p16, p21, and p53 proteins play an important role in development of pancreatic intraepithelial neoplastic

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Abstract

Background Deregulation of cell cycle takes place during the development of many cancers as well as pancreatic ductal adenocarcinoma (PDA), which develops from precursor lesions, most frequently including pancreatic intraepithelial neoplasia (PanIN).

Aims The aim of this study was to evaluate and compare the expression of p16, p21, and p53 proteins taking part in the regulation of the cell cycle in normal pancreatic ducts and pancreatic intraepithelial neoplasia at its various advancing stages.

Methods The expressions of p16, p21, and p53 were assessed immunohistochemically in 70 patients with different pancreatic diseases (pancreatic ductal adenocarcinoma, pancreatitis, and pancreatic cysts), showing also pancreatic intraepithelial neoplasia. The results correlated with chosen clinicopathological parameters.

Results Our study revealed a difference in p16, p21, and p53 expressions between normal pancreatic ducts and various stages of PanIN. p16 expression progressively decreased, whereas p21 and p53 increased from normal pancreas to PanIN 1, 2, and 3. The expression of p21 was associated with age, p53 with PanIN location in the pancreas and p16 with the type of primary diseases. Simultaneously, we observed a directly proportional relationship between the expression of p21 and p53 proteins and inversely proportional between the p16 and the p21 and p53 proteins.

Conclusions p16, p21, and p53 proteins play an important role in the deregulation of the cell cycle and participate in the development of pancreatic intraepithelial neoplasia. Immunohistochemical evaluation of their expressions may be helpful in the diagnosis of PanIN.

Keywords Cell cycle · Immunohistochemistry · Pancreatic cancer · Pancreatic intraepithelial neoplasia · PanIN

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the most common epithelial cancers of the exocrine pancreas representing more than 95% of malignant tumors of this organ [1]. It is classified on the 12th position in terms of cancer incidence in the world but is the fourth leading cause of cancer deaths. According to the latest reports, if by 2030 year a

significant progress is not reported in the diagnosis and treatment of pancreatic cancer, it will be classified on the 2nd position among all cancers in terms of mortality [2]. Therefore, it is important to detect cancer early, before it changes into an invasive form, when the possibility of complete cure is minimal. In order to improve a severe course of disease and survival of patients, routine diagnostic tests aimed at detecting precursor lesions of PDAC should be developed and applied. Early detection and treatment of precursor lesions would prevent the further progression and development of invasive cancer as observed in the case of colorectal, breast, and cervical cancer, where the screening test significantly reduced the percentage of morbidity and mortality due to cancer [3].

One of the most commonly occurring precursor lesions of pancreatic ductal adenocarcinoma is pancreatic intraepithelial neoplasia (PanIN). Pancreatic intraepithelial neoplasias are

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defined as microscopic, flat, or papillary noninvasive lesions developing in small pancreatic ducts (< 5 mm diameter). They are composed of cuboidal or columnar cells with varying amounts of mucin facilitating their distinction from normal ductal epithelium composed of cuboidal or low columnar with amphophilic cytoplasm and without any evidence of mucinous cytoplasm [4]. According to the degree of architectural and cytological atypia in pancreatic ducts, PanINs are categorized into two grades: the low-grade PanIN (PanIN 1A, 1B and PanIN 2) and high-grade PanIN (PanIN 3), which is also referred to as carcinoma in situ [5].

Deregulation of the cell cycle, one of the characteristic features of cancer, is also present during the development of pancreatic ductal adenocarcinoma, during which, as it has been demonstrated, many mutations of tumor suppressor genes, such as *p16* or *TP53* gene have also been found. These mutations have also been observed in pancreatic intraepithelial neoplasia. Inactivation of the *p16* or *TP53* gene mutation results in the deregulated cell cycle and may cause inactivation of other proteins involved in the process [6].

The aim of this study was to assess the expression of cell cycle regulatory proteins such as p16, p21, and p53 proteins and investigate their role in the development of pancreatic intraepithelial neoplasia. Moreover, considering the role of these proteins in the cell cycle, the objective of the current study was to determine whether there was a correlation between p16, p21, and p53 proteins.

