ORIGINAL PAPER

FASCIN OVEREXPRESSION IS ASSOCIATED WITH HIGHER GRADES OF BREAST CANCER

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In the present study, we tried to evaluate the relationship between Fascin molecule expression and other histopathologic parameters, especially tumor grade and hormone profile. Sixty-one malignant breast tumors were enrolled in this study. Fascin marker staining was performed on paraffin blocks of breast samples using immunohistochemistry (IHC) method. The age range of patients was 26-87 years, with an average of 48.41 ±1.68. Our results showed that fascin expression was significantly higher in tumors with high grade histology, lymph node involvement and larger tumor size (p = 0.04, 0.04, 0.04, respectively). Also fascin expression in triple negative tumors was significantly higher than other molecular subtypes (p = 0.001). fascin can be used as an important marker in evaluating breast cancer especially in triple negative tumors.

Key words: breast cancer, fascin, immunohistochemistry, histopathologic, tumors, IHC.

Introduction

Breast cancer is the most common type of cancer and the second leading cause of cancer-related death among women [1]. Its incidence has increased over the last decade in the Asian countries [2, 3]. Breast cancer is the most common cancer among Iranian women and more than 30 percent of patients are less than 30 years old [4]. Considering its high frequency and increasing incidence rate, there has been an ongoing effort to find new methods for patients treatment but considering remarkable improvements, significant mortality and morbidity still exist [5].

Fascin is a 55-kDa actin-bundling protein which is normally expressed in neuronal, mesenchymal, and endothelial cells, and is low or absent in normal epithelial cells [6, 7]. Recent studies have reported overexpression of fascin in several types of malignancies, including lung [8], colon [9], stomach [10], ovary [11] and breast {12]. Previous studies on breast cancer have shown that fascin expression was associated with poor prognosis and some studies have suggested to use fascin as a potential therapeutic target especially in triple negative tumors [13, 14, 15]. In this study, we tried to evaluate the expression of fascin in human breast cancer using immunohistochemistry (IHC) method and explore its correlation with clinicopathological findings.

Material and methods

In this study, 61 paraffin embedded samples of breast cancer collected by pathology department of Urmia University of medical sciences, Urmia, Iran were enrolled. All embedded paraffin blocks were

sectioned at 4 micrometer and sections were stained by hematoxylin and eosin (HE) method for routine histologic examination and tumor grading. Tumor grading was performed according to Nottingham modification of Bloom-Richardson system.

IHC staining

For IHC staining 5 consecutive sections were obtained and stained for estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor 2 (HER 2/neu), ki67 and Fascin according to manufacturer's guidelines.

All antibodies and associated reagents were obtained from DAKO Corporation, Glostrup, Denmark. We also used Hodgkin lymphoma and Reedsternberg cells as positive control for Fascin and stained in each staining run. Histologic evaluation was performed using a light microscope (Olympus CX41, Japan).

ER and PR IHC reporting

The immunohistochemistry results for ER, PR and Her2/neu were interpreted according to the College of American Pathologists (CAP) protocols as following: For ER and PR staining, nuclear positivity was scored 0 to 5 as following: 0 (0%), 1 (< 1%), 2 (1-10%), 3 (11-33%),4 (34-66%), and 5 (> 67%).

The intensity of staining (IS) was graded as 0, 1, 2 and 3 when there was none, mild, moderate or strong staining, respectively. Finally Allred score was calculated by summing up the values for proportion and intensity of staining. The Allred score range was between 0-8 and the results higher than 2 was considered as positive [16].

Her2/neu IHC reporting

Her2/neu staining results were reported as following: 0 (negative) if there was no immunoreactivity, 1+ (negative) faint or weak incomplete membranous immunoreactivity in > 10% of tumor cells, 2+(equivocal) weak to moderate complete membrane immunoreactivity in > 10% of tumor cells or circumferential (complete) intense membranous staining < 10% of cells and 3+ (positive) more than 10% of the tumoral cells showed circumferential (complete) intense and uniform membranous staining with homogenous chickenwire pattern [16].

Ki67 IHC reporting

Percent of positive cells (nuclear staining) in hot spot areas were reported as ki67 index. The cases were divided into two groups: index ≤ 14% and more than 14%.

