HHS Public Access

Author manuscript

Hum Pathol. Author manuscript; available in PMC 2018 December 01.

Published in final edited form as:

Hum Pathol. 2017 December ; 70: 62–69. doi:10.1016/j.humpath.2017.10.010.

T-complex-associated-testis-expressed 3 (TCTE3) is a Novel Marker for Pancreatobiliary Carcinomas

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Abstract

Several markers of pancreatobiliary lineage have been described in the literature. However, none have demonstrated sufficient specificity and sensitivity to warrant diagnostic use. We evaluated the utility of T-complex-associated-testis-expressed 3 (TCTE3) as a pancreatobiliary marker. A set of 247 adenocarcinomas from the gastrointestinal (GI) tract was identified including 18 from the gastroesophageal junction (GEJ), 29 stomach, 17 ampullary, 62 pancreatic, and 16 common bile duct and gallbladder (CBD/GB), 13 non-ampullary small intestine, 32 colon, and 24 rectum. The remainder consisted of 16 cholangiocarcinomas, and 20 hepatocellular carcinomas (HCC). Additionally, 163 adenocarcinomas from the breast, gynecologic tract, prostate, urothelium, kidney, and lung were stained for comparison. Immunohistochemistry for TCTE3 and other gastrointestinal markers was performed. Positive expression of TCTE3 was characterized by a strong, well-defined membranous pattern with or without weak cytoplasmic staining. Expression was identified in the normal epithelial cells of pancreatobiliary tree, but staining was absent in normal epithelial cells of esophagus, stomach, and intestine. Hepatocytes, pancreatic acini and islets and other non-epithelial cells were also negative for staining. TCTE3 was expressed in 93.5% of pancreatic ductal adenocarcinomas, 37.5% of CBD/GB adenocarcinomas, 50% of cholangiocarcinomas, 76.4% of ampullary adenocarcinomas, and 33.3% of GEJ adenocarcinomas. Only 3.5% of the gastric, 7.7% of non-ampullary small intestinal and 6.25% of colonic tumors exhibited positive staining. Expression was absent in rectal carcinomas and HCCs. These results suggest that TCTE3 is a useful marker of pancreatobiliary differentiation and may aid in distinguishing these tumors from gastric and intestinal primary tumors.

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Disclosure/Duality of Interest

The authors do not have potential conflicts of interest to disclose.

Keywords

TCTE3; pancreatobiliary adenocarcinoma; diagnostic marker

1. Introduction

Pancreatobiliary adenocarcinomas are comprised of cholangiocarcinomas, gallbladder adenocarcinomas, and pancreatic ductal carcinomas. These tumors typically consist of malignant glands with surrounding markedly desmoplastic stroma. The tumor cells are usually positive for CK7 and CK19 and negative for CDX2 and CK20. However, this immunophenotype is neither sensitive nor specific. Other pancreatobiliary markers, including mucins and CK17, have been proposed and summarized by Lin et al [1]. Gastroesophageal junction (GEJ) and gastric adenocarcinomas often share overlapping immunophenotype with pancreatobiliary cancer. In the diagnostic workup of adenocarcinomas involving the liver, the distinction between upper GI tract and pancreatobiliary primary site based on immunohistochemistry is often difficult. While there is presently no specific immunohistochemical marker to support pancreaticobiliary origin in metastatic carcinomas, a marker of pancreaticobiliary differentiation may aid in this diagnostic workup.

T-complex-associated-testis-expressed 3 (TCTE3) was originally described as the outer arm of the dynein light chain in cilia. Defects in TCTE3 have been associated with in asthenozoospermia in mice [2]. TCTE3 is also proposed as a candidate gene for congenital diaphragmatic hernia and primary ciliary dyskinesia [3–4]. Its expression in the gastrointestinal epithelium as well as other epithelial tissues has not been investigated. In the present study, we examine the expression of TCTE3 in normal tissues of the GI tract, GI adenocarcinomas and non-GI carcinomas and assess its utility as a differential diagnostic marker.

