Acta Biomed 2021; Vol. 92, N. 5: e2021254 DOI: 10.23750/abm.v92i5.10954

Original Article

Invasive Breast Carcinoma with Abundant Collagenous Stroma Shows Lower Level of CD68-Positive Tumor Associated Macrophages than Those of Invasive Carcinoma without Abundant Collagenous Stroma

Canan Kelten Talu[1], Ezgi Hacihasanoglu[2*], Cem Leblebici[1], Mehmet Ali Nazli[3], Esra Arslan[4], Didem Can Trabulus[5]*

[1]Department of Pathology, University of Health Sciences, Istanbul, Turkey; [2]Department of Pathology, Yeditepe University, School of Medicine , Istanbul, Turkey; [3]Department of Radiology, University of Health Sciences, Istanbul, Turkey; [4]Department of Nuclear Medicine, University of Health Sciences, Istanbul, Turkey; [5]Department of General Surgery, University of Health Sciences, Istanbul, Turkey; *equally-contributing first authors.

Abstract.

Background and aim of the work: The significance of association between cancer and its stromal microenvironment has been recognized. We aimed to investigate the immunohistochemical staining features of D2-40 (podoplanin), SMA (smooth muscle actin) and CD68 (pan-macrophage marker) in patients with early stage invasive breast cancer with/out peritumoral PASH-like stroma. *Methods:* The H&E sections of core needle biopsy specimens of invasive breast carcinomas diagnosed during one-year time period were reviewed in terms of the presence of accompanying PASH-like stroma retrospectively. Cases with similar pattern of growth in their surgical excision materials were included. Eight cases were grouped as 'Invasive tumor with PASH-like stroma' and 21 cases as 'Invasive tumor without PASH-like stroma', consecutively. The results of immunohistochemical staining for D2-40, SMA and CD68 were noted semiquantitatively as 'negative','weak', moderate' or 'strong'. *Results:* CD68 was found significantly lower in invasive tumor with peritumoral PASH-like stroma than those of tumor without PASH-like stroma. No significant differences were found for SMA and D2-40 between two groups. *Conclusions:* Tumor-associated macrophages (CD68 positive) in tumor stroma have been demonstrated in association with tumor behavior in several studies. The presence of peritumoral PASH-like stroma, which is poorly staining for CD68, might be a morphological clue for the behavior of tumor. (www.actabiomedica.it)

Key words: Breast cancer, pseudoangiomatous stromal hyperplasia, tumor microenvironment, tumor-associated macrophages, CD68

Introduction

The tumor microenvironment (TME) is comprised of tumor cells, tumor stroma, fibroblasts, immune cells, endothelial cells, infiltrating inflammatory cells and a variety of associated tissue components. (1, 2) The association between cancer and its stromal microenvironment, and the importance of this association in cancer progression and spread, have been recognized for a long time. (1, 3-7) Collagen is the main component of extracellular matrix (ECM) surrounding solid tumors, and structural changes of col-

lagen fibers such as alterations in orientation at the tumor boundary may facilitate the invasion of tumor cells. (8) Structural changes of collagen in tumor tissue have been found to correlate with a poor prognosis in human breast cancer. (9)

Cancer-associated fibroblasts (CAFs) are a group of cells associated with TME. There is substantial data on the role CAFs play in promoting tumor progression. (4) CAFs are a heterogeneous group, and a subset expresses smooth muscle actin (SMA) while others have been shown to express podoplanin (D2-40), p53, CD10, fibroblast activation protein, matrix metalloproteinases, tenascin-C and platelet-derived growth factor (PDGFR), desmin, S100A4 and Thy-1. (2, 4)

Macrophages that infiltrate and surround the tumor nests are defined as tumor-associated macrophages (TAMs). (10) TAMs are major constituents of TME and are characterized by expression of the CD68 cell surface marker. (11) They release several growth factors, pro-angiogenic factors, proteases and cytokines, and also interact with neoplastic cells, therefore contributing to cancer initiation and progression. (6, 12) CD68 positive TAMs in tumor stroma have been demonstrated to be associated with poor prognosis in various types of tumors. (6, 13-20)

In this study, we aimed to evaluate the immunohistochemical staining features of CD68, SMA and D2-40, three biomarkers associated with TME, in tumor stroma with and without abundant collagenous features in CNBS taken from early stage breast carcinomas (stage IA-IB-IIA-IIB-IIIA cancers).

