RESEARCH ARTICLE

Overexpression of Matrix Metalloproteinase 11 in Thai Prostatic Adenocarcinoma is Associated with Poor Survival

Nongnuch Nonsrijun[1], Jumphol Mitchai[2], Kamoltip Brown[3], Ratana Leksomboon[4], Panya Tuamsuk[1]*

Abstract

Background: The incidence of prostate cancer, one of the most common cancers in elderly men, is increasing annually in Thailand. Matrix metalloproteinase 11 (MMP-11) is a member of the extracellular matrix metalloproteases which has been associated with human tumor progression and clinical outcome. Aim: To quantify MMP-11 expression in prostatic adenocarcinoma tissues and to determine whether its overexpression correlates with survival outcome, and to assess its potential as a new prognostic marker. Materials and Methods: Expression of MMP-11 was analyzed using immunohistochemistry in 103 Thai patients with prostatic adenocarcinoma. Overall survival was analyzed using Kaplan-Meier methods and Cox regression models. Results: Immunoreactivity of MMP-11 was seen in the stroma of prostatic adenocarcinoma tissue samples, high expression being significantly correlated with poor differentiation in Gleason grading, pathologic tumor stage 4 (pT4), and positive-bone metastasis ($p<0.05$), but not age and prostatic-specific antigen (PSA) level. Patients with high levels of MMP-11 expression demonstrated significantly shorter survival ($p<0.001$) when compared to those with low levels. Multivariate analysis showed that MMP-11 expression and pT stage were related with survival in prostatic adenocarcinoma [hazard ratio (HR)=0.448, 95% confidence interval (95%CI)=0.212-0.946, HR=0.333, 95%CI=0.15-0.74, respectively]. Conclusions: Expression of MMP-11 is significantly associated with survival in prostatic adenocarcinoma. High levels may potentially be used for prediction of a poor prognosis.

Keywords: Matrix metalloproteinase 11 - prostatic adenocarcinoma - survival time - prognostic significance

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Introduction

Prostate cancer is a major problem in elderly men and is the second most common male cancer worldwide, especially in developed countries (Jemal et al., 2011). In Thailand, this cancer is the fourth most common cause of male cancer, but its incidence is increasing annually (Khuhaprema et al., 2010). Almost all patients with prostate cancer are advanced and at metastatic stage at the time of initial diagnosis and have been related to poor survival time more than early stage (Tuamsuk et al., 2011). Currently, serum prostatic-specific antigen (PSA) is used for both screening and monitoring of prostate cancer during treatment. However, PSA is more sensitive, but not more specific and for diagnosis because common pathologic condition such as benign prostatic hyperplasia (BPH) and prostatitis can also present abnormality of PSA levels (Madu and Lu, 2010). Consequently, there are requirements for accurate prognostic markers, that could be combined with recently those available in order to improve survival time and management to the most effective treatment of prostate cancer.

Various studies have shown that matrix metalloproteinase 11 (MMP-11) was significant factor in progression of such cancers as pulmonary cancer (Kren, 2006), head (Thorns, 2003), neck carcinoma (Wasenius, 2003), and breast cancer (Mellick, 2003). In addition, MMP-11 mRNA and protein were correlated with a poor clinical outcome in breast cancer (Chenard et al., 1996; Ahmad et al., 1998). Moreover, the expression of MMP-11 in prostate cancer tissues was associated with high probability of biochemical recurrence that means PSA level at >0.2 ng/ml (Escaff et al., 2010). However, there has been a lack of correlation data between survival time of patients and prostate cancer. Therefore, the aim of this study was to quantify MMP-11 expression in normal and prostatic adenocarcinoma tissues and to determine whether overexpression correlates with survival outcome and so might serve as a new prognostic marker.

Materials and Methods

Human prostatic adenocarcinoma tissue

Paraffin embedded human prostatic adenocarcinoma

*[1]Department of Anatomy, [2]Department of Pathology, Faculty of Medicine, [3]International College, Khon Kaen University, Khon Kaen, [4]College of Medicine and Public Health Ubon Ratchathani University, Ubon Ratchathani, Thailand *For correspondence: panya@kku.ac.th*

tissues (n=103) were obtained from the Pathology unit, Srinagarind Hospital, Faculty of Medicine, Khon Kaen University (Khon Kaen, Thailand) during January 2003 to December 2008. None of patients received any anticancer therapy prior to sample collection. Time of follow-up was from January 2003 to December 2012. All tissue samples were clinicopathologically assessed such characters as age, Gleason score, serum PSA, Bone metastasis, pathological tumor classification (pT), and survival outcome. Sample of normal prostate was used as negative controls and obtained from autopsied case at Forensic Medicine, Faculty of Medicine, Khon Kaen University. This study was approved by Ethic Committee for Human Research, Khon Kaen University (HE551272).

