

Metalloproteinase-7 (MMP-7) and their clinical relevance in urinary bladder cancer

Wasan A. Bakir, Nahi, Y. Yaseen, Noor H. Ismail, Dina W. Abd, Haidar A. Hasson

Iraqi centre for cancer and medical genetic research, Al- Mustansiriya University, Baghdad, Iraq.

Abstract:

Background: metalloproteinase-7 (MMP-7) is produced by stromal cells and by tumor cells, and overexpressed in a variety of epithelial and mesenchymal tumors. The aim of this study was to assess the association of MMP-7 with clinical-pathological factors and to evaluate its diagnostic value in patients with bladder carcinoma.

Methods: Expression of MMP-7 was evaluated in tumor tissues of 32 urinary bladder cancer cases and 30 control groups were fixed in formalin and embedded in paraffin blocks, then analyzed by in situ hybridization technique.

Results: Expression of MMP-7 was significantly higher in tumor compared to normal samples. The expression significantly associated with tumor grade and muscle invasion.

Conclusion: MMP-7 may play an essential role in pathogenesis of bladder cancer. And may be helpful to conventional diagnostic tools in malignancy as diagnosis.

Keywords: MMP-7, Bladder carcinoma

Introduction:

Bladder cancer is the ninth most common cancer throughout the world and is considerably more common in developed than developing countries (1). Bladder cancer ranges from mild disease with a low mortality rate to manifestations as numerous high-grade tumors associated with high mortality and it has an evident correlation with environmental exposure (2)

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- κ B and oxidative stress (3).

Increased MMP activity has an important role in several pathological conditions, such as arthritis, cardiovascular disease and cancer (4, 5). There is clear evidence that different MMPs are causally involved in tumor invasion and metastasis (4, 6).

Matrix metalloproteinase (MMPs) family consists of more than 26 endopeptidases that share homologous protein sequences, with conserved domain structures and specific do-

main related to substrate specificity and recognition of other proteins (6, 7).

Matrix metalloproteinase-7 (MMP-7) is the smallest member of the MMP family. Its broad substrate specificity indicates its versatile role in neoplastic transformation and in tumor progression and is able to cleave E-cadherin and Fas receptor at the cell surface, disrupting cell-cell interactions and inhibiting apoptosis (8). Furthermore, MMP-7 has been shown to release RANKL (receptor activator of NF- κ B ligand), which induces osteolysis by activating osteoclasts (8, 9). It is produced by stromal cells and by tumor cells and overexpressed in a variety of epithelial and mesenchymal tumors (10).

It has been found to be over-expressed in several tumors, such as those associated with esophageal, cholangio carcinoma, gastric, colon, prostate and bladder cancer (11, 12). MMP-7 also known as matrilysin, is the smallest MMP; its molecular weight as proenzyme is 28 kDa, which reduces to 19 kDa after an activation step induced by plasmin and trypsin (14).

In the present study, it was aimed to investigate the effect of MMP-7 on the tumorigenesis of human bladder cancer as well as its association with markers and clinico-pathologic variables, to evaluate the diagnostic value of (MMP-7) in patients with bladder transitional cell carcinoma.

Corresponding address:

Wasan A. Bakir

Iraqi Center for Cancer and Medical Genetic Research/ Al-Mustansiriya University.

Email: wasan.altaie@iccmgr.org

Material and Methods:

Fifty urinary bladder carcinoma with paraffin embedded tissue samples were obtained from the files of the Department of Pathology at Al-Yarmouk and Baghdad Teaching Hospitals. In addition 30 apparently normal bladder autopsies were collected from the Forensic Medicine Institute archives. The histopathogenic section were evaluated and reviewed by a pathologist to diagnose the carcinoma of the bladder. The histological grade of the tumors was reviewed and classified according to the WHO classification for urological tumors. The median age of the patients was 60 year. The clinical stage was defined according to the 1997 International Union against Cancer tumor staging system.

In situ hybridization (ISH) for detection of MMP-7mRNA.