Material and Methods

The Hematoxylin-Eosin (H&E) stained sections of CNBS of cases with invasive breast carcinoma submitted to our department during a one-year period were retrospectively reviewed in terms of accompanying abundant collagenous stroma. Among 117 cases, abundant collagenous stroma accompanying the tumor was present in 21 cases. Patients with early stage breast carcinomas (stage IA-IB-IIA-IIB-IIIA) from these 21 cases were included in the study. All cases had subsequent surgical excision materials in our department. Twenty-one consecutive early stage invasive breast carcinoma cases without abundant collagenous stroma diagnosed within the same time period were used as the control group.

Nuclear and histologic grades and staining results for estrogen receptor (ER), progesterone receptor (PR) and CerbB2 were noted for all cases. Immunohistochemical stains for D2-40, SMA and CD68 were applied in all 29 cases with early stage breast carcinoma (8 cases with and 21 cases without abundant collagenous stroma). The immunohistochemical stainings in tumor stroma were evaluated at low power (x10) and the results were noted semiquantitatively as 'negative-0', 'weak-1', 'moderate-2' or 'strong-3'. The Pearson Chi-square test was used for the comparison of the immunohistochemical staining results.

Immunohistochemical assays were performed on 5-µm-thick formalin-fixed paraffin-embedded sections. Assays for CD68 (clone KP1 mouse monoclonal, 1/250 dilution, Cell Marque), SMA (clone 1A4 mouse monoclonal, 1/250 dilution, Cell Marque), and D2-40/podoplanin (clone D2-40 mouse monoclonal, 1/50 dilution, Cell Marque) were used. Immunohistochemical staining was performed on a Ventana NexES Automated immunostainer (Ventana Medical Systems, Inc., Tucson, AZ) with adequate positive and negative controls. A negative control was carried out by omission of the primary antibody in each case. All immunohistochemical stains were evaluated in correlation with H&E stained slides of the cases in order to avoid misinterpretation resulting from staining of other cell types, especially granulocyte staining seen with CD68 (KP1 clone). All patient identifiers were kept confidential.

Results

Among 117 cases diagnosed as invasive breast carcinoma in CNBS in one-year period, abundant collagenous stroma accompanying the tumor was present in 21 cases (17.9%). Among 21 cases with abundant collagenous stroma, 8 cases were early stage breast carcinomas. Collagenous tumor stroma has been demonstrated in the surgical excision material and CNBS in Figure 1 and Figure 2, respectively. Table 1 summarizes the information on age, tumor size, nuclear

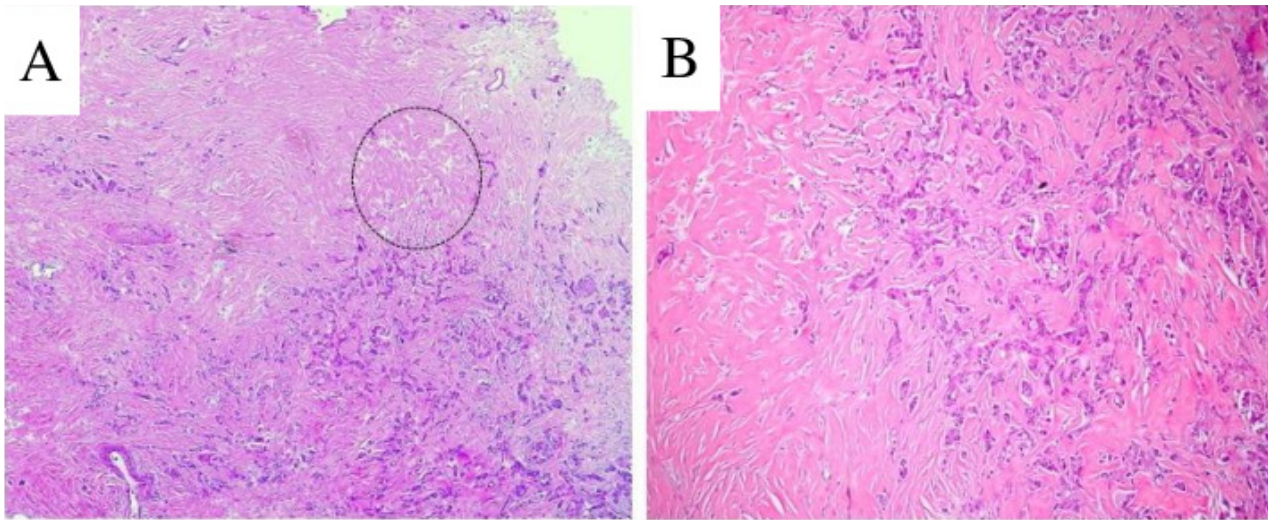

Figure 1. A, B- Invasive breast carcinoma with abundant collagenous stroma in surgical excision material (A:H&E x 40, B: H&E x 100).

