RESEARCH ARTICLE

Preoperatıve Levels of Matrıx Metalloproteinase-7 and -9 and Tissue Inhibitor of Matrix Metalloproteinase-1 Relation to Pathologic Parameters in Bladder Carcinoma Patients

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Abstract

Our aim was to test the hypothesis that preoperative serum levels of matrix metalloproteinase-7 (MMP-7) and -9 (MMP-9) and tissue inhibitor of matrix metalloproteinase (TIMP-1) levels correlate with pathological features. Serum levels of MMP-7, and MMP-9 and TIMP-1 were determined in 90 bladder cancer patients and 40 healthy controls using an enzyme linked immunosorbent assay. Preoperative serum MMP-7 and MMP-9 levels were significantly higher in cancer patients than control groups (p<0.001). In contast, serum TIMP-1 levels were lower (p<0.001). Alteration in MMP-7, and MMP-9, and TIMP-1 production may contribute to tumor angiogenesis and be associated with clinic-pathological features.

Keywords: MMP-7 - MMP-9 - TIMP-1 - bladder cancer - pathological parameters

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Introduction

Bladder cancer is a widespread urologic cancer. All bladder cancers originate in the urothelium, which is a 3-7-cell mucosal layer within the muscular bladder. In North America, Europe, and Asia, the most common type of urothelial tumor diagnosed is transitional cell carcinoma. Primary transitional cell carcinoma of the bladder is a comparatively common tumor, comprising about 90% of all bladder cancer cases in western countries (Anton-Culver et al., 1992). It is the second most common genitourinary malignancy (Johansen et al., 1997).

Bladder cancer ranges from mild disease with a low mortality rate to manifestations as numerous high-grade tumors associated with high mortality. It has an evident correlation with environmental exposure (Sharma et al., 2009).

In recent years, there has been controversy about the role of urine-based tumor markers in the diagnosis and surveillance of bladder cancer. The tumor marker tests available include the bladder tumor antigen (BTA) stat test and the BTA Trak test; subjectsluorescence in situ hybridization analysis; the ImmunoCyt test; the nuclear matrix protein 22 (NMP 22) test; the NMP 22 Bladder Check test; and the telomeric repeat amplification protocol (Koss et al., 1985; Sharma et al., 2009). No tumor markers have the specificity of conventional urine cytology for the detection of bladder cancer; therefore, tumor markers should not be used for diagnosis (Sharma et al., 2009).

If bladder cancer is diagnosed at an early stage in development when the tumor is confined to the bladder, the 5-year survival rate is 94%; however, if the cancer malignancy is not determined until it has disseminated beyond the bladder or as distal metastases, the survival ratio drops to 48 and 6%, respectively (Andersen et al., 2006). The invasion of bladder tumor cells into the sub-mucosa is accompanied by markers (such as growth factors, matrix metalloproteinases, cytokines) and is thought to promote tumor dissemination. These markers facilitate tumor detection and destruction.

The matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases with proteolytic activity. Their activity can be regulated by various factors such as NF-KB and oxidative stress. The strongest evidence for such activity has been in vitro experiments concerning matrilysin (MMP-7) Vargo-Gogola et al. (2002). MMP-7 has been found to be over-expressed in several tumors, such as those associated with esophageal, cholangiocarcinoma, gastric, colon, prostate and bladder cancer (Hashimoto et al.,1998; Martinez-Fernandez et al., 2009; Leelawat et al., 2010; Yeh et al., 2010; Szarvas et al., 2011; Zhou et al., 2011). MMP-7 levels are included in the evaluation of cancer invasion and metastases.

Tumor cells have the capacity to produce and release matrix metalloproteinase-9 (MMP-9), a proteolytic enzyme capable of degrading the basement membrane

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and type IV collagen in the cells. Degradation by MMP-9 is required for both tumor invasion and metastases (Murray et al., 2004). Increased invasion and expression of MMP-9 in human colorectal lines by a CD44-dependent mechanism (Hashimoto et al., 1998; Murray et al., 2004; Martinez-Fernandez et al., 2009; Leelawat et al., 2010; Yeh et al., 2010; Szarvas et al., 2011).

All members of the MMP family can be regulated by their inhibitors, tissue inhibitor of matrix metalloproteinases (TIMPs), which bind MMP non-covalently in a 1:1 complex TIMP-1 inhibits the activity of MMP-7 and MMP-9 Ray, Stetler-Stevenson (1994) The role of matrix metalloproteinase and their inhibitors in tumor invasion, metastasis and angiogenesis (Ray et al., 1994; Lipton et al., 2007).

Recently, it has been observed that TIMP-1 plays other roles in the cell. TIMP-1 is reported to mediate many complicated effects in the growth and dissemination of cancer cells, including the inhibition of apoptosis (Li et al., 1999), the promotion of growth (Hayakawa et al., 1992), and the regulation of angiogenesis (Laleur et al., 2002). Higher concentrations MMP-9 and lower TIMP-1 levels have also been associated with worse prognoses in several cancer types, including gastric cancer , breast cancer (Wu et al., 2008) and colorectal cancer (Yukawa et al., 2007; Park et al., 2012; Yang et al., 2012).

