REVIEW

Mucins in neoplasms of pancreas, ampulla of Vater and biliary system

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Abstract

Tumors of the pancreas, the ampulla of Vater, and the extrahepatic and intrahepatic bile ducts have significant histological similarities due to the common embryonic origin of the pancreatobiliary system. This obviates the need for discovery of biomarkers with diagnostic and prognostic value for these tumors. Mucins, especially MUC-1, -2, -4 and -5AC, are important candidates for developing into such reliable biomarkers. Increased expression of MUC1 occurs in pancreatic ductal adeno-carcinomas and is associated with increased degrees of dysplasia in pancreatic intraepithelial neoplasia (PanIN). Positive expression of MUC2 in intraductal papillary mucinus neoplasms (IPMN) of the intestinal type indicates high potential progression to invasive carcinoma with *de novo* expression of MUC1, while absence of MUC2 expression in IPMNs of gastric type implies low potential to malignant evolution. *De novo* MUC4 expression correlates to the severity of dysplasia in PanIN and is associated with a poor prognosis in patients with pancreatic ductal adenocarcinomas. In biliary intraepithelial neoplasia (BilIN), increased expression of MUC1 is associated with higher degrees of dysplasia. Intrahepatic cholangiocarcinomas (ICC) are characterized by increased expression of all glycoforms of MUC1. Positive MUC2 expression in intraductal papillary neoplasm of the bile ducts (IPNB) of the intestinal type indicates high malignant potential with *de novo* expression of MUC1 in the invasive element. Absent MUC2 expression in any degree of BilIN may prove useful in differentiating them from IPNB. *De novo* expression of MUC4 is associated with poor prognosis in patients with ICC or carcinoma of the extrahepatic bile ducts (EHBDC). High *de novo* expression of MUC5AC is found in all degrees of BilIN and all types of IPNB and ICC. The MUC5AC is useful in the detection of neoplastic lesions of the bile duct at an early stage. Increased expression of mucin MUC1 in carcinoma of the ampulla of Vater associated with unfavorable behavior of the tumor, such as lymph node metastasis, infiltration of the pancreas and duodenum, advanced TNM classification and worse prognosis. Patients with

intra-ampullary papillary-tubular neoplasm (IAPN) of the pancreatobiliary immunophenotype did not show MUC2, while those of the intestinal immunophenotype are MUC2 positive. The expression of MUC4 is associated with poor prognosis in patients with carcinoma of the ampulla of Vater favoring metastasis and making them resistant to apoptosis. Moreover, it appears that MUC4 positivity correlates with recurrence of the tumor. Expression of MUC5AC is associated with the invasive potential of the tumor.

Key words: Ampulla Vater neoplasms; Biliary system neoplasms; Mucins; Pancreatic neoplasms

Core tip: The combined status of mucin expression may be useful in the differential diagnosis of pancreatobiliary neoplasms, the detection of pre-malignant lesions and the evaluation of their malignant behavior. Besides their diagnostic and prognostic role, their involvement in carcinogenesis reveals their importance as putative therapeutic targets in the future.

INTRODUCTION

All mucosal surfaces of the human body are covered by a thick viscous layer which protects them from external insults. A major component of this defensive barrier is a class of epithelial-derived glycoproteins known as "mucins". The structural signature of a mucin is a dense network of oligosaccharide side chains comprising of N-acetyl-galactosamine, which bind *via* an O-glycosidic linkage to specific amino acid residues (serine, threonine and proline), that occur in repetitive short stretches in the protein backbone termed "tandem repeats"[1,2]. The side chains make up to 50%-90% of the weight of a mucin. Mucins are distinguished from proteoglycans (another major class of glycoproteins) by the lack of uronic acid and xylose and the presence of the aforementioned O-glycosidic links.