2. Materials and Methods

2.1. Case selection

With institutional approval, cases of gastrointestinal adenocarcinomas and carcinomas from other sites accessioned from 2000 through 2015 were retrieved from the archives of the Department of Pathology at Rhode Island Hospital and The Miriam Hospital (Providence, RI). 247 adenocarcinomas from the gastrointestinal (GI) tract were identified. This cohort included 18 gastroesophageal junction (GEJ) tumors, 29 gastric tumors, 17 ampullary tumors, 62 pancreatic tumors, 16 common bile duct and gallbladder tumors, 16 cholangiocarcinomas, 20 hepatocellular carcinomas, 13 non-ampullary small intestinal tumors, 32 colonic tumors, and 24 rectal tumors. 163 adenocarcinomas from the breast, gynecological tract, prostate, urothelium, kidney, and lung were also included (Table 1).

2.2. Tissue microarray construction

Formalin-fixed paraffin-embedded tissue blocks with representative tumor areas were identified through review of corresponding hematoxylin and eosin–stained sections. Areas of

interest were identified and marked on each selected block. The block was cored using a 1-mm core needle. For each case four representative cores of tumor and 1 core of adjacent normal tissue were arrayed. The cores were transferred to the recipient “master block” using a Beecher Tissue Microarrayer (Beecher Instruments, Silver Spring, MD).

2.3. Immunohistochemical staining

Immunohistochemical staining for TCTE3 and eight other markers including CDX2, CK7, CK20, MUC1, MUC2, MUC4, MUC5AC, and MUC6 was performed. The characteristics of the primary antibodies are presented in Table 2. Sections from paraffin-embedded tissue microarrays were cut to 4 μm sections, deparaffinized, and rehydrated with xylene and graded alcohols. Microwave antigen retrieval was performed in 10 mM citrate buffer (pH 6.0) or EDTA (pH 9.0) for 40 minutes followed by cooling for 15 minutes at room temperature. Immunohistochemical staining was run on a Discovery Autostainer using the DabMap Detection Kit (Ventana Medical Systems, Tucson, AZ, USA). Slides were counterstained with hematoxylin. Appropriate positive and negative controls were stained simultaneously. Positive expression was defined by the presence of ≥5% tumor cells showing membranous staining with or without cytoplasmic expression. Normal human testis was used as a positive control (Figure 1).

2.4. Statistical methods

Chi-square analysis was used to assess associations between TCTE3 expression and tumor primary sites. All tests were 2-sided using a p-value of 0.05 as the threshold for statistical significance. Analyses were performed using SAS software, JMP Base version 12.0.1 (SAS, Cary, NC, USA).

3. Results

3.1. Expression of TCTE3 in adenocarcinomas of the GI tract and other organs

Expression TCTE3 in GI tract was predominantly membranous with or without weak cytoplasmic staining. Normal epithelial cells of pancreatic ducts, bile ducts, and gallbladder mucosa demonstrated positive staining. In contrast, pancreatic acinar cells, hepatocytes, gastric epithelium, small and large intestinal epithelium, endothelial cells and stromal cells showed no expression of TCTE3 (Figure 2).

Of the 62 pancreatic ductal carcinomas, 58 (93.5%) showed positive membranous expression of TCTE3 (Figure 3A). TCTE3 expression was detected in 6 of 16 (37.5%) CBD and GB tumors, 8 of 16 (50%) cholangiocarcinomas, and 13 of 17 (76.4%) ampullary tumors (Figures 2B–2F and Table 1).

Among non-pancreaticobiliary adenocarcinomas of the GI tract, 9 out of 137 expressed TCTE3 including 6 of 18 (33.3%) GEJ tumors, 1 of 29 (3.5%) gastric carcinomas, 1 of 13 (7.7%) small intestinal tumors, and 2 of 32 (6.25%) colonic tumors. TCTE3 expression was absent in hepatocellular carcinomas and rectal tumors (Table 1) (Figure 3).

Among non-GI adenocarcinomas, TCTE3 was expressed in 6 of 16 (37.5%) breast carcinomas including 3 invasive ductal tumors, 1 lobular tumor, and 2 mixed lobular and

ductal tumors. Most of gynecological carcinomas (17 out of 18) expressed TCTE3, including 11 serous, 2 endometrioid, and 4 clear cell carcinomas. TCTE3 was negative in 1 serous carcinoma. Fourteen of 16 lung adenocarcinomas expressed TCTE3. Only 3 out of 43 renal tumors expressed TCTE3. Three of 5 papillary renal cell carcinomas demonstrated positive expression. Staining was absent in clear cell renal cell carcinomas, urothelial carcinomas, and prostatic adenocarcinomas (Table 1).

