Differential Expression of Cancer-Associated Fibroblast-Related Proteins in Ductal Carcinoma in situ According to Molecular Subtype and Stromal Histology

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Keywords

Breast · Cancer-associated fibroblast · Ductal carcinoma in situ · Stroma

Abstract

Objective: The purpose of this study is to investigate the expression of cancer-associated fibroblast (CAF)-related proteins and their implication in ductal carcinoma in situ (DCIS).

Methods: We constructed a tissue microarray of 223 cases of DCIS and examined immunohistochemical staining for the 7 CAF-related proteins. We classified DCIS into luminal type, human epidermal growth factor receptor-2 (HER-2) type, and triple negative breast cancer (TNBC) according to the immunohistochemical results for estrogen receptor, progesterone receptor, and HER-2. We also classified DCIS into desmoplastic, normal-like, and inflammatory type according to stromal histology. **Results:** There were significant differences in the expression of S100A4, podoplanin, prolyl 4-hydroxylase subunit alpha 3, NG2, and PDGFR α in stromal cells of DCIS when classified according to molecular subtype. The expression rate of all CAF-related proteins in stromal cells was higher in the HER-2 type and TNBC than in the luminal type ($p < 0.001$). When classified according to stromal sub-

type, there were significant differences in the expression of all CAF-related proteins in stromal cells, with the inflammatory stromal type showing higher expression of CAF-related proteins than other stromal types. **Conclusion:** The expression of CAF-related proteins in stromal cells of DCIS varies according to molecular subtype and stromal type.

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Introduction

Ductal carcinoma in situ (DCIS) is a pre-invasive lesion of breast cancer. As normal epithelium progresses to atypical ductal hyperplasia, ductal carcinoma, and invasive carcinoma during the pathogenesis of breast cancer, DCIS might be considered a direct precursor of invasive carcinoma [1]. Also, approximately half of invasive carcinomas are accompanied by DCIS [1], and the nearly identical genetic changes of atypical ductal hyperplasia, ductal hyperplasia in situ, and invasive ductal carcinoma further support the notion that DCIS is a direct precursor of invasive carcinoma [2]. However, it is reported that only 20–50% of patients with untreated DCIS show progression to invasive carcinoma, suggesting that additional

events are needed for DCIS to progress to invasive ductal carcinoma [3–5]. The factors required for DCIS to progress to invasive carcinoma might be intrinsic such as genetic alteration of tumor cells in DCIS and/or extrinsic, which include the effects of the tumor microenvironment or tumor stroma [6]. The tumor microenvironment consists of non-transformed elements located in the area of tumor but that are not tumor cells and includes immune system elements (such as macrophages and lymphocytes), blood vessel cells, fibroblasts, myofibroblasts, mesenchymal stem cells, adipocytes, and extracellular matrix. Among these, cancer-associated fibroblasts (CAFs) are the most important and have been widely studied [7]. It is reported that CAFs located around cancer cells are associated with tumor initiation, tumor-stimulatory inflammation, metabolism, metastasis, drug response, and immune surveillance [8]. CAFs have been shown to affect the initial progression of DCIS to invasive carcinoma through their interaction with cancer cells. There are various suggested markers of CAFs, such as α -SMA [9], tenascin-C [10], chondroitin sulfate proteoglycan (NG2) [11], platelet-derived growth factor α receptor (PDGFR- α) and PDGFR- β [12], fibroblast activation protein (FAP) [13], podoplanin [14], prolyl 4-hydroxylase subunit alpha 3 (P4HA3) [15], and FSP-1 (S100A4) [11]. Therefore, we expect that there are different functional subtypes among CAFs. A recent research study divided CAFs into 4 subsets – FAP type, FSP1 type, PDGFR- α type, and PDGFR- β type – and reported that each has distinct characteristics [16], supporting the hypothesis that CAFs have various phenotypes. However, there are no studies on the phenotypes of CAFs in DCIS. The purpose of this study is to investigate the expression of CAF-related proteins and their implications in DCIS.