In situ hybridization (ISH) is a technique that makes use of the high specificity of complementary nucleic acid binding to detect specific DNA or RNA sequence in the cell. The use of Biotin – Labeled DNA probe for mmp-7 / DNA (Maxim Biotec, USA) 216bp, MMP-7 (8 µg/100 µl) in distilled H₂O (Maxim Biotech, Inc., U.S.A). For detection of this marker, the biotinylated DNA probe hybridizes to the target sequence (mmp-7 mRNA sequence) then a streptavidin-AP (streptavidin-alkaline phosphatase) conjugate is applied followed by addition of the substrate prometho-chloro – indolyl – phosphate / nitro-blue tetrazolium (BCIP/NBT) which yields an intense blue – black signal at the specific site of the hybridized probe. This streptavidin – AP conjugate like the biotinylated probe provides a rapid and highly sensitive detection method. Hybridization / Detection System will give an

intense blue – black color at the specific sites of the hybridization probe in both positive test tissues. Evaluation of the in situ staining was done with assistance of a histopathologist.

Scoring

A scoring system that includes evaluation of the staining percentage of stained bladder cells was employed for the expression of MMP7. Counting the number of the positive cells in the bladder tissue which gave a blue-black nuclear staining under the light microscope. The extent of the ISH signaling in cells of the examined tissue was determined in 10 fields under high power microscope (100X). In each field, the total staining score was divided by the number of whole cells per field in 10 fields, so the percentage of positively stained cells in the 10 fields was calculated for each case by taking the mean of the percentage of the positively stained cells in the 10 fields (15).

Statistical analysis: Student test (t-test) was used for the quantitative data. The lowest level of significance was when the probability ($p < 0.05$) and the highly significant was ($p < 0.01$).

Result:

The expression of MMP-7 mRNA in bladder cancer patients was different from that of cystitis (control group), (table 1). Overexpression of MMP-7 mRNA was detected in $\geq 50\%$ of the cells appearing as positive of the urinary bladder cancer samples. Higher MMP-7 mRNA expressions were found in bladder cancer patients than in controls (58.35 ± 4.65 versus 4.70 ± 0.756 , $p < 0.01$).

The expression of MMP-7 was heterogeneous dark brown staining in the tissues (Figure – 1).

Table 1: MMP – 7 expressions in patients and normal control with urinary bladder carcinoma.

Groups	No	MMP-7 ISH expression (mean \pm SE)	Comparison of significant <i>p</i> -value
Bladder carcinoma	32	58.35 \pm 4.65	(P < 0.01)*
Control	30	4.70 \pm 0.76	

*= highly significant difference ($p < 0.01$)

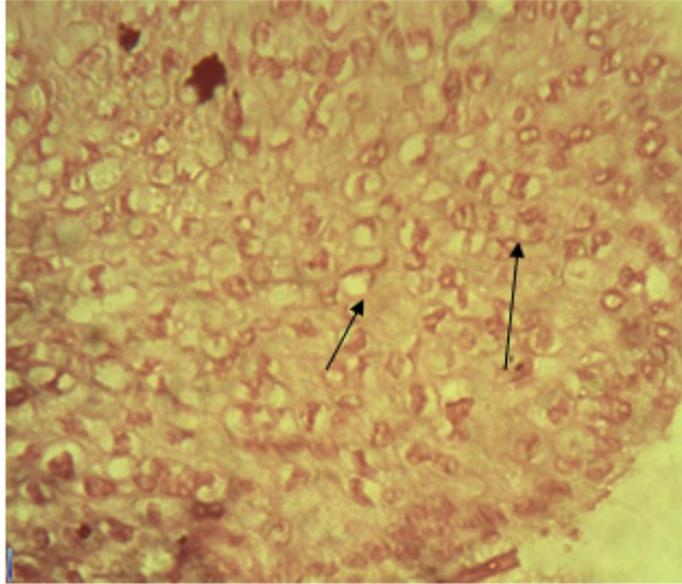


Figure-1: Detection of MMP-7 in patient with urinary bladder carcinoma by in situ hybridization.

Staining of MMP-7 mRNA by BCIP/ NBT (dark – brown) counterstained with nuclear fast red. Tissue from patients with urinary bladder carcinoma shows positive MMP-7 by hybridization signals (arrows).

Analysis of the positivity of the different of expression in tissue is in Table 2. There was a significant difference between the overexpression of MMP-7 and tumor grade or with muscle invasion ($P < 0.01$).

Table 2: Clinical and pathological feature of 32 patients with urinary bladder carcinoma.

Variable	No.	MMP-7 ISH expression (mean \pm SE)	<i>p</i> -value
Tumor grade			
- Low grade	18	40.30 \pm 4.88	<i>P</i> < 0.01*
- High grade	14	63.69 \pm 8.78	
Muscle invasion			
- With Muscle invasion	15	65.60 \pm 8.08	<i>P</i> < 0.01*
- Without Muscle invasion	17	34.65 \pm 5.07	

* Significant at the < 0.01 level.

