RESEARCH ARTICLE

Correlation between Microvascular Density and Matrix Metalloproteinase 11 Expression in Prostate Cancer Tissues: a Preliminary Study in Thailand

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Abstract

Background: Prostate cancer is a major concern of public health. Microvascular density (MVD) is one of the prognostic markers for various solid cancers. Matrix metalloproteinase 11 (MMP11) plays an important role in angiogenesis and changes in its expression level are known to be associated with tumor progression and clinical outcome. Aim: To investigate the relationship between MVD and MMP11 expression in prostatic adenocarcinoma tissues. Materials and Methods: The expression levels of MMP11 and MVD were analyzed immunohistochemically for 50 specimens of prostatic adenocarcinoma. Results: MMP11 was mainly expressed in stromal cells but rarely seen in epithelial cells. Mean MVD was 36/mm^2, and it was correlated significantly only with bone metastases. MVD was also significantly correlated with MMP11 expression (r=0.29, p=0.044). Conclusions: MMP11 may alter the stromal microenvironment of prostate cancer to stimulate tumor angiogenesis.

Keywords: Matrix metalloproteinase 11 - prostatic adenocarcinoma - Microvascular Density angiogenesis

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Introduction

Prostate cancer is a major concern of public health especially in Western countries. In Asia, prevalence of prostate cancer is low at the moment (Globocan, 2012), although it is increasing yearly associated with the increase of elderly population. In Thailand, among the leading cancer of men, prostate cancer is ranked the fourth common cancer (ASR 7.7) (Khuhaprema et al., 2013).

Angiogenesis is important not only for the solid tumor growth but also for providing a microenvironment for tumor cells to penetrate into circulation (Gimbrone et al., 1972; Folkman, 1992; Weidner et al., 1993). Microvascular density (MVD) has been used as the marker for angiogenesis and can be detected using immunohistochemical technique on tissue sections (Weidner et al., 1993). Several studies showed MVD correlated with the clinical outcome of renal (Iakovlev et al., 2012) and colorectal cancer (Yodavudh et al., 2008). In prostate cancer, MVD correlated with the stage (Kaygusuz et al., 2007) and with the progression after radical prostatectomy (Taille 2000). In particular, MVD is considered as the significant predictor of PSA recurrence after radical prostatectomy (Erbersdobler et al., 2010). Angiogenesis is a complicated multistep process, and its basic elements are consisted of endothelial cell, extracellular matrix, and soluble factors (van Moorselaar and Voest, 2002).

The matrix metalloproteinases (MMPs) play important roles in the degradation of both vascular basement membrane and extracellular matrix (ECM) in association with angiogenesis (Rundhaug 2005; Deryugina and Quigley, 2015). Among MMPs, MMP11, also known as stromelysin-3, was first identified from stromal cells of breast cancer (Basset et al., 1990). MMP11 was expressed in fibroblast cells, but was not present in cancer cells (Min et al., 2013; Nonsrijun et al., 2013; Roscilli et al., 2014). Compared with other MMPs, MMP11 has relatively weak proteolytic potential (Murphy et al., 1993; Pei et al., 1994; Manes et al., 1997). In prostate cancer, high expression of MMP11 or -13, or a cluster thereof, was significantly associated with higher probability of biochemical recurrence (Escaff et al., 2010). We also revealed previously that overexpression of MMP11 in prostate cancer tissue is associated with the survival outcome (Nonsrijun et al., 2013). In this study, we investigated the correlation between MVD and MMP11 expression in prostate cancer tissues.

Materials and Methods

Human prostatic adenocarcinoma tissue

Formalin-fixed and paraffin-embedded human prostatic adenocarcinoma tissues (n=50) collected between January 2003 to December 2008 were obtained from the Pathology unit, Srinagarind Hospital, Faculty of

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Medicine, Khon Kaen University (Khon Kaen, Thailand). Clinicopathological data of all the patients such as age, Gleason's score, pathological tumor classification (pT), serum PSA and bone metastasis were obtained from the clinical records. This study was approved by the Ethics Committee for Human Research, Khon Kaen University (HE541202).