3.2. Diagnostic utility of TCTE3 and correlation with histologic features

Among tumors identified in the GI tract, the sensitivity of TCTE3 for pancreatic ductal carcinoma was 93.5% with a specificity of 80.0%. The positive predictive value (PPV) was 61.1% and the negative predictive value (NPV) was 97.4% (Table 3). The sensitivity of TCTE3 for pancreatobiliary tumors was 76.6% with a specificity of 92.6%. The positive predictive value was 89.5% and the negative predictive value was 82.9% (Table 3).

The seventeen ampullary cancers in the study consisted of 12 pancreatobiliary type, 3 intestinal type, one invasive papillary, and one with signet ring differentiation. Eleven out of 12 pancreatobiliary type tumors were positive for TCTE3 while 2 of 3 intestinal type tumors were negative for TCTE3 ($p=0.0379$). The papillary type tumor was positive for TCTE3 and the tumor with signet ring differentiation was negative. Overall, there were no associations between histologic differentiation and TCTE3 expression ($p=0.8929$) (Table 4).

TCTE3 expression showed no correlation with histological grade in GB and CBD carcinomas ($p=0.3154$). However, expression was associated with the biliary histologic type ($p=0.0055$). Expression of TCTE3 in cholangiocarcinoma was not associated with histological grade ($p=0.1255$) and location ($p=0.0803$). Histologic differentiation was not associated with TCTE3 expression in cancer of the GEJ ($p=0.1778$) (Table 4).

3.3. Association between TCTE3 and other frequently used GI markers

In comparison to other markers, TCTE3 was negatively associated with CDX2 and MUC6 ($p<0.05$) and positively associated with CK7 and MUC4 ($p<0.01$) when analyzing the GI tumors together. Individually, TCTE3 was positively associated with CK7 in ampullary cancers ($p<0.01$), GB and CBD tumors ($p<0.05$). Furthermore, there was a negative association between TCTE3 and CK20 in GB and CBD tumors ($p<0.05$), positive association with MUC1 and MUC4 in pancreatic tumors ($p<0.05$ and $p<0.01$ respectively), and positive association with MUC5AC in cholangiocarcinomas ($p<0.01$) and GEJ tumors ($p<0.05$) (Table 5).

4. Discussion

4.1. TCTE3 as a marker for pancreatobiliary differentiation

TCTE3 was first described as a protein associated with dynein arms involved in spermatogenesis and sperm motility. Given its role in the cytoskeleton of spermatozoa, it is postulated that cells with motile projections such as cilia should express TCTE3. This theory is corroborated by the presence of TCTE3 in ciliated tissue from lung, Müllerian tract and mesothelium in the current series. For cells lacking cilia, the role of TCTE3 expression on

the plasma membrane is unknown. Microvilli in the GI tract lack TCTE3 while expression is present in pancreatobiliary epithelial cells. From a diagnostic viewpoint, the absence of TCTE3 expression in foveolar and intestinal mucosa suggests that it may represent an ideal marker for pancreatobiliary differentiation. Indeed, our findings demonstrate that TCTE3 is expressed in most pancreatic cancers, extrahepatic bile duct tumors and cholangiocarcinomas with loss of expression only in some poorly differentiated cholangiocarcinomas. As expected, hepatocellular carcinomas do not express TCTE3. Gastric and intestinal tumors are only rarely positive. Based on these differential staining features, TCTE3 may aid in the determination of primary sites for metastatic carcinomas. Correspondingly, lack of TCTE3 expression may aid in excluding pancreatobiliary differentiation while positive staining may support pancreatobiliary origin provided breast, lung and Müllerian tumors can be excluded using their respective lineage markers.

Currently, no reliable markers are available to distinguish cholangiocarcinoma and other pancreaticobiliary tumors from gastric adenocarcinoma. CK7 and CK19 often show overlapping expression in both tumors. MUC5AC has been proposed as a gastric marker with good prognostic utility [5]. However, it is expressed in over a half of pancreatic ductal carcinomas (Table 5). Therefore, the absence of expression in gastric tissue makes TCTE3 a more attractive marker in this context. In cases of liver metastases with unknown primary, positive IHC for TCTE3 would aid in excluding a gastric primary.