Immunohistochemical detection of MMP-11

The paraffin-embedded prostatic tissues were deparaffinized with xylene, and the rehydrated with stepwise descending concentration of ethanol. The sections were submerged into 10 mM citrate buffer (pH 6.0) and boiled for 5 min for antigen retrieval, and were the treated for 10 min in 3% (v/v) hydrogen peroxide in methanol to block endogenous hydrogen peroxide activity. Sections were incubated for 30 min with 3% normal horse serum (NHS) to block non-specific binding, then incubated with anti-MMP-11 (rabbit polyclonal, 1:500; Abcam, USA) overnight at 4˚C. NHS and normal human prostate tissue were used as internal and external negative controls, respectively. After washing, tissue sections were treated with 50% horseradish peroxidase (HRP) polymer detection (Thermo, USA). The color was developed with 3, 3'-diaminobenzidine tetrahydrochloride (DAB) as substrate, then counterstained with Mayer's hematoxylin, dehydrated, and mounted (Jamnongkan et al., 2012).

Evaluation of immunostaining sections

The staining frequency of MMP-11 was semi-quantitatively scored by 2 observers based on the percentage of positive cells as follows: 0 (0%), +1 (1-25% positive cells), +2 (26-50% positive cells), and +3 (>50% positive cells). The staining intensity was graded as follows: 0 (no staining), 1 (weak staining=light yellow), 2 (moderate staining=yellow brown), and 3 (strong staining=brown). Low and high expression scores were calculated by multiplying the frequency score with intensity score. They were categorized as three subgroups: negative (0), low (1-4), and high (5-9) (Zhao et al., 2010; Jamnongkan et al., 2012).

Statistical analysis

All statistical analyses were performed using IBM SPSS 19.0 software. Data were shown as median±SD. The survival curves were established using the Kaplan-Meier method. The correlation coefficient between MMP-11 expression and clinicopathological data of patients were analyzed by the Pearson correlation method. Univariate and multivariate analyses were considered by the Cox proportional hazards model to assess the prognosis values of MMP-11 expression. Statistical significance was set at P<0.05 (Zhao et al., 2010; Jamnongkan et al., 2012).

Results

Expression of MMP-11 in prostatic adenocarcinoma tissue

The median age of 103 patients with prostatic adenocarcinoma under study was 70±9 years (range of 42-91 years). Positive immunostaining for MMP-11 was mainly localized in the cytoplasm of stromal cells, not shown in cancer cells (Figure 1). High expression of MMP-11 was detected in 48 (46.6%) cases, and low expression was detected in 55 (53.4%) cases. More than 50% samples with a classification of pT4 and a high Gleason grading also show high staining of MMP-11, while samples with a grading of pT1 and a low Gleason grading showed low expression levels of MMP-11 (Table 1).

MMP-11 and clinicopathologic variables

High expression of MMP-11 staining correlated with Gleason score, pT, and bone metastasis (p<0.05), whereas, MMP-11 expression did not correlate with age and PSA (levels) (p>0.05) (Table 1).

MMP-11 and patient prognosis

The time of follow-up of patients was January 2003 to December 2012. The overall median of survival time was 69 months. A total of 43 of 103 cases died within the follow-up period, due to prostate cancer. Generally, patients whose prostate samples showed high levels of MMP-11 expression had significantly shorter survival (p<0.001) (Figure 2). The Kaplan-Meier analysis established that the survival rates of patients with high expression of MMP-11 levels was 41.7%, but 72.7% in patients with low expression of MMP-11 levels, after five years of follow-up (p<0.01, log rank analysis; Figure 2). For univariate analysis, MMP-11 expression, Gleason grading, pT, and bone metastasis were significantly associated with survival, whereas age and PSA levels were not associated with survival (Table 2). Multivariate analysis

