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Original Article MEDICAL ONCOLOGY

Fibulin1 and mesothelin expressions in pancreas ductal adenocarcinoma

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ABSTRACT

OBJECTIVE: The balance between malignant tumor cells and the connective tissue surrounding them determines the aggressiveness of the tumor. We aimed to understand the effects of mesothelin (MSLN) and fibulin1 (FBLN1) expressions on survival in pancreas ductal adenocarcinoma (PDCA), and also whether these proteins have prognostic value for PDCA.

METHODS: Of 80 patients in total, 40 who underwent the Whipple procedure for diagnosed PDCA between 2009 and 2016, and 40 patients with diagnosed pancreatitis as the control group, were included in the present study. Immunohistochemically, MSLN, and FBLN1 expressions were evaluated retrospectively. We assessed the relationship between the degree of MSLN, FBLN1 expression, clinical-pathological features, and survival rates in PDCA cases.

RESULTS: The median follow-up duration was 11.4 (3–41) months. All of the patients for MSLN and FBLN1 were immune reactive. We detected a significant difference in MSLN expression between patients with PDCA and control groups, but not in FBLN1 expression. MSLN, FBLN1 expressions were categorized as lower-higher (L/H) groupings. There was no difference in the median overall survival (OS) of patients in the MSLN groups. The L-FBLN1 group had a median OS of 18 months (95% CI: 9.51–26.48) versus 14 months (95% CI: 13.021–14.97) in the H-FBLN1 group (interconnective tissue) (p=0.035). According to Kaplan–Meier analysis, L-FBLN1 expression in the tumor microenvironment was associated with longer survival in PDCA. The FBLN1 expression in the tumor microenvironment was shown to be significantly inversely related to OS (p=0.05).

CONCLUSION: The FBLN1 expression, which is in the tumor microenvironment of PDCA, may serve as a prognostic biomarker.

Keywords: Fibulin1; mesothelin; pancreas ductal adenocarcinoma; prognosis; tumor microenvironment.

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Pancreas ductal adenocarcinoma (PDCA) is an aggressive cancer and the fourth leading cause of death worldwide [1]. Despite many targeted molecular therapies available today, 5-year overall survival rates are around 7–8%. Although 20–25% of the patients are resectable, their course often involves local and distant metastases and relapses. PDCA consists of 85% of cases [2].

RAS mutations, which are important oncogenes in the etiopathogenesis of PDCA, Her2/Neu/ERB, epidermal growth factor receptor (EGFR), fibroblast growth factor (FGF), insulin growth factor-1, and many other growth receptors are highly expressed in these patients, as they are in many cancers [2, 3].

The identification of molecular alterations and activities that happen in the initiation and progression

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of PDCA, as well as early diagnosis and treatment of PDCA, are important targets for PDCA treatment.

The connective tissue component surrounding primary tumor tissue may be responsible for the long-term stability of certain cancers despite their aggressiveness [3, 4]. Fibulin (FBLN), a constituent of the extracellular component, has been reported to serve as a tumor suppressor gene in several carcinomas [5, 6]. Mesothelin (MSLN), a surface antigen, is prominent in normal peritoneal and pericardial cells. This glycoprotein has been shown to be overexpressed in several malignancies, especially ovarian cancer, mesothelioma, and pancreatic cancer, and to have a prognostic value in cancerogenesis through the Wnt pathway [7, 8]. The aim of this study was to investigate how MSLN and FBLN1 expressions affect survival in PDCA patients and whether these proteins have prognostic value for PDCA.

Highlight key points

- The lower FBLN1 (in the acinar tissue) expression has a favorable factor for estimating longer survival in pancreas ductal adenocarcinoma.
- Low FBLN1 expression in the pancreatic ductal adenocarcinoma tumor microenvironment indicates longer survival.
- Immunohistochemically, MSLN expressions can be evaluated in the diagnosis of pancreas ductal adenocarcinoma.

