Histopathological and immunohistochemical study of matrix metalloproteinase-2 and matrix metalloproteinase-9 in breast carcinoma

Naglaa F. Abbas[a], Marwa El-Araby Shabana[a], Fahima M. Habib[b], Ahmed A. Soliman[b]

[a]Department of Pathology, Division of Medical, National Research Centre, [b]Department of Pathology, Faculty of Medicine, Cairo University, Giza, Egypt

Correspondence to Marwa El-Araby Shabana, MD, Reasercher at NRC, Department of Pathology, Division of Medical, National Research Centre, Al Bouhouth Street, Dokki, Giza, Egypt Tel: +20 237 408 974/ 20 122 430 80808;
e-mail: marwashabana999@gmail.com

Received 24 January 2017
Accepted 26 February 2017

Journal of The Arab Society for Medical Research 2017, 12:6–12

Background/aim
Breast cancer is the most harmful tumor among women around the world, with increasing incidence rates. Invasion and metastasis are the most insidious and life-threatening parts of the cancer. Efforts have been made to understand the mechanisms that regulate and facilitate the metastatic process. This step in metastasis of malignant cells requires the association of proteolytic catalysts that degrade protein segment of the extracellular matrix. Matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) are the members of degrading enzymes required in tumor advancement, invasion, and metastasis. This work aims to examine the utility of MMPs in breast carcinoma to assess their usefulness in growth, invasion, and metastasis.
Patients and methods
A total of 60 samples of cases of breast cancer with positive and negative lymph nodes were collected randomly as paraffin blocks which was previously prepared material. The samples from the cases were immunostained for MMP-2 and MMP-9, and their expression was correlated with various clinicopathological parameters.
Results
Most cases of cancer were presented in the age group 51–60 years. The most common type was invasive duct carcinoma Not Otherwise Specified (NOS), representing 70% of cases. Overall, 51 (85%) cases were positive for MMP-2 whereas 54 (90%) cases were positive for MMP-9. Presence of MMP-2 and MMP-9 in peritumoral stroma was in the ratio of 60 and 64%, respectively. Both markers were significantly elevated in malignant tissues of patient with lymph node metastasis as compared with those without lymph node metastasis (P=0.029 and 0.048, respectively). The expression of MMP-2 and MMP-9 increased with advanced clinical staging and grading (P=0.015 and 0.011).
Conclusion
Expression of MMP-2 and MMP-9 in breast cancer is closely correlated with positive lymph node, high histological grade, and advanced clinical stage. More studies with a huge sample size are needed to assess the prognostic role of MMPs in breast cancer. The use of MMP inhibitors as an adjuvant treatment for breast carcinoma is recommended.

Keywords:
breast carcinoma, metalloproteinase-2, metalloproteinase-9

J Arab Soc Med Res 12:6–12
© 2017 Journal of The Arab Society for Medical Research
1687-4293

Introduction

All over the world, millions of women are determined to have breast disease [1,2]. In Egypt, breast malignancy is the most understood female cancer, which ranks first according to Cancer Pathology Registry 2003–2004 with 18%, and is considered the most widely recognized reason for mortality in Egypt [3–5]. In addition, both invasion and metastasis are considered the most common life-threatening factors in carcinoma. Cancer cells are outfitted with many proteolytic enzymes which appear to have a part in cancer dissemination [6]. The most significant point in cancer is spreading of tumor cells, and once it happens, the patient cannot be cured by just nearby therapy = usual therapy [7].

The procedure of metastasis includes intravasation, extravasation of tumor cells, and then implantation at distant sites accompanied by formation of new angiogenesis. These steps in metastasis require proteolytic enzymes that cause extracellular matrix protein degradation. Matrix metalloproteinase (MMP) is a class of these proteins and thought to assume critical part in this process [8].

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work noncommercially, as long as the author is credited and the new creations are licensed under the identical terms.

MMPs are group of homologous, zinc (Zn^{2+})-dependent endopeptidases and calcium-dependent extracellular enzymes that assume important role in extracellular matrix degradation related to tumor cell infiltration, metastasis, and angiogenesis. Matrix metalloproteinase A and B (MMP-2 and MMP-9) are two related entities of the MMP family, with a capacity to degrade collagens or gelatins, but they are different from other MMPs as they can decay gelatinase and type IV collagen, the principal part of basement membrane [8,9].

