Acta Biochim Biophys Sin 2011, 43: 840–848 | © The Author 2011. Published by ABBS Editorial Office in association with Oxford University Press on behalf of the Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. DOI: 10.1093/abbs/gmr085.
Advance Access Publication 3 October 2011

Original Article

Matrix metalloproteinase 2 promotes cell growth and invasion in colorectal cancer

Wei Dong[1,2†], Hong Li[1†], Yan Zhang[2], Heng Yang[2], Min Guo[2], Li Li[2], and Tongjun Liu[1*]

[1]Colorectal and Anal Surgery, China–Japan Union Hospital of Jilin University, Changchun 130031, China
[2]General Surgery, Jilin Academy of Chinese Medicine Sciences, Changchun 130021, China
[†]These authors contributed equally to this work.
*Correspondence address. Tel: +86-431-89876792; Fax: +86-431-84641026; E-mail: Tongjunliu2000@163.com

Colorectal cancer (CRC) is the third leading cause of cancer-related death in the western world. In this study, we evaluated the expression of matrix metalloproteinase 2 gene (*MMP2*) in CRC and analyzed its correlation with clinicopathological features. We found that the expression of *MMP2* was significantly higher in CRC tissues than in the colorectal tissues. In addition, high levels of MMP2 protein were positively correlated with the status of tumor size, lymph node metastasis, distant metastasis, Dukes' stage, and tumor invasion. Moreover, patients with higher MMP2 levels had markedly shorter overall survivals than those with low MMP2 levels. Multivariate analysis results suggested that the level of *MMP2* expression is an independent prognostic indicator for the survival of patients with CRC. Silencing *MMP2* expression in CRC cell lines with lentiviral-mediated shRNA markedly suppressed cell proliferation, colony formation, and invasion. Furthermore, we observed that vascular endothelial growth factor (VEGF) and membrane type 1 (MT1)-MMP protein levels were decreased in MMP2-down-regulated colorectal cells. Therefore, our study demonstrated that MMP2 is an important factor related to carcinogenesis and metastasis of CRC, and MMP2 promotes CRC cell growth and invasion by up-regulating VEGF and MT1-MMP expression, which makes this pathway a potential target for cancer treatment.

Keywords MMP2; colorectal cancer; clinicopathological features; survival prognosis; RNAi

Received: April 20, 2011 Accepted: July 14, 2011

Introduction

With 655, 000 deaths worldwide per year, colorectal cancer (CRC) is the third leading cause of cancer-related death in the western world (http://www.cancer.gov/cancertopics/commoncancers). Although the incidence of CRC in China is lower than that in the west countries, it has been increased in recent years and has already become one of the most common malignant diseases in China [1]. Despite intensive investigation having been made toward understanding the signaling pathway related to CRC, a few useful biomarkers for diagnosis, prognosis, and targets for treatment are available.

Matrix metalloproteinase-2 (MMP2) is a member of the matrix metalloproteinase (MMP) family, which is involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, angiogenesis [2,3], and tissue remodeling, as well as in disease processes, such as arthritis and tumor invasion and metastasis [4]. In the past few years, MMPs overexpression has been intensively investigated as potential biomarkers and unfavorable factors in a range of types of cancer including CRC [5–10]. *MMP2* gene encodes an enzyme which degrades the major structural component of basement membranes, type IV collagen, and thus plays an important role in promoting the invasion and metastasis of tumor cell. Overexpression of MMP2 has been detected in bladder cancer [11], oral carcinoma [12], CRC [13,14], lung cancer [15], prostate cancer [16], as well as gastric cancer [17]. However, the correlation between MMP2 expression and clinicopathological features in CRC as well as its function in CRC carcinogenesis has not been fully elucidated yet.

In this study, we found that overexpression of MMP2 protein was positively correlated with tumor size, lymph node metastasis, distant metastasis, Dukes' stage, and tumor invasion. Moreover, we observed that patients with higher MMP2 levels had markedly shorter overall survivals than those with low MMP2 levels. Silencing *MMP2* expression in CRC cell lines markedly suppressed cell proliferation, colony formation, and invasion by regulating vascular endothelial growth factor (VEGF) and membrane type 1 (MT1)-MMP in colorectal cells.

Materials and Methods

Sample collection

Eight freshly paired CRC and adjacent non-cancerous colorectal samples, 172 paraffin-embedded CRC, and 45

colorectal samples were obtained from China–Japan Union Hospital of Jilin University. In the 172 CRC cases, there were 116 males and 56 females with age ranging from 23 to 83 years (median, 56.5 years). The CRC samples were obtained from patients without any neoadjuvant therapy. A complete 5-year follow-up was available in all 172 patients. For the use of these clinical materials for research purposes, prior consents from the patients and approval from the Ethics Committees of our hospital were obtained. Histological classification and clinicopathological staging of the samples were performed according to the Japanese General Rules for Clinical and Pathological Studies on Cancer of the Colon, Rectum, and Anus along with the International Union against Cancer classification.

