Am J Transl Res 2015;7(11):2412-2422
www.ajtr.org /ISSN:1943-8141/AJTR0015177

Original Article

Increased matrix metalloproteinase-2 expression and reduced tissue factor pathway inhibitor-2 expression correlate with angiogenesis and early postoperative recurrence of pancreatic carcinoma

Lu-Lu Zhai[1,2*], Yang Wu[1,2*], Da-Wei Huang[1,2], Zhi-Gang Tang[1,2]

[1]Department of General Surgery, Affiliated Provincial Hospital, Anhui Medical University, Hefei 230001, People's Republic of China; [2]Anhui Province Key Laboratory of Hepatopancreatobiliary Surgery, Hefei 230001, People's Republic of China. [*]Equal contributors.

Received August 27, 2015; Accepted October 13, 2015; Epub November 15, 2015; Published November 30, 2015

Abstract: Matrix metalloproteinase (MMP)-2 and tissue factor pathway inhibitor (TFPI)-2 are known to influence tumor angiogenesis and progression. This work aimed to describe the levels of MMP-2 and TFPI-2 expression associated with tumor angiogenesis and early postoperative recurrence in patients with pancreatic carcinoma. Expression of MMP-2 and TFPI-2 in carcinoma tissues and paracarcinomatous tissues was assayed by immunostaining. Expression of vascular endothelial growth factor (VEGF) and CD34 in tumor tissues was also assayed by immunostaining. The correlations of MMP-2 and TFPI-2 with VEGF, microvessel density (MVD), and early postoperative recurrence were analyzed. The results showed that MMP-2 expression was significantly increased ($P < 0.05$) and TFPI-2 expression was significantly decreased ($P < 0.001$) in carcinoma tissues compared with paracarcinomatous tissues. MMP-2 expression was positively correlated with VEGF ($r = 0.594$, $P < 0.001$) and MVD ($r = 0.432$, $P < 0.001$) in carcinoma tissues. TFPI-2 expression was negatively correlated with VEGF ($r = -0.654$, $P < 0.001$) and MVD ($r = -0.360$, $P < 0.001$) in carcinoma tissues. Multivariate logistic regression analysis showed that up-regulated MMP-2 and down-regulated TFPI-2 were independent predictors of early postoperative recurrence of pancreatic carcinoma. Receiver operating characteristic curve analysis showed that the combination of MMP-2 and TFPI-2 was a reliable predictive model of early recurrence. We conclude that increased MMP-2 expression and reduced TFPI-2 expression are closely linked to angiogenesis and early postoperative recurrence of pancreatic carcinoma. Immunohistochemical assay of MMP-2 and TFPI-2 may be useful for predicting early relapse of pancreatic carcinoma after surgery.

Keywords: MMP-2, TFPI-2, angiogenesis, recurrence, pancreatic carcinoma

Introduction

Pancreatic carcinoma is a highly aggressive and rapidly progressive disease. It is the ninth most common malignant tumor and the fourth leading cause of cancer deaths, with an average 5-year survival rate of about 4% in the United States [1, 2]. Currently, radical surgery is the only treatment for cure or long-term survival of patients with pancreatic carcinoma. Unfortunately, the majority of the patients present with extensive tumor infiltration and/or distant metastasis when the disease is first diagnosed and are no longer candidates for curative resection [3]. Substantial efforts toward improving the diagnosis and treatment of pancreatic carcinoma have not produced significant improvement in 5-year survival [4]. There is therefore an urgent need to deepen the understanding of the mechanisms by which pancreatic carcinoma develops and progresses in order to improve its prognosis.

Angiogenesis is the biological process through which new capillaries sprout from preexisting vascular structures. It has an active role in many physiological and pathological conditions, including embryogenesis, tissue repair, and tumor progression [5]. Angiogenesis is regulated by a balance of proangiogenic and antiangiogenic factors and involved proliferation and migration of endothelial cells [6]. Angiogenesis

Table 1. Clinicopathological data of 126 patients with pancreatic carcinoma

Characteristic		Number	%
Age (years)	< 60	67	53.2
	≥ 60	59	46.8
Gender	Male	67	53.2
	Femal	59	46.8
Tumor diameter (mm)	< 20	60	47.6
	≥ 20	66	52.4
Tumor location	Head	86	68.3
	Body/tail	40	31.7
Serum CA19-9 (U/mL)	≤ 37	46	36.5
	> 37	80	63.5
Differentiation	Mod-poor	89	70.6
	Well	37	29.4
LNM	Absent	41	32.5
	Present	85	67.5
PNI	Absent	49	38.9
	Present	77	61.1
Tumor stage	I	37	29.4
	II	89	70.6
Early postoperative recurrence	No	37	29.4
	Yes	89	70.6

CA19-9, carbohydrate antigen19-9; LNM, lymph node metastasis; PNI, perineural invasion.

also requires degeneration and remodeling of extracellular matrix (ECM), both of which depend on the action of various proteases, particularly matrix metalloproteinases (MMPs) [7, 8]. MMPs comprise a large family of zinc-dependent endopeptidases that play a pivotal role in ECM degradation related to tumor invasion, metastasis, and angiogenesis [7]. One of them, MMP-2 stimulates the angiogenic responses of endothelial cells [8-10]. Previous studies have shown that MMP-2 expression is increased in various types of human solid tumors, including pancreatic carcinoma [11-14]. Tissue factor pathway inhibitor (TFPI)-2 is a broad-spectrum Kunitz-type serine proteinase repressor that is abundantly synthesized by the cells of normal human tissues and secreted into their ECM [15]. TFPI-2 can suppress the activity of ECM-associated proteases, including MMPs [16, 17]. Therefore, TFPI-2 may help to maintain the integrity of the ECM, thereby combating tumor invasion, metastasis and angiogenesis [17-20]. Reduced TFPI-2 expression has been correlated with the development and progression of some malignant diseases including pancreatic carcinoma [21, 22].