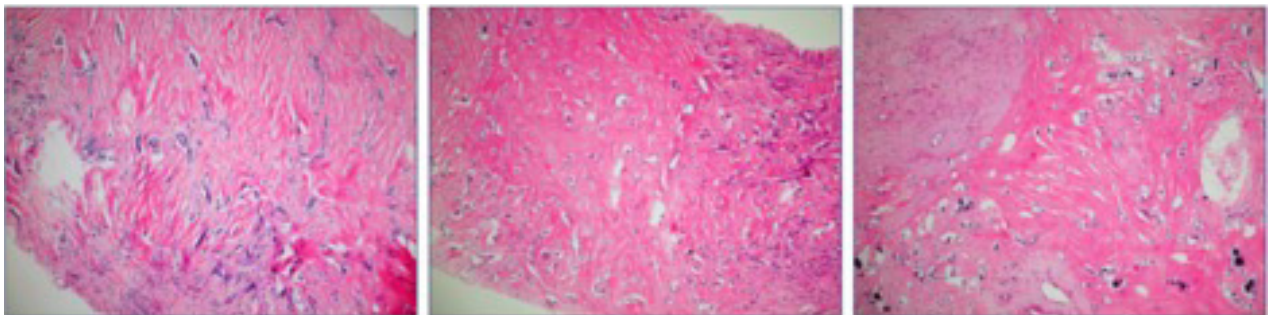

Figure 2. Invasive breast carcinoma with abundant collagenous stroma within core needle biopsy (respectively: H&E x 100, H&E x 100, H&E x 200)

grade, histological grade, presence of lymphovascular invasion, presence of PASH areas, T category, N category and corresponding stage, ER, PR and CerbB2 status and molecular subtype in cases with and without abundant collagenous stroma.

For the cases with abundant collagenous tumor stroma, the mean age was 57.3 years (range 45 to 75). Tumor sizes were between 15 mm and 40 mm (mean=25.1, median=25). Nuclear grade was 2 in five cases and 3 in three cases. Histologic grade was 2 in six cases and 3 in two cases. Peritumoral LVI was detected in the surgical excision material in 7 out of 8 cases (87.5%). All breast carcinoma cases with abundant collagenous tumoral stroma showed areas of pseudoangiomatous stromal hyperplasia (PASH), at least focally, either in CNBS or in the surgical excision materials. In terms of T category, three cases were T1 and five cases were T2. N category was N0 in four cases, N1 in three cases and N2 in one case. All cases showed positive immunohistochemical staining for both ER and PR receptors. There were 6 cases classified as Luminal A and 2 cases as Luminal B.

For the cases without abundant collagenous tumor stroma, the mean age of the patients was 60.4 years (range 33 to 82). Tumor sizes were between 10 mm and 40 mm (mean=22.6, median=22). Nuclear grade was 1 in two cases, 2 in ten cases, and 3 in nine cases. Histologic grade was 2 in eleven cases and 3 in ten cases. Peritumoral LVI was detected in the surgical excision material in 3 out of 21 cases (14.3%). T category was T1 in eight cases and T2 in thirteen cases. Regarding N category, fourteen cases were N0 and seven cases were N1. Approximately half of the cases showed ER/PR expression. Eleven cases in this group were of the Luminal

Table 1. Clinicopathological findings of early stage breast carcinoma cases with and without abundant collagenous stroma