The size of MMP-2 is 72 kDa whereas MMP-9 is 92 kDa, which can separate extracellular matrix that ties to gelatin or distinctive types of collagens. Also, both markers possess various extracellular matrix atoms including type IV, V, and XI collagens, laminin, and aggrecan. MMP-2 digests collagens I, II, and III in a way similar to collagenases, yet MMP-9 cannot do this. It was postulated that both MMP-2 and MMP-9 were not synthesized by the tumor epithelium itself but rather by the encompassing tumor stroma, mainly fibroblasts [10].

Numerous clinical and preclinical information proposed that MMPs assume a critical role in the process of cancer progress. Many reports have demonstrated that MMP inhibitors were required in the treatment of various kinds of tumors, for example, leukemias, lymphomas, testicular cancer, lung cancer, gastrointestinal cancer, and oropharyngeal cancer [11].

At present, nodal status is the most important prognostic parameter in breast cancer, so the higher expression of MMPs in negative lymph node tissue points to increased danger of metastasis. Therefore, it may be important to use MMP inhibitors as an adjuvant therapy in breast cancer [12].

The present work aims to investigate the expression of MMP-2 and MMP-9 in breast carcinoma and correlate their expression with various clinicopathological parameters to assess their role in growth and metastasis.

Patients and methods

The present study included 60 samples of cases, which were collected randomly from the Pathology Department, Faculty of Medicine, Cairo University, from 2010 to 2012. Most of collected cases blocks gained by subtotal mastectomy. The ethical committee on human research at NRC (Egypt) approved the protocol for the study.

Patients' data

The available clinical data were obtained from patients' medical reports, including age, size of the tumor (largest diameter), clinical staging, histopathological grade and lymph node status, and results of hormonal state, which were routinely examined.

Overall, three sections 4-μm thick were cut from each block: one section was stained with hematoxylin and eosin to revise the diagnosis and tumor histological type by routine histopathologic study, and the other sections were mounted on a positively charged glass slide for immunohistochemical staining.

Immunohistochemical staining was performed according to the streptavidin biotin peroxidase complex. Sections were deparaffinized with xylene and rehydrated with graded ethanol. Antigen retrieval was done by incubating the sections in 10-ml sodium citrate buffer (pH 6.0) for 10 min in a conventional microwave oven. The endogenous peroxidase activity was blocked by immersing the tissue sections for 10–20 min in 3% hydrogen peroxide with methanol. The nonspecific reactivity to other proteins was blocked by nonimmune protein-blocking serum. The primary antibody for both markers used was the mouse myeloma at a dilution 1 : 40–1 : 80 for 60 min at 25°C. Detection was performed using ultravision detection system (Lab Vision Corporation, United State).

Immunostaining results were interpreted blindly to the specific diagnosis for each individual case based on the percentage of positive tumor cells that was evaluated in 10 fields at original magnification ×200.Tumor cells that showed cytoplasmic staining were considered positive [13].

Cytoplasmic expression of MMP-2 and MMP-9 was evaluated semiquantitatively in tumor and stromal cells according to the percentage of positivity and assigned to one of the four following grades: 0: negative, 1: focally positive (1–10% positive cells in the lesion), 2: moderately positive (11–50%), and 3: markedly positive more than 50%. The intensity of staining was semiquantitatively assessed as weak, moderated, or strong [14].

Statistical analysis

Data were statistically described in terms of frequencies (number of six cases) and relative frequencies (percentages). χ^2-Test was performed to compare the different study variables between the study groups. Yates correction and Fisher's exact tests were used only when the expected frequency was found to be

less than five. A *P* value of less than 0.05 was considered statistically significant.

All statistical calculations were done using computer programs Microsoft Excel, version 7 (Microsoft Corporation, New York, New York, USA) and statistical package for the social science (SPSS; SPSS Inc., Chicago, Illinois, USA) statistical program.

