Immunohistochemical Fascin-1 expression correlate with lymph node and distant metastases in pancreatic ductal adenocarcinoma

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ABSTRACT

Introduction: Pancreatic cancer is characterized by its aggressiveness and poor prognosis. Furthermore, mortality is one of the highest among all types of cancers. It has been observed that the expression of Fascin-1 (the actin-bundling protein which enables the motility of cells) is higher in cancer cells and is correlated with invasiveness and metastasis.

Purpose: To investigate the expression of Fascin-1 in histopathological specimens from patients treated for pancreatic cancer and its relationship with histopathological parameters.

Materials and methods: The study was performed on a group of 52 patients diagnosed with pancreatic cancer in the Medical University of Bialystok Clinical Hospital. The expression of Fascin-1 was evaluated in tissue samples using the immunohistochemical method and was rated as "present" or "absent". The analysis of histopathological parameters was performed in correlation with Fascin-1 expression.

Results: Fascin-1 expression was observed in the cytoplasm of cancer cells in 42/52 cases (80.8%). Fascin-1 expression occurred more frequently among patients with lymph node metastases (92.6%) than without this type of metastases (68%) (p=0.024). Likewise, the expression of the investigated protein was observed more often with the presence of distant metastases (100%) than without those metastases (74.4%) (p=0.043). There were no statistically significant differences about age, sex, histological type of cancer, grade of histological differentiation, desmoplasia, inflammatory infiltration, foci of hemorrhage, necrosis, and MVD.

Conclusion: The expression of Fasicn-1 is correlated with the presence of metastases and can be a useful marker of pancreatic cancer involvement.

Keywords: Fascin-1, metastases, pancreatic ductal adenocarcinoma

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INTRODUCTION

Pancreatic ductal adenocarcinoma is rare cancer but one with the worst prognosis. According to GLOBOCAN data, 338 000 people have had pancreas cancer in the world in 2012, which is the 11th place among malignant tumors. In the same year 331 000 people died as a result of this disease, making this cancer the seventh most common cause of death due to cancer [1].

The average 5-year survival is around 6%. It causes that pancreatic cancer is one of the most deadly types of cancer [2].

There are several reasons for the high mortality of this cancer. The most important of which is the high stage of cancer at the time of diagnosis, due to the lack of symptoms at the early stage of the disease, as well as the lack of markers for early diagnosis of this Cancer [3]. Metastasis is associated with a process called EMT - epithelial-mesenchymal transition. In this process, the cell loses properties characteristic of the epithelial phenotype, and gains the mesenchymal phenotype enabling its movement and invasion. The epithelial cells are characterized by an apical-basal polarization, strong intercellular interactions, and adhesion to the basement membrane. When the cell undergoes the EMT process, the intercell conjunctions are impaired, the polarity of the cell disrupts and the arrangement characteristic for epithelial tissue disappears. Under physiological conditions, EMT takes place during embryonic development, while in pathological processes it is important in the regeneration of damaged tissues, organ fibrosis and cancer development. During tumor progression, the EMT process may cause a change in a benign tumor into a locally aggressive and invasive form. In the epithelial-mesenchymal transition, adhesion proteins and cellular matrix proteins such as cadherins, integrins, ECM proteins, FAK family proteins or Rho family proteins participate [4]. Decreasing the expression of adhesion particles is often the first characteristic marker of EMT.

Recent reports indicate that Fascin is one of the proteins also involved in the epithelial-mesenchymal transition [5,6].

Fascin is a monomeric actin filament bundling protein that participates in the formation of actin-based cell surface protrusions. These protrusions allow to cell migration and cell-matrix adhesion. Fascin occurs in the 3 isoforms: the most common Fascin-1 present in nerve cells, fibroblasts, vascular endothelial and smooth muscle cells as well as dendritic and mesenchymal cells; Fascin-2 present in photoreceptor cells of the retina and Fascin-3 localized in the testis [7,8].

In physiological conditions, Fascin-1 is not present in columnar epithelial cells, however, overexpression of this protein has been observed in the course of tumors originating from this tissue, such as breast, lung, kidney, ovarian, pancreatic or gastric cancer [9,10].