A few studies have shown that TFPI-2 overexpression strongly inhibits the activity and expression of MMP-2 and that the anti-invasive properties of TFPI-2 are closely linked to MMP-2 inhibition [19, 23, 24]. A significant negative correlation of MMP-2 and TFPI-2 expression has been seen in pancreatic carcinoma, and the differential expression of MMP-2 and TFPI-2 was closely associated with poor prognosis [25]. The available evidence indicates that MMP-2 and TFPI-2 have opposite effects on tumor invasiveness, metastasis and angiogenesis and that these processes are modulated by the relative levels of expression of these two proteins.

Although the roles of MMP-2 and TFPI-2 in tumor growth, invasiveness and metastasis have been described, there have been no reports on the influence of MMP-2, TFPI-2, on angiogenesis in pancreatic carcinoma. Consequently, we assayed MMP-2, TFPI-2, vascular endothelial growth factor (VEGF) and CD34 expression by immunostaining of pancreatic carcinoma tissues, and investigated the association of MMP-2 and TFPI-2 staining with VEGF expression and microvessel density (MVD). The relationships of MMP-2 and TFPI-2 with early postoperative relapse of pancreatic carcinoma were also determined.

Materials and methods

Patients and tissue samples

Pancreatic carcinoma and adjacent paracarcinomatous tissues were obtained from 126 patients who underwent curative resection at the Affiliated Provincial Hospital of Anhui Medical University (Hefei, China) between 2008 and 2011. Pathological diagnosis of pancreatic carcinoma was made in formalin-fixed, paraffin-embedded tissues. None of the patients had received chemotherapy, radiotherapy, or biologically targeted therapy before surgery. Pancreatic tissue located 2 cm beyond the tumor margin was defined as paracarcinomatous. Patients or their relatives gave written informed consent; the study was conducted in accordance with the principles of the Helsinki Declaration and was approved by the Human Scientific Ethics Committee of Anhui Medical University.

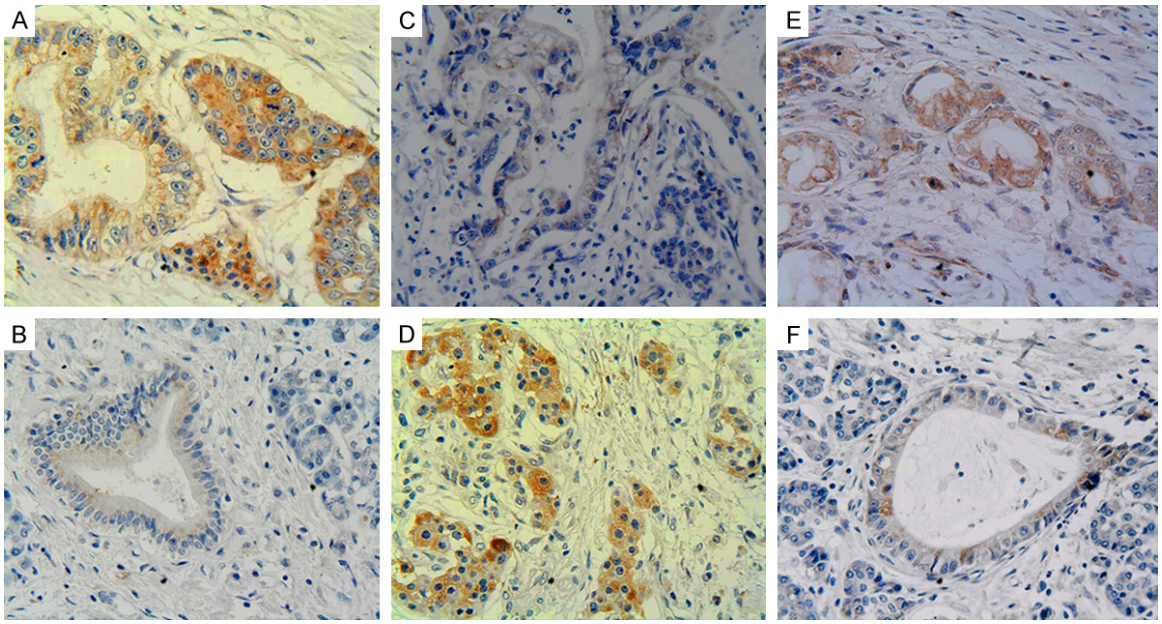

Figure 1. Immunohistochemical staining for MMP-2, TFPI-2 and VEGF. MMP-2, TFPI-2 and VEGF are primarily localized in cytoplasm of cells and have varying staining intensity. A. High MMP-2 expression. B. Low MMP-2 expression. C. Low TFPI-2 expression. D. High TFPI-2 expression. E. High VEGF expression. F. Low VEGF expression. All images, ×400.

Table 2. Differential expression of MMP-2 and TFPI-2 in pancreatic carcinoma tissues and paracarcinomatous tissues (N = 126)

Tissue	MMP-2 expression			TFPI-2 expression		
	Low (%)	High (%)	*P*-value	Low (%)	High (%)	*P*-value
Carcinoma, N (%)	34 (27.0%)	92 (73.0%)	0.019	90 (71.4%)	36 (28.6%)	< 0.001
Paracarcinomatous, N (%)	90 (71.4%)	36 (28.6%)		27 (21.4%)	99 (78.6%)	

MMP, matrix metalloproteinase; TFPI, tissue factor pathway inhibitor.

Table 3. Association of MMP-2 and TFPI-2 expression with VEGF and MVD in pancreatic carcinoma

Immunoreactivity	MMP-2 expression				TFPI-2 expression			
	Low	High	r-value	*P*-value	Low	High	r-value	*P*-value
VEGF								
Low	20	5	0.594	< 0.001	3	22	-0.654	< 0.001
High	14	87			87	14		
MVD								
Low	25	24	0.432	< 0.001	25	24	-0.360	< 0.001
High	9	68			65	12		

MMP, matrix metalloproteinase; TFPI, tissue factor pathway inhibitor; VEGF, vascular endothelial growth factor; MVD, microvessel density. The MVD was calculated after staining for CD34. The MVD was categorized as either low or high based on the median value of 15.