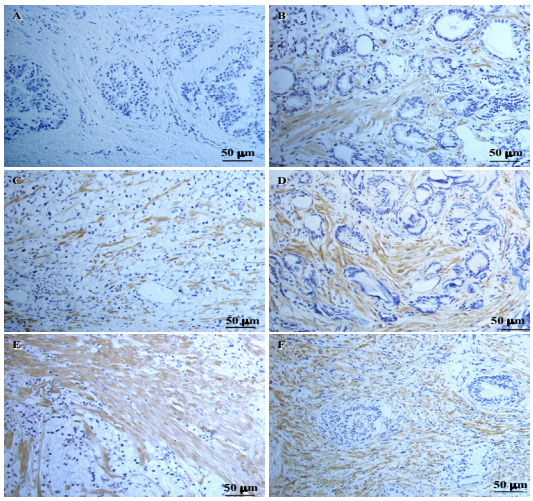

Figure 1. Immunohistochemical Staining for MMP-11 in Prostate Adenocarcinoma Tissues. Positive label was found in cytoplasm of stromal cells, and normal prostate tissue. (A) Normal prostate tissue is MMP-11 negative. The expression of MMP-11 was classified as low level (B-D) and high level (E-F) according to the staining intensity and frequency score (Original magnification x200)

Table 1. Correlation of MMP-11 Expression with Clinicopathologic Parameters in Prostatic Adenocarcinoma

Clinicopathologic parameters	Total cases		MMP11 expression (n=103) Low (n=55) No. (%)	High (n=48) No. (%)	p-value
Age (y)	≤70	52	32 (61.5)	20 (38.5)	0.960
	>70	51	23 (45.1)	28 (54.9)	
PSA (ng/ml)	≤10	21	9 (42.9)	12 (57.1)	0.282
	>10	82	46 (56.1)	36 (43.9)	
Gleason grading	2-4	13	8 (61.5)	5 (38.5)	0.044*
	5-6	26	19 (73.1)	7 (26.9)	
	7-10	64	28 (43.8)	36 (56.3)	
Pathologic Tumor stage					
	T1	25	19 (76.0)	6 (24.0)	0.007*
	T2	42	21 (50.0)	21 (50.0)	
	T3	17	9 (52.9)	8 (47.1)	
	T4	19	6 (31.6)	13 (68.4)	
Bone Metastasis	Negative	55	37 (67.3)	18 (32.7)	0.002*
	Positive	48	18 (37.5)	30 (62.5)	

* p<0.05, PSA=prostatic-specific antigen

Table 2. Univariate and Multivariate Analyses of Clinicopathologic Parameters with Prostatic Adenocarcinoma Survival

Clinicopathologic parameters	Univariate; HR(95% CI)	p-value	Multivariate; HR (95%CI)	p-value
Age	0.612 (0.332-1.127)	0.115	0.936 (0.443-1.976)	0.0861
PSA (ng/ml)	0.499 (0.210-1.186)	0.116	0.766 (0.288-2.039)	0.5930
Gleason grading	0.161 (0.490-0.526)	0.002*	0.301 (0.080-1.128)	0.0750
pT stage	0.276 (0.131-0.583)	0.001*	0.333 (0.150-0.740)	0.0070*
Bone metastasis	0.304 (0.162-0.573)	<0.001*	0.487 (0.235-1.010)	0.0530
MMP-11 expression	0.343 (0.183-0.645)	0.001*	0.448 (0.212-0.946)	0.0350*

*p<0.05, CI=confidence interval; HR=hazard ratio; PSA=prostatic-specific antigen; MMP=matrix metalloproteinase

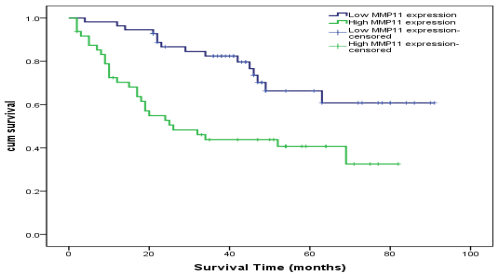

Figure 2. Kaplan-Meier curves of survival time differences between patients with low and high levels of MMP-11 expression in prostatic adenocarcinoma. P-value was determined using the log-rank test (p<0.01)

by Cox regression model revealed that the expression of MMP-11 and pT were independent prognostic factors in patients with prostatic adenocarcinoma [hazard ratio (HR)=0.448, 95% confidence interval (95%CI)=0.212-0.946 and HR=0.333, 95%CI=0.15-0.74, respectively]. However, age, PSA levels, Gleason grading, and bone metastasis were not prognostic values (Table 2).