Materials and Methods

Patient Selection and Histologic Evaluation

This study targeted patients who were diagnosed with DCIS and underwent surgery from January 2005 to December 2010 in Severance Hospital. Patients who previously underwent chemotherapy or hormonal therapy were excluded. The study was approved by the Institutional Review Board of Yonsei University Severance Hospital, which exempted the need for informed consent from patients. All cases were reviewed through hematoxylin and eosin (H&E)-stained slides by a breast pathologist (J.S.K.). Histologic grade was assessed using the Nottingham grading system [17]. Clinicopathological parameters evaluated in each case included patient age at initial diagnosis, lymph node metastasis, tumor recurrence, distant metastasis, and patient survival. We classified the stromal type of DCIS through microscopic examination as follows: *desmoplastic type*, cellular fibroblast/myofibroblast

proliferation; *normal-like type*, normal-like stromal feature without stromal reaction; *inflammatory type*, mainly inflammatory cells such as lymphocytes.

Tissue Microarray

A representative area showing tumor and tumor stroma was selected on the H&E-stained slide, and a corresponding spot was marked on the surface of the paraffin block. Using a biopsy needle, the selected area was punched out, and a 3-mm tissue core was transferred to a 6 × 5 recipient block. Two tissue cores of the tumor were extracted to minimize extraction bias. Each tissue core was assigned a unique tissue microarray location number that was linked to a database containing other clinicopathological data.

Immunohistochemistry

The following antibodies were used for immunohistochemistry: S100A4, podoplanin, P4HA3, NG2, FAP-1, PDGFR- α , PDGFR- β , estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor-2 (HER-2), Ki-67. Immunohistochemistry was performed with formalin-fixed, paraffin-embedded tissue sections. Briefly, 5- μ m-thick sections were obtained with a microtome, transferred onto adhesive slides, and dried at 62 °C for 30 min. After incubation with primary antibodies, immunodetection was performed with biotinylated anti-mouse immunoglobulin, followed by peroxidase-labeled streptavidin using a labeled streptavidin biotin kit with 3,3'-diaminobenzidine chromogen as the substrate. The primary antibody incubation step was omitted in the negative control. Positive control tissue was used according to the manufacturer's recommendation. Slides were counterstained with Harris hematoxylin.

Interpretation of Immunohistochemical Staining

All immunohistochemical markers were assessed by using light microscopy. A cut-off value of $\geq 1\%$ positively stained nuclei was used to define ER and PR positivity [18]. HER-2 staining was analyzed according to the American Society of Clinical Oncology/College of American Pathologists guidelines using the following categories: 0 = no immunostaining; 1+ = weak incomplete membranous staining in $<10\%$ of tumor cells; 2+ = complete membranous staining, either uniform or weak, in at least 10% of tumor cells; and 3+ = uniform intense membranous staining in at least 30% of tumor cells [19]. HER-2 immunostaining was considered positive when strong (3+) membranous staining was observed, whereas cases with 0 to 1+ staining were regarded as negative. Cases showing 2+ HER-2 expression were evaluated for HER-2 amplification by fluorescent in situ hybridization.

Immunohistochemical markers for podoplanin, P4HA3, FAP α , S100A4, PDGFR- α , PDGFR- β , and NG2 were assessed by using light microscopy. The stained slides were evaluated semi quantitatively according to previously reported methods [20]. Tumor and stromal cell staining was assessed as 0, negative or weak immunostaining in $<1\%$ of the tumor/stroma; (1) focal expression in 1–10% of tumor/stroma; (2) positive in 11–50% of tumor/stroma; and (3) positive in 51–100% of tumor/stroma. These evaluations were performed throughout the entire area of the tumor, and a score greater than 2 was considered positive.

Tumor Phenotype Classification

For this study, we classified phenotypes of DCIS according to the immunohistochemistry results for ER, PR, HER-2, Ki-67, and fluorescent in situ hybridization results for HER-2 as follows [21]:

Table 1. Basal characteristics of ductal carcinoma in situ

Parameters	Total (<i>n</i> = 223), <i>n</i> (%)	Molecular subtype, <i>n</i> (%)			<i>p</i> value
		luminal (<i>n</i> = 126)	HER-2 (<i>n</i> = 84)	TNBC (<i>n</i> = 13)	
Age, years					0.059
≤50	128 (57.4)	81 (64.3)	41 (48.8)	6 (46.2)	
>50	95 (42.6)	45 (35.7)	43 (51.2)	7 (53.8)	
Type					0.004
Cribriform	104 (46.6)	73 (57.9)	28 (33.3)	3 (23.1)	
Solid	85 (38.1)	38 (30.2)	39 (46.4)	8 (61.5)	
Micropapillary	23 (10.3)	9 (7.1)	12 (14.3)	2 (15.4)	
Papillary	8 (3.6)	6 (4.8)	2 (2.4)	0 (0.0)	
Apocrine	3 (1.3)	0 (0.0)	3 (3.6)	0 (0.0)	
Nuclear grade					0.001
Low	16 (7.2)	11 (8.7)	5 (6.0)	0 (0.0)	
Intermediate	118 (52.9)	75 (59.5)	42 (50.0)	1 (7.7)	
High	89 (39.9)	40 (31.7)	37 (44.0)	12 (92.3)	
Necrosis					<0.001
Absent	106 (47.5)	78 (61.9)	23 (27.4)	5 (38.5)	
Focal	35 (15.7)	22 (17.5)	11 (13.1)	2 (15.4)	
Comedo	82 (36.8)	26 (20.6)	50 (59.5)	6 (46.2)	
Stromal type					<0.001
Desmoplastic	142 (63.7)	96 (76.2)	41 (48.8)	5 (38.5)	
Sclerotic	27 (12.1)	18 (14.3)	7 (8.3)	2 (15.4)	
Inflammatory	54 (24.2)	12 (9.5)	36 (42.9)	6 (46.2)	

Luminal A type, ER or/and PR positive, HER-2 negative, and Ki-67 labeling index <14%; *Luminal B type*, (HER-2 negative), ER or/and PR positive, HER-2 negative, and Ki-67 labeling index ≥14%; *HER-2 overexpression type*, ER and PR negative and HER-2 overexpressed or/and amplified; triple negative breast cancer (*TNBC type*, ER, PR, and HER-2 all negative).

Statistical Analysis

Data were analyzed using SPSS for Windows, Version 12.0 (SPSS Inc., Chicago, IL, USA). For the determination of statistical significance, Student's *t* and Fisher's exact tests were used for continuous and categorical variables, respectively. When analyzing data with multiple comparisons, a corrected *p* value with application of the Bonferroni multiple comparison procedure was used. Statistical significance was set to *p* < 0.05. Kaplan-Meier survival curves and log-rank statistics were employed to evaluate time to tumor recurrence and overall survival. Multivariate regression analysis was performed using the Cox proportional hazards model.

Results

Basal Characteristics of DCIS

Basal characteristics of the 223 cases of DCIS included in this study are shown in Table 1. Among 223 cases, 126 (56.5%) were luminal type, 84 (37.7%) were HER-2 type, and 13 (5.8%) were TNBC. These molecular subtypes

showed differences in histologic type (*p* = 0.004), nuclear grade (*p* = 0.001), necrosis pattern (*p* < 0.001), and stromal type (*p* < 0.001). In particular, the luminal type showed a high rate of cribriform type, no necrosis, and desmoplastic stromal type, whereas HER-2 and TNBC showed a high rate of solid type, comedo necrosis, and inflammatory stromal type.

Expression of CAF-Related Proteins in DCIS According to Molecular Subtype

Immunohistochemical staining of CAF-related proteins showed positivity in tumor cells, fibroblasts, and immune cells in a case-by-case manner. Examination of the expression of CAF-related proteins according to molecular subtype in DCIS showed no difference in the expression in tumor cells (Table 2). However, the expression of CAF-related proteins – S100A4, podoplanin, P4HA3, NG2, and PDGFRα – in stromal cell showed significant differences according to molecular subtype (Table 3, and *p* < 0.001). The expression of these 5 CAF-related proteins in stromal cells was higher in HER-2 type and TNBC than in luminal type (Fig. 1). For immunohistochemistry-positive stromal cells, positivity rate was higher in immune cells than in fibroblasts, except for PDGFRβ.

Table 2. CAF-related protein expression in tumor cells of ductal carcinoma in situ according to the molecular subtype

Parameters	Total (<i>n</i> = 223), <i>n</i> (%)	Molecular subtype, <i>n</i> (%)			<i>p</i> value
		luminal (<i>n</i> = 126)	HER-2 (<i>n</i> = 84)	TNBC (<i>n</i> = 13)	
S100A4					0.354
Negative	217 (97.3)	124 (98.4)	81 (96.4)	12 (92.3)	
Positive	6 (2.7)	2 (1.6)	3 (3.6)	1 (7.7)	
Podoplanin					0.349
Negative	15 (6.7)	9 (7.1)	4 (4.8)	2 (15.4)	
Positive	208 (93.3)	117 (92.9)	80 (95.2)	11 (84.6)	
P4HA3					0.191
Negative	18 (8.1)	8 (6.3)	10 (11.9)	0 (0.0)	
Positive	205 (91.9)	118 (93.7)	74 (88.1)	13 (100.0)	
NG2					0.347
Negative	28 (12.6)	13 (10.3)	12 (14.3)	3 (23.1)	
Positive	195 (87.4)	113 (89.7)	72 (85.7)	10 (76.9)	
FAP-1					0.055
Negative	159 (71.3)	97 (77.0)	52 (61.9)	10 (76.9)	
Positive	64 (28.7)	29 (23.0)	32 (38.1)	3 (23.1)	