Discussion:

Matrilysin (MMP-7), the smallest member of the MMP family, is involved in the regulation of cellular processes, such as apoptosis, cell growth, and angiogenesis, beside of its primary ECM-degradation effect (14, 15). In present study, the expression of MMP-7 was associated in tissue of urinary bladder cancer of patients in relation to their clinicopathological features. It was shown that MMP-7 expression is sig-

nificantly higher in tissue samples of urinary bladder cancer compared to those with controls.

In situ hybridization expression of MMP-7 in UBC tissue is more common in high grade than in low grade and in muscle invasion than in non-invasion, thus indicating that its expression is correlated with the presence of metastasis. This result is supported by the finding of a previous report, in which MMP-7 expression was found to be greater in UBC than in normal

mucosal tissues, and expression of MMP-7 increasing with Dukes stage(16).

Elevated of MMP-7 levels are associated with the presence of metastatic disease. MMP-7 was increased according to stage. This is in accordance with previous reports showing that high MMP-7 tissue expressions are associated with presence and later metastasis Szarvas, et al. (2011), Szarvas et al(2010) and also were in agreement with Becker et al. (2002), that elevated MMP-7 level in samples from patients with urinary bladder cancer.

the cells through the secretion of MMP-7 to degrade the surrounding extracellular matrix, so that their own local walk more easily, into neighboring lymphatic capillaries and small veins leading to local invasion and distant metastasis diaphragm (20). matrilysin specifically induces tumor cell aggregation due to processing of membrane proteins. These aggregated cells showed a dramatically enhanced metastatic potential (18, 21).

Early genetic alterations or gene expression changes may occur. Therefore, normal-appearing epithelium from a cancerous bladder does not necessarily represent normal epithelial cells (14, 22). Furthermore, tumor cells can influence the

mRNA and protein expression of normal cells in a paracrine-manner (23).

MMP-7 muffles the immune response to tumors through processes involving chemokine deactivation (3). Matrix metalloproteinase activity inactivates the CXC chemokine stromal cell-derived factor-1 and T-lymphocyte suppression (12).

These findings together suggest a clear relevance for MMP-7 in predicting patients' prognosis in bladder cancer MMP-7 may play a role in tumor progression, as tumor invasion and progression are a multifactorial process promoted by micro-environmental changes that include overexpression of matrix(19).

Conclusions

Metalloproteinase -7 is present in detectable amounts in the tissue of patients with bladder cancer. Elevated of urinary matrilysin tissue of patients with metastasis could help to detect bladder cancer metastasis and indicates that this marker may be a valuable tool for the identification of patients with present and/or with high risk (11, 22) and may therefore provide a more reliable prognosis and influence therapy decisions.

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ماتركس ميتالوبروتينيز - 7 الالهية الطبية والسريية في سرطان المثانة

وسن عبد الاله باقر، ناهي يوسف ياسين، نور هاشم اسماعيل، دينا وائل عبد، حيدر عدنان حسون

المركز العراقي لبحوث السرطان والوراثة الطبية / الجامعة المستنصرية

الخلاصة:

تنتج ماتركس ميتالوبروتينيز - 7 في خلايا الانسجة والخلايا السرطانية حيث تكون بتعبير عالي غير طبيعي في انواع من الخلايا الطلائية والسرطانية. **الهدف من الدراسة:** هو تقييم ظهور ماتركس ميتالوبروتينيز - 7 مع العوامل السريية وتحديد القيم التشخيصية في مرضى سرطان المثانة. **طريقة العمل:** تم تقييم ظهور ماتركس ميتالوبروتينيز - 7 في 32 حالة سرطان المثانة و 30 حالة طبيعية ثبتت بالفورمالين ثم استخدمت طريقة التهجين الموضعي لغرض الفحص. **النتائج:** زيادة ظهور ماتركس ميتالوبروتينيز - 7 يرتفع معنويا في الورم مقارنة بالحالات الطبيعية. كما وجد ان هناك علاقة وثيقة بين مع درجة الورم وغزو العضلات. **الاستنتاج:** اثبتت هذه الدراسة ان ماتركس ميتالوبروتينيز - 7 لعب دورا اساسيا في تحديد الاصابة سرطان المثانة. وربما تعتبر مساعدات لطرق التشخيص التقليدية في الاورام الخبيثة.