Molecular subtyping

Samples were also classified on molecular subtypes according to the following:

Luminal A (ER+ and/or PR+, HER-2 negative, Ki-67 < 14%), Luminal B with HER-2 negative (ER+ and/or PR+, HER-2 negative, Ki-67 ≥ 14%), Luminal B with HER-2 positive (ER+ and/or PR+, HER-2+, any Ki-67), HER-2 positive (ER–, PR–, HER-2+), and basal-like (triple negative) (ER–, PR–, HER-2 negative) [17].

Fascin IHC reporting

Cytoplasmic staining pattern was considered as positive result. Reed Sternberg cells in a Hodgkin lymphoma were used as positive control in every staining run.

Statistical analysis

The results are expressed as mean ±SD. Statistical analysis was performed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). Normality of data was evaluated with the Kolmogorov-Smirnov test. The statistical differences between proportions were determined by χ^2 analysis. Numerical data were evaluated using analysis of variance, followed by Tukey's *post hoc* test. P < 0.05 was considered as significant.

Results

Patients' mean age was 48.4 ±1.56 years. Twenty five (41%) masses were on the right and 36 (59%) were on the left breast. Two (3.3%) of the evaluated tumors were grade I, 33 (54.5%) were grade II, and 26 (42.2%) were grade III.

ER, PR and Her2/neu staining

Of 61 cases, 22 (36.1%) were luminal A, 12 (19.7%) were Her2 positive, 19 (31.1%) were luminal B, and 8 (13.1%) Were triple negative (Basal-like). Patients' demographic data and tumor characteristics are mentioned in Table I in details.

Fascin staining

IHC staining for Fascin marker revealed that 16 cases (26.2%) were positive and 45 cases (73.8%) were negative for this marker (Fig. 1). There was a statistical significant relationship between fascin expression and histological grade of tumor, tumor size, axillary lymph node involvement and molecular subtype (p = 0.04, p = 0.03, p = 0.04 and p = 0.0001, respectively). Moreover, there was an inverse correlation between fascin expression and both estrogen receptor (p = 0.003) and progesterone receptor (p = 0.0001) expressions. However no correlation was found between fascin expression and tumor

Table I. Tumor characteristics and Fascin expression in examined breast cancer cancers

	N (%)	FASCIN EXPRESSION		
		NEGATIVE (N = 45)	POSITIVE (N = 16)	P VALUE
Histological type				
Invasive ductal carcinoma	55 (90.2)	40	15	0.099
Invasive lobular carcinoma	5 (8.2)	5	0	
Medullary carcinoma	1 (1.6)	0	1	
Histologic grade				
Grade I	2 (3.3)	2	0	< 0.05
Grade II	33 (54.5)	28	5	
Grade III	26 (42.2)	15	11	
Tumor Side				
Right	25 (40.9)	18	7	0.51
Left	36 (59.1)	27	9	
Tumor Size				
< 2 cm	3 (4.9)	1	2	< 0.05
2-5 cm	44 (72.1)	36	8	
> 5cm	14 (22)	8	6	
Lymph-vascular invasion				
Present	43 (70.5)	33	10	0.376
Not identified	18 (29.5)	12	6	
Perineural invasion				
Present	19 (31.1)	13	6	0.367
Not identified	42 (68.9)	32	10	
Nipple involvement				
Present	12 (18.6)	10	2	0.496
Not identified	49 (81.4)	35	14	
Skin involvement				
Present	13 (20.3)	10	3	0.642
Not identified	48 (79.7)	35	13	
Axillary lymph node Involvement				
Present	49 (81.4)	38	11	< 0.05
Not identified	12 (18.6)	7	5	
Estrogen receptor				
Positive	35(57.4)	31	4	0.003
Negative	26 (42.6)	14	12	
Progesterone Receptor				
Positive	36 (59)	35	1	0.0001
Negative	25 (41)	10	15	
HER 2				
Positive	12 (19.7)	6	6	0.345
Negative	49 (80.3)	38	11	

Table I. Cont.