Complete clinicopathological data, including age, gender, tumor diameter, tumor location, preoperative serum carbohydrate antigen 19-9 (CA19-9) concentrations, tumor differentiation, lymph node metastasis (LNM), perineural invasion (PNI), tumor stage and early postoperative recurrence were obtained from the patients' medical records. The patients ranged from 45 to 66 years of age (mean of 55 years) and included 67 men and 59 women. Tumor differentiation was histologically defined using the World Health Organization (WHO) classification. Tumor stage was determined using the seventh edition of the American Joint Committee on Cancer (AJCC) tumor-node-metastasis (TNM) staging system

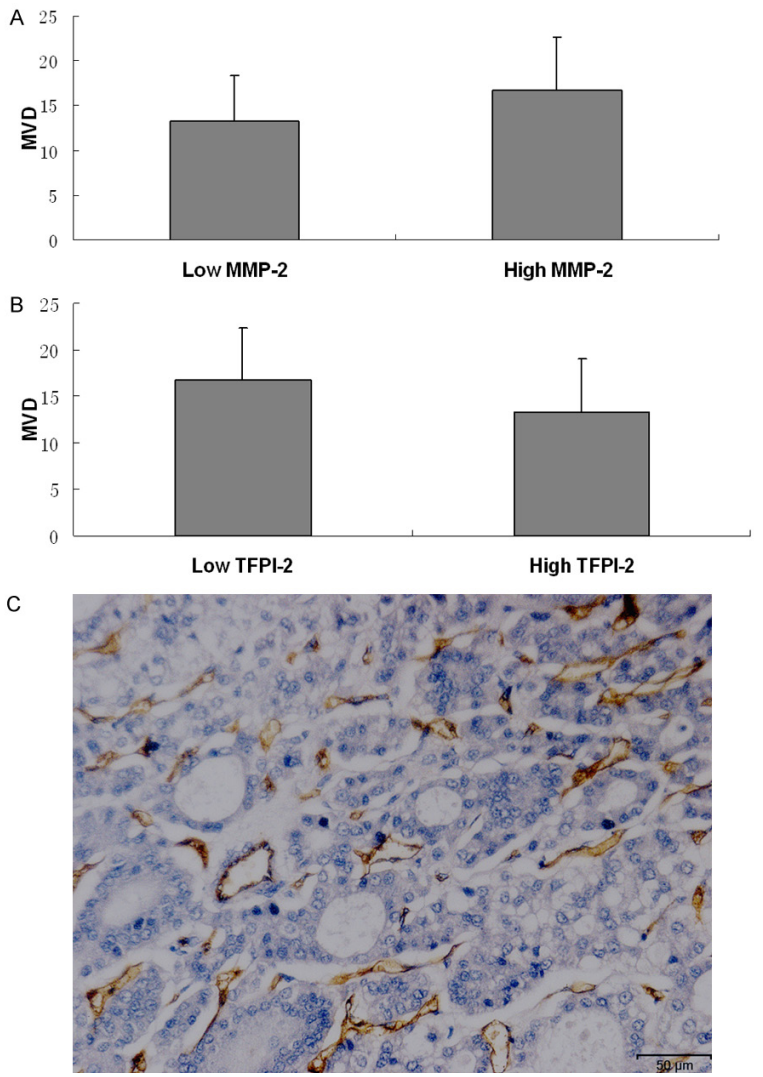

Figure 2. CD34 staining of MVD in pancreatic carcinoma tissue. A. Tumors with high MMP-2 expression had a significantly greater MVD than tumors with low MMP-2 expression (P = 0.002). B. Tumors with high TFPI-2 expression had a significantly smaller MVD than tumors with low TFPI-2 expression (P = 0.004). C. Representative section of pancreatic carcinoma tissues with immunohistochemical staining of CD34 (×200).

[26]. Tumor recurrence within 12 months of surgery was defined as early postoperative recurrence [27]. A summary of the clinicopathological data is given in **Table 1**.

Immunohistochemical staining and assessment

Immunohistochemical staining was performed using a two-step protocol following the instructions provided by the kit manufacturer. Paraffin embedded specimens were sectioned at 4 mm, mounted on slides, heated at 60°C for 20 minutes, dewaxed with xylene, rehydrated through an ethanol series (100%, 95%, 75%), and rinsed in phosphate-buffered saline (PBS) [25]. Antigen retrieval was then performed by heating tissue sections immersed in 0.01 mol/L sodium citrate buffer (pH 6.0) at 80°C for 20 minutes in a microwave oven. After endogenous peroxidase activity was blocked with 0.3% H_2O_2 for 10 minutes, the sections were incubated overnight (at least 10 hours) with monoclonal rabbit anti-human MMP-2 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), monoclonal sheep anti-human TFPI-2 (Fitzgerald Industries International, Inc., MA, USA), polyclonal rabbit anti-human VEGF (Zhongshan Golden Bridge Biotechnology Co., Ltd., Beijing, China) or polyclonal rabbit anti-human CD34 (R&D systems, Inc., Minneapolis, MN, USA) antibody in a humid chamber at 4°C. After washing with PBS, the sections were incubated with a biotinylated secondary antibody (mouse anti-rabbit-IgG or mouse anti-sheep-IgG; Zhongshan Golden Bridge Biotechnology Co., Ltd., Beijing, China) for 10 minutes at 37°C. Immunoreactivity was visualized with 3,3-diaminobenzidine (DAB; Sigma-Aldrich Corp. St. Louis, MO, USA) substrate and the sections were counterstained with hematoxylin, dehydrated and mounted. Tissue sections that were not incubated with primary antibodies served as negative controls.

