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The role of matrix metalloproteinase -2 in bladder cancer

***Noor H.Ismail[1], Wassan A.Bakir[1] , Ekram A. Hassan[2], Hayder A. Hasoon[1], Zena Z. Saeed[1]**

[1]Iraqi Center for Cancer and Medical Genetics Research , Al-Mustansiriyah University
[2]Department of pathology ,College of Medicine , Al-Mustansiriyah University

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Abstract: Transitional cell carcinoma of the bladder is the most common malignancy of the urinary tract.This study was designed to assess the expression of matrix metalloproteinase -2 in transitional cell carcinoma of the bladder using in situ hybridization technique.Formalin-fixed, paraffin embedded blocks tissue for thirty urinary bladder carcinoma patients were obtained from the archives of the Pathology laboratories of Al-Yarmouk and Baghdad Teaching Hospital from January 2011 to July 2012.In addition twenty apparently normal bladder autopsies were collected from the Forensic Medicine Institute archivesas control group.The blocks were subjected to cut as serial thin sections of (4µm) thickness and were sticked on positive charge slides to be used for *In situ* hybridization for the detection of MMP-2. Over expression of matrix metalloproteinase -2 was detected in 70.1923% (26 out of 30) of transitional cell carcinoma of the bladder samples (> 50% of the cells appearing as positive). The remaining 4 samples (42.775%) showed a weak- moderately expression (< 50% of the cells appearing as positive). In conclusion matrix metalloproteinase -2 play an important role in transitional cell carcinoma of the bladder.

Key words: Bladder cancer, matrix metalloproteinase -2, invasion, carcinogenesis,*in situ* hybridization technique.

***Corresponding author**: should be addressed (Email: nooraldhahir@yahoo.com)

دور ماتريكس ميتالوبروتينيز -2 في سرطان المثانة

نور هاشم اسماعيل[1] ، وسن عبد الاله باقر[1] ، اكرام عبد اللطيف حسن [2]، حيدر عدنان حسون [1] ، زينة زكي سعيد [1]

[1] المركز العراقي لبحوث السرطان والوراثة الطبية / الجامعة المستنصرية
[2] فرع علم الامراض –كلية الطب / الجامعة المستنصرية

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الخلاصة: سرطان الخلية الانتقالية للمثانة اكثر السرطانات شيوعا للمجاري البولية . عائلة انزيمات matrix metalloproteinase (MMP) تتالف من انزيمات بروتينية مهمة في اعادة تشكيل الارضية خارج خلوية وتشترك خلال عملها في عدد من العمليات الرئيسية الطبيعية والمرضية ، هذه الاخيرة تشمل نمو الورم ، تقدمه وانتشاره وتكوين اوعية دموية جديدة مقترنة مع هذه الاحداث . ونتيجة لذلك اصبحت هذه الانزيمات البروتينية تمثل اهدافا مهمة علاجية وتشخيصية في العلاج والكشف عن سرطانات الانسان. وتدخل matrix metalloproteinase -2 ضمن مجموعة من اهم هذه الانزيمات . وصممت هذه الدراسة لتقييم التهجين الموضعي لتعبير matrix

metalloproteinase -2 واهميته في سرطان الخلية الانتقالية للمثانة وتم الحصول على ثلاثين خزعة سرطان مثانة مثبتة بالفورمالين ومطمورة بالبرافين من ارشيف مختبر الامراض في مستشفى اليرموك التعليمي ومستشفى بغداد التعليمي للفترة من كانون الثاني 2011 الى تموز 2012 . فضلا عن عشرين عينة مثبتة بالفورمالين ومطمورة بالبرافين لخزع طبيعية من المثانة تم الحصول عليها من معهد الطب العدلي واستخدامها كمجموعة سيطرة . حضرت مقاطع نسيجية بسمك (4µm) على شرائح زجاجية موجبة الشحنة لتستخدم في التهجين الموضعي للتحري عن MMP-2 .ووجدت زيادة التعبير لـ MMP-2 في 70.1923% (26 من 30) لعينات سرطان الخلية الانتقالية للمثانة (> 50% من الخلايا كانت موجبة) وأظهرت العينات الاربعة الباقية تعبيرا ضعيف ومتوسطا″ (42.775%) (< 50% من الخلايا كانت موجبة) . **وقد** استنتج من الدراسة الحالية انmatrix metalloproteinase-2 يلعب دورا مهما في سرطان الخلية الانتقالية للمثانة .