Real-time polymerase chain reaction (PCR)

Total RNA was extracted from CRC and normal colorectal tissues with TRIzol reagent (Invitrogen, Carlsbad, USA) according to the manufacturer's protocol. cDNA was synthesized using RT reagents (Takara, Dalian, China) from 1 μg of total RNA using oligo$(dT)_{15}$ in 20 μl reaction volume. Real-time PCR was used to measure the expression of *MMP2* mRNA using SYBR Pre-mix Ex Taq according to the manufacturer's protocol (Takara) with an Mx3000P real-time PCR system (Stratagene, La Jolla, USA) as described previously [18]. The sequence for sense primer was 5′-ACCAGCTGGCCTAGTGATGATG-3′, and for anti-sense primer was 5′-GGCTTCCGCATGGTCTCG ATG-3′. *GAPDH* gene was used as an internal control using the sense primer 5′-GCACCGTCAAGGCTGAGA AC-3′ and anti-sense primer 5′-TGGTGAAGACGCCAG TGGA-3′. Real-time PCR was performed in a final reaction volume of 20 μl containing 10 μl 2× SYBR pre-mixture, 0.4 μl Rox reference dye (Takara) 1 μl templates, 2 μl each of the primer pair (10 pmol), and 6.6 μl sterilized water. PCR conditions were as follows: pre-heating for 3 min at 95°C, 45 cycles of 94°C for 15 s, 55°C for 20 s, and 72°C for 15 s.

Immunohistochemistry

All the reagents of immunohistochemistry including IHC Biotin Block Kit, SP Detection Kit, and DAB Visualization Kit were purchased from Maxim Inc. (Fuzhou, China). The experiments were performed according to the instruction of manufacturers. Paraffin sections (4 μm) from samples of 172 CRCs and 45 colorectal tissues were deparaffinized in 100% xylene and re-hydrated in descending ethanol series and water according to standard protocols. Heat-induced antigen retrieval was performed in 10 mM citrate buffer for 2 min at 100°C. Endogenous peroxidase activity and non-specific antigen were blocked with peroxidase blocking reagent containing 3% hydrogen peroxide and serum, followed by incubation with mouse anti-human MMP2 antibody (1:100; Santa Cruz, Santa Cruz, USA) overnight. After washing, the sections were incubated with biotin-labeled goat anti-mouse IgG antibody (Maxin) for 10 min at room temperature, and subsequently were incubated with streptavidin-conjugated horseradish peroxidase (HRP) (Maxin). Sections were visualized with diaminobenzidine (DAB) and counterstained with hematoxylin, mounted in neutral gum, and analyzed using a bright field microscope (vanoxah-fl-2; Olympus Inc., Tokyo, Japan).

Evaluation of staining

The immunohistochemically stained tissue sections were reviewed and scored separately. The extent of the staining, defined as the percentage of positive staining areas of tumor cells or normal nasopharyngeal epithelial cells related to the whole tissue area, was scored on a scale of 0–4 as follows: 0, <10%; 1, 10–25%; 2, 26–50%; 3, 51–75%; and 4, >75%. The sum of the staining-intensity and staining-extent scores was used as the final staining score for MMP2 (0–7). For statistical analysis, a final staining score of 0–5 and 6–7 were considered to be low and high expression [19], respectively.

Establishment of CRC cell lines with stably expressing short hairpin RNA (shRNA) shRNA-*MMP2*

The construction of lentiviral vector of shRNA targeting *MMP2* and establishment of CRC cell lines stably expressing shRNA had been described previously [20]. In brief, we selected one sequence (*MMP2* 1917: sense, 5′-**CGCGTCCCCGC**GGAGACAAATTCTGGAGAT**TTC AAGAGA**ATCTCCAGAATTTGTCTCC**GCTTTTTGGA AAT-**3′; anti-sense: 5′-**CGATTTCCAAAAAGC**GGAGA CAAATTCTGGAGAT**TCTCTTGAA**ATCTCCAGAATTT GTCTCC**GCGGGGA**-3′) for targeting the *MMP2* gene using the BLOCK-It RNAi Designer (Invitrogen). The preparation of lentiviral vectors expressing human *MMP2* shRNA was performed using the pLVTHM-green fluorescent protein (GFP) Lentiviral RNAi Expression System endowed by Trono Laboratory (Lausanne, Switzerland). Replication-incompetent lentivirus was produced by cotransfection of the pLVTHM/*MMP2*-shRNA expression vector and ViraPower packaging mix containing an optimized mixture of two packaging plasmids: psPAX2 and pMD2.G into 293FT cells. CRC HT29 and SW480 cells were infected with lentiviral particles containing specific or negative control vectors, and the polyclonal cells with strong GFP expression were selected by flow cytometry sorting using anti-GFP monoclonal antibody (BD Company, New York, USA) to culture and expand.

Cell proliferation analysis

Cell growth was determined by MTT assay (Sigma, St. Louis, USA). Briefly, 1×10^3 cells were seeded into a 96-well plate with quadruplicate for each condition. Seventy-two hours later, MTT reagent was added to each well at 5 mg/ml in 20 μl and incubated for another 4 h. The formazan crystals formed by viable cells were then dissolved in dimethyl sulfoxide and measured at 490 nm for the absorbance (A) values. Each experiment was performed in triplicate.

Plate colony formation assay

The cells (1×10^2) were added to each well of a six-well culture plate. After incubation at 37°C for 13 d, cells were washed twice with phosphate-buffered saline (PBS) and stained with Giemsa solution. The number of colonies containing >50 cells was counted under a microscope (plate clone formation efficiency = number of colonies/number of cells inoculated×100%). Each experiment was performed in triplicate.

In vitro invasion assay

Cells in log phase was treated with trypsin and re-suspended as single-cell solution. A total of 1×10^5 cells were seeded on a fibronectin-coated polycarbonate membrane insert with pre-coated ECMatrix in a transwell apparatus (Corning Inc., New York, USA). In the lower chamber, 600 μl RPMI 1640 medium with 10% NBCS was added as chemoattractant. After cells were incubated for 14 h at 37°C in 5% CO_2, the insert was washed with PBS, and cells on the top surface of the insert were removed by a cotton swab. Cells adhering to the lower surface were fixed by methanol, stained by Giemsa and counted under a microscope in five pre-determined fields (×200). All assays were independently repeated at least three times.