Experimental studies carried out on cell lines and mice have shown that silencing of the Fascin-1 gene significantly reduces the number of filopodia and thus inhibits cell migration. In mice with implanted tumors, these treatments resulted in suppression of tumor growth and metastasis inhibition [11].

Immunohistochemical studies assessing Fascin-1 expression in cancers have shown that its overexpression often correlates with invasive tumor phenotype, poor prognosis and shortened disease-free survival, which suggests that this protein may be used in the future as a biomarker in the screening of cancer diseases [12].

The overexpression of Fascin-1 may lead to EMT, which, as previously mentioned, plays a key role in the development and progression of cancer.

Therefore the aim of the study was to assess the expression of Fascin-1 in pancreatic cancer and to demonstrate the relationship between the expression of this protein and selected clinicopathological parameters.

MATERIALS AND METHODS

Study group

The study group consisted of 52 patients (33 men and 19 women, aged 41-84 years, the average age was 62.3) treated surgically for pancreatic ductal adenocarcinoma in the 2nd Clinical Department of General and Gastroenterological Surgery at the University Hospital in Bialystok, in the years 2006-2014.

More than half of the patients (51.9% of patients) had metastases to regional lymph nodes, while 13 patients (25.0%) had distant metastases to the liver and lungs.

In accordance with the GCPs (Guidelines for Good Clinical Practice), this research was approved by the Bioethics Committee of the Medical University of Bialystok (Resolution No.: R-I-002/12/2017).

Histopathological examination

The postoperative material was fixed in 10% buffered formalin and paraffin-embedded. From paraffin blocks, 5-µm sections were cut and stained with hematoxylin-eosin (H+E).

The histopathological analysis included the histological type and histological grade of the tumor. Forty-nine patients (94.2%) had adenocarcinoma without mucosal component, while three patients (5.8%) had adenocarcinoma with the mucous component. In 46 patients (90.4%), the tumor was moderately differentiated, while in the remaining 6 (9.6%), the tumor was low differentiated. Inflammatory cell infiltration in the tumor tissue was

assessed as a weak: <10 inflammatory cells; medium: 10 to 50 inflammatory cells; and strong: >50 inflammatory cells per hpf (high power field) at 100x magnification. The degree of desmoplasia was classified as poor if desmoplasia occupied <25% of the tumor area and prominent when it occupied >25% of the tumor area. Foci of hemorrhage was rated at 400x magnification and was classified as absent, single and numerous. In contrast, necrosis was assessed as: absent, weak (<10% of tumor area), moderate (10-30% of tumor area) and strong (>30% of tumor area). In addition, microvessel density (MVD) was evaluated using immunohistochemical staining with CD34 for blood vessels. They were counted as a number of intratumoral microvessels per unit area of the tumor, subjectively selected from the most vascularized areas (5 hpf under 200x magnification). The PDAC tumors were divided into two groups, with low or high MVD. The cutoff value was the mean MVD. The mean MVD of the tumor tissue was 14.03 vessels (range: 1-43), confirming the histopathological features of hypovascular pancreatic tumors [13].

Immunohistochemical analysis

Fascin-1 expression has been evaluated using immunohistochemistry in both normal and cancerous tissue.

Normal parts of the pancreas (control group) were obtained during resection from unchanged parts of the pancreas.

Sections has been fixed in formalin and embedded in paraffin.

Tissue blocks were cut using a microtome into 4-µm-thick sections on silanized glasses.

Slides were incubated at 60°C for 1 hour. Next, the sections were deparaffinized in a series of xylene and rehydrated in alcohols of decreasing concentrations.

In order to exhibit an antigen, the tissue sections were heated in a water bath in citrate buffer (pH=6.0) at 95°C for 20 min and next cooled for 20 min in room temperature.

Then, they were incubated with 3% hydrogen peroxide to block endogenous peroxidase (10 minutes).

Subsequently, incubation was carried out with Protein Block to prevent non-specific binding antibodies (20 minutes).

In the next step, the preparations were incubated with the monoclonal anti-Fascin-1 antibody (R&D Systems, clone #833223), diluted 1:50, for 1 hour at room temperature.

The NovoLink Polymer detection kit (Novocastra, Poland) and DAB chromogen (Novocastra, Poland) were used to visualize the reaction.