Result

In this study, the mean age of patients with breast carcinomas was 53.2 years. The common age group was within the fifth decade. Most of the cases were of low grade. Intraductal component was present in only 25 (42%) cases, whereas the remaining 35 (58%) cases were devoid of it. The group in which tumor size ranged from 2 to 5 cm (pT2) was the most common group (56%), with mean maximal dimension was 3.9 cm. A total of 60 cases were tested for estrogen-receptor (ER) and progesterone receptor (PR) reaction, and their reports were available. More than half of cases (43, 72%) were estrogen positive, and 45 (75%) cases were progesterone positive.

Immunostaining of MMP-2 and MMP-9 in breast cancer

Staining of MMP-2 and MMP-9 in this study was detected in the cytoplasm of malignant cells and in the peritumoral stroma. In addition, vascular endothelium stained positively for both markers (Figs 1–4). Number of cases expressing MMP-9 was 51 (85%) whereas cases expressing MMP-2 was 54 (90%).

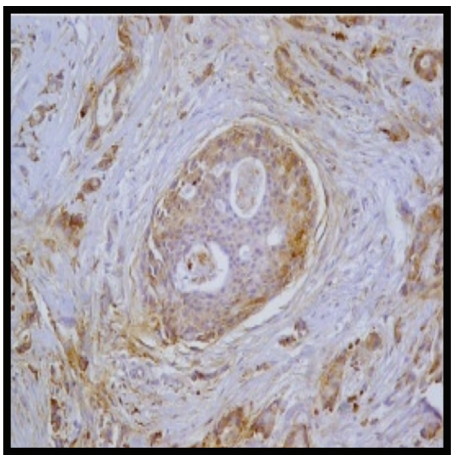
The immunostaining of MMP-2 and MMP-9 was correlated with clinic-pathological parameters, including age, clinical staging of the tumor, histological grade, lymph node status, and histological grade.

Both markers' expression in the tumor cells was high, with median value of 85% for MMP-9 and 90% for MMP-2. MMP-2 expression was observed in 60% of stromal cells whereas MMP-9 positivity was observed in 64% of stromal cells (Fig. 5).

From the age group 50 years or younger, 91% showed positive immunostaining, and only four cases showed negative reaction. On the contrary, 13 patients older than 50 years were positive for both markers. However, the correlation between age and both MMP-9 and MMP-2 was statistically insignificant (*P*=0.659).

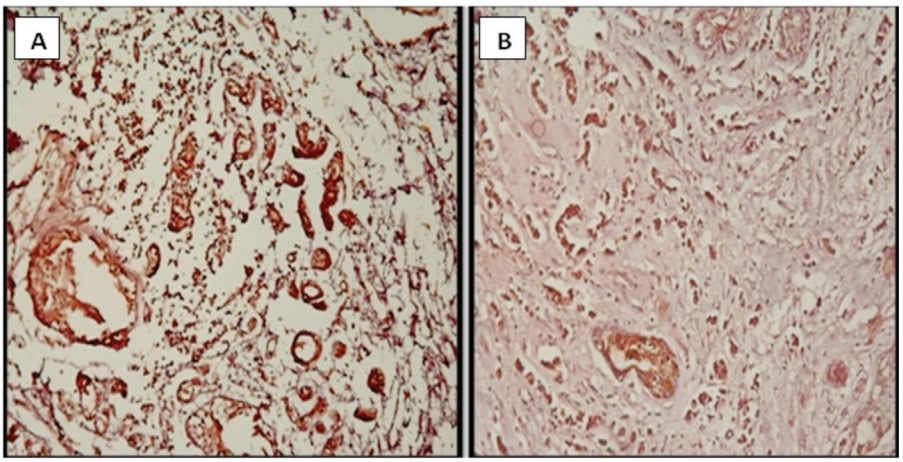
Significant increase in the positivity of MMP-2 and MMP-9 was seen with each progressed clinical phase of disease, demonstrating increase expression in tumor

Figure 2



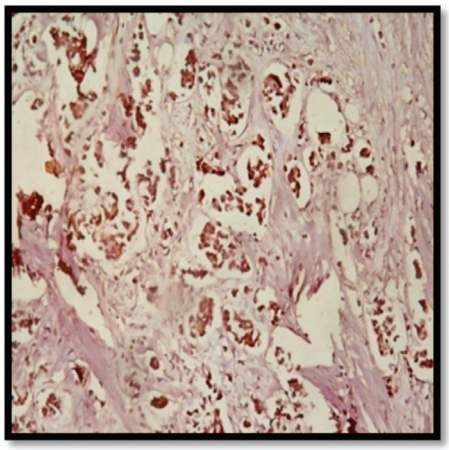
Invasive duct carcinoma, with prominent intraductal component, shows MMP-9 immunostaining (immunoperoxidase ×200). MMP-9, matrix metalloproteinase-9