Western blot analysis

Cells were lysed in RIPA buffer [50 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0, 5 mM dithiothreitol, 2% sodium dodecyl sulfate (SDS)], and protein concentration was determined using bicinchoninic acid assay (Beyotime Inc., Shanghai, China). Total protein (30 μg) was resolved using a 10% SDS-polyacrylamide gel electrophoresis and electro-transferred to polyvinylidene fluoride membranes (Invitrogen) [21], and blocked with 5% non-fat dry milk in Tris-buffered saline, pH 7.5. Membranes were immunoblotted overnight at 4°C with goat anti-human polyclonal MMP2 antibody (1:800; Abcam, Cambridge, UK), polyclonal rabbit anti-human polyclonal ACTB antibody (1:500; Santa Cruz), rabbit anti-human monoclonal VEGF (1:1000), and rabbit anti-human polyclonal MT1-MMP (1:1000; Epitomics, Burlingame, USA). An HRP-conjugated anti-rabbit or goat IgG antibody was used as the secondary antibody (Zhongshan Inc., Beijing, China).

Statistical analyses

All statistical analyses were performed using SPSS 13.0 software. The χ^2 and Kruska and Wallis tests were used to analyze the correlation between the levels of *MMP2* expression and clinicopathological characteristics. Survival curves were plotted using the Kaplan–Meier method and compared using the log-rank test. The significances of various variables in survival were analyzed using univariate and multivariate Cox proportional-hazards model. One-way analysis of variance was used to determine the differences between groups for all *in vitro* and *in vivo* analyses. $P < 0.05$ was considered statistically significant.

Results

Up-regulated expression of *MMP2* in CRC

In order to assess the role of *MMP2* in CRC, we performed real-time PCR to measure the expression of *MMP2* mRNA in eight-paired CRCs and their adjacent colorectal tissues. As shown in **Fig. 1(A)**, all eight CRC tissues showed elevated expression of *MMP2* compared with their matched normal tissues. Among them, five CRC tissues showed markedly increased changes (>2 fold).

Immunohistochemical analysis of MMP2 protein in CRC and normal colorectal tissues

We measured the level and subcellular localization of MMP2 in 172 archived paraffin-embedded CRC samples and 45 normal colorectal samples using immunohistochemical staining [**Fig. 1(B), a,b,c**]. Specific MMP2 protein staining was mainly found in the cytoplasm of non-cancerous epithelial and malignant tumor cells [**Fig. 1(B), b,c**]. Furthermore, we observed that in 61.2% (116 of 172) CRC samples, MMP2 was highly expressed. In contrast, only 31.1% (14 of 45) normal colorectal samples had highly expressed MMP2, significantly lower than those in the CRC samples ($P < 0.001$) (**Table 1**).

Correlation between clinicopathological features and MMP2 level in CRC patients

The relationships between clinicopathological characteristics and MMP2 levels in individuals with CRC were analyzed. We did not find any significant association of MMP2 levels with patient's age, sex, smoking, tumor site, and differentiation degree in 172 CRC cases. However, we observed that the level of MMP2 was positively correlated with tumor size ($P < 0.001$), tumor invasion ($P = 0.016$), lymph node metastasis ($P < 0.001$), distant metastasis ($P = 0.003$), and Dukes' stage ($P < 0.001$) in CRC patients (**Table 2**).