Genes encoding for the protein backbone of mucins are denoted by the three letter code "MUC", followed by a number (MUC1-21), which corresponds to the chronological order of discovery[3]. Mucins are classified according to their structure and function as either "membrane bound" or "secreted"[4]. The first class includes mucins MUC1, MUC3, MUC4, MUC12, MUC13, MUC16, MUC17, and MUC21. These mucins possess a large N-terminal extracellular portion, a transmembrane part

and a small C-terminal cytoplasmic one. The cytoplasmic segment is important for signal transduction, as it contains several phosphorylation sites that are important for interaction with other scaffolding and signaling proteins. The extracellular portion of mucins includes several specific domains of unknown function, which include epidermal growth factor (EGF)-like, AMOP (adhesion associated domain in MUC4 and other proteins), VWD (Factor Von Willebrand), SEA (sea-urchin sperm protein enterokinase and agrin) and NIDO (Nidogen-like)[5]. Another unique feature of the membrane bound mucins is the existence of multiple isoforms with modified structure and function, which are generated by alternative splicing[4].

Secreted or gel-forming mucins are encoded by genes located contiguously on chromosome 11 and include MUC2, MUC5AC, MUC5B, MUC6 and MUC7. This genetic clustering indicates a close functional association of the relevant proteins. Secreted mucins also have "tandem repeats", the number of which is a unique feature with potential diagnostic, prognostic and possibly therapeutic significance[6]. Two unique domains that characterize secreted mucins are the VWD domain and the cysteine knot (CK) motif[7]. The first appears to be important for connecting the mucins into oligomeric structures, while the second for the formation of mucin homo- or heterodimers.

EXPRESSION OF MUCINS IN NORMAL PANCREATOBILIARY SYSTEM

The mucin profile in normal pancreatobiliary system has been identified mostly through immunostaining studies with the use of mucin-specific antibodies. However, it should be noted, that different antibodies have been used across separate studies, leading to heterogeneous expression patterns. A typical example is the work of Yamashita *et al*[8] who reported that immunoexpression of MUC1 in normal bile duct was dissimilar between three separate MAbs (DF3, MUSE11 and 139H2), that were tested. In particular, MUC1/139H2 was expressed in normal bile ducts of any size at the luminal surface of the biliary cells and/or in the cytoplasm. MUC1/MUSE11 was detected only in small-sized ducts (S < 100 mm), predominantly in the apical surface of the cells, while MUC1/DF3 was undetectable in normal biliary epithelium. In the same study, MUC2 staining in normal bile ducts also varied and was dependent upon the use of the specific antibody (polyclonal anti-MUC2/MRP or monoclonal CCP58 antibody). MUC2/anti-MRP was not expressed in normal bile duct epithelium, whereas MUC2/CCP58 exhibited supranuclear expression in 60% of the cases[9]. Finally, MUC4 was undetectable in normal bile ducts, while MUC5AC immunostaining was present in a small number of cases[9].

In another study, it was shown that in normal pancreatic tissue, MUC1 and all of its glycoforms were expressed at the apices of centroacinar cells, intercalated and intralobular ducts, and focally in the interlobular pancreatic ducts. MUC1 and MUC1 glycoforms were

Table 1 Expression of various glycoforms of mucin 1 in normal pancreatic tissue				
Cells/structure	**MUC1/ CORE**	**MUC1/ DF3**	**MUC1/ MY.1E12**	**MUC1/ HMFG1**
Central areas of acini	-/+	-/+	++	++
Intercalated ducts	+	+	+	+
Intralobular ducts	+	+	+	-/+
Interlobular ducts	-	-/+	-/+	-
Main pancreatic ducts	-	-	-	-

MUC: Mucins.

undetectable in the main pancreatic duct[10]. In the same study there was no detectable pancreatic expression of MUC2, MUC4, and MUC5AC. The expression of various glycoforms of MUC1 (MUC1/CORE, MUC1/DF3, sialylated MUC1/MY.1E12 and fully glycosylated MUC1/HMFG-1) in normal pancreatic tissue, is summarized in Table 1[11].

