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Maspin expression and subcellular localization in invasive ductal carcinoma of the breast: Prognostic significance and relation to microvessel density

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ABSTRACT

Maspin (Mammary serine protease inhibitor) is a tumor suppressor serine. Its clinical significance and role in breast carcinoma are contradictory and inconclusive. Researches demonstrated that the function of maspin differs according to its subcellular localization. This study was conducted to investigate the expression of maspin in invasive ductal carcinoma (IDC) of the breast with special emphasis on its subcellular localization and to evaluate its prognostic role in relation to clinicopathological parameters and microvessel density (MVD) of the tumor. The expression of maspin was evaluated immunohistochemically in 45 IDC cases. The positive rate of maspin expression was 73.3%. Maspin positivity was significantly related to higher tumor grade (p value = 0.041), nodal metastasis (p value = 0.044), perineural invasion (p value = 0.047), and high CD34+MVD (p value = 0.002). Nuclear maspin was detected in 36.6% whereas cytoplasmic maspin was detected in 63.4% of maspin positive cases. A significant inverse relationship was observed between nuclear maspin and high tumor grade (p value = 0.016), and nodal metastasis (p value = 0.047). These results suggest that maspin expression has a prognostic role in breast cancer. Maspin expression is related to increased angiogenesis. Subcellular localization of maspin can strongly affect cancer prognosis. Cytoplasmic maspin relates to poor prognostic parameters whereas nuclear maspin relates to good prognostic ones.

Introduction

Breast carcinoma is the most frequently diagnosed women cancer [1]. Although breast carcinoma has many targeted biomarkers for its treatment, it is considered a heterogeneous disease with different outcomes and this shows the need for new markers to individualize the treatment for better prognosis [2].

Maspin (mammary serine protease inhibitor) is a member of serine protease inhibitors (serpins). Maspin was first identified in normal breast in myoepithelial cells not in luminal cells, and later in breast cancers [3]. The mechanism of maspin function and role in cancer is still unclear. Maspin is classified as a tumor suppressor gene. Previous studies showed that maspin plays a role in the inhibition of invasiveness and metastasis of cancer cells. Also; maspin showed an inhibitory effect on angiogenesis in cancers [4,5].

Studies on the role and clinical significance of maspin in human breast cancer are contradictory and inconclusive. Some researchers have demonstrated an inverse relation between maspin expression and poor clinical course of the disease [5,6]. On the contrary, others found that strong maspin expression is related to poor prognosis in breast carcinoma [7,8]. There is insufficient data on the prognostic importance of maspin in breast carcinoma.

Maspin protein was detected in the nucleus and the cytoplasm of cancer cells. The conflicting observations regarding maspin role and significance can be explained by distinct subcellular localization of maspin in cancer cells. An unclear topic about maspin expression in breast cancer is the relevance of subcellular localization of maspin, as it may indicate different functions [9]. Previous studies showed that nuclear maspin expression is associated with well-differentiated tumors and good prognosis, whereas cytoplasmic maspin is associated with poor prognosis in many cancers [10,11].

Angiogenesis is the process of new vessel budding from pre-existing ones, it is crucial for tumoral growth and survival. Angiogenesis plays a critical role in breast carcinogenesis and metastasis. The tumor microvessel density (MVD) of breast cancers has shown

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to be a prognostic parameter for recurrence and overall survival. Therefore, angiogenesis is considered a potential therapeutic target for breast carcinoma [12].

The aim of the present work was to assess the role of maspin in invasive ductal carcinoma and to study its subcellular localization (nuclear – cytoplasmic). Also, we aimed to examine relations between maspin expression and prognostic parameters in breast cancer mainly MVD.

Material and methods

Patients and tissue specimens

This retrospective study included 45 patients with primary breast cancers operated upon by modified radical mastectomy. Paraffin-embedded tissue blocks were obtained from archives of pathology department, Tanta University during the period from January 2016 to December 2016. All breast cancers were histologically classified as invasive ductal carcinoma (IDC) according to the criteria of the World Health Organization (WHO) [13]. Patients who had undergone chemotherapy or radiotherapy prior to surgery were excluded.

The histologic tumor grade were assigned according to the criteria of Elston and Ellis [14]. They were also classified in 4 categories (luminal A, luminal B, Her2-enriched and triple-negative) according to the modern molecular classification [15].

