

Expression of matrix metalloproteinase -2 in Breast cancer

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Abstract:

Formalin-fixed , paraffin embedded blocks tissue for thirty one breast cancer patients were obtained from the archive of the Pathology laboratory of Al-Yarmouk Teaching Hospital from January 2011 to July 2012 . In addition Formalin-fixed , paraffin embedded blocks tissue for ten fibroadenoma of breast were collected and used as control group . These blocks were subjected to cut as serial thin sections of (4µm) thickness and were stucked on positive charge slides to be used for in situ hybridization for the detection of matrix metalloproteinase -2 in breast cancer (MMP-2) .

Overexpression of MMP-2 was detected in 90.3% (28 out of 31) of breast cancer samples (> 50% of the cells appearing as positive) . The remaining 3 samples (9.7%) showed a weak- moderately expression (< 50% of the cells appearing as positive) . The statistical analysis demonstrated a highly significant differences in MMP-2 expression among patients with breast cancer when compared with fibroadenoma of breast patients (control group) . In conclusion MMP-2 plays an important role in pathogenesis of breast cancer and supports the evidence of its role in evolution , progression and cell survival of breast cancer .

Keywords: Breast cancer; matrix metalloproteinase-2, invasion, carcinogenesis, metastasis, in situ hybridization technique.

Introduction:

Breast cancer is the most frequently diagnosed cancer in women worldwide with an estimated 1.4 million new cases in 2008. About half of these cases occurred in economically developing countries (1). Breast cancer incidence rates varied internationally .The factors that contribute to the international variation in incidence rates largely stem from differences in reproductive and hormonal factors and the availability of early detection services .The incidence has grown rapidly during the last decades in many developing countries, and slowly in developed countries (2) . In Iraq according to the 2004 Iraqi Cancer Registry report , breast cancer, in terms of incidence and mortality , it is the first among the commonest ten leading cancers in females in 2004 (3).

In breast cancer, like most solid tumors, metastatic disease

rather than the primary tumor itself is responsible for death (4). The metastatic process involves a complex cascade of events, including the organized breakdown of the extracellular matrix (ECM) by matrix metalloproteinases (MMPs) (5). Together, the MMPs are able to process or degrade all ECM components. Each ECM element is cleaved by a specific MMP or MMP group (6) .The activity of these proteases is tightly regulated by specific inhibitors, known as tissue inhibitors of MMPs (TIMPs) (7) .

Gelatinase A , or matrix metalloproteinase-2 (MMP-2) , is one of a family of neutral, zinc-metalloendopeptidases , which collectively degrade extracellular matrix proteins, glycoproteins, and proteoglycans . MMP-2 is secreted in a latent zymogen (pro-enzyme) form , and must be activated before it can degrade substrate (8,9) Like MMP-9, MMP-2 can degrade the type IV collagen which is unique to basement membranes, and thus has been implicated in carcinoma dissemination (6). Of the various MMPs thought to be involved in cancer, attention has focused on the gelatinases because : (i) they are overexpressed in a variety of malignant tumors and (ii) their expression and activity are often associated with tumor ag-

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gressiveness and a poor prognosis (10). The objective of the current study is to determine the expression of MMP-2 in breast cancer .

Materials and Methods:

Tissue samples

Formalin-fixed , paraffin embedded blocks tissue for thirty one breast cancer patients were obtained from the archive of the Pathology laboratory of Al-Yarmouk Teaching Hospital from January 2011 to July 2012 . The diagnosis of these tissue blocks were primarily based on the obtained histopathological records of breast biopsy samples that accompanied in the hospital laboratory . The patients age included in this retrospective study ranged from 26 to 65 years. In addition , formalin-fixed , paraffin embedded blocks tissue for ten fibroadenoma of breast were collected and used as control group with an age range the same as the patients' group .

Tissue sectioning and slide preparation:

Sections from paraffin embedded blocks were taken from the archive of department of pathology of Al-Yarmouk Teaching Hospital . Each formalin-fixed paraffin-embedded block was subjected to cut as serial thin sections of 4µm thickness and were stuck on positive charge slides to be used for in situ hybridization for the detection of MMP-2 .