EXPRESSION OF MUCINS IN PANCREATOBILIARY NEOPLASMS

Pancreatic carcinogenesis develops through the accumulation of several genetic and epigenetic lesions, some of which affect genes encoding for mucins. It has been shown that certain mucins are expressed *de novo* during neoplastic transformation of the epithelium, while specific mucin patterns have been recognized in different pre-malignant and malignant neoplasms of ductal origin. In recent years, mucins have gained significant value for the diagnostic approach to pancreatic neoplasms. At the same time, their high specificity has rendered them potential candidates for therapeutic interventions[12]. Finally, mucin expression patterns have become a major criterion for the subclassification of intraductal papillary mucinus neoplasms (IPMN).

According to the WHO classification of 2010, IPMNs are histologically classified as pancreatobiliary, intestinal, gastric or oncocytic type[13]. Distinction of IPMN subtypes is very important from the clinical perspective, because they demonstrate differences in their malignant potential. IPMN of pancreatobiliary type with MUC1 expression and intestinal type with MUC2 expression are located mainly in the main pancreatic duct and exhibit a high frequency of carcinoma development[11]. In contrast, IPMN of gastric type with MUC5A expression is usually located in the pancreatic branch ducts and rarely shows malignant transformation. These findings are consistent with the clinicopathological description of the "International guideline for management of IPMN/MCN". According to this classification, IPMNs are classified as "IPMN-main duct type" and "IPMN-branch duct type"[14].

A similar subclassification based on the aforementioned parameters (cell type, mucin profile and prognostic data), has been introduced for biliary and ampullary neoplasias. According to the WHO classification of 2010, intraductal papillary neoplasm of the bile ducts (IPNB) are classified as pancreatobiliary, intestinal and gastric type, while the oncocytic type is referred as a variant of the pancreatobiliary[15]. The most frequent histopathologic types are pancreatobiliary and intestinal.

MUC1

In 1993 it was reported for the first time, that pancreatic ductal adenocarcinomas (PDAC) with aggressive biological behavior usually express MUC1[16]. Furthermore, the pattern of MUC1 expression in PDAC is different from that in the normal pancreas. In well differentiated PDAC, MUC1 is detected not only along the luminal cell surface of glandular formations, but also in the baso-lateral surface. In poorly differentiated PDAC, cytoplasmic expression of MUC1 is demonstrated[17]. In addition, PDAC show high expression rates for MUC1 glycoforms (MUC1/CORE 66%, MUC1/DF3 96%, MUC1/MY.1E12 98%, MUC1/HMFG1 76%)[18]. Regarding pancreatic intraepithelial neoplasia (PanIN), the expression profile of MUC1 is associated with the grade of dysplasia[19]. Indeed, increase of MUC1 expression has been correlated with increase of PanIN grade[20].

MUC1 is exclusively associated with pancreatobiliary type IPMN and its malignant variant[13,21]. Moreover, underglycosylated MUC1 is absent or rarely seen in IPMN-intestinal type and IPMN-gastric type. On the other hand, glycosylated MUC1 is undetectable or occasionally detectable in IPMN-intestinal type, but is frequently observed in IPMN-gastric type[11]. The invasive carcinomas, which develop in intestinal type IPMN, show *de novo* MUC1 expression[20,22].

There are only few references to the expression of MUC1 in mucinous cystic neoplasms (MCN), oftentimes with conflicting results. In one study, MUC1 appeared to be rarely expressed in MCN[23], while in another the majority of MCN was found positive for MUC1/DF3[24]. On the other hand, MUC1 expression is detectable in up to 90% of intraductal tubulopapillary neoplasms (ITPN)[25].

Regarding the mucin expression in neoplasms of the biliary system, MUC1 profile in biliary intraepithelial neoplasia (BilIN) was similar to that in PanIN, characterized by progressive increase with increasing severity of dysplasia[26]. In the intraductal papillary neoplasm of the bile ducts (IPNB) of pancreatobiliary type, MUC1 expression rates were high in invasive, but low in non-invasive lesions[9]. On the other hand, IPNB-intestinal type showed *de novo* expression of MUC1, particularly in cases with frequent invasive growth and significant poorer survival[9].

