Pathol. Oncol. Res. (2009) 15:589–596
DOI 10.1007/s12253-009-9158-9

ORIGINAL PAPER

Prognostic Value of Tumor-Related Molecular Expression in Gastric Carcinoma

Zhong-Sheng Zhao · Yuan-Yu Wang · Zai-Yuan Ye · Hou-Quan Tao

Received: 5 November 2008 / Accepted: 17 February 2009 / Published online: 18 March 2009

Abstract In order to identify reliable molecular markers for prognostic prediction in gastric carcinoma, we evaluated the expression of six molecular markers, namely bFGF, IGF-2, HGF, MMP-9, integrin β3 and uPA in gastric cancer. There was a significant correlation between the expression of these markers and the depth of tumor invasion, vessel invasion, lymph node and distant metastasis, TNM stage and micro-vessel density. The average survival time and 5-year survival rate of patients with positive expression of molecular markers was higher than those with negative expression. Multivariate analysis showed that abnormal expression of bFGF, MMP-9 and uPA, as well as depth of invasion, lymph node and distant metastasis and TNM stage were independently related to poor prognosis of gastric cancer. MMP-9, bFGF and uPA are potential candidates for development as clinically applicable molecular prognostic markers for gastric carcinoma, and may be effective therapeutic targets for the disease in the future.

Keywords Gastric cancer · Basic fibroblast growth factor · Matrix metalloproteinase-9 · Urokinase plasminogen activator · Prognosis · Molecular markers

Support by a grant from Zhejiang Provincial Medical Science Research Foundation (No.2007A013)

Z.-S. Zhao (✉)
Department of Pathology, Zhejiang Provincal People's Hospital,
158 shangtang road,
310014 Hangzhou, China
e-mail: lywyy1979@hotmail.com

Y.-Y. Wang · Z.-Y. Ye · H.-Q. Tao
Department of Surgery, Zhejiang Provincal People's Hospital,
Hangzhou, China

Introduction

According to global estimates of cancer incidence in the year 2002, gastric cancer is the second most frequent cancer-related cause of death after lung cancer. The incidence of gastric cancer is estimated to be 934,000 cases, with 56% of the new cases occurring in East Asia, including 41% in China and 11% in Japan [1]. Although the global incidence of gastric cancer has decreased in recent years, its mortality rate in China is the highest among all tumors and represents 25% of gastric cancer mortality worldwide. Despite recent advances in chemotherapy and surgical techniques, the overall 5-year survival rate in China is low at 40%, most gastric cancer is diagnosed at stage III or IV, and the rate of lymph node metastasis is higher (50–75%) [2]. The development of tumor invasion and metastasis is a very complicated and continuous process with multiple steps. During this process, tumor cells break through several tissues barriers, which involves the extracellular matrix and the basement membrane of the epithelium. Subsequently, tumor cells penetrate into blood and lymphatic vessels. Finally, the cells leak out from the vessels and build up new secondary cancer cell colonies at distant sites [3]. At the molecular level, multiple regulating genes including adhesion molecules, protein catabolic enzymes, cell growth factors and various angiogenesis factors contribute to this process. At present, numerous studies have only examined the expression of one or more of these genes in gastric carcinoma, but only a few have examined the expression of multiple regulating genes altogether.

Here, we analyzed the prognostic significance of six molecular markers in gastric carcinoma, namely basic fibroblast growth factor (bFGF), insulin-like growth factor-2

(IGF-2), hepatocyte growth factor (HGF), matrix metalloproteinase-9 (MMP-9), urokinase plasminogen activator (uPA) and integrin β3. Our results show that bFGF, MMP-9 and uPA have independent prognostic significance in gastric carcinoma, and their combined expression profile may be a useful molecular marker.