Cell nuclei were stained with hematoxylin, after which preparations were dehydrated in a series of alcohols with increasing concentration, a series of xylenes and closed in DPX medium.

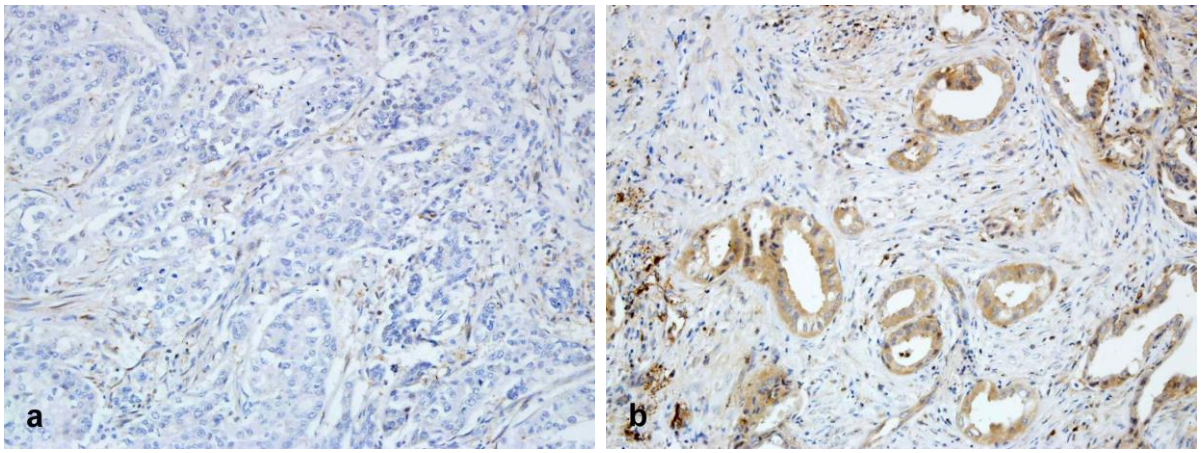
Positive and negative staining control was carried out in accordance with the manufacturer's recommendations.

The cytoplasmic expression of Fascin-1 was observed in all tumor cells.

The stained preparations were evaluated using an Olympus CX41 light microscope. Fascin-1 expression was assessed as negative (lack of reaction) or positive. Each positive reaction was considered positive regardless of its intensity (medium or strong) (Figure 1).

Statistical analysis

All calculations were carried out using STATISTICA 12.0 (POLAND). Statistical analyses were performed with Spearman's correlation coefficient test. A *p-value* of <0.05 was considered to be statistically significant.



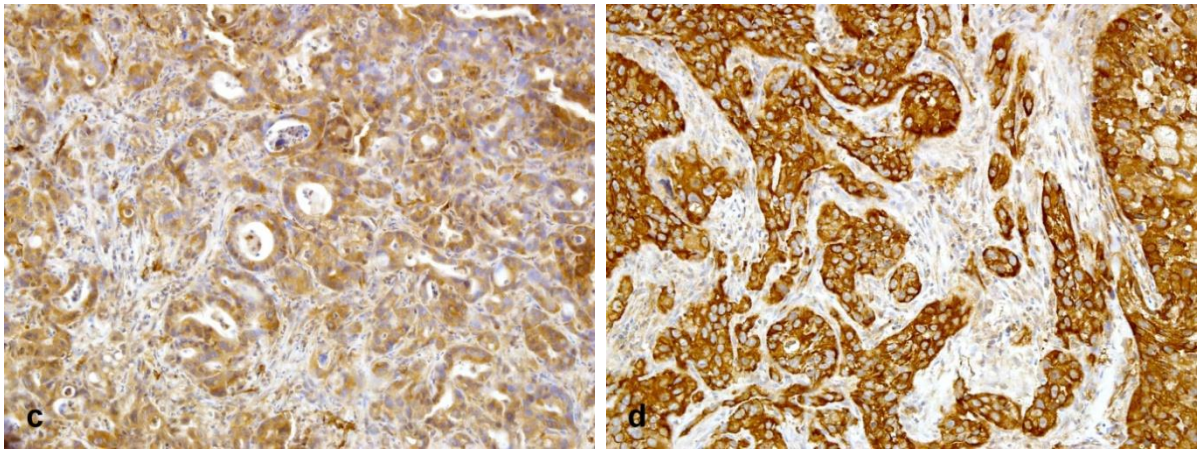


Figure 1. Negative (a) and positive: (b,c) medium, (d) strong intesivity of Fascin-1 protein expression in pancreatic ductal adenocarcinoma cells (IHC staining, magnification x200).