Intrahepatic cholangiocarcinoma (ICC) with aggressive biological behavior and poor outcome usually presents a MUC1+/MUC2- profile, while bile duct cystadenocarcinoma (BDCC) with moderate aggressiveness and favorable prognosis demonstrates a MUC1-/MUC2+ pattern[8]. The frequency of expression of MUC1 in ICC was significantly higher than that in BDCC[27]. In general, positive staining for MUC1 has been associated with poor prognosis, regardless of the glycosylation status, in both ICC and

BDCC[27].

The expression pattern of MUC1 in tumors of the ampulla of Vater appears to have predictive value based on results of recent studies[28,29]. In particular, MUC1 positivity was associated with unfavorable behavior of the tumor, such as lymph node metastasis, vascular invasion, infiltration of the pancreas and duodenum, advanced TNM classification and worse prognosis[29].

Utility of MUC1 as biomarker in pancreatobiliary neoplasia: A strong association has been observed between elevated expression of MUC1 in pancreatic neoplasms and decreased survival. The negative predictive value was high (95%), as 19 out of 20 patients with no MUC1 expression survived more than 30 mo[30]. Out of 13 candidate genes, only those of MUC1, mesothelin (MSLN), and MUC2 showed statistically significant differences in the expression pattern between the groups with aggressive and less aggressive carcinomas[31]. Mesothelin/MSLN is a protein that is present on normal mesothelial cells and is overexpressed in mesothelioma and ovarian and pancreatic adenocarcinoma. Both MSLN and MUC1 appeared to be strong predictors of survival thus acquiring prognostic significance. In fact, the prognostic significance of MUC1 positivity exceeded that of conventional pathological features[31]. Accordingly, the National Cancer Institute has identified MUC1 and MSLN among the most promising targets for cancer vaccine development[32]. A radiolabeled monoclonal antibody against MUC1 was investigated recently in a phase Ⅰ/Ⅱ trial, and phase Ⅲ study will follow[33]. Finally, promoter-driven gene therapy, which exploits overactive MUC1 and MSLN promoters in various cancer types has been extensively studied in pre-clinical cancer models using viral vectors[34].

Much of the oncogenic role of MUC1 may be attributed to the participation of the small cytoplasmic tail of MUC1 (MUC1.CT) in signal transduction and transcriptional events[35]. MUC1.CT regulates the recruitment and activity of transcription factors, thereby regulating transcription of the corresponding genes[36]. Other studies have implicated MUC1 in tumour growth, invasion and metastasis in pancreatic cancer[37]. MUC1 participates in the regulation of pancreatic cancer cell metabolism[38]. It was shown, that MUC1 physically occupies the promoter regions of genes involved in glycolysis and glucose metabolism in pancreatic cancer cells. The MUC1 selectively enhances the transcription of some of the glycolytic genes and this effect is more pronounced under conditions of hypoxia in a HIF-dependent manner. Furthermore, MUC1 expression is associated *in vivo* with increased glucose uptake in xenograft model of orthotopic implantation of pancreatic cancer. Increased tumour cell metabolism has been identified as a hallmark of cancer requirements for rapid cell growth and is consistent with previous studies which consider MUC1 as a modulator of growth and invasive properties in multiple cancer types[39].

An association of MUC1 with Platelet-Derived Growth Factor-A (PDGF-A) has also been reported[38]. PDGF-A is one of the several regulators of tumor growth, angiogenesis and metastasis in pancreatic carcinoma. MUC1 regulates the expression and secretion of PDGF-A. In particular, the increase of MUC1 expression induces expression of PDGF-A in multiple human and mouse cell lines *in vitro* and *in vivo*, as well as in pancreatic cancer models with or without expression of MUC1. Both PDGF-A and MUC1 are considered unfavorable prognostic markers and potential targets for therapeutic intervention in pancreatic cancer. Moreover, expression of hypoxia-inducible factor 1-alpha (HIF1-α) as a marker of pancreatic cancer progression correlates with the expression of PDGFA and with poor prognosis[40]. Therefore, it is hypothesized, that MUC1 promotes nuclear translocation of this transcription factor, which is a known regulator of PDGFA[41].