Immunohistochemistry

Microvessels in tumor tissue sections were visualized immunohistochemically using anti-CD31 and anti-MMP11 antibodies. After deparaffinization and rehydration, sections were boiled for 5 min to retrieve antigens. The sections were incubated in 3% (v/v) hydrogen peroxide in methanol for 10 min to block endogenous peroxidase activity, washed, and then they were incubated in 3% normal horse serum (NHS) for 30 min to block non-specific binding. The sections were then incubated with anti-MMP11 (rabbit polyclonal, 1:500; Abcam, USA) or anti-CD31 antibody (1:100, Diagnostic Biosystems, USA) at 4˚C overnight. Tonsil tissue section was used as a positive control. After washing, sections were treated with 50% horseradish peroxidase (HRP) polymer detection (Thermo, USA). The color was developed by 3, 3'-diaminobenzidine tetrahydrochloride (DAB), counterstained with Mayer's hematoxylin, dehydrated, and mounted (Nonsrijun et al., 2013).

Evaluation

The staining frequency of MMP11 was semi-quantitatively scored by two observers based on the percentage of positive cells as follows: +1 (0-25% positive cells), +2 (26-50% positive cells), and +3 (>50% positive cells) (Nonsrijun et al., 2013). Microvessel density (MVD) was quantified according to the methods established by Weidner et al. (1993). Firstly, all section slides were scanned by the auto-microscope equipped with the camera (10-200 magnifications). Then, the area of the most heavy distribution of microvessels (called "hot spot") was identified and captured by the Aperio Image scope program version 12.1 (Leica Biosystems) (×40 power field magnification). Microvessels in each field were counted at high-power magnification (×200). Brown-colored endothelial cells or endothelial cluster clearly separated from the adjacent microvessel was counted as the single vessel. Endothelial cell fragments or a vessel lumen-like structure were not counted as microvessels. Finally, the mean number of microvessels per mm2 was calculated and expressed as the MVD score as follows: +1 (1-30 MVD), +2 (31-60 MVD), and +3 (≥61 MVD).

Statistical analysis

All statistical analyses were performed using IBM SPSS 19.0 software. The relationship between MMP11 expression or MVD and the clinicopathological data was analyzed using the Pearson correlation method. Statistical significance was set at $P < 0.05$.

Results

Clinical characteristics of the patients

The median-age of 50 patients with prostate cancer was 73-year-old with the range of 56 to 91 years. The median-serum PSA level was 27.33 ng/ml with the range

Table 1. Characteristics of the 50 Patients with Prostate Cancer

Median age in years		73 (56-91)
Median PSA in ng/ml		27.33 (3-100)
Age	≤70	17 (34%)
	>70	33 (66%)
PSA	≤10	12 (24%)
	>10	38 (76%)
Pathological tumor stage	pT2	27 (54%)
	pT3	12 (24%)
	pT4	11 (22%)
Gleason score	2-4	3 (6%)
	5-7	28 (56%)
	8-10	19 (38%)
Bone metastasis	Negative	20 (40%)
	Positive	30 (60%)

Table 2. Association between MVD and MMP11 Expression with Cliniopathologic Parametes (n=50) (*p<0.05)

Variable	No.	Microvascular density (in 1 mm^2)			P-value	MMP11 expression			P-value
		1(≤30)	2(31-60)	3(≥61)		1(<25%)	2(25-50%)	3(≥51%)	
Age					0.4				0.01*
≤70	17	9	7	1		9	5	3	
>70	33	14	15	4		5	17	11	
PSA					0.4				0.66
≤10	12	6	6	0		4	3	5	
>10	38	17	16	5		10	19	9	
Pathological tumor stage					0.1				0.9
pT2	27	13	13	1		9	8	10	
pT3	12	6	6	0		4	6	2	
pT4	11	4	3	4		1	8	2	
Gleason score					0.9				0.33
2-4	3	1	2	0		0	0	3	
5-7	28	13	12	3		9	13	6	
8-10	19	9	8	2		5	9	5	
Bone metastasis					0.035*				0.05*
Negative	20	13	6	1		9	7	4	
Positive	30	10	16	4		5	15	10	

of 3 to 100 ng/ml. There were 33 patients with the age of >70-year-old, and 38 patients had high serum PSA (>10 ng/ml). Just over a half (27cases) of the patients were reported to be pT2, and 28 cases were Gleason score 5-7 scores. Bone metastasis was seen in 30 cases (Table 1).

MMP11 expression

In prostate cancer tissues, MMP11 was expressed in stromal cells but not in the cancer cells (Figure 1). The degree of MMP11 expression was semiquantified by scoring and its correlation to clinicopathological features were analyzed (Table 2). As can be seen in the Table 2, 22 (44%) out of 50 tissue samples showed +2 MMP11 expression, whereas 14 (28%) each showed +1 and +3 MMP11 expressions. Among various clinicopathological features, only the age and bone metastasis were significantly correlated with the degree of MMP11 expression (Table 2).