Material and methods

Patients and identification of duct lesions

The study involved 70 patients treated surgically due to different diseases of the pancreas (ductal adenocarcinoma, cysts, pancreatitis) in the 2nd Clinical Department of General and Gastroenterological Surgery at the University Hospital in Białystok, in the years 2006–2014. The characteristics of the study group are shown in Table 1. The postoperative material was fixed in buffered and paraffin-embedded formalin. From paraffin blocks, 5-μm sections were cut off and stained with hematoxylin-eosin (H+E). Histopathological analysis included not only the diagnosis of primary disease but also the presence and stage of pancreatic intraepithelial neoplasia. All slides were reviewed by two independent pathologists for the presence and grade of PanIN lesions in accordance with the guidelines developed by international group of experts from various disciplines on the consensus meeting organized by Drs. Ralph H. Hruban and David S. Klimstra and convened at The Johns Hopkins University School of Medicine from June 17 to 18, 2014, under the auspices of The Sol Goldman Pancreatic Cancer Research Center [5].

Table 1 Characteristics of the study group

Clinicopathological features	Number of cases <i>N</i> (%)
Sex	
Male	35 (50%)
Female	35 (50%)
Age	
< 60 years	33 (47.2%)
≥ 60 years	37 (52.8%)
Diagnosis	
Pancreatitis	23 (32.9%)
Pancreatic ductal adenocarcinoma	38 (54.3%)
Pancreatic cysts	9 (12.8%)
Location of disease	
Head	33 (47.1%)
Body	5 (7.1%)
Tail	20 (28.6%)
Body and tail	12 (17.2%)

Briefly, PanIN 1A is an epithelial flat lesion whereas PanIN 1B is a papillary or micropapillary lesion composed of tall columnar cells with basally located nuclei and abundant supranuclear mucin without cytologic atypia. PanIN 2 is a mucinous, epithelial flat or papillary lesion with some nuclear abnormalities including loss of polarity, crowding, enlargement, nuclear stratification, and hyperchromatism. PanIN 3 usually is a papillary or micropapillary architecture with abnormal cribriforming, budding, and luminal necrosis with cytologic abnormalities, such as loss of nuclear polarity, dystrophic goblet cells, atypical mitotic figures, and macronucleoli [7, 8]. PanIN 1A, 1B, and 2 have been recognized as low-grade lesions whereas PanIN 3 as high-grade lesions [5].

The presence of PanINs was evaluated on the slides of normal pancreatic tissue at least 5 mm away from the carcinoma, while in the non-neoplastic lesions, PanINs were assessed in the site of ongoing disease process. In the group of 70 patients, the following lesions were found: normal pancreatic ducts were observed in 35 patients, PanIN 1A in 65 patients, PanIN 1B in 67 patients, PanIN 2 in 51 patients and only 21 patients had PanIN 3 (Table 2).

Immunohistochemistry

Tissue blocks were cut using a microtome into 5-μm-thick sections on silanized glasses. The sections were deparaffinized in xylenes and hydrated in alcohols. In order to exhibit an antigen, the tissue sections were heated in a water bath at 99 °C for 20 min and next cooled for 20 min in room temperature in citrate buffer (pH = 6.0). Then, they were incubated with 0.5% hydrogen peroxide in methanol to block endogenous peroxidase and, next, with protein block (Novocastra)

Table 2 Number of lesions assessed in the group of 70 patients

	Normal pancreatic ducts (%)	Low-grade lesions				High-grade lesions	Total (%)
		PanIN 1a (%)	PanIN 1b (%)	PanIN 2 (%)	Total low-grade lesions	PanIN 3 (%)	
Pancreatitis	14 (18.2%)	21 (27.3%)	21 (27.3%)	16 (20.8%)	58 (75.4%)	5 (6.4%)	77
Pancreatic ductal adenocarcinoma	14 (10.8%)	35 (26.9%)	37 (28.5%)	29 (22.3%)	101 (77.7%)	15 (11.5%)	130
Pancreatic cysts	7 (21.9%)	9 (28.1%)	9 (28.1%)	6 (18.8%)	24 (75.0%)	1 (3.1%)	32
Total number of lesions	35 (14.7%)	65 (27.2%)	67 (28.0%)	51 (21.3%)	183 (76.5%)	21 (8.8%)	239

for 5 min. Incubation with mouse anti-p16 antibody (clone G175-405, Biogenex, ready to use), rabbit anti-p21 antibody (clone 3B6, Novocastra; 1: 50 dilution) and rabbit anti-p53 antibody (clone 36B5, Novocastra, 1:50 dilution) for 1 h in room temperature. Following streptavidin-biotin reaction (biotinylated secondary antibody, streptavidin-HRP; Novocastra), the antigen antibody complex was visualized by application of chromogen 3,3'-diaminobenzidine (DAB, Novocastra).