Discussion

Matrix matelloproteinases (MMPs) are zinc-dependent endopeptidases. More than 25 MMP family members have been identified. MMPs are divided into 4 subgroups, based on characteristic domains or substrate specificities, including gelatinases, collagenases, stromelysin, and membrane type MMPs. The role of MMPs is in degradation of extracellular matrix (ECM) proteins in both physiological and pathological processes, such as gelatinases (MMP-2 and MMP-9) (Alexander and Werb, 1991; Parks and Mecham, 1998). In cancer progression, the roles of MMPs lie in the degradation of ECM components, among other roles (Egeblad and Werb, 2002; Freije et al., 2003; Hojilla et al., 2003). MMP-9 regulates cancer cells growth (Coussens et al., 2000) and associates with angiogenesis via the angiogenic switch during carcinogenesis (Bergers et al., 2000), while MMP-2 positively regulates angiogenesis (Fang et al., 2000).

MMP-11 or stromelysin-3 was first characterized from stromal cells of breast cancer (Basset et al., 1990). It was unique among the MMPs since it was shown in stromal fibroblast cells adjacent to tumor cells, rather than within tumor cells themselves (Basset et al., 1990, 1993). MMP-11 is also different from other MMPs in that it is secreted from cells in potential active form, while its inactive form is produced by furin-dependent proteolytic cleavage intracellularly (Pei and Weiss, 1995). Interestingly, MMP-11 was not able to degrade in major component of extracellular matrix when compared with other MMPs (Murphy et al., 1993; Pei et al., 1994; Manes et al., 1997). This study clearly showed that immunoreactivity of MMP-11 was present within stromal cell of prostate cancer tissue but did not occur in samples of normal prostate, which seem to involve in an important mechanism in the molecular biology of malignancy. In addition, MMP-11 protein was only shown in stromal cells while it was not present in tumor epithelial cells, similarly to the study of Basset et al. (1990). This phenomenon may indicate that MMP-11 promotes cancer progression by tumor microenvironment inducement that association with embedding in the extracellular host tissue at the time of cancer cells invasion (Masson et al., 1998; Boulay et al., 2001).

Our results reveal that MMP-11 expression is positively correlated with Gleason grading, pT, and bone metastasis. Further high expression of MMP-11 correlated significantly with advanced stage of prostate cancer (poor differentiation, pT4, and positive-bone metastasis categories). Our data thus reflects several studies of other human cancers, which showed that high expression of MMP-11 were found in human solid cancers including oral cancers (Arora, et al., 2005), desmoid tumors (Denys et al., 2004), non-small cell lung cancer (Kettunen et al., 2004), esophageal adenocarcinoma (Hourihan et al., 2003), and skin cancer (Chen et al., 2012). In addition, Sunil Kumar et al. (2013) showed that MMP-11 could play a significant role in the degradation of extracellular matrix in canine mammary tumor. Moreover, some studies demonstrated that MMP-11 protein related with cancer progression including gastric cancer (Zhao et al., 2009), oral cancer (Soni et al., 2003). Escaff et al. (2010) showed that prostate cancer with high MMP-11 expression was significantly associated with higher probability of biochemical recurrence of the disease.

Subsequently, we found that patients with low-level MMP-11 expression had significantly longer survival

time compared with high levels of MMP-11. Accordingly, overexpression of MMP-11 gene is related with increased aggressiveness of cancers and a poor clinical outcome (Boulay et al., 2001; Andarawewa et al., 2003). Yan et al. (2011) demonstrated that serum levels of MMP-11 in patients with gastric adenocarcinoma correlated with those advanced stages. Clinical studies have shown that high expression of MMP-11 is correlated with a lower survival time among patients with breast, non-small cell lung cancer, and colon cancer (Têtu et al., 2006; Cheng et al., 2010). Moreover, Boulay et al. (2001) stated that the cellular function of MMP-11 is closely related to decreasing cancer cells death via apoptosis and necrosis during malignancy. Inactivation of MMP-11 function may represent a new strategy for preventing cancer cell invasion and metastasis.