Table 3. CAF-related protein expression in stromal cells of ductal carcinoma in situ according to the molecular subtype

Parameters	Total (<i>n</i> = 223), <i>n</i> (%)	Molecular subtype, <i>n</i> (%)			<i>p</i> value
		luminal (<i>n</i> = 126)	HER-2 (<i>n</i> = 84)	TNBC (<i>n</i> = 13)	
S100A4					<0.001
Negative	148 (66.4)	101 (80.2)	40 (47.6)	7 (53.8)	
Positive (F)	6 (2.7)	3 (2.4)	3 (3.6)	0 (0.0)	
Positive (I)	69 (30.9)	22 (17.5)	41 (48.8)	6 (46.2)	
Podoplanin					<0.001
Negative	158 (70.9)	109 (86.5)	43 (51.2)	6 (46.2)	
Positive (F)	11 (4.9)	4 (3.2)	5 (6.0)	2 (15.4)	
Positive (I)	54 (24.2)	13 (10.3)	36 (42.9)	5 (38.5)	
P4HA3					<0.001
Negative	150 (67.3)	102 (81.0)	41 (48.8)	7 (53.8)	
Positive (F)	8 (3.6)	5 (4.0)	3 (3.6)	0 (0.0)	
Positive (I)	65 (29.1)	19 (15.1)	40 (47.6)	6 (46.2)	
NG2					<0.001
Negative	117 (52.5)	82 (65.1)	30 (35.7)	5 (38.5)	
Positive (F)	30 (13.5)	18 (14.3)	11 (13.1)	1 (7.7)	
Positive (I)	76 (34.1)	26 (20.6)	43 (51.2)	7 (53.8)	
FAP-1					0.074
Negative	172 (77.1)	104 (82.5)	58 (69.0)	10 (76.9)	
Positive (F)	16 (7.2)	8 (6.3)	7 (8.3)	1 (7.7)	
Positive (I)	35 (15.7)	14 (11.1)	19 (22.6)	2 (15.4)	
PDGFRα					<0.001
Negative	91 (40.8)	69 (54.8)	20 (23.8)	2 (15.4)	
Positive (F)	30 (13.5)	20 (15.9)	8 (9.5)	2 (15.4)	
Positive (I)	102 (45.7)	37 (29.4)	56 (66.7)	9 (69.2)	
PDGFRβ					0.091
Negative	139 (62.3)	86 (68.3)	47 (56.0)	6 (46.2)	
Positive (F)	84 (37.7)	40 (31.7)	37 (44.0)	7 (53.8)	
Positive (I)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	

F, fibroblast, I, immune cell.

Fig. 1. Expression of CAF-related proteins in DCIS according to molecular subtype. The expression of CAF-related proteins – S100A4, podoplanin, P4HA3, NG2, and PDGFR α – was higher in HER-2 type and TNBC than in luminal type.

Expression of CAF-Related Proteins in DCIS According to Stromal Subtype

Examination of the expression of CAF-related proteins according to stromal subtype in DCIS showed no difference in the expression of tumor cells (Table 4). In

contrast, the expression of all CAF-related proteins in stromal cells showed significant differences according to stromal subtype (Table 5). For all CAF-related proteins, the expression rate was higher in the inflammatory stromal type than in the other two stromal types (Fig. 2).

