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Thrombomodulin expression impacts the recurrence and long-term survival in pancreatic cancer

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Abstract

Background: Pancreatic cancer is one of the most aggressive digestive cancers. The tumor expression of thrombomodulin (TM) is correlated with favorable prognosis in several types of cancer. However, this correlation has not been confirmed in hepato-pancreato-biliary cancer. The aim of this study was to evaluate the prognostic value of TM expression in resected pancreatic ductal adenocarcinoma.

Methods: The data of patients who underwent pancreatic resection for pancreatic invasive ductal adenocarcinoma were obtained from a prospectively maintained database. A total of 131 patients were included. Paraffin sections of tumor tissues were stained immunohistochemically using TM antibody. The patients were divided into two groups: the TM-positive or TM-negative group.

Results: The specimens were TM-positive in 72 cases. TM expression was a significant factor of favorable prognosis in univariate analysis for disease-free (DFS) and overall survival (OS). The median OS in the TM-positive patients was 32.9 mo, which was better than the 20.0 mo in TM-negative patients (P =.006). TM positivity retained its significance on multivariate analysis for DFS (hazard ratio [HR] 0.651, 95% confidence interval [CI] 0.433–0.979, P =.039) and OS (HR 0.569, 95% CI 0.376–0.862, P =.008).

Conclusions: The tumor expression of TM is a favorable factor for OS in resected pancreatic invasive ductal adenocarcinoma.

KEYWORDS

epithelial-mesenchymal transition, immunohistochemical staining, metastasis, pancreatic cancer, thrombomodulin

Hiroshi Sugano and Yoshihiro Shirai contributed equally to the work.

1 | INTRODUCTION

Pancreatic cancer is among the most aggressive types of cancer and the fourth leading cause of cancer-related deaths in developed countries.[1] Despite the recent developments in cancer therapy, the median survival time (MST) of patients with pancreatic cancer after pancreatic resection and adjuvant chemotherapy is only 22.8–54.4 mo.[2-4] Because distant metastasis is the main cause of poor prognoses, predicting distant metastasis is important for treatment strategies.

Thrombomodulin (TM) is a membrane protein that binds to thrombin (TM-thrombin complex) and controls anticoagulation and the fibrinolytic system.[5,6] TM consists of 496 amino acids in its extracellular component, 23 amino acids in its cell membrane, and 38 amino acids in its intracellular component, which are separated into five domains (D1–D5).[7] The lectin-like domain (D1) located in the N-terminal domain has antiinflammatory roles by adsorbing high mobility group box 1 (HMGB1) or lipo-polysaccharide (LPS).[8] D2 consists of six epithelial growth factor (EGF)-like components, and thrombin binds to these components and loses its coagulant activity.[9] The TM-thrombin complex suppresses coagulation by activating protein C and inhibiting coagulation factors V and VIII. In addition, the TM-thrombin complex has an antifibrinolytic effect through the activation of thrombin-activatable fibrinolysis inhibitor (TAFI).[10] TM helps to maintain a balance between coagulation and fibrinolysis.

The role of TM in malignant tumors has not been fully elucidated. The correlation between TM expression and malignant tumors was first reported in 1993. TM expression was different in various lung cancer histological types, and the relationship between TM expression and cancer malignancy was suggested.[11] In hepatocellular carcinoma, decreased TM expression increased the lymph node metastasis rate and cancer recurrence after tumor resection.[12] In lung squamous cell carcinoma (SCC), TM expression was reduced in the metastatic tumor compared to the primary tumor,[13] and a lower TM expression in the primary tumor contributed to poor patient survival.[14] Since then, similar pathological studies have been performed in several types of cancers such as breast cancer,[15] oral SCC,[16] and esophageal SCC.[17] There are few reports involving gastrointestinal adenocarcinomas. In colorectal tissue, TM expression gradually decreased in the order of normal mucosa, adenoma, and adenocarcinoma.[18] This trend was the same in colorectal cancer and correlated with the postoperative prognosis.[19] However, there is no analysis of TM in pancreatic cancer. Therefore, we examined TM expression in resected tissue from patients with pancreatic ductal adenocarcinoma (PDAC) and analyzed the correlation between cancer prognosis and other clinicopathological factors.

2 | METHODS

We enrolled 168 patients with pancreatic ductal adenocarcinoma who underwent pancreatic resection at the Department of Surgery of the Jikei University Hospital, Tokyo, Japan between 2000 and 2014. Only patients who had been followed up for at least 5 y after pancreatic resection were included. Twenty-one patients were lost to follow up. Patients with specimens not suitable for immunohistochemical staining (n = 7), intrahospital death (n = 2), and other causes of death (n = 7) were excluded. A total of 131 patients were included and their specimens obtained for further evaluation.