Degradation of the basement membrane and neo-vascularization are characteristic of the progression of bladder cancer. MMP-7 and 9 and TIMP-1 are involved in this process and might therefore represent potential biomarkers in bladder cancer.

In the present study, we aimed to investigate the effect of MMP-7, MMP-9 and TIMP-1 on the tumorigenesis and angiogenesis of human bladder cancer as well as the associations among these markers and clinico-pathologic variables

Materials and Methods

Patients were recruited at the Department of Urology, Yuzuncu Yıl University Medical Faculty, and archived data on patients newly diagnosed between January 2000 and November 2011 were reviewed. All of the patients were ethnically Turkish. Informed consent was obtained from all patients.

All patients were initially evaluated by history-taking, clinical examination, standard laboratory investigations, chest radiographs, excretory urography and/or abdominal ultrasonography. Abdominal and pelvic computed tomography and radioisotope bone scans were performed for patients with evidence of advanced disease. The pathologic stage was assigned according to the 2002 American Joint Committee on Cancer TNM staging system. The pathologic grade was classified according to the 1998 WHO/International Society of Urological Pathology classification system.

Biochemical measurements

Blood samples were drawn from all patients before surgical treatment. None of the bladder cancer patients had received chemo- or radiotherapy before the blood samples were collected. To standardize the clotting conditions, all sera were separated within 1 h after blood collection, separated into aliquots and stored at -80° C untilneeded. The levels of MMP-7, MMP-9 and TIMP-1 were measured using enzyme-linked immunosorbent assay kits (ELISA) (R&D Systems, MN, USA) and a Luminex BioAnalyzer. The samples were diluted 100-fold before determination. Each sample was tested in duplicate.

Statistical analysis

Shapiro-Wilk test-of-normality control charts and histograms were drawn. The median values are presented as minima and maxima. Group comparison was performed by Kruskal-Wallis one-way analysis of variance. Bilateral (post-hoc) comparisons were performed with the Bonferroni-corrected Mann-Whitney U test. The SPSS 17.0 statistical package program was used for analysis. The limit of significance was taken as $p<0.05$.

Results

Serum MMP-7, MMP-9 and TIMP-1 levels were measurable for all 90 patients (57 men and 33 women) with a median age of 57 (40-67) years who were analyzed. Table 1 provides the details of the demographic and clinico-pathological data for the entire group of patients. Levels were also measured in 40 healthy control groups (25 men and 15 women) with a median age of 55 (39-65) years.

Table 1. Demographic and Clinico-Pathologic Variables of all Patients

Clinico-pathologic variables		All Patients	Controls
Age (years)		57±15 (40-67)	55±10 (39-65)
Gender (M/F)		57/33	35/15
Lymphovascular involvement (A/P)		25/65	
T Stage	T1/T2	11/17	
	T3/T4	25/37	
Distant metastasis (M0/M1)		30/60	
Lymph-node metastasis (N0/N1/N2)		13/30/47	
Tumor diameter	<3 cm	18	
	≥3 cm	72	

Table 2. The Serum Levels of All MMPs in Patients with Bladder Cancer According to Clinico-Pathologic Variables (Mean±SD) (Min.-Max.)

Variables	MMP-7 levels	MMP-9 levels	TIMP-1 levels
Lymphovascular involvement			
Absent	1250±455 (938-2274)	1000±227 (688-1830)	88±11 (53-99)
Present	4705±1366 (1620-7305)	7580±2725 (2040-9859)	65±13 (41-78)
T Stage			
T1	2139±1235(1096-4258)	1900±384(1015-2531)	1900±384 (1015-2531)
T2	3786±788 (2517-4839)	2675±493 (2100-3618)	70±4 (60-78)
T3	4337±1048 (2317-5905	5425±810 (3780-7100)	54±2 (50-60)
T4	4337±1048 (2317-5905	5425±810 (3780-7100)	54±2 (50-60)
Distant metastasis			
M0	106±50 (42-190)	1172±163 (990-1790)	90±5 (80-100)
M1	4376±1791 (1026-8321)	9850±1445 (1025-12605)	51±4 (35-60)
Lymph-node metastasis			
N0	110±48 (90-215)	1090±110 (910	95±5 (80-100)
N1	2909±904 (2095-4537)	2430±473 (1450-3450)	75±5 (65-80)
N2	5143±672 (3097-6285)	8135±2110 (1180-11900)	53±4 (40-60)
Tumor diameter (cm)			
<3	1090±538 (190-2740)	1250±278 (990-2340)	75± 5 (60-90)
≥3	4382±1562 (2316-8535)	9461±2965 (4560-11950)	40±5 (30-50)

Table 3. Serum Levels of All MMPs in Patients and Healthy Controls (Mean±SD) (Min.–Max.)