All slides were stained simultaneously with appropriate specimens, which served as positive controls. We used as a positive control cervical cancerous tissue for p16, colon cancerous tissue for p21 and colon and breast cancerous tissue were used as positive control for p53. Negative controls were performed by incubating the sample without the primary antibodies.

The expression of the proteins was assessed in the pancreatic ductal epithelial cells using the quantitative method and determined as a percentage. The authors counted average number of positively stained nuclei in each pancreatic duct with PanIN. Assessment was performed in all pancreatic ducts in each specimen. In the case of more than one type of PanIN lesions present in many ducts in each patient, the authors described a mean value of expression of protein.

Statistical analysis

We used STATISTICA 10.0 (Statsoft, Cracow, Poland) for statistical analysis. The data were analyzed using Spearman's rank correlation test. Correlations between proteins expression depending on PanIN stage were tested with the use of Mann-Whitney's test. A p value of <0.05 was considered statistically significant. Missing data were removed in pairs.

Results

The positive immunohistochemical reaction of the proteins p16, p21, and p53 was evaluated in the nucleus of pancreatic ductal epithelial cells (Figs. 1, 2, and 3). The expression

of p16 in pancreatic intraepithelial neoplasia ranged from 0 to 100%, while its average value equaled 45.9%. The median was 50% and the modal was 0%. The expression of p21 protein was in the range of 0 to 80%. The mean value of p21 expression was 1.4% and the median was 5%; the modal was equal to 0%. p53 expression values were within the range of 0–90%, whereas the mean value was 8.6%. The median and modal amounted to 0% (Table 3).

p16, p21, and p53 expression in correlation to clinicopathological parameters in pancreatic intraepithelial neoplasia

The higher expression of p16 was shown to be associated with the type of primary disease pancreatic cysts ($p = 0.015$). Patients above the age of 60 had an increased p21 expression ($p = 0.023$). An increase in the expression of p53 was observed in the corpus of the pancreas in comparison to other locations ($p = 0.040$). Statistical analysis revealed correlations of the p16, p21, and p53 expressions and the presence and grade of pancreatic intraepithelial neoplasia ($p < 0.001$) (Table 4).

Comparison of the p16, p21, and p53 expressions between normal pancreatic ducts and different grades of pancreatic intraepithelial neoplasia

Normal pancreatic ducts vs. low-grade and high-grade pancreatic intraepithelial neoplasia

In comparison with normal pancreatic ducts, low-grade and high-grade pancreatic intraepithelial neoplasia expressed p16, p21, and p53 significantly more frequently. The expressions of p21 and p53 proteins were significantly higher whereas p16 expression was significantly lower in pancreatic intraepithelial neoplasia compared to the normal tissue. The mean expression of p16 was significantly lower in low-grade PanIN (45.9%) and high-grade PanIN (21.9%) compared to normal pancreatic ducts, where the mean protein expression was 60.0% ($p = 0.018$, $p = 0.005$,

Fig. 1 Nuclear and cytoplasmic immunoreactivity of p16 in PanIN 1A (1a), in PanIN 1B (1b), in PanIN 2 (1c), in PanIN 3 (1d). Typical examples are shown

respectively). On the other hand, the mean p21 expression was significantly higher in low-grade PanIN (9.8%) and high-grade PanIN (32.4%) in comparison with normal pancreatic ducts (0.1%) ($p < 0.0001$). Similarly, the mean expression of p53 was significantly higher ($p < 0.0001$) in low-grade PanIN (8.4%) and high-grade PanIN (25.0%) compared to normal pancreatic ducts (0.0%) (Figs. 4, 5, and 6).

Low-grade vs. high-grade pancreatic intraepithelial neoplasia

The mean expression of p16 protein was significantly higher in low-grade PanIN in comparison with high-grade PanIN ($p = 0.003$). Also, p21 and p53 had a higher expression in high-grade PanIN compared to low-grade PanIN ($p < 0.001$) (Figs. 4, 5, and 6).