Microvascular density (MVD)

Table 3. Distribution of MMP11 Expression and Microvascular Density in 1 mm²

MMP11 expression	No.	Microvascular density (in 1 mm²)	
		Range	Mean(SD)
1	14	12-47	28±2.8
2	22	13-69	38±3.5
3	14	12-90	41±5.8

Mean microvascular density (MVD) was 36/mm2, and its intensity distribution among the patients was as follows: 23 cases (46%) were +1, 22 cases (44%) were +2, and 5 cases (10%) were +3. In terms of the correlation between the degree of MVD and clinicopathological features, MVD was significantly correlated with the pT stage (p=0.014) and also with bone metastasis (p=0.035), but not correlated with any other features (Table 2).

Correlation between MMP11 expression and microvascular density (MVD)

When correlation between MVD and MMP11 expressions was examined, statistically significant correlation (r=0.29, p=0.044) was observed. There were 14 cases with +1 MMP11 expression that exhibited the mean of MVD at 28±2.8/mm2. In contrast, those who had +2 and +3 MMP11 expressions showed the mean of MVD at 38±3.5/mm2 and 41±5.8/mm2, respectively (Table 3). Although the mean MVD of both +2 and +3 MMP11 expression groups were higher than that of +1 MMP11 expression group, the difference between those groups was statistically not significant (p=0.1).

Discussion

Generally, a solid tumor is able to grow up to a diameter of 2-3 mm receiving nutrients by diffusion from the pre-existing vasculature. Tumors need new capillaries for further expansion and survival (Folkman et al., 1989; Folkman 1990, Fidler and Ellis, 1994). Angiogenesis is the