This research was approved by the Ethics Committee of the Jikei University School of Medicine [27–177 (8062)] and it conformed to the provisions of the Declaration of Helsinki. We analyzed the relationship between clinicopathological variables including TM expression, disease-free (DFS), and overall (OS) survival after pancreatic resection by univariate and multivariate analysis of nine factors: American Society of Anesthesiologists physical status (ASA-PS), CA 19-9 levels, tumor size, resectability, nodal involvement, tumor differentiation, lymphatic invasion, venous invasion, resection margin status, adjuvant chemotherapy, and TM. In addition, the relationship between TM expression and clinicopathological variables were analyzed by univariate analysis.

2.1 | Immunohistochemical staining

Formalin-fixed and paraffin-embedded tissues were sectioned at a thickness of 4 μm. The paraffin sections were stained with anti-TM mouse monoclonal antibody (1:25 dilution; Agilent Technologies, Santa Clara, CA) for 30 min at 37°C and visualized using BenchMark-XT (Ventana Medical System). Given that TM expression is normal in adjacent normal vascular endothelial cells, they were used as internal positive controls. The specificity of the immunostaining for TM was confirmed by a negative control using isotype-matched control Mouse IgG in place of the primary antibody. The immunohistochemistry was quantitatively evaluated by two pathologists (S.S. and H.S.) independently, blinded to patient characteristics and outcome. The TM-positive rate was evaluated by the eyeball method under microscopic examination among all tumor cells in one entire slide, which was the largest section in tumor size in each case. When there was a discrepancy between the pathologists, a consensus opinion was achieved. The interpathologist concordance rate was analyzed using the Kappa–Cohen method, and the kappa value was 0.94. Cases were categorized as TM-positive (TM+) if 1% or more of cancer cells showed positive staining, and otherwise TM-negative (TM-) based on a previous report.[20] In addition, the percentage of cells that stained positive for TM were assessed in <1%, <5%, and 10% increments. Simultaneously, positivity for TM was classified into three categories: –, <1%; 1+, 1%–50%, and 2+, 51%–100% positive cells.

2.2 | Clinicopathological data

Clinicopathological data were obtained from the hospital's prospectively maintained database. Performance status was evaluated using the ASA-PS system. Pathological diagnosis was based

on the 8th edition of the Union for International Cancer Control (UICC) guidelines and was done at the hospital's pathology department. Serum CA 19-9 was evaluated immediately before surgery. The regimen of adjuvant chemotherapy was gemcitabine or S1 for 6 mo. Recurrence of pancreatic cancer was defined as newly detected local or distant metastatic tumors using computed tomography (CT) or magnetic resonance imaging (MRI) reviewed by radiologists.

2.3 | Statistical analysis

IBM SPSS Statistics software version 23.0 was used for statistical analysis. Continuous variables were expressed as median and interquartile range. Categorical variables were expressed as absolute numbers. Univariate analyses for categorical data were analyzed using the chi-squared test. The CA 19-9 cutoff value for predicting 5-y postoperative survival was determined by receiver operating characteristic (ROC) analysis. The DFS and OS rates were calculated using the Kaplan–Meier method. Comparison of DFS and OS were evaluated using the log-rank test for univariate analysis and Cox proportional regression model for multivariate analysis. All *P* values were considered statistically significant when the associated probability was less than .05.

TABLE 1 Patient characteristics (n = 131)

Factor	Median or Rate	Range (IQR)
Age (y)	68	62–74
Gender (male : female)	77:54	—
ASA-PS (1:2)	51:80	—
CA19-9 (U/mL)	114.5	36.0–350.5
Operation (PD : DP: TP)	88:40 : 3	—
Disease free survival (mo)	11.8[a]	9.0–14.5[b]
Overall survival (mo)	25.5 [a]	19.2–31.8[b]
Thrombomodulin (positive : negative)	72:59	0.4–14.8

ASA-PS, American Society of Anesthesiologists-physical status; DP, distal pancreatectomy; IQR, interquartile range; PD, pancreaticoduodenectomy; TP, total pancreatectomy.
[a]Median survival time.
[b]95% CI.

