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Mucin 16 (cancer antigen 125) expression in human tissues and cell lines and correlation with clinical outcome in adenocarcinomas of the pancreas, esophagus, stomach, and colon^{★,★★}

Mirte M. Streppel, MD^{a,b,c}, Audrey Vincent, PhD^a, Radha Mukherjee, MSc^a, Nathaniel R. Campbell, MSc^a, Shih-Hsun Chen, MSc^d, Konstantinos Konstantopoulos, PhD^d, Michael G. Goggins, MD^{a,e}, Isabelle Van Seuning, PhD^{f,g}, Anirban Maitra, MBBS^a, and Elizabeth A. Montgomery, MD^{a,*}

^aDepartment of Pathology, Johns Hopkins University, Baltimore, MD 21231-2410, USA

^bDepartment of Gastroenterology and Hepatology, University Medical Center Utrecht, Utrecht, The Netherlands ^cDepartment of Pathology, University Medical Center Utrecht, Utrecht, The Netherlands ^dDepartment of Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, MD, USA ^eDepartment of Gastroenterology and Hepatology, Johns Hopkins University, Baltimore, MD, USA ^fInserm, UMR837, Jean-Pierre Aubert Research Center, Team 5 “Mucins, Epithelial Differentiation and Carcinogenesis”, rue Polonovski, 59045 Lille cedex, France ^gUniversité Lille-Nord de France, 1 place de Verdun, 59045 Lille Cedex, France

Summary

Mucin 16 (cancer antigen 125) is a cell surface glycoprotein that plays a role in promoting cancer cell growth in ovarian cancer. The aims of this study were to examine mucin 16 expression in a large number of digestive tract adenocarcinomas and precursors and to determine whether mucin 16 up-regulation is correlated with patient outcome. Tissue microarrays were constructed using surgical resection tissues and included pancreatic (115 normal, 29 precursors, 200 pancreatic ductal adenocarcinomas), esophageal (86 normal, 104 precursors, 95 esophageal adenocarcinomas, 35 lymph node metastases), gastric (211 normal, 8 precursors, 119 gastric adenocarcinomas, 62 lymph node metastases), and colorectal (34 normal, 17 precursors, 39 colorectal adenocarcinomas) tissues. Mucin 16 was detected in 81.5%, 69.9%, 41.2%, and 64.1% of the pancreatic ductal adenocarcinomas, esophageal adenocarcinomas, gastric adenocarcinomas, and colorectal adenocarcinomas, respectively. Mucin 16 was seen in a subset of the precursors. On multivariate analysis, moderate/diffuse mucin 16 in pancreatic ductal adenocarcinomas was strongly associated with poor survival ($P < .001$), independent of other prognosis predictors. A similar trend was observed for esophageal adenocarcinomas ($P = .160$) and gastric adenocarcinomas ($P = .080$). Focal mucin 16 in colorectal adenocarcinomas was significantly correlated ($P = .044$) with a better patient outcome, when compared with mucin 16–negative cases.

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*Corresponding author: emontgom@jhmi.edu (E. A. Montgomery).

Supplementary data

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Using Western blot analysis, we found mucin 16 expression in 3 of 6 pancreatic ductal adenocarcinoma and 1 of 2 esophageal adenocarcinoma cell lines. We conclude that most of the digestive tract adenocarcinomas and a subset of their precursors express mucin 16. Mucin 16 expression is an independent predictor of poor outcome in pancreatic ductal adenocarcinomas and potentially in esophageal adenocarcinomas and gastric adenocarcinomas. We propose that mucin 16 may function as a prognostic marker and therapeutic target in the future.

Keywords

MUC16; CA125; Digestive tract adenocarcinoma

1. Introduction

The digestive tract is the second most common site affected by cancer in the United States. Cancers of the digestive tract cause approximately 140 000 deaths annually, which accounts for 25% of all cancer-related deaths. Colorectal, pancreatic, and esophageal cancer are among the top 10 deadliest cancer types [1]. Most patients are diagnosed at an advanced stage because of nonspecific clinical symptoms. There is a pressing need for discovery of new markers because such markers cannot only function as prognostic tools but can potentially also offer new therapeutic targets for these aggressive tumors.