Correlation between p16, p21, and p53 protein expression

Statistical analysis of the expressions of p16, p21, and p53 proteins showed a statistically significant correlation between p21 and p53. This relationship was directly proportional, which means that if the patient had a positive expression of p21 protein, he would also have a positive expression of p53 protein. An inversely proportional relationship was proved between the expression of p16 and p21 and p53 proteins. This means that in case of a decrease in p16 expression, the increased expression of other proteins was observed (Table 5).

Discussion

One of characteristic abnormalities accompanying the development of cancers, including pancreatic cancer, is deregulation of the cell cycle. According to Domagała [9], cancer is a disease of the cell cycle defined as “an abnormal tissue that originates from a single cell and grows as a consequence of dynamism disorders, correct cell cycle progression and impaired both cell differentiation as well as intracellular, inter-cellular and extracellular communication (between the cell and stroma - the extracellular matrix) of its clonal progeny. Deregulation of the cell cycle also occurs during the development of pancreatic ductal adenocarcinoma, in which, as it was demonstrated, there is a series of mutation tumor suppressor genes—*p16*, *TP53*, *DPC4*, *BRCA2*—and oncogenes such as *KRAS* and *c-erbB-2* [9]. These mutations have also been observed in pancreatic intraepithelial neoplasia, which is a precursor lesion of pancreatic ductal adenocarcinoma. The *p16* gene inactivation and *TP53* mutation result in deregulation of the cell cycle and may affect the inactivation of other proteins involved in this process. Therefore, it was justified to assess the expression of p16, p21, and p53 proteins in pancreatic intraepithelial neoplasia.

Statistical analysis of the expressions of cell cycle regulatory proteins such as p16, p21, and p53 showed significant correlations with the presence and stage of pancreatic intraepithelial neoplasia. It has been observed that the expression of p21 and p53 increased with the increasing stage of pancreatic intraepithelial neoplasia, whereas the expression

Fig. 2 Nuclear immunoreactivity of p21 in PanIN 1A (2a), in PanIN 1B (2b), in PanIN 2 (2c), in PanIN 3 (2d). Typical examples are shown

of p16 decreased with increasing of PanIN dysplasia. An increase in the expressions of p21 and p53 proteins with the stage of pancreatic intraepithelial neoplasia was also revealed in the work of Karamitpoulou et al. [10]. The research demonstrated that p53 overexpression might be associated with mutation or inactivation of *TP53* gene, which occurs in about

55–75% of pancreatic cancers. Due to its short half-life (approximately 15 min), a “wild type” of p53, which is a proper form, is not detected in the tissue using immunohistochemistry. A “mutant type” p53 is much more stable and accumulates in large amounts in the nuclei of tumor cells. The anti-p53 antibody used for immunohistochemistry is

Fig. 3 Nuclear immunoreactivity of p53 in PanIN 1A (3a), in PanIN 1B (3b), in PanIN 2 (3c), in PanIN 3 (3d). Typical examples are shown

Table 3 The values of proteins expression: p16, p21, and p53 in pancreatic intraepithelial neoplasia

Protein	Average	Minimum	Maximum	Median	Modal
p16	45.9%	0%	100%	50%	0%
p21	10.4%	0%	80%	5%	0%
p53	8.6%	0%	90%	0%	0%

directed against the two forms of p53—both the “wild” type and the mutant type. The positive expression of p53 in the tissue indicates the presence of the mutant form devoid of a suppressive function resulting in loss of cell cycle control in the G1/S checkpoint and inability to induce apoptosis [11]. Our research revealed that mean expression of p53 was 8.4% in low-grade PanIN while in high-grade lesions, this value equaled 25.0%. The positive expression of this protein was not observed in normal pancreatic ducts. The gradual increase in the nuclear accumulation reaching the maximum values in PanIN 3 confirms the theory stating that the inactivation of the p53 gene is a late phenomenon in the progression of pancreatic cancer. Similarly, Norfadzilah [12] observed that the overexpression of p53

increases with the stage of pancreatic intraepithelial neoplasia. This phenomenon was confirmed in the work of Abe et al. [6] and Biankin et al. [12]. They showed an increased incidence of p53 positive cases with the increasing stage of PanIN. Abe [6] found a positive expression of p53 with a cut-off more than 10% staining of nuclei in 11.1% of PanIN 1, 46.7% of PanIN 2, and 88.9% of PanIN 3. In Biankin's work [13], the positive p53 expression with a cut-off more than 25% staining of nuclei was present in 20% of PanIN 2 cases and 57% of PanIN 3 cases as well as in normal pancreatic ducts, PanIN 1A and PanIN 1B. On the basis of our and other authors' research [6, 12, 13], the p53 protein is considered to play an important role in the transformation of normal epithelium lining of the pancreatic ducts in pancreatic ductal adenocarcinoma.