In situ hybridization procedure :

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) . 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 (11). For 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 (12). 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 10 to 50% of cells, and 3 if strong staining in more than 50% of cells was detected (13) .

Statistical analysis:

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

Results :

Histopathological classification:

Thirty one formalin-fixed, paraffin embedded blocks were collected from breast carcinoma patients and histopathological re-examination with hematoxylin and eosin stain was done by histopathologist for histological typing and grading of the tumors . The patients' age included in this retrospective study ranged from 26 to 65 years , of these patients , 26 (83.87%) were infiltrative ductal carcinoma , 4 (12.9%) were infiltrative lobular carcinoma and 1 (3.23%) was infiltrative mammary carcinoma . Histological grade was as follow : 5(16.13 %) were well differentiated (grade I) , 21 (67.74 %) were moderately differentiated (grade II) and 5 (16.13 %) were poorly differentiated (grade III) . Ten patients with fibroadenoma of breast were used as control group .

In situ hybridization detection of MMP-2

The results of in situ hybridization detection of MMP-2 revealed (Table 1 and Figure 1) an overexpression of MMP-2 in 90.3% (28 out of 31) of the breast cancer samples (> 50% of the cells appearing as positive) . The remaining 3 samples (9.7%) 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 breast cancer when compared with fibroadenoma of breast patients (control group) .

Table 1. The expression of MMP-2 mRNA in tissue of studied groups depending on the scoring level .

Group	No.	MMP-2 score N(%)				Comparison of significance	
		0	1	2	3	p-value	significance
Breast cancer	31	0	0	3(37.3667)	28(74.1893)	0.01	Highly significant
Fibroadenoma of breast	10	0	7(7.2)	3(23.233)	0		

