The Stromal Overexpression of CD10 in Invasive Breast Cancer and its Association with Clincophathologic Factors

Ali Taghizadeh-Kermani[1, 2], Amir Hossein Jafarian[3], Reza Ashabyamin[4], Mehdi Seilanian-Toosi[2], Leila Pourali[5], Mehdi Asadi[1], Leila Mashhadi[6]

Abstract

Background: Breast carcinoma is the most common non-skin malignancy in women. More recently, it has been suggested that extracellular proteinase has also regulated growth factors and cytokines that might contribute to tumor progression. CD10 is a 90-110kd cell surface zinc-dependent metalloproteinase. Since CD10 is structurally similar to matrix metalloproteinase and stromelysin, it might facilitate cancer cell invasion and/or metastasis. The aim of this study was investigation the rate of CD10 expression in the stromal cells of invasive ductal breast carcinomas, Immunohistochemical aspects, then any other aspects to be able to clarify its correlation with other clinicopathological factors of this disease.
Methods: One hundred patients with histopathologic diagnosis of invasive ductal carcinoma and 50 patients with fibroadenoma of breast (as the control group) have selected, then 150 paraffin blocks have obtained. The stained slides by immunohistochemistry method for CD10 marker have examined separately by two pathologists, and discrepancies have reviewed in common session to get the final result.
Results: Stromal CD10 has detected in 28% of the IDC. No kind of immunoreactivity has identified in the stromal cells of normal breast. Stromal CD10 expression in IDC has significantly correlated with increasing tumor size (p<0.001), increasing histologic grade (p<0.001), the presence of nodal metastases (p<0.001) and estrogen receptor negative status (p=0.003).
Conclusion: Stromal CD10 expression in IDC has closely correlated with invasion and metastasis and it might play an important role in the pathogenesis of IDC.

Keywords: CD10; Immunohistochemistry; Breast carcinoma; Stromal cell

1. Surgical Oncology Research Center, Imam Reza Hospital, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
2. Cancer Research Center, Omid Hospital, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
3. Cancer Molecular Pathology Research Center, Ghaem Hospital, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
4. Dept. of Pathology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
5. Women's Health Research Center, Ghaem Hospital, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
6. Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Corresponding Author:
Amir Hossein Jafarian, MD; Assistant Professor of Pathology
Tel: (+98) 511 801 23 95
Email: JafarianAH@mums.ac.ir

Introduction

Breast cancer is the most common malignant tumor among women worldwide. There have been many progresses in treatment of this malignancy. However, each one of malignant tumor has expressed distinct behavior in regards to metastatic potency, then the response to treatment. In addition to traditional prognostic factors including tumor and nodal stage and tumor grade, molecular markers such as estrogen and progesterone receptors and HER-2/neu expression have emerged for survival prediction. The detection of these molecular markers has played a major role in planning treatment strategy [1].

The recognition of new markers which have been able to predict invasive and metastatic tumors potency, could be helpful in making proper treatment decision. Macromolecules in Extracellular Matrix (ECM) have played a crucial role in proliferation, invasion and metastasis of tumor cells. The degradation of collagen and other proteins in ECM has mediated through a family of Matrix Metalloproteinase (MMP) that is zinc dependant for their activity. The tumor invasion to ECM has required degradation of ECM components, as not just the

result of physical pressure by tumor growth. Tumor cells have produced proteolytic enzymes or promote stromal fibroblasts to release proteases. The activity of proteases has modulated by anti-proteases; however, in vicinity of tumors, the balance between proteases and anti-proteases has altered in favor of proteases. One of stromal changes at the invasion site is appearance of myofibroblasts. The transformation of fibroblasts to myofibroblasts has mediated by cancer cell which derived cytokines such as Transforming Growth Factor-β (TGF-β). There were evidences that show myofibroblasts had a great role in tumor growth, invasion and metastasis [2-5].