Sütun1	Healthy control group	All Patients	p
MMP-7	750±120 (42-935)	3890±1120 (42-8535)	<0.0001
MMP-9	1105±114 (50-1250)	8583±3290 (688-12685)	<0.0001
TIMP-1	100±11 (80-120)	57±19 (30-100)	<0.0001

The levels of MMPs increased with tumor grade (p<0.001). However, the levels of TIMP-1 decreased with tumor grade (p<0.001). Serum MMP levels were significantly higher in patients with bladder cancer with metastatic disease, lymphovascular involvement, lymph-node metastasis, and higher tumor burden (p<0.001). However, serum TIMP-1 levels were lower in patients with bladder cancer with metastatic disease, lymphovascular involvement, lymph-node metastasis, and more severe tumor burden (p<0.001).

Discussion

To elucidate the molecular pathways and the biological role of tumorigenesis and angiogenesis in bladder carcinoma, several studies have investigated MMP-7 and MMP-9 levels in bladder cancer. The concentrations of MMP-7 and MMP-9 in the sera of 90 bladder cancer patients were assessed in relation to clinic-pathological variables associated with tumor stage. Our study demonstrated that serum MMP-7 and MMP-9 levels were increased in patients with bladder cancer and that TIMP-1 levels were decreased in patients compared to controls.

To elucidate the molecular-cell pathways and biologic role of angiogenesis in urothelial carcinoma, our studies investigated MMP-7 and MMP -9 and TIMP-1 levels.

MMP-7 is a small protein in the MMP family that lacks a C-terminal hemopexin domain common to other MMP members. MMP-7 concentration may play a role in tumor progression. In bladder cancer, high levels of MMP-7 gene expression and serum and urine concentrations proved to be independent prognostic indicators of metastasis and lymph-node metastasis (Szarvas et al., 2010). MMP-7 activity is pro-angiogenic and can cleave plasminogen to form the angiogenesis inhibitor angiostatin (Cornelius et al., 1998). MMP-7 muffles the immune response to tumors through processes involving chemokine deactivation (McQuibban et al., 2001). Matrix metalloproteinase activity inactivates the CXC chemokine stromal cell-derived factor-1 (McQuibban et al., 2000; 2001) and T-lymphocyte suppression (Sheu et al., 2001; Cheng et all., 2012). MMP-9 enzyme activity is thought to exist in bladder cancer cells but not in normal epithelium (Durkan et al., 2003). Alteration in urinary matrix metalloproteinase-9 to tissue inhibitor of metalloproteinase-1 ratio predicts recurrence in nonmuscle-invasive bladder cancer (Durkan et al., 2003; Vasala et al., 2008). High MMP-9 expression is a predictor of disease recurrence and poor survival (Slaton et al., 2004). Its serum levels were fundamentally increased in patients with end-stage and high-grade bladder cancer (Guan et al., 2003).

Depleted plasma concentrations of TIMP-1 have been observed in patients with bladder cancer (Naruo et al., 1994).TIMPs have several different functions. They inhibit the catalysis of MMPs, and they are able to act as growth factors (Hayakawa et al., 1992). The TIMP-1-mediated growth of bladder cell carcinoma, however, has not yet been described. TIMP-1 may promote tumor cell growth and progression rather than inhibiting matrix degradation. Additionally, TIMP-1 levels may limit aggressive tumor growth. We have demonstrated that MMP-7 and -9 and TIMP-1 are correlated with tumor grade. MMP-9 was observed in the crew stroma and bladder cancer (Wallard et al., 2006).

This study highlights the significance of MMPs levels and reveals an important increase in expression with increasing tumor grade. We found that low-grade tumors (T1 and T2) appear similar to normal tissue, independent of tumor stage. If the analysis is narrowed to non-invasive stage Ta/T1 tumors, MMP-7 and MMP-9, there is a correlation with increasing grade. We found TIMP-1 to be expressed at lower levels in tumor cells and high-grade tumors. Similar reports have found MMPs and TIMPs to be involved in tumor recurrence or progression in bladder cancer (Vasala et al., 2008). An elevation in MMP-7 and -9 levels and a reduction in TIMP-1 levels may designate an increase in proteolytic activity. This may represent the delicate balance between the pro-angiogenic and tumorigenic activity of MMP-7 and -9 and he effects of their endogenous inhibitor (TIMP-1) (Egeblad et al., 2009).

This study demonstrated that MMP-9 and TIMP-1 levels are related to tumor stage, tumor grade, tumor size, tumor multiplicity, and tumor progression. It is recognized that TIMP-1 is a multifunctional protein that is independently responsible for MMP-inhibition, including the capacity to stimulate the growth of bladder cancer cells when TIMP-1 is present in excess of MMP-9 levels.

In conclusion, we evaluated the diagnostic value of serum MMP-7 and -9 and TIMP-1 levels in bladder cancer. Functional research will help to resolve the roles of these proteins in cancer cells and highlight their potential as early diagnostic markers in bladder cancer.

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