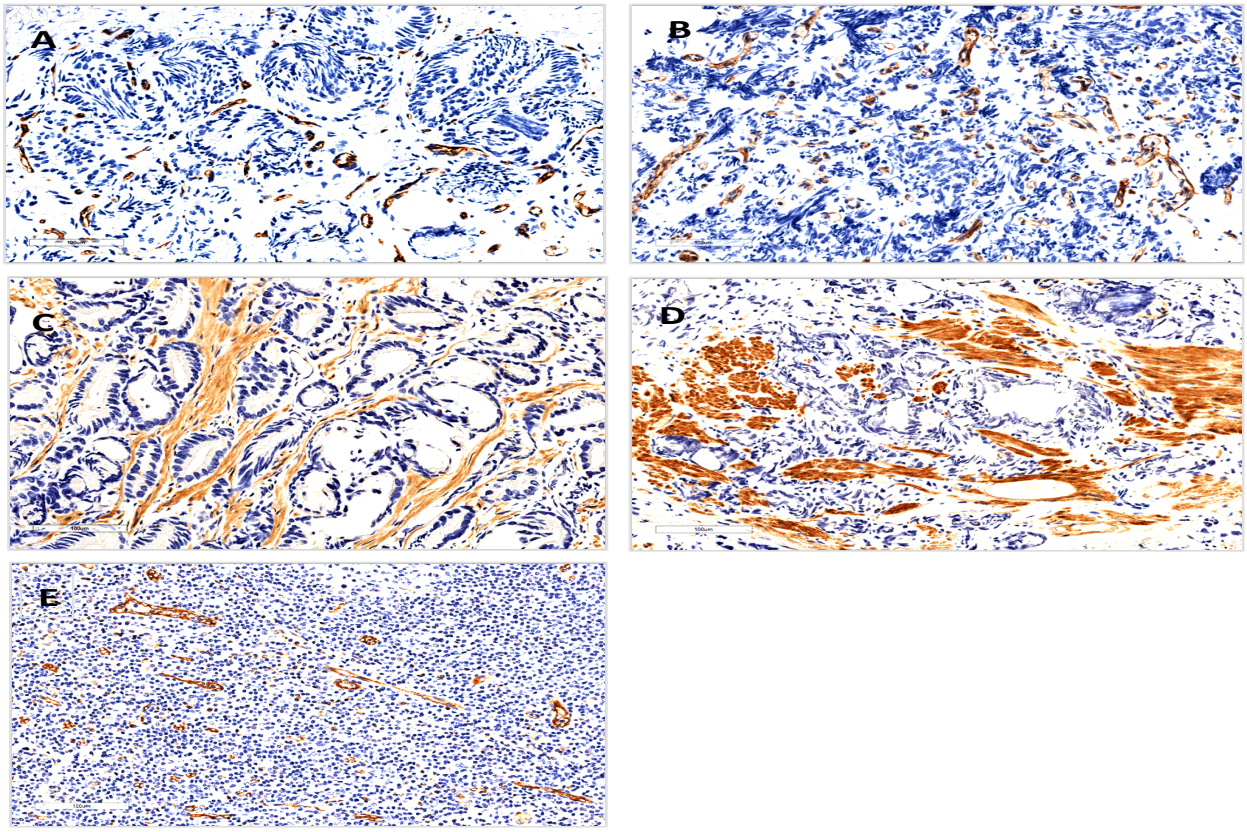

Figure 1. "Hot Spot" for Microvascular Density as Demonstrated by CD31 Immunostaining (A,B), MMP11 Expression in Stromal Cells (C,D), Tonsil tissue for positive control in microvascular (E) (Original magnification x200).

process of new blood vessel formation from the existing vessels, and it is a critical process for cancer growth and metastasis (Folkman, 1971). For progression of prostate cancer, the angiogenesis is a critical step involved in development from early to advanced stage (Choy and Rafii, 2001; Nicholson and Theodorescu, 2004).

Microvessel density (MVD) is the quantification of new capillaries via histological observation (Montironi et al., 1996). The evaluation of MVD in prostate cancer revealed a significant correlation of MVD with cancer progression and high-Gleason grade (Bono et al., 2002; Sinha et al., 2004; Concato et al., 2009). In the present study, MVD was significantly correlated only with bone metastasis, but not with any other parameters. Bone metastasis, especially to the femur and pelvis, is the most common feature of prostate cancer (Tuamsuk et al., 2010). Possibly, cancer cells spread through nearest blood and lymphatic vessels such as pelvic venous plexus.

Matrix matelloproteinases (MMPs) are zinc-dependent enzymes that are able to degrade in components of an extracellular matrix (ECM). MMPs-family members are composed of gelatinases, collagenases, stromelysin, and membrane-bound type MMPs depending on their structure and substrate specificities (Nagase and Woesser, 1999). Minimally, the domain structure comprises of a signal peptide, a pro-domain, and a catalytic domain with a zinc-binding site (Rundhaug 2005).

MMP11, also known as stromelysin-3, was first identified in the stromal cells, but not in the tumor cells, of breast cancer (Basset et al., 1990, 1993). In prostate tissues, MMP11 was only localized in prostate cancer tissue, but not in the normal tissue (Nonsrijun et al., 2013; Roscilli et al., 2014). MMP11 expression is known to associate with cancer progressions in various cancers such as gastric (Zhao et al., 2009), oral (Lin et al., 2014), and breast (Min et al., 2013) cancers. For prostate adenocarcinoma, MMP11 expression was significantly related to the tumor recurrence monitored by prostate specific-antibody (PSA) level (Escaff et al., 2010). In our previous study, we found that MMP11 expression was positively correlated to Gleason's grading, pT, and bone metastasis, and its overexpression was associated with the survival outcome (Nonsrijun et al., 2013). In the present study, MMP11 expression was correlated with the patients' age and bone metastasis. The discrepancy between our previous and present study might be due to the small sample size of this study. More extensive study including the samples of the previous study is necessary.

MMPs play important roles in angiogenesis not only via degradation of basement membrane and ECM components but also via stimulate the release of pro-angiogenic factors such as bFGF, VEGF, and TGFb (Rundhaug, 2005). Since MMP11 expression is restricted to the stromal fibroblast cells in prostate cancer tissues, MMP11 possibly modify the stromal microenvironment suitable for the prostate cancer cells for their growth, survival, and metastasis (Condon, 2005; Kaygusuz et al., 2007, Kessenbrock et al., 2010). In the present study, MMP11 expression was only identified in fibroblast cells and related to bone metastasis; it also correlated to MVD in prostate cancer tissue. These results suggest that tumor-associated extracellular MMPs inducer functionally mediates tumor-stroma interactions and directly contributes to tumor angiogenesis and growth via stimulation of VEGF and MMP expression (Tang et al., 2005).

In conclusion, This study indicated that MMP11 expression level was significantly correlated with the degree of MVD, suggesting the important role of MMP11 to alter the stromal microenvironment suitable for prostate cancer via angiogenesis. Further studies are required to investigate the role of MMP11 in angiogenesis in order to develop anti-angiogenesis medicine for the management of prostate cancer..

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