MUC2

MUC2 is not expressed in PDAC[16]. This was originally reported in 1993[17] and was later confirmed by immunohistochemistry[18]. Similarly, MUC2 was not detected in PanIN independently of the grade of dysplasia[19]. In contrast, MUC2 expression has been reported in IPMNs. More specifically IPMN-intestinal type, that expresses MUC2, is primarily located at the main pancreatic duct and shows high frequencies of malignant transformation and invasive carcinoma (usually mucinous/colloid carcinoma)[11]. On the other hand, MUC2 has not been detected in MCN[23,24].

Regarding expression in biliary neoplasms, in an important study by Zen *et al*[26], MUC2 expression was absent in all BilIN, while it was expressed in the majority of IPNB. In a more recent study, it was also reported that MUC2 was detectable in 50% of IPNB[15], although quantitative data on the relation to specific subtypes are missing. This was however addressed in another study, in which MUC2 expression was observed in 95% of IPNB intestinal type and in 50% of IPNB pancreatobiliary type[9]. Expression of MUC2 in extrahepatic bile ducts carcinoma (EHBDC) was related to tumor progression and the expression of the mucins was associated with poor prognosis[42]. ICC with aggressive biological behavior and poor outcome usually demonstrates a MUC2 negative profile while BDCC with moderate aggressiveness and favorable prognosis exhibits MUC2 positive profile[8,26]. Overall, MUC2 expression is considered to be a favorable prognostic marker in neoplastic lesions of the bile ducts[26,27]. Regarding the carcinoma of ampulla of Vater, expression of MUC2 is associated with non-invasive pancreatobiliary papillary neoplasia of intestinal subtype which is more frequently associated with invasive neoplasia[43]. In a study of 105 adenocarcinomas of the ampulla of Vater, intestinal immunophenotype was characterized by MUC2 and caudal-related homeobox transcription factor-2 (CDX2) positivity, while pancreatobiliary immunophenotype was characterized by MUC2 and CDX2 negative expression[43].

MUC4

Recently, it was found that high expression of MUC4 was an independent poor prognostic factor in adenocarcinomas of the pancreas and bile ducts[9]. In a study of 135 PDAC, MUC4 was expressed in 32% of the cases while intense expression was associated with significantly poorer survival[29]. In the studies of Swartz *et al* and Park *et al* MUC4 was detected in 89% and 79% of PDAC respectively[44,45]. These discrepancies may be ascribed to the application of a lower threshold-value for MUC4 positivity in the latter two studies. There are no current data available regarding the expression of MUC4 in IPMN and MCN. In sharp contrast, PanIN is associated with *de novo* expression of MUC4. Moreover, MUC4 expression intensity increases in parallel with the degree of dysplasia[44,45].

MUC4 is also expressed in neoplasms of the biliary system. In one study MUC4 was expressed in 63% of IPNB. The MUC4 showed more frequent expression in IPNB-intestinal relative to IPNB-pancreatobiliary type[9]. In intrahepatic and in extrahepatic cholangiocarcinoma MUC4 is expressed *de novo* mainly in the cytoplasm of cancer cells and is correlated with poor prognosis. The survival rate of patients with MUC4 positivity is significantly poorer compared to patients with negative MUC4 expression[9,46]. There is no information concerning MUC4 expression in BDCC.

MUC4 is less frequently expressed in the normal epithelium of the ampulla and is an independent marker of the pancreatobiliary type adenocarcinoma of the ampulla[28] associated with poor prognosis and recurrence of the tumor[28,47].

Utility of MUC4 as biomarker in pancreatobiliary neoplasia: MUC4 expression is associated with poor prognosis in patients with pancreatobiliary neoplasia. There has been a strong correlation between the expression of MUC4 and carcinoma recurrence[28,47]. In a retrospective study of biliary tract carcinoma in 2006, it was observed that patients with positive MUC4 in bile had reduced survival (5.0 mo *vs* 11.5 mo)[46], while a similar difference in survival rate has been reported in a Japanese population, which underwent surgery for extrahepatic biliary tract carcinoma[46].