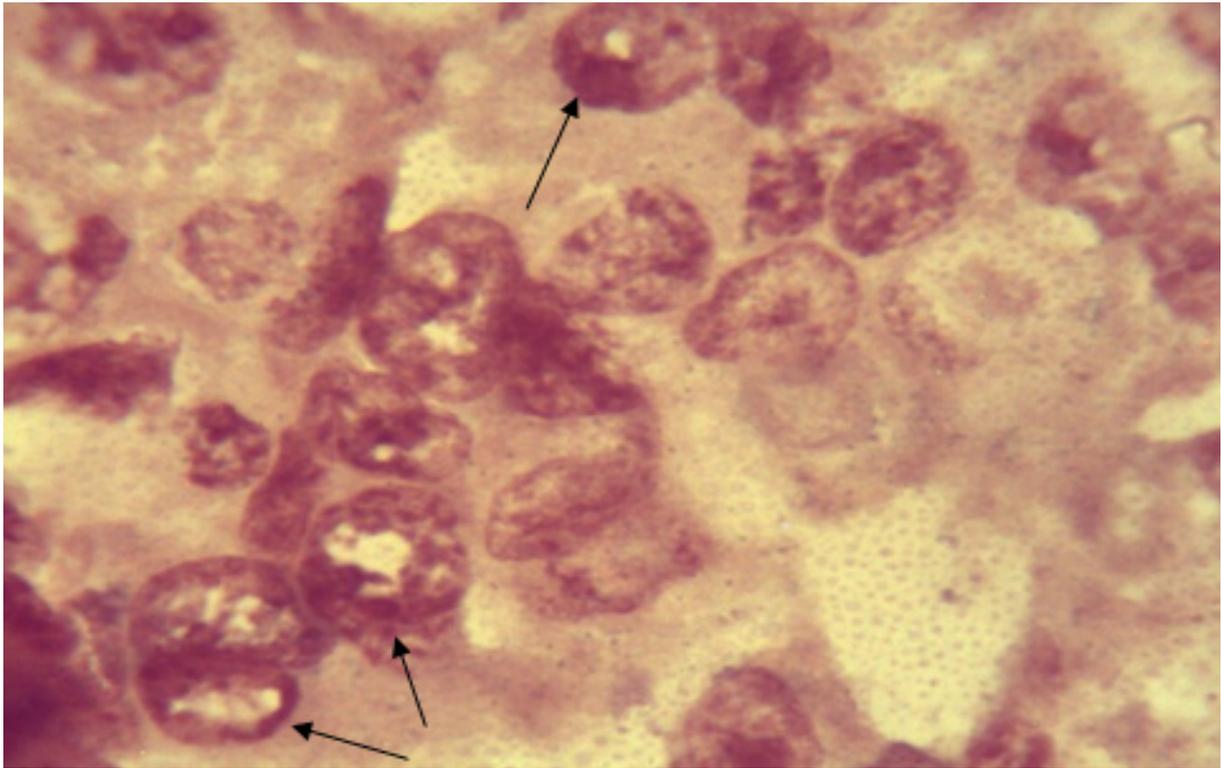


Figure 1. In situ hybridization for MMP-2 of patient with breast cancer shows positive MMP-2 by hybridization signals , stained by BCIP/NBT-Chromogen and counter stained with Hematoxylin , magnification power 1000X .

Discussion:

This study described the expression of MMP-2 in non-malignant (fibroadenoma of breast) and malignant breast tissues . Using in situ hybridization , the results showed that MMP-2 was a statistically highly significant overexpressed in breast cancer when compared with control group (fibroadenoma of breast) . These results might possibly reflect the association between cellular expression of MMP-2 and breast cancer genesis .

Matrix metalloproteinases selectively degrade various components of the extracellular matrix (ECM) and release growth factors and cytokines that reside in the ECM (14 , 15). The MMPs are also capable of activating various latent growth factors, cytokines and chemokines and cleaving cell surface proteins (cytokine receptors, cell adhesion molecules, the urokinase receptor, etc.) (16-19). Through their proteolytic activity, MMPs play critical roles in invasion , metastasis and regulate signaling pathways that control cell growth, survival, invasion, inflammation and angiogenesis (20-22). Of the various MMPs thought to be involved in cancer, attention has focused on the gelatinases because : (i) they are overexpressed in a variety of malignant tumors and (ii) their expression and activity are of-

ten 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 (10). It was initially suggested that gelatinases played a dominant role in basement membrane invasive events because of their ability to degrade collagen IV.(23) .However, studies (24-26) strongly suggest that gelatinases do not promote basement membrane invasion . In fact, recent evidence shows that gelatinases play major but indirect roles in cell signaling by controlling the bioavailability and bioactivity of molecules that target specific receptors regulating cell growth, migration, inflammation and angiogenesis . By degrading the ECM, gelatinases generate or release bioactive molecules that influence tumor progression. Gelatinase activity can cause the release of cryptic information from the ECM, leading to cell migration and angiogenesis . For example, cleavage of laminin-5 by MMP-2 results in the exposure of a cryptic epitope that enhances endothelial cell migration (27, 28) . The results are consistent with result of previous studies suggesting that MMP-2 may play an important role in breast cancer or could facilitate its progression .

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التعبير عن matrix metalloproteinase-2 في سرطان الثدي

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الخلاصة:

تم الحصول على واحد وثلاثون خزعة سرطان ثدي مثبتة بالفورمالين ومطمورة بالبرافين من ارشيف مختبر الامراض في مستشفى اليرموك التعليمي للفترة من كانون الثاني 2011 الى تموز 2012 . فضلا عن عشرة عينات مثبتة بالفورمالين ومطمورة بالبرافين من ورم الثدي الحميد fibroadenoma تم الحصول عليها واستخدامها كمجموعة سيطرة . حضرت مقاطع نسيجية بسماك 4µm على شرائح زجاجية موجبة الشحنة لتستخدم في التجهيز الموضعي للتحري عن MMP-2 (matrix metalloproteinase-2) في سرطان الثدي . اظهرت النتائج زيادة في التعبير عن MMP-2 في 90,3 (28 من 31) من عينات سرطان الثدي (< 50% من الخلايا كانت موجبة) . العينات الثلاثة الباقية (9,7%) اظهرت تعبيراً ضعيفاً- متوسط (> 50% من الخلايا كانت موجبة) . التحليل الاحصائي اظهر فرق معنوي عالي في التعبير عن MMP-2 لدى مرضى سرطان الثدي مقارنة مع المرضى بالاورام الحميدة (مجموعة السيطرة) . استنتج ان MMP-2 يلعب دوراً مهماً في امراضية سرطان الثدي ويدعم دليلاً على دوره في تطور , تقدم وبقاء خلايا سرطان الثدي .