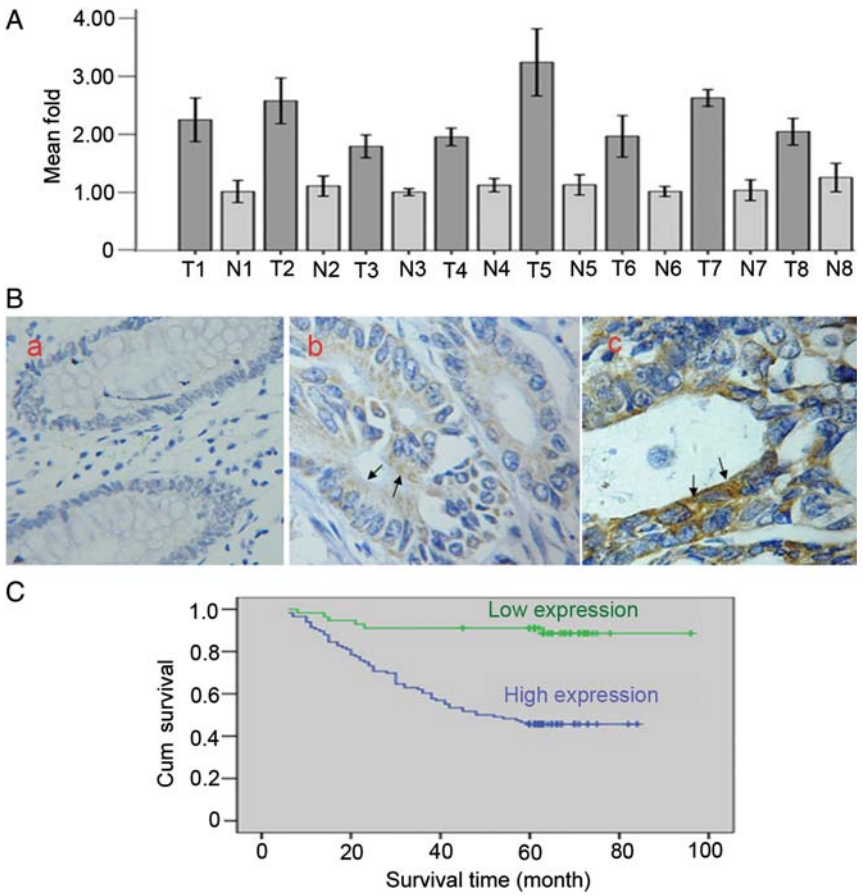

Figure 1 Overexpression of *MMP2* is unfavorable for CRC patients' survival (A) *MMP2* mRNA level was markedly up-regulated in CRC tissues compared with their matched normal tissues. T, CRC sample; N, normal sample. (B) (a) Negative expression of *MMP2* in normal colorectal sample; (b) weak expression of *MMP2* in CRC samples (magnification ×400); (c): strong staining of *MMP2* in CRC samples (magnification ×400). Arrows means MMP2 positive staining. (C) Kaplan–Meier survival analysis of overall survival duration in 172 CRC patients according to MMP2 protein level. The log-rank test was used to calculate *P* values.

Table 1 Protein level of MMP2 between CRC and colorectal samples

Group	Protein level			*P* value
	Cases	High level	Low level	
Cancer	172	116 (61.2%)	56 (38.8%)	
Normal	45	14 (31.1%)	31 (68.9%)	<0.001

Survival analysis

To investigate the prognostic value of *MMP2* expression for CRC, we assessed the association between the levels of *MMP2* expression and patients' survival using Kaplan–Meier analysis with the log-rank test. In 172 CRC cases with prognosis information, we observed that the level of MMP2 protein was significantly correlated with the overall survival of CRC patients [**Fig. 1(C)**]. Patients with high *MMP2* expression level had poorer survival than those with lower level of *MMP2* expression. In univariate analysis, using Cox's proportional-hazard regression model, tumor size, tumor invasion, lymph node metastasis, distant metastasis, Dukes'stage, and *MMP2* expression were found to be significantly correlated with patients' survival ($P < 0.001$, $P = 0.041$, $P < 0.001$, $P < 0.001$, $P < 0.001$, and $P < 0.001$, respectively) (**Table 3**). Further Cox's proportional-hazard regression model was then used to determine which factors were jointly predicative of overall survival. All variables with $P < 0.05$ in univariate analysis (**Table 3**) were included in this analysis. Tumor size, lymph node metastasis, distant metastasis, and *MMP2* expression were significantly related to overall survival of CRC patients ($P < 0.001$, $P = 0.011$, $P < 0.001$, $P = 0.007$, respectively). *MMP2* expression might potentially be prognostic indicator in CRC patients (**Table 3**).

Reduced expression of *MMP2* in CRC cells

To study the biological functions of *MMP2*, we introduced a lentiviral vector containing shRNA specifically targeting *MMP2* to stably knock down the endogenous expression of *MMP2* in CRC HT29 and SW480 cells, and stably infected polyclonal cells were obtained. Western blot assay results showed that MMP2 protein in two shRNA-*MMP2*

Table 2 Correlation between the clinicopathological characteristics and level of MMP2 protein in CRC

Characteristics	*n*	MMP2 (*n*)		*P* value
		High level	Low level	
Gender				0.624
Male	116	66	50	
Female	56	29	27	
Age				1.000
≥50	81	55	26	
<50	91	61	30	
Smoking				0.241
Yes	39	30	9	
No	133	87	46	
Tumor site				0.093
Colon	109	79	30	
Rectum	64	38	26	
Tumor size (cm)				<0.001
<5	81	13	68	
≥5	91	48	43	
Tumor differentiation				0.216
Good + moderate	33	19	14	
Poor	139	97	42	
Tumor invasion				0.016[a]
Expansive	34	16	18	
Intermediate	82	61	21	
Infiltrative	56	39	17	
Lymph node metastasis				<0.001
Positive	96	78	18	
Negative	76	39	37	
Distant metastasis				0.003
Positive	46	39	7	
Negative	126	77	49	
Dukes' stage				<0.001
A + B	60	20	40	
C + D	112	95	17	

[a]Kruska–Wallis test.

polyclonal cells was markedly reduced expression, while two control empty vector clone cells did not show any change in MMP2 protein level based on densitometry analysis (**Fig. 2**).

Decreased expression of *MMP2* suppressed the expression of VEGF and MT1-MMP in colorectal cells

To further study the mechanism by which MMP2 regulates cell growth and invasion, we examined the protein levels of VEGF and MT1-MMP after *MMP2* expression was inhibited by lentiviral-mediated shRNA in CRC HT29 and SW480 cells. Using western blot analysis, we found that VEGF and MT1-MMP levels were significantly decreased in both MMP2-suppressed CRC HT29 and SW480 cells compared with their corresponding control cells (**Fig. 2**).

Suppressed expression of *MMP2* reduces cell proliferation *in vitro*

To analyze the function of *MMP2*, we studied the rate of cell proliferation of shRNA-*MMP2* CRC cells. The growth curves determined by MTT assay showed that decreased *MMP2* expression inhibited cell proliferation of HT29 and SW480 cells in comparison with control cells [**Fig. 3(A)**]. The results from plate colony formation assay showed that *MMP2*-down-regulating HT29 and SW480 (53 ± 4; 48 ± 5) cells formed significantly less colonies than control clone cells (71 ± 4; 66 ± 3) ($P < 0.05$ for both cell types) [**Fig. 3(B)**], suggesting the proliferation effect of *MMP2* on anchorage-dependent growth of CRC cells.

Down-regulated *MMP2* expression suppresses cell invasion

Using a boyden chamber coated with matrigel, we determined changes in cell invasiveness after 14-h incubation. Compared with the negative control Ctr-HT29 and Ctr-SW480 cells (175 ± 13; 183 ± 10), *MMP2*-down-regulating HT29 and SW480 cells (82 ± 16; 99 ± 9) both showed significantly decreased cell invasiveness (for both $P < 0.05$) [**Fig. 3(C)**].