MUC4 is a membrane associated mucin which inhibits intercellular adhesion and adhesion between cells and stroma favoring extravasation and metastasis[48,49]. It has also been implicated as promoter in infiltrative growth and metastasis of pancreatic cancer by facilitating cancer cell motility[50-52]. In various *in vitro* and *in vivo* studies, selective suppression of MUC4 expression highlighted its role in cell adhesion and epithelial mesenchymal transition[53] and in restriction of cancer cell growth and metastasis[54]. In pancreatic tumor cells selective downregulation of MUC4 led to suppression of pERK1/2 and increased expression of E-cadherin. These findings suggest that MUC4 inhibits the function of E-cadherin as well as of N-cadherin and EGFR1 by activation of Akt and

JNK/2 signaling pathways, respectively[55]. Decreased expression of matrix metalloproteinase-9 (MMP-9) has also been identified after downregulation of MUC4[56]. Inversely, activation of ERK1/2 signaling pathway *via* MUC4, activates MMP-9, which in turn causes degradation of E-cadherin resulting in alteration of intercellular contacts. These data demonstrate that MUC4-mediated repression of E-cadherin may enhance the invasive capacity of tumor cells. Suppression of MUC4 is also correlated with changes in the shape of cancer cells indicating its involvement in epithelial-mesenchymal transition[56,57]. Finally, cells expressing MUC4 are resistant to immune mediated apoptotic cell death by natural killer cells (NK)[58]. Mucins, including MUC4, which are connected with cell membrane, have become a point of interest as they act as endogenous ligands and modulators of the tyrosine kinase receptor ErbB2[59], which is part of an antiapoptotic pathway utilized by most common epithelial tumors[60]. The therapeutic blockage of ErbB2 with the monoclonal antibody trastuzumab improves the effectiveness of chemotherapy and survival in breast cancer[61]. In breast carcinoma cells, MUC4 has anti-aggregatory action and appears to promote tumor growth[62]. These ongogenic properties of MUC4 point out its significance as a potential therapeutic target.

MUC5AC

High *de novo* expression of MUC5AC has been observed in many types of pancreatobiliary neoplasms including all grades of PanIN[19,22] and BilIN[26], PDAC[11], ICC[15], as well as all types of IPMN[11] and IPMB[9]. In particular, IPMN of gastric type with MUC5AC expression is usually located in the pancreatic branch ducts, and rarely shows malignant transformation[14]. Since MUC5AC is not expressed in normal pancreatobiliary epithelium[26], it seems to be a specific marker of early neoplastic lesions of the pancreatobiliary system[11,26] which correlates with poor prognosis[63]. Diverse expression rates (37.5% *vs* 100%) have been reported in MCN and are attributed to the use of different antibodies[23,24].

MUC5AC expression was frequent in both BilIN and IPNB[26] and showed high expression in both IPNB-intestinal and IPNB-pancreatobiliary type[9,15]. In a study in Northern Thailand, a region with high incidence of BTC (biliary tract cancer), MUC5AC protein was detected in the serum of 63% of the patients with BTC, whereas it was absent in healthy controls[64]. In the same study in benign biliary epithelium MUC4 and MUC5AC were undetectable. There are no literature data for MUC5AC expression in BDCC.

Expression of MUC5AC in neoplasias of the ampulla of Vater depends on the histological subtype. Positive expression of MUC5AC is related to the pancreatobiliary phenotype and participates in subsequent stages of carcinoma extension such as invasion and metastasis[65,66]. In intestinal type carcinomas a weak expression of the neoplastic epithelium was observed. In very few cases it was also detected in the center of the tumor, while in

lymph node metastases and vascular infiltrates it was mostly absent[67].

Utility of MUC5AC as biomarker in pancreatobiliary neoplasia: In a study from Thailand[63], detection of MUC5AC in cholangiocarcinomas was associated with larger-sized tumors (> 5 cm), and advanced-stage of disease. Patients who had positive serum MUC5AC status had a significantly poorer prognosis compared to patients with undetectable serum MUC5AC (P < 0.001). Multivariate analysis showed that patients with positive serum MUC5AC status had a 2.5-fold higher risk of death compared to patients who had negative serum MUC5AC status (P < 0.001). Moreover, the diagnostic specificity of MUC4 and MUC5AC for BTC was found superior of CA19-9 (93% MUC4, 96% MUC5AC *vs* 65% CA19-9)[68]. This high specificity, however, was weakened by the comparatively low sensitivity (27% MUC4 and 44% MUC5AC), which increased to 58% by combining MUC4 and MUC5AC[68].

