# TM4SF1 as a prognostic marker of