Significance of mucin expression in pancreatic intraepithelial neoplasia (PanIN) – precursor lesions of pancreatic ductal adenocarcinoma (PDAC)

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ABSTRACT

Purpose: To evaluate chosen mucins (mucin 1, 4, 5AC) expression in pancreatic intraepithelial neoplasia, which is a precursor lesion of pancreatic ductal adenocarcinoma.

Materials and methods: The study group included 70 patients operated on due to inflammation, cysts and pancreatic ductal adenocarcinoma with pancreatic intraepithelial neoplasia revealed additionally. Mucin 1, 4 and 5AC expression was assessed by immunohistochemical method using polyclonal antibodies.

Results: Statistical analysis proved a positive correlation between the expression of mucin 1, 4 and 5AC proteins and the presence and staging of pancreatic intraepithelial neoplasia ($p<0.001$). Statistically significant correlations were determined between mucin 1, 4 and 5AC and the location of PanIN lesion in the pancreas. Positive correlations were found between mucin 5AC expression and the type of a basic disease ($p=0.014$). Differences in the expression of MUC 1, 4 and 5AC proteins between healthy pancreatic ducts and various stages of pancreatic intraepithelial neoplasia were statistically significant ($p<0.001$).

Conclusions: Overexpression of mucin 1, 4 and 5AC is related to the presence of pancreatic intraepithelial neoplasia. This suggests that overproduction of mucus is a phenomenon occurring early in the process of carcinogenesis in the pancreas and has its beginning in precancerous lesions of an early stage.

Keywords: **M**ucin, immunohistochemistry, pancreatic intraepithelial neoplasia, PanIN

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INTRODUCTION

Despite significant progress in diagnostics and treatment of pancreatic cancer, it still remains the most deadly cancer with the worst prognosis. According to the data published by Rahiba et al. [1], this cancer takes up the third rank with regard to mortality among all neoplasms in the United States, but in accordance with the latest prognosis up to 2030 year it will be classified as the second one. It has been estimated that in 2017 year in the USA the number of new cases of pancreatic cancer will total 53,670 and deaths approximately 43,090 [2].

Based on the numerous studies into the biology of pancreatic ductal adenocarcinoma, it has been proved that it develops due to cancerous transformation of epithelial cells in pancreatic ducts. In this process, numerous mutations of suppressive genes and oncogenes transform the normal non-mucinous, cuboidal epithelium lining up the pancreatic ducts into noninvasive, cancerous cylindrical epithelial cells possessing the ability to produce mucus [3]. Mucins – high-molecular-weight glycoproteins are one of the components of the mucus secreted by epithelial cells in pancreatic intraepithelial neoplasia. They are built up of a protein core (apomucin) combined with numerous sugar chains by means of O-glycosidic bonds formed between threonine and serine radicals of the polypeptide chain and N-acetylgalactosamine radicals of the sugar chain. Based on the structure and function of a molecule, mucin is subdivided into 3 categories: membrane-bound mucins (MUC1, MUC3 MUC4, MUC12, MUC16, MUC17), gel-forming (secreted) mucins (MUC2, MUC5AC, MUC5B, MUC6, MUC19), and soluble mucin (MUC7). Since membrane-bound mucins can function as ligands for adhesive cells from the selectin family, they are believed to play an important role in intracellular reactions. Gel-forming mucins contribute to mucus production via formation of 3-D network by means of oligomerization domains, thus protecting the epithelium from various damages, that is, injuries, inflammation, bacteria, viruses, pH changes, etc. [4, 5].

Changes in both the protein and sugar structure accompany inseparably cancer cells and cause an increase or decrease in the expression of a given apomucin or/and changes in the type of mucins produced. Based on the examinations assessing MUC1 expression, a significant increase in the expression of this glycoprotein was found as well as a relation was proved with invasiveness of cancerous cells, among others, in breast cancer, invasive pancreatic ductal adenocarcinoma, invasive cholangiocarcinoma, intraductal papillary mucinous neoplasm (IPMN) of the pancreas and adenocarcinoma of the bile ducts [6]. An increase in mucin 4 expression was observed in ductal adenocarcinoma, though no positive expression of this protein was established in chronic pancreatitis, which suggests that this protein may be used as a marker differentiating both diseases.

Overexpression of 5AC mucin was found not only in pancreatic neoplasia but also in precursor lesions: in intraductal papillary mucinous neoplasms (IPMN) and pancreatic mucinous cystic neoplasms (MCN). Additionally, it was observed that the patients with pancreatic cancer with a positive expression of mRNA MUC5AC had better survival rates than the patients without any mRNA MUC5AC expression [7].

