

ORIGINAL PAPER

CLINICAL IMPORTANCE OF FIBULIN-5 IMMUNOHISTOCHEMICAL STAINING IN BREAST LESIONS

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The aim of this study was to evaluate the clinical importance of fibulin-5, which has been shown to display tumour-promoting and tumour-protective functions in breast lesions. Sixty-two breast cancer patients, 19 patients with fibroadenoma, and 15 healthy breast tissues were enrolled. Forty-seven patients had invasive ductal carcinoma (IDC) (12 of them also had *in situ* carcinoma DCIS), and 15 had invasive lobular carcinoma (ILC). A scoring system from 0 to 4 was used to evaluate the fibulin-5 staining according to the percentage of stained cells. The median values of fibulin-5 staining scores of the breast cancer, fibroadenoma, and healthy breast tissues were 2 (range: 0-4), 3 (range: 3-4), and 4 (range: 1-4), respectively, and the difference is statistically significant ($p = 0.0001$). There was no significant difference between the fibulin-5 scores of IDC, ILC, and DCIS. Fifteen patients with triple-negative breast cancer (TNBC) had the lowest fibulin-5 score ($p < 0.0001$). The median fibulin-5 scores of the patients according to Ki-67 index $\geq 14\%$ and $< 14\%$ were 2 (range: 1-5) and 4 (range: 1-5), respectively, and the difference is statistically significant ($p = 0.001$). These data can be explained by the inhibitory effect of fibulin-5 on epithelial cell proliferation, which is closely related to differentiation and prognosis.

Key words: fibulin-5, breast cancer, fibroadenoma.

Introduction

Fibulins can act as molecular bridges in the extracellular matrix, and they are mediators of cellular processes. These processes are cell adhesions and motility, cell-cell and cell-extracellular matrix communications and elastogenesis [1]. Fibulin-5, an elastogenic short fibulin, is a glycoprotein that belongs to the fibulin family. Its major roles are development and growth of the tissues. It interacts with different extracellular matrix proteins including tropoelastin, fibrillin, and elastin microfibril interface 1, lysyl oxidase-like I, or apolipoprotein A [2, 3, 4, 5].

The roles of fibulin-5 in both tumorigenesis and angiogenesis have been emphasised in previous studies [3]. It plays a role in both suppression and promo-

tion of tumorigenesis. In epithelial cells, it inhibits proliferation [3, 6]. In endothelial cells, it reduces proliferation, invasion, and development of angiogenesis. There are also studies showing fibulin-5 as an angiogenesis antagonist [3, 7]. It prevents endothelial cells' angiogenic sprouting and it also reduces cell response to the proangiogenic factor, vascular endothelial growth factor, and enhances endothelial cell expression of the antiangiogenic factors and thrombospondin [3, 7, 8, 9].

Fibulin-5-related experiences in breast cancer patients are very limited [1, 10, 11].

In the study by Mohamedi *et al.* [1], Ki-67, a nuclear protein associated with cell proliferation, was found to be low in patients with high fibulin-5, whereas Ki-67 was high in patients with low fibulin-5

expression in breast cancer cells. In another study on fibulin-5 and breast cancer performed in mice by Lee *et al.* [10], fibulin-5 was found to promote growth both in normal and malignant mammary epithelial cells. The authors concluded that fibulin-5 initiates an epithelial-mesenchymal transition and has tumour-promoting function in the development and progression of breast cancers.

As seen above, the studies on breast cancer and fibulin-5 are either in breast cancer cells or experimental animal studies. The aim of this study was to evaluate the clinical importance of fibulin-5, which has been shown to display in both tumour-promoting and tumour-protective functions, in breast lesions.

Material and methods

In this study, 62 breast cancer patients, 19 patients with fibroadenoma, and 15 healthy breast tissues obtained from reduction mammoplasty specimens were enrolled. The breast cancer patients' ages ranged from 30 to 80 years (median age 52 years). Of the patients with breast cancer, 47 were IDC (12 of them had also DCIS), and 15 were ILC. The DCIS component in the slides was also evaluated separately. The patients' age with FA ranged from 15 to 40 years (median, 19 years). The patients' age with normal

breast tissue ranged between 17 and 54 years (median 38 years). The patients' tumour size, nodal status, clinical stage, hormone receptors status, and Ki-67 index were recorded. Ki-67 index was defined as high ($\geq 14\%$) and low ($< 14\%$). Fifteen patients with IDC were TNBC.