3 | RESULTS

3.1 | Patient characteristics

The patient characteristics are shown in Table 1. The median age of the patients was 68 (62–74) y, and 77 of them were male. A total of 67% of the patients underwent pancreaticoduodenectomy. Figure 1 shows TM-negative and -positive tumors. TM expressed the surface of the tumor cell membrane. The TM-positive patients were 55%. TM-positive patients were categorized into TM1+ or TM2+ as 61 cases and 11 cases, respectively, whose TM-positive rates were distributed as shown in Figure S1. Median DFS and OS after pancreatic resection were 11.8 and 25.5 mo, respectively, while the 5-y DFS and OS rates were 13.6% and 22.9%, respectively.

3.2 | Relationship between clinicopathological variables and TM

The relationship between clinical variables and TM status is shown in Table 2. The TM-negative group had higher nodal involvement (*P* =.032). Tumor differentiation had no association with TM expression.

3.3 | Comparison of clinicopathological variables in relation to DFS

The relationship between clinicopathological variables including TM expression and DFS after pancreatic resection for PDAC is shown in Table 3. In univariate analysis, high CA 19-9 levels (*P* <.001), larger tumor size (*P* =.014), nodal involvement (*P* <.001), moderately and poor tumor differentiation (*P* <.001), lymphatic invasion (*P* =.010), venous invasion (*P* =.042), and TM negativity

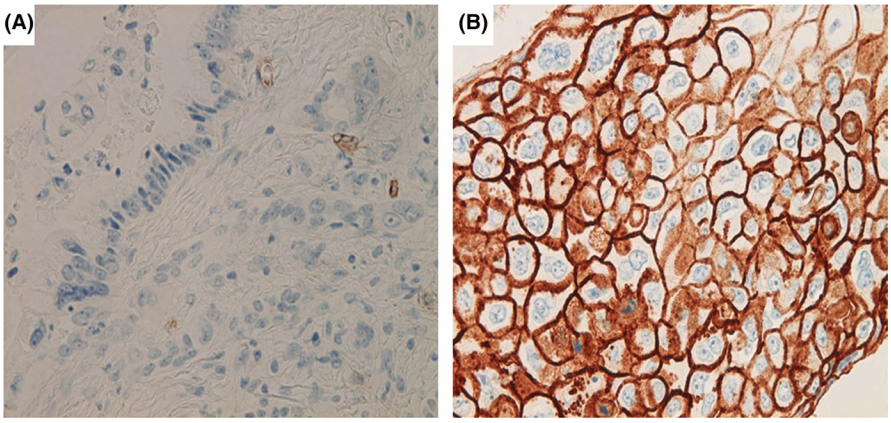

FIGURE 1 Immunohistochemical staining of TM in pancreatic ductal adenocarcinoma. A, The specimen is negative for TM. B, The specimen is positive for TM. The surface of the cell membrane was stained

TABLE 2 Univariate analysis of clinicopathologic variables in relation to thrombomodulin (n = 131)

Factor	Thrombomodulin		*P*-value
	Positive (n = 72)	Negative (n = 59)	(univariate)
ASA-PS (1:2)	24:48	27:32	0.147
CA19-9, U/mL (<322 : ≥322)[a]	54:17	39:16	0.515
Tumor size, mm (<40 : ≥40)	51:21	36:23	0.237
Resectability (R : BR)	63:9	54:5	0.458
Nodal involvement (Positive : Negative)	38:34	42:17	0.032
Tumor differentiation (Well : Moderate/ Poor)	25:47	22:37	0.761
Lymphatic invasion (Positive : Negative)	51:21	41:18	0.867
Venous invasion (Positive : Negative)	53:19	38:21	0.255
Resection margin status (R0 : R1)	53:19	41:18	0.602
Adjuvant chemotherapy (Yes : No)	61:11	48:11	0.608

ASA-PS, American Society of Anesthesiologists-physical status; BR, borderline resectable; R, resectable.

[a]Data for 126 patients.

(*P* =.007, Figure 2A) were significant prognostic factors for cancer recurrence. The median DFS of the TM-positive and TM-negative patients was 13.6 and 7.9 mo, respectively. In multivariate analysis, CA 19-9 (*P* =.006), nodal involvement (*P* =.011), tumor differentiation (*P* =.039), and TM (*P* =.039) were independent risk factors for cancer recurrence. The TM-positive patients had 40% lower recurrence rates compared with the TM-negative patients. TM-positive rates were also correlated with DFS (TM- : TM 1+ : TM 2+ = 7.3 [95% confidence interval [CI] 4.7–10.1] : 12.7 [95% CI 8.6–16.8] : 35.8 [95% CI 33.3–38.3] mo, *P* =.012 [Figure 3A]). In the patients with nodal involvement (n = 80), the median DFS in the TM-positive patients were longer than in the TM-negative patients (TM-positive vs TM-negative = 11.8 vs 6.7 mo, *P* =.029 [Figure S2A]).