In response to DNA damage, the p53 protein may inhibit cell proliferation by increasing intracellular levels of p21 protein, which is responsible for the arrest and repair of cell cycle and apoptosis initiation. Furthermore, the p21 protein prevents Rb phosphorylation by inhibiting the activity of cyclin E-CDK2—necessary to initiate phosphorylation. A statistically significant increase in the expression of p21 protein following the increasing stage of pancreatic intraepithelial neoplasia was

Table 4 Associations of p16, p21, and p53 expressions with chosen clinicopathological parameters

Variables	Mean (%) p16 expression	<i>p</i> value	Mean (%) p21 expression	<i>p</i> value	Mean (%) p53 expression	<i>p</i> value
Age		0.125		0.023		0.235
≤ 60	42.6		8.0		7.2	
> 60	49.9		12.4		9.8	
Gender		0.457		0.291		0.177
Male	49.2		10.2		7.3	
Female	42.5		10.6		10.0	
Location		0.003		0.163		0.040
Head	35.9		12.1		10.4	
Corpus	61.2		15.0		12.6	
Tail	60.9		4.6		6.4	
Corpus and tail	44.3		12.6		5.4	
Diagnosis		< 0.0001		0.661		0.907
Pancreatitis	48.6		5.9		4.8	
Pancreatic ductal	34.8		14.6		12.4	
Adenocarcinoma						
Pancreatic cysts	84.7		4.0		2.3	
PanIN		< 0.0001		< 0.0001		< 0.0001
Normal pancreatic ducts	60.0		0.1		0.0	
Low-grade lesions	45.9		9.8		8.4	
• PanIN 1A	54.2		2.2		1.9	
• PanIN 1B	46.9		9.2		7.2	
• PanIN 2	34.3		20.4		18.1	
high-grade lesions						
• PanIN 3	21.9		32.4		25.0	

p < 0.05 was considered statistically significant

Fig. 4 Comparison of the mean expression of p16 in various degrees of PanIN

observed in our study. The average expression was 9.8% in low-grade PanIN and 32.4% in high-grade PanIN. In normal pancreatic ducts, p21 expression was practically undetectable and oscillated on the border of 0.1%. The differences in protein expression in normal pancreatic ducts and between the various grades of pancreatic intraepithelial neoplasia were statistically significant. Karamitpoulou et al. [10] demonstrated similar results in their study. Also, Biankin et al. [13] showed a gradual increase in the expression of this protein in PanIN. The positive expression of p21 was found in 5/53 (9%) of normal pancreatic ducts, 7/43 (16%) of PanIN 1A, 9/28 (32%) of PanIN 1B, 19/34 (56%) of PanIN 2, and 24/30 (80%) of PanIN 3. Additionally, these authors demonstrated statistically significant differences in the mean p21 protein expression in normal pancreatic ducts and between the various grades/stages of PanIN. Biankin [13] revealed that the overexpression of p21 was a relatively early phenomenon

Fig. 5 Comparison of the mean expression of p21 in various degrees of PanIN

Fig. 6 Comparison of the mean expression of p53 in various degrees of PanIN

appearing in the development of pancreatic intraepithelial neoplasia and might play an important role in the deregulation of the cell cycle, which affects the process of carcinogenesis in the pancreas. In his work, the author showed that the increasing intracellular concentration of p21 might be caused by mutations in the *KRAS* gene which were present in over 80% of pancreatic cancer cases. Similarly, Hermanova et al. [14] observed the increasing p21 expression together with the advancing stage of PanIN. In their work, the authors confirmed that a p53 protein had a significant impact on the expression of this protein in PanIN 2 and 3 and invasive cancer. The overexpression of p21 is a result of the stabilization of wild-type p53, which occurs due to a genotoxic stress. Mutant-type p53 will not have the ability to induce p21 expression. If there is no activation of the *KRAS* gene, the overexpression of p21 may lead to the overexpression of *HER-2/neu*, which acts through the Ras signal. Other theory assumes that the increased expression of p21 resulting in the subsequent activation of D-CDK4 cyclin is responsible for activation of the Ras / Raf / MEK / ERK [15].