Semi-quantitative analysis of MMP-2, TFPI-2 and VEGF staining included determining the percentage of stained cells and scoring the intensity of staining, as described previously [25]. The percentage of stained cells was scored as 0 for none; 1 for < 10%, 2 for 10-30%, and 3 for > 30%. Staining intensity was scored

Table 4. Logistic regression analysis of factors associated with early postoperative recurrence of pancreatic carcinoma

Variables	Univariate model			Multivariate model		
	Coefficient	OR (95% CI)	*P*-value	Coefficient	OR (95% CI)	*P*-value
Age (years) (< 60 vs. ≥ 60)	-0.681	0.506 (0.229-1.119)	0.092			
Gender (male vs. female)	-0.723	0.485 (0.222-1.058)	0.069			
Tumor diameter (mm) (< 20 vs. ≥ 20)	-0.249	0.779 (0.360-1.686)	0.526			
Tumor location (head vs. body/tail)	-0.218	0.804 (0.357-1.811)	0.599			
Serum CA19-9 (U/mL) (≤ 37 vs. > 37)	1.212	3.360 (1.513-7.462)	0.003	0.123	1.131 (0.306-4.178)	0.853
Differentiation (mod–poor vs. well)	-1.790	0.167 (0.072-0.389)	< 0.001	-0.291	0.748 (0.170-3.290)	0.701
LNM (absent vs. present)	3.414	30.390 (10.774-85.718)	< 0.001	0.740	2.095 (0.073-59.855)	0.665
PNI (absent vs. present)	1.381	3.981 (1.777-8.915)	0.001	-0.119	0.887 (0.207-3.810)	0.872
Tumor stage (I vs. II)	3.603	36.703 (12.617-106.771)	< 0.001	2.102	8.180 (2.002-33.431)	0.003
MMP-2 expression (low vs. high)	3.454	31.629 (10.966-91.225)	< 0.001	1.834	6.258 (1.478-26.503)	0.013
TFPI-2 expression (low vs. high)	-2.693	0.068 (0.027-0.172)	< 0.001	-1.985	0.137 (0.040-0.467)	0.001

CA19-9, carbohydrate antigen19-9; LNM, lymph node metastasis; PNI, perineural invasion; MMP, matrix metalloproteinase; TFPI, tissue factor pathway inhibitor; OR, odds ratio; CI, confidence interval.

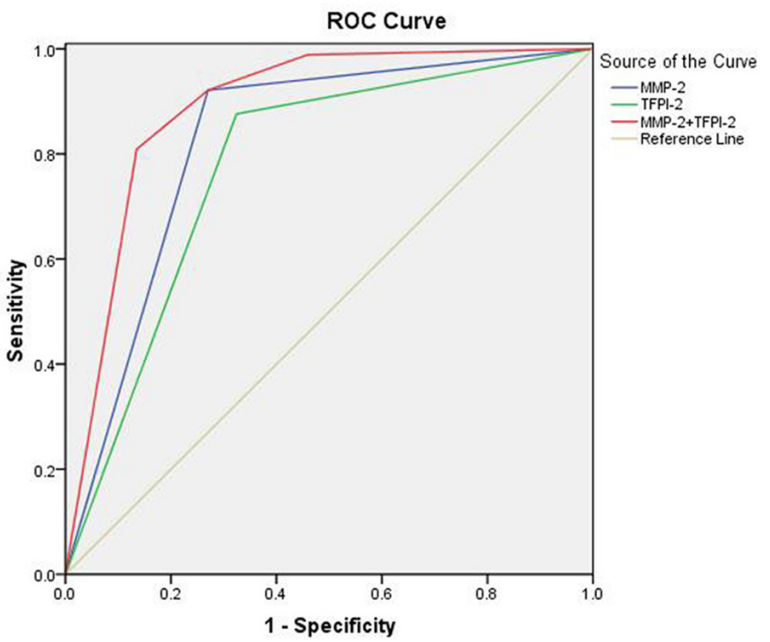

Figure 3. Receiver operating characteristic (ROC) curves of MMP-2, TFPI-2, and MMP-2+TFPI-2 for prediction of early postoperative recurrence in pancreatic carcinoma. The areas under the curve (AUC) for MMP-2, TFPI-2 and MMP-2+TFPI-2 were 0.826, 0.776 and 0.890, respectively.

as 0, none; 1, weak; 2, intermediate; 3, strong. The total immunoreactivity score was the product of the staining percentage and intensity scores. Low expression was reported as a final score ≤ 3 and high expression as a score > 3. MVD was calculated after staining for CD34. Microvessels were preliminarily evaluated at low magnification (×200) to identify the most vascularized areas (hot spots) and then the highest MVD of each hot spot was calculated at high magnification (×400) [28]. The final MVD was the average value of measurements made for three hot spots in each tumor tissue. All sections were assessed independently by two experienced pathologists without knowing each other's results. Differences in scoring and microvessel count were resolved by joint evaluation.

Statistical analysis

Statistical analyses were performed using SPSS version 17.0 (SPSS, Inc., Chicago, IL, USA). Results for continuous variables were presented as mean ± standard deviation (SD). Statistically significant differences in the total immunoreactivity scores of pancreatic carcinoma tissues and the surrounding paracarcinomatous tissues were evaluated using Pearson's chi-square test. The associations of MMP-2 and TFPI-2 with VEGF expression and MVD were determined using Spearman rank correlation coefficient. Univariate and multivariate analyses of factors associated with early postoperative recurrence were performed by logistic regression analysis. The sig-

Table 5. Predictive ability of MMP-2 and TFPI-2 for early postoperative recurrence of pancreatic carcinoma

Variable	AUC (95% CI)	*P*-value
MMP-2	0.826 (0.734-0.917)	< 0.001
TFPI-2	0.776 (0.678-0.875)	< 0.001
MMP-2+TFPI-2	0.890 (0.816-0.963)	< 0.001

MMP, matrix metalloproteinase; TFPI, tissue factor pathway inhibitor; AUC, area under the curve.

nificant variables in univariate model were introduced into multivariate model in a stepwise manner (i.e., backward elimination) to determine the independent risk factors for early postoperative recurrence of pancreatic carcinoma. Logistic regression combined with receiver operating characteristics (ROC) curve analysis was used to evaluate the ability of MMP-2 and TFPI-2 alone and in combination to predict early postoperative recurrence of pancreatic carcinoma. All analyses were two-tailed and $P < 0.05$ was defined as statistically significant.

Results

Immunohistochemical characteristics of MMP-2 and TFPI-2

MMP-2 and TFPI-2 staining were mainly localized in the cytoplasm of cells. MMP-2 expression was greater in pancreatic carcinoma than in paracarcinomatous tissues (**Figure 1A**, **1B**). In contrast, TFPI-2 expression was lower in pancreatic carcinoma than in paracarcinomatous tissues (**Figure 1C**, **1D**). The differences in expression of MMP-2 ($P = 0.019$) and of TFPI-2 ($P < 0.001$) in carcinoma compared with paracarcinomatous tissues were significant (**Table 2**).