3.4 | Comparison of clinicopathological variables in relation to OS

The relationship between clinicopathological variables including TM expression and DFS after pancreatic resection for PDAC is shown in Table 4. In univariate analysis, high CA 19-9 level (*P* <.001), larger tumor size (*P* =.030), nodal involvement (*P* <.001), moderately and poor tumor differentiation (*P* <.001), lymphatic invasion (*P* =.0010), no adjuvant chemotherapy (*P* =.043), and TM negativity (*P* =.006, Figure 2B) were significant prognostic factors for poor patient survival. The median OS of the TM-positive and TM-negative patients were 32.9 and 20.0 mo, respectively. In multivariate analysis, CA 19-9 (*P* =.002), nodal involvement (*P* =.026), tumor differentiation (*P* =.005), lymphatic invasion (*P* =.019), adjuvant chemotherapy (*P* =.008), and TM (*P* =.008) were independent risk factors for poor patient survival. TM-positive rates were also correlated with OS (TM- : TM 1+ : TM 2+ = 20.0 [95% CI 15.1–24.8] mo : 31.3 [95% CI 22.0–40.6] mo: not reached, *P* =.011 [Figure 3B]). In the patients with nodal involvement, the median OS in the TM-positive patients were longer than in the TM-negative (TM-positive vs TM-negative = 20.5 vs 16.6 mo, *P* =.042 [Figure S2B]).

4 | DISCUSSION

Several mechanisms may be involved in the correlation between malignant features and TM. The main cause of favorable prognosis by TM expression is suppression of metastasis. Cancer cell metastasis is established through multiple steps such as dissociation of cell-to-cell attachment in the primary site, infiltration by degradation of the extracellular matrix, vascular invasion, immune evasion, adhesion to endothelial cells of the target organ, extravasation, and proliferation.[21] TM is involved in each of these processes. TM is localized in tumor epithelial cells, and is strongly expressed in the intercellular bridge.[13] TM has a function of directly adhering cells to each other using a lectin-like domain (D1).[22] In poorly differentiated cancers, TM expression is not only reduced, but the localization of TM changes from the cell surface to the cytoplasm, which causes detachment of cancer cells from the primary site due to decrease in the adhesion ability. A recent study demonstrated that TM knockdown downregulates the expression of E-cadherin, which is an adhesion factor that induces epithelial-mesenchymal transition (EMT),[23] and conversely upregulates EMT-related proteins such as vimentin, ZEB1, and Snail.[24] In addition, administration of TM activates TAFI and suppresses plasmin, which is a protease, and subsequently inhibits cell invasion and migration by blocking the degradation of type IV collagen.[25] These studies suggested that TM plays an important role in inhibiting escape from the primary site. Furthermore, also in blood vessels, TM binds to thrombin and inactivates platelets, and acquires a mechanism for immune evasion by platelet aggregation.[26] In this study, TM expression reduced nodal involvement and cancer recurrence. Its original functions such as antifibrinolysis and adhesion prevent cancer metastasis. In addition, TM positivity tended to prevent a distant metastasis, but this association did not reach a significant difference (HR 0.680, 95% CI 0.401–1.152, *P* =.142, data not shown in Table). A further number of cases is required to clarify this relationship.

Several studies reported that TM has an antiproliferative effect. In melanoma cells, the expression levels of TM are less in highly aggressive phenotypes.[27] This growth modulatory effect of TM depends on the inactivation of thrombin-induced cell proliferation. In addition, the correlation between external TM and cancer cells was studied. Administration of purified soluble TM inhibited

TABLE 3 Univariate and multivariate analysis of clinicopathologic variables in relation to disease-free survival (n = 131)

		Univariate analysis		Multivariate analysis	
Factor	N	Hazard ratio (95% CI)	*P*-value	Hazard ratio (95% CI)	*P*-value
ASA-PS					
2	80	0.9052	.606		
1	51	(0.616–1.326)			
CA19-9 (U/mL) [a]					
≥322	33	2.299	<.001	1.897	.006
<322	93	(1.824–5.176)		(1.206–2.984)	
Tumor size					
≥40 mm	44	1.715	.014	1.113	.624
<40 mm	87	(1.117–2.631)		(0.727–1.703)	
Resectability					
Borderline resectable	14	1.564	.125		
Resectable	117	(0.862–3.447)			
Nodal involvement					
Positive	80	1.985	<.001	1.784	.011
Negative	51	(1.379–2.894)		(1.144–2.781)	
Tumor differentiation					
Moderate/ Poor	84	1.971	<.001	1.635	.039
Well	47	(1.356–2.850)		(1.024–2.609)	
Lymphatic invasion					
Positive	92	1.716	.010	1.400	.149
Negative	39	(1.133–2.425)		(0.886–2.211)	
Venous invasion					
Positive	91	1.522	.042	1.083	.755
Negative	40	(1.016–2.182)		(0.657–1.785)	
Resection margin status					
R1	37	1.413	.117		
R0	94	(0.918–2.176)			
Adjuvant chemotherapy					
No	22	1.283	.309		
Yes	109	(0.777–2.226)			
Thrombomodulin					
Positive	72	0.585	.007	0.651	.039
Negative	59	(0.396–0.863)		(0.433–0.979)	

