Matrix metalloproteinase-1 expression in human breast cancer: morphological analysis using *in situ* hybridization technique

Ahmed Basim Shehab, Alhindawi Mohanad Mahdi* and Alsikafi Mays Ibrahim
Department of Pathology and Forensic Medicine, College of medicine, Mustansiriyah University, Baghdad, IRAQ
*alhindawi_mohanad@uomustansiriyah.edu.iq

Abstract
Breast cancer is the most frequent malignancy of women worldwide. Matrix metalloproteinase-1 (MMP-1) a member of the MMPs protease family has been considered a factor of decisive importance in cell invasion and metastasis of breast cancer. MMP-1 mRNA expression was assessed by in situ hybridization (ISH) technique in 56 retrospective breast carcinoma cases and 30 fibroadenoma cases.

Overexpression of MMP-1 was detected in 58.86% of the breast cancer cases and 26.37% of fibroadenoma tissue. The highest scoring expression of MMP-1 mRNA was in breast cancer tissue (41.04%) and in Grade III carcinoma (50%). MMP-1 mRNA was significantly overexpressed in breast cancer cases in comparison with Fibroadenoma cases and achieve significantly higher expression score in cancer cases particularly in Grade III carcinomas.

Keywords: MMP-1, Breast Cancer, *In situ* hybridization.

Introduction

Breast cancer is a pathologically and clinically heterogeneous disease.[10] It is the most frequent malignancy of women worldwide. In Iraq, breast cancer ranks first among cancers diagnosed in women with the Iraqi cancer registry data during the period 2000-2009, showing rising incidence of all female cancers across all age groups.[1]

Metastatic disease rather than primary tumor itself is responsible for death in most solid tumors.[17] There are many events for the metastatic process including the organized destruction of the extracellular matrix (ECM) by matrix metalloproteinases (MMPs).[9]

The MMPs have the ability for processing and degradation of all ECM components[16] and the activity of these proteins is highly regulated by certain inhibitors known as tissue inhibitors of MMPs (TIMPs).[11]

Matrix metalloproteinase family members have been associated with advanced-stage cancer and contribute to tumor progression, invasion and metastasis as determined by inhibitor studies.[7,8] There is a very tight and complex regulation in the expression of this family of proteases in breast cancer that generally represents a host response to tumor.[7] In this regard, MMPs represent an important factor for supporting cancer cell metastasis because they are responsible for degradation and digestion of the ECM components of the basement membrane.[12] One of these proteins is matrix metalloproteinase-1 (MMP-1) that is known also as interstitial collagenase and fibroblast collagenase, which is an enzyme that is encoded by the MMP-1 gene in humans.[4] This gene is part of a cluster of MMP genes that localizes to chromosome 11q22.3.[14]

In breast cancer, matrix metalloproteinase-1 (MMP-1) has been considered a factor of decisive importance especially in cell invasion and metastasis.[19] The expression of this protein has been reported to inversely correlate with survival in advanced cancers and it is often up regulated in breast cancer especially in basal-type breast tumors.[3] Expression of MMP genes is transcriptionally regulated by a variety of extracellular contacts to ECM.[18]

The expression of MMP-1 in breast cancer and fibroadenomatous breast tissue has been established by several previous studies, however we conducted to address this issue by using *in situ* hybridization to detect the MMP-1 mRNA expression. An extended understanding of the expression of this protein may provide an important information about the role of MMP-1 in pathological process of breast tumorigenesis.

Material and Methods

Fifty-six breast carcinoma cases with paraffin embedded tissue samples were obtained from the files of the Department of Pathology at Al-Yarmouk and Baghdad Teaching Hospitals. The samples were evaluated to represent the carcinoma of the breast. The median age of the patients was (53.6) and ranged between 30-77 years. The control group included (30) fibroadenoma tissue with a mean of age (49.3) and range between 33 and 75 years. For each patient included in this study, tissue sections were cut into 4μm thickness and put on Fisher brand positively charged slides.