Discussion

CRC is a malignant tumor arising from the mucosal epithelium of the large intestine. Genetic alterations, together with epigenetic components and dietary factors, play a fundamental role in the initiation and progression of CRC by causing a number of deregulations, such as the activation of oncogenes and inactivation of tumor suppressors [22–27]. However, the molecular mechanism of CRC pathogenesis remains incompletely understood.

MMP2, a member of Zn^{2+}-dependent endopeptidase family, is capable of cleaving most extracellular matrix and basement membrane, which promotes the invasion and metastasis of tumor cells, and thus involves in the progression and metastasis of many tumors. The positive expression of *MMP2* was associated with higher grade glioma. Patients with *MMP2* expression had the lower survival rate [28]. In endometrial carcinoma, a positive correlation was found between *MMP2* overexpression and vascular and lymphatic invasion, respectively [29]. *MMP2* expression was increased with the elevated grade of cervical intra-epithelial neoplasia (CIN). However, upon transition from CIN to invasive cancer, major up-regulation of *MMP2* expression will be observed in invasive cancer [30].

Table 3 Summary of univariate and multivariate Cox regression analysis of overall survival duration

Parameter	Univariate analysis			Multivariate analysis		
	P	HR	95%CI	*P*	HR	95%CI
Age						
≥50 vs. <50 years	0.368	0.805	0.502–1.291	–	–	–
Gender						
Male vs. female	0.348	1.254	0.782–2.010	–	–	–
Smoking						
Yes vs. no	0.680	0.891	0.515–1.541	–	–	–
Tumor site						
Colon vs. rectum	0.061	0.608	0.361–1.023	–	–	–
Tumor size						
<5 vs. ≥5	0.000	6.025	3.382–10.732	0.000	4.066	2.174–7.603
Lymph node metastasis						
Positive vs. negative	0.000	3.693	2.081–6.553	0.011	2.443	1.229–4.854
Distant metastasis						
Positive vs. negative	0.000	0.172	0.106–0.278	0.000	0.300	0.177–0.509
Dukes' classification						
A + B vs. C + D	0.000	5.239	2.546–11.154	0.231	0.532	0.189–1.493
Tumor differentiation						
Good + moderate vs. poor	0.551	1.217	0.639–2.318	–	–	–
Extent of tumor invasion						
Expansive vs. intermediate vs. infiltrative	0.041	1.423	1.015–1.994	0.316	1.214	0.177–0.509
MMP2 expression						
High vs. low	0.000	6.685	2.889–15.471	0.007	3.758	1.429–9.879

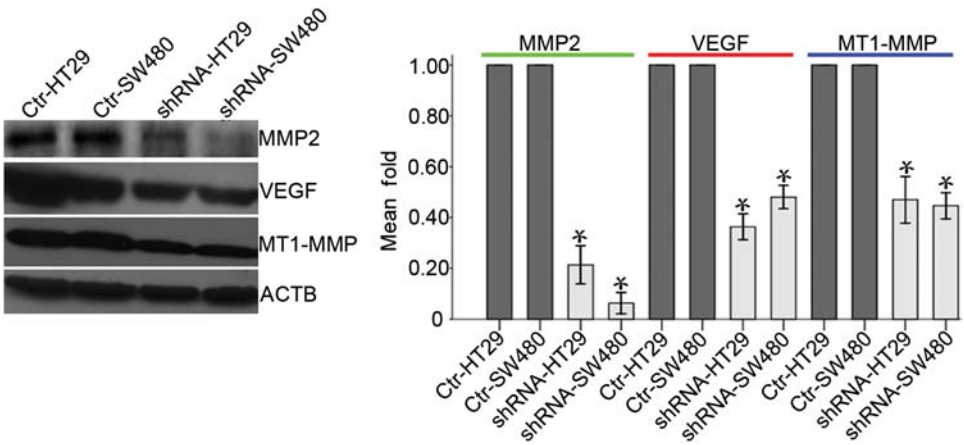

Figure 2 MMP2 regulated the expression of VEGF and MT1-MMP in CRC cells Western blot showed that VEGF and MT1-MMP levels were significantly reduced in *MMP2* expression-suppressed CRC HT29 and SW480 cells compared with their control-vector cells.

In this study, we demonstrated that *MMP2* was predominantly expressed in the cancer cells and epithelial cells, which was consistent with Lipari's and Jeffery' reports [12,13]. Similar to the report from Wang *et al*. [14], we confirmed that MMP2 protein levels was markedly increased in CRC tissues compared with colorectal tissues, suggesting that MMP2 was involved in the pathogenesis of CRC.

Using immunohistochemical assay, Li *et al*. [31] have found that increased expression of MMP2 is significantly related to higher tumor grade and larger tumor size in node-negative breast cancer. In addition, Danilewicz *et al*. [32] also have discovered that MMP2 immunostaining in laryngeal cancer patients with lymph node metastases is markedly increased compared with those patients without

