Iraqi Journal of Biotechnology, 2014,Vol.13, No.2 , 266-272

Prevalence of Matrix metalloproteinase-9 in Breast Cancer As a Marker of Prognosis

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Abstract: Matrix metalloproteinase-9 (MMP-9) is regarded as important for degradation of the basement membrane and extracellular matrix during cancer invasion and other tissue-remodeling events. the aim of this study is to investigate and compare the expression of MMP-9 in human breast adenocarcinoma with fibroadenoma. In this study we evaluate the prognostic value of MMP-9, by *in situ* hybridization staining in a series of 30 breast cancer tissues. Both mean percentage of MMP-9 MRNA and scoring of it،s expression in normal and cancer, showing an increased expression of MMP-9 was significantly high in tumor compared with control samples, with extensive score among breast cancer tissues. This study shows that MMP-9 expression in breast cancer is associated with better prognosis.

Key word : MMP-9, Breast cancer, fibroadenoma, adenocarcinoma.

فحص البروتينيز المعدني الحشوي -9 (MMP-9) كمؤشر حيوي في تشخيص سرطان الثدي

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الخلاصة: يمثل البروتينيز المعدني الحشوي -9 انزيماً مهما في عملية تحلل الغشاء القاعدي والحشوات بين الخلايا خلال مرحلة انتشار السرطان. هدفت هذه الدراسة لمقارنة مستوى تعبير البروتينيز MMP-9 في سرطان الثدي الغدي والاورام المتليفة وذلك من خلال استخدام تقنية التهجين خارج الخلايا لثلاثين نسيجاً تعود الى عينات من سرطان الثدي وأخرى طبيعية. بينت النتائج بأن مستوى تعبير البروتينيز MMP-9 كان عاليا معنويا في عينات السرطان مقارنة مع العينات الطبيعية مما يجعل هذا البروتين مؤشر حياتي مهم في التشخيص المبكر لسرطان الثدي.

Introduction

Breast cancer is the most frequently diagnosed cancer in woman worldwide. About half of the new cases occurred in economically developing countries (1). Breast cancer incidence rates varied internationally. 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 the breast cancer, in terms of incidence and mortality, it is the first among the commonest ten leading cancers in males and females in 2004 (3).

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 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).

Matrix metalloproteinases (MMPs) are a family of proteinase enzymes that breakdown a broad range of extracellular matrix and basement membrane components. Invasion and metastasis of tumor cells require adherence to ECM and passage through it's components (8). In this context, MMPs play a key role, because they can either degrade or digest the ECM components and thereby support cancer cell metastasis to the other tissues (9). Degradation of the extracellular matrix and components of the basement membrane by proteases facilitates the detachment of tumor cells, their crossing of tissue boundaries, and invasion into adjacent tissue compartments (10). These gelatinases are secreted as zymogens and cleaved to the active form, and their function is tightly regulated by several different mechanisms (11, 12). In breast cancer, both MMP-2 and MMP-9 seem to be expressed in cancer tissue (13, 14, 15, 16). In zymography studies, the expression of MMP-2 and MMP-9 has been detected in benign tissue, and enzyme activities have been less than that in malignant tissue (17). Therefore, we conducted a study to explore whether MMP-9 is involved in breast cancer and indicate it as a prognostic marker in breast carcinoma.

Materials and Methods

Thirty breast carcinoma cases with paraffin embedded tissue samples were obtained from the files of the Department of Pathology at Al-Yarmouk and Baghdad Teaching Hospital. The samples were evaluated by a pathologist to represent the carcinoma of the breast. The median age of the patients was (53.7) years. In addition formalin-fixed, paraffin embedded blocks tissue for fibroadenoma of breast were collected and used as control group with median age (49.4) years.

In Situ hybridization (ISH) for detection of MMP-9 gene

A Biotin – Labeled DNA probe for MMP-9 / DNA (Maxim Biotic, USA) 216bp, MMP-9 (8 µg/ 100 µl) litter dd H2O (Maxim Biotech, Inc., USA).,was used.

In situ hybridization (ISH) is a technique makes use of the high

specificity of complementary nucleic acid binding to detect specific DNA or RNA sequence in the cell. For detection of this marker, the biotinylated DNA probe hybridized to the target sequence (MMP-9 mRNA sequence) then a streptavidin-AP (streptavidin-alkaline phosphatase). Conjugate was applied followed by addition of the substrate promo-chloro-indolyl-phosphatel / nitro-blue tetrazolium (BCIP/NBT) which yielded an intense blue-black signal that appeared at the directly specific site of the hybridized probe. This strepteividin-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

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 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 (18).

Statistical Analysis

The suitable statistical methods were used in order to analyze and assess the results. The associations between the person's of MMP -9 in different groups (breast cancer patients and fibroadenoma group) were assessed by using Pearson's Chi – Square test. *P* - Value < 0.05 and P < 0.05 was considered statistically significant (19).

Results

The expression of MMP -9 was detected by ISH technique. Tables 1 and 2 show the percentage of frequency scoring for MMP -9 mRNA expressions among study groups, respectively. Chi-square test was conducted to examine the association between MMP -9 mRNA expression in the tissue in the two groups of investigation ,it was found that highly significant association ($p<0.01$) between them among the four scoring levels. The mean percentages of the MMP -9 was significantly higher ($p<0.001$) in malignant breast cancer compared with fibroadenoma tumor as demonstrated in (Table 1). The results showed that percentages of mRNA expression of MMP - 9 were in (≥ 10-50%) for malignant breast cancer. This research also showed that (60%) of fibroadenoma breast tumor were expression less than (<10%) and (10%) of these patients between (10 – 50 %) for MMP -9 mRNA. The expression of MMP - 9 was heterogeneous blue-black staining in the tissue, as shown in Figure (1).

