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MMP26: A Potential Biomarker for Prostate Cancer*

Teng CHENG (程 腾), Fei LI (李 飞), Rui WEI (魏 睿), Meng-qin LV (吕梦琴), Yin ZHOU (周 颖), Yun DAI (代 芸), Yuan YUAN (袁 圆), Gui-ying JIANG (蒋桂英), Ding MA (马 丁), Qing-lei GAO (高庆蕾)#
Cancer Biology Research Center (Key Laboratory of the Ministry of Education), Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

Summary: The application of prostate-specific antigen (PSA) in the screening and diagnosis of prostate cancer (PCa) has improved the clinical management of PCa patients. However, the PSA assay has been faced with criticism due to its potential association with over-diagnosis and subsequent overtreatment of indolent patients. Matrix metalloproteinase-26 (MMP26) is a member of matrix metalloproteinases (MMPs) and has been reported to be highly expressed in many cancers. This investigation evaluated the potential of serum MMP26 as a biomarker for PCa. The level of serum MMP26 was measured by enzyme-linked immunosorbent assay (ELISA) in 160 subjects including PCa group (n=80), benign prostatic hyperplasia (BPH) group (n=40) and control group (n=40). Furthermore, we evaluated the expression of MMP26 in tissues by immunohistochemistry. The results showed the serum MMP26 levels were significantly higher in PCa group than in BPH group and control group. Similarly, the MMP26 protein was positive in PCa tissues and negative in BPH tissues and control tissues. In conclusion, these results suggested MMP26 could be used as a potential serum biomarker in the diagnosis of PCa.
Key words: prostate cancer; matrix metalloproteinase-26; serum biomarker

Prostate cancer (PCa) is the most frequent type of malignancy and one of the leading causes of death due to cancer in male[1]. With the introduction of serum prostate-specific antigen (PSA) test in the late 1980s, the PSA test has been widely used for screening and monitoring of PCa. However, in patients with PSA values between 3–10 ng/mL, the PSA test has a low specificity for PCa, resulting in a highly negative biopsy rate of 60%–75%[2, 3]. The elevated PSA levels can be caused by a number of benign conditions [such as benign prostatic hyperplasia (BPH) and prostatitis], in addition to PCa, resulting in low specificity[4]. Although PSA-based screening reduces PCa mortality by 20%, it has a high risk of diagnosing clinically insignificant PCa that would not have been diagnosed in the patient's lifetime in the absence of screening[5, 6].

The early differential diagnosis between BPH and PCa is essential, because both the outcome and the treatment of these two diseases are distinct. Currently, the prognosis of PCa is evaluated in terms of age, the elevated levels of PSA, and a prostatic digital rectal examination (DRE) often followed by prostate biopsy, none of which can distinguish between BPH and PCa[7]. Therefore, there is an urgent need for novel biomarkers that can effectively distinguish between patients with BPH or PCa.

Recently, several biomarkers have been correlated with aggressive phenotypes in matrix metalloproteinases (MMPs) that comprise a family of structurally and functionally related zinc-dependent enzymes. MMP26 expression is tightly controlled by transcription levels, cellular localization, zymogen activation, and endogenous inhibition[8]. MMPs directly modulate a wide variety of physiologic processes such as embryo implantation, bone remodeling, cell proliferation, migration, apoptosis, and nerve development through cleavage of bioactive molecules[9]. MMP26, also called endometase or matrilysin-2, is a partially characterized proteinase, containing 261 amino acids[10]. Structurally, it shares features with other MMPs such as a signal peptide, a propeptide domain with unique cysteine switch, and a catalytic domain[11]. MMP26 has been reported to be highly expressed in several cancers[12]. In this study, we measured serum MMP26 levels in PCa, BPH and healthy people.

1 MATERIALS AND METHODS

1.1 Serum Sampling Collection

Between December 2014 and March 2016, 80 blood samples from PCa, 40 samples from healthy males and 40 samples from BPH were obtained from the clinical laboratory of Tongji Hospital. Blood samples were centrifuged at 3000 r/min at 4°C for 10 min and the serum supernatant was stored at –80°C until further processing. All samples were collected from patients within a week before surgery. The clinical data of patients were recorded, including age, Gleason score,

Teng CHENG, E-mail: 364509773@qq.com
#Corresponding author, E-mail: qingleigao@hotmail.com
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histopathological findings, PSA levels, positive lymph nodes and medication history. Ethical approval was obtained from the ethical management committee of Tongji Hospital. All participants provided written informed consent before sampling in our study.