Figure 1



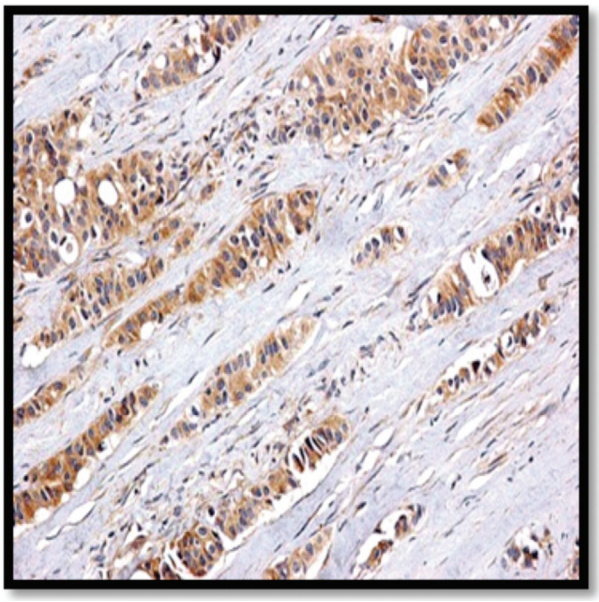
Microscopic pictures show (a) invasive duct carcinoma with strong positivity for MMP-9 and (b) moderate positivity for MMP-2 (immunoperoxidase ×200). MMP-2, matrix metalloproteinase-2; MMP-9, matrix metalloproteinase-9

Figure 3



Mucinous carcinoma of the breast shows strong MMP-2 immunostaining (immunoperoxidase ×200). MMP-2, matrix metalloproteinase-2

Figure 4



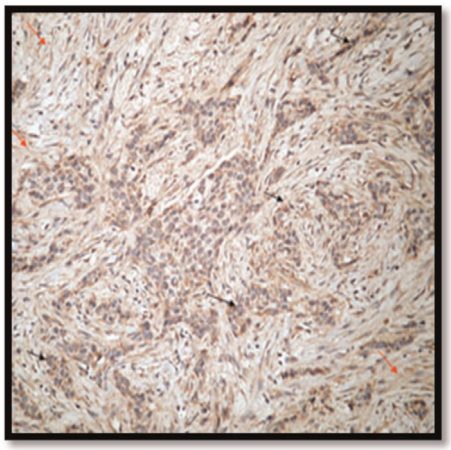
Invasive lobular carcinoma shows strong MMP-9 positivity (immunoperoxidase ×400). MMP-9, matrix metalloproteinase-9

tissue of the breasts with clinical stage III in contrast with those with clinical stages I and II ($P \leq 0.001$).

By considering correlation between MMP-2 and MMP-9 positivity and tumor grade, which is divided into low grade and high grade, our results shows that significant statistical association could be found when comparing the expression of both markers (MMP-2 and MMP-9) with grades of all studied ($P=0.015$ and 0.011).

From all malignant cases with positive lymph node, all cases were positive (100%) for MMPs, whereas among

Figure 5



Invasive duct carcinoma shows strong stromal positivity for MMP-2 (red arrows), and moderate tumor cell positivity (black arrow) (immuoperoxidase ×100). MMP-2, matrix metalloproteinase-2

the nodal negative cases, only one showed weak positive staining. Both MMP-2 and MMP-9 were essentially lifted in malignant tissues of patient with positive lymph node when contrasted with tumor tissues of patients without lymph node metastasis ($P=0.029$ and 0.048, respectively). Both matrix metalloproteinase 2 and 9 expression indicated a highly significant with ER positive and PR positive cases with ($P=0.014$ and 0.034) (Table 1).