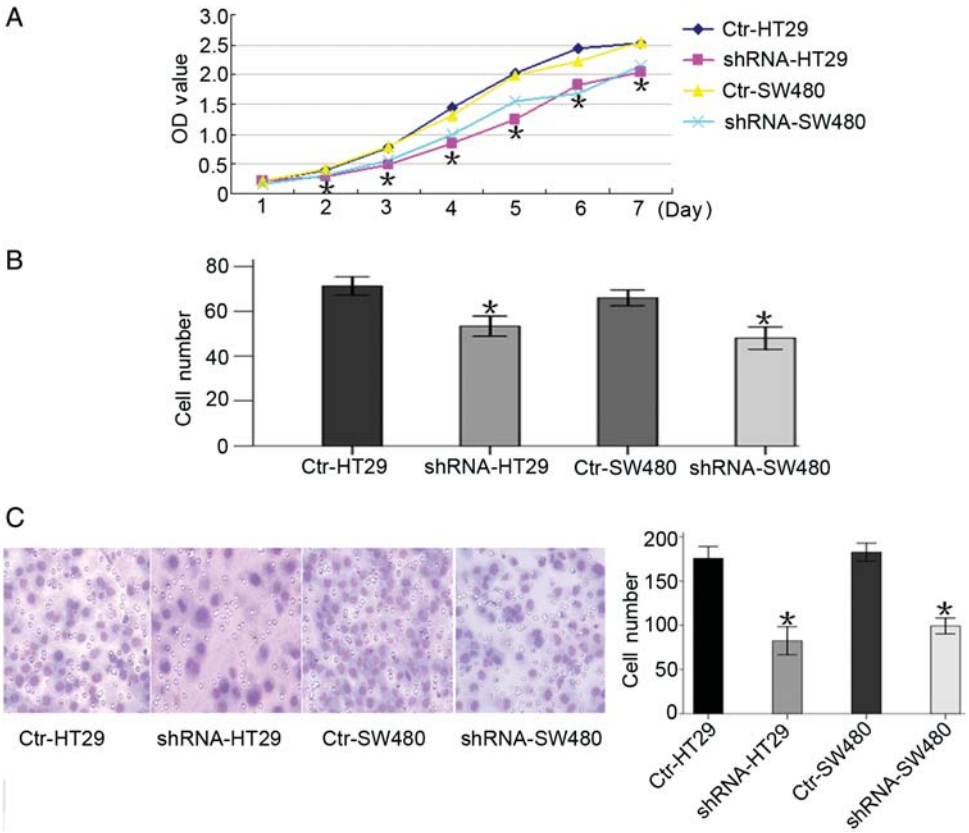

Figure 3 Suppressed *MMP2* expression inhibited cell proliferation and invasiveness *in vitro* (A) The growth of control-vector cells and shRNA-HT29 and SW480 cells was examined by MTT assay over a 7-day period. $*P < 0.05$, compared with their corresponding control-vector cells. (B) The growth of control-vector cells and shRNA-HT29, and SW480 cells, was examined by plate colony formation assay. $*P < 0.05$, compared with their corresponding control-vector cells. (C) Cell invasion capabilities of two control-vector cells, shRNA-HT29 and SW480, were examined using boyden chamber assay. Data were presented as mean $\pm$ SD for three independent experiments. $*P < 0.05$, as compared with their corresponding control-vector cells.

lymph node involvement. In this study, we also found that *MMP2* overexpression was significantly associated with tumor size, lymph node metastasis, tumor invasion, distant metastasis, and Dukes' stage of CRC patients. This result is similar to Wang's report [14], suggesting that overexpressed MMP2 promoted the progression in CRC patients and played an unfavorable role in CRC pathogenesis. However, the correlation between *MMP2* expression and the survival of CRC patients has been never reported.

Elevated expression of *MMP2* in tumor cells has been shown to be an unfavorable prognostic factor in a few tumors. In renal cell carcinoma, the rate of increased expression of *MMP2* is up to 67%. Its overexpression is not only correlated with high tumor grade, but also as an expression marker that correlated with shortened survival [33]. Interestingly, similar results were revealed in lung cancer, breast cancer, laryngeal cancer, and so on. By investigation of 189 patients with early-stage non-small-cell lung carcinoma, Passlick *et al*. [34] have found a significant association between *MMP2* overexpression and shortened overall survival and disease-free survival. Yamamura *et al*. [15] also demonstrate that high expression of MMP2 protein significantly associates with poor overall survival in non-small cell lung carcinomas. In lymph node-negative breast cancer, overexpressed MMP2 as an unfavorable prognostic factor is not correlated with overall survival of patients, but relates to patients' shortened relapse-free survival [31]. This result is similar to Leppä *et al.*'s investigation. However, the latter further presented MMP2 level of serum as an independent predictor for overall survival in node-positive breast carcinoma [35]. Furthermore, Danilewicz *et al*. [32] have reported in laryngeal cancer that the survival time of patients with higher *MMP2* expression tumor is significantly shorter than that with low *MMP2* expression.

In the current study, we presented the evidence that MMP2 protein expression in CRC was inversely associated with patient's overall survival. According to Kaplan–Meier curve analysis, there was a significant difference in overall survival between MMP2 negative and MMP2 positive patients, and the patients with higher level of MMP2 protein had shorter overall survival time than those of patients with lower MMP2 level.

In univariate analysis, using Cox's proportional-hazard regression model we found *MMP2* expression was significantly associated with patients' overall survival ($P < 0.001$). In multivariate analysis, using Cox proportional-hazard model we found elevated expression of *MMP2*, larger tumor size, lymph node metastasis, and distant metastasis were unfavorable for shortened overall survival time ($P = 0.007$, <0.001, $=0.011$, and <0.001 for each). We concluded that MMP2 was an unfavorable prognostic factor in CRC patients. Therefore, MMP2 expression might be potential predictive factor in CRC patients.