CD10 which has also called Neprilysin and Common Acute Lymphoblastic Leukemia/Lymphoma Antigen (CALLA) was a zinc-dependant metalloproteinase which has produced by myofibroblasts. This enzyme has caused ECM degradation and has an activity similar to matrix metalloproteinases. CD10 has highly expressed in kidney and lung tissues and could be found in small intestine, placenta, choroid plexus, gonads, adrenal cortex and leukocytes [6]. CD10 has identified in stromal myoepithelial cells from normal breast tissues [7]. CD10 positive cells have also detected in stroma of various malignancies including gastric, lung, breast, prostate and colorectal carcinomas [8-12].

Multiple studies have shown that the expression of CD10 in stromal cells have associated with more biologically aggressive tumors [8-12]. This study has designed to assess the association between the expression of CD10 in stroma of breast carcinoma and some clinic pathological parameters.

Materials and Methods

This study has performed at the departments of Pathology and Oncology, Ghaem hospital, Mashhad, Iran. The biopsy specimens from 100 patients with Invasive Ductal Carcinoma (IDC) and 50 samples from patients with fibroadenoma as control group have selected. All patients have diagnosed between April 2010 and April 2012. The following data have collected: age, menstrual status, tumor and nodal stage, tumor grade (according to Bloom-Richardson grading system), estrogen and progesterone receptor status (based on Allred method) [10]. The specimens have separately re-examined by two pathologists. In case of discrepancy, a consensus has reached by reviewing the samples. The study has approved by the institutional ethics committee.

Immunohistochemical Staining for CD10:

We have used 5 μm slices for the immunohistochemical staining. The sections have deparaffinized in xylene and r antigenic retrieval has done by incubation with molar citrate buffer 1% (pH=6) in a microwave oven for 12 min dehydrated in alcohol and washed in Phosphate Buffered Saline (PBS). The endogenous peroxidase activity has blocked using 3% H_2O_2 for 5 minutes. Then, the sections have incubated with protein block (containing 0.4% Casein in PBS, with stabilizers, surfactant, and 0.2% Bronidox L as a preservative) for 5 minutes. Thereafter, the sections have incubated with CD10 antibody (monocolonal IgG1 colon (56c6) Novocastra, United Kingdom) as primary antibody for 60 minutes followed by incubation with post primary block containing 10% animal serum in Tris Buffered Saline (TBS) 0.09% for 30 minutes. Then the sections have incubated with NovoLink polymer anti mouse/rabbit IgG-Poly-HRP for 30 minutes. Between each step, the sections have washed twice in TBS. Sections have further incubated with the substrate/chromogen, 3,3'-diaminobenzidine (DAB), prepared from Novocastra DAB Chromogen and NovoLink DAB Substrate Buffer (polymer). Reactions with the peroxidase have produced a visible brown precipitate at the antigen site. Sections have then counterstained with Novocastra hematoxylin, rinsed in running tap water, dehydrated in ethanol (70%, 90%, and 100%, consecutively) and cleared with xylene. Then, the slides have cover-slipped. The normal breast tissue has positive control and primary antibody has omitted for negative control. The staining has scored as negative (0; no staining), weak (1; diffuse light brown or strong dark brown in less than 30% of the stromal cells), and strong (2; defined as strong staining in more than 30% of the stromal cells) according to Marketsov study [10].
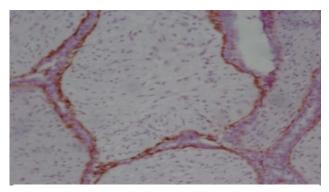
Data analysis has performed using SPSS 16.0 statistical analysis software. The Correlation between stromal cells CD10 expression and clinicopathological features including tumor size, nodal status, tumor grade, Estrogen/progesterone receptor status, age and menstrual status has evaluated using the chi-square test. P-values <0.05 have considered as significant.