Materials and Methods

Patients and Tissue Samples

Gastric cancer tissues were collected from gastrectomy specimens of 105 patients (mean age 57.6, range 38–78 years; 70 male, 35 female) from the Department of Surgery, Zhejiang Provincial People's Hospital from 1986–1998. All patients had follow-up records for over 5 years. The follow-up deadline was October 2002. The survival time was counted from the date of surgery to the follow-up deadline or date of death, which was mostly caused by carcinoma recurrence or metastasis. The bobtail value was defined as 0. According to the WHO histological classification of gastric carcinoma formulated in 1999, there were 37 tubular adenocarcinomas, 17 papillary adenocarcinomas, 34 poorly differentiated adenocarcinomas, eight signet-ring cell carcinomas, nine mucinous adenocarcinomas; 63 were classified as well or moderately differentiated adenocarcinomas and 42 as poorly differentiated and undifferentiated adenocarcinomas; 63 intestinal types, 42 diffuse types. Based on the 5th Edition of the UICC TNM system, there were 20 at stage pT_1, 24 at stage pT_2, 39 at stage pT_3, and 22 at stage pT_4. In 76 cases, cancer invasion of the blood or lymph vessels was found. There were 42 cases with distant metastasis, including 24 with peritoneal metastasis and 18 with hepatic metastasis. Finally, 15 cases were categorized as stage I, 10 as stage II, 35 as stage III and 45 as stage IV.

General Histological Procedures

Tissue specimens fixed in 10% neutral formalin and embedded in paraffin blocks. Serial 5-μm sections were cut and spread on slides using 0.1% diethyl pyrocarbonate, and polylysine was used to prevent tissue sections coming off the slides. The slides were baked in an oven at 70°C for 2–3 h and overnight at 58°C. Finally, baked slides were wrapped in tin foil and stored in a 4°C refrigerator or kept at room temperature. These slides were prepared for H&E staining, *in situ* hybridization and immunohistochemical staining.

Reagents

Digoxin-labeled probes and sensitivity-enhanced *in situ* hybridization kits were provided by Boster Biological Technology (Wuhan, China). The mRNA sequences (length of probe: 29–30 base pairs) are shown in Table 1. Immunohistochemical reagents including CD34 (mouse anti-human immunoglobulin antibody) and streptavidin-peroxidase (SP) kit were purchased from Maixin-Bio (Fuzhou, China).

In Situ Hybridization

In situ hybridization was performed according to manufacturer's instructions (Boster Biological Technology) [4, 5]. Tissue sections (5 μm) were deparaffinized, dehydrated and incubated in 0.2 mol/L HCl for 20 min. After washing with

Table 1 The mRNA sequences of of bFGF, IGF-2, HGF, MMP-9, Integrin beta-3 and uPA

Probe	sequence 5′~3′					
IGF-2	TGGCC	TTCGC	CTCGT	GCTGC	ATTGC	TGCTT
	GCGTT	CAGGG	AGGCC	AAACG	TCACC	GTCCC
HGF	TGCAG	CATGT	CCTCC	TGCAT	CTCCT	CCTGC
	TTGGA	ATGGA	ATTCC	ATGTC	AGCGT	TGGGA
	TGGGA	AATGA	GAAAT	GCAGC	CAGCA	TCATC
MMP-9	TCCCT	GCCCG	AGACC	GGTGA	GCTGG	ATAGC
	CAACT	CGGCG	GGAGA	GCTGT	GCGTC	TTCCC
	CCAGG	TGGAC	CAAGT	GGGCT	ACGTG	ACCTA
bFGF	GCCGT	CGGGG	TGGAT	GCGCA	GGAAG	AAGCC
	TTGAT	AGACA	CAACT	CCTCT	CTCTT	CTGCT
	ACCGG	TAAGT	ATTGT	AGTTA	TTAGA	TTCCA
Integrin beta-3 gene	GACAC	CTGTG	AGAAG	TGCCC	CACCT	GCCCA
	GGATG	ACTGT	GTCGT	CAGAT	TCCAG	TACTA
	GCTAA	ATTTG	AGGAA	AGGCG	CGCCA	GAGC
uPA	CTAGG	CCTGG	GGAAA	CACAA	TTACT	GCAGG
	TGTCT	ACACG	AGGGT	CTCAC	ACTTC	CTGGA

Table 2 Relationship between pathologic parameters and bFGF, IGF-2, HGF, MMP-9, integrin beta 3 and u PA expression in gastric carcinoma (n=105)