In conclusions, MMP-11 expression in prostatic adenocarcinoma correlated positively with Gleason grading, pT, and bone metastasis and is closely associated with survival time. Therefore, high levels of MMP-11 expression may potentially be used to predict decreased survival in prostatic adenocarcinoma and be used for new prognostic marker in combination with currently available markers. Further studies are required to investigate whether serum MMP-11 levels correlate with survival outcome in patient with prostatic adenocarcinoma to determine whether the molecule should be developed as a prognostic marker.

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References

Ahmad A, Hanby A, Dublin E, et al (1998). Stromelysin 3: an independent prognostic factor for relapse-free survival in node-positive breast cancer and demonstration of novel breast carcinoma cell expression. *Am J Pathol*, **152**, 721-8.

Alexander CM, Werb Z (1991). Extracellular matrix degradation. In 'Cell Biology of Extracellular Matrix', Ed. Hay ED. Plenum Press, New York, 255-302.

Andarawewa KL, Boulay A, Masson R, et al (2003). Dual stromelysin-3 function during natural mouse mammary tumor virus-ras tumor progression. *Cancer Res*, **63**, 5844-9.

Arora S, Kaur J, Sharma C, et al (2005). Stromelysin 3, Ets-1, and vascular endothelial growth factor expression in oral precancerous and cancerous lesions: correlation withmicrovessel density, progression, and prognosis. *Clin Cancer Res*, **11**, 2272-84.

Basset P, Bellocq JP, Wolf C, et al (1990). A novel Metalloproteinase gene specifically expressed in stromal cells of breast carcinomas. *Nature*, **348**, 699-704.

Basset P, Wolf C, Chambon P (1993). Expression of the stromelysin-3 gene in fibroblastic cells of invasive carcinomas of the breast and other human tissues: a review. *Breast Cancer Res Treat*, **24**, 185-93.

Bergers G, Brekken R, McMahon G, et al (2000). Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. *Nature Cell Biol*, **2**, 737-44.

Boulay A, Masson R, Chenard MP, et al (2001). High cancer cell death in syngeneic tumors developed in host mice deficient for the stromelysin-3 matrix metalloproteinase. *Cancer Res*, **61**, 2189-93.

Chen YT, Chen WT, Huang WT, et al (2012). Expression of MMP-2, MMP-9 and MMP-11 in dermatofibroma and dermatofibrosarcoma protuberans. *Kaohsiung J Med Sci*, Oct; **28**,545-9.

Chenard MP, O'Siorain L, Shering S, et al (1996). High levels of stromelysin-3 correlate with poor prognosis in patients with breast carcinoma. *Int J Cancer*, **69**, 448-51.

Cheng CW, Yu JC, Wang HW, et al (2010). The clinical implications of MMP-11 and CK-20 expression in human breast cancer. *Clin Chim Acta*, **411**, 234-41.

Coussens LM, Tinkle CL, Hanahan D, et al (2000). MMP-9 supplied by bone marrow-derived cells contributes to skin carcinogenesis. *Cell*, **103**, 481-90.

Denys H, De Wever O, Nusgens B, et al (2004). Invasion and MMP expression profile in desmoid tumours. *Br J Cancer*, **90**, 1443-9.

Egeblad M, Werb Z, (2002). New functions for the matrix metalloproteinases in cancer progression. *Nat Rev Cancer*, **2**, 161-74.

Escaff s, Fernandez JM, Gonzalez LO, et al (2000). Study of matrix metalloproteinases and their inhibitors in prostate cancer. *Bri J Cancer*, **102**, 922-9.

Fang J, Shing Y, Wiederschain D, et al (2000). Matrix metalloproteinase-2 is required for the switch to the angiogenic phenotype in a tumor model. *Proc Natl Acad Sci USA*, **97**, 3884-9.

Freije JM, Baibin M, Pendas AM, et al (2003). Matrix metalloproteinases and tumor progression. *Adv Exp Med Biol*, **532**, 91-107.

Jamnongkan W, Techasen A, Thanan R, et al (2013). Oxidized alpha-1 antitrypsin as a predictive risk marker of opisthorchiasis-associated cholangiocarcinoma. *Tumor Biol*, **34**,695-704.

Hojilla CV, Mohammed FF, Khokha R, (2003). Matrix metalloproteinases and their tissue inhibitors direct cell fate during cancer development. *Br J Cancer*, **89**, 1817-21.