Cases w collagenous stroma stroma stroma	Age	Tm size* (mm)	NG	HG*	LVI*	PASH areas	T category	N category	Stage	ER (%)	PR (%)	CerbB2 (score)	Molecular Subtype
1	59	25	3	2	Neg	Pos	T2	N0	IIA	80	10-15	3	B
2	75	15	2	2	Pos	Pos	T1c	N1a	IIA	90	15	3	B
3	45	36	3	3	Pos	Pos	T2	N0	IIA	30	60	0	A
4	59	30	2	2	Pos	Pos	T2	N1a	IIB	100	50	0	A
5	62	40	3	3	Pos	Pos	T2	N0	IIA	90	30	0	A
6	48	25	2	2	Pos	Pos	T2	N0(i+)	IIA	75	100	0	A
7	46	15	2	2	Pos	Pos	T1c	N2a	IIIA	80	70	0	A
8	65	15	2	2	Pos	Pos	T1c	N1mi	IB	80	70	0	A
Cases w/o collagenous stroma	**Age**	**Tm size* (mm)**	**NG**	**HG***	**LVI***					**ER (%)**	**PR (%)**	**CerbB2 (score)**	**Molecular Subtype**
1	76	20	2	2	Neg	Neg	T1c	N0	IA	100	90	0	A
2	67	15	2	2	Neg	Neg	T1c	N1a	IIA	95	80	0	A
3	81	30	3	3	Neg	Neg	T2	N0	IIA	0	0	1	TNBC
4	61	35	3	3	Neg	Neg	T2	N0	IIA	0	0	0	TNBC
5	54	10	2	2	Pos	Neg	T1b	N0	IA	90	40	0	A
6	82	22	2	2	Neg	Neg	T2	N0	IIA	90	5	0	A
7	76	40	2	2	Pos	Neg	T2	N1a	IIB	100	100	0	A
8	41	11	3	3	Neg	Neg	T1c	N0	IA	1	0	0	TNBC
9	49	23	1	2	Neg	Neg	T2	N1a	IIB	90	70	0	A
10	52	22	3	3	Neg	Neg	T2	N1a	IIB	0	0	3	HER2
11	50	15	2	2	Neg	Neg	T1c	N0	IA	100	100	0	A
12	33	35	3	3	Neg	Neg	T2	N0	IIA	0	0	0	TNBC
13	52	10	2	2	Neg	Neg	T1b	N1mi	IB	40	80	0	A
14	61	22	3	3	Neg	Neg	T2	N0	IIA	0	0	0	TNBC
15	77	27	2	2	Pos	Neg	T2	N1a	IIB	70	30	0	A
16	57	25	2	3	Neg	Neg	T2	N0	IIA	0	0	1	TNBC
17	59	20	3	3	Neg	Neg	T1c	N0	IA	0	0	0	TNBC
18	45	24	3	3	Neg	Neg	T2	N0	IIA	0	0	0	TNBC
19	37	26	1	2	Neg	Neg	T2	N1a	IIB	60	80	0	A
20	79	35	3	3	Neg	Neg	T2	N0(i+)	IIA	0	0	0	TNBC
21	80	8	2	2	Neg	Neg	T1b	N0	IA	90	90	0	A

***:** *Tm size, Histological grade and the knowledge of LVI were obtained from the surgical excision materials. NG: Nuclear grade, HG: Histological grade, TNBC: Triple Negative Breast Carcinoma, A: Luminal A, B: Luminal B, HER2: HER2 subtype, pos: positive, neg: negative*

A molecular subtype, 9 cases were triple negative, and 1 case was of the HER2 subtype. There were no cases classified as Luminal B in this group.

Table 2 shows the immunohistochemical staining results of CD68, D2-40 and SMA in early stage breast carcinomas with and without abundant collagenous stroma. Figure 3 shows examples of score 0-1-2-3. CD68 staining density was found to be significantly lower ($p<0.0001$) in invasive tumors with abundant collagenous stroma compared to cases without this feature.

Table 2. Chi-square analysis of CD68, D2-40 and SMA immunohistochemical staining results in early stage breast carcinomas with and without abundant collagenous stroma

		Score (0-1-2-3)				Total	p value
		0*	1*	2*	3*		
CD68	W abundant collagenous stroma	7	1	0	0	8	<0,0001
	W/o abundant collagenous stroma	1	8	4	8	21	
D2-40	W abundant collagenous stroma With abundant collagenous stroma	4	3	1	0	8	0,44
	W/o abundant collagenous	6	5	9	1	21	
SMA	W abundant collagenous stroma	1	2	0	5	8	0,49
	W/o abundant collagenous	1	4	5	11	21	