Correlation of MMP-2 and TFPI-2 with VEGF in pancreatic carcinoma tissues

High VEGF expression was found in 101 of 126 carcinoma samples (80.2%; **Figure 1E**, **1F**). The relationships of MMP-2 and TFPI-2 with VEGF expression were evaluated by using Spearman's rank correlation test. There was a significant positive correlation between MMP-2 and VEGF expression in pancreatic carcinoma tissues ($r = 0.594$, $P < 0.001$). Conversely, there was a significant negative correlation between TFPI-2 and VEGF expression ($r = -0.654$, $P < 0.001$) (**Table 3**).

Correlation of MMP-2 and TFPI-2 with MVD in pancreatic carcinoma tissues

CD34 staining was used to assess MVD in tumor tissues. The mean value of MVD was 15.7 per field (median, 15; range, 4-31). Tumor tissues with high MMP-2 expression had significantly greater MVD values than those with low MMP-2 expression (16.7 ± 5.9 vs. 13.2 ± 5.2; $P = 0.002$; **Figure 2A**). Conversely, tumor tissues with high TFPI-2 expression had significantly smaller MVD values than those with low TFPI-2 expression (13.3 ± 5.7 vs. 16.7 ± 5.7; $P = 0.004$; **Figure 2B**). Representative CD34 staining results in pancreatic carcinoma tissues are shown in **Figure 2C**.

An MVD value greater than or equal to the median value of 15 was considered to be high and a value < 15 was regarded as a low density. MMP-2 expression was positively correlated with MVD (Spearman's $r = 0.432$, $P < 0.001$), whereas TFPI-2 expression was negatively correlated with MVD (Spearman's $r = -0.360$, $P < 0.001$) (**Table 3**).

Logistic regression analysis of early postoperative recurrence of pancreatic carcinoma

Logistic regression analysis was used to identify variables that had a significant influence on early postoperative recurrence of pancreatic carcinoma. Univariate analysis found that early postoperative recurrence was significantly associated with high preoperative serum CA19-9 concentrations ($P = 0.003$), poor tumor differentiation ($P < 0.001$), LNM ($P < 0.001$), PNI ($P = 0.001$), high tumor stage ($P < 0.001$), increased MMP-2 expression ($P < 0.001$) and decreased TFPI-2 expression ($P < 0.001$) (**Table 4**).

In multivariate analysis, high MMP-2 expression was found to be an independent risk factor for early postoperative recurrence of pancreatic carcinoma [odds ratio (OR) = 6.258; 95% confidence interval (CI) 1.478-26.503; $P = 0.013$]. However, high TFPI-2 expression was an independent protective factor against early postoperative recurrence (OR = 0.137; 95% CI 0.040-0.467; $P = 0.001$). High tumor stage was

also an independent predictor of early postoperative recurrence (OR = 8.180; 95% CI 2.002-33.431; *P* = 0.003) (**Table 4**).

MMP-2, TFPI-2, and prediction of early postoperative recurrence of pancreatic carcinoma

ROC curve (**Figure 3**) was constructed to evaluate the predictive value of MMP-2 and TFPI-2 for early postoperative recurrence. The area under the curve (AUC) was 0.826 (95% CI 0.734-0.917) for MMP-2 and 0.776 (95% CI 0.678-0.875) for TFPI-2. The combination of MMP-2 and TFPI-2 increased the specificity and sensitivity over that of MMP-2 or TFPI-2 alone for predicting early postoperative recurrence, with an AUC of 0.890 (95% CI 0.816-0.963) (**Table 5**).

Discussion

In this study, we used immunochemical staining to assess MMP-2 and TFPI-2 expression in pancreatic carcinoma and paracarcinomatous tissues and investigated the association of MMP-2 and TFPI-2 expression with angiogenesis and early postoperative recurrence. As reported previously, MMP-2 expression was significantly up-regulated in pancreatic carcinoma relative to paracarcinomatous tissues [11-14, 25]. TFPI-2 expression was significantly down-regulated in pancreatic carcinoma compared with paracarcinomatous tissues, which is also consistent with previous findings [21, 22, 25]. Tumors with high MMP-2 or low TFPI-2 expression had a significantly greater MVD than those with low MMP-2 or high TFPI-2 expression. We found that there was a significant positive correlation of MMP-2 expression with VEGF expression and MVD. However, TFPI-2 expression had a significant negative correlation with VEGF expression and MVD in tumor tissues. Increased MMP-2 expression and decreased TFPI-2 expression were independent predictors of early recurrence of pancreatic carcinoma after curative resection. Including both MMP-2 and TFPI-2 in ROC curve analysis increased the AUC.

Angiogenesis is recognized as essential for tumor growth, invasion and metastasis, and is regulated by several key promoters and inhibitors [29, 30]. VEGF, a specific proangiogenic factor, has long been regarded as a crucial mediator of tumor angiogenesis [31, 32]. Accumulating evidence indicates that MMP-2 plays a prominent role in angiogenesis and progression of tumor [33-38]. Itoh *et al*. [33] reported that the volume and number of blood vessels in transplanted tumors were reduced in MMP-2-deficient compared with wild-type mice, and proposed a role for MMP-2 in angiogenesis and tumor growth. Fang *et al*. [34] reported that suppression of MMP-2 activity by antisense oligonucleotides led to the loss of tumor microvasculature and decrease in tumor weight. MMP-2 is thus required for development of the angiogenic phenotype during tumor progression. Moreover, both endogenous and synthetic MMP-2 inhibitors have been shown to strongly inhibit tumor angiogenesis both in *vivo* and in *vitro* [34-38]. In this group of patients, MMP-2 expression had a significant positive correlation with both VEGF expression and MVD. Together with previous findings, this suggests a facilitative role of MMP-2 in VEGF-mediated angiogenesis of pancreatic carcinoma. The correlation with VEGF expression could explain the rapid progression of tumors that express MMP-2.