Therefore, the aim of this study was to assess and compare the expression of mucin 1, 4 and 5AC between the various degree of pancreatic intraepithelial neoplasia constituting the precursor lesions of pancreatic cancer and the normal pancreatic ducts.

MATERIALS AND METHODS

Patients

The study group consisted of 70 patients with different pancreatic diseases (pancreatic ductal adenocarcinoma, cysts, pancreatitis) operated on in the 2nd Clinical Department of General and Gastroenterological Surgery at the University Hospital in Bialystok, in the years 2006-2014. The characteristics of the study group are shown in Table 1. The study was performed in conformity with the Declaration of Helsinki for Human Experimentation and received approval by the Local Bioethics Committee of the Medical University of Bialystok (Nr R-I-002/142/2016).

Histopathological examination and identification of ductal lesions

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 diagnosis of a primary disease, but also the presence and stage of pancreatic intraepithelial neoplasia. All slides were reviewed by two independent pathologists. PanIN lesions were classified according to the guidelines established by the international group of pathologists on Pancreas Cancer Think Tank meeting sponsored by the National Cancer Institute and held in Park City, Utah in September 16-19, 1999 [8]. 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 cytological abnormalities, such as loss of nuclear polarity, dystrophic goblet cells, atypical mitotic figures and macronucleoli. The presence of PanINs was evaluated on the slides of the normal pancreatic tissue at least 5 mm away from the carcinoma, while, in the non-neoplastic lesions, PanINs were evaluated in the site of an 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).

Table 1. Characteristics of the study group

Clinicopathological features	**Frequency n (%)**
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	
head	33 (47.1%)
body	5 (7.1%)
tail	20 (28.6%)
body and tail	12 (17.2%)

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

Normal pancreatic ducts (%)	**PanIN 1a (%)**	**PanIN 1b (%)**	**PanIN 1 (1A + 1B) (%)**	**PanIN 2 (%)**	**PanIN 3 (%)**	**Total (%)**
14 (18.2%)	21 (27.3%)	21 (27.3%)	42 (54.6%)	16 (20.8%)	5 (6.4%)	77
14 (10.8%)	35 (26.9%)	37 (28.5%)	72 (55.4%)	29 (22.3%)	15 (11.5%)	130
7 (21.9%)	9 (28.1%)	9 (28.1%)	18 (56.2%)	6 (18.8%)	1 (3.1%)	32
35 (14.7%)	65 (27.2%)	67 (28.0%)	132 (55.2%)	51 (21.3%)	21 (8.8%)	239

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) for 5 min. Incubation with mouse anti human monoclonal CD227 antibody (mucin 1) (clone VU-3C6, AbDSerotec, 1:200 dilution), mouse anti human monoclonal mucin 4 antibody (clone 5B12, AbDSerotec; 1:1000 dilution) and mouse anti human monoclonal mucin 5AC antibody (clone 1-13M1, AbDSerotec, 1:200 dilution) for properly 30, 10 and 30 minutes 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).

Analysis of immunohistochemical data

Immunohistochemical staining of mucins 1, 4, and 5AC in each tissue was independently assessed by two senior pathologists who have been blinded to the clinicopathological data. In case of disagreement, the scoring was discussed by the pathologists until they agreed on a final result. Expression of mucin 1, 4 and 5AC was found in cytoplasm of pancreatic ductal epithelial cells. The results of the staining were semiquantitatively assessed for the percentage and intensity of the positively stained cells according to the method proposed by Kim et al. [7].

Expression of mucin 1, 4 and 5AC was evaluated based on 4-point scale:

- 0- lack of expression
- 1 (weak) - positive reaction present in <25% of pancreatic ductal epithelial cells
- 2 (moderate) - positive reaction present in 25-50% of pancreatic ductal epithelial cells
- 3 (strong) - positive reaction present in >50% of pancreatic ductal epithelial cells.

Statistical analysis

STATISTICA 10.0 (Statsoft, Cracow, Poland) was used for statistical analysis. The data were analyzed using Spearman's rank correlation test.

Correlations between proteins expression depending on PanIN stage were examined 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

Mucin 1, 4 and 5AC expressions in correlation with clinicopathological parameters in pancreatic intraepithelial neoplasia

The positive immunohistochemical reaction of the mucins 1, 4 and 5AC was evaluated in the cytoplasm of pancreatic ductal epithelial cells (Figure 1a-3d).