MATERIALS AND METHODS

Patient Selection

The present study includes a total of 80 patients. Our study was conducted by the departments of medical oncology, pathology, and histology, following the approval of the Faculty of Medicine Board of Ethics at Firat University (10.11.2016, no: 17/04). For all situations, written informed permission was provided, which was in accordance with the Statement of Helsinki.

This study includes 40 patients with PDCA who underwent Whipple surgery between 2009 and 2016, and 40 patients who were operated for non-malignant reasons as the control group and whose last pathology was pancreatitis. Case clinical information was obtained from the hospital's computer data center, and patients with missing medical data were excluded from the study.

Patients' Features

Patients' baseline information (age, gender, staging, dates of diagnosis and death/final control, pathology reports, pre-operative (pre-op) and post-operative (post-op) computed tomography views, Carsinoembrionic antigen (CEA), and Ca19-9 readings as above and below median values) were recorded. The American Joint Committee on Cancer-8 guidelines were used to stage patients diagnosed with PDAC [9]. The therapies were planned in accordance with the standards of the National Comprehensive Cancer Network [10]. This study included patients with PDAC who had received gemcitabine/5 FU, cisplatin-based cytotoxic drugs as adjuvant CT and were diagnosed with local advanced or advanced disease [9, 10].

Pathological Evaluation

Using polyclonal antibodies and immunological histochemical techniques, we graded biopsy samples based on the degree of MSLN and FBLN1 expression. The pathologist used polylysine slices 5–6 m thick to convert paraffin blocks to slides. After passing through a graded alcohol series for antigen retrieval, embedded in paraffin tissues in a citrate buffer solution at pH six, they were cooked in a microwave oven (750W) for 7–5 min. After blocking the base stain with Ultra V Block (TA-125-UB, Lab Vision Corporation, USA), primary antibodies (Anti-e NOS Antibody PA1712-1, Boster Bio, USA) were incubated for 60 min. The tissues were treated for 30 min with a secondary antibody (anti-mouse/rabbit IgG with biotin, Diagnostic Bio Systems, KP 50A, Pleasanton, USA) and then for 30 min with Streptavidin Alkaline Phosphatase (TS-060-AP, Lab Systems).

The final preparations were evaluated and photographed by an independent pathologist using a Leica DM500 microscope. The FBLN1 and MSLN antibody control sample subjects were evaluated using endometrial carcinoma specimens. The severity (0: none, +0.5: very few, +1: few, +2: moderate, +3: severe) and extent of immunological response in the stain (0.1: 25%, 0.4: 26–50%, 0.6: 51–75%, 0.9: 76–100%) were used to calculate the histoscore. The histoscore was derived using the formula (extent X severity). Based on values lower (L) and higher (H) than that of the median, we separated the histoscore into two categories: Lower and higher groups [11]. MSLN and FBLN1 values were classified into two groups based on whether they were lower or higher than the median (□2.7 H-MSLN group, ≤2.7 L- MSLN group, ≤1.8 L-FBLN1 group, and □1.8 H-FBLN1 group).

Table 1. The relationships between FBLN1 and MSLN expressions and features of patients

Variables	FBLN1			MSLN		
	L-FBLN1, n	H-FBLN1, n	p	L-MSLN, n	H-MSLN, n	p
Age			1			0.545
≤65	11	10		11	10	
>65	10	9		8	11	
Gender			1			1
Woman	8	7		7	8	
Man	13	12		12	13	
Stage			1			0.407
Local advanced	17	16		15	18	
Advanced	3	3		4	2	
PNI			0.596			1
(-)	1	2		1	2	
(+)	20	17		18	19	
LVI			0.538			0.750
(-)	11	12		10	13	
(+)	10	7		9	8	

LVI: Lymphovascular invasion; PNI: Per neural invasion; (+): Present; (-): Absent; L: Low; H: High; MSLN: Mesothelin; FBLN: Fibulin.