Fibulin-5 antibody for immunohistochemistry (UniProtKB, Inc., Q9UBX5 (FBLN5-HUMAN)) was used for immunohistochemical staining. The detailed protocol was obtained from the product description. Finally, the slides were counterstained with haematoxylin and then examined under a light microscope by an experienced pathologist. Only the cytoplasmic staining in tumour cells (approximately 1000 cells in 3-4 hpf) was calculated. The results for fibulin-5 staining were classified according to the percentage of positively stained cells in five quantitative categories: score 0: negative staining; score 1, 25% or less positive cells; score 2, 26% to 50% positive cells; score 3, 51% to 75% positive cells; and score 4, 76% or more positive cells (Fig. 1).

Ethical approval for this study was obtained from the Ethics Committee of Selcuk University, Faculty of Medicine (2018/283). The Ethics Committee waived informed consent because of the retrospective nature of the study procedure.

	HE	SCORES				
		0	1	2	3	4
Healthy				No patients		
FA		No patients	No patients	No patients		
DCIS						
IDC						
ILC			No patients			

Fig. 1. All groups' haematoxylin-eosin and fibulin-5-stained features

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (version 22.0; Chicago, IL, USA). Median values (with minimum and maximum values) were used to analyse the demographic characteristics. Chi-square test or Fishers exact and Kruskal-Wallis tests were used to assess the homogeneity for other parameters within these groups and subgroups. The groups' fibulin-5 scores were evaluated with Kruskal-Wallis test. After that, the Bonferroni-corrected Mann-Whitney U test was used. A Bonferroni-corrected significance level of $0.05/3 = 0.017$ was used instead of the conventional level of 0.05. In the other statistical evaluations, a p-value < 0.05 was regarded as statistically significant.

Results

The median fibulin-5 scores of the specimen from the breast cancer patients ($n = 62$), fibroadenoma ($n = 19$), and healthy breast tissue ($n = 15$) were 2 (range: 0-4), 3 (range: 3-4), and 4 (range: 1-4), respectively. When these three groups were evaluated, a statistically significant difference was found in terms of fibulin-5 staining characteristics ($p = 0.0001$) (Table I).

When the specimens of patients with breast cancer according to subgroups were examined for fibulin-5 staining, the median scores of the IDC, ILC, and DCIS were 2 (range: 0-4), 3 (range: 0-4), and 3.5 (range: 1-4), respectively. The difference between the groups was not statistically significant ($p > 0.05$).

The median fibulin-5 scores of the patients' Ki-67 index $\geq 14\%$ and $< 14\%$ were 2 (range: 1-4) and 4 (range: 1-4), respectively. There was a statistically significant difference between these groups ($p = 0.001$) (Table I).

There was no statistically significant difference in fibulin-5 staining properties according to tumour size and nodal status or clinical stage ($p > 0.05$) (Table I).

When fibulin-5 staining properties were evaluated according to hormone receptor status, there was no difference in patients with or without ER, PR, and HER-2 positivity (Table 1). The median fibulin-5 staining scores of the TNBC patients and the others were 1 (1-3) and 4 (1-5), respectively. The patients with TNBC had lower fibulin-5 score than the others (< 0.0001).

Discussion

Breast cancer is the most common cancer among women worldwide, but its causes are not fully known. The known causes are age, birth history, family history, previous breast cancer or lump, dense breast

tissue, hormone therapy (exposure to oestrogen, hormone replacement therapy and oral contraceptive), lifestyle factors (overweight or obesity), alcohol abuse, and radiation exposure.

The known prognostic factors in breast cancer usually determine the growth-promoting, invasion and metastatic abilities of the tumour. Both younger (< 35 years) and older patients' (> 65 years) poor survival rates are well known. Pathologic features including tumour stage, tumour morphology, histologic grade, peritumoral lymphovascular invasion, and hormone receptors status play an important role in determining the prognosis [12].

Although it is not clear if Ki-67 provides additional prognostic information, it is widely used as a marker of proliferation, especially in early breast cancer [13, 14]. In recent years, the genetic profile studies including luminal subtypes, HER2-enriched, and ER-negative subtypes are used to determine the prognosis. These classifications are helpful in order to determine personal targeted therapy, but unfortunately it is not easy to perform them in every single centre.

Recently, different prognostic factors including survivin, mitochondrial-derived activator of caspase, androgen receptor expression, lymphoid environment, and Treg cell population have been used in breast cancer [15, 16, 17, 19]. Adamkov *et al.* [15] examined the survivin expression patterns in malignant and benign breast masses. In this study, nuclear accumulation of survivin was associated with proliferative phenotype, and survivin was shown to be a worse prognostic marker in breast ductal carcinoma. In another study from Pluta *et al.* [16], the patients with high second mitochondrial-derived activator of caspase had longer disease-free survival, and second mitochondrial-derived activator of caspase expression was shown to be a favourable prognostic factor.