Table 4. CAF-related protein expression in tumor cells of ductal carcinoma in situ according to the stromal subtype

Parameters	Total (<i>n</i> = 223), <i>n</i> (%)	Stromal subtype, <i>n</i> (%)			<i>p</i> value
		desmoplastic (<i>n</i> = 142)	normal-like (<i>n</i> = 27)	inflammatory (<i>n</i> = 54)	
S100A4					0.260
Negative	217 (97.3)	140 (98.6)	26 (96.3)	51 (94.4)	
Positive	6 (2.7)	2 (1.4)	1 (3.7)	3 (5.6)	
Podoplanin					0.396
Negative	15 (6.7)	12 (8.5)	1 (3.7)	2 (3.7)	
Positive	208 (93.3)	130 (91.5)	26 (96.3)	52 (96.3)	
P4HA3					0.312
Negative	18 (8.1)	9 (6.3)	4 (14.8)	5 (9.3)	
Positive	205 (91.9)	133 (93.7)	23 (85.2)	49 (90.7)	
NG2					0.688
Negative	28 (12.6)	19 (13.4)	2 (7.4)	7 (13.0)	
Positive	195 (87.4)	123 (86.6)	25 (92.6)	47 (87.0)	
FAP-1					0.191
Negative	159 (71.3)	103 (72.5)	22 (81.5)	34 (63.0)	
Positive	64 (28.7)	39 (27.5)	5 (18.5)	20 (37.0)	

Table 5. CAF-related protein expression in stromal cells of ductal carcinoma in situ according to the stromal subtype

Parameters	Total (<i>n</i> = 223), <i>n</i> (%)	Stromal subtype, <i>n</i> (%)			<i>p</i> value
		desmoplastic (<i>n</i> = 142)	normal-like (<i>n</i> = 27)	inflammatory (<i>n</i> = 54)	
S100A4					<0.001
Negative	148 (66.4)	113 (79.6)	25 (92.6)	10 (18.5)	
Positive (F)	6 (2.7)	3 (2.1)	1 (3.7)	2 (3.7)	
Positive (I)	69 (30.9)	26 (18.3)	1 (3.7)	42 (77.8)	
Podoplanin					<0.001
Negative	158 (70.9)	115 (81.0)	27 (100.0)	16 (29.6)	
Positive (F)	11 (4.9)	9 (6.3)	0 (0.0)	2 (3.7)	
Positive (I)	54 (24.2)	18 (12.7)	0 (0.0)	36 (66.7)	
P4HA3					<0.001
Negative	150 (67.3)	115 (81.0)	26 (96.3)	9 (16.7)	
Positive (F)	8 (3.6)	7 (4.9)	0 (0.0)	1 (1.9)	
Positive (I)	65 (29.1)	20 (14.1)	1 (3.7)	44 (81.5)	
NG2					<0.001
Negative	117 (52.5)	88 (62.0)	23 (85.2)	6 (11.1)	
Positive (F)	30 (13.5)	24 (16.9)	2 (7.4)	4 (13.5)	
Positive (I)	76 (34.1)	30 (21.1)	2 (7.4)	44 (81.5)	
FAP-1					<0.001
Negative	172 (77.1)	120 (84.5)	22 (81.5)	30 (55.6)	
Positive (F)	16 (7.2)	10 (7.0)	2 (7.4)	4 (7.4)	
Positive (I)	35 (15.7)	12 (8.5)	3 (11.1)	35 (15.7)	
PDGFR α					<0.001
Negative	91 (40.8)	71 (50.0)	16 (59.3)	4 (7.4)	
Positive (F)	30 (13.5)	21 (14.8)	6 (22.2)	3 (5.6)	
Positive (I)	102 (45.7)	50 (35.2)	5 (18.5)	47 (87.0)	
PDGFR β					<0.001
Negative	139 (62.3)	96 (67.6)	24 (88.9)	19 (35.2)	
Positive (F)	84 (37.7)	46 (32.4)	3 (11.1)	35 (64.8)	
Positive (I)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	

F, fibroblast, I, immune cell.

Fig. 2. Expression of CAF-related proteins in DCIS according to stromal subtype. The expression of all CAF-related proteins in stromal cells was higher in the inflammatory stromal type than in the other two stromal types.

Fig. 3. Correlations between clinicopathological factors and expression of CAF-related proteins. Stromal PDGFR-α positivity showed a significant association with tumor cell necrosis. In addition, S100A4 positivity, podoplanin positivity, P4HA3 positivity, and NG2 positivity in stromal cells was associated with ER negativity, PR negativity, and HER-2 positivity.

For immunohistochemistry-positive stromal cells, positivity rate was higher in immune cells than in fibroblasts.