MUC5AC has been shown to be a major component of gastric and bronchial mucous, while in other normal tissues the expression is low or absent. MUC5A is known to be a marker of and associated with poor prognosis in diffuse type gastric carcinoma[69].

According to a recent study in MUC5AC knockdown human pancreatic cancer cell lines that were generated by the introduction of siRNA, ectopic expression of MUC5AC did not affect cell proliferation *in vitro*, but was directly involved in tumor progression *in vivo* by dramatically reducing growth and metastatic potential[70].

All the results described above concerning the expression of mucins 1, 2, 4 and 5AC in neoplasms of pancreas, biliary system and ampulla Vater are formatting in Tables 2-4 respectively.

Other mucins
MUC3: MUC3 is a predictor of poor prognosis in gastric and breast cancer. In pancreatobiliary neoplasms, a strong independent association was identified in the setting of periampullary carcinomas between cytoplasmic expression of MUC3 and favorable prognosis, in contrast to poor outcome of carcinomas showing membranous expression[71]. The cysteine-rich domains within MUC3 are able to inhibit apoptosis and promote migration of MUC3-expressing cells, favouring cancer progression[72]. However, neither vascular nor perineural invasion correlated with membranous expression of MUC3, suggesting that an alternative mechanism may be responsible for poor survival in these patients[71].

MUC6: MUC6 had no effects on survival of patients with pancreatic cancer[73].

MUC16 (CA 125): MUC16 (CA 125) is known as a tumor marker in ovarian and pancreatic cancer. Studies have investigated the expression of MUC16 in the initiation, progression and metastasis of pancreatic cancer[74-76]. Its participation in the diagnosis, prognosis and treatment of pancreatic cancer and its relation to the stage and degree of differentiation has also been explored. MUC16 is not expressed in the normal pancreatic ducts, but it was detected in pancreatitis[74]. It was also detected in pre-cancerous lesions, suggesting that its overexpression is already detectable at the early stages of carcinogenesis. In one study 20% of PanIN-Ⅰ, 28% of PanIN-Ⅱ and 42% of PanIN-Ⅲ were found positive[75]. The expression of MUC16 was significantly higher in high-grade dysplasia PanIN-Ⅲ compared to low grade dysplasia, while there was no significant difference in MUC16 expression between PanIN-Ⅰ and Ⅱ[75]. The expression of MUC16 seems to be stronger in metastatic foci as compared to the primary tumor, thus, possibly playing an important role in metastasis of adenocarcinoma[74].

In the same study MUC16 was detected in 65% of pancreatic carcinomas. Progressive increase of expression was found to be parallel to the loss of tumor differentiation with positivity being 50% in well differentiated, 59% in moderately differentiated and 66% in undifferentiated adenocarcinomas[74]. Whereas the expression of MUC16 was not significantly different between the three degrees of differentiation, the expression was significantly higher in moderately differentiated and undifferentiated carcinomas compared with the well differentiated. The observation that MUC16 is overexpressed in pancreatic carcinoma may be associated with a similar upregulation of other mucins associated to the cell membrane such as MUC4 and MUC1[1,76]. Overall, these results suggest a possible involvement of MUC16 in the pathogenesis of pancreatic adenocarcinoma and provide the basis for future studies aimed at unraveling the role of this glycoprotein.

CONCLUSION
Mucins in epithelial neoplasias of the pancreatobiliary system, in particular MUC-1, 2, 4 and 5AC are gaining importance due to their potential roles as diagnostic, predictive and prognostic markers.