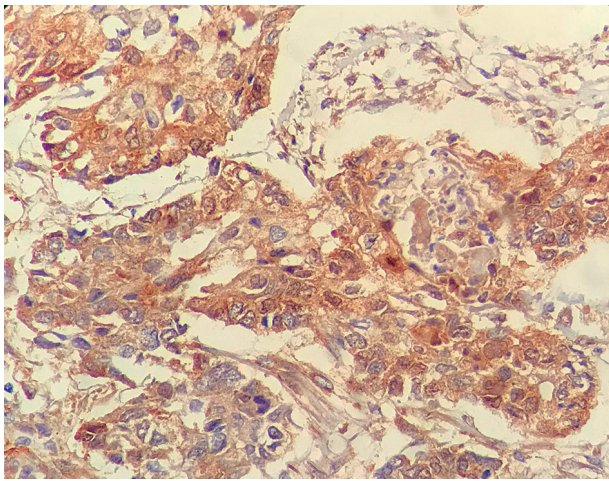|	N (%)	Fascin Expression		
		Negative (N = 45)	Positive (N = 16)	P value
Molecular Subtype				
Luminal A	22 (36.1)	21	1	0.0001
Luminal B	19 (31.1)	17	2	
HER-2/neu	12 (19.7)	6	6	
Triple negative	8 (13.1)	1	7	
Ki 67				
Ki67 ≤ 14 %	22 (36.1)	11	1	0.115
Ki67 >14%	39 (63.9)	14	7	

Fig. 1. Immunohistochemical staining for FASCIN MOLECULE showing cytoplasmic staining in tumoral cell (IHC, 20×)

histological subtype, tumor side, Lymph-vascular invasion, perineural invasion, nipple involvement, skin involvement, HER 2/neu and Ki 67 status. The relationship between fascin expression and clinicopathological parameters is shown in Table I.

Discussion

Breast cancer is the most common malignant tumour as well as the most common cause of death by carcinomas in the female population [1]. The incidence of breast carcinoma is lower in Iran compared to most other countries. However an increased incidence has been recorded making it the most prevalent carcinoma in our country [18, 19]. Additionally, an estimated 30% of the affected population in our country are younger than 40 years old comparing to Western countries in which below 6% of patients are younger than 40 [18].

Currently, the mainstay of diagnosis of neoplastic diseases is the employment of biomarkers. Although many prognostic factors such as clinical staging,

invasion of the lymphatic system, histological grading and oestrogen and progesterone receptor presentation are available for breast cancer, the use of specific markers yields more information regarding the evaluation of disease course and treatment of choice. One of the major prognostic determinants in breast carcinoma is invasion and metastasis which is the result of multiple processes which could enable the malignant cells to possess high motility and overcome the intercellular and cell to matrix adhesion [20]. The increased motility is the result of cytoskeletal microfilament rearrangement and actin cross-linking [20, 21]. Fascin, a 55 kDa protein regulates the rearrangement of cytoskeletal components and plays an important role in adjusting actin-based structures. Immunohistochemical analyses have revealed that the expression of fascin was related to clinical progression and invasion of the tumour, and decreased short term survival [8].

The present study showed a significant relationship between fascin expression with tumours of high histologic grading, axillary lymph node metastasis and a larger tumour size. In addition, we also observed higher expression of fascin molecule in triple negative tumours.

In a study performed by Chao-Qung Wang and et al on 457 breast cancer patients in China, they found that the expression of fascin is significantly elevated in triple negative tumours compared to Luminal A and B and HER2 enriched subtypes of breast cancer (p < 0.01), [22].

In another study performed by Hiromichi *et al.*, on 301 cases of invasive ductal carcinoma, they also revealed that the expression of fascin in triple negative breast cancers was significantly higher than other tumour subtypes (p = 0.0056). Our results were in line with these mentioned studies showing that Fascin molecule expression is increased in triple negative cases. Although in contrast to our study, they have found no meaningful relationship between

the expression of fascin molecule and tumour size or lymphatic invasion [23].

In a study performed by Nermeen Salah Yussef and *et al*. they have discovered that expression of fascin was related to lymph node metastasis (p = 0.001), higher tumour stage (p = 0.004) and negative expression of ER (p = 0.002) and PR (p = 0.003) [24].

Similar to our findings, Monther Al-Alwan and *et al*. have reported a strong relationship between the expression of fascin and basal-like breast cancer (p < 0.001), negative expression of ER (p < 0.001) and PR (p < 0.020), larger tumour size (p = 0.091) and distant metastasis (p = 0.017) [25]. Some other studies have also reported higher expression of Fascin in triple negative or high grade tumours [13, 14, 26].

Conclusions

In conclusion, based on the present findings it could be suggested that the expression of fascin is related to higher histologic grading of breast cancer, lymph node metastasis, tumour size, and triple negative breast cancer subtype. This marker is able to provide valuable information as well as being a prognostic factor, on cancer grading system based on AJCC criteria [27]. This marker may also be useful in the treatment of breast cancers, especially in those with triple negative subtypes which are less responsive to hormonal treatments.

Authors declare no conflict of interest.

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