Table 1: Comparison of mean percentages of MMP-9 mRNA among studied groups

Studied groups	No.	Mean ± SE	*P* value
Fibroadenoma of breast	30	11.72 ± 4.86	0.00**
Breast cancer	30	70.58 ± 3.32	

****** Highly significant difference $P< 0.001$

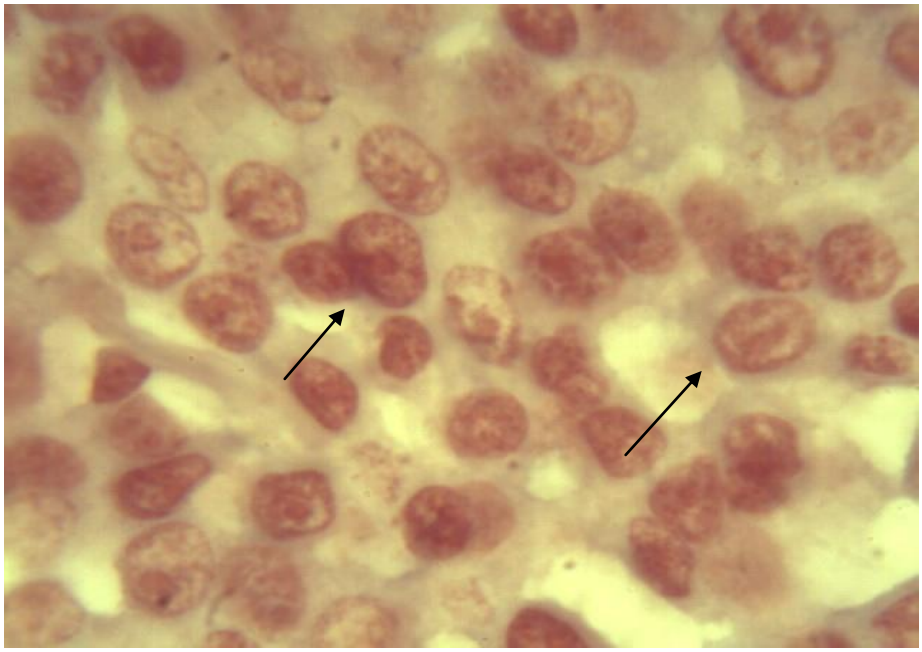

Figure (1): **Detection of MMP -9 in studied groups by in situ hybridization (ISH)**. Staining of MMP -9 mRNA by BCIP/NBT (blue-black) counterstained with nuclear fast red. Tissue from breast cancer patients shows positive MMP -9 hybridization signals (X400)

Table 2: Scoring of MMP-9 expression in Fibroadenoma and patients with breast adenocarcinoma

Groups	No.	0	Low < 10%	Medium 10 – 50 %	Extensive > 50%	P value
Fibroadenoma of breast	30	9 (10%)	18 (6.657%)	3 (17.633%)	0	< 0.001
Breast cancer	30	0	0	2 (32.35%)	28 (71.7%)	

Discussion

Invasive breast cancer varies widely in biologic aggressiveness, from fairly indolent tumors to rapidly disseminating carcinomas. Matrix metalloproteinase have enzymatic activity and assist in tumor invasion by degrading basement membranes and extracellular matrix (16). The key event of invasive growth of malignant epithelial tumors is the dissolution of the peritumoral basement membrane (BM) (20). Matrix metalloproteinases selectively degrade various components of the extracellular matrix (ECM) and release growth factors and cytokines that reside in the ECM (21, 22). 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.) (23, 24). Through their proteolytic activity, MMPs play crucial roles in invasion and metastasis and regulate signaling pathways that control cell growth, survival, invasion, inflammation and angiogenesis (25, 26).
Our results revealed high frequency of MMP-9 activities in breast cancer as compared to control (fibroadenoma) of breast (P<0.001), and showed an overexpression (71.7 %) of MMP-9 mRNA in breast carcinoma, and these results agree with some previous studies, in which MMP-9 protein and mRNA have been mostly localized into stromal cells (16, 27), and have been detected in carcinoma cells (16, 17, 27, 28, 29, 30).
Previously, it has been suggested that stromal fibroblasts secrete MMPs, which are stored and activated in carcinoma cells (31).
In zymography studies, the expression of MMP-2 and MMP-9 has been detected in benign tissue, and enzyme activities have been less than in malignant tissue (17, 30, 31).
There are many explanations for increasing MMP-9 expression in breast cancer tissue, in which expression of MMP-2 and MMP-9 in breast cancer seems to be partly related to expression of activator protein-2(AP-2) and HER 2. Positive stromal MMP-9 expression predicts poor survival in the hormone-responsive small tumors, whereas MMP-9 expression in carcinoma cells favors survival (29).
There is a factor known as Krüppel-like factor 8 (KLF8), which plays a key role in oncogenic transformation and is highly overexpressed in several types of invasive human cancer, including breast cancer (32), and it's overexpression induced a strong increase in MMP-9 expression (28).
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, galatinases generate or release bioactive molecules that influence tumor progression (33, 34). The overexpression of MMP-9 in breast cancer may be used as a marker to subdivide node negative breast cancer patients in order to determine the optimal treatment modality (18). The MMps including MMP-9 are likely to be involved in mediating breast cancer progression and may thus be good targets for designing specific MMP inhibitors for the treatment of breast cancer (35).
In conclusion, the results are consistent with results of previous studies suggesting that MMP-9 may play important role in breast cancer or could facilitate its progression.

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