Statistical Analysis

To analyze the data, we utilized the IBM Statistical Package for the Social Sciences 24.0 (SPSS Inc., Chicago, IL, USA) software system from International Business Machines (IBM). Every piece of data was calculated using the mean and standard deviation. The time from diagnosis to death or the final visit was defined as median survival (OS). For evaluating OS, we utilized Kaplan–Meier analysis and log-rank analysis. To identify the variables that influence OS, we performed multivariate and univariate analysis with the Cox proportional hazards model. The parameters influencing the OS were evaluated utilizing Spearmen/Pearson correlation and the Student's t-test. $P<0.05$ was accepted to be statistically significant.

RESULTS

Table 1 shows the demographic and histological characteristics of the patients. The degree of FBLN1-MSLN expression is unrelated to patients' demographic or histological characteristics.

In light microscopy examination of immune histochemical staining in PDCA acinar cells compared to the control group, MSLN immune reactivity was statistically significantly higher as shown in Figure 1A, B, but not in FBLN1 immune reactivity, as shown in Table 2 and Figure 1B–D.

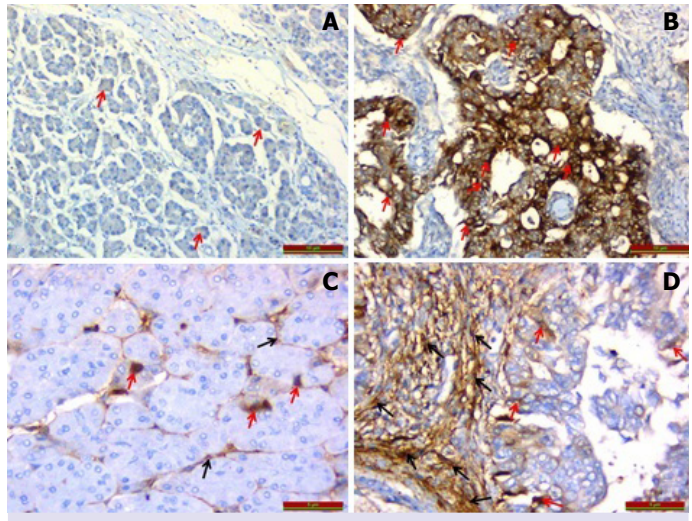

Figure 1. Histopatholiogical evaluation. **(A)** Mesothelin immune reactivity in control pancreas tissue, **(B)** Mesothelin immune reactivity in pancreas ductal adenocarcinoma, **(C)** Fibulin1 immune reactivity in control pancreas tissue, **(D)** Fibulin1 immune reactivity in pancreas ductal adenocarcinoma.

TABLE 2. Histoscore (area × intensity)

Groups	Mesothelin		Fibulin1			
	Acinar cells	Cancer cells	Acinar cells	Cancer cells	ICT acinar	ICT malignant
Control	0.45±0.21	–	0.71±0.20	–	0.65±0.19	
PDCA	–	2.17±0.5[a]	–	0.85±0.23		2.11±0.90[a]

Values are given as mean±standard deviation. a: Compared to the control group, (p<0.05); ICT: Inter connective tissue; PDCA: Pancreas ductal adenocarcinoma.