Mucins, encoded by *MUC* genes, are glycoproteins that contain a variable number of tandem repeat structures. These variable number of tandem repeat structures constitute a prolines-, threonines-, and serines- (PTS) rich domain, which, in turn, carries the mucin characteristic O-glycosylation. Mucins can be divided into 4 categories: secreted gel forming (MUC2, -5AC, -5B, -6, and -19), secreted non-gel forming (MUC7, -8, and -9), transmembrane (TM) (MUC1, -3A, -3B, -4, -12, -13, -14, -15, -16, -17, -20, and -21), and unclassified (MUC11) mucins [2]. TM mucins are found on the apical membrane of epithelial cells, as well as in the mucus layers of the respiratory and gastrointestinal tracts [3]. Cell surface overexpression of TM mucins has been identified in cancers including digestive tract lesions, and these mucins are believed to promote tumor cell growth and tumor cell survival [4–6].

Molecular cloning of the cancer antigen 125 [CA125] led to the discovery of mucin 16 (MUC16) in 2001 [7]. MUC16 is the largest TM mucin and is used worldwide for monitoring patients with ovarian cancer [4,8]. Several research groups have attempted to elucidate the role of MUC16 in promoting ovarian cancer cell growth and survival [9–14]. At present, no studies have been performed attempting to clarify mechanisms via which MUC16 can encourage tumor progression in cancers other than ovarian cancer.

MUC16 (CA125) was initially believed to be a specific biomarker for ovarian cancer, but over the past 15 years, it has become clear that this marker can also be detected in sera of patients with other types of cancers including gastric, colorectal, and pancreatic adenocarcinomas. Although it is already known that MUC16 plays a role in at least a subset of the digestive tract adenocarcinomas and elevated serum MUC16 levels are associated with poor survival [15–21], the presence of MUC16 in digestive tract adenocarcinoma tissues, precursor lesions, and normal digestive tract epithelia has not been comprehensively evaluated.

In the present study, we show that MUC16 is up-regulated in a large percentage of the digestive tract adenocarcinomas and in a subset of noninvasive precursor lesions. Furthermore, we propose that MUC16 may function as a prognostic marker and therapeutic

target for pancreatic ductal adenocarcinomas (PDAC) and, potentially, for esophageal adenocarcinomas (EAC) and gastric adenocarcinomas (GAC) in the future.

2. Materials and methods

2.1. Patient material

Paraffin-embedded material obtained from digestive tract adenocarcinoma patients who underwent surgical resection at the Johns Hopkins Hospital, Baltimore, Maryland, between the years 1984 and 2004 was used for this study. Tissue microarrays (TMA) were constructed using these specimens. Each cancer case was represented by three to twelve 1.5-mm cores on the TMAs to obtain adequate representation of different regions of the tumor, precursor lesions, and normal epithelium.

Tissue cores included pancreatic (115 normal acinar tissue, 76 obstructive ducts, 10 pancreatic intraepithelial neoplasia [PanIN], 200 PDACs), esophageal (86 normal squamous epithelium, 37 nondysplastic Barrett esophagus [BE], 21 low-grade dysplasia [LGD], 46 high-grade dysplasia [HGD], 95 EACs, and 35 lymph node metastases [LNM], and 3 distant metastases [DM]), gastric (176 normal columnar lining of the stomach, 35 normal gastric cardia, 8 HGDs, 119 GACs, and 62 LNMs), colorectal (34 normal colonic mucosa, 10 LGD adenomas, 7 HGD adenomas, and 39 colorectal carcinomas [CRC]) tissues. Normal and precursor lesions were primarily obtained from adjacent regions of the cancer resection specimen. In addition to the PanIN lesions present in the TMAs, 19 low- and high-grade PanIN lesions were randomly selected and also included in this study. All PanIN lesions were categorized as follows: low-grade (PanIN IA and IB) and high-grade (PanIN II and III) PanINs. *BE* was defined as nondysplastic columnar mucosa with intestinal metaplasia (goblet cells) in the esophagus as per the guidelines of both the American College of Gastroenterologists and the American Gastroenterological Association [22,23]. Nondysplastic columnar mucosa with gastric foveolar type surface cells and gastric cardiac type glands without goblet cells was classified as cardiac mucosa.