It is well known that MMP2 plays an important role in survival, growth, and invasion of malignant cells. To better understand the mechanisms of MMP2 promotes tumor cell growth and invasion, we decreased *MMP2* expression in CRC cell lines with lentiviral-mediated shRNA. Analogous to previous studies [36,37], we found that inhibition of *MMP2* expression could markedly reduce the ability of cell proliferation and invasiveness *in vitro*. These results suggested a promoting role of MMP2 in the development and progression of CRCs. Furthermore, we found that the protein levels of VEGF and MT1-MMP were markedly reduced in *MMP2*-suppressed colorectal cells compared with their control cells, suggesting that *MMP2* can positively regulate the expression of VEGF and MT1-MMP to promote cell proliferation and invasion [3,38,39].

In summary, our investigation demonstrated that the expression level of *MMP2* was significantly increased in CRC and correlated with the malignant progression status of CRC. In addition, our data suggested that increased expression of *MMP2* was an important adverse prognosis factor for CRC. To our knowledge, our study reported, for the first time, the association of *MMP2* expression with the survival in CRC. Furthermore, we found that suppression of *MMP2* could reduce the ability of cell growth and invasion by regulating the expression VEGF and MT1-MMP.

References

1 Xu AG, Yu ZJ, Jiang B, Wang XY, Zhong XH, Liu JH and Lou QY, *et al*. Colorectal cancer in Guangdong Province of China: a demographic and anatomic survey. World J Gastroenterol 2010, 16: 960–965.

2 Giannopoulos G, Pavlakis K, Parasi A, Kavatzas N, Tiniakos D, Karakosta A and Tzanakis N, *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.

3 Chetty C, Lakka SS, Bhoopathi P and Rao JS. MMP-2 alters VEGF expression via alphaVbeta3 integrin-mediated PI3K/AKT signaling in A549 lung cancer cells. Int J Cancer 2010, 127: 1081–1095.

4 Volland S, Kugler W, Schweigerer L, Wilting J and Becker J. Stanniocalcin 2 promotes invasion and is associated with metastatic stages in neuroblastoma. Int J Cancer 2009, 125: 2049–2057.

5 Murray GI, Duncan ME, O'Neil P, Melvin WT and Fothergill JE. Matrix metalloproteinase-1 is associated with poor prognosis in colorectal cancer. Nat Med 1996, 2: 461–462.

6 Leeman MF, Curran S and Murray GI. New insights into the roles of matrix metalloproteinases in colorectal cancer development and progression. J Pathol 2003, 201: 528–534.

7 Curran S, Dundas SR, Buxton J, Leeman MF, Ramsay R and Murray GI. Matrix metalloproteinase/tissue inhibitors of matrix metalloproteinase phenotype identifies poor prognosis colorectal cancers. Clin Cancer Res 2004, 10: 8229–8234.

8 Wilson S, Wakelam MJ, Hobbs RF, Ryan AV, Dunn JA, Redman VD and Patrick F, *et al*. Evaluation of the accuracy of serum MMP-9 as a test for colorectal cancer in a primary care population. BMC Cancer 2006, 6: 258.

9 Lyall MS, Dundas SR, Curran S and Murray GI. Profiling markers of prognosis in colorectal cancer. Clin Cancer Res 2006, 12: 1184–1191.

10 Liu Z, Li L, Yang Z, Luo W, Li X, Yang H and Yao K, *et al*. Increased expression of MMP9 is correlated with poor prognosis of nasopharyngeal carcinoma. BMC Cancer 2010, 10: 270.

11 Mohammad MA, Ismael NR, Shaarawy SM and El-Merzabani MM. Prognostic value of membrane type 1 and 2 matrix metalloproteinase expression and gelatinase A activity in bladder cancer. Int J Biol Markers 2010, 25: 69–74.

12 Lipari L, Mauro A, Tortorici S, Burruano F, Leone A, Spatola GF and Gerbino A, *et al*. Immunohistochemical and transcriptional expression of the matrix metalloproteinases MMP-2 and MMP-9 in normal and pathological human oral mucosa. J Biol Regul Homeost Agents 2009, 23: 259–267.

13 Jeffery N, McLean MH, El-Omar EM and Murray GI. The matrix metalloproteinase/tissue inhibitor of matrix metalloproteinase profile in colorectal polyp cancers. Histopathology 2009, 54: 820–828.

14 Wang K, Sun XJ, Li SG, Shang WH, Jia PB and Feng HA. Expressions of matrix metalloproteinase 2 and carbohydrate antigen 50 in colorectal carcinoma, transitional mucosa and normal colorectal mucosa and its clinical significance. Chin J Bases Clin Gen Surg 2006, 13: 417–420.

15 Yamamura T, Nakanishi K, Hiroi S, Kumaki F, Sato H, Aida S and Kawai T. Expression of membrane-type-1-matrix metalloproteinase and metalloproteinase-2 in nonsmall cell lung carcinomas. Lung Cancer 2002, 35: 249–255.

16 Trudel D, Fradet Y, Meyer F, Harel F and Têtu B. Membrane-type-1 matrix metalloproteinase, matrix metalloproteinase 2, and tissue inhibitor of matrix proteinase 2 in prostate cancer: identification of patients with poor prognosis by immunohistochemistry. Hum Pathol 2008, 39: 731–739.

17 Feng G and Tan Y. Expression and significance of MMP2 and type IV collagen in gastric cancer. Zhonghua Wai Ke Za Zhi 2000, 38: 775–777.

18 Xie SM, Fang WY, Liu Z, Wang SX, Li X, Liu TF and Xie WB, *et al*. Lentivirus-mediated RNAi silencing targeting ABCC2 increasing the sensitivity of a human nasopharyngeal carcinoma cell line against cisplatin. J Transl Med 2008, 6: 55.