The fibulin family, as of now consists of seven members of calcium-binding glycoproteins. The role of fibulin-5 on tumourigenesis and angiogenesis has been emphasised recently [3]. Unfortunately, its role is still unclear. In epithelial cells, it inhibits proliferation. It is involved in targeted signalling or pathways of extracellular signal-regulated kinase 1/ extracellular signal-regulated kinase 2, p38 mitogen-activated protein kinase and activator protein-1 activity, and activity of cyclin A expression [3, 6]. In endothelial cells, it reduces proliferation, invasion, and development of the angiogenesis. The pathways or targeted signalling that play a role here are decreased activities of the extracellular signal-regulated kinase 1/ extracellular signal-regulated kinase 2, p38 mitogen-activated protein kinase and vascular endothelial growth factor, and increased activity of the thrombospondin expression [3]. Fibulin-5 stimulates the fibroblasts

Table I. The patients' fibulin-5 staining features

	FIBULIN-5 STAINING FEATURE MEDIAN VALUE, (RANGE)	P
All patients		0.0001
Breast cancer	2 (0-4)	
Fibroadenoma	3 (3-4)	
Healthy breast tissue	4 (1-4)	
	Breast cancer and fibroadenoma	0.0003
	Breast cancer and healthy breast tissue	0.0033
	Fibroadenoma and healthy breast tissue	0.048
Breast cancer patients		NS
Invasive ductal carcinoma	2 (0-4)	
Invasive lobular carcinoma	3 (0-4)	
Ductal carcinoma <i>in situ</i>	3.5 (1-4)	
Breast cancer patients, Ki-67		0.001
< 14%, (n = 27)	4, (1-5)	
≥ 14%, (n = 35)	2, (1-5)	
Breast cancer patients, TNM stage, T status		NS
1, (n = 19)	4, (1-5)	
2, (n = 39)	3, (1-5)	
3, (n = 4)	4.5, (1-5)	
Breast cancer patients, TNM stage, N status		NS
0, (n = 21)	3, (1-5)	
1, (n = 27)	4, (1-5)	
2, (n = 9)	2, (2-5)	
3, (n = 5)	3, (1-5)	
Breast cancer patients, clinical stage		NS
I, (n = 9)	3, (1-5)	
II, (n = 37)	3, (1-5)	
III, (n = 16)	3, (1-5)	
Breast cancer, receptor status		
Oestrogen		NS
Positive, (n = 43)	4, (1-5)	
Negative, (n = 4)	4, (4-4)	
Progesterone		NS
Positive, (n = 40)	4, (1-5)	
Negative, (n = 7)	4, (1-5)	
HER-2		NS
Positive, (n = 18)	2, (2-5)	
Negative, (n = 29)	3, (1-5)	
Triple-negative breast cancer, (n = 15)	1, (1-3)	<0.0001
The others, (n: 47)	4, (1-5)	

IDC – invasive ductal carcinoma; ILC – invasive lobular carcinoma; DICS – ductal carcinoma *in situ*

through increased activities of extracellular signal-regulated kinase 1/extracellular signal-regulated kinase 2, and p38 mitogen activated protein kinase [3, 6]. There are also studies showing fibulin-5 as an angiogenesis antagonist [3, 7].

Fibulin-5-related experiences in breast cancer patients are very limited [1, 10, 11].

In our study, median fibulin-5 staining scores of breast cancer patients were lower than fibroadenoma and normal breast tissue staining scores. Interestingly, there was no difference between the staining scores of normal breast tissue and fibroadenoma. Maybe these findings can be explained by the inhibitory effect of fibulin on epithelial cell proliferation. When the pathological subgroups of breast cancer patients including IDC, ILC, and DCIS were examined, no difference was found between the staining scores of the pathological subgroups. The median fibulin-5 staining score was low in patients with high Ki-67 score. This can be explained by the higher proliferation in patients with high Ki-67 score.

No positive or negative effect of hormone receptor status including oestrogen, progesterone, and HER2 was observed on the median fibulin-5 staining score in breast cancer patients. However, the median fibulin-5 staining score was found to be very low in TNBC patients compared with other patients.

In conclusion, the fibulin-5 immunohistochemical staining score was lower in breast cancer than fibroadenoma and healthy breast tissue specimens. Among patients with cancer, it was seen that TNBC patients and patients with higher Ki-67 index had the lowest score. These data suggest that the inhibitory effect of fibulin on epithelial cell proliferation could be indirectly related to prognosis. However, disease staging using the Tumour-Node-Metastasis (TNM) system is the most commonly used method in determining prognosis, and as the stage increases, it adversely affects the prognosis; however, also fibulin-5 immunohistochemical staining may act as an additional prognostic marker in light of these findings.

The authors declare no conflict of interest.

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