There is increasing evidence that TFPI-2 also has a key role in the process of tumor angiogenesis [39-42]. Chand *et al* [39] demonstrated that HT-1080 fibrosarcoma cells transfected with the human *TFPI-2* gene, and actively expressing TFPI-2 protein, regulate tumor angiogenesis by down-regulating the VEGF receptor, with subsequent inhibition of tumor growth and metastasis. Yanamandra *et al*. [40] indicated that infection with recombinant adenovirus expressing TFPI-2 inhibited tumor growth, invasion, and angiogenesis in a human glioblastoma cell line. Ran *et al*. [41] demonstrated that TFPI-2 protein significantly inhibited tumor-related angiogenesis and tumor invasion both in *vitro* and in *vivo*. Zhang *et al*. [42] found that expression of TFPI-2 was negatively correlated with VEGF and MVD in cervical cancer, and that decreasing TFPI-2 was significantly correlated with tumor angiogenesis during the progression of cervical cancer. In these pancreatic carcinoma patients, TFPI-2 expression had a significant negative correlation with both VEGF expression and MVD in tumor tissues. These findings revealed that TFPI-2 had an inhibiting effect on angiogenesis in pancreatic carcinoma tissues. The mechanism may involve down-regulation of VEGF expression due to inhibition of

MMP-2 expression thereby resulting in suppression of uncontrolled tumor growth.

MMP-2 expression was found to be closely related to tumor recurrence in various types of human tumors, such as tongue squamous cell carcinoma, ovarian adenocarcinoma, hepatocellular carcinoma, non-small cell lung cancer, and prostate carcinoma [43-49]. Yoshizaki *et al*. [43] reported that increased MMP-2 expression was significantly correlated with local and distant metastatic tumor recurrence in tongue squamous cell carcinoma. Zhang *et al*. [45] found that the MMP-2 in the stromal compartment of hepatocellular carcinoma was significantly associated with recurrence. Leinonen *et al*. [47] reported that high MMP-2 expression was associated with increased tumor recurrence in non-small cell lung cancer. In our patients, elevated MMP-2 expression was an independent predictor of early relapse following curative resection. However, other series did not find a significant association between the expression of MMP-2 and the risk of local and/or distant recurrence in early-stage oral squamous cell carcinoma [50] or rectal cancer [51]. In addition, Ekinci *et al*. [52] found that tumor recurrence in ovarian epithelial cancer was not significantly associated with the staining intensity and total score of MMP-2 expression, but was associated with stromal staining of MMP-2. Additional studies are thus required to confirm our results.

It has been reported that negative or low expression of TFPI-2 was significantly associated with aggressive clinicopathologic features, tumor recurrence, and unfavorable outcome in breast cancer [53]. This is consistent with our finding that decreased TFPI-2 expression was an independent predictor of early relapse of pancreatic carcinoma after surgery. ROC curve analysis showed that taken together, high MMP-2 plus low TFPI-2 expression had increased predictive value. Thus, increased MMP-2 and decreased TFPI-2 expression might be a useful indicator of early postoperative relapse, and might help clinicians to identify patients at increased risk.

Currently, very little additional data are available on the roles of MMP-2 and TFPI-2 in angiogenesis and in identifying patients at risk of early postoperative relapse of pancreatic carcinoma. This, and the possibility of selection bias in any retrospective study, indicates that additional, prospective studies enrolling larger cohorts of subjects are needed to support our findings.

Taken together, we found that increased MMP-2 expression and reduced TFPI-2 expression were significantly correlated with tumor angiogenesis, and were independent predictors of early postoperative relapse in patients with pancreatic carcinoma. MMP-2 and TFPI-2 may have opposing influences on angiogenesis and progression of pancreatic carcinoma. The results support the value of immunohistochemical evaluation of MMP-2 and TFPI-2 in pancreatic carcinoma for predicting early recurrence following curative resection.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (No. 81272740).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Zhi-Gang Tang, Department of General Surgery, Affiliated Provincial Hospital, Anhui Medical University; Anhui Province Key Laboratory of Hepatopancreatobiliary Surgery, 17 Lujiang Road, Hefei 230001, Anhui Province, People's Republic of China. Tel: +86-13866112279; Fax: +86-551-62283280; E-mail: tzg567@163.com; jackyzhai123@163.com

References

[1] Siegel R, Ma J, Zou Z and Jemal A. Cancer statistics, 2014. CA Cancer J Clin 2014; 64: 9-29.
[2] Gong ZH, Holly EA and Bracci PM. Survival in Population-based Pancreatic Cancer Patients: San Francisco Bay Area, 1995-1999. Am J Epidemiol 2011; 174: 1373-1381.
[3] Niederhuber JE, Brennan MF and Menck HR. The National Cancer Data Base report on pancreatic cancer. Cancer 1995; 76: 1671-1677.
[4] Vincent A, Herman J, Schulick R, Hruban RH and Goggins M. Pancreatic cancer. Lancet 2011; 378: 607-620.
[5] Folkman J. Angiogenesis. Annu Rev Med 2006; 57: 1-18.
[6] Folkman J. Role of angiogenesis in tumor growth and metastasis. Semin Oncol 2002; 29: 15-18.