Discussion

Breast cancer is the most widely recognized tumor affecting women in the world today. It is the leading cause of death in women worldwide; millions of women are determined to have breast cancer consistently, accounting for a tenth of all new cancers [1]. In Egypt, the National Cancer Institute 2002 cancer registry outlined that the incidence of breast cancer was 19%, ranking first among common cancers in women, representing 37.5% of all women cancer cases [2]. Despite breast cancer consider a disease of developed countries, Most brearst cancer death ocuur in developing countries (69%) [15,16]. Metastasis is considered the essential cause of disease mortality, this is why the need to focus on the manner which direct this procedure. It is understood that development and spread of cancer cell to other areas to a great extent relies on the response between tumor cells and their environment. Likewise, turn over from ductal carcinoma *in situ* to invasive breast carcinoma is a poorly understood event in the progression of breast carcinoma. A portion of the potential organic markers of progression to invasion in ductal carcinoma *in situ* are the particles involved in the stromal connective

Table 1 The correlation of MMP-2 and MMP-9 expression with clinical and pathological parameters

Factors	MMP-2 [*n* (%)]	*P* value	MMP-9 [*n* (%)]	*P* value
All patient	54 (90)		51 (85)	
Size (cm)				
<2	14 (26)		14 (27)	
2–5	34 (63)		34 (67)	
>5	6 (11)		3 (6)	
Grade		0.015		0.011
Low grade	14		16	
Mild	2 (14)		3 (19)	
Moderate	5 (36)		5 (31)	
Marked	7 (50)		8 (50)	
High grade	40		35	
Mild	12 (30)		11 (31.4)	
Moderate	11 (27.5)		9 (25.7)	
Marked	17 (42.5)		15 (42.9)	
Nodal state				
Positive cases [*n* (%)]		0.029		0.048
Positive LN	54 (100)		51 (100)	
Negative LN	0%		0%	
Negative cases				
Positive LN	1 (16.7)		1 (11)	
Negative LN	5 (83.3)		8 (88)	
Hormonal state				
ER		0.014		0.014
Negative	17		17	
Positive	34		34	
PR		0.034		0.034
Negative	10		10	
Positive	41		41	

ER, estrogen-receptor; LN, lymph node; MMP-2, matrix metalloproteinase-2; MMP-9, matrix metalloproteinase-9; PR, progesterone receptor.

tissue degradation and invasion of the basement membrane; some of these segments, especially the interstitial collagens, are exceptionally impervious to proteolytic attack and can be corrupted just by MMPs. This reality was the principal purpose behind examining metalloproteinase expression in the tumor cells and in the tumor tissue stroma [17].

It was recognized that MMP-2 and MMP-9 have gotten more prominent consideration of late as implicated as causative part in disease attack and metastasis. Various preclinical and clinical data suggested that MMPs assume a critical part in the process of cancer progression in many organs. Increase level of MMP-2 had been seen in invasive region of colorectal tumors and connected with Dukes stage and in addition increase level of MMP-2 had been seen in invasive region of colorectal tumors and this connected with DUKES stage, in addition increase level of MMP-2 in cases of polyp progress to adenocarcinoma [18,19]. It was shown that extended articulation of MMP-9 was a critical indicator of poor prognosis for patients with nasopharyngeal carcinoma, particularly at late stage, encouraging pharmaceutical industries for synthesizing MMP inhibitors as antimetastatic therapy [20].

For this reason, in our study, we aim to evaluate prognostic value of MMP-2 and MMP-9 in breast carcinoma. Therefore, 60 cases from Cairo University were collected and studied by using immunohistochemistry.

In the present study, age of patients range from 28 to 82 years with a mean age 53.67±11.01 years. Patients aged 50 years and older constituted the greater part of the studied cases (60.87%). Past review of Omar *et al.* [21] detected that in Egypt breast cancer is common among younger age and majority of cases occurring at 30–60y of age with middle age 46y; one decade more youthful than the corresponding age in Europe and North America. The one decade younger incidence was also reported by Middle East Cancer Consortium [15]. This was not the case in our study. Most of patients were 50 years and older with a link or trend to postmenopausal status. The increased incidence after 50 years has been reported recently in Egyptian patients [22,23].