ASA-PS, American Society of Anesthesiologists-physical status; CI, confidence interval.
[a]Data for 126 patients.

cell proliferation and invasion in murine melanoma.[28] Furthermore, recombinant TM suppressed tumor growth by inhibiting thrombin-induced activation of protease activate receptor-1 (PAR1) and nuclear factor kappa B (NF-κB) activation in pancreatic cancer cells.[29] Kuo et al reported that the lectin-like domain recognized and adhered the Lewis-Y antigen of the EGF receptor (EGFR) on the surface of the cancer cell and subsequently inhibited the binding of EGF to EGFR and suppressed cell proliferation.[30] On the contrary, our study showed no correlation between tumor size and differentiation and TM expression. Thus, further studies are needed. Although there are presently many unclear points, these studies suggest the antiproliferative effects of TM.

We found that TM expression was strongly correlated with cancer prognosis. Survival analysis was performed in accordance with the intensity of TM-staining, but there was no significant relation with survival rates. On the contrary, TM-positive rates

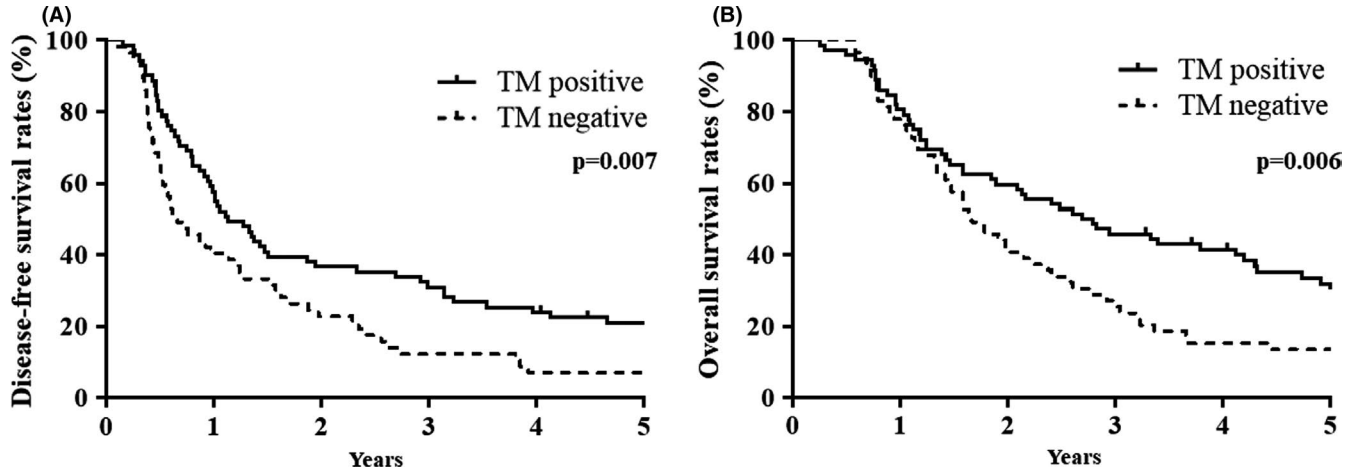

FIGURE 2 Relationship between disease-free survival and overall survival after pancreatic resection and TM expression. A, Disease-free survival (DFS). TM negativity is an independent risk factor of cancer recurrence (5-y DFS: TM-positive vs TM-negative = 23.8% vs 7.0%, *P* =.007). B, Overall survival (OS). TM-negative is an independent risk factor of cancer-related death (5-y OS: TM-positive vs TM-negative = 30.4% vs 13.6%, *P* =.006)

