Malaysian J Pathol 2018; 40(2) : 137 – 142

ORIGINAL ARTICLE

Stromal podoplanin expression and its clinicopathological role in breast carcinoma

Emrah KUMCU[1], Hatice UNVERDI[2], Esin KAYMAZ[3], Onur ORAL[4], Duygu TURKBEY[5] and Sema HUCMENOGLU[6]

[1]Bingol State Hospital, Bingol, Turkey, [2]Gaziantep Ersin Arslan State Hospital, Gaziantep, Turkey [3]Zonguldak Bulent Ecevit University, Zonguldak, Turkey [4]Viransehir State Hospital, Sanliurfa, Turkey [5]Ankara Baskent University, Ankara, Turkey and [6]Ankara Education and Research Hospital, Ankara, Turkey.

Abstract

Introduction: Breast cancer is still a serious health problem in 21st century and diagnosis, treatment and prognosis of this malignant disease are subject to many research. While cancer research has been focused on tumour cells primarily, recent studies showed that tumour stroma contribute to carcinogenesis as well as tumour cells. Especially fibroblasts adjacent to epithelial tumour cells are not ordinary fibroblasts and play the critical role. Studies showed that these cancer associated fibroblasts (CAFs) have different genetic profile and protein expression. One of the differently expressed molecules recently found is podoplanin. Podoplanin, utilised as a lymphatic endothelial marker, is found to be expressed in CAFs. The aim of this study is to evaluate the relationship between the stromal expression of podoplanin in invasive breast carcinoma and clinicopathological parameters. *Materials & Methods:* Podoplanin expression was evaluated immunohistochemically in 153 breast cancers. Tumours with ≥ 10% distinct cytoplasmic podoplanin staining in CAFs were considered as positive. *Results:* In 65.3% of analysed tumours, podoplanin expression was found positive in CAFs. According to our results, podoplanin positive CAFs correlated significantly with tumour size (p= 0.012), tumour grade (p= 0.032) and cerbB2 score (p= 0.032). *Discussion:* Our results suggest that podoplanin expression by CAFs could predict poor patient outcome in breast carcinoma.

Keywords: Breast cancer, tumour stroma, podoplanin, prognosis

INTRODUCTION

Breast cancer is the second most common cancer in the world and the most common cancer in women. One of the four females with cancer is diagnosed with breast cancer. It is the second leading cause of cancer-related death in women worldwide after lung cancer.[1] For these reasons, diagnosis, treatment and prognosis of breast cancer are subject of interest to many research.

While cancer research has been focused on tumour cells primarily, recent studies shown that tumour stroma contribute to carcinogenesis as well as tumour cells. This stroma consists of cancer associated fibroblasts (CAFs), which are different from stromal ordinary fibroblasts genetically and functionally, affect patient prognosis and treatment. The mucin-type sialoglycoprotein podoplanin, due to its expression on lymphatic endothelium, is widely used as an immunohistochemical marker for the discrimination between lymphatic and blood vessels. However, recently podoplanin was also reported to be expressed in various cancer cells, dendritic cells and CAFs. The aim of this study is to evaluate the relationship between the stromal expression of podoplanin in invasive breast carcinoma and clinicopathological parameters.

MATERIALS AND METHODS

Between 2009 and 2016, specimens of 153 patients who were diagnosed as invasive breast carcinoma from Ankara Education and Research

Address for correspondence: Dr. Emrah Kumcu, Bingol State Hospital, Bingol, Turkey. Tel: 905321747536. Fax: 904262132560. Email:emrahkumcu85@hotmail.com

Hospital were included in our study. According to the WHO 2012 classification,[2] 119 invasive ductal carcinomas, 16 lobular carcinomas, 11 mucinous carcinomas, 4 medullary carcinomas and 3 tubular carcinomas were diagnosed. Paraffin blocks, which best represent the invasive tumour with wide desmoplastic stroma, were chosen for whole-cut serial section and immunohistochemical staining of podoplanin. Briefly, 3-μm sections were obtained using a microtome, transferred onto adhesive slides, deparaffinized in xylene and rehydrated through graded ethanol. For antigen retrieval, slides were heated in a pressure cooker in citrat buffer for 20 minute. Subsequently, for blocking of peroxidase activity, sections were treated with peroxide block for 10 minute. Slides were incubated with mouse monoclonal anti-podoplanin (clone D2-40, 1:100 dilution, Abcam, Cambridge, United Kingdom) at room temperature for 1 hour. Staining was visualised with Diaminobenzidine chromogen and counter stained with Haematoxylin. For evalution, two pathologist (E.K. and H.U) who were blinded to the clinical information and the diagnosis, examined the whole section of slides using a multi headed microscope. Cytoplasmic podoplanin staining in 10% or more of the tumour stroma was considered positive.[3] Lymphatic endothelial cells were selected as positive internal control (Fig. 1 & 2).