Characteristics of the patients with cancer including age, sex, race, and survival time were collected. In addition, tumor features such as differentiation of the tumor, the presence of LNMs and DMs, and angiolymphatic invasion were, if available, obtained from the original pathologic surgical resection reports.

2.2. Immunohistochemistry

Five-micrometer sections were deparaffinized, endogenous peroxidase was blocked, and antigen retrieval was performed using routine techniques. After blocking of nonspecific binding sites using serum free Protein-block (DAKO North America, Carpinteria, CA), the slides were incubated with a primary monoclonal antibody against MUC16 (dilution 1:200, 1 hour, room temperature [RT], X325; Abcam, Cambridge, MA). The slides were subsequently incubated with Post-antibody blocking and PowerVision+ Poly-HRP-anti-mouse/rabbit IgG (Leica Biosystems, Newcastle, United Kingdom) for 15 and 30 minutes, respectively. 3,3'-diaminobenzidine tetrachloride plus (Leica Biosystems) was used as the chromogen for visualization. Finally, the slides were counterstained with hematoxylin.

2.3. Scoring of immunolabeling

Staining patterns were evaluated by 3 authors (E. A. M., A. M., and M. M. S.). MUC16 protein was considered present if epithelial cells exhibited brown membranous staining. Because cytoplasmic and nuclear staining were rarely present, only membranous staining was scored. Staining was categorized as follows: "negative" (no detectable labeling), "focal labeling" (<25% of the epithelial cells showing positivity), "moderate labeling" (25%-50%),

and “diffuse labeling” (>50%). Staining intensity was not scored because no variability in staining intensity was observed. Normal tissues obtained from noncancerous patients were included in all TMAs and were used as negative controls. Samples of nonneoplastic mucosa from the same patients whose tumors appeared in the arrays were also included in the TMAs. Randomly selected ovarian cancer specimens functioned as positive controls in this study.

2.4. Cell culture and protein extraction

Six PDAC cell lines (Panc3.014, Panc5.04, Panc8.13, Panc10.05, Panc10.7, and BxPC-3) and 2 EAC cell lines (OE33, JHesoAD1) were analyzed for the presence of MUC16 protein. OVCAR3, a commonly used ovarian cancer cell line in which MUC16 expression has been confirmed by Goodell et al [24], was used as a positive control. Human pancreatic normal epithelial (HPNE) cells functioned as a negative control. OE33, JHesoAD1, and OVCAR3 cells were grown in RPMI containing 15% to 20% fetal bovin serum, and the PDAC cell lines were all cultured in DMEM supplemented with 10% to 20% fetal bovin serum.

Total protein was extracted from cell lines using lysis buffer supplemented with protease and phosphatase inhibitors. After undergoing 2 freeze thaw cycles, the lysates were centrifuged for 10 minutes at full speed. Protein concentrations were measured following the bicinchoninic acid method, as described in the manufacturer’s instruction manual (Thermo Fisher Scientific, Landford, IL).

2.5. Western blots

Forty micrograms of total protein extracts were separated on 3% to 8% Tris-acetate and 4% to 12% Bis-Tris gels for detection of MUC16 (500–5000 kDa) and β -actin (42 kDa), respectively. Proteins were transferred to 0.45- μ m nitrocellulose membranes. The membranes were subsequently incubated in 5% fat-free milk at 4°C overnight to block nonspecific binding. A mouse monoclonal antibody against MUC16 (M11, dilution 1:1000, 2 hours at RT; DAKO North America) was used for MUC16 detection. The membrane was subsequently incubated with an antimouse secondary antibody (dilution 1:4000, 1 hour at RT; Sigma Aldrich, St. Louis, MO). The loading control β -actin was detected using a goat antibody against β -actin (dilution 1:1000, 1 hour, RT; Santa Cruz, Santa Cruz, CA), and a secondary antigoat antibody (dilution 1:2000, 1 hour, RT; Santa Cruz). Blots were developed using PICO reagents (Bio-Rad).