Introduction

Bladder cancer is a worldwide health problem, more common in industrial countries than in developing countries and 77% of the tumors occur in men (1). In Iraq according to the 2004 Iraqi Cancer Registry report the bladder cancer, in terms of incidence and mortality is the fifth among the commonest ten leading cancers in males and females in 2004 (2).

The major risk factors related to the development of bladder cancer are smoking and occupational exposures in Western countries, whereas chronic infection with *Schistosoma hematobium* in developing countries particularly in Africa and the Middle East . A majority of bladder cancers associated with schistosomiasis are squamous cell carcinoma while those associated with smoking are transitional cell carcinoma (3).

More than 90% of bladder carcinomas are transitional cell carcinomas (TCC), and most of these carcinomas (75%–85%) are non-muscle invasive tumors at first diagnosis (4). One of the fundamental modifications that occur in malignancy is tissue invasion and metastasis (5). Degradation of the basement membrane and the extra cellular matrix (ECM) is a prerequisite for tumor invasion. Matrix metalloproteinases (MMPs) belong to the group of ECM degradation enzymes, which selectively degrade different components of the ECM and release growth factors and cytokines that reside in the ECM (6,7). The proteolytic activity of MMPs plays a critical role in invasion and metastasis and regulate signaling pathways that control cell growth, survival, invasion, inflammation and angiogenesis (6).

The degrading activity of MMPs is regulated by tissue inhibitors of metalloproteinases (TIMPs). The balance of secreted MMPs and their specific TIMPs plays an important role in maintaining connective tissue homeostasis in normal tissue (8). In neoplastic diseases an imbalance of MMPs and TIMPs leading to an excess of degradative activity is supposed to be linked to the invasive and metastatic potential of tumor cells (8).

Gelatinase A or matrix metalloproteinase-2 (MMP-2) is one of a family of neutral zinc-metalloendopeptidases which collectively degrade extra cellular matrix proteins, glycoproteins and proteoglycans. MMP-2 is secreted as a latent pro-enzyme form and must be activated for catalytic activity before it can degrade substrate (9,10). Like MMP-9, MMP-2 can degrade the type IV collagen which is a major

component of all basement membranes, and thus has been involved in carcinoma dissemination (11).Various MMPs thought to be involved in cancer but attention has focused on the gelatinases (MMP-2 and MMP-9) because they are over expressed in a variety of malignant tumors and their expression and activity are often associated with tumor aggressiveness and a poor prognosis (12). In urothelial carcinoma several of the well characterized MMPs including MMP-2, MMP-9 and MMP-14 demonstrate the increase expression and activity (13). The present study was designed to evaluate the expression of mmp-2 in the transitional cell carcinoma of the bladder.

Materials and Methods

Tissue samples

Formalin-fixed and paraffin embedded blocks tissue for thirty urinary bladder carcinoma patients were obtained from the archives of the Pathology laboratories of Al-Yarmouk and Baghdad Teaching Hospital from January 2011 to July 2012. The diagnosis of these tissue blocks were been primarily based on the obtained histopathological records of bladder biopsy samples that had been accompanied in the relevant hospital laboratory. The patients included in this retrospective study were 18 male (60%) and 12 female (40%) with a mean age (61.6667) ranged from 49 to 80 years. In addition twenty normal bladder autopsies were collected from the Forensic Medicine Institute archives. They were 10 males and 10 female and the range of the age was the same as patients group. Confirmatory histopathological re-examination of freshly prepared hematoxylin and eosin-stained slides of each obtained tissue blocks was done by specialist pathologistand classified according to criteria outlined by the World Health Organization (WHO).

Tissue sectioning and slide preparation

Formalin-fixed and paraffin embedded blocks tissue were subjected to cut as serial thin sections of (4μm) thickness and were sticked on positive charge slides to be used for *In situ* hybridization for the detection of MMP-2 .