Immunohistochemical staining

Sections were deparaffinized, rehydrated, and then heated for 9 min in citrate buffer (0.01 M [pH 6.5]) in an 800-watt microwave oven for antigen retrieval. The sections were treated with 2% hydrogen peroxide for 10 min to inactivate endogenous peroxidase and blocked with 3% normal goat serum in 0.2 M phosphate-buffered saline (PBS) (pH 7.4). This was followed by incubating the slides for 2 h with rabbit polyclonal antibody to maspin (Santa Cruz Biotechnology, Santa Cruz, USA at a dilution of 1:400) and CD34 (mouse monoclonal CD34, QBEnd10, Dako, USA at a dilution of 1:50). Ultravision detection kit (TA-015-HD, Labvision, USA) was then used. The slides were incubated with biolinylated goat anti-polyvalent then streptavidin peroxidase for 10 min each. DAB (diaminobenzidine) tetrachloride was used as a chromogen and the slides were counterstained with Mayer's haematoxylin.

Evaluation of immunostaining

Maspin immunostaining

Maspin positivity was determined as a definite positive reaction in the cytoplasm or the nucleus. The results were determined as positive if more than 10% staining of tumor cells was noted [16]. Positivity and subcellular localization were both recorded for maspin results.

CD34 immunostaining (MVD)

CD34 immunostaining was used to determine tumor MVD. The three most hypervascular areas were selected under low power field. Any single endothelial cell or cluster of endothelial cells identified by positive CD34 staining was counted as a single microvessel.

MVD was counted as the number of vessels per 200 high power field (hpf ×200). The mean value for the three fields was recorded as the MVD for each tumor sample [17]. In the present study, low CD34 +ve MVD was defined as a value of MVD ≤15/hpf, whereas a value of MVD >15/hpf was considered as a high CD34 +ve MVD [18].

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Science (SPSS version 16). Data were expressed in terms of frequencies and percentages for categorical variables. For age, me an + standard deviation (+std) were calculated. For comparing categorical data, Chi-square (X^2) test was performed. Fisher's exact test was performed when one of the expected frequencies was equal to zero or if 20% of them was 5 or less. P values of <0.05 were considered statistically significant.

Results

This study included 45 cases of invasive ductal carcinoma of the breast. The age of the studied cases ranged from 29 to 72 years with a mean of 57.58 + 10.56 years. Grade II carcinomas constituted 40% whereas the remaining cases (60%) were grade III. Luminal A carcinomas (86.67%) was the most prevalent category, whereas the remaining cases (13.33%) were triple negative breast cancer (TNBC). Clinicopathologic characters are shown in Table 1.

Immunohistochemical expression of maspin

In this study, the positive rate of maspin expression was 73.33% of the studied cases (33 cases). Maspin expression was detected as homogenous brownish staining either in the cytoplasm only [21 out of 33 maspinpositive cases (63.64%)]) or in the nucleus [12 out of 33 maspin positive cases (36.36%)]. All cases positive for nuclear maspin co-expressed maspin in the cytoplasm as well. No case was found to express nuclear maspin only.

Relation between maspin positivity and clinicopathological parameters (Table 2)

Maspin expression was detected in 85.2% of grade III cases, 82.3% of cases positive for nodal metastasis, and in 87% of cases positive for perineural invasion. Also, the 6 TNBC cases included in this study were positive for maspin expression. Maspin

Table 1
Clinicopathologic features of the studied invasive ductal carcinoma cases.

Clinicopathologic features	Total n = 45 (100%) n (%)
Age	
≤50 years	13 (28.9)
>50 years	32 (71.1)
Size	
≤5 cm	38 (84.44)
>5 cm	7 (15.56)
Tumor grade	
Grade II	18 (40)
Grade III	27 (60)
Lymph node	
Negative	11 (24.4)
Positive	34 (75.6)
Vascular invasion	
Present	27 (60)
Absent	18 (40)
Perineural invasion	
Present	23 (51.11)
Absent	22 (48.89)
Molecular type	
Luminal A	39 (86.67)
TNBC[#]	6 (13.33)

[#]TNBC = Triple negative breast cancer.

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