The lack of TCTE3 expression in pancreatobiliary tumors with intestinal, foveolar and pyloric metaplasia is a potential pitfall in its use as a diagnostic marker. This may limit its utility in limited biopsy specimens. We recommend that other lineage markers be used in conjunction with TCTE3 if lung, breast or Müllerian origin is suspected since TCTE3 expression may be found in tumors from these sites. In the absence of evidence indicating origin from one of these other sites, TCTE3 expression would support pancreaticobiliary differentiation.

4.2. TCTE3 expression in ampullary carcinomas

Ampullary cancers of pancreatobiliary type carry a significantly worse prognosis than the intestinal variant [6–7] and have differing genomic landscapes. The pancreatobiliary type shares mutations commonly found in pancreatic carcinomas while intestinal type shares mutations with colorectal carcinoma. This suggests that there are biological differences present that may have therapeutic and prognostic implications [8]. CDX2, CK20, MUC1 and MUC2 have been employed to help subtype ampullary tumors with some success [9]; [10]. In the current study, TCTE3 is positive in 11 out of 12 pancreatobiliary type tumors and negative in 2 out of 3 intestinal type tumors. While these numbers are limited, our findings lend credence to the use of TCTE3 for this application.

4.3. TCTE3 expression in gastroesophageal junction carcinomas

Submucosal glands at the GEJ express TCTE3 (Supplemental figure 1) while expression is absent in the gastric mucosa (Figure 2). Submucosal glands at the GEJ have been demonstrated to be a source of BE associated dysplasia [11]. However, gastric cardiac mucosa adjacent to the GEJ has been proposed as a site of origin for GEJ malignancies [12].

TCTE3 expression in a subset GEJ adenocarcinomas suggest that these may arise from dysplastic BE. In contrast, those lacking TCTE3 may arise from the gastric cardiac mucosa. Together, these findings support the theory suggesting a heterogenous cellular origin for adenocarcinomas arising from the GEJ.

4.4 Conclusion

In summary, we identified a specific immunohistochemical marker for pancreatobiliary differentiation in the GI tract. TCTE3 is expressed in malignant tumors of the pancreatobiliary epithelium, but is absent in hepatocytes, pancreatic acinar cells, and normal and neoplastic tissue with gastric and intestinal differentiation. While its expression is not exclusive to tumors of the GI tract, TCTE3 supports pancreaticobiliary differentiation when utilized with other markers to rule out non-gastrointestinal primary sites. Overall, our findings suggest that this novel marker may aid in the diagnostic workup for cholangiocarcinoma and pancreatic ductal tumors in their respective primary organs or metastatic sites.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The study was supported by Rhode Island Foundation (No. 20144126) and NIH/NIGMS (1P30GM110759)