RESULTS

In normal pancreatic tissue expression of Fascin-1 was not observed. In pancreatic cancer cells cytoplasmic expression of Facin-1 was observed in 42 cases (80.8%).

Statistical analysis did not reveal any significant differences between Fascin-1 expression and gender, patient age, histological type of tumor or degree of histological differentiation. There was no correlation with additional histopathological parameters such as desmoplasia, inflammatory cell infiltration, foci of hemorrhage, necrosis, and MVD. However, a positive correlation was observed between the expression of this protein and the presence of metastases to local lymph nodes (p=0.024). Fascin-1 expression was demonstrated in 25/27 patients (92.6%) with metastases to the lymph nodes, whereas in non-metastatic patients, expression was present in 17/25 cases (68%). In addition, a positive correlation occurred between Fascin-1 expression and the presence of distant metastases (p=0.043). Fascin-1 expression was present in all 13 patients with distant metastases (100%), whereas in patients without such metastases expression appeared in 29/39 cases (74%). Correlation results are presented in Table 1.

Table 1. Correlations between immunohistochemical expression of Fascin-1 in tumor tissue and clinicopathological parameters in pancreatic cancer patients

		Fascin-1 expression		
Parameters	Patients N(%)	Absent	Present	*P* value
Age				0.873
<60	22 (42.3%)	4 (18.2%)	18 (81.8%)	
≥60	30 (57.7%)	6 (20%)	24 (80%)	
Sex				0.805
Male	33 (63.5%)	6 (18.2%)	27 (81.8%)	
Female	19 (36.5%)	4 (21.1%)	15 (78.9%)	
Adenocarcinoma type				0.533
Nonmucinosus	49 (94.2%)	9 (18.4%)	40 (81.6%)	
Mucinosus	3 (5.8%)	1 (33.3%)	2 (66.7%)	
Grade of malignancy				0.233
Medium-differentiated	47 (90.4%)	8 (17%)	39 (83%)	
Poor-differentiated	5 (9.6%)	2 (40%)	3 (60%)	
Lymph node metastasis				**0.024**
Absent	25 (48.1%)	8 (32%)	17 (68%)	
Present	27 (51.9%)	2 (7.4%)	25 (92.6%)	
Distant metastasis				**0.043**
Absent	39 (75%)	10 (25.6%)	29 (74.4%)	
Present	13 (25%)	0	13 (100%)	

Desmoplasia				0.150
Poor	31 (59.6%)	8 (25.8%)	23 (74.2%)	
Prominent	21 (40.4%)	2 (9.5%)	19 (90.5%)	
Inflamatory infiltration				0.630
Absent	1 (1.9%)	0	1 (100%)	
Weak	18 (34.6%)	4 (22.2%)	14 (77.8%)	
Medium	19 (36.6%)	2 (10.5%)	17 (89.5%)	
Strong	14 (26.9%)	4 (28.6%)	10 (71.4%)	
Foci of hemorrhage				0.457
Absent	22 (42.3%)	4 (18.2%)	18 (81.8%)	
Single	17 (32.7%)	2 (11.8%)	15 (88.2%)	
Numerous	13 (25%)	4 (30.8%)	9 (69.2%)	
Necrosis				0.290
Absent	23 (44.2%)	4 (17.4%)	19 (82.6%)	
Weak	15 (28.9%)	2 (13.3%)	13 (86.7%)	
Moderate	10 (19.2%)	2 (20%)	8 (80%)	
Strong	4 (7.7%)	2 (50%)	2 (50%)	
MVD				0.739
Low	34 (65.4%)	7 (20.6%)	27 (79.4%)	
High	18 (34.6%)	3 (16.7%)	15 (83.3%)	

Spearman's correlation coefficient test. Significant relationship is marked in bold.