Statistical analysis revealed correlations of the mucins 1, 4 and 5AC expressions with the presence and degree of pancreatic intraepithelial neoplasia ($p<0.001$). Expressions of these proteins increased with a degree of advancement of PanIN. The higher expression of mucin 1 ($p=0.001$), mucin 4 ($p=0.007$) and mucin 5AC ($p=0.027$) was shown to be associated with the location of pancreatic intraepithelial neoplasia in the pancreas (Table 3). The highest expression of mucin 1 was found in the corpus (2.18±1.08), mucin 4 in the head (1.18±1.06) and mucin 5AC in the corpus and tail of the pancreas (1.82±1.14). Expression of mucin 5AC was significantly higher ($p=0.014$) in pancreatic intraepithelial neoplasia accompanied by pancreatic ductal adenocarcinoma (1.53±1.26) than in case of pancreatitis (1.52±1.18) and pancreatic cysts (1.21±1.11) (Table 3).

Comparison of the mucins 1, 4 and 5AC expression between normal pancreatic ducts and different degrees of pancreatic intraepithelial neoplasia

Normal vs PanIN 1, PanIN 2 and PanIN 3

Pancreatic intraepithelial neoplasia expressed mucins 1, 4 and 5AC significantly more frequently in comparison with normal pancreatic ducts. The expressions of mucins 1, 4 and 5AC were significantly higher in pancreatic intraepithelial neoplasia compared to the normal tissue ($p<0.001$). The mean mucin 1 expression was significantly higher in PanIN 1 (0.69±0.74), PanIN 2 (1.85±1.11) and PanIN 3 (2.79±0.80) in comparison with normal pancreatic ducts (0.04±0.19) ($p<0.001$). Similarly, the mean expression of mucin 4 was significantly higher in PanIN 1 (0.70±0.74), PanIN 2 (1.77±0.94) and PanIN 3 (2.17±1.03) compared to normal pancreatic ducts (0.04±0.18). Mucin 5AC also had a higher expression in PanIN 1 (1.28±0.82), PanIN 2 (2.50±1.06) and PanIN 3 (2.63±1.06) in comparison with normal pancreatic ducts (0.00±0.00) (Figure 4, 5, 6).

PanIN 1 vs PanIN 2 and PanIN 3

The mean expression of mucins 1, 4 and 5AC was significantly higher in PanIN 2 (1.85±1.11, 1.77±0.94, 2.50±1.06 respectively) and PanIN 3 (2.79±0.80, 2.17±1.03, 2.63±1.06 respectively) in comparison with PanIN 1 (0.69±0.74, 0.70±0.74, 1.28±0.82 respectively) (Figure 4, 5, 6).

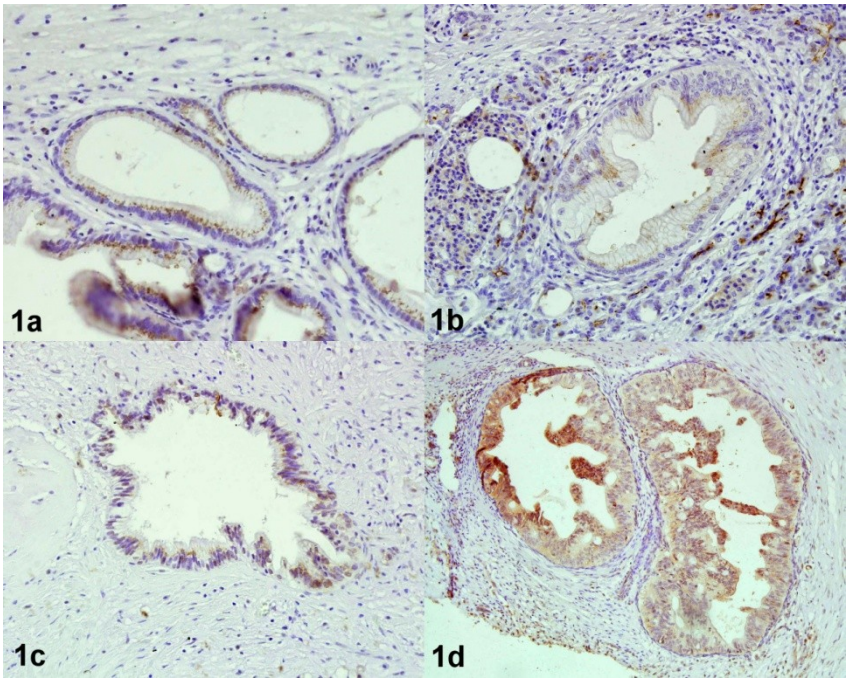

Figure 1. Cytoplasmic immunoreactivity of mucin 1 in PanIN 1A (1a), in PanIN 1B (1b), in PanIN 2 (1c), in PanIN 3 (1d). Typical examples are shown

Table 3. Correlations of mucins 1, 4 and 5AC expressions with chosen clinicopathological parameters