1.2 Tissues

Slides were cut from paraffin blocks of patients who underwent prostatectomy at the Pathology Department of Tongji Hospital (China). A prostate cancer tissue microarray TC0204 (Auragene, China) contains prostate tissues, BPH tissues, normal adjacent tissues and normal tissues as controls. All these tissues were divided into three groups: PCa group, BPH group, and control group. The characteristics including TNM, Gleason score, clinical stage and pathology grade are summarized in table 1.

Table 1 Clinicopathological characteristics of tissue samples

Characteristics	No. (%) of total population
Age, years	
Median	54
Range	25–88
Normal	20
Hyperplasia	30
PCa	60
Clinical stage	
T1	1
T2	23
T3	17
T4	19
Gleason score	
⩽6	14
7	27
⩾8	19

1.3 Enzyme-linked Immunosorbent Assay

The level of serum MMP26 was measured by a human MMP26 enzyme-linked immunosorbent assay (ELISA) kit (SEB256HU, Cloud-Clone Corp, USA). The ELISA was performed as follows. 100 μL standards, blank samples and serum samples (diluted 1:5) were added to the appropriate wells and covered with the plate sealer. The assay was conducted using ELISA kits according to the manufacturer's instructions. Absorbance at 450 nm (A_{450}) was determined for each well using an automated microplate reader (SpectraMax 190; Molecular Devices, USA). The concentration of MMP26 was calculated based on the standard curve generated.

1.4 Immunohistochemistry

Immunohistochemistry (IHC) was used to detect MMP26 protein in the tissues. Briefly, sections were deparaffinized and rehydrated with xylene and a series of grades of alcohol. Epitopes were retrieved by heating in a microwave oven with 10 mmol/L citrate buffer (pH 6.0) for 10 min at 100°C, followed by cooling down at room temperature. Endogenous peroxidase activity was inhibited by 3% hydrogen peroxide for 30 min at 37°C. Unspecific binding was blocked with 10% goat serum for 30 min at 37°C. Then, the slides were incubated with primary antibody (MMP26, 1:100, Proteintech, China) overnight at 4°C, followed by incubation with horseradish peroxidase-linked secondary antibody, and finally developed using a DAB kit (BD Bioscience, USA) for optimal staining intensity. IHC scoring was evaluated according to staining intensity and positively stained areas by three independent individuals, as previously described[13].

1.5 Statistical Analysis

Statistical analyses were performed using Prism 5 software (GraphPad) and SPSS 13.0 software. Two-sides P values of <0.05 were considered significant.

2 RESULTS

2.1 Characteristics of Patients

A total of 160 cases of sera were collected in this study, including 40 cases of normal males, 40 cases of BPH and 80 cases of PCa. The median age of these people was 68 years old (ranging from 55–82 years). In PCa group, serum PSA was under 10 ng/mL (3.75%) in 3 patients, 10 to 20 ng/mL (15%) in 12 patients and above 20 ng/mL (81.25%) in 65 patients respectively. Other clinical details are listed in table 2.

Table 2 Clinicopathological characteristics of serum samples

Parameters	Median (range) or *n* (%)
Age, *n* (years)	160 (55–82)
Normal	40 (25)
Hyperplasia	40 (25)
PCa	80 (50)
Serum PSA (ng/mL)	
<10	3
10–19.9	12
⩾20	65
Clinical stage	
T1	8
T2	25
T3	27
T4	20
Metastasis	
Negative	34
Positive	46
Gleason score	
⩽6	6
7	21
⩾8	53

2.2 Expression of Serum MMP26 in Each Group

Univariate analysis of the ELISA results demonstrated that the level of sera MMP26 was significantly higher in PCa group than in healthy control group and BPH group (both P<0.0001, fig. 1). However, there was no significant correlation of MMP26 with PSA, Gleason scores, prostate volume, clinical and pathologic T-stage, and metastasis.

2.3 Up-regulation of MMP26 in PCa Tissues

The expression of MMP26 in prostate tissues was detected by IHC in control group (n=20), BPH group (n=30), and PCa group (n=60). MMP26 was positively

stained in PCa group, and it was negative in control group and BPH group (fig. 2A). The IHC scores for MMP26 in PCa group were significantly higher than in control group and BPH group (P<0.0001) (fig. 2B).