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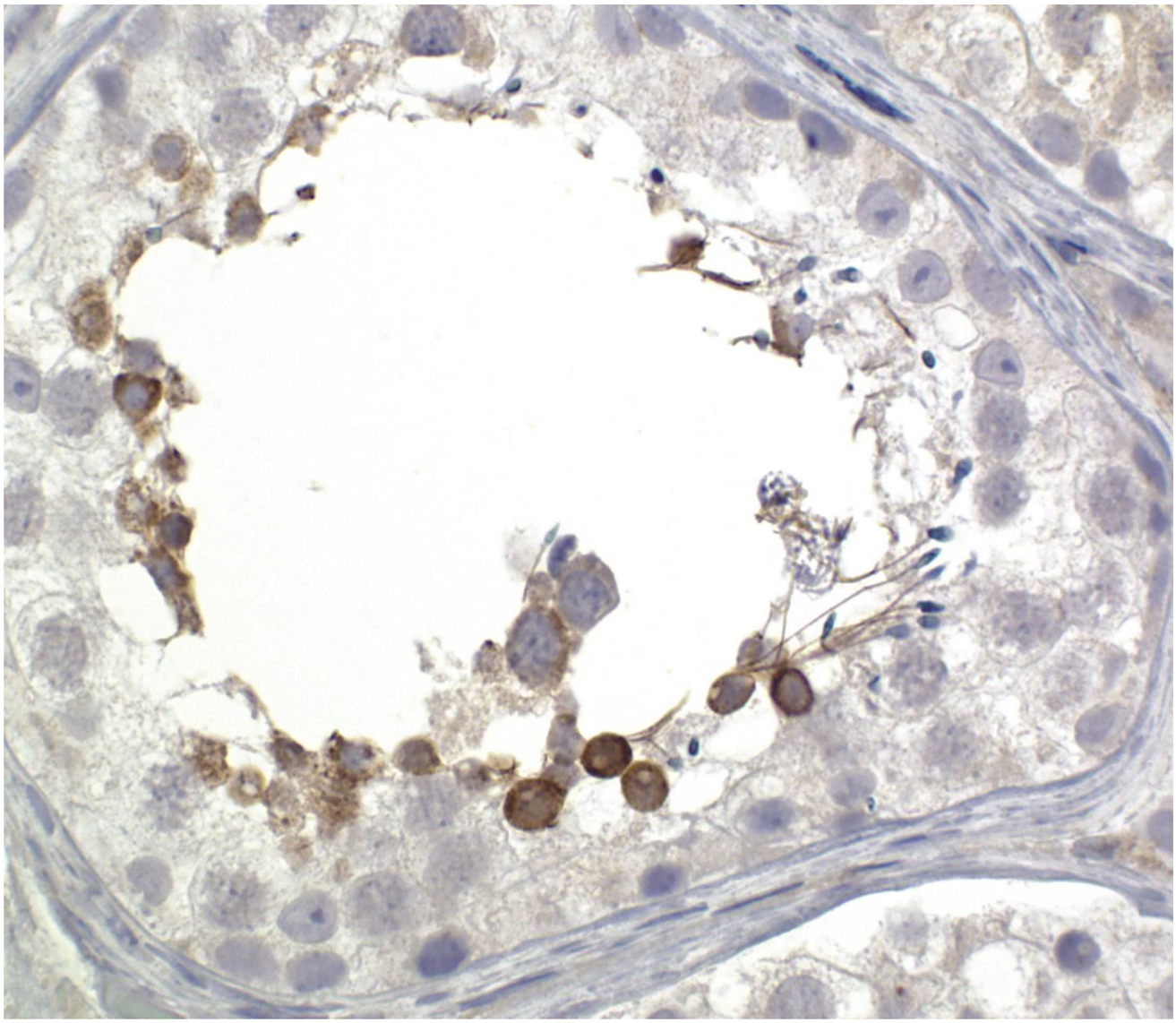

Figure 1.
TCTE3 expression in testis (600×). Strong stain can be seen in the tails of spermatozoa and in the cytoplasm of spermatids where cytoskeletal components of axoneme are made.