In situ hybridization procedure

In situ hybridization (ISH) is a technique used the high specificity of complementary nucleic acid binding to detect specific DNA or RNA sequence in the cell (14).For *in situ* hybridization technique (ISH), DNA Probe Hybridization/Detection System in situ kit (Maxim Biotech, Inc., USA , cat # IH-60001(IH-0050) , high sensitivity type was used. The probe was biotinylated long DNA probe for human mmp-2 (191bp.) (Maxim Biotech, Inc., USA , cat # IH-60025). For the detection of this marker, the biotinylated DNA probe hybridize the target sequence (mmp-2 mRNA sequence) then a streptavidin-AP (streptavidin - alkaline phosphatase) conjugate is applied followed by addition of the substrate promo-chloro-indolyl-phosphate /nitro-blutetrazolium (BCIP/NBT) which yields an intense blue- black signal appears at the specific site of the hybridized probe (15). This directly streptavidin-AP conjugate linked to the biotinylated probe provides a rapid and highly sensitive detection method. Evaluation of ISH signal was done with the assistance of a specialist pathologist.

Scoring

The expression of mmp-2 mRNA was measured by the counting of the number of the positive cells in the tissue that has given a blue-black (BCIP/NBT) nuclear staining under the light microscope. The score was the average from 10 distinct high-power fields observed under ×100 magnification. The percentage of positively stained cell was calculated for each case by taking the mean of the percentages of the positively stained cell in the 10 fields. A score of (0) was given when no staining was detected (1) if there was weak to moderate staining in less than or equal to 10% of cells (2) if moderate to strong staining was present in 11 to 50% of cells and (3) if strong staining in more than 50% of cells was detected (16).

Statistical analysis

Statistical analysis was done using Chi-Square test to determine the difference in the *in situ* expression of MMP-2 between different groups (bladder cancer patients group and control group). Values were considered statistically significant when $p<0.05$.

Results and Discussion

Histopathpological Classification

Thirty formalin-fixed and paraffin embedded blocks were collected from bladder carcinoma patients and histopathologial re-examination with hematoxylin and eosin stain was done. The specimens were graded according to world health organization classification (17). As follows low grad: well –moderately differentiated transitional cell carcinoma of the bladder (n=19) (63.3%) and high Grade: poorly differentiated transitional cell carcinoma of the bladder (n=11) (36.7%). Muscle invasion was seen in 15 case (50%) while non-invasion was seen in the other 15 cases (50%).

***In situ* hybridization detection of MMP-2**

The results of *in situ* hybridization detection of MMP-2 showed in table 1 and figure 1 which were demonstrated that over expression of MMP-2 was detected in 86.67% (26 out of 30) of the urinary bladder cancer samples (> 50% of the cells appearing as positive). The remaining 4 samples (13.33%) showed a weak- moderately expression (< 50% of the cells appearing as positive). Statistical analysis demonstrated a highly significant differences in MMP-2 expression among patients with transitional cell carcinoma of the bladder when compared with healthy control group. These results might possibly reflect the important role of MMP-2 cellular expression in the pathogenesis of bladder cancer. Over expression of MMP-2 in transitional cell carcinoma of the bladder have been reported in several studies (16,18,19,20). The MMPs are proteolytic enzymes and their basic mechanism of action degradation of proteins regulates various cell functions related to cancer biology. These include cancer-cell growth, differentiation, apoptosis, migration and invasion as well as the regulation of tumour angiogenesis and immune surveillance (21). MMPs can regulate the tumor microenvironment, and their expression and activation is increased in almost all human cancers compared with normal tissue (22).Various MMPs thought to be involved in cancer but attention has focused on the gelatinases (MMP-2 and MMP-9) because they are over expressed in a variety of malignant tumors and their expression and activity are often associated with tumor

aggressiveness and a poor prognosis. Elevated levels of MMP-2 and /or MMP-9 are found in breast, brain, ovarian, pancreas, colorectal, bladder, prostate and lung cancers and melanoma (12).

Table (2) demonstrated the association between expression of MMP-2 score with different variables. The results showed that there were no significant differences between *in situ* hybridization expression of MMP-2 with age, gender, grade and muscle invasive.