RESULTS

The mean age of the cases was 52.6 ± 12.3. The age of the patients ranged from 26 to 87 years. Histological grade of tumours; 21 cases (13.7%) were grade 1, 84 cases (54.9%) were grade 2 and 48 cases (31.4%) were grade 3. In all of the cases, 68% ER, 56.2% PR and 34% cerbB2 positivity were observed. Immunohistochemical study showed that 100 (65.3%) of 153 cases were positive for cytoplasmic stromal staining with podoplanin. 90 of 100 podoplanin positive cases were grade 2 and 3 (Fig. 3 & 4). Also, 84 of 100 positive stained cases were found to be T stage 2 and 3 (t > 2 cm). Forty of 52 cerbB2 positive cases (76.9%) were also found to be positive for stromal podoplanin. As a result, podoplanin positive CAFs correlated significantly with larger tumour size (p=0.012), higher tumour grade (p=0.032) and higher cerbB2 score (p=0.032).

We also evaluated overall survival with Kaplan Meier method, but the effect of podoplanin to survival was not statistically different in our cases. Relationship between podoplanin expression by tumour stroma and clinicopathological parameters are shown in Table 1.

DISCUSSION

Recent studies related to cancer pathogenesis have focused on the relationship between cancer cells and its microenvironment. Tumour microenvironment consist of extracellular matrix, fibroblasts, neuroendocrine cells, adipose cells, immune-inflamatory cells and the blood and lymphatic vascular networks. All of the components of tumour microenvironment play a crucial role for tumour initiation, progression and metastasis, when they are in an unhealthy state.[4] In this tumour stroma, cancer-associated fibroblasts (CAFs), a subpopulation of fibroblasts with a myofibroblastic phenotype in tumour environment, have been shown to take part in proliferation, survival and migration of cancer cells and extracellular matrix remodeling by releasing cytokines, growth factors, matrix metalloproteinases.[5]

Researchers focused on to find out molecular pathways and protein expressions for revealing the relationship between tumour cells and CAFs. They found a lot of different protein expressions and one of them is podoplanin. Podoplanin, regarded as a marker of lympangiogenesis, also shows expression in CAFs of several types of tumour. In recent studies, podoplanin expression in CAFs was found to be associated with tumours with different localisations such as lung adenocarcinoma, colorectal carcinoma, hepatocellular carcinoma, pancreatic carcinoma, malignant mesothelioma, aero-digestive localised squamous cell carcinomas, cutaneous squamous cell carcinoma.[6-13]

In lung adenocarcinoma, positive immunoreactivity for podoplanin in CAFs was associated with poor patient outcome.[6] In contrast, in colorectal carcinoma, podoplanin-expressing CAFs have been identified as a favourable prognostic factor.[7] In most of the studies, podoplanin positivity in CAFs correlated with poor prognosis. However, Yamanashi *et al*[7] suggest the opposite and claimed that podoplanin could play a protective role against colorectal cancer invasion. In literature, Nakayama *et al*.[14] concluded that the presence of podoplanin positive pericryptal stromal cells was associated with epithelial tumourigenesis in the colorectum in their research. Studies also found that there were other proteins in CAFs effecting tumour behaviour differently in different tumour types.[15] Research might show us the complex