2.6. Statistical analysis

Kaplan-Meier survival curves were prepared. Log-rank tests were used to determine whether MUC16 expression correlated with survival. Univariate and multivariate analyses were run using Cox regression analysis to examine whether MUC16 was an independent prognostic indicator. $P < .05$ was considered statistically significant.

3. Results

Clinicopathologic features of the patients with cancer are detailed in Table 1.

3.1. PDAC

Examples of the presence of MUC16 in pancreatic tissues are provided in Fig. 1 (row 1). Membranous MUC16 was present in 81.5% (163/200) of the surgically resected PDACs (Fig. 2A). Moderate or diffuse staining, which was seen in 59.5% (119/200) of all PDACs, was strongly associated with poor patient outcome in the Kaplan-Meier log-rank test ($P < .001$, Fig. 3A), the univariate and multivariate analyses ($P < .001$, hazard ratios [HR] of 1.9 and 2.0, respectively [Supplementary Table S1]). White ethnicity, poor tumor

differentiation, and LNMs were statistically significant negative prognostic factors in addition to moderate/diffuse MUC16 positivity in the multivariate analysis (Supplementary Table S1). The mean survival times for the different staining intensity groups were as follows: 33.6, 30.7, 19.3, and 18.5 months, for patients with no, focal, moderate, and diffuse MUC16 staining, respectively ($P = .001$, log-rank test [Fig. 3A]). Furthermore, MUC16 expression was observed in only 2.7% (3/115) of the normal pancreatic tissues, and none of the low-grade (0/19) and high-grade PanIN lesions (0/10) exhibited MUC16 (Fig. 2A). This strongly indicates that MUC16 expression is a late event during the carcinogenesis of PDAC.

3.2. EAC

MUC16 immunostaining (Fig. 1, row 2) was seen in 69.5% (66/95) of the primary EACs, 51.4% (18/35) of the LNMs, 28.2% (13/46), and 4.8% (1/21) of the HGD and LGD lesions, respectively, and was absent in BE (0/37). MUC16 was focally present on cell membranes of 4 of 86 normal squamous epithelial tissues. Among the 66 EAC tissues expressing MUC16, only 14.7% (14/95) of the tumors demonstrated a pattern of moderate or intensive staining (Fig. 2B). There was a trend of worse outcome for patients with moderate or diffuse MUC16 expression (Fig. 3B). On average, patients with no or only focal labeling in EAC tissue lived 15.6 months longer than those with moderate/diffuse MUC16 expression (39.2 months for no/focal staining versus 23.6 months for moderate/diffuse staining). However, this difference did not reach statistical significance ($P = .30$). The multivariate Cox regression survival test (Supplementary Table S1) suggested that a patient with moderate/diffuse MUC16 was 2.2 times (HR) more prone to die from EAC; however, still no statistically significant difference was found ($P = .16$). Poor tumor differentiation ($P = .005$), positive surgical margins ($P = .009$), and the presence of LNMs ($P = .023$) and DMs ($P < .001$) were predictors of poor patient outcome in univariate analysis.

3.3. GAC

MUC16 was detected in a sizable minority (41.2%, 49/119) of the GACs and LNMs (40.3%, 25/62). Of the MUC16-positive tumors, 27 (55.1%) showed only focal membranous labeling (Fig. 1 [row 3] and Fig. 2C). The survival time after diagnosis was on average 94.9, 57.1, 47.8, and 26.8 months for the absent, focal, moderate, and diffuse staining groups, respectively ($P = .082$, Kaplan-Meier log-rank [Fig. 3C]). Using multivariate analysis, we confirmed that the presence of staining was of borderline significance for a poor prognosis ($P = .084$, HR of 1.714) (Supplementary Table S1). On univariate analysis, positive surgical margins and the presence of LNMs and DMs were identified as predictors of poor survival. However, only DMs were significantly associated with a shorter life time in multivariate analysis ($P = .004$) (Supplementary Table S1). MUC16 protein expression was uncommonly encountered in noninvasive precursor lesions of GAC; only 25% (2/8) of the HGD tissues labeled with MUC16. Furthermore, MUC16 was not detected in normal gastric mucosa (Fig. 2C).