Protein expression Variables	**Mean value of mucin 1 expression + SD**	***p-value***	**Mean value of mucin 4 expression + SD**	***p-value***	**Mean value of mucin 5AC expression + SD**	***p-value***
AGE						
≤60	0.84±1.04	*p=0.392*	0.72±0.84	*p=0.515*	1.32±1.17	*p=0.984*
>60	1.17±1.14		1.05±1.08		1.66±1.16	
GENDER						
male	1.12±1.17	*p=0.336*	0.81±0.88	*p=0.338*	1.53±1.21	*p=0.746*
female	0.91±1.04		0.95±1.04		1.31±1.12	
LOCATION						
head	0.93±1.03	*p=0.001*	1.18±1.06	*p=0.007*	1.62±1.14	*p=0.027*
corpus	2.18±1.08		0.44±0.73		0.23±0.37	
tail	0.74±1.07		0.62±0.84		1.19±1.15	
corpus and tail	1.25±1.11		0.87±0.97		1.82±1.14	
DIAGNOSIS						
pancreatitis	0.91±1.02	*p=0.167*	0.89±0.93	*p=0.415*	1.52±1.18	*p=0.014*
pancreatic ductal adenocarcinoma	1.17±1.14		1.01±1.08		1.53±1.26	
pancreatic cysts	0.75±1.11		0.67±0.87		1.21±1.11	
PanIN						
Normal pancreatic ducts	0.04±0.20	*p<0.001*	0.04±0.21	*p<0.001*	0.00±0.00	*p<0.001*
1A	0.41±0.61		0.36±0.57		0.93±0.60	
1B	0.96±0.77		1.07±0.74		1.62±0.86	
2	1.85±1.11		1.77±0.94		2.50±1.06	
3	2.79±0.80		2.17±1.03		2.63±1.06	

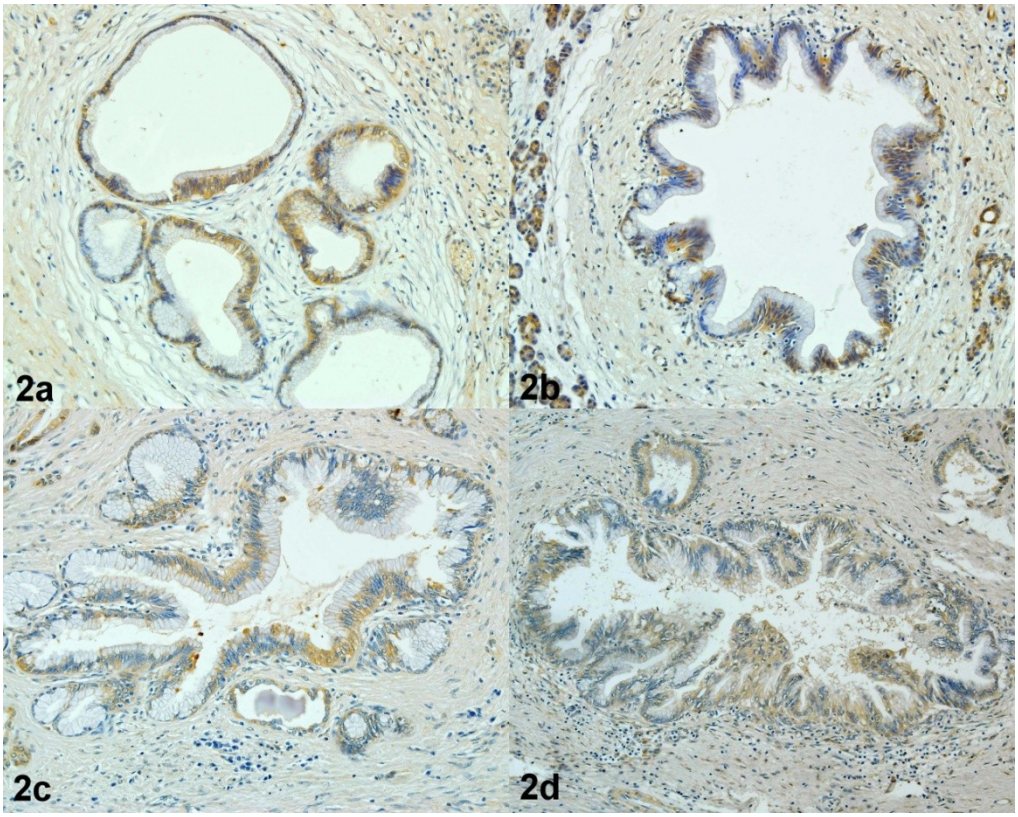

Figure 2. Cytoplasmic immunoreactivity of mucin 4 in PanIN 1A (2a), in PanIN 1B (2b), in PanIN 2 (2c), in PanIN 3 (2d). Typical examples are shown