Correlations between Clinicopathological Factors and Expression of CAF-Related Proteins

Analysis of the association between the expression of CAF-related proteins and clinicopathological factors revealed that PDGFR-α positivity in stromal cells was associated with tumor cell necrosis ($p < 0.001$). S100A4 pos-

itivity, podoplanin positivity, P4HA3 positivity, and NG2 positivity in stromal cells were associated with ER negativity, PR negativity, and HER-2 positivity, respectively ($p < 0.001$, Fig. 3).

Impact of CAF-Related Proteins in DCIS on Prognosis

In univariate analysis of the effect of CAF-related proteins in patients with DCIS on disease-free survival, there was no factor that showed statistical significance.

Discussion

We investigated the expression of CAF-related proteins in DCIS. Our study showed differences in the expression of CAF-related proteins (S100A4, podoplanin, P4HA3, NG2, and PDGFR- α) in the stromal component of DCIS according to molecular subtype. Specifically, the expression rate was higher in HER-2 type and TNBC than in luminal type. As molecular subtypes of invasive breast cancer differ from one another in clinical, histological, molecular, and therapeutic aspects, we conclude that cancer cells of different molecular subtypes have different characteristics [22, 23]. It is reported that DCIS also has different tumor biology, according to its molecular subtype [24–26]. Therefore, there appears to be cross talk between cancer cells and tumor stroma through several signaling pathways. Because such cross talks affect the tumor microenvironment [27], we hypothesize that characteristics of the tumor stroma would also be different according to molecular subtype of DCIS, as confirmed through this study. Previous studies also reported different expressions of CAF genes in different molecular subtypes of breast cancer [28]. They reported that the expression of genes associated with cancer cell migration was higher in HER-2 type CAFs than in ER-positive type CAFs, similar to the results of this study. The functional pathways of genes of which expression increased in CAFs of HER-2 positive breast cancer were actin cytoskeleton signaling and integrin signaling. It has been reported that actin cytoskeleton signaling is associated with NG2 [29] and PDGFR [30], while integrin signaling is associated with S100A4 [31–33] and NG2 [34–36]. It has also been reported that molecular features that induce disease progression are different in each intrinsic molecular subtype of DCIS [24] and have an important role in the tumor microenvironment and progression of DCIS [37, 38]. CAF-related proteins also have an intrinsic function. Each CAF-related protein has an association with each function as follows: FAP-1; extracellular matrix modulation and cell invasion, S100A4; metastatic colonization, PDGFR- α ; tumor cell growth and angiogenesis, PDGFR- β ; metastatic spread, and NG2; tumor cell invasion and metastasis [16]. Therefore, it is possible that differences in the expression of CAF-related proteins in stromal cells of DCIS affect disease progression according to the intrinsic subtype, and further study is needed to address this.

In this study, the expression of all CAF-related proteins in the stromal component was different according to the histological phenotype in the order of inflammatory type > desmoplastic type > normal-like type. One possible

mechanism for the different expression of CAF-related proteins by stromal histologic phenotype is the influence of molecular subtype. HER-2 type and TNBC DCIS had a high proportion of inflammatory stroma. Previous studies also showed frequent stromal inflammation in HER-2 positive DCIS [25] and a high expression ratio of immune response-related genes in basal type DCIS [24]. Therefore, we suggest that the molecular subtype of DCIS can affect the stromal histology. Another possible mechanism is the correlation between CAF-related proteins and immune cells. It has been reported that FAP α is associated with immunomodulatory function [16], S100A4 induces massive infiltration of immune cells through serum amyloid A in breast cancer [39], and NG2 induces macrophage recruitment in brain tumor [40]. These results indicate that College of American Pathologists-related proteins are associated with immune reaction. This study also shows that CAF-related proteins are expressed not only in stromal fibroblasts, but also in immune cells. The higher expression rate of immune cells compared to that of stromal fibroblasts supports the relationship between CAF-related proteins and immune cells.

In this study, the expression of CAF-related proteins showed no association with the prognosis of DCIS. A previous study of mammary invasive carcinoma reported that tumoral S100A4 negativity and stromal S100A4 positivity were associated with poor prognosis [41]. However, there is a difficulty in the statistical analysis of DCIS owing to the lack of recurrence or death. A multi-center study would be suitable for investigating associations with prognosis of DCIS.

In conclusion, our study showed different expressions of CAF-related proteins in stroma according to molecular subtype and stromal type of DCIS. The expression of CAF-related proteins was higher in HER-2 type and TNBC than in luminal type and was also higher in inflammatory stromal type than in other stromal types.

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Disclosure Statement

The authors declare no conflicts of interest.

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