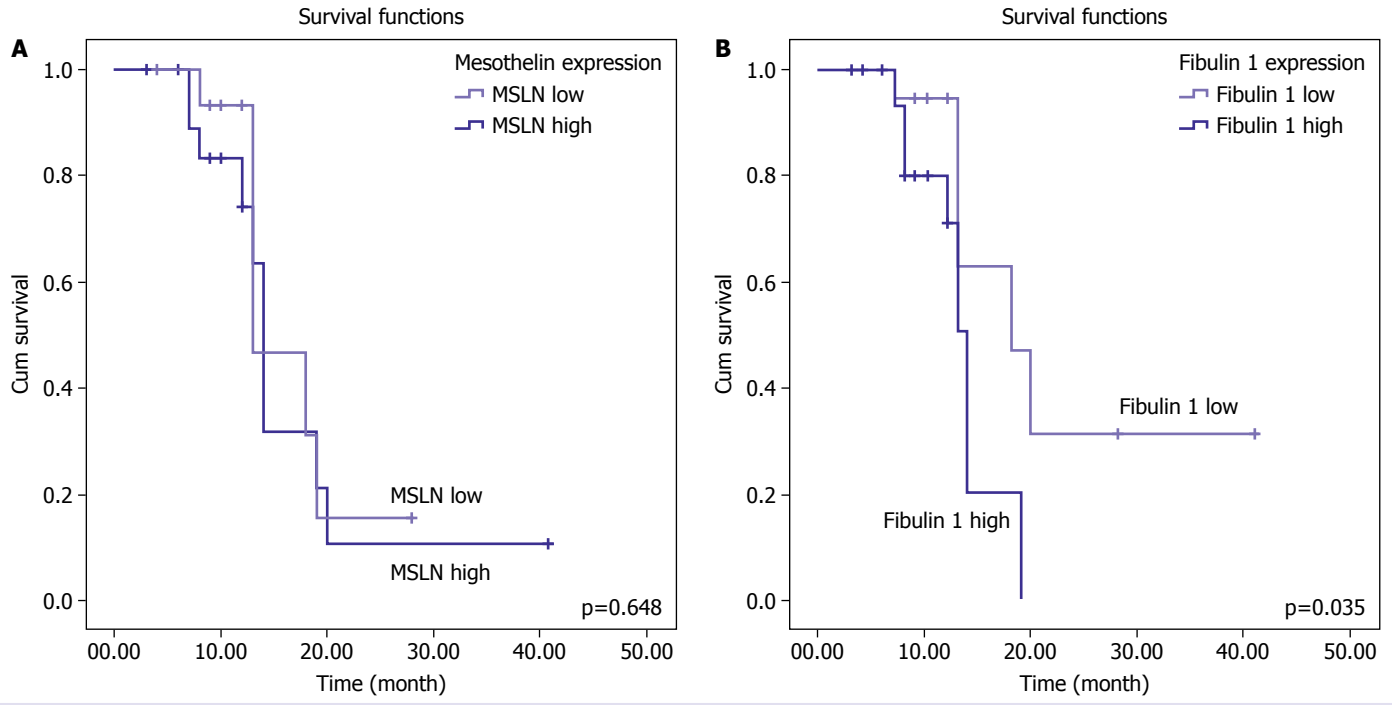

FIGURE 2. Overall survival graphics. **(A)** Overall survival graphic according to levels of mesothelin expression in pancreas ductal adenocarcinoma, **(B)** Overall survival graphic according to level of fibulin1 expression in connective tissue in pancreas ductal adenocarcinoma.

Immune reactivity was statistically significantly higher in the FBLN1 group than in the control group, as shown in Table 2 and Figure 1B–D, but not in the FBLN1. In contrast, FBLN1 immuno reactivity was significantly more in connective tissue between PDCA cells than in connective tissue between acinar structures (ICT) in the control group (p=0.05) (Fig. 1C and D).

The median period of follow-up was 11.4 months (range 3–41 months).

The H-MSLN group had a median OS of 14 months (95% CI: 13.09–14.91), whereas the L-MSLN group had a median OS of 13 months (95% CI: 6.91–19.86). The Kaplan-Meier method and log-rank analyses showed no association between the MSLN groups (p=0.648) (Fig. 2A).

The median OS in the connective tissue was 14 months (95% CI: 13.021–14.97) for the H-FBLN1 group and 18 months (95% CI: 9.51–26.48) for the L-FBLN1 group, as shown in Figure 2B. L-FBLN1 group had a considerably longer OS, as seen in (p=0.035).

When the factors affecting OS were evaluated in terms of MSLN and FBLN1 expressions in univariate and multivariate analysis, it was observed that only the age factor was effective as a favorable prognostic factor on OS (p<0.05) as shown in Tables 3 and 4.