***In Situ* hybridization (ISH) for detection of MMP-1 mRNA:** The use of Biotin – Labeled DNA probe for MMP-9 / DNA (Maxim Biotic, USA) 216bp, MMP-9 (8μg/ 100 μl) litter dd H_2O (Maxim Biotech, Inc., USA).

In situ hybridization (ISH) is a technique making 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 hybridizes to the target sequence (MMP-1 mRNA

sequence), then a streptavidin-AP (streptavidin-alkaline phosphatase) conjugate is applied followed by addition of the substrate promo-chloro-indolyl-phosphatel / nitro-blue tetrazolium (BCIP/NBT) which yields an intense blue-black signal appearing 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.

The expression of MMP-1 mRNA was measured by counting of the number of the positive cells in the tissue that has given a blue-black (BCIP/NBT) 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 staining distribution below 10% was regarded as negative. For statistical analysis, score of (0) was given when no staining was detected, (1) if there was weak staining in less than or equal to 10% of cells, (2) if moderate staining was present in cells and (3) if strong staining 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-1between different groups (breast cancer patients group and control group). Values were considered statistically significant when $p < 0.05$.

Results

The results of *in situ* hybridization detection of MMP-1 are shown in table 1 which demonstrated that overexpression of MMP-1 was detected in 58.86% of the breast cancer tissue (figure 1B) and 26.37% of fibroadenoma tissue (figure 1A), with highly significant difference ($P< 0.01$) in the mean percentage of this protein mRNA expression in tissue of breast carcinoma and breast fibroadenoma.

Table 2 showed the distribution of MMP-1 mRNA scoring expression among studied groups, in which the strong expression was the most frequent score in breast cancer tissue 41.04% (21 out of 56) followed by moderate expression in 30.14% (18 out of 56) and then 19.64% (11 out of 56) represent the weak score of expression in breast carcinoma tissue and when compared with fibroadenoma breast tissue, they showed highly significant difference ($P<0.01$).

Histopathological examination of 56 breast carcinoma tissue included in this study showed that there was 22 of grade II breast carcinoma tissue and 34 of grade III breast carcinoma tissue. Comparison between the expression of MMP-1 mRNA among histopathological variables in breast carcinoma tissue has been shown in table 3, in which the strong score was expressed in 50% of grade III breast carcinoma and the results showed highly significant difference between the two grades (P value <0.01).