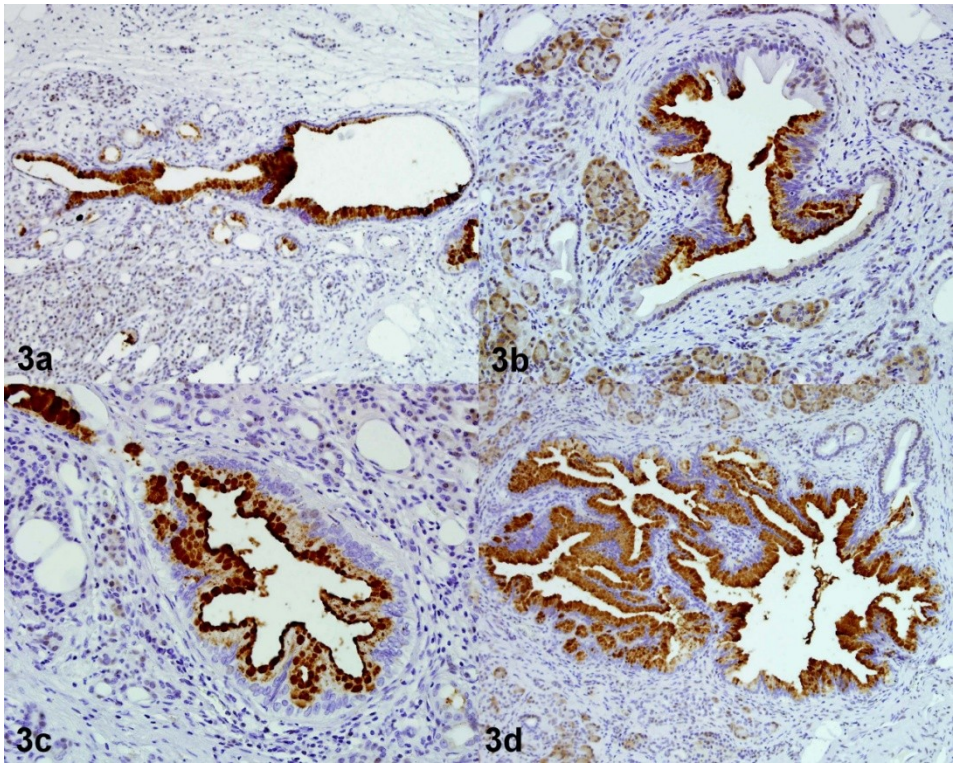

Figure 3. Cytoplasmic immunoreactivity of mucin 5AC in PanIN 1A (3a), in PanIN 1B (3b), in PanIN 2 (3c), in PanIN 3 (3d). Typical examples are shown

Pan IN 2 vs PanIN 3

The mean mucin 1 expression was significantly higher in PanIN 3 (2.79±0.80) compared to PanIN 2 lesions (1.85±1.11). (Figure 4).

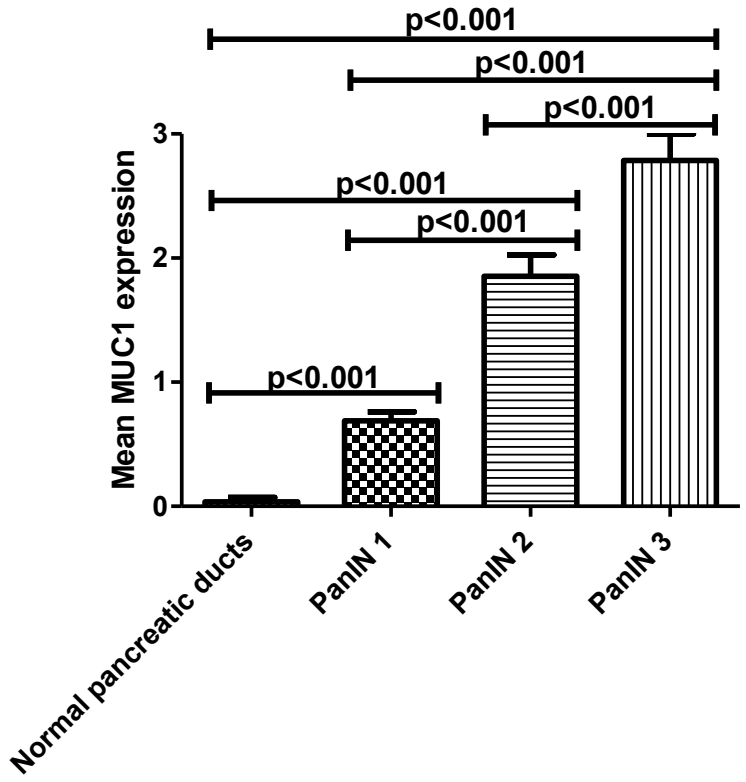

Figure 4. Comparison of the mean expression of mucin 1 between various degrees of PanIN