Table 3. Statistical analysis of variables with MSLN affecting survival (n=40)

Variables	Univariate analyses		Multivariate analyses		
	(%)	p	p	HR	95% CI
Age	62±11*	**0.008**	**0.002**	0.088	0.019–0.404
Gender		0.252	–	–	–
Woman	37.5				
Man	67.5				
Stage		**0.038**	0.986	0.00	0.00–0.00
Local advanced (II-III)	92.5				
Advanced (IV)	7.5				
MSLN		0.648	–	–	–
H-MSLN	52.5				
L-MSLN	47.5				
LVI		**0.046**	0.100	4.380	0.752–25.498
(+)	42.5				
(-)	57.5				
PNI	0.715	–	–	–	
(+)	92.5				
(-)	7.5				
Pre-op CEA	2.50±15.81*	0.193	–	–	–
L-CEA	52.5				
H-CEA	47.5				
Post-op CEA		0.165	–	–	–
L-CEA	50				
H-CEA	50				
Pre-op Ca19-9	24.43±21.96	**0.038**	0.145	2.852	0.697–11.670
L-Ca19-9	30				
H-Ca19-9	70				
Post-op Ca19-9		0.560	–	–	–
L-Ca19-9	52.5				
H-Ca19-9	42.5				
Status of CT		0.207	0.929	1.058	0.306–3.664
(+)	62.5				
(-)	37.5				
Status of RT		0.163	–	–	–
(+)	45				
(-)	55				

LVI: Lymphovascular invasion; PNI: Per neural invasion; CT: Chemotherapy; RT: Radiotherapy; CEA: Carsinoembrionic antigen; *: Median meaning; HR: Hazard ratio; MSLN: Mesothelin; (+): Present; (-): Absent; Pre-op: Pre operatuar; Post-op: Post operatuar.

According to Spearman/Pearson correlation and Student's t-test, there was no correlation between degrees of MSLN, FBLN1 in acinar cells, and OS (r: 0.094, p: 0.282), (r: -0.233, p: 0.074), respectively, but there was a weak, negative correlation between OS and the degree of FBLN1 expression in connective tissue (r: -0.283, p=0.038).

DISCUSSION

Tumor tissue is made up of tumor tissues surrounded, sustained, and nested by a mixture of cellular and non-cellular connective tissue components. The tumor microenvironment plays an important role in tumor

spread. Fibronectin, laminin, angiogenin, tropoelastin, and proteoglycans are all found in the extracellular matrix (ECM). The PDCA micro-environment has two essential components: Severe desmoplasia and severe immunological suppression [4]. In studies, the FBLN family has been established to have a significant role in the establishment of an ECM [12–14]. The 19 homologous members of FGF have been proven to have proangiogenic effects and limit tumor development rate in a variety of cancer types as well as tissue healing [5, 15]. FBLN1 has four distinct splice variants and is also located on the 22nd chromosome. FBLN1 was activated when FGF receptors (FGFR) were phosphorylated. FBLN1 has been known to play a role in the RAS-mitogen-activated protein (MAPK), phosphatidylinositol-3'-kinase (PI3K)-Akt signaling (PI3K-Akt), signal transducer, and activator of transcription pathways [16]. According to recent research, FBLN1 is overexpressed in various carcinomas and is implicated in the tumor processing mechanism [15, 17–19]. Preventing extracellular-signal-regulated kinase (ERK) stimulation may suppress cancer cell migration and metastasis through fibronectin [20, 21]. Although there is insufficient information about the role of FBLN1 in PDCA, tumors, overexpressing FBLN1, act as a tumor suppressor gene, which also offers good prognostic properties [22–25].

In their researches, some academics came up with various outcomes. The overexpression of FBLN 1 inhibited epithelial growth factor (EGFR) activation in non-small cell lung cancer cell lines, according to Harikrishnan et al. [26]. FBLN1 was down-regulated by promoter hypermethylation in gastric cancer cell lines [27]. The FGFR inhibitors, such as erdafitinib, have been studied in clinical and non-clinical studies in many solid malignancies with different phase levels [28–30]. Researchers have created treatment alternatives by targeting the FGFR with the down regulation of the FBLN [31, 32].

The demographic and histopathologic features of patients with low and high rates of FBLN1 expression (in acinar) were not correlated in this study.