Clinicopathologic Index	n	bFGF			IGF-2			HGF			MMP-9			Integrin beta3			uPA		
		-	+	p	-	+	p	-	+	p	-	+	p	-	+	p	-	+	p
Growth pattern				0.023			1.10			0.22			0.002			0.002			0.001
Expansive	48	27	21		29	19		24	24		27	21		24	24		29	19	
Invasive	57	14	43		24	33		21	36		17	40		17	40		15	42	
Depth of invasion				0.001			0.003			0.001			0.004			0.015			0.001
T_1-T_2	44	31	13		31	13		29	15		26	18		24	20		27	17	
T_3-T_4	61	10	51		22	39		16	45		18	43		17	44		17	44	
Vessel invasion				0.001			0.025			0.04			0.024			0.55			0.006
No	29	25	4		19	10		19	10		17	12		14	15		16	13	
Ye	76	16	60		34	42		26	50		27	49		27	49		28	48	
Lymph node metastasis				0.001			0.019			0.013			0.01			0.025			0.001
No	35	25	10		22	13		22	13		21	14		19	16		21	14	
Yes	70	16	54		31	39		23	47		23	47		22	48		23	47	
Distant metastasis				0.001			0.001			0.001			0.001			0.035			0.004
No	63	37	26		43	20		40	23		40	23		35	28		39	24	
Yes	42	4	38		10	32		5	37		4	38		6	36		5	37	
TNM stage				0.01			0.001			0.001			0.001			0.001			0.001
I	15	15	0		14	1		13	2		15	0		13	2		15	0	
II	10	9	1		8	2		10	0		9	1		8	2		10	0	
III	35	12	23		20	15		21	14		15	20		12	23		12	23	
IV	45	5	40		11	34		6	39		5	40		8	37		7	38	
Intestinal types	63	35	28		40	23	0.001	38	25	0.001	39	24	0.001	34	29	0.001	40	23	0.001
Diffuse types	42	6	36		13	29		7	35		5	37		7	35		4	38	

2 × SSC, the sections were incubated with proteinase K for 10 min at 37°C, fixed with PBS containing 4% paraformaldehyde for 5 min, washed with 2 × SSC, and then prehybridized for 2 h at 63°C in a buffer containing 50% deionized formamide, 4 × SSC, 2 × Denhardt's solution and 250 μg/mL RNA. Hybridization was performed in 50% deionized formamide, 4 × SSC, 2 × Denhardt's solution, 10% dextran sulfate and 500 μg/mL RNA. The final concentration of DIG-labeled probes was ~500 ng/mL. The probes were placed on the section, covered with parafilm and incubated at 63°C overnight in a moisture chamber. After hybridization, excess probes were removed by washing in 2 × SSC, followed by RNase treatment with 100 U/mL RNase T1 at 37°C for 30 min. The sections were washed at 65°C in 2 × SSC for 10 min, washed three times in 0.2 × SSC and 50% deionized formamide (10 min each time), and incubated with an anti-DIG antibody. Detection was accomplished using the streptavidin/biotin/peroxidase method and the reaction was developed with 3, 3′-diaminobenzidine (DAB) chromogen. All antibodies were purchased from Boster Biological Technology.

Immunohistochemistry

Immunohistochemistry was performed according to the manufactures' instructions (Maixin-Bio) [6, 7]. Briefly, the tissue sections were deparaffinized in xylene at 37°C for 20 min. Endogenous peroxide was blocked by incubating the sections with 30 mL/L H_2O_2 for 10 min at 37°C. The sections were incubated with primary and secondary antibodies to bFGF, IGF-2, HGF, MMP-9, integrin β3 and uPA at 4°C overnight. Staining was visualized with DAB for 10 min at room temperature.