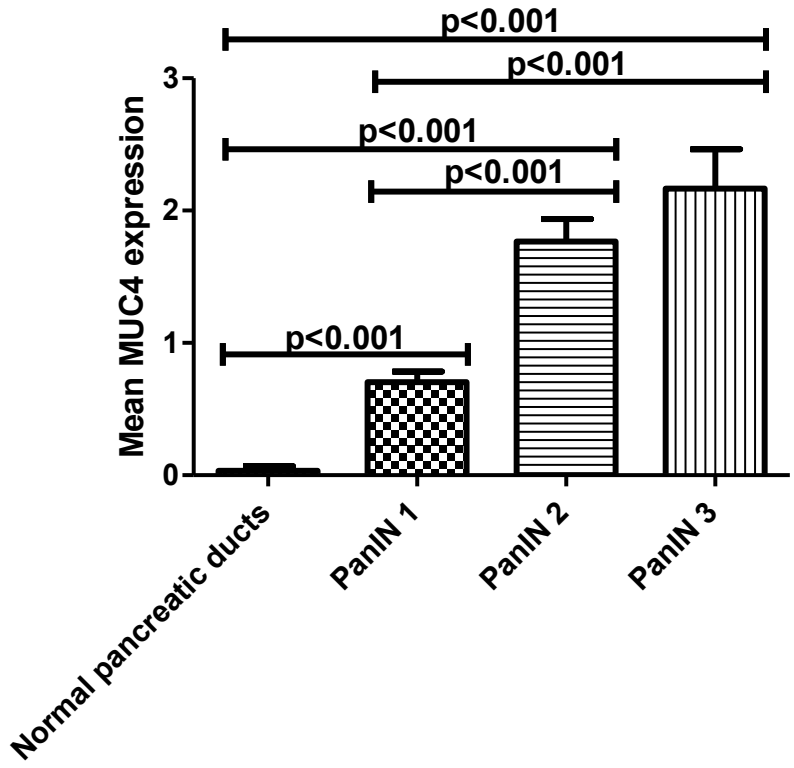

Figure 5. Comparison of the mean expression of mucin 4 between various degrees of PanIN

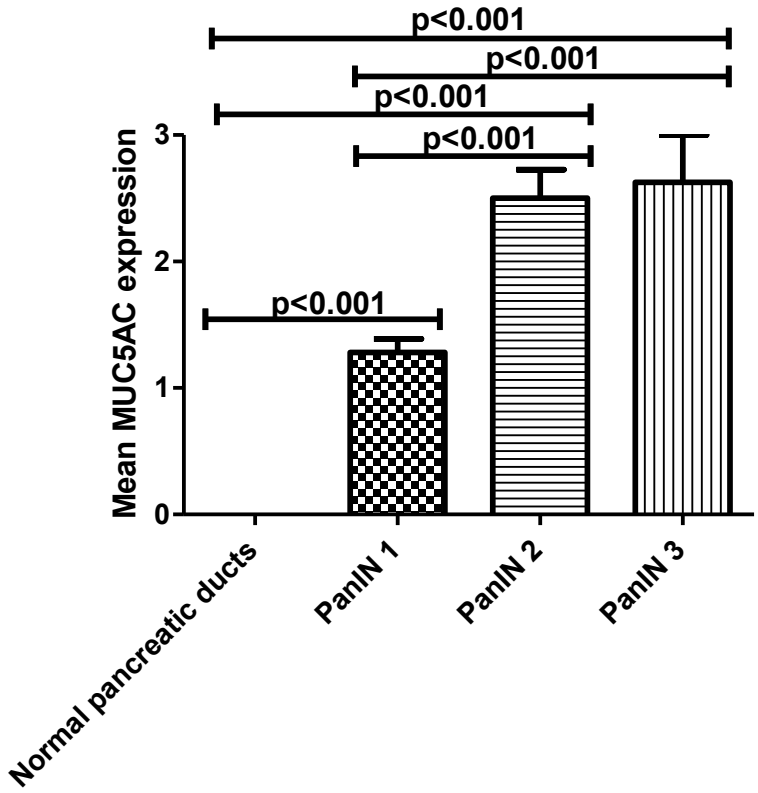

Figure 6. Comparison of the mean expression of mucin 5AC between various degrees of PanIN