3.4. Colorectal adenocarcinoma

Among the 39 CRCs examined, 25 (64.1%) expressed MUC16. Fifteen of these cases showed focal staining (Fig. 1 [row 4] and Fig. 2D). Direct comparison of the cases with absent expression to those with focal expression revealed a significant survival advantage for patients whose tumors displayed focal MUC16 expression ($P = .044$, HR of 3.0). Patients whose tumors lacked staining ($n = 14$) had an average postoperative survival of 87.3 (95% CI, 34.0–140.5) months, compared with 182.6 (95% CI, 143.1–222.1) months for patients whose CRCs had focal staining (Kaplan-Meier log-rank, Fig. 3D). The mean survival time for all MUC16-positive CRC patients was 157.5 months, which surprisingly did not reach statistical significance when compared with MUC16-negative CRC patients ($P = .13$, Fig.

3D). Among all possible predictors of prognosis, only the presence of DMs was significantly correlated with poor patient outcome in the multivariate analysis ($P = .008$, HR of 46.4, Supplementary Table S1). In addition, MUC16 was present in 42.9% (3/7) of the adenomas with HGD and 10% (1/10) of the adenomas without HGD, reiterating that aberrant expression can be observed in noninvasive precursor lesions in the colon. In contrast, normal colon mucosa lacked MUC16 expression (Fig. 2D).

3.5. MUC16 in pancreatic and esophageal cell lines

The Western blot results appear in Fig. 4. MUC16 was detected in the positive control OVCAR3. The size of MUC16 in OVCAR3 was above the 500 kDa, which is within the range of the predicted size of MUC16 (500–5000 kDa). MUC16 was undetectable in our negative control HPNE. MUC16 was found in 3 of 6 examined PDAC cell lines, with strong expression in Panc8.13 and Panc10.7 and weak expression in Panc5.04. In addition, MUC16 was present in JHesoAD1, whereas it was undetectable in OE33. β -actin was used as a control for presence of protein and was strongly expressed in all cell lines (Fig. 4).

4. Discussion

Herein, we provide an overview of MUC16 protein expression in normal epithelia, precursor lesions, adeno-carcinomas, and metastases of the digestive tract, including the esophagus and the stomach, which have not been evaluated for this protein to date. MUC16 has been previously studied in CRCs and PDACs; however, precursor lesions and direct correlation with patient survival have not been assessed.

In 1988, Macdonald et al [25] were the first to report immunolabeling for MUC16 in PDACs. These authors detected MUC16 in 14%, 55%, and 91% of poorly, moderately, and well-differentiated tumors, respectively [25]. Their results contrast with ours, as we found a strong correlation between MUC16 expression and aggressive tumor features. Recently, Einama et al [26] also reported MUC16 expression in PDACs, and their data are similar to ours. However, we were able to examine MUC16 in a much larger PDAC cohort and to include precursor lesions in our study. Our results imply that MUC16 up-regulation is a late event in the carcinogenesis of PDAC. We identified MUC16 as a negative prognostic factor ($P < .001$), independent of other well-known clinicopathologic prognosis predictors; our study indicates that PDAC patients with no/focal MUC16 immunostaining have a survival benefit of 12.2 months. Although MUC16 protein is present in most of our PDAC cases, high MUC16 concentrations in sera of PDAC patients are uncommon; elevated MUC16 levels were found in only 45% to 63% of PDAC patients [15–17]. Detection of MUC16 in sera may be of limited value in diagnosing PDAC; however, we show in the current study that MUC16 immunolabeling of tissues can function as an excellent prognostic marker in PDAC tissues. Considering its direct correlation with aggressive tumor behavior and surface expression, MUC16 could potentially also be used as a therapeutic target in the future.

Because MUC16 was observed in most of the EACs and only a subset of the nondysplastic and dysplastic precursors, it is likely that up-regulation of MUC16 is also a late event in the carcinogenesis of EAC. Although not statistically significant, Kaplan-Meier analysis showed a clear trend of worse survival for patients whose tumors manifested moderate/diffuse MUC16 expression. Multivariate analysis strengthened this correlation, indicating that MUC16 can potentially function as a predictor of survival independent of known prognostic factors. The lack of statistical significance of these analyses most probably reflects the relatively low number of samples revealing moderate/diffuse MUC16 expression. Larger cohort studies are required to confirm that moderate/focal MUC16 correlates with a poorer patient outcome.