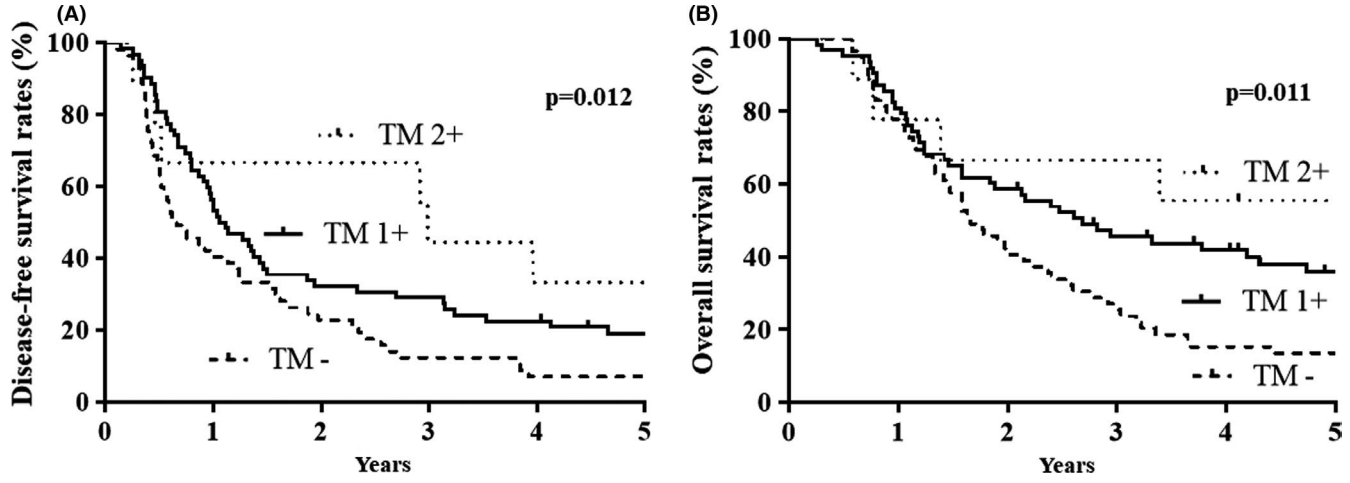

FIGURE 3 Relationship between disease-free survival and overall survival after pancreatic resection and TM expression rates. A, Disease-free survival (DFS). TM-positive rates correlated with cancer recurrence (5-y DFS: TM 2 + vs TM1 + vs TM- = 33.3% vs 18.9% vs 7.0%, *P* =.012). B, Overall survival (OS). TM-positive rates correlated with cancer survival (5-y OS: TM 2 + vs TM 1 + vs TM- = 55.6% vs 28.7% vs 13.6%, *P* =.011)

were more correlated with patients' survival than the intensity of staining. A similar tendency was reported for oral squamous cell carcinoma,[16] indicating that TM-positive rates are important for evaluation of cancer prognosis. Previous reports categorized a small number of TM-positive cell less than 5%[16] or 10%[31] into the TM-negative group. To evaluate the significance of a small number of TM-positive cells, we analyzed survival analysis between negative (<1%) and less than 5% of TM-positive. As a result, less than 5% of TM-positive showed better prognosis than that of negative (TM < 5% vs TM- = 51.7 [95% CI 42.0–61.5] mo vs 20.0 [95% CI 15.1–24.8] mo, *P* =.002). Therefore, we assigned less than 5% of TM-positive to the TM + group.

Our data also showed the correlation between TM expression and lymph node metastasis, suggesting that TM expression inhibited not only cancer recurrence, but also translymphatic cancer metastasis. Previous reports showed that TM-positive intensity of the main tumor and metastatic lymph nodes were approximately the same status[13,17]. Additional research is needed for pancreatic cancer. The TM-negative patients with nodal involvement relapsed in about half a year (Figure S2). These patients may need more powerful adjuvant chemotherapy. Predicting immediate postoperative prognosis using TM expression is useful in making a choice of adjuvant chemotherapy. These results suggest that TM expression may help for new therapeutic strategies in pancreatic cancer.

The limitation of this study was that it was a retrospective study and had a small sample size. TM status of metastatic lymph nodes was an important point to discuss the relationship between TM and translymphatic metastasis. However, the TM status of metastatic lymph nodes was not shown. Although we discussed antifibrinolysis and adhesion, pathological data of fibrosis were not shown.