[7] Deryugina EI and Quigley JP. Tumor angiogenesis: MMP-mediated induction of intravasation- and metastasis-sustaining neovasculature. Matrix Biol 2015; 44-46C: 94-112.
[8] Pratheeshkumar P and Kuttan G. Andrographolide inhibits human umbilical vein endothelial cell invasion and migration by regulating MMP-2 and MMP-9 during angiogenesis. J Environ Pathol Toxicol Oncol 2011; 30: 33-41.
[9] Zheng H, Takahashi H, Murai Y, Cui Z, Nomoto K, Niwa H, Tsuneyama K and Takano Y. 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.
[10] Gialeli C, Theocharis AD and Karamanos NK. Roles of matrix metalloproteinases in cancer progression and their pharmacological targeting. FEBS J 2011; 278: 16-27.
[11] Iki K, Tsutsumi M, Kido A, Sakitani H, Takahama M, Yoshimoto M, Motoyama M, Tatsumi K, Tsunoda T and Konishi Y. Expression of matrix metalloproteinase 2 (MMP-2), membrane-type 1 MMP and tissue inhibitor of metalloproteinase 2 and activation of proMMP-2 in pancreatic duct adenocarcinomas in hamsters treated with N-nitrosobis (2-oxopropyl) amine. Carcinogenesis 1999; 20: 1323-1329.
[12] Määttä M, Soini Y and Liakka A, Autio-Harmainen H. Differential expression of matrix metalloproteinase (MMP)-2, MMP-9, and membrane type 1-MMP in hepatocellular and pancreatic adenocarcinoma: implications for tumor progression and clinical prognosis. Clin Cancer Res 2000; 6: 2726-2734.
[13] Ellenrieder V, Alber B, Lacher U, Hendler SF, Menke A, Boeck W, Wagner M, Wilda M, Friess H, Büchler M, Adler G and Gress TM. Role of MT-MMPs and MMP-2 in pancreatic cancer progression. Int J Cancer 2000; 85: 14-20.
[14] Roy R, Zurakowski D, Wischhusen J, Frauenhoffer C, Hooshmand S, Kulke M and Moses MA. Urinary TIMP-1 and MMP-2 levels detect the presence of pancreatic malignancies. Br J Cancer 2014; 111: 1772-1779.
[15] Udagawa K, Yasumitsu H, Esaki M, Sawada H, Nagashima Y, Aoki I, Jin M, Miyagi E, Nakazawa T, Hirahara F, Miyazaki K and Miyagi Y. Subcellular localization of PP5/TFPI-2 in human placenta: a possible role of PP5/TFPI-2 as an anti-coagulant on the surface of syncytiotrophoblasts. Placenta 2002; 23: 145-153.
[16] Herman MP, Sukhova GK, Kisiel W, Foster D, Kehry MR, Libby P and Schönbeck U. Tissue factor pathway inhibitor-2 is a novel inhibitor of matrix metalloproteinases with implications for atherosclerosis. J Clin Invest 2001; 107: 1117-1126.
[17] Rao CN, Cook B, Liu Y, Chilukuri K, Stack MS, Foster DC, Kisiel W and Woodley DT. HT-1080 fibrosarcoma cell matrix degradation and invasion are inhibited by the matrix-associated serine protease inhibitor TFPI-2/33 kDa MSPI. Int J Cancer 1998; 76: 749-756.
[18] Rao CN, Reddy P, Liu Y, O'Toole E, Reeder D, Foster DC, Kisiel W and Woodley DT. Extracellular matrix-associated serine protease inhibitors (Mr 33,000, 31,000, and 27,000) are single-gene products with differential glycosylation: cDNA cloning of the 33-kDa inhibitor reveals its identity to tissue factor pathway inhibitor-2. Arch Biochem Biophy 1996; 335: 82-92.
[19] Izumi H, Takahashi C, Oh J and Noda M. Tissue factor pathway inhibitor-2 suppresses the production of active matrix metalloproteinase-2 and is down-regulated in cells harboring activated ras oncogenes. FEBS Lett 2000; 481: 31-36.
[20] Konduri SD, Rao CN, Chandrasekar N, Tasiou A, Mohanam S, Kin Y, Lakka SS, Dinh D, Olivero WC, Gujrati M, Foster DC, Kisiel W and Rao JS. A novel function of tissue factor pathway inhibitor-2 (TFPI-2) in human glioma invasion. Oncogene 2001; 20: 6938-6945.
[21] Jiang P, Watanabe H, Okada G, Ohtsubo K, Mouri H, Tsuchiyama T, Yao F and Sawabu N. Diagnostic utility of aberrant methylation of tissue factor pathway inhibitor 2 in pure pancreatic juice for pancreatic carcinoma. Cancer Sci 2006; 97: 1267-1273.
[22] Tang Z, Geng G, Huang Q, Xu G, Hu H, Chen J and Li J. Expression of tissue factor pathway inhibitor 2 in human pancreatic carcinoma and its effect on tumor growth, invasion, and migration in vitro and in vivo. J Surg Res 2011; 167: 62-69.
[23] Zhao B, Luo X, Shi H and Ma D. Tissue factor pathway inhibitor-2 is downregulated by ox-LDL and inhibits ox-LDL induced vascular smooth muscle cells proliferation and migration. Thromb Res 2011; 128: 179-185.
[24] Gessler F, Voss V, Seifert V, Gerlach R and Kögel D. Knockdown of TFPI-2 promotes migration and invasion of glioma cells. Neurosci Lett 2011; 497: 49-54.
[25] Zhai LL, Cai CY, Wu Y and Tang ZG. Correlation and prognostic significance of MMP-2 and TFPI-2 differential expression in pancreatic carcinoma. Int J Clin Exp Pathol 2015; 8: 682-691.
[26] Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL and Trotti A. American Joint Committee on Cancer (AJCC) cancer staging manual, 7th ed. Chicago, IL: Springer; 2010.
[27] Yamamoto Y, Ikoma H, Morimura R, Konishi H, Murayama Y, Komatsu S, Shiozaki A, Kuriu Y,

Kubota T, Nakanishi M, Ichikawa D, Fujiwara H, Okamoto K, Sakakura C, Ochiai T and Otsuji E. Optimal duration of the early and late recurrence of pancreatic cancer after pancreatectomy based on the difference in the prognosis. Pancreatology 2014; 14: 524-529.

[28] Weidner N, Semple JP, Welch WR and Folkman J. Tumor angiogenesis and metastasis-correlation in invasive breast carcinoma. N Engl J Med 1991; 324: 1-8.

[29] Folkman J. Role of angiogenesis in tumor growth and metastasis. Semin Oncol 2002; 29: 15-18.

[30] Carmeliet P and Jain RK. Molecular mechanisms and clinical applications of angiogenesis. Nature 2011; 473: 298-307.

[31] Veikkola T, Karkkainen M, Claesson-Welsh L and Alitalo K. Regulation of angiogenesis via vascular endothelial growth factor receptors. Cancer Res 2000; 60: 203-212.

[32] Hoeben A, Landuyt B and Highley MS. Vascular endothelial growth factor and angiogenesis. Pharmacol Rev 2004; 56: 549-580.

[33] Itoh T, Tanioka M, Yoshida H, Yoshioka T, Nishimoto H and Itohara S. Reduced angiogenesis and tumor progression in gelatinase A-deficient mice. Cancer Res 1998; 58: 1048-1051.