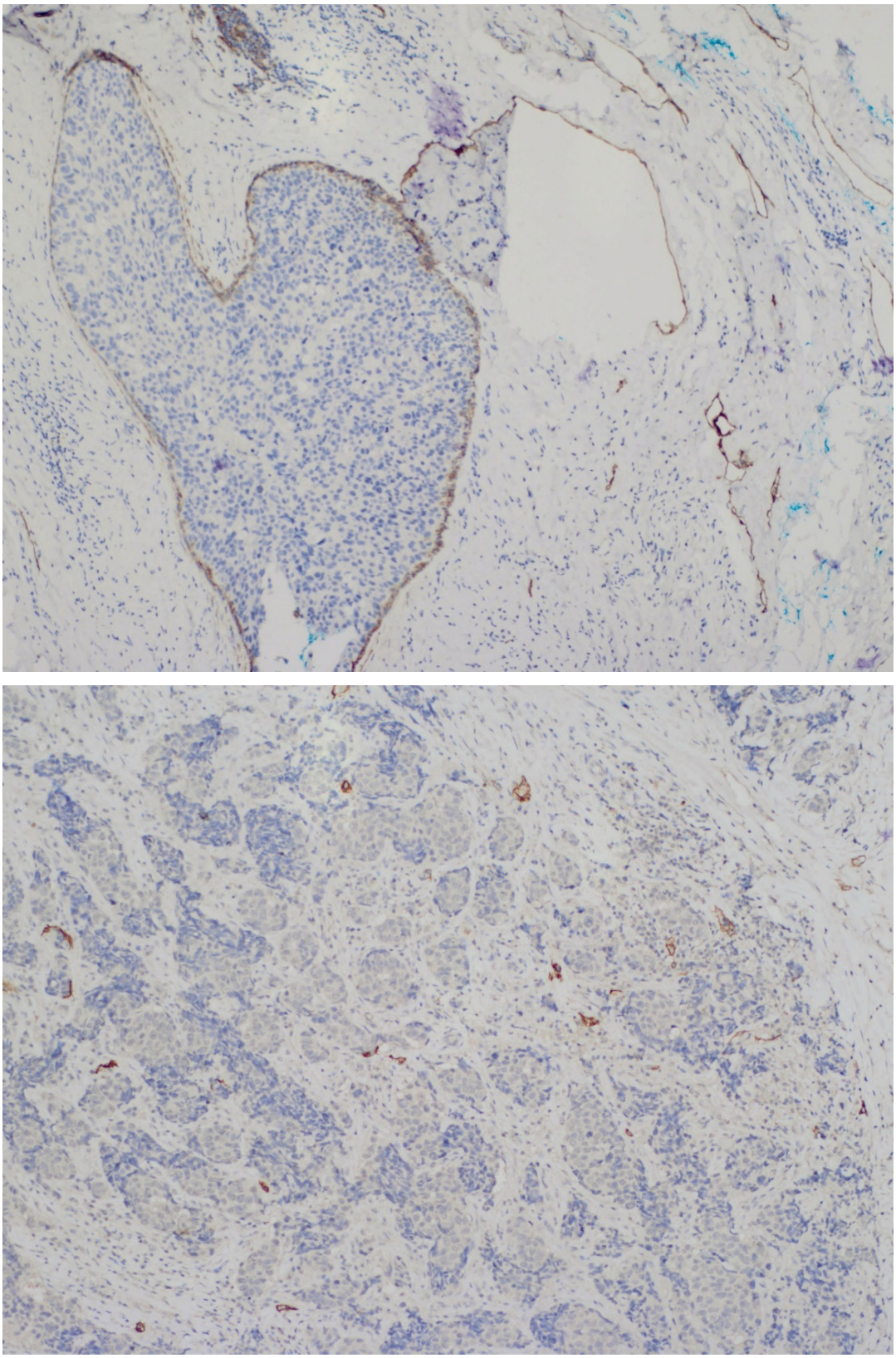

FIG. 1 & 2: Podoplanin stained lymphatic endothelial cells and myoepithelial cells around DCIS while it shows negativity in tumour stroma of grade 2 tumour. (IHC, x100)