Correlation between MUC 1, MUC 4 and MUC 5AC expression

Statistical analysis of the expressions of MUC 1, MUC 4 and MUC 5AC showed positive correlations between these proteins ($p<0.001$). This relationship was directly proportional, which means that if the patient had an increased positive expression of e.g. mucin 1 protein he would also have an increased expression of mucin 4 and mucin 5AC (Table 4)

Table 4. Correlations between MUC1, MUC4 and MUC5AC proteins

Protein	MUC1	MUC4	MUC5AC
MUC1	_____	R= 0.4702 *p<0.0001*	R= 0.4849 *p<0.0001*
MUC4	R= 0.4702 *p<0.0001*	_____	R=0.8369 *p<0.001*
MUC5AC	R= 0.4849 *p<0.0001*	R=0.8369 *p<0.001*	_____

DISCUSSION

An extremely aggressive course as well as high mortality of pancreatic ductal adenocarcinoma induce still ongoing intensive research into biology of this neoplasm. Based on these studies, it has been proved that pancreatic ductal adenocarcinoma develops from precursor lesions similarly to colon cancer developing from polyps or adenocarcinomas as well as cervix cancer developed due to transformation of epithelium into intraepithelial neoplasia and then into invasive cancer. Intraepithelial neoplasia defined as micropapillary or flat noninvasive lesions of epithelium with the diameter not exceeding 5 mm developed in the pancreatic ducts belong to the most frequent precancerous lesions. They are built up of cylindrical cells with the changing quantity of mucus, which facilitates its distinction from the

normal epithelium lining up the pancreatic ducts and consisting of cubical cells without the mucus in the cytoplasm [6]. Mucins – high-molecular-weight glycoproteins are the main component of the mucus. They are secreted by specialized epithelial cells and engaged in the renewal and differentiation of epithelium, modulation of cellular adhesion as well as signalization. These molecules are divided into 3 groups: the membrane bound, secreted and soluble mucin with regard to their structure and the function they have [4]. Mucin 1 belongs to the membrane bound mucin and takes part in cellular signalization as well as epithelial differentiation and proliferation [9]. Its biological features, changed expression and post-translational modifications contribute significantly to progression of a tumor and metastases [10]. In our study, mucin1 expression was assessed in pancreatic intraepithelial neoplasia – the most frequent precursor lesion of pancreatic ductal adenocarcinoma. Statistically significant correlations were proved between the mucin 1 expression and the grade of pancreatic intraepithelial neoplasia ($p<0.001$). Expression of this protein increased with PanIN staging: normal pancreatic ducts, PanIN 1A, 1B, 2 and 3, reaching the following values: 0.04±0.20, 0.41±0.61, 0.96±0.77, 1.85±1.11, 2.79±0.80, respectively. Gold [11] described a positive reaction of MUC1 in 87% of pancreatic ductal adenocarcinoma cases, though, similarly to our study, he observed no positive expression of this protein in normal pancreatic ducts. In the lesions of PanIN 1A and 1B type, the author revealed a strong, spilt reaction of which intensity and tissue distribution decrease in PanIN 2 and 3. According to Gold [11], this decrease can be caused by markedly fewer lesions of PanIN 2 and 3 compared to other stages of PanIN, in which the reaction was assessed. Similarly, in his study, Nagata [12] reported a significant increase in mucin 1 expression accompanying the higher staging of PanIN. Matsuyama [13] also assessed MUC1 expression in pancreatic intraepithelial neoplasia and proved statistically significant correlations between an increase in mucin 1 and PanIN staging. Additionally, he compared mucin 1 expression in pancreatic intraepithelial neoplasia of various staging in the samples of pancreas without lesions (material obtained from autopsies) with PanIN lesions accompanying pancreatic ductal adenocarcinoma and observed a higher expression of this protein in the lesions accompanying pancreatic cancers. Although PanIN present in patients with pancreatic cancers is characterized by the same spectrum of morphological lesions as in PanIN found in the unchanged pathologically pancreas, these lesions have other features referring to mucin expression. Matsuyama [13] suggested that PanIN lesions present in normal pancreas and accompanying pancreatic ductal adenocarcinoma might present various stadia of neoplasia. Lo [14] showed other MUC1 expression in his study. He observed no positive expression of mucin 1 in PanIN 1 and PanIN 2, whereas this protein was determined in PanIN 3 and early stages of pancreatic ductal adenocarcinoma. Mucin 1 was proved to be expressed more intensively in neoplasia of a low stage and decreased with its progression. According to this author, a decrease in MUC1 expression was associated with the loss of the ductal tissue architecture and low differentiation of carcinoma. Thus MUC1 expression in the early stages of neoplasia may be considered as a potential biomarker of this cancer.