In pancreatic neoplasms, increased expression of MUC1 occurs in pancreatic ductal adenocarcinomas and correlates with increasing degree of dysplasia in PanIN. Positive expression of MUC2 in IPMN-intestinal type indicates high biological potential for progression to invasive carcinoma with *de novo* expression of MUC1. IPMN-gastric type with negative MUC2 expression implies low probability for malignant evolution. *De novo* MUC4 expression seems to increase with enhancement of dysplasia in PanIN and is related to poor prognosis in patients with pancreatic ductal adenocarcinomas.

In biliary carcinogenesis expression of MUC1 correlates with the degree of BilIN dysplasia while increased expression of all MUC1 glycoforms was observed almost uniformly in ICC. Positive MUC2 expression in IPNB-intestinal type indicates high malignant potential associated with *de novo* expression of MUC1 in the invasive component. Absence of MUC2 in BilIN may be useful in the differential diagnosis from IPNB. Appearance of MUC4 in patients with ICC or EHBDC is associated

Table 2 Mucins expression in pancreatic neoplasms

	Normal	PanIN			PDCA	IPMN				MCN	ITPN
		PanIN-I	PanIN-II	PanIN-III		Gastric-type		Intestinal-type			
						Non-invasive	Invasive	Non-invasive	Invasive		
MUC1	2	2	3	4	4	1	4	1	4	CR	4
MUC2	1	1	1	1	1	1	1	4	4	1	1
MUC4	1	2	3	4	4	NA	NA	NA	NA	NA	NA
MUC5AC	1	4	4	4	4	4	4	4	4	CR	1

NA: Non available data; CR: Conflicting results; PanIN: Pancreatic intraepithelial neoplasia; IPMN: Intraductal papillary mucinus neoplasms; MCN: Mucinous cystic neoplasms; ITPN: Intraductal tubulopapillary neoplasms; MUC: Mucins. 1: No expression; 2: Low expression; 3: Moderate expression; 4: High expression.

Table 3 Mucins expression in neoplasms of biliary system

	BilIN			EHBDC	IPNB			
	BilIN-I	BilIN-II	BilIN-III		Pancreatobiliary-type		Intestinal-type	
					Non-invasive	Invasive	Non-invasive	Invasive
MUC1	2	3	4	4	2	4	1	3
MUC2	1	1	1	3	NA	4	NA	3
MUC4	NA	NA	NA	4	NA	4	NA	3
MUC5AC	4	4	4	4	4	4	4	4

NA: Non available data; BilIN: Biliary intraepithelial neoplasia; EHBDC: Extrahepatic bile ducts; IPNB: Intraductal papillary neoplasm of the bile ducts; MUC: Mucins. 1: No expression; 2: Low expression; 3: Moderate expression; 4: High expression.

Table 4 Mucins expression in ampulla Vater neoplasms

	Ampulla vater	
	Pancreatobiliary-type	Intestinal-type
MUC1	4	4
MUC2	1	4
MUC4	4	4
MUC5AC	4	3

MUC: Mucins. 1: No expression; 2: Low expression; 3: Moderate expression; 4: High expression.

with poor prognosis. *De novo* expression of MUC5AC occurs in all grades of BilIN and in all types of IPNB and ICC. MUC5AC is useful in detection of bile duct neoplastic lesions at early stage.

Finally, in carcinoma of the ampulla of Vater, increased expression of mucin MUC1 is associated with unfavorable behavior of the tumor, such as lymph node metastasis, infiltration of the pancreas and duodenum, advanced TNM classification and worse prognosis. IAPN with pancreatobiliary-immunophenotype show no MUC2 expression while those with intestinal-immunophenotype are positive. The expression of MUC4 is linked with poor prognosis in patients with carcinoma of the ampulla of Vater favoring metastasis and tumor recurrence. Expression of MUC5AC correlates with carcinoma invasive capacity.

In conclusion, the combined status of mucin expression may be useful in the differential diagnosis of pancreatobiliary neoplasms, the detection of pre-malignant lesions and in the evaluation of their malignant behavior. Besides their diagnostic and prognostic role, their involvement in carcinogenesis reveals their importance as putative therapeutic targets in the future. Further studies are needed to clearly define the exact positioning of these evaluations in the diagnostic and therapeutic algorithms of neoplastic lesions of the pancreas and biliary system.

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