For patients with GAC, we found a borderline significant correlation ($P = .084$) between MUC16 expression and shorter survival. We observed a gradually decreased survival time with the increase of staining intensity. Multivariate analysis suggested that MUC16 as a prognostic marker is independent of other prognostic indicators. Consistent with our results, others have found that elevated MUC16 in sera of GAC patients is significantly correlated with the presence of peritoneal metastases, LNM, and shorter survival time [18–21]. Our data show that MUC16 could be a candidate marker for predicting patient outcome, and future studies should indicate whether it could be targeted to treat patients with advanced GAC.

Some observers have suggested that MUC16 immunolabeling can be used for distinguishing between primary ovarian adenocarcinoma and ovarian metastases from a primary CRC [27–29]. However, we found MUC16 expression in most of the primary CRCs, which indicates that MUC16 is not specific for ovarian cancers. Our results suggest that CRC patients whose tumors display MUC16 expression, in particular, those whose tumors have focal MUC16 expression, have a statistically significant better prognosis than CRC patients whose tumors lack MUC16 expression. This might indicate that MUC16 has a protective role in CRC. Additional studies are required to confirm this and to elucidate potential mechanisms behind it.

We also demonstrated the presence of MUC16 protein in pancreatic and esophageal cell lines. MUC16 was present in 50% of the PDAC and EAC cell lines. These data are useful for future functional studies targeting MUC16.

It is known that MUC16 harbors adhesive and anti-adhesive properties [9]. Over the past 10 years, several MUC16-binding receptors have been identified in ovarian cancer cells. Protein-protein interactions between MUC16 and its various receptors may influence cell-cell interactions, allowing cancer cells to evade apoptosis [10–14]. The first identified binding site is localized on mesothelin, a glycosylphosphatidylinositol (GPI)-anchored glycoprotein classically detected on mesothelial cells. The N-terminus of the extracellular domain of mesothelin has specific affinity for MUC16 and might represent a therapeutic target because it mediates cancer cell adhesion and possibly plays a role in the implantation and metastatic spread of tumors [10–12]. It has been demonstrated that mesothelin is expressed in 90% to 100% and 29% of PDACs and EACs, respectively, whereas mesothelin is absent in adjacent normal epithelia [30–33]. Recently, Einama et al [26] reported that coexpression of mesothelin and MUC16 is associated with poor patient outcome in PDAC. Siglec-9 is the second known binding domain for MUC16 and is found on immune cells. As Siglec-9 seems to be an inhibitory receptor, binding of MUC16 to Siglec-9 may lead to immune response escape and thus, in the case of ovarian cancer, cell survival instead of cell death due to increased immune recognition [13]. Galectin-1 is the most prominent member of the lectin protein family and is the third identified receptor for MUC16 [14]. Further research is required to investigate whether immunotoxic antibodies against these binding sites can be exploited for future therapies.

In conclusion, we propose that MUC16 expression is a late event in the carcinogenesis of digestive tract adenocarcinomas and that it is present on the cell surface of a large percentage of these neoplasms. We have presented evidence that MUC16 can potentially function as a prognostic indicator; moderate/diffuse MUC16 expression was an individual predictor of poor patient outcome in PDACs and might also be in GACs and EACs. In contrast, focal MUC16 staining in CRCs was significantly correlated with a longer patient survival. Although additional studies are required to confirm this, MUC16 or MUC16-binding sites may offer some promise as therapeutic targets for MUC16-positive PDACs, GACs, and EACs.

Supplementary Material

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Fig. 1.

Examples of MUC16 expression in lesions in the pancreas, esophagus, stomach, and colon are shown in rows 1, 2, 3, and 4, respectively. The brown color indicates the presence of MUC16 protein. Row 1 (pancreas): A, High-grade PanIN lesion (PanIN II) with focal MUC16 expression (10×); B, Staining in obstructed ductules adjacent to a carcinoma (20×); C, Diffuse staining in a PDAC (20×). Row 2 (esophagus): Images illustrate focal-moderate MUC16 expression in HGD (A) and focal (B) and diffuse expression (C) in EAC. Row 3 (stomach): GACs showing no (A), moderate (B), and diffuse MUC16 immunostaining. Row 4 (colon): Absent staining in an adenoma with LGD (A), whereas focal and diffuse staining is observed in carcinomas in images B and C, respectively.