**: number of cases*

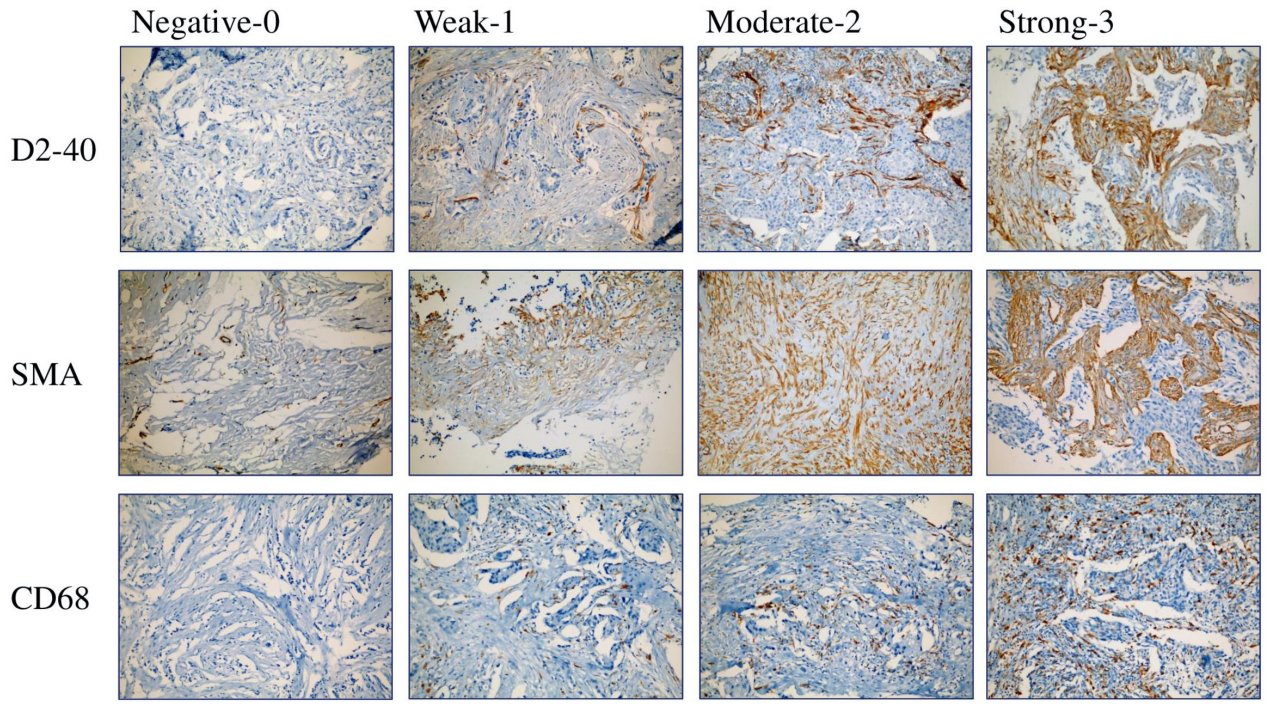

Figure 3. Examples for semiquantative evaluation of immunohistochemical stainings for D2-40, SMA and CD68

No significant difference was found in terms of SMA or D2-40 staining density between the two groups.

Discussion and Conclusion

The association between cancer and tumor microenvironment which composes primarily of tumor stroma, fibroblasts and inflammatory cells, and the importance of this association in cancer progression and spread, have been recognized for a long time. (1, 3-7) Cancer-associated fibroblasts are a group of cells associated with TME and in the light of today's studies, it is known that these cells promote tumor progression. (4) Another major element of tumor microenvironment are the macrophages that infiltrate and surround the tumor nests, which are defined as tumor-associated macrophages (TAMs). (10) It is also known that collagen is the main component of

tumor stroma and structural changes of collagen fibers may facilitate the invasion of tumor cells. (8)

In this study, abundant collagenous tumor stroma was present in 17.9% (21 out of 117 cases) of invasive breast carcinoma cases. All breast carcinoma cases with abundant collagenous tumoral stroma showed PASH areas, at least focally. Early stage invasive breast carcinoma with abundant collagenous tumor stroma showed higher LVI and a lower level of CD68 (+) TAMs expression than carcinomas without this stroma. Regarding the CAFs, no significant differences were found for SMA and D2-40 staining between the two groups. All cases with abundant collagenous stroma were positive for ER and PR hormone receptors (luminal subtype).

The association between cancer and its stromal microenvironment, and the importance of this association in cancer progression and spread, have been recognized for a long time. TME consists of tumor cells and a variety of noncancerous cells present in the tumor. (2) These cells, generally referred as stromal elements, include fibroblasts, immune cells, endothelial cells, inflammatory cells, adipocytes, signaling molecules and extracellular matrix components. (1, 2) Collagen is the principal structural component of the ECM surrounding tumoral proliferation. The abundant collagenous stroma can be detected by microscopic examination and we believe that this type of tumor stroma could potentially reflect a specific interaction between the tumor and its stroma.