Evaluation of Results

The cytoplasm with IGF-2, HGF, MMP-9, bFGF, integrin β3 and uPA mRNA was stained as buffy. The staining results were estimated by two pathologists, based on the percentage of positive cells: (−) no staining or positive cell number <10%, (+) positive cell number 11–50%, (++) positive cell number 51–75%, (+++) positive cell number >75%. Tumor microvessel density (MVD) value was determined as follows [8]: Microvessel counting was performed twice. Each slide was first scanned at 100× magnification to determine three "hot spots", defined as areas with the maximum number of CD34 + vessels. The CD34 + vessel density was determined by counting all the immunostained vessels at 200× magnification, and the mean number of positive vessels was calculated in the three selected areas for each case. The average count in the three hot spots was taken as the MVD and expressed as the mean±SD.

Statistical Analysis

All statistical analyses were performed using SPSS11.0 software. Measurement data were analyzed using the Student's *t* test, while categorical data were studied using χ^2 or Fisher exact tests. Survival curves were estimated using the Kaplan-Meier method, and the log-rank test was used to compute differences between the curves. Multivariate analysis using the Cox proportional hazards regression model was performed to assess the prognostic values of protein expression. Correlation coefficients between protein expression and clinicopathological findings were estimated using the Pearson correlation method. Statistical significance was taken as $P<0.05$.

Results

Tumor-Related Gene Expression in Gastric Carcinoma

The positive expression rate of bFGF, IGF-2, HGF, MMP-9, integrin β3 and uPA mRNA in gastric carcinoma was 60.95, 49.52, 57.14, 58.10, 60.95 and 58.10%, respectively. Expression of IGF-2, HGF and MMP-9 mRNA was negative in normal gastric mucous membrane, whereas integrin β3 and bFGF mRNA was expressed in 30% (6/20) and 10% (2/20) of non-tumor gastric mucous membrane, respectively.

Table 3 Correlation between expression of tumor-related genes and gastric carcinoma angiogenesis

Group	N	MVD	t	*P*
IGF-2			4.92	0.001
+-+++	52	43.01±15.38		
-	53	35.92±14.62		
HGF			3.57	0.012
+-+++	60	44.30±13.31		
-	45	32.94±13.54		
bFGF			3.21	0.002
+-+++	64	46.09±11.51		
-	41	29.05±12.47		
MMP-9			7.31	0.001
+-+++	61	43.75±13.41		
-	44	33.45±13.92		
integrin beta 3			11.25	0.025
+-+++	64	41.02±8.55		
-	41	25.26±11.25		
uPA			8.95	0.032
+-+++	61	44.08±6.15		
-	44	27.25±7.85		

Table 4 Relationship between tumor MVD value and various pathologic parameters in gastric carcinoma

Groups	N	MVD	t	*P*
Depth of tumor invasion(T)			5.96	0.001
T_1~T_2	44	30.84±13.66		
T_3~T_4	61	45.64±11.69		
Vessel invasion			7.39	0.001
NO	29	25.69±10.11		
YES	76	44.68±12.33		
Lymph node metastasis			3.82	0.01
NO	35	27.07±11.33		
YES	70	45.62±11.69		
Distant metastasis			8.52	0.001
NO	63	31.30±12.97		
YES	42	51.69±5.21		

Correlation Between mRNA Expression of Tumor-Related Genes and Pathological Parameters of Tumor Progression

Positive expression of bFGF, IGF-2, HGF, MMP-9, uPA and integrin β3 correlated with depth of invasion, vessel invasion, lymph node and distant metastasis, and Lauren's classification ($P<0.05$), as shown in Table 2, The positive expression rate of bFGF, IGF-2, HGF, MMP-9, uPA and integrin β3 in diffuse types GC was significantly higher than that in Intestinal types GC ($P<0.05$) (Table 2). The positive expression rate of bFGF, MMP-9, uPA and integrin β3 in invasive tumor tissue was significantly higher than that in expansive tumor tissue ($P<0.05$) (Table 2).

Correlation Between Tumor-Related Genes and Gastric Carcinoma Angiogenesis

MVD in patients with positive expression of bFGF, IGF-2, HGF, MMP-9 and integrin β3 was significantly higher than that in those with negative expression (Table 3). Meanwhile, positive expression of these genes correlated well with MVD. In our study, when mRNA for the aforementioned genes was expressed positively, MVD increased significantly.