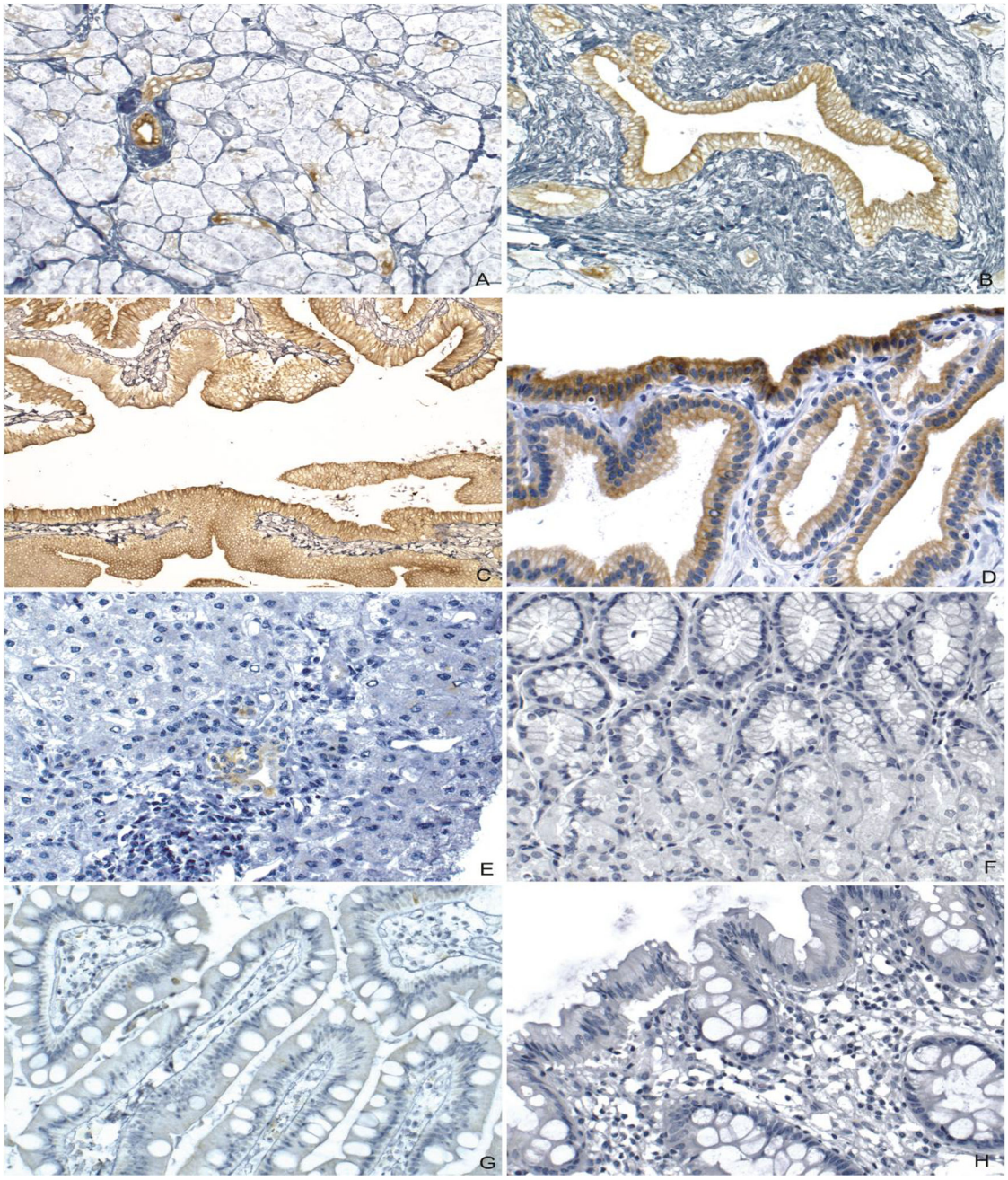

Figure 2.

TCTE3 expression in GI tract (400×). A. Pancreatic ductules. B. Pancreatic interlobular duct. C. Common pancreatic duct. D. Gall bladder. E. Liver portal triad with ductular reaction. F. Stomach. G. Small intestine. H. Colon.