Results

The median age for patients with fibroadenoma was 30 years (range, 18-48). Fifty patients were premenopausal and 50 patients were post-menopausal. Twenty two patients were grade 1, 50 were grade 2 and 28 were grade 3. Tumor

Table 1. The correlation between CD10 expression and clinicopathological parameters

	Total number	CD10 expression			p value
		Negative N (%)	Week N (%)	Strong N (%)	
Menstrual Status:					
Premenopausal	50	22 (44)	17 (34)	11 (22)	0.28
Postmenopausal	50	14 (28)	19 (38)	17 (34)	
Age:					
≤ 40	18	10 (55.6)	6 (33.3)	2 (11.1)	0.21
41-60	67	23 (36.3)	23 (34.3)	21 (31.3)	
> 60	15	3 (20)	7 (46.7)	5 (33.3)	
Tumor grade:					
Grade 1	22	15 (68.2)	5 (22.7)	2 (9.1)	<0.001
Grade 2	50	19 (38)	20 (40)	11 (22)	
Grade 3	28	2 (7.1)	11 (39.3)	15 (53.6)	
Tumor size:					
≤ 2 cm	19	8 (42.1)	10 (52.6)	1 (5.3)	0.04
> 2 cm	81	28 (34.6)	26 (32.1)	27 (33.3)	
Nodal Status:					
Negative	46	26 (56.5)	14 (30.4)	6 (13)	< 0.001
1-3	21	4 (19)	11 (52.4)	6 (28.6)	
> 4	33	6 (18.2)	11(33.3)	16 (48.5)	
ER status:					
Negative	36	6 (16.7)	14 (38.9)	16 (44.4)	0.003
Positive	64	30 (46.9)	22 (34.4)	12 (18.8)	
PR status:					
Negative	46	14 (30.4)	22 (47.8)	10 (21.7)	0.07
Positive	54	22 (40.7)	14 (25.9)	18 (33.3)	

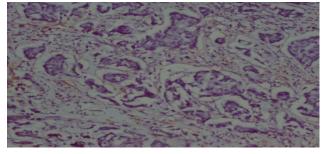**Figure 1.** It shows negative staining of stromal cell for CD10 immunohistochemical staining in fibroadenoma×400.

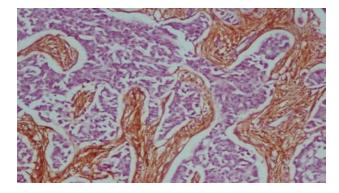**Figure 2.** It shows strong staining of stromal cell for CD10 immunohistochemical staining ×400.

Figure 3. It shows weak staining of stromal cell for CD10 immunohistochemical staining ×400.

size was ≤3.5cm in 19 patients and was >3.5cm in 81 patients.

Weak expression of CD10 in stroma has shown in 2 out of 50 (4%) fibro adenoma samples (Figure 3). The median age of patients with invasive ductal carcinoma was 50 years (range, 26-85). The stromal expression of CD10 in 100 patients with invasive ductal carcinoma was as follows: 36 negative, 36 weak and 28 strong (Figure 1, 2, 3).

The correlations between stromal CD10 expression and clinicopathological characteristics of

patients are illustrated in table 1. Age and menstrual status had no significant correlation with stromal CD10 expression. Meanwhile, larger tumor size (p=0.04), higher tumor grade (p<0.001), the number of positive nodes (p<0.001) and estrogen receptor negativity (p=0.003) have associated with stronger stromal CD10 staining. Progesterone receptor status has not correlated with CD10 expression.

Discussion

There were multiple recognized prognostic factors in breast carcinoma; however, many genetic factors involving in biological behavior of this cancer have remained to be elucidated. The ability of a malignant tumor to invade adjacent tissues was the first step for metastasis. Changes in adhesion molecules including Cadherins have facilitated tumor cell detachment from primary growth and eventually metastasis. Tumor cells not only have produced proteolytic enzymes but also induced stromal cells including fibroblasts to produce these enzymes. Modulation of stromal cells including fibroblast-to-myofibroblast transdifferentiation has played an important role in tumor invasion. CD10 which is a zinc-dependent metalloproteinase has expressed by myofibroblasts and has involved in ECM cleavage [2]. Previous studies have shown stromal CD10 expression in some breast cancer tumors [7, 10, 13]. Considering the CD10 proteolytic activity, we might hypothesize that its expression in a tumor is suggested as associated with increased invasive and metastatic potential.