19 Liu Z, Li X, He X, Jiang Q, Xie S, Yu X and Zhen Y, *et al*. Decreased expression of updated NESG1 in nasopharyngeal carcinoma: its potential role and preliminarily functional mechanism. Int J Cancer 2011, 128: 2562–2571.

20 Wu A, Wu B, Guo J, Luo W, Wu D, Yang H and Zhen Y. Elevated expression of CDK4 in lung cancer. J Transl Med 2011, 9: 38.

21 Wang S and Fang W. Increased expression of hepatoma-derived growth factor correlates with poor prognosis in human nasopharyngeal carcinoma. Histopathology 2011, 58: 217–224.

22 Zhang C, Fang Z, Xiong Y, Li J, Liu L, Li M and Zhang W, *et al*. Copy number increase of aurora kinase A in colorectal cancers: a correlation with tumor progression. Acta Biochim Biophys Sin 2010, 42: 834–838.

23 Ashktorab H, Schäffer AA, Daremipouran M, Smoot DT, Lee E and Brim H. Distinct genetic alterations in colorectal cancer. PLoS One 2010, 5: e8879.
24 Gan L, Chen S, Zhong J, Wang X, Lam EK, Liu X and Zhang J, *et al*. H.ZIC1 is downregulated through promoter hypermethylation, and functions as a tumor suppressor gene in colorectal cancer. PLoS One 2011, 6: e16916.
25 Willis ND, Cox TR, Rahman-Casañs SF, Smits K, Przyborski SA, van den Brandt P and van Engeland M, *et al*. Lamin A/C is a risk biomarker in colorectal cancer. PLoS One 2008, 3: e2988.
26 Peng Y, Li H, Wu M, Wang X, Fan S, Liu F and Xiang B. NGX6 inhibits AP-1 and Ets-1 expression and down-regulates cyclin D1 in human colorectal cancer. Acta Biochim Biophys Sin 2009, 41: 504–514.
27 Choi JS, Kim KH, Jeon YK, Kim SH, Jang SG, Ku JL and Park JG. Promoter hypermethylation of the ADAM23 gene in colorectal cancer cell lines and cancer tissues. Int J Cancer 2009, 124: 1258–1262.
28 Gu J, Zhang C, Chen R, Pan J, Wang Y, Ming M and Gui W, *et al*. Clinical implications and prognostic value of EMMPRIN/CD147 and MMP2 expression in pediatric gliomas. Eur J Pediatr 2009, 168: 705–710.
29 Karahan N, Güney M, Baspinar S, Oral B, Kapucuoglu N and Mungan T. Expression of gelatinase (MMP-2 and MMP-9) and cyclooxygenase-2 (COX-2) in endometrial carcinoma. Eur J Gynaecol Oncol 2007, 28: 184–188.
30 Branca M, Ciotti M, Giorgi C, Santini D, Di Bonito L, Costa S and Benedetto A, *et al*. HPV-PathogenISS Study Group. Matrix metalloproteinase-2 (MMP-2) and its tissue inhibitor (TIMP-2) are prognostic factors in cervical cancer, related to invasive disease but not to high-risk human papillomavirus (HPV) or virus persistence after treatment of CIN. Anticancer Res 2006, 26: 1543–1556.
31 Li HC, Cao DC, Liu Y, Hou YF, Wu J, Lu JS and Di GH, *et al*. Prognostic value of matrix metalloproteinases (MMP-2 and MMP-9) in patients with lymph node-negative breast carcinoma. Breast Cancer Res Treat 2004, 88: 75–85.
32 Danilewicz M, Sikorska B and Wagrowska-Danilewicz M. Prognostic significance of the immunoexpression of matrix metalloproteinase MMP2 and its inhibitor TIMP2 in laryngeal cancer. Med Sci Monit 2003, 9: MT42–MT47.
33 Kallakury BV, Karikehalli S, Haholu A, Sheehan CE, Azumi N and Ross JS. Increased expression of matrix metalloproteinases 2 and 9 and tissue inhibitors of metalloproteinases 1 and 2 correlate with poor prognostic variables in renal cell carcinoma. Clin Cancer Res 2001, 7: 3113–3119.
34 Passlick B, Sienel W, Seen-Hibler R, Wöckel W, Thetter O, Mutschler W and Pantel K. Overexpression of matrix metalloproteinase 2 products unfavorable outcome in early-stage non-small cell lung cancer. Clin Cancer Res 2000, 6: 3944–3948.
35 Leppä S, Saarto T, Vehmanen L, Blomqvist C and Elomaa I. A high serum matrix metalloproteinase-2 level is associated with an adverse prognosis in node-positive breast carcinoma. Clin Cancer Res 2004, 10: 1057–1063.
36 Rojiani MV, Alidina J, Esposito N and Rojiani AM. Expression of MMP-2 correlates with increased angiogenesis in CNS metastasis of lung carcinoma. Int J Clin Exp Pathol 2010, 3: 775–781.
37 Jang HS, Lal S and Greenwood JA. Calpain 2 is required for glioblastoma cell invasion: regulation of matrix metalloproteinase 2. Neurochem Res 2010, 35: 1796–1804.
38 Zhou J, Gan N, Zhang W, Lu W and Xie X. Proliferation suppression and apoptosis of ovarian carcinoma cells induced by small interfering RNA against vascular endothelial growth factor. J Obstet Gynaecol Res 2010, 36: 232–238.
39 Wu M, Shi Y, Xi L, Li Q, Liao GN, Han ZQ and Lu YP, *et al*. Construction of antisense MT1-MMP vector and its inhibitory effects on invasion of human ovarian cancer cells. J Huazhong Univ Sci Technol Med Sci 2005, 25: 715–717.