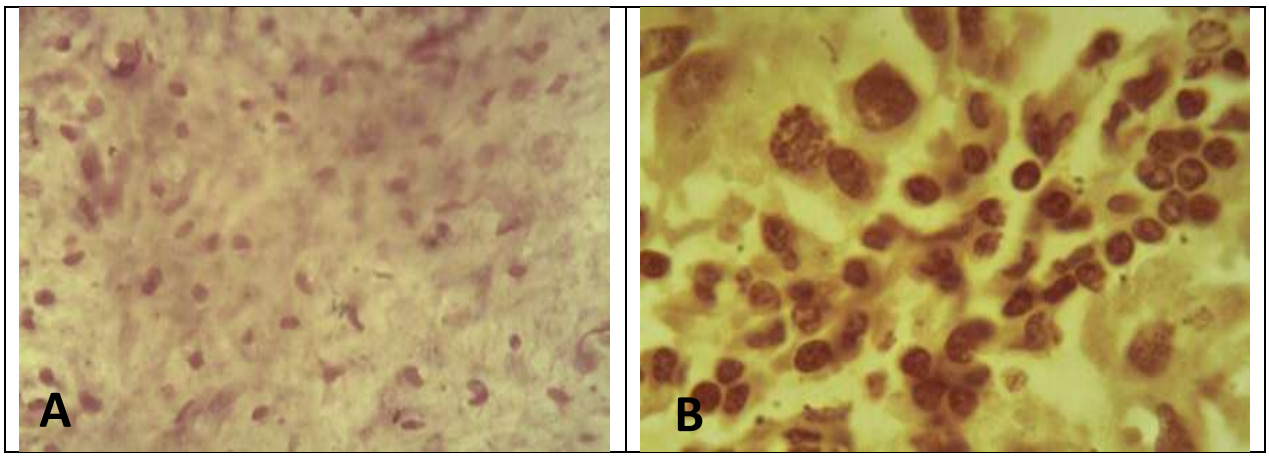

Figure 1: Detection of MMP-1 in studied groups by in situ hybridization (ISH). Staining of MMP-1 mRNA by BCIP/NBT (blue-black) counterstained with nuclear fast red. (A) Tissue from fibroadenoma patients shows positive MMP-1 hybridization signals (X400). (B) Tissue from breast cancer patients shows positive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

Table 1
Comparison of mean percentage of MMP-1 mRNA in Breast cancer and Fibroadenoma patients.

Studied group	No.	Mean ± SE	Comparison of Sig.	
			P value	Sig
Breast cancer	56	58.86± 3.45	0.00	Highly sig (p<0.01)
Fibroadenoma	30	26.37± 2.27		

Table 2
Distribution of MMP-1 mRNA expression among studied group

ISH MMP-1		Studied group		Comparison of Sig.	
		Breast cancer no. 56	Fibroadenoma no.32	p value	Sig.
0	No	6	14	0.00	Highly sig. (p<0.01)
	%	7.14%	43.75%		
1	No	11	13		
	%	19.64%	40.62%		
2	No	18	5		
	%	30.14%	15.62		
3	No	21	0		
	%	41.07%	0		

Table 3
Comparison between the expressions of MMP-1 mRNA among histopathological variables in breast cancer patients.

ISH MMP-1		Studied group			Comparison of Sig.
		Grade II No.22	Grade III No. 34	p value	Sig.
0	No	6	0	0.00	Highly sig. (p<0.01)
	%	27.27%	0		
1	No	8	3		
	%	36.36%	8.82%		
2	No	4	14		
	%	18.18%	41.17%		
3	No	4	17		
	%	18.18%	50%		

Discussion

Matrix metalloproteinase proteins (MMPs) were previously proved to be expressed in nearly all human tumors, facilitating tumor growth, invasion and metastasis.[9,16] The study by Xuan et al[19] using immunohistochemical staining, provided a novel discovery that MMP-1expression is higher in non-specific invasive ductal carcinoma and lymph node metastatic carcinoma compared to cancer-adjacent normal breast tissue and normal lymph node tissue.

The present study showed the overexpression of MMP-1 in breast carcinoma tissue compared to breast fibroadenoma tissue and this result is compatible to higher expression of this protein in breast cancer closely associated with its invasion and metastasis.[5,6] Poola et al[15] identified that MMP-1 is a candidate marker that may be useful for identification of breast lesions that can develop into cancer.

The first step of stroma generation is of pivotal importance for carcinogenesis because at this stage angiogenesis is initiated which is important for continuing the growth of tumor and the proliferation of stromal fibroblasts. These developments contribute to the onset of tumor invasion and this invasion is caused by secreting several matrix-degrading proteases. These two processes are tightly controlled at several levels of significant importance in transcription. There is certain factor affect involved in both tumor vascularization and invasion, like Ets-1 transcription factor transactivates several genes encoding matrix-degrading proteases.[2]

Conclusion

MMP-1 mRNA was significantly overexpressed in breast cancer cases in comparison with Fibroadenoma cases and achieves significantly higher expression score in cancer cases particularly in grade III carcinomas

Acknowledgement

The authors would like to thank Mustansiriyah University (www.uomustansiriyah.edu.iq) Baghdad, Iraq for its support in the present work.