Relationship Between Tumor MVD and Various Pathological Parameters in Gastric Carcinoma

The vascular endothelial cells were positively immunostained for CD34. Many microvessels inside tumor and adjacent tissue were deeply stained from buffy to dark brown . There was a significant difference between tumor MVD and various pathological parameters such as depth of invasion ($P=0.001$), blood or lymph vessel invasion ($P=0.001$), and lymph node ($P=0.01$) or distant ($P=0.001$) metastasis, as described in Table 4. However, no statistically significant difference was found between tumor MVD and histological type ($P>0.05$). In addition, there was no

Table 5 Correlation between mRNA expression of bFGF, IGF-2, HGF, MMP-9, uPA and integrin beta 3 with MVD and postoperative survival time

Group	N	mean survival time(m)	five-year survival rate(%)	*P*
IGF-2				0.001
-	53	94.30±6.38	76.88 (40/53)	
+-+++	52	40.38±7.48	15.91 (8/52)	
HGF				0.002
-	45	113.33±7.03	83.57 (36/45)	
+-+++	60	43.43±6.04	19.89 (12/60)	
MMP-9				0.002
-	44	107.38±7.21	81.82 (36/44)	
+-+++	61	42.56±8.52	19.67 (12/61)	
integrin beta 3				0.003
-	41	94.28±6.21	85.36 (35/41)	
+-+++	64	42.32±7.51	20.31 (13/64)	
bFGF				0.001
-	41	118.04±6.52	87.06 (38/41)	
+-+++	64	33.06±3.57	15.66 (10/64)	
uPA				0.005
-	44	115.99±6.70	86.03 (38/44)	
+-+++	61	40.32±5.50	16.24 (10/61)	
MVD value				0.015
<39.5	45	85.50±6.8	78.4 (35/45)	
≥39.5	60	38.70±4.8	21.56 (13/60)	

significant difference between MVD of well or moderately differentiated adenocarcinoma and that of poorly differentiated or undifferentiated adenocarcinoma ($P>0.05$).

Correlation Between Expression of bFGF, IGF-2, HGF, MMP-9, Integrin β3, uPA, MVD and Postoperative Survival

Statistical analysis revealed that mean overall survival and 5-year survival rate of patients with positive expression of the aforementioned factors and MVD≥39.5 were significantly lower than those in patients with negative expression and MVD <39.5 (Table 5 and Figs. 1, 2 and 3).

Multivariate Analysis of Clinicopathological Parameters and Prognosis

The factors with possible prognostic effect in gastric carcinoma were analyzed by Cox regression analysis. The study revealed that tumor size ($P=0.011$), depth of tumor invasion ($P=0.022$), lymph node ($P=0.004$) and distant ($P=0.003$) metastasis, expression of bFGF ($P=0.001$), MMP-9 ($P=0.007$), uPA ($P=0.014$) and tumor MVD ($P=0.02$) were independent prognostic factors in patients with gastric carcinoma, whereas expression of IGF-2 ($P=0.083$), HGF ($P=0.087$) and integrin β3 ($P=0.676$) had no prognostic value.

Discussion

Stage is the most important factor in the survival, management and prognosis of gastric cancer patients, as well as in those with other carcinomas. The prognosis of these patients who have undergone curative resection remains poor because of high rates of local recurrence and early lymph node and systemic metastases. Gastric cancer is usually diagnosed at later stages (stage III and IV) in China, and the overall 5-year survival rate is low at 40%. Surgical resection remains the primary curative treatment option in gastric cancer, with 5-year survival rates of 58–78% and 34% reported for stage I and II disease, respectively. Despite this, the overall 5-year survival rate for all patients remains poor and ranges between 15 and 38% [9]. Thus, identification of reliable molecular prognostic markers is more important in gastric cancer than in other malignancies, and their measurement in serum or small biopsy samples should provide important prognostic information.