Our study investigated the immunohistochemical expression profile of MMP-2 and MMP-9 in breast carcinoma. For interpretation of both markers positivity, tumor cells were examined for their expression in the cytoplasm of both malignant and stromal cells. This is similar to the interpretation used by Ranogajec *et al.* [23] in their previous study. We found that cytoplasmic MMP-2 and MMP-9 expression was seen at 60 and 64% of stromal cells, respectively. Positive expression of MMP-9 and MMP-2 in stromal cells showed significant correlation with clinicopathological parameters related to aggressive disease. Also, MMP-2 and MMP-9 expression was found in the cytoplasm of tumor cells at 90 and 85%, respectively. This goes with what was seen by Ranogajec *et al.* [23] who suggested the significance of stromal cells as treatment target for breast cancer.

Previous studies reported that MMP-9 is useful as an independent unfavorable prognostic component for breast cancer growth, demonstrating that presence of MMPs in the stroma as a host response to presence of tumor cells may assume a part in the control of tumor prognosis [24].

The current study showed that patients with large tumor size had significantly greater activity for MMP-2 and MMP-9 in comparison with those with small tumors. Similar findings were obtained by Kiris *et al.* [24] who proved that expression of MMP-2 basically associated with tumor size (P=0.012). Additional studies on this issue gave same results, as Chen *et al.* [25] proved that expression of MMP-2 significantly correlated with tumor size (P=0.012). In contrast, Ranogajec *et al.* [23] proved that MMP-2 and MMP-9 had no statistical significant correlation between tumor and stromal MMP-2/MMP-9 positivity and other standard prognostic factors except for tumor size.

Past study of Li *et al.* [12] announced that MMP-2 and MMP-9 expression extended in high-grade tumors more than that in low grade ones. This was in agreement with the current study, which demonstrated that patients with high-grade tumor show high expression of MMP-2 and MMP-9 (P=0.015 and 0.011). Also, these results was in agreement with Mylona *et al.* [26] who confirmed the same result. In contrast, Yurdanur *et al.* [27] proved that although MMP-9 expression is significantly increased in high-grade tumors (P=0.001), no significant correlation was found between MMP-2 and tumor grade (P=0.121).

As tumor grade and tumor size were well-known prognostic factors and their prognostic values are stronger than others, the correlations of MMP-2 and MMP-9 with higher tumor grade and larger tumor size suggest that both markers may be considered as unfavorable prognostic factors especially in node-negative breast carcinoma [19,26].

Axillary lymph node status is the most basic prognostic factor in patients with breast cancer, so those with negative lymph node have more favorable prognosis [26]. In the present study, immunostaining for MMP-2 and MMP-9 was significantly correlated with lymph nodes status of the tumor (P=0.029 and 0.048). Different studies on this issue gave markedly the same outcome, such as that seen by Yurdanur *et al.* [27]. In this work, MMP-2 negativity in node-positive patients was related to good prognosis. Also, it showed that duration of survival was longer in MMP-2 node-negative breast tumor group than MMP-2 node-positive patients. Also Shah and Li *et al.* [12] demonstrated that the expression of MMP-2 and MMP-9 has huge prognostic value in node-negative patients [8]. Both results confirmed that the high proportion of MMP-2 in lymph node-negative tissue proposed expanded danger of metastasis and therefore it could be used to screen patients without lymph node involvement. It was confirmed that the expression of MMP-2 and MMP-9 was corresponding with tumor invasion and lymph node metastasis [28].

In spite of the fact that ER and PR markers may have limited value as prognostic pointers, they may mix with different parameters which have predictive value for reaction to treatment [29]. An affiliation was accounted between steroid hormones and metalloproteinase action, and the initiation of ER receptors by estrogen had been recommended to prompt both tumor metastasis, by mimicking cell development, and attack by growing MMPs [27]. In this study, we obtained significant statistical result between both MMP-9 and MMP-2 with ER and PR positive cases (P=0.014 and 0.034). This in agreement with Ranogajec *et al.* [23] and Razandi *et al.* [30] who found high relation between both markers and ER and PR and confirmed that estrogen causes stimulation of transduction cascade from plasma membrane to G protein and at last leads to MMP-2 and MMP-9 activation. Our study is in contrast with that of Wang *et al.* [31] who confirmed that MMP-9 had negative relation with PRs and Jinga *et al.* [32] who found no relation between MMP-2 and MMP-9 and ER and PR expression.