TABLE 4 Univariate and multivariate analysis of clinicopathologic variables in relation to overall survival (n = 131)

		Univariate analysis		Multivariate analysis	
Factor	N	Hazard ratio (95% CI)	*P*-value	Hazard ratio (95% CI)	*P*-value
ASA-PS					
2	80	0.9722	.887		
1	51	(0.658–1.436)			
CA19-9 (U/mL) [a]					
≥322	33	2.434	<.001	2.115	.002
<322	93	(1.973–5.814)		(1.325–3.377)	
Tumor size					
≥40 mm	44	1.540	.030	0.959	.850
<40 mm	87	(1.049–2.464)		(0.621–1.480)	
Resectability					
Borderline resectable	14	1.228	.500		
Resectable	117	(0.653–2.403)			
Nodal involvement					
Positive	80	1.895	<.001	1.664	.026
Negative	51	(1.290–2.762)		(1.063–2.603)	
Tumor differentiation					
Moderate/ Poor	84	2.168	<.001	1.910	.005
Well	47	(1.447–3.102)		(1.211–3.011)	
Lymphatic invasion					
Positive	92	2.201	.001	1.793	.019
Negative	39	(1.333–3.508)		(1.100–2.923)	
Venous invasion					
Positive	91	1.306	.213		
Negative	40	(0.865–1.931)			
Resection margin status					
R1	37	1.409	.099		
R0	94	(0.933–2.267)			
Adjuvant chemotherapy					
No	22	1.642	.043	2.058	.008
Yes	109	(1.022–3.244)		(1.210–3.498)	
Thrombomodulin					
Positive	72	0.595	.006	0.569	.008
Negative	59	(0.387–0.853)		(0.376–0.862)	

ASA-PS, American Society of Anesthesiologists-physical status; CI, confidence interval.
[a]Data for 126 patients.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest for this article.

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REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin. 2019;69:7–34.