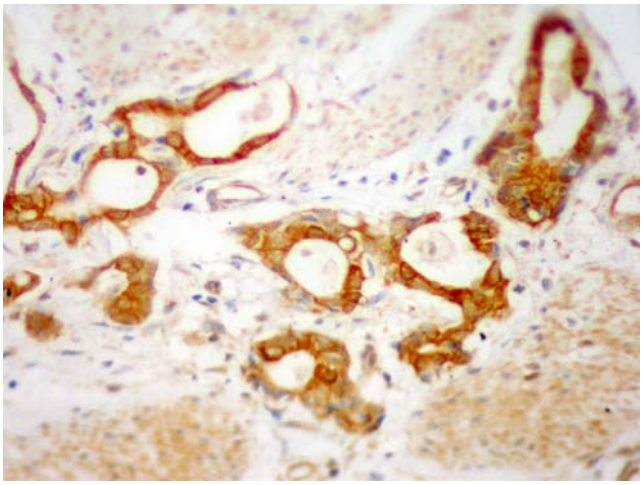

Fig. 2 HGF protein was positively expressed in moderately differentiated adenocarcinoma. In immunohistochemistry and visualized with SP method. Magnification × 200

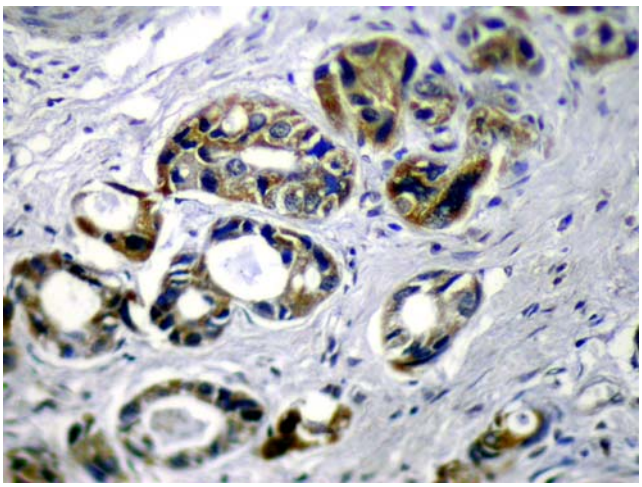

Fig. 1 HGF mRNA was positively expressed in moderately differentiated adenocarcinoma. In situ hybridization and visualization with DAB. Magnification × 200

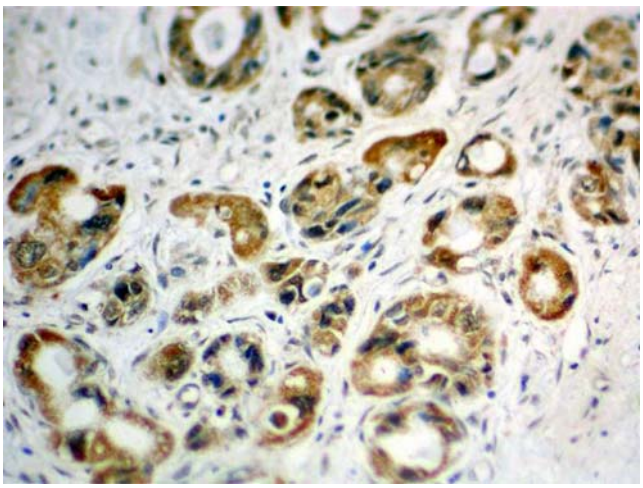

Fig. 3 uPA mRNA was positively expressed in moderately differentiated adenocarcinoma. In situ hybridization and visualization with DAB. Magnification × 200

All of the molecular markers screened in this study were correlated with depth of invasion, blood or lymph vessel invasion, lymph node and distant metastasis, advanced tumor stage, MVD, Lauren's classification and survival in gastric cancer patients, but none of the markers have any relationship with tumor histological type or differentiation. These findings, along with previous results from other genetic studies, have identified that HGF may promote cell proliferation and migration, through inducing tyrosine phosphorylation of Met [10]. It may also promote tumor invasion and metastasis, and can be used as a predictive marker for recurrence of gastric carcinoma [11]. IGF-2 is an autocrine growth factor, and overexpression of IGF-2 mRNA may play an important role in the initiation, progression and metastasis of gastric cancer [12]. Alpha v beta 3 and alpha v beta 5 integrins and their ligands Del-1 and L1 play an important role in the process of tumor cells moving from the original place [13]. The vascular expression level of alpha(v)beta(3) integrin is correlated with the presence of liver metastases, and vascular expression of alpha(v)beta(3) integrin is a prognostic indicator for colon carcinoma [14].