Brabrand et al. investigated individual fiber straightness and collagen fiber alignment in various regions of breast carcinoma using second harmonic generation (SHG) microscopy. (21) They found differences in the collagen structure between the two tumoral regions (intratumoral and juxtatumoral) and also between collagen in the tumor and collagen located at a distance. (21) Intratumoral collagen fibres were seen to be straightened, whereas juxtatumoral collagen fibers had a tendency to realign and run parallel to each other. However, collagen fibers at a distance from the tumor were unaffected. The authors therefore suggested that the realignment of collagen structure facilitates the migration of epithelial tumor cells during the invasion process. (21) Interestingly, no differences were found between the high-grade and low-grade breast cancers in terms of collagen pattern. In our study, we selected early stage breast carcinoma cases that showed invasive carcinoma intermingled with abundant collagenous stroma and compared them to cases without this feature as regards the LVI status in the subsequent excision materials. Peritumoral LVI was identified in 7 out of 8 (87.5%) cases of invasive breast carcinoma with abundant collagenous stroma and 3 out of 21 cases (14.3%) without this stroma. Since all the cases consisted of early stage breast carcinoma, we believe that LVI might indicate a preference for dissemination of the tumors that include abundant collagenous stroma. In other words, reconstruction of the abundant collagen bundles may facilitate LVI of the tumor.

Pseudoangiomatous stromal hyperplasia (PASH) is a common stromal lesion and frequently encountered on microscopic examination. (22) Microscopically, PASH is recognized on H&E stained slides by its unique morphology consisting of a network of slit-like channels that resemble vascular spaces. (23) The lesion can be multicentric in 60% of the cases. (24) The frequency of PASH has been reported as 6.4% to 23% in breast specimens resected for various conditions, and 24-47% in men with gynecomastia. (25-27) PASH is mostly detected in association with benign breast lesions such as fibroepithelial lesions, gynecomastia and areas adjacent to fat necrosis and inflammation. (22) Malignant breast lesions associated with PASH such as invasive ductal carcinoma and lymphoma (diffuse large B-cell lymphoma and follicular lymphoma) have also been identified occasionally. (22, 28, 29) Although the pathogenesis of this lesion is still unknown, a proliferative response of myofibroblasts to hormonal stimuli has been assumed. (30)

Tumor-associated macrophages, defined as macrophages that infiltrate and surround the tumor nests, have been demonstrated to be associated with aggressive tumor behavior and poor prognosis in several studies. (2, 13-15, 31-36) In a recently published meta-analysis of 16 relevant studies, it was concluded that a high density of TAMs was associated with a poor breast cancer survival rate, as well as younger patient age, larger tumor size, high histological grade, negative hormone receptor status, and higher frequency of vascular invasion. (37) In the current study, we found that early stage invasive breast carcinomas with abundant collagenous tumor stroma had lower density of CD68+ TAMs than carcinomas without this feature. In addition, all cases with abundant collagenous stroma (low density of CD68+ TAMs) showed hormone receptor positivity and were of

the luminal phenotype. None of them showed HER2 or triple negative phenotype. This finding was consistent with the results of the meta-analysis. In contrast, almost half of the cases without a collagenous stroma had the triple negative (9 / 21 cases) or HER2 molecular phenotype (1 / 21).

Cancer-associated fibroblasts (CAFs) are also present in the TME. CAFs have been reported to play an important role in promoting tumor progression. (4) αSMA is one of the well-known markers expressed by CAFs. Some studies have reported an increased role of αSMA in the CAFs of both primary tumors and metastases. (38-40) Mundim et al. have reported that the αSMA expression levels are similar for breast carcinoma-associated fibroblasts of both the primary tumor and the involved axillary lymph nodes but no expression was detected in cancer-free lymph nodes. (38) Similarly, Rozenchan et al. found no difference for αSMA expression between the primary breast carcinoma and its metastatic axillary lymph nodes. (39) The authors therefore suggested that the fibroblasts continued to be the active element in the metastases of cancer cells to the lymph nodes. In another study, elevated levels of CXCL-1, a chemotactic cytokine, which also overlapped with the expression levels of αSMA and FSP1 proteins in the stroma of breast cancer, were found to be correlated with tumor grade, disease recurrence and decreased patient survival. (40) There are only a few studies on the presence of podoplanin-expressing CAFs and its significance in invasive breast carcinomas. (41, 42) The presence of podoplanin-expressing CAFs has been found to be associated with shorter disease-free survival in one study. (41) Increased expression of podoplanin-expressing CAFs has also been found to be associated with desmoplastic stroma and metastatic sites (specifically bone metastasis) in another study. (42) We found no significant difference for the expression levels of SMA and D2-40 between the two groups.

In conclusion, the presence of abundant collagenous stroma in the tumor may provide a morphological clue regarding the biological behavior of the tumor. PASH-like areas may coexist within the collagenous tumoral stroma, at least focally. This type of tumor may show hormone receptor expression (ER, PR positivity) and a low level of TAMs, and have a tendency to involve lymphovascular channels at the early stage of the tumor.