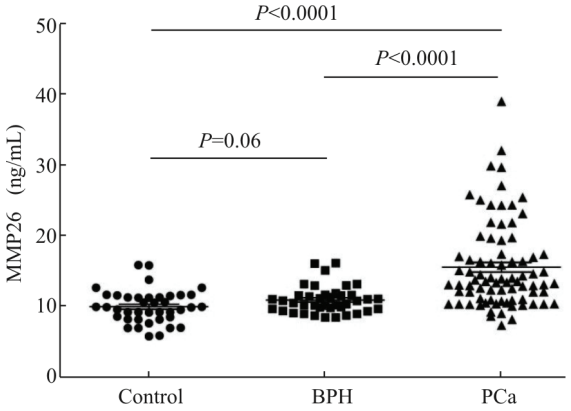

Fig. 1 The serum MMP26 concentration (ng/mL) in PCa group, BPH group and control group
MMP26 was higher in PCa group than in BPH group and control group (both P<0.0001).

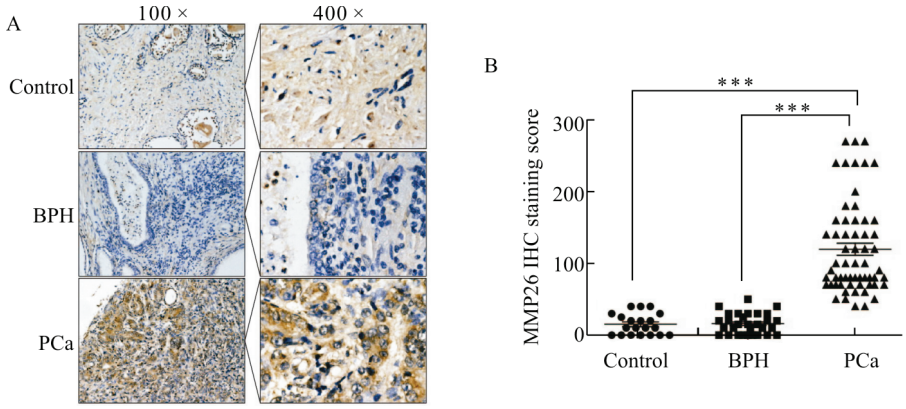

Fig. 2 The expression of MMP26 in tissues detected by IHC staining
A: MMP26 was positively stained in cancer tissues, but negatively in BPH and control tissues; B: Scoring of MMP26 in the above IHC stained tissues; $^{***}P$<0.0001

In the past three decades, the use of PSA as a routine examination has increased the proportion of patients with early tumor stage at the time of diagnosis and reduced PCa mortality[19, 20]. However, PSA-based screening is also associated with overtreatment, especially unnecessary biopsies. In fact, PSA was also elevated in the serum of patients with BPH[21, 22]. Thus, PSA is not the ideal biomarker for PCa detection and management. Thus, it is meaningful to discover new serum biomarker. The present study screened 80 PCa sera from PCa patients and tested sera from two control groups, including 60 BPH patients and 60 normal human individuals. The ELISA data showed that the level of serum MMP26 in PCa patients was significantly higher than that in BPH patients and normal human individuals. In addition, there was no statistically significant difference between BPH group and normal group. These results suggested that MMP26 could be used as a potential serum biomarker in PCa detection. Furthermore, we detected the MMP26 protein in tissue specimens. The IHC results showed that MMP26 was positively stained in PCa specimens and negatively in BPH and control specimens.

This study was designed as a tentative investigation and thus included a limited number of samples. Since all subjects only underwent a general health check, this could contribute to a potential bias for the serum MMP26 expression. We will verify it in a larger sample pool in future work.

In summary, our study showed the up-regulation of MMP26 expression in PCa patients, indicating that the serum MMP26 may be used as a novel diagnostic biomarker for identifying PCa patients from BPH patients and normal human.

3 DISCUSSION

The key finding of this study is that MMP26 acts as a potent biomarker in PCa screening. We found that MMP26 was upregulated in serum of PCa group as compared with control group and BPH group. Moreover, we observed that MMP26 was also positively stained in PCa tissue and negative in control and BPH tissues. Taken together, our data demonstrate that MMP26 is upregulated in serum and tissue of PCa and suggest MMP26 to be a potential biomarker in PCa.

In previous studies, elevated expression of MMP26 has been found in multiple carcinomas such as colorectal cancer, lung cancer, chondrosarcoma and breast cancer[14–17]. Recently, Khamis*et al*[18] reported a pro-apoptotic role of MMP26 in invasion and progression of PCa. This study verifies the level of MMP26 in serum and tissue samples among PCa patients, BPH patients and healthy people.

Conflict of Interest Statement

The authors confirm that this article content has no conflict of interest.

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