Cancer cells can secrete various proteolytic enzymes to dissolve extracellular matrix adjacent to the tumor and form a pathway for movement of tumor cells. MMPs and uPA are the most important among these enzymes. MMP-9 and tissue inhibitor of metalloproteinase-1 play a critical role in maintaining the degradation and synthesis of extracellular matrix. Loss of such balance is associated with tumor invasion and metastasis [15]. Abnormal expression of bFGF, MMP-9 and uPA, as well as depth of invasion, lymph node and distant metastasis, and tumor stage, was related independently to poor prognosis of gastric cancer, and bFGF, MMP-9 and uPA were able to promote angiogenesis. Our results are consistent with earlier reports on the prognostic significance of uPA, MMP-9 and bFGF in cancer. The study of Kaneko et al. has demonstrated that depth of tumor invasion, lymph node involvement and uPA expression are independent prognostic factors. uPA is a key factor in the plasminogen activator system, and is associated with poor outcome of gastric cancer, and contributes to invasive activity and angiogenesis [16]. MMP-9 and uPA might play an important role in the invasion and metastasis of non-small cell lung cancer (NSCLC) [17]. When gastric carcinoma expresses a high level of MMP-9 and vascular endothelial growth factor (VEGF), with high MVD, the level of infiltration and metastasis is enhanced [18]. bFGF has a very important role in promoting vascular endothelial cell mitosis and in increasing chemotaxis of endothelial cells. The measurement of plasma levels of such angiogenic factors as VEGF, bFGF and MMP-9 in advanced NSCLC is helpful for predicting metastatic tendency and prognosis [19].

Tumor invasion and metastasis are dependent on the synergistic function of proteolytic enzymes [20]. bFGF can significantly induce uPA expression [21]. uPA can rapidly upregulate MMP-9 in a dose-dependent manner [22]. uPA-mediated direct activation of MMP-9 may promote glioblastomas cell invasion [23]. *In vitro inhibition* tests on human umbilical artery smooth muscle cells have shown the direct influence of bFGF on the activity, production and expression of MMP-2 and MMP-9 [24]. In summary, adenovirus-mediated inhibition of uPA-uPAR interaction and MMP-9 on the cell surface may be a promising anti-invasive and anti-metastatic strategy for cancer gene therapy [25]. The expression of bFGF, MMP-9 and uPA can promote tumor angiogenesis and contributes to tumor invasion and metastasis in gastric carcinoma. MMP-9, bFGF and uPA are potential candidates for development as clinically applicable molecular prognostic markers of gastric carcinoma, and may be effective therapeutic targets for this disease in the future.

References

1. Inoue M, Tsugane S (2005) Epidemiology of gastric cancer in Japan. Postgrad Med J 81:419–424
2. Wenhua Z, Fanghai H (2008) Surgical therapy of gastric cancer in china. Journal of Practical oncology 23:91–93
3. Egeblad M, Werb Z et al (2002) New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer 2:161–174
4. Moon WS, Park HS, Yu KH et al (2006) Expression of angiopoietin 1, 2 and their common receptor Tie2 in human gastric carcinoma: in implication for angiogenesis. Journal of Korean Medical Science 21:272–278
5. Niedergethmann M, Hildenbrand R, Wostbrock B et al (2002) High expression of vascular endothelial growth factor predicts early recurrence and poor prognosis after curative resection for ductal adenocarcinoma of the pancreas. Pancreas 25:122–129
6. Kolev Y, Uetake H, Iida S et al (2007) Prognostic significance of VEGF expression in correlation with COX-2, microvessel density, and clinicopathological characteristics in human gastric carcinoma. Annals of Surgical Oncology 14:2738–2747
7. Mizokami K, Kakeji Y, Oda S et al (2006) Clinicopathologic significance of hypoxia-inducible factor 1 alpha overexpression in gastric carcinomas. Journal of Surgical Oncology 94:149–154
8. Weidner N, Semple JP, Welch WR, Folkman J (1991) Tumor angiogenesis and metastasis-correlation in invasive breast carcinoma. N Engl J Med 324:1–8
9. Hundahl SA, Phillips JL, Menck HR (2000) The National Cancer Data Base Report on poor survival of U.S. gastric carcinoma patients treated with gastrectomy: fifth edition American Joint Committee on cancer staging, proximal disease, and the 'different disease' hypothesis. Cancer 88:921–932
10. Park M, Park H, Kim WH, Cho H, Lee JH (2005) Presence of autocrine hepatocyte growth factor-Met signaling and its role in proliferation and migration of SNU-484 gastric cancer cell line. Exp Mol Med 37:213–219
11. Niki M, Iso Zaki H, Toyoda M et al (1999) Serum human hepatocyte growth factor is elevated in patients with metastatic gastric carcinoma. Hepatogastroenterology 46:568–573