Conflict of Interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

References

1. Whiteside TL. The tumor microenvironment and its role in promoting tumor growth. Oncogene. 2008;27(45):5904-12.
2. Mittal S, Brown NJ, Holen I. The breast tumor microenvironment: role in cancer development, progression and response to therapy. Expert Rev Mol Diagn. 2018;18(3):227-43.
3. Yang J, Li X, Liu X, Liu Y. The role of tumor-associated macrophages in breast carcinoma invasion and metastasis. Int J Clin Exp Pathol. 2015;8(6):6656-64.
4. Place AE, Jin Huh S, Polyak K. The microenvironment in breast cancer progression: biology and implications for treatment. Breast Cancer Res. 2011;13(6):227.
5. Pollard JW. Trophic macrophages in development and disease. Nat Rev Immunol. 2009;9(4):259-70.
6. Soysal SD, Tzankov A, Muenst SE. Role of the Tumor Microenvironment in Breast Cancer. Pathobiology. 2015;82(3-4):142-52.
7. Lee CH, Liu SY, Chou KC, et al. Tumor-associated macrophages promote oral cancer progression through activation of the Axl signaling pathway. Ann Surg Oncol. 2014;21(3):1031-7.
8. Provenzano PP, Inman DR, Eliceiri KW, et al. Collagen density promotes mammary tumor initiation and progression. BMC Med. 2008;6:11.
9. Conklin MW, Eickhoff JC, Riching KM, et al. Aligned collagen is a prognostic signature for survival in human breast carcinoma. Am J Pathol. 2011;178(3):1221-32.
10. Wang YC, He F, Feng F, et al. Notch signaling determines the M1 versus M2 polarization of macrophages in antitumor immune responses. Cancer Res. 2010;70(12):4840-9.
11. Wu P, Wu D, Zhao L, et al. Inverse role of distinct subsets and distribution of macrophage in lung cancer prognosis: a meta-analysis. Oncotarget. 2016;7(26):40451-60.
12. Chen XJ, Han LF, Wu XG, et al. Clinical Significance of CD163+ and CD68+ Tumor-associated Macrophages in High-risk HPV-related Cervical Cancer. J Cancer. 2017;8(18):3868-75.
13. Medrek C, Ponten F, Jirstrom K, Leandersson K. The presence of tumor associated macrophages in tumor stroma as a prognostic marker for breast cancer patients. BMC Cancer. 2012;12:306.
14. Steidl C, Lee T, Shah SP, et al. Tumor-associated macrophages and survival in classic Hodgkin's lymphoma. N Engl J Med. 2010;362(10):875-85.
15. Ruffell B, Coussens LM. Macrophages and therapeutic resistance in cancer. Cancer Cell. 2015;27(4):462-72.
16. Charpin C, Bergeret D, Garcia S, et al. ELAM selectin expression in breast carcinomas detected by automated and

