
13. Li B, Zhao P, Liu S, Yu Y, Han M, Wen J. Matrix metalloproteinase-2 and tissue inhibitor of metallo-proteinase-2 in colorectal carcinoma invasion and metastasis. *World J Gastroenterol*. 2005;11:3046–3050.
14. Giannopoulos G, Pavlakis K, Parasi A et al. The expression of matrix metalloproteinases-2 and -9 and their tissue inhibitor 2 in pancreatic ductal and ampullary carcinoma and their relation to angiogenesis and clinicopathological parameters. *Anticancer Res*. 2008;28:1875–1881.
15. Hoikkala S, Pääkkö P, Soini Y, Mäkitaro R, Kinnula V, Turpeenniemi-Hujanen T. Tissue MMP-2 and MMP-9 are better prognostic factors than serum MMP-2/TIMP-2-complex or TIMP-1 in stage I-III lung carcinoma. *Cancer Lett*. 2006;236:125–132.
16. Zheng H, Takahashi H, Murai Y et al. Expressions of MMP-2, MMP-9 and VEGF are closely linked to growth, invasion, metastasis and angiogenesis of gastric carcinoma. *Anticancer Res*. 2006;26:3579–3583.
17. Wu Z, Li J, Zhan W, He Y. Lymph node micrometastasis and its correlation with MMP-2 expression in gastric carcinoma. *World J Gastroenterol*. 2006;12:2941–2944.
18. Mönig S, Baldus S, Hennecken J et al. Expression of MMP-2 is associated with progression and lymph node metastasis of gastric carcinoma. *Histopathology*. 2001;39:597–602.

19. Lochhead P, El-Omar E. Gastric cancer. *Br Med Bull*. 2008;85:87–100.
20. Samson P, Escovidal L, Yrastorza S, Veneracion R, Nerves M. Re-study of gastric cancer: analysis of outcome. *World J Surg*. 2002;26:428–433.
21. Alakus H, Grass G, Hennecken J et al. Clinicopathological significance of MMP-2 and its specific inhibitor TIMP-2 in gastric cancer. *Histol Histopathol*. 2008;23:917–923.
22. Kubben F, Sier C, van Duijn W et al. Matrix metalloproteinase-2 is a consistent prognostic factor in gastric cancer. *Br J Cancer*. 2006;94:1035–1040.
23. Jass JR, LH S. *WHO International histological classification of tumors. Histological typing of intestinal tumors.* New York: Springer-Verlag, 1989.
24. Mroczko B, Szmitkowski M, Okulczyk B. Granulocyte-colony stimulating factor (G-CSF) and macrophagecolony stimulating factor (M-CSF) in colorectal cancer patients. *Clin Chem Lab Med*. 2002;40:351–355.
25. Roberts L, Visser J, Ingraham H. Involvement of a matrix metalloproteinase in MIS-induced cell death during urogenital development. *Development*. 2002;129:1487–1496.
26. Linask K, Han M, Cai D, Brauer P, Maisastry S. Cardiac morphogenesis: matrix metalloproteinase coordination of cellular mechanisms underlying heart tube formation and directionality of looping. *Dev Dyn.* 2005;233:739–753.
27. Cho W, Chow A, Schulz R, Daniel E. Matrix metalloproteinase-2, caveolins, focal adhesion kinase and c-Kit in cells of the mouse myocardium. *J Cell Mol Med*. 2007;11:1069––1086.
28. Koyama S. Enhanced cell surface expression of matrix metalloproteinases and their inhibitors, and tumor-induced host response in progression of human gastric carcinoma. *Dig Dis Sci*. 2004;49:1621–1630.
29. Waas E, Wobbes T, Ruers T, Lomme R, Hendriks T. Circulating gelatinases and tissue inhibitor of metalloproteinase-1 in colorectal cancer metastatic liver disease. *Eur J Surg Oncol*. 2006;32:756–763.
30. Endo K, Maehara Y, Baba H et al. Elevated levels of serum and plasma metalloproteinases in patients with gastric cancer. *Anticancer Res*. 1997;17:2253–2258.
31. Mroczko B, Groblewska M, Łukaszewicz-Zajac M, Bandurski R, Kedra B, Szmitkowski M. Pre-treatment serum and plasma levels of matrix metalloproteinase 9 (MMP-9) and tissue inhibitor of matrix metalloproteinases 1 (TIMP-1) in gastric cancer patients. *Clin Chem Lab Med*. 2009;47:1133––1139.
32. Mroczko B, Wereszczyńska-Siemiątkowska U, Groblewska M et al. The diagnostic value of hematopoietic cytokines measurement in the sera of gastric cancer and gastric ulcer patients. *Clin Chim Acta*. 2006;374:165–167.

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