Mucin 4 likewise mucin 1 belongs to the group of membrane-bound mucins and is responsible for inhibition of intercellular adhesion as well as adhesion between cells and the stroma, which enhances mobility of the cells and aids the process of cancer metastasizing and infiltrating [15]. Numerous studies conducted in vivo and in vitro have confirmed the role of MUC4 in cellular adhesion and epithelial – mesenchymal transition (EMT) and proved that inhibition of this protein expression resulted in limitation of growth and mobility of cancer cells [16]. In our study, expression of this protein was assessed in precursor lesions of pancreatic ductal adenocarcinoma and an increase in MUC4 expression following a higher grade of pancreatic intraepithelial neoplasia was determined ($p<0.001$). A mean expression of this protein in normal pancreatic ducts PanIN 1A, 1B, 2 and 3 was as follows: 0.04±0.21, 0.36±0.57, 1.07±0.74, 1.77±0.94, 2.17±1.03. In the study, Swartz et al [17] presented similar results, showing a positive, focal reaction of MUC4 in 17% of PanIN 1, 36% of PanIN 2, 85% of PanIN 3 and 89% of pancreatic ductal adenocarcinoma cases. However, he observed no positive expression of this protein in normal pancreatic ducts. Park [18] proved a positive expression of this protein only in pancreatic intraepithelial neoplasia Type 3 and in ductal adenocarcinoma. The results of our study are in concordance with Swartz's et al. results [17] stating that an increasing expression of mucin 4 in pancreatic intraepithelial neoplasia supports the model of pancreatic cancer progression. Additionally, according to this author, immunohistochemical method is the best method to determine mucin 4 in various lesions in the pancreas. Mucin 5AC belongs to the group of secreted mucins, which forms long polymers with the disulphide bond end-to-end, which gives molecules of high adhesiveness in a solution. This mucin is often defined as gastric surface mucous epithelial mucin present on the surface of the cardiac orifice, fundus and pylorus of the stomach [7]. In our study, mucin 5AC expression was found in pancreatic intraepithelial neoplasia and increased together with the higher grade of PanIN. The mean

expression of this protein in specific grades of PanIN was the following: 0.93±0.60 in PanIN 1A, 1.62±0.86 in PanIN 1B, 2.50±1.06 in PanIN 2 and 2.63±1.06 in PanIN 3. No expression of this protein was established in normal pancreatic ducts. Kim et al. [7] showed similar results in their study. In case of normal pancreatic ducts, mucin 5AC expression was determined in 4%, 71% in PanIN 1A, 89% in PanIN 1B , 88% - PanIN 2, and 90% - PanIN 3. In ductal adenocarcinoma, a mean expression equaled 85%. Additionally, they assessed the expression of MUC5AC gene using fluorescent hybridization technique in situ and revealed positive correlations between hybridization in situ and the results of immunohistochemical method, proving that activation of transcription starts as early as in pancreatic intraepithelial neoplasia. Additionally, in ductal adenocarcinoma, the percentage of results with a positive transcription of MUC5AC was lower than in case of positive results with a positive expression of protein MUC5AC, which confirms that changed glycosylation of MUC5AC occurs in neoplasia. These authors have concluded that a positive expression of mucin 5AC is a phenomenon found early in pancreatic intraepithelial neoplasia [7].

Matsuyama et al. [13] examined mucin 5AC expression in pancreatic intraepithelial neoplasia in the pancreases without lesions obtained from autopsies as well as in pancreases with lesions PanIN accompanying pancreatic cancers. They proved statistically significant higher expression of MUC5AC in the lesions of PanIN 1A and PanIN 2 type occurring in patients with pancreatic ductal adenocarcinoma. Thus, it has been found that the advanced stage of cancer has an effect on the expression of this mucin in intraepithelial neoplasia coexisting with pancreatic ductal adenocarcinoma. Moreover, in the studies of Matsuyama et al. [13], samples of a tumor were obtained from big cancerous masses, which suggests that the pancreatic parenchyma with cancerous lesions affects the entire environment of the pancreas inducing differences in mucin expression. Additionally, glycosylation of mucins is changed via proinflammatory signalization in pancreatic cancerous cells and released cytokines may change mucin expression [19].

CONCLUSIONS

Summing up, mucin 1, 4 and 5AC expression increased together with the increased staging of pancreatic intraepithelial neoplasia and was not found in normal pancreatic ducts.

This suggests that it is a phenomenon occurring early in the process of carcinogenesis in the pancreas and develops as early as precursor lesions of a low staging – starting from pancreatic intraepithelial neoplasia of type 1A and ending at the advanced lesions of PanIN 3.

Acknowledgments
Not applicable

Conflicts of interest
The authors declare that they have no conflicts of interest.

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