Previous studies confirmed that expression of both MMP-2 and MMP-9 in patients with breast tumor

demonstrated 13.9 times higher danger of death as compared with the negative patient groups, suggesting their role in predicting aggressive disease and worse outcome [24].

It is possible that therapeutic strategies targeting interaction of tumor cells with their surrounding may be more beneficial in inhibiting tumor progression than focusing on the tumor epithelial cell alone.

Conclusion

The present study demonstrated that the expression of MMP-2 and MMP-9 in tumor and stromal cells could serve as a parameter of poor prognosis in breast cancer. Both play a critical role in degradation of extracellular matrix to enhance the invasive and metastatic capacity of breast cancer. Their high expression was significantly correlated with high histological grades, advanced clinical staging, and positive lymph node status. Further studies with a large sample size can clarify the role of MMP-9 and MMP-2 expression as prognostic factor in the breast carcinoma and the role of coexpression of MMP-2 and MMP-9 as prognostic value in node-negative patients.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflict of interest.

References

1 Daniele A, Divella R, Mazzocca A. Expression of metalloproteinases MMP-2 and MMP-9 in sentinel lymph node and serum of patients with metastatic and non-metastatic breast cancer. Anticancer Res 2010; 30:3521–3528.

2 Elattar IA, Hassan NM, Lamee MM, Elbasmy AA. Cancer Profile at National Cancer Institute, Egypt, 2002–2003. J Clin Oncol 2005; 23:9653.

3 Mokhtar N, Gouda I, Adel I. Breast cancer. In: Cancer Pathology Registry 2003–2004 and time trend analysis.Cairo, Egypt: Department of Pathology, NCI: 2007. pp. 40–45.

4 Ibrahim AS, Mikhail NN. Breast cancer. In: Egypt National Cancer Registry Aswan Profile–2008. Egypt: National Cancer Registry Program of Egypt (publications no. RR1). 2010. pp. 50–53

5 Ibrahim AS, Mikhail NN, Darwesh H, Heikel T. Breast cancer. In: Egypt National Cancer Registry. Damietta Profile-2009. Egypt: National Cancer Registry Program of Egypt (publications no. RR2). 2011. pp. 64–67

6 Quaranta M, Venneri MT, Daniele A. MMP-2, MMP-9, VEGF and CA 15.3 in breast cancer. Anticancer Res 2011; 27:3593–3600.

7 Sen T, Moulik S, Dutta A, Choudhury PR, Banerji A, Das S, *et al.* Multifunctional effect of epigallocatechin-3 gallate (EGCG) in down regulation of gelatinase-A (MMP-2) in human breast cancer cell line MCF-7. Life Sci 2009; 84:194–204.

8 Shah FD, Shah PM, Patel PS. Clinical significance of matrix metalloproteinase 2 and 9 in breast cancer. BMC Cancer 2010; 46: 194–202.

9 Patel S, Sumitra G, Alpana S, Koner BC. Role of serum matrix metalloproteinase-2 and –9 to predict breast cancer. Arch Intern Med 2011; 2:125–133.

10 Chang KW, Liu CJ, Cheng HW. Pre-surgical serum levels of matrix metalloproteinase-9 and vascular endothelial growth factor in oral squamous cell carcinoma, Oral Oncol 2009; 45:920–925.

11 Liu Z, Li L, Yang Z, Luo W, Li X, Yang H, *et al.* Increased expression of MMP9 is correlated with poor prognosis of nasopharyngeal carcinoma. BMC Cancer 2010; 10:270.

12 Li CI, Daling JR, Porter PL, Malone KE. Adjuvant hormonal therapy for breast cancer and risk of hormone receptor-specific subtypes of contralateral breast cancer. Cancer Res 2009; 69:6865–6870.

13 Vu-Nishino H, Tavassoli FA, Ahrens WA, Haffty BG. Clinicopathologic features and long-term outcome of patients with medullary breast carcinoma managed with breast-conserving therapy (BCT). Int J Radiat Oncol Biol Phys 2005; 62:1040–1047.