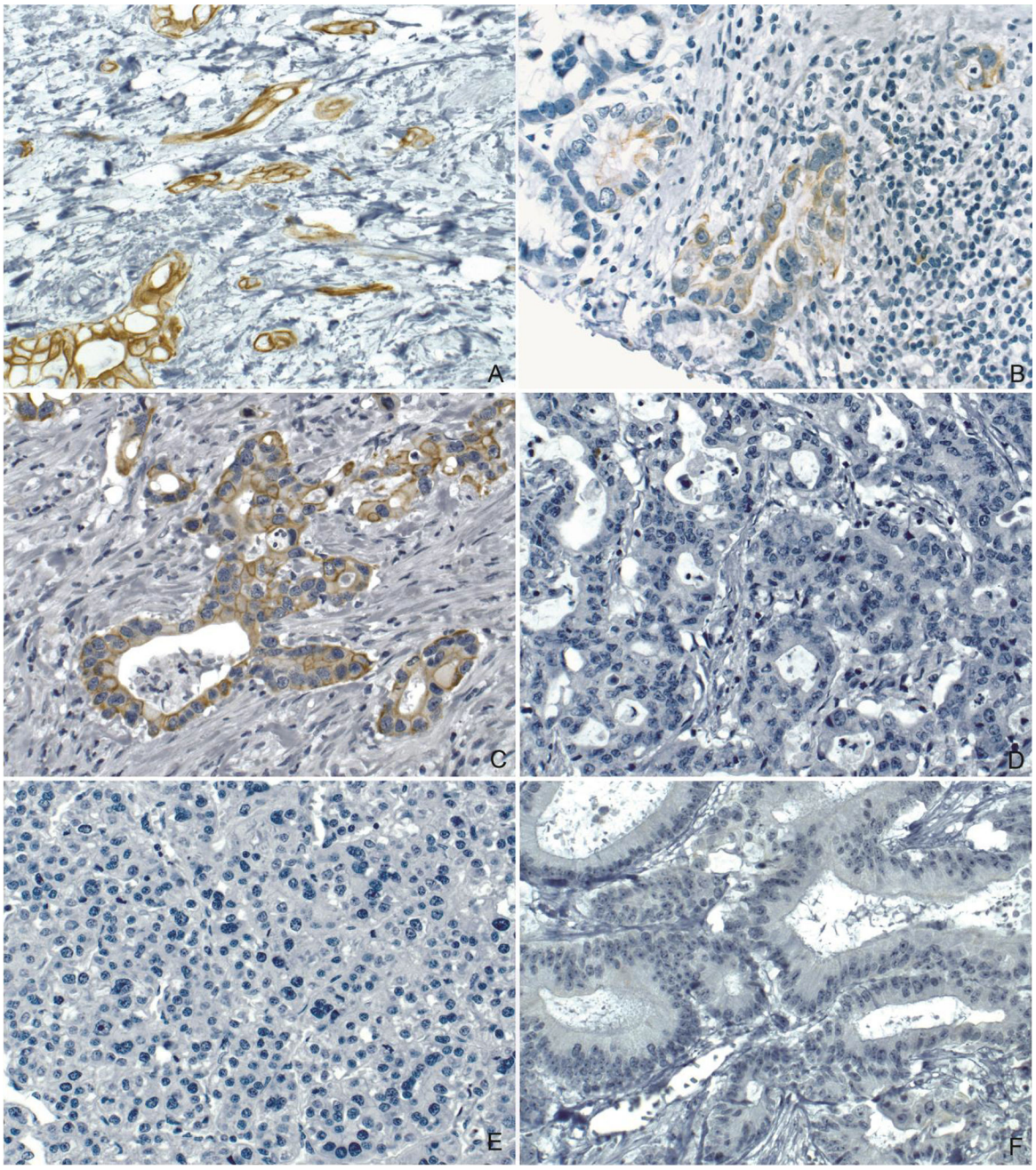

Figure 3.

TCTE3 expression in GI tract adenocarcinoma (400×). A. Pancreatic ductal carcinoma. B. Gall bladder carcinoma. C. Cholangiocarcinoma. D. Gastric adenocarcinoma. E. Hepatocellular carcinoma. F. Small bowel adenocarcinoma.

Table 1

Immunohistochemical expression of TCTE3.

	TCTE3 positive	TCTE3 negative
Pancreatobiliary cancer		
Pancreatic ductal	58 (93.5%)	4 (6.5%)
Cholangiocarcinoma	8 (50%)	8 (50%)
CBD & GB	6 (37.5%)	10 (62.5%)
Ampullary	13 (76.4%)	4 (23.5%)
Other GI adenocarcinoma		
GEJ	6 (33.3%)	12 (66.7%)
Stomach	1 (3.5%)	28 (96.5%)
HCC	0	20 (100%)
Small bowel	1 (7.7%)	12 (92.3%)
Colon	2 (6.25%)	30 (93.75%)
Rectum	0	24 (100%)
Non-GI adenocarcinoma		
Breast	6 (37.5%)	10 (62.5%)
Gynecology	17 (94.4%)	1 (5.6%)
Prostate	0	55 (100%)
Bladder	0	15 (100%)
Kidney	3 (6.9%)	40 (93%)
Lung	14 (87.5%)	2 (12.5%)

CBD – common bile duct; GB – gallbladder; GEJ – gastroesophageal junction; HCC – hepatocellular carcinoma

Table 2

Antibodies and methods used for immunohistochemistry:

Antibody	Source	Host and clone	Antigen Retrieval	Dilution	Detection Method
TCTE3	Sigma-Aldrich St. Louis, MO	Rabbit Polyclonal	10 mM Citrate; pH 6; 100°C, 40 min	1:300	Ventana Discovery DabMap Kit
CDX2	BioGenex Fremont, CA	Mouse Monoclonal, Clone CDX2-88	10 mM Citrate pH 6 100°C, 40 min	1:50	Ventana Discovery DabMap Kit
CK7	Cell Marque Rocklin, CA	Mouse Monoclonal, Clone OV-TL	EDTA pH 9 100°C, 40 min	1:300	Ventana Discovery DabMap Kit
CK20	Cell Marque Rocklin, CA	Mouse Monoclonal, Clone Ks20.8	EDTA pH 9 100°C, 40 min	1:200	Ventana Discovery DabMap Kit
MUC1	Abcam Cambridge, MA	Mouse Monoclonal, Clone HMFG1	EDTA pH 9 100°C, 40 min	1:200	Ventana Discovery DabMap Kit
MUC2	Thermo-Fisher Waltham, MA	Mouse Monoclonal, Clone M53	EDTA pH 9 100°C, 40 min	1:100	Ventana Discovery DabMap Kit
MUC4	Abcam Cambridge, MA	Rabbit Monoclonal, Clone EPR9308	EDTA pH 9 100°C, 40 min	1:200	Ventana Discovery DabMap Kit
MUC5AC	Abcam Cambridge, MA	Mouse Monoclonal, Clone 1-13M1	EDTA pH 9 100°C, 40 min	1:500	Ventana Discovery DabMap Kit
MUC6	Dako Carpinteria, CA	Mouse Monoclonal, Clone M11	EDTA pH9 100°C, 40 min	1:100	Ventana Discovery DabMap Kit

Table 3

TCTE3 expression in pancreatic or pancreatobiliary versus other GI tumors.

	Pancreatic tumor	**Other GI tumor**	
TCTE3 positive	58	37	PPV=61.1%
TCTE3 negative	4	148	NPV=97.4%
	Sensitivity=93.5%	Specificity=80.0%	
	Pancreatobiliary tumor	**Other GI tumor**	
TCTE3 positive	85	10	PPV=89.5%
TCTE3 negative	26	126	NPV=82.9%
	Sensitivity=76.6%	Specificity=92.6%	

PPV: positive predictive value; NPV=negative predictive value.

Table 4

Association between TCTE3 expression, histologic type and histologic grade in pancreatobiliary tumors.

	TCTE3 Positive	TCTE3 Negative	P-value
Ampullary adenocarcinoma			
Histologic type *			P=0.0379
Pancreatobiliary	11	1	
Intestinal	1	2	
Histologic differentiation			P=0.8929
Well-moderately	6	2	
Poorly	7	2	
GB & CBD adenocarcinoma			
Histologic type			P=0.0055
Biliary	6	4	
Others	0	6	
Histologic differentiation			P=0.3154
Well-moderate	5	6	
Poor	1	4	
Cholangiocarcinoma			
Histologic differentiation			P=0.1255
Well-moderate	6	3	
Poor	2	5	
Location			P=0.0803
Hilar	2	0	
Peripheral	6	8	
GEJ adenocarcinoma			
Histologic differentiation			P=0.1778
Well-moderate	4	4	
Poor	2	8	

CBD – common bile duct; GB – gallbladder; GEJ – gastroesophageal junction

* Two ampullary tumors are excluded in the analysis. One is papillary type and the other is poorly differentiated. Both are difficult to be classified as either pancreatobiliary or intestinal type.

Table 5

Comparison of TCTE3 against other frequently-used markers in GI adenocarcinomas.

Tumor (n)	TCTE3	CDX2	CK7	CK20	Muc1	Muc2	Muc4	Muc5AC	Muc6
Pancreatic (64)	93.4%	1.6%	96.7%	22.4%	98.4%*	12.3%	73.7%**	54.2%	8.2%
Ampullary (17)	76.5%	17.7%	76.5%**	28.6%	100.0%	37.5%	58.8%	41.2%	12.5%
Cholangiocarcinoma (18)	55.6%	0.0%	100.0%	18.8%	100.0%	37.5%	6.7%	43.8%**	0%
CBD& GB (13)	37.5%	0.0%	75%*	43.8%†	100.0%	33.3%	53.3%	53.3%	40.0%
Gastric (20)	3.5%	20.7%	69.0%	42.9%	96.6%	44.8%	34.5%	58.6%	31.0%
GEJ (18)	33.3%	5.6%	82.4%	23.5%	88.9%	33.3%	41.2%	50.0%*	35.3%
Total (150)	58.0%	7.0%†	85.4%**	28.7%	97.40%	29.0%	52.3%**	51.6%	18.1%†

*Positively correlated with TCTE3, P<0.05.

**Positively correlated with TCTE3, P<0.01.

†Negatively correlated with TCTE3, P<0.05.

CBD – common bile duct; GB – gallbladder; GEJ – gastroesophageal junction