References

1. Al-Hashimi M.M. and Wang X.J., Breast cancer in Iraq, incidence trends from 2000-2009, *Asian Pac J Cancer Prev*, **15(1)**, 281-6 **(2014)**

2. Behrens P., Rothe M., Wellmann A., Krischler J. and Wernert N., The Ets-1 transcription factor is up-regulated together with MMP 1 and MMP 9 in the stroma of pre-invasive breast cancer, *J Pathol*, **194(1)**, 43-50 **(2001)**

3. Bostrom P., Soderstrom M., Vahlberg T., Soderstrom K.O., Roberts P.J., Carpen O. and Hirsimaki P., 'MMP-1 expression has an independent prognostic value in breast cancer, *BMC Cancer*, **11**, 348 **(2011)**

4. Brinckerhoff C.E., Ruby P.L., Austin S.D., Fini M.E. and White H.D., Molecular cloning of human synovial cell collagenase and selection of a single gene from genomic DNA, *J Clin Invest*, **79(2)**, 542-6 **(1987)**

5. Chabottaux V. and Noel A., Breast cancer progression: insights into multifaceted matrix metalloproteinases, *Clin Exp Metastasis*, **24(8)**, 647-56 **(2007)**

6. Egeblad M. and Werb Z., New functions for the matrix metalloproteinases in cancer progression, *Nat Rev Cancer*, **2(3)**, 161-74 **(2002)**

7. Hemati S., Sadeghi M., Motovali-Bashi M., Sahebi A. and Malekshahi Z.V., Higher plasma MMP-9 level in breast cancer patients with MMP-9 promoter T allele, *Journal of Biological Research*, **13**, 113 **(2010)**

8. Heppner K.J., Matrisian L.M., Jensen R.A. and Rodgers W.H., Expression of most matrix metalloproteinase family members in breast cancer represents a tumor-induced host response, *Am J Pathol*, **149(1)**, 273-82 **(1996)**

9. Itoh Y. and Nagase H., Matrix metalloproteinases in cancer, *Essays Biochem*, **38**, 21-36 **(2002)**

10. Jemal A., Siegel R., Ward E., Hao Y., Xu J. and Thun M.J., Cancer statistics, 2009, *CA Cancer J Clin*, **59(4)**, 225-49 **(2009)**

11. Lambert E., Dasse E., Haye B. and Petitfrere E., TIMPs as multifacial proteins, *Crit Rev Oncol Hematol*, **49(3)**, 187-98 **(2004)**

12. Nagase H. and Woessner J.F., Matrix metalloproteinases, *J Biol Chem*, **274(31)**, 21491-4 **(1999)**

13. Ozden F., Saygin C., Uzunaslan D., Onal B., Durak H. and Aki H., Expression of MMP-1, MMP-9 and TIMP-2 in prostate carcinoma and their influence on prognosis and survival, *J Cancer Res Clin Oncol*, **139(8)**, 1373-82 **(2013)**

14. Pendas A.M., Santamaria I., Alvarez M.V., Pritchard M. and Lopez-Otin C., Fine physical mapping of the human matrix metalloproteinase genes clustered on chromosome 11q22.3, *Genomics*, **37(2)**, 266-8 **(1996)**

15. Poola I., DeWitty R.L., Marshalleck J.J., Bhatnagar R., Abraham J. and Leffall L.D., Identification of MMP-1 as a putative breast cancer predictive marker by global gene expression analysis, *Nat Med*, **11(5)**, 481-3 **(2005)**

16. Visse R. and Nagase H., Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function and biochemistry, *Circ Res*, **92(8)**, 827-39 **(2003)**

17. Welch D.R., Steeg P.S. and Rinker-Schaeffer C.W., Molecular biology of breast cancer metastasis. Genetic regulation of human breast carcinoma metastasis, *Breast Cancer Res*, **2(6)**, 408-16 **(2000)**

18. Westermarck J. and Kahari V.M., Regulation of matrix metalloproteinase expression in tumor invasion, *Faseb J*, **13(8)**, 781-92 **(1999)**

19. Xuan J., Zhang Y., Zhang X. and Hu F., Matrix metalloproteinase-1 expression in breast cancer and cancer-adjacent tissues by immunohistochemical staining, *Biomed Rep*, **3(3)**, 395-397 **(2015)**.