2. Oettle H, Neuhaus P, Hochhaus A, Hartmann JT, Gellert K, Ridwelski K, et al. Adjuvant chemotherapy with gemcitabine and long-term outcomes among patients with resected pancreatic cancer: the CONKO-001 randomized trial. JAMA. 2013;310:1473–81.
3. Uesaka K, Boku N, Fukutomi A, Okamura Y, Konishi M, Matsumoto I, et al. Adjuvant chemotherapy of S-1 versus gemcitabine for resected pancreatic cancer: a phase 3, open-label, randomised, non-inferiority trial (JASPAC 01). Lancet. 2016;388:248–57.
4. Conroy T, Hammel P, Hebbar M, Ben Abdelghani M, Wei C, Raoul L, et al. FOLFIRINOX or Gemcitabine as adjuvant therapy for pancreatic cancer. N Engl J Med. 2018;379:2395–406.
5. Esmon CT, Owen WG. Identification of an endothelial cell cofactor for thrombin-catalyzed activation of protein C. Proc Natl Acad Sci U S A. 1981;78:2249–52.
6. Esmon CT, Esmon NL, Harris KW. Complex formation between thrombin and thrombomodulin inhibits both thrombin-catalyzed fibrin formation and factor V activation. J Biol Chem. 1982;257:7944–7.
7. Suzuki K, Kusumoto H, Deyashiki Y, Nishioka J, Maruyama I, Zushi M, et al. Structure and expression of human thrombomodulin, a thrombin receptor on endothelium acting as a cofactor for protein C activation. EMBO J. 1987;6:1891–7.
8. Abeyama K, Stern DM, Ito Y, Kawahara K-I, Yoshimoto Y, Tanaka M, et al. The N-terminal domain of thrombomodulin sequesters high-mobility group-B1 protein, a novel antiinflammatory mechanism. J Clin Invest. 2005;115:1267–74.
9. Zushi M, Gomi K, Yamamoto S, Maruyama I, Hayashi T, Suzuki K. The last three consecutive epidermal growth factor-like structures of human thrombomodulin comprise the minimum functional domain for protein C-activating cofactor activity and anticoagulant activity. J Biol Chem. 1989;264:10351–3.
10. Bajzar L, Nesheim ME, Tracy PB. The profibrinolytic effect of activated protein C in clots formed from plasma is TAFI-dependent. Blood. 1996;88:2093–100.
11. Tamura A, Matsubara O, Hirokawa K, Aoki N. Detection of thrombomodulin in human lung cancer cells. Am J Pathol. 1993;142:79–85.
12. Suehiro T, Shimada M, Matsumata T, Taketomi A, Yamamoto K, Sugimachi K. Thrombomodulin inhibits intrahepatic spread in human hepatocellular carcinoma. Hepatology. 1995;21:1285–90.
13. Ogawa H, Yonezawa S, Maruyama I, Matsushita Y, Tezuka Y, Toyoyama H, et al. Expression of thrombomodulin in squamous cell carcinoma of the lung: its relationship to lymph node metastasis and prognosis of the patients. Cancer Lett. 2000;149:95–103.
14. Hamatake M, Ishida T, Mitsudomi T, Akazawa K, Sugimachi K. Prognostic value and clinicopathological correlation of thrombomodulin in squamous cell carcinoma of the human lung. Clin Cancer Res. 1996;2:763–6.
15. Kim SJ, Shiba E, Ishii H, Inoue T, Taguchi T, Tanji Y, et al. Thrombomodulin is a new biological and prognostic marker for breast cancer: an immunohistochemical study. Anticancer Res. 1997;17:2319–23.
16. Tabata M, Sugihara K, Yonezawa S, Yamashita S, Maruyama I. An immunohistochemical study of thrombomodulin in oral squamous cell carcinoma and its association with invasive and metastatic potential. J Oral Pathol Med. 1997;26:258–64.
17. Tezuka Y, Yonezawa S, Maruyama I, Matsushita Y, Shimizu T, Obama H, et al. Expression of thrombomodulin in esophageal squamous cell carcinoma and its relationship to lymph node metastasis. Cancer Res. 1995;55:4196–200.
18. Takebayashi Y, Yamada K, Maruyama I, Fujii R, Akiyama S, Aikou T. The expression of thymidine phosphorylase and thrombomodulin in human colorectal carcinomas. Cancer Lett. 1995;92(1):1–7.
19. Hanly AM, Redmond M, Winter DC, Brophy S, Deasy JM, Bouchier-Hayes DJ, et al. Thrombomodulin expression in colorectal carcinoma is protective and correlates with survival. Br J Cancer. 2006;94:1320–5.
20. Tamura A, Hebisawa A, Hayashi K, Sagara Y, Fukushima K, Kurashima A, et al. Prognostic significance of thrombomodulin expression and vascular invasion in stage I squamous cell carcinoma of the lung. Lung Cancer. 2001;34:375–82.
21. The American Society of Anesthesiologist performance status classification system. Accessed on June 13, 2020. https://www.asahq.org/standards-and-guidelines/asa-physical-status-classification-system
22. Massague J, Obenauf AC. Metastatic colonization by circulating tumour cells. Nature. 2016;529:298–306.
23. Zheng N, Huo Z, Zhang B, Meng M, Cao Z, Wang Z, et al. Thrombomodulin reduces tumorigenic and metastatic potential of lung cancer cells by up-regulation of E-cadherin and down-regulation of N-cadherin expression. Biochem Biophys Res Commun. 2016;476:252–9.
24. Kao YC, Wu LW, Shi CS, Chu C-H, Huang C-W, Kuo C-P, et al. Downregulation of thrombomodulin, a novel target of Snail, induces tumorigenesis through epithelial-mesenchymal transition. Mol Cell Biol. 2010;30:4767–85.
25. Bazzi ZA, Lanoue D, El-Youssef M, Romagnuolo R, Tubman J, Cavallo-Medved D, et al. Activated thrombin-activatable fibrinolysis inhibitor (TAFIa) attenuates breast cancer cell metastatic behaviors through inhibition of plasminogen activation and extracellular proteolysis. BMC Cancer. 2016;16:328.
26. Nierodzik ML, Plotkin A, Kajumo F, Karpatkin S. Thrombin stimulates tumor-platelet adhesion in vitro and metastasis in vivo. J Clin Invest. 1991;87:229–36.
27. Zhang Y, Weiler-Guettler H, Chen J, Wilhelm O, Deng Y, Qiu F, et al. Thrombomodulin modulates growth of tumor cells independent of its anticoagulant activity. J Clin Invest. 1998;101:1301–9.
28. Hanly AM, Winter DC. The role of thrombomodulin in malignancy. Semin Thromb Hemost. 2007;33:673–9.
29. Shirai Y, Uwagawa T, Shiba H, Shimada Y, Horiuchi T, Saito N, et al. Recombinant thrombomodulin suppresses tumor growth of pancreatic cancer by blocking thrombin-induced PAR1 and NF-kappaB activation. Surgery. 2017;161:1675–82.
30. Kuo CH, Chen PK, Chang BI, Sung M-C, Shi C-S, Lee J-S, et al. The recombinant lectin-like domain of thrombomodulin inhibits angiogenesis through interaction with Lewis Y antigen. Blood. 2012;119:1302–13.
31. Chang YJ, Cheng YW, Lin RK, Huang C-C, Chen W-L, Ke T-W, et al. Thrombomodulin influences the survival of patients with non-metastatic colorectal cancer through epithelial-to-mesenchymal transition (EMT). PLoS One. 2016;22:e0160550.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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