Hourihan RN, O'Sullivan GC, Morgan JG, (2003). Transcriptional gene expression profiles of oesophageal adenocarcinoma and normal oesophageal tissues. *Anticancer Res*, **23**, 161-5.

Jemal A, Bray F, Center MM, et al (2011). Global Cancer Statistics. *Ca Cancer J Clin*, **61**. 69-90.

Kettunen E, Anttila S, Seppänen JK, et al (2004). Differentially expressed genes in nonsmall cell lung cancer: expression profiling of cancer-related genes in squamous cell lung cancer. *Cancer Genet Cytogenet*, **149**, 98-106.

Khuhaprema T, Srivatanakul P, Attasara P, et al (2010). Cancer in Thailand Volume V 2001-2003. *National Cancer Institute*, **5**, 7-62.

Kren L, Goncharuk VN, Krenova Z, et al (2006). Expression of matrix metalloproteinases 3, 10 and 11 (stromelysins 1, 2 and 3) and matrix metalloproteinase 7 (matrilysin) by cancer cells in non-small cell lung neoplasms. Clinicopathologic studies. *Cesk Patol*, **42**, 16-9.

Madu Co, Lu Y (2010). Novel diagnostic biomarkers for prostate cancer. *J Cancer*, **1**, 150-77.

Manes S, Mira E, Barbacid MD, et al (1997). Identification of insulin-like growth factor-binding protein-1 as a potential physiological substrate for human stromelysin-3. *J Biol Chem*, **272**, 25706-12.

Masson R, Lefebvre O, Noël A, et al (1998). In vivo evidence that the stromelysin-3 metalloproteinase contributes in a paracrine manner to epithelial cell malignancy. *J Cell Biol*. **140**, 1535-41

Mellick AS, Blackmore D, Weinstein SR, at al (2003). An assessment of MMP and TIMP gene expression in cell lines and stroma-tumour differences in microdissected breast cancer biopsies. *Tumour Biol*, **24**, 258-70.

Murphy G, Segain JP, O'Shea M, et al (1993). The 28-kDa N-terminal domain of mouse stromelysin-3- has the general properties of a weak metalloproteinase. *J Biol Chem*, **268**, 15435-41.

Parks WC, Mecham RP (1998). Matrix metalloproteinases. New York: Academic Press.

Pei D, Majmudar G, Weiss SJ (1994). Hydrolytic inactivation of a breast carcinoma cell-derived serpin by human stromelysin-3. *J Biol Chem*, **269**, 25849-55.

Pei D, Weiss SJ (1995). Furin-dependent intracellular activation of the human stromelysin-3 zymogen. *Nature*, **375**, 244-7.

Soni S, Mathur M, Shukla NK, et al (2003). Stromelysin-3 expression is an early event in human oral tumorigenesis. *Int J Cancer*, **107**, 309-16.

Sunil Kumar BV, Kumar KA, Padmanath K, et al (2013). Heterologous expression and functional characterization of matrix metalloproteinase-11 from canine mammary tumor. *Anim Biotechnol*, **24**, 31-43.

Têtu B, Trudel D, Wang CS, (2006). Proteases by reactive stromal cells in cancer: an attractive therapeutic target. *Bull Cancer*, **93**, 944-8.

Thorns V, Walter GF, Thorns C, (2003). Expression of MMP-2, MMP-7, MMP-9, MMP-10 and MMP-11 in human astrocytic and oligodendroglial gliomas. *Anticancer Res*, **23**, 3937-44.

Tuamsuk P, Nonsrijun N, Pachirat K, et al (2011). Prostate cancer in Srinagarind Hospital. *Srinagarind Med J*, **26**, 373-6.

Wasenius VM, Hemmer S, Kettunen E, et al (2003). Hepatocyte growth factor receptor, matrix metalloproteinase-11, tissue inhibitor of metalloproteinase-1, and fibronectin are up-regulated in papillary thyroid carcinoma: a cDNA and tissue microarray study. *Clin Cancer Res*, **9**, 68-75.

Yan D, Dai H, Liu JW, (2011). Serum levels of MMP-11 correlate with clinical outcome in Chinese patients with advanced gastric adenocarcinoma. *BMC Cancer*, **11**, 151.

Zhao ZS, Chu YQ, Ye ZY, et al (2010). Overexpression of matrix metalloproteinase 11 in human gastric carcinoma and its clinicopathologic significance. *Hum Pathol*, **41**, 686-96.