DISCUSSION

Pancreatic ductal adenocarcinoma (PDAC) is a cancer characterized by very poor prognosis. The overall survival time is only a few months. The late diagnosis is still a big problem. Therefore it is important to search for mechanisms responsible for the development and invasiveness of this cancer.

In our studies, we observed a positive reaction of Fascin-1 in 42 patients suffered from pancreatic cancer (80.8%) while we did not observe this protein expression in normal pancreatic tissue. It was also confirmed by the results of Li et al. [11] in study on PDAC lesions. In addition, Xu et al. [9] conducted studies on the human non-transfected and Fascin-1 transfected cell line MIA PaCa-2 and found that this protein promotes cell migration, invasion and scattering, thus contributes to the aggressive behaviour of pancreatic cancer cells. These studies confirm the contribution of Fascin-1 to the development of pancreatic cancer.

There are many reports about role of Fascin-1 in the development and metastasis of many neoplasms such as melanoma, breast, lung and stomach cancer [14-17]. Several studies have shown that increase in expression of Fascin-1 correlates with tumours characterized by high metastatic potential and poor prognosis as well as shorter overall survival [12]. In our study, we did not have data of T stage of pancreatic cancer, however, the current reports indicate the relationship between expression of Fascin-1, stage of cancer advancement and overall survival time. Research by both Li et al. [11] and Tsai et al. [18,19] indicate that the increase of Fascin-1 expression occurs in more advanced tumours (T3 and T4) compared to less advanced tumours (T1 and T2) and is associated with shorter overall survival time. Additionally, Li et al. [11] showed that stronger expression of Fascin-1 correlates with short time to recurrence (<6 months) compared to longer time when cancer is not detectable (>6 months). However, the results of our study indicate significant relationship between Fascin-1 expression with lymph node metastases (pN) and with distant metastases (pM). In tumours represent both lymph node metastases and distant metastases, significant increase of Fascin-1 protein expression was observed ($p=0.024$ and $p=0.043$, respectively). In turn, Li et al. [11] and Tsai et al. [18, 19] did not observe such correlation between expression of Fascin-1 and lymph node metastases. In addition, Tsai et al. [18,19] did not show any relationship between the expression of this protein and distant metastases.

Moreover, we have not shown any correlation between Fascin-1 expression and patient's age, gender, histological type of cancer as well as the grade of malignancy. By contrast, Tsai et al. [18] showed that an increase of Fascin-1 expression is associated with histological grade of a tumour. Their studies present statistically significant elevated Fascin-1 expression ($p=0.038$) in low-differentiated tumours compared to medium- and high-differentiated tumours. It indicates the relationship of Fascin-1 expression with worse histological type of pancreatic cancer.

We also examined the relationship between Fascin-1 expression and degree of inflammatory infiltration, foci of hemorrhage, necrosis and microvessel density. It is known that Fascin-1 participates in the process of inflammation, but our results did not show correlation between its expression and extent of inflammatory infiltration in pancreas cancer tissue. Moreover, expression of

Fascin-1 did not correlate with the degree of necrosis, foci of hemorrhage and microvessel density. However, one research showed that Fascin-1 may play a synergic role with angiogenesis in progression of sinonasal inverted papilloma (SNIP). The authors of this report revealed a positive correlation between the expression of Fascin-1 and microvessel density counts in SNIP [20].

In pancreatic cancer, extensive fibrosis occurs in the primary tumor, referred as a desmoplasia. Symptoms of desmoplasia are overexpression of ECM proteins and extensive conversion of fibroblast-type cells into the myofibroblast phenotype. Desmoplasia is associated with poor prognosis by promoting progression of pancreatic cancer and resistance to chemotherapy [21,22]. However, we did not observe any relationship between Fascin-1 expression and degree of desmoplasia.

CONCLUSIONS

Based on the results from our research and current reports we conclude that the increase of Fascin-1 expression is associated with worse prognostic pancreatic tumours.

Higher Fascin-1 expression in tumours with both lymph nodes and distant metastases and lack of expression in normal tissue may suggest its potential role as a marker of PDAC advancement.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Financial disclosure/funding

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