Several studies have assessed the stromal CD10 expression in various malignant tumors and its correlation with adverse pathological features and/or prognosis. Haung et al. in a study on 116 cases with gastric carcinoma has shown that CD10 expression in stromal cells has associated with tumor depth, lymph node metastasis and vascular invasion [8]. The results of an investigation by Oqawa et al. revealed a significant difference in stromal CD10 expression between invasive and non-invasive tumors indicating a crucial part for stromal CD10 expression in colorectal carcinogenesis [12]. A study by Braham et al. on 47 patients with nasopharyngeal carcinoma has shown that in comparison with early stages, cases with advanced stages have more frequently associated with CD10 expression by fusiform stromal cells (23% vs. 56%; p=0.04) [14]. Bilalovic et al. has evaluated CD10 expression in primary tumor and stromal cell in 70 samples from primary and 28 with metastatic melanoma. More advanced tumors have associated with higher CD10 expression in tumor cell. The expressions of CD10 and Ki67 have significantly

higher in the metastatic than the primary tumors. Primary tumors with higher Clark levels had also significantly higher CD10 expression in intratumoral stromal cells [15].

Prognostic significance of CD10 expression in stromal cells of breast carcinoma has investigated by Iwaya et al. None of 13 samples from patients with non-invasive ductal carcinoma had CD10 expression in the stromal cells. Meanwhile, 20 out of 110 (18%) cases with invasive ductal carcinoma have stained positive for CD10 expression in stromal cells which was more significantly frequent in patients with lymph node metastasis. In their study, CD10 expression was a significant independent predictive factor for shorter time to recurrence and significant adverse prognostic factor for overall survival [13]. In a trial conducted by Makretsov et al., a significant correlation has proven between CD10 stromal expression in invasive ductal carcinoma and some adverse prognostic factors including high tumor grade, estrogen receptor negativity; however, they have not shown any significant association between CD10 stromal expression and lymph node status, tumor size, Her-2 status and progesterone receptors. CD10 expression has significantly associated with decreased disease specific and overall survival in entire cohort and in patients with negative lymph nodes [10]. Kim et al. investigating the immunohistochemical expression of stromal CD10, E-cadherin and beta-catenin in 104 invasive ductal carcinoma and 10 cases of Ductal Carcinoma Insitu (DCIS), found stromal CD10 expression in 49.5% of IDC samples; meanwhile, no immunoreactivity has found in stromal cells of DCIS cases. The results of their study have shown a significant association between CD10 stromal expression and higher histological grade, larger tumor size, presence of nodal and distant metastasis, estrogen-negative status, cytoplasmic beta-catenin accumulation and lower overall survival rate [16]. These results have suggested that CD10 stromal expression might be a novel prognostic factor in breast carcinoma.

In the present study, stromal CD10 expression has shown in 64/100 (36 week, 28strong staining) of cases with invasive ductal carcinoma and 2/50 cases (week staining) of cases with fibroadenoma. This result has suggested the probable role of stromal CD10 expression in breast carcinoma development and carcinogenesis. The stromal CD10 expression has also significantly correlated with recognized adverse pathological prognostic factors including larger tumor size, positive auxiliary lymph nodes, negative estrogen receptor and higher tumor grade.

Conclusion

As compatible with previous studies, these findings reaffirm that stromal CD10 expression has enhanced the invasive and metastatic potential of breast carcinoma. Cases with stromal CD10 expression have been more likely to show unfavorable pathological features.

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Conflict of Interest

The authors have no conflict of interest in this article.

Authors' Contribution

Ali Taghizadeh-Kermani, Amir Hossein Jafarian, and Reza Ashabyamin designed the study, analyzed the data and wrote the paper. Mehdi Seilanian-Toosi, Leila Pourali, Mehdi Asadi and Leila Mashhadi contributed to the data entry, literature review and writing-up process. All authors read and approved the final manuscript.

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