14 Kanthan R, Senger J, Diudea D. Malignant mixed Mullerian tumors of the uterus: histopathological evaluation of cell cycle and apoptotic regulatory proteins. World J Surg Oncol 2010; 1:8–60.

15 Gonzalez LO, Corte MD, Vazquez J, Vizoso FJ. Androgen receptor expression in breast cancer: Relationship with clinicopathological characteristics of the tumors, prognosis, and expression of metalloproteases and their inhibitors. BMC Cancer 2008; 8:149.

16 Kayser K, Radziszowski D, Bzdyl P, Sommer R, Kayser G. Towards an automated virtual slide screening: theoretical considerations and practical experiences of automated tissue-based virtual diagnosis to be implemented in the internet. Diagn Pathol. 2006; 1:10.

17 Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: Globocan 2008. Int J Cancer 2010; 127:2893–2917.

18 Nelson AR, Fingleton B, Rothenberg ML. Matrix metalloproteinases: biological and clinical implications. J Clin Oncol 2000; 18:1135–1149.

19 Yong S, Beom Y, Jung H. Matrix metalloproteinase-9 activity is associated with poor prognosis in T3-T4 node negative colorectal cancer. Hum Pathol 2007; 38:1603–1610.

20 Liu Z, Li L, Yang Z, Luo W, Li X, *et al.* Increased expression of MMP9 is correlated with poor prognosis nasopharyngeal carcinoma. BMC Cancer 2010; 10:270.

21 Omar S, Khaled H, Gaafar R, Zekry AR, Eissa S, el-Khatib O. Breast cancer in Egypt: a review of disease presentation and detection strategies. East Mediterr Health J 2003; 9:448–463.

22 Sandra J, Frederic M, Roy R. Florid lobular carcinoma in-situ: molecular profiling and comparison to classic lobular carcinoma in-situ and pleomorphic lobular carcinoma in-situ. Human Pathology 2013; 44: 1998–2009.

23 Ranogajec I, Puzovic V, Gabrilovac J. Prognostic value of matrix metalloproteinase-2 (MMP-2), matrix metalloproteinase-9 (MMP-9) and aminopeptidase N/CD13 in breast cancer patients. Med Oncol 2012; 29:561–569.

24 Kiris M, Johanna M, Jari K, Kosma VM. Expression of matrix metalloproteinase MMP-2 and MMP-9 in breast cancer with a special reference to activator protein-2, HER2, and prognosis. Clin Cancer Res 2004; 10:7621–7628.

25 Chen CD, Welsbie DS, Tran C, Baek SH, Chen R, Sawyers CL. Molecular determinants of resistance to antiandrogen therapy. Nat Med 2004; 10:33–39.

26 Mylona E, Nakopoulou L, Magkou C, Nomikos A. The clinicopathological and prognostic significance of membrane type 1 matrix metalloproteinase (MT1-MMP) and MMP-9 according to their localization in invasive breast carcinoma. Histopathology 2007; 50:338–347.

27 Yurdanur S, Guzin G, Yildirim A. Matrix metalloproteinase-2 (MMP-2) and MMP-9 expression in invasive ductal carcinoma of the breast. Pathol Res Pract 2011; 207:747–753.

28 Lu LS, Chen L, Ding WX. Elevated expression of both MDR1 and MMP-2 genes in metastasized lymph node of invasive ductal cancer. Eur Rev Med Pharmacol Sci 2012; 16:2037–2043.

29 Arpino G, Bardou VJ, Clark GM, Elledge RM. Infiltrating lobular carcinoma of the breast: tumor charactericts and clinical outcome. Breast Cancer Res 2004; 6:R149–R156.

30 Razandi M, Pedram A, Park ST. Proximal events in signaling by plasma membrane estrogen receptor. J Biol Chem 2003; 24:2710–2712

31 Wang HY, Zhang XB, Wang M. Expression of matrix metalloproteinase 9 (MMP9) & laminin receptor in breast carcinoma and their correlation with tumor metastasis. Ai Zheng 2003; 22:529–532.

32 Jinga DC, Blidaru A, Szegli G. MMP9 and MMP2 gelatinases and TIMP-1 and TIMP-2 inhibitors in breast cancer: correlation with prognostic factors. J Cell Mol Med 2006; 10:499–510.