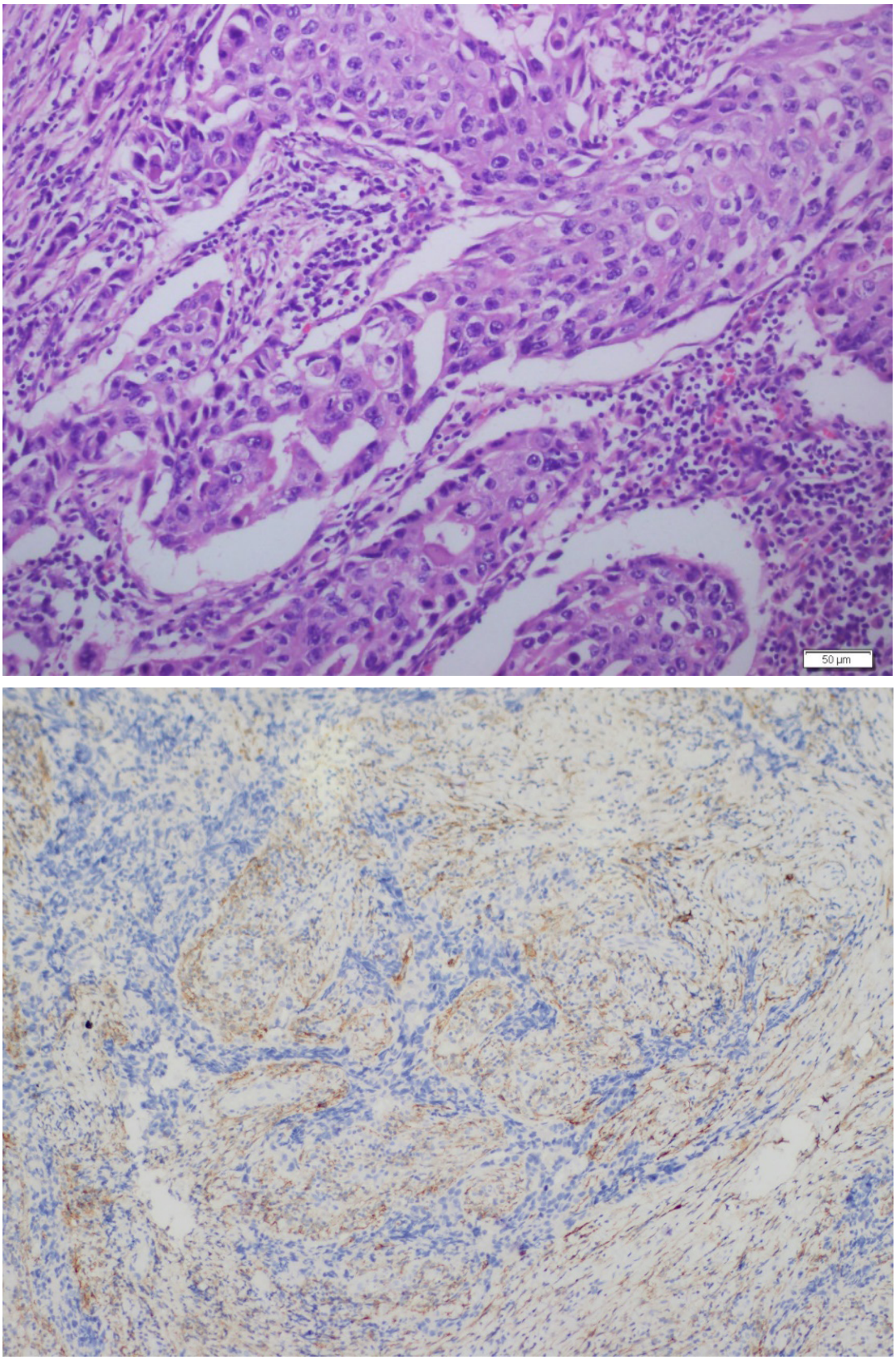

FIG. 3 & 4: Solid, high grade invasive breast carcinoma and podoplanin positive stromal CAFs in high grade tumour. (FIG. 3: H&E, x400 and FIG. 4: IHC, x100)

TABLE 1: Patient characteristics from 153 patients with statistical analyse of podoplanin immunohistochemistry

Variables	Negative (%)	Positive (%)	p-value
Age (years)	51.6±12.0	51.2±11.4	0.755
T stage			0.012
I	16 (30.2)	16 (16)	
II	35 (66)	71 (71)	
III	2 (3.8)	13 (13)	
Grade			0.032
I	11 (20.8)	10 (10)	
II	30 (56.6)	54 (54)	
III	12 (22.6)	36 (36)	
N stage			0.266
N0	17 (32.1)	44 (44)	
N1	17 (32.1)	23 (23)	
N2	9 (16.9)	18 (18)	
N3	10 (18.9)	15 (15)	
Necrosis			0.159
Absent	50 (94.4)	87 (87)	
Present	3 (5.6)	13 (13)	
Ki-67 n:75			0.080
≤20	20 (86.9)	31 (59.7)	
>20	3 (13.1)	21 (40.3)	
Lymphovascular invasion			0.233
Absent	27 (50.9)	61 (61)	
Present	26 (49.1)	39 (39)	
Perineural invasion			0.998
Absent	44 (83.1)	83 (83)	
Present	9 (16.9)	17 (17)	
ER			0.474
Negative	15 (28.3)	34 (34)	
Positive	38 (71.7)	66 (66)	
PR			0.943
Negative	23 (43.4)	44 (44)	
Positive	30 (56.6)	56 (56)	
cerbB2			0.032
Negative	41 (77.3)	60 (60)	
Positive	30 (23.7)	40 (40)	

multiple-sided relationship between tumour and its microenvironment (CAFs). Additionally, we have little knowledge about effect of podoplanin into carcinogenesis. Further studies are needed, especially CAFs in colorectal carcinomas.

In our study, presence of podoplanin positive CAFs is not associated with overall survival statistically. However, there are studies that observe significant correlation between podoplanin expression in CAFs and survival in breast cancer.[3] Our study might not reach statistical significance due to low number of patients in our cohort.

Evidence mostly suggest that CAFs have a crucial role in tumour progression and actively involved in cancer initiation, proliferation, invasion and metastasis. However, the origin, definition and specific markers of CAFs remains to be elucidated. Further investigation into this critical component of the cancer microenvironment is needed.[16]

In the present study, we demonstrated that podoplanin-expressing CAFs seemed to have a significant clinicopathological role in patients with invasive breast cancer. The presence of podoplanin-expressing CAFs was further significantly correlated with histological grading, tumour size and cerbB2 score in our collective. Our results suggest that podoplanin expression by CAFs could predict poor patient outcome in breast carcinoma.

Ethics Review: This study was approved by the Research Ethics committe of Ankara Education and Research Hospital on the 12th of August 2015 (Meeting number: 605 Provision number: 5078)

Conflict of interest: Authors declared no conflict of interest or financial support.

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