[34] Fang J, Shing Y, Wiederschain D, Yan L, Butterfield C, Jackson G, Harper J, Tamvakopoulos G and Moses MA. Matrix metalloproteinase-2 is required for the switch to the angiogenic phenotype in a tumor model. Proc Natl Acad Sci U S A 2000; 97: 3884-3889.

[35] Gonzalez-Villasana V, Fuentes-Mattei E, Ivan C, Dalton HJ, Rodriguez-Aguayo C, Fernandez-de Thomas RJ, Aslan B, Del C Monroig P, Velazquez-Torres G, Previs RA, Pradeep S, Kahraman N, Wang H, Kanlikilicer P, Ozpolat B, Calin G, Sood AK and Lopez-Berestein G. Rac1/Pak1/p38/MMP-2 Axis Regulates Angiogenesis in Ovarian Cancer. Clin Cancer Res 2015; 21: 2127-2137.

[36] Gatto C, Rieppi M, Borsotti P, Innocenti S, Ceruti R, Drudis T, Scanziani E, Casazza AM, Taraboletti G and Giavazzi R. BAY 12-9566, a novel inhibitor of matrix metalloproteinases with antiangiogenic activity. Clin Cancer Res 1999; 5: 3603-3607.

[37] Ma YL, Lin SW, Fang HC, Chou KJ, Bee YS, Chu TH, Chang MC, Weng WT, Wu CY, Cho CL and Tai MH. A novel poly-naphthol compound ST104P suppresses angiogenesis by attenuating matrix metalloproteinase-2 expression in endothelial cells. Int J Mol Sci 2014; 15: 16611-16627.

[38] Liu G, Liu M, Wei J, Huang H, Zhang Y, Zhao J, Xiao L, Wu N, Zheng L and Lin X. CS5931, a novel polypeptide in Ciona savignyi, represses angiogenesis via inhibiting vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs). Mar Drugs 2014; 12: 1530-1544.

[39] Chand HS, Du X, Ma D, Inzunza HD, Kamei S, Foster D, Brodie S and Kisiel W. The effect of human tissue factor pathway inhibitor-2 on the growth and metastasis of fibrosarcoma tumors in athymic mice. Blood 2004; 103: 1069-1077.

[40] Yanamandra N, Kondraganti S, Gondi CS, Gujrati M, Olivero WC, Dinh DH and Rao JS. Recombinant adeno-associated virus (rAAV) expressing TFPI-2 inhibits invasion, angiogenesis and tumor growth in a human glioblastoma cell line. Int J Cancer 2005; 115: 998-1005.

[41] Ran Y, Pan J, Hu H, Zhou Z, Sun L, Peng L, Yu L, Sun L, Liu J and Yang Z. A novel role for tissue factor pathway inhibitor-2 in the therapy of human esophageal carcinoma. Hum Gene Ther 2009; 20: 41-49.

[42] Zhang Q, Zhang Y, Wang SZ, Wang N, Jiang WG, Ji YH and Zhang SL. Reduced expression of tissue factor pathway inhibitor-2 contributes to apoptosis and angiogenesis in cervical cancer. J Exp Clin Cancer Res 2012; 31: 1.

[43] Yoshizaki T, Maruyama Y, Sato H and Furukawa M. Expression of tissue inhibitor of matrix metalloproteinase-2 correlates with activation of matrix metalloproteinase-2 and predicts poor prognosis in tongue squamous cell carcinoma. Int J Cancer 2001; 95: 44-50.

[44] Torng PL, Mao TL, Chan WY, Huang SC and Lin CT. Prognostic significance of stromal metalloproteinase-2 in ovarian adenocarcinoma and its relation to carcinoma progression. Gynecol Oncol 2004; 92: 559-567.

[45] Zhang Q, Chen X, Zhou J, Zhang L, Zhao Q, Chen G, Xu J, Qian F and Chen Z. CD147, MMP-2, MMP-9 and MVD-CD34 are significant predictors of recurrence after liver transplantation in hepatocellular carcinoma patients. Cancer Biol Ther 2006; 5: 808-814.

[46] Suh YG, Lee EJ, Cha H, Yang SH and Seong J. Prognostic values of vascular endothelial growth factor and matrix metalloproteinase-2 in hepatocellular carcinoma after radiotherapy. Dig Dis 2014; 32: 725-732.

[47] Leinonen T, Pirinen R, Böhm J, Johansson R and Kosma VM. Increased expression of matrix metalloproteinase-2 (MMP-2) predicts tumour recurrence and unfavourable outcome in non-small cell lung cancer. Histol Histopathol 2008; 23: 693-700.

[48] Oguić R, Mozetič V, Cini Tešar E, Fučkar Čupić D, Mustać E and Dorđević G. Matrix metalloproteinases 2 and 9 immunoexpression in prostate carcinoma at the positive margin of radical prostatectomy specimens. Patholog Res Int 2014; 2014: 262195.

[49] Aparna M, Rao L, Kunhikatta V and Radhakrishnan R. The role of MMP-2 and MMP-9 as prognostic markers in the early stages of tongue squamous cell carcinoma. J Oral Pathol Med 2015; 44: 345-352.
[50] Katayama A, Bandoh N, Kishibe K, Takahara M, Ogino T, Nonaka S and Harabuchi Y. Expressions of matrix metalloproteinases in early-stage oral squamous cell carcinoma as predictive indicators for tumor metastases and prognosis. Clin Cancer Res 2004; 10: 634-640.
[51] Schwandner O, Schlamp A, Broll R and Bruch HP. Clinicopathologic and prognostic significance of matrix metalloproteinases in rectal cancer. Int J Colorectal Dis 2007; 22: 127-136.
[52] Ekinci T, Ozbay PO, Yiğit S, Yavuzcan A, Uysal S and Soylu F. The correlation between immunohistochemical expression of MMP-2 and the prognosis of epithelial ovarian cancer. Ginekol Pol 2014; 85: 121-130.
[53] Xu C, Wang H, He H, Zheng F, Chen Y, Zhang J, Lin X, Ma D and Zhang H. Low expression of TFPI-2 associated with poor survival outcome in patients with breast cancer. BMC Cancer 2013; 13: 118.