Fig. 2. Distribution of MUC16 over different tissue types are shown in A, B, C, and D, for the pancreas, esophagus, stomach, and colon, respectively. MUC16 was detectable in most of the adenocarcinomas and LNMs and in only a subset of the precursor lesions. Furthermore, MUC16 was found in all pancreatic ducts in areas of obstructive pancreatitis.

Fig. 3. Kaplan-Meier survival curves of 4 digestive tract adenocarcinomas. PDAC patients whose tumors displayed no/focal MUC16 expression lived significantly longer than patients with moderate/diffuse MUC16 expression (log-rank test, $P < .001$) (A). GAC (C) and EAC (B) patients with detectable MUC16 in their adenocarcinomas also tended to have a worse prognosis (log-rank test, $P = .080$ and $P = .300$, respectively). Notably, although not statistically significant (log-rank test, $P = .130$) (D), a trend of better patient outcome was seen for MUC16-positive CRC patients compared with MUC16-negative CRC patients.

Fig. 4.

MUC16 Western blot analysis results. MUC16 (predicted size range, 500–5000 kDa) was strongly expressed in 2 PDAC (Panc8.13 and Panc10.7) and 1 EAC cell line (JHesoAD1). Weak expression of MUC16 was found in Panc5.04, whereas there is no evidence of MUC16 presence in the remaining PDAC cell lines (Panc3.014, Panc10.05, and BxPC3) and OE33. OVCAR3 and HPNE were used as positive and negative controls, respectively. The loading control β -actin was present in all cell lines.

Table 1
Patient characteristics and tumor features of all digestive tract adenocarcinoma patients

	PDAC (n = 200)		EAC (n = 95)		GAC (n = 119)		CRC (n = 39)	
	Absolute	%	Absolute	%	Absolute	%	Absolute	%
Age (y)								
Mean	66.4	NA	66.0	NA	63	NA	63.6	NA
Median	67	NA	68.0	NA	63.5	NA	63.0	NA
Sex								
Male	108	54	84	88	78	66	23	59
Female	92	46	11	12	41	34	16	41
Race								
White	171	86	92	97	69	58	31	79
Black	16	8	2	2	38	32	8	21
Asian	0	0	1	1	0	0	0	0
Other/unknown	13	7	0	0	12	10	0	0
Differentiation grade								
Well	6	3	2	2	0	0	0	0
Moderate	106	53	35	37	26	22	30	77
Poor	88	44	55	58	73	61	6	15
Unknown	0	0	3	3	20	17	3	8
Tumor dimension (cm)								
Mean	3.0	NA	3.9	NA	–	–	4.8	NA
Median	3.0	NA	3.5	NA	–	–	5.0	NA
Surgical margins								
Negative	88	44	80	84	80	67	34	87
Positive	111	56	15	16	39	33	3	8
Unknown	1	1	0	0	0	0	2	5
Angiolymphatic invasion								
Absent	80	40	–	–	4	3	5	13
Present	106	53	–	–	33	28	4	10
Unknown	14	7	–	–	82	69	30	77
Tumor stage (TNM)								

	PDAC (n = 200)		EAC (n = 95)		GAC (n = 119)		CRC (n = 39)	
	Absolute	%	Absolute	%	Absolute	%	Absolute	%
1	5	3	25	26	3	3	2	5
2	17	9	16	17	16	13	6	15
3	176	88	54	57	52	44	29	74
4	2	1	0	0	48	40	2	5
Lymph node stage								
0 LNMs	31	16	39	41	29	24	21	54
1–2 LNMs	58	29	24	25	22	18	7	18
3–6 LNMs	64	32	21	22	35	29	5	13
7 LNMs	47	24	11	12	33	28	6	15
Distant metastases								
Absent/unknown	157	79	78	82	88	74	29	74
Present	43	22	17	18	31	26	10	26

Abbreviations: NA indicates not applicable.