p16 protein is encoded by a gene *p16INK4a*, which is inactivated by homozygous deletion accompanied by loss of heterozygosity (LOH) and mutation of a gene promoter methylation in 95% of cases of pancreatic ductal adenocarcinoma [16]. Recent reports suggest that similar mechanisms of *p16* gene inactivation take part in the development of non-invasive lesions in pancreatic ducts. In our research the mean expression of p16 was 60.0% in normal pancreatic ducts, 45.9% in low-grade lesions, and 21.9% in high-grade PanIN. In their study, Rosty et al. [17] demonstrated that the expression of p16 protein was observed in all cases of PanIN 1A, while its loss was observed in 11% of PanIN 1B, 16% of PanIN 2, and 40% of PanIN 3. Moreover, they showed statistically significant differences in the expression of this protein between all stages of PanIN. Maitra et al. [18] showed a loss of p16 expression in 31% of PanIN 1A, 44% of PanIN 1B, 50% of

Table 5 Correlations between p16, p21, and p53 proteins in pancreatic intraepithelial neoplasia

Protein	p16	p21	p53
p16	_____	$R = -0.2311 < 0.001$	$R = -0.2540 < 0.001$
p21	$R = -0.2311 < 0.001$	_____	$R = 0.4913 < 0.001$
p53	$R = -0.2540 < 0.001$	$R = 0.4913 < 0.001$	_____

PanIN 2, and 85% of PanIN 3. Similar results were observed by Wilentz et al. [19]. The authors showed a loss of this protein expression in 30% of the flat-lesions in pancreatic ducts without significant atypia (currently PanIN 1A), 27% of papillary lesions without significant atypia (now PanIN 1B), 55% of papillary lesions without atypia (now PanIN 2) and 71% of carcinoma in situ (now PanIN 3). In Biankin's study [20], the expression of p16 protein was present in 71% of normal pancreatic ducts, 74% of PanIN 1A, 76% of PanIN 1B, 61% of PanIN 2, 29% of PanIN 3, and only in 17% of invasive cancer cases. Based on the findings described in this study and the research carried out by Wilentz et al. [19], Maitra et al. [18], and Biankin et al. [20], it can be concluded that inactivation of *p16INK4a* gene resulting in loss of p16 expression is common and can be regarded as an early indicator occurring in the development of pancreatic cancer. In addition, Maitra [18] stated that loss of p16 expression increased with increasing dysplasia in pancreatic ducts and preceded inactivation of *p53* and *DPC4*. The author also suggested that loss of the expression of the protein involved in the regulation of one cell cycle checkpoint was not sufficient to initiate the uncontrolled division of cells in pancreatic intraepithelial neoplasia. Moreover, studies performed on mouse models revealed that deletion and consequently inactivation of *p16INK4A* greatly accelerated the malignant progression of mutant *K-ras*-triggered PanIN lesions into highly invasive or metastatic PDAC after activation of *K-ras* gene which is responsible for the development of pancreatic intraepithelial neoplasia. It suggest that activation of *K-ras* serves to initiate precursor lesions of pancreatic ductal adenocarcinoma and the *p16/INK4A* tumor suppressors normally function to inhibit the malignant transformation potential of mutant *K-ras* [21, 22].

In addition to examining the relationship between the presence and the stage of pancreatic intraepithelial neoplasia and the expression of selected proteins involved in the cell cycle regulation (p16, p21, p53) and apart from the assessment of differences in protein expression between different stages of PanIN, we also examined the interactions between the expressions of these proteins. A directly proportional correlation was proved between p21 and p53, which means that the increased expression of one protein caused the increased expression of the other. In the case of p16 protein, an inverse proportional relationship was demonstrated. The decreased expression of p16 was associated with the increased expression of p21 and p53. We did not find reports describing the correlations between p16, p21, and p53 proteins.

So far, the exact mechanisms involved in the development of pancreatic intraepithelial neoplasia and pancreatic ductal adenocarcinoma have not been explained completely, yet. Therefore, further investigation of the disorders of the cell cycle as basic agents underlying the development of cancers must be carried out in the future.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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