quantitative immunohistochemical assays. Int J Oncol. 1998;12(5):1041-8.
17. Nguyen M, Corless CL, Kraling BM, et al. Vascular expression of E-selectin is increased in estrogen-receptor-negative breast cancer: a role for tumor-cell-secreted interleukin-1 alpha. Am J Pathol. 1997;150(4):1307-14.
18. Muller AM, Weichert A, Muller KM. E-cadherin, E-selectin and vascular cell adhesion molecule: immunohistochemical markers for differentiation between mesothelioma and metastatic pulmonary adenocarcinoma? Virchows Arch. 2002;441(1):41-6.
19. Staal-van den Brekel AJ, Thunnissen FB, Buurman WA, Wouters EF. Expression of E-selectin, intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 in non-small-cell lung carcinoma. Virchows Arch. 1996;428(1):21-7.
20. Bhaskar V, Law DA, Ibsen E, et al. E-selectin up-regulation allows for targeted drug delivery in prostate cancer. Cancer Res. 2003;63(19):6387-94.
21. Brabrand A, Kariuki, II, Engstrom MJ, et al. Alterations in collagen fibre patterns in breast cancer. A premise for tumour invasiveness? APMIS. 2015;123(1):1-8.
22. Kelten Talu C, Boyaci C, Leblebici C, Hacihasanoglu E, Bozkurt ER. Pseudoangiomatous Stromal Hyperplasia in Core Needle Biopsies of Breast Specimens. Int J Surg Pathol. 2017;25(1):26-30.
23. Vuitch MF, Rosen PP, Erlandson RA. Pseudoangiomatous hyperplasia of mammary stroma. Hum Pathol. 1986;17(2):185-91.
24. Powell CM, Cranor ML, Rosen PP. Pseudoangiomatous stromal hyperplasia (PASH). A mammary stromal tumor with myofibroblastic differentiation. Am J Surg Pathol. 1995;19(3):270-7.
25. Degnim AC, Frost MH, Radisky DC, et al. Pseudoangiomatous stromal hyperplasia and breast cancer risk. Ann Surg Oncol. 2010;17(12):3269-77.
26. Ibrahim RE, Sciotto CG, Weidner N. Pseudoangiomatous hyperplasia of mammary stroma. Some observations regarding its clinicopathologic spectrum. Cancer. 1989;63(6):1154-60.
27. Milanezi MF, Saggioro FP, Zanati SG, Bazan R, Schmitt FC. Pseudoangiomatous hyperplasia of mammary stroma associated with gynaecomastia. J Clin Pathol. 1998;51(3):204-6.
28. Damiani S, Eusebi V, Peterse JL. Malignant neoplasms infiltrating pseudoangiomatous' stromal hyperplasia of the breast: an unrecognized pathway of tumour spread. Histopathology. 2002;41(3):208-15.
29. Drinka EK, Bargaje A, Ersahin CH, et al. Pseudoangiomatous stromal hyperplasia (PASH) of the breast: a clinicopathological study of 79 cases. Int J Surg Pathol. 2012;20(1):54-8.
30. Zubor P, Kajo K, Dussan CA, Szunyogh N, Danko J. Rapidly growing nodular pseudoangiomatous stromal hyperplasia of the breast in an 18-year-old girl. APMIS. 2006;114(5):389-92.
31. Gwak JM, Jang MH, Kim DI, Seo AN, Park SY. Prognostic value of tumor-associated macrophages according to histologic locations and hormone receptor status in breast cancer. PLoS One. 2015;10(4):e0125728.
32. Yuan ZY, Luo RZ, Peng RJ, Wang SS, Xue C. High infiltration of tumor-associated macrophages in triple-negative breast cancer is associated with a higher risk of distant metastasis. Onco Targets Ther. 2014;7:1475-80.
33. Zhang Y, Cheng S, Zhang M, et al. High-infiltration of tumor-associated macrophages predicts unfavorable clinical outcome for node-negative breast cancer. PLoS One. 2013;8(9):e76147.
34. Jensen TO, Schmidt H, Moller HJ, et al. Macrophage markers in serum and tumor have prognostic impact in American Joint Committee on Cancer stage I/II melanoma. J Clin Oncol. 2009;27(20):3330-7.
35. Komohara Y, Hasita H, Ohnishi K, et al. Macrophage infiltration and its prognostic relevance in clear cell renal cell carcinoma. Cancer Sci. 2011;102(7):1424-31.
36. Lima L, Oliveira D, Tavares A, et al. The predominance of M2-polarized macrophages in the stroma of low-hypoxic bladder tumors is associated with BCG immunotherapy failure. Urol Oncol. 2014;32(4):449-57.
37. Zhao X, Qu J, Sun Y, et al. Prognostic significance of tumor-associated macrophages in breast cancer: a meta-analysis of the literature. Oncotarget. 2017;8(18):30576-86.
38. Mundim FG, Pasini FS, Nonogaki S, et al. Breast Carcinoma-associated Fibroblasts Share Similar Biomarker Profiles in Matched Lymph Node Metastasis. Appl Immunohistochem Mol Morphol. 2016;24(10):712-20.
39. Rozenchan PB, Pasini FS, Roela RA, et al. Specific upregulation of RHOA and RAC1 in cancer-associated fibroblasts found at primary tumor and lymph node metastatic sites in breast cancer. Tumour Biol. 2015;36(12):9589-97.
40. Zou A, Lambert D, Yeh H, et al. Elevated CXCL1 expression in breast cancer stroma predicts poor prognosis and is inversely associated with expression of TGF-beta signaling proteins. BMC Cancer. 2014;14:781.
41. Cai D, Wu X, Hong T, Mao Y, Ge X, Hua D. CD61+ and CAF+ were found to be good prognosis factors for invasive breast cancer patients. Pathol Res Pract. 2017;213(10):1296-301.
42. Kim HM, Jung WH, Koo JS. Expression of cancer-associated fibroblast related proteins in metastatic breast cancer: an immunohistochemical analysis. J Transl Med. 2015;13:222.

Correspondence:
Received: 14 November 2020
Accepted: 12 January 2021
Ezgi Hacihasanoglu, MD
Department of Pathology
Yeditepe University Medical Faculty
Atasehir
Istanbul, 34744, Turkey
Phone: 0905368501160
E-mail: ezgihaci@yahoo.com