While FBLN1 levels were assessed directly for tumor acinar tissue in the previous research, we assessed both acinar and connective tissue in the tumor microenvironment. We observed that patients with L-FBLN1 in the tumor microenvironment had a longer duration of OS. The tumor microenvironment has quite a desmoplastic structure in PDCA. In this desmoplastic area, ECM proteins are profoundly overexpressed, resulting in a poor prognosis or even CT resistance. ECM proteins consist of tumor growth factor-β (TGF-β), FBLN growth factor 2, and connective tissue growth factor that support secretion in molecular structures. TGF-β plays an important role in cancer initiation and progression. Tumor cell proliferation rises as a result of nucleus-stimulated tumor cell transcription. The activation of processing mechanisms induces invasion in a hypoxic environment, increasing desmoplasia, and the following pancreatic tumor tissue becomes more aggressive. Indeed, these desmoplastic structural components have been explored as a potential target of PDCA in recent years [21, 25, 33]. In this study, PDCA patients with L-FBLN1 expression had a less desmoplastic microenvironment and had a better prognostic pattern.

MSLN, a glycoprotein epithelial surface antigen, is a tumor differentiation antigen that talks back to mesothelium cells with the monoclonal antibody (mAb) K1. MSLN is located on chromosome 16p13.3 and is made up of 17 human exons. Experimental investigations have demonstrated that this soluble molecule acts as a megakaryotic potentiating factor [27]. MSLN is more commonly expressed in mesothelioma and gastrointestinal malignancies. It suppresses proapoptotic gene expression while promoting anti-apoptotic gene expression (through the PI3K/Act and MAPK/ERK signal transduction pathways), leading to IL-6 production and activating nuclear factor-B in the proliferation pathway [21].

As a result of these strategies, tumor cells gradually multiply and evading apoptosis. There are conflicting results on this issue in the literature. In some studies, patients with advanced ovarian cancer and high-density MSLN expression had a better prognosis, while patients with other cancers with high-density MSLN expression had a worse prognosis [34, 35]. The expression of MSLN has been shown to be overexpressed in 80–85% of PDAC patients. In our investigation, MSLN expression was revealed to be diagnostic in PDAC. In ovarian cancer, MSLN interacts with MUC 16 and uses this method to communicate between cells [36]. In addition, MSLN was also shown to have an influence on MUC 16 in PDCA patients in a study conducted by Chen et al. [37] in 2013. According to other researchers, MSLN overexpression has not been shown to promote cellular proliferation or serve as a tumor progression factor in cell line studies. MSLN overexpression, it turns out and suppresses tumor development in immune-compromised animals [38]. The level of MSLN expression had no effect on survival in this investigation. Clinical studies have also revealed that increased MSLN expression in cancer patients is an unfavorable prognostic factor for survival [8, 39].

There are various limitations to our study. To begin with, the number of PDCA cases was restricted. Second, in this investigation, we exclusively looked at MSLN and FBLN1 using only immune histochemistry on tumor tissue and the tumor microenvironment. If we could have carried out the blood levels of MSLN and FBLN1, we could have achieved statistical significance regarding PDCA's proliferation processing mechanism.

Conclusion

The expression of MSLN in PDCA's acinar structures can be used for diagnostic purposes, but that is not predictive. L-FBLN1 expression in the tumor microenvironment is a significant predictive factor for OS in patients with PDCA. FBLN1 in the ECM may be a potential treatment target for PDCA. More comprehensive studies to be done in the future will further strengthen our judgment.

Ethics Committee Approval: The Firat University Clinical Research Ethics Committee granted approval for this study (date: 10.11.2016, number: 17/04).

Conflict of Interest: No conflict of interest was declared by the authors.

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Authorship Contributions: Concept – AA; Design – AA; Supervision – AA, GA; Fundings – AA; Materials – AA, GA; Data collection and/or processing – AA, GA, TK; Analysis and/or interpretation – AA, TK; Literature review – AA, MK; Writing – AA, MK; Critical review – AA, MK.

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