12. Yi HK, Hwang PH, Yang DH, Kang CW, Lee DY (2001) Expression of the insulin-like growth factors (IGFs) and the IGF-binding proteins (IGFBPs) in human gastric cancer cells. Eur J Cancer 37:2257–2263
13. Niu JX, Zhang WJ, Ye LY (2007) The role of adhesion molecules, alpha v beta 3, alpha v beta 5 and their ligands in the tumor cell and endothelial cell adhesion. Eur J Cancer Prev 16:517–527
14. Vonlaufen A, Wiedle G, Borisch B (2001) Integrin alpha(v) beta (3) expression in colon carcinoma correlates with survival. Mod Pathol 14:1126–32
15. Zhang S, Li L, Lin JY, Lin H (2003) Imbalance between expression of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in invasiveness and metastasis of human gastric carcinoma. World J Gastroenterol 9:899–904
16. Kaneko T, Konno H, Baba M (2003) Urokinase-type plasminogen activator expression correlates with tumor angiogenesis and poor outcome in gastric cancer. Cancer Sci 94(1):43–49
17. Yang SF, Hsieh YS, Lin CL (2005) Increased plasma levels of urokinase plasminogen activator and matrix metalloproteinase-9 in nonsmall cell lung cancer patients. Clin Chim Acta 354:91–99
18. Gao ZL, Zhang C, Du GY, Lu ZJ (2007) Clinical significance of changes in tumor markers, extracellular matrix, MMP-9 and VEGF in patients with gastric carcinoma. Hepatogastroenterology 54:1591–1595
19. Zhao J, Liu XY, Zhang QY, Jiang W (2005) Plasma level and prognostic significance of VEGF, bFGF and MMP-9 in patients with advanced non-small-cell lung cancer. Zhonghua Zhong Liu Za Zhi 27:676–679
20. Ramos-Desimone N, Haha-Dantona E, Siply J et al (1999) Activation of matrix metalloproteinases 9 Via a converging plasmin/stromelysin-1 cascade enhances tumor cell invasion. J Biol Chem 274:13066–13076
21. Kim MH (2003) Flavonoids inhibit VEGF/bFGF-induced angiogenesis in vitro by inhibiting the matrix-degrading proteases. J Cell Biochem 89:529–538
22. Lee SR, Guo SZ, Scannevin RH (2007) Induction of matrix metalloproteinase, cytokines and chemokines in rat cortical astrocytes exposed to plasminogen activators. Neurosci Lett 417:1–5
23. Zhao Y, Lyons CE Jr, Xiao A (2008) Urokinase directly activates matrix metalloproteinases-9: a potential role in glioblastoma invasion. Biochem Biophys Res Commun 369:1215–1220
24. Sapienza P, di Marzo L, Borrelli V (2004) Basic fibroblast growth factor mediates carotid plaque instability through metalloproteinase-2 and -9 expression. Eur J Vasc Endovasc Surg 28:89–97
25. Rao JS, Gondi C, Chetty C (2005) Inhibition of invasion, angiogenesis, tumor growth, and metastasis by adenovirus-mediated transfer of antisense uPAR and MMP-9 in non-small cell lung cancer cells. Mol Cancer Ther 4:1399–1408