Table (1) :The expression of MMP-2 in patients with transitional cell carcinoma of the bladder

Group	No.	MMP-2 score N(%)				Comparison of significance	
		0	1	2	3	p-value	significance
Transitional carcinoma patients	30	0	0	4(42.775%)	26(70.1923%)	0.01	Highly significant
Control	20	10(0%)	3(9%)	7(28.657%)	0		

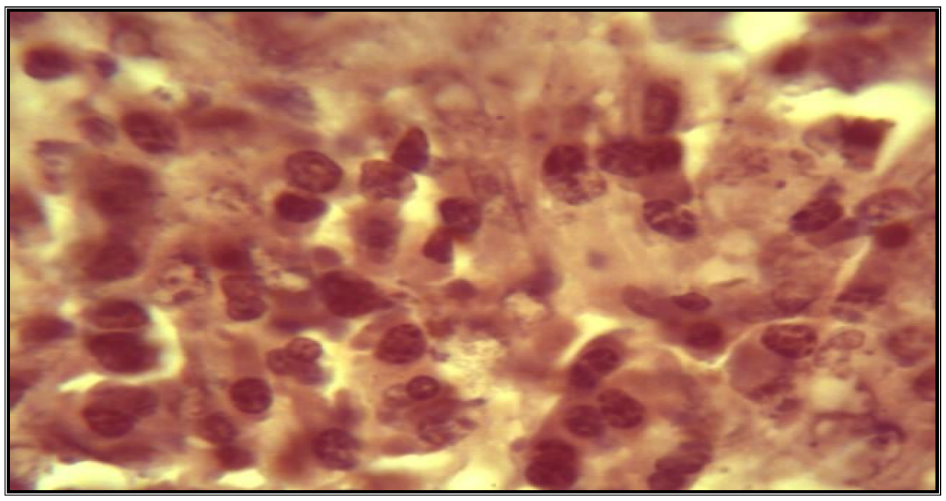

Figure (1) *In situ* hybridization for MMP-2 of patient with TCC of the bladder shows positive MMP-2 by hybridization signals and stained by BCIP/NBT-Chromogen and counter stained with Hematoxylin , magnification power 1000X

Table (2) :The association of MMP-2 scores with different variables of patients with TCC

Variable	MMP-2 score N(%)				significance
	0	1	2	3	
Gender					
Male	0(0%)	0(0%0)	2(41.25%)	16(71.5%)	Not significant (*P*>0.05)
Female	0(0%)	0(0%)	2(44.3%)	10(68.08)	Not significant (*P*>0.05)
Age					
≤60 y.	0(0%)	0(0%)	2(45.3%)	14(68%)	Not significant (*P*>0.05)
>60 y.	0(0%)	0(0%)	2(40.25%)	12(72.7%)	Not significant(*P*>0.05)
Grade					
Low grade	0(0%)	0(0%)	3(41.53%)	16(65.575%)	Not significant(*P*>0.05)
High grade	0(0%)	0(0%)	1(46.5)	10(77.58%)	Not significant(*P*>0.05)
Muscle Invasion					
Invasive	0(0%)	0(0%)	2(45.5%)	13(74.446%)	Not significant (*P*>0.05)
Non invasive	0(0%)	0(0%)	2(40.05%)	13(65.939%)	Not significant(*P*>0.05)

The results of current study showed no association between MMP-2 expression and age, gender also no association with tumor grade and muscle invasion observed . In the previous studies (23,24) investigators reported that the biomarker MMP-2 did not correlate with bladder cancer grade or stage. Kanayama *et al.* (25) reported that reverse transcriptase-polymerase chain reaction analysis of the levels of MMP-2 correlated with cancer grade. Gerhards *et al.* (26) also reported that MMP-2 urinary excretion was associated with a high stage and grade of bladder carcinoma. The inconsistent result may be attributed to the method of analysis or the sample size. MMPs have functions other than promotion of invasion have substrates other than components of the extra cellular matrix and that they function before invasion in the development of cancer . It was initially suggested that gelatinases played a dominant role in basement membrane invasive events because of their ability to degrade collagen IV (21). However, studies have demonstrated that gelatinases do not promote basement membrane invasion. In fact, recent evidence shows that gelatinases have main but

indirect functions in cell signaling by controlling the bio activaty of molecules that target specific receptors regulating cell growth, migration, inflammation and angiogenesis. By degrading the ECM, gelatinases generate or release bioactive molecules that effect tumor progression (27, 28, 29).

Thus gelatinases , MMP-2 and MMP-9, are involved in several steps of cancer development and are potential markers of malignant tumors (30) .

In conclusion, the results of our study suggest that MMP-2 may plays a significant role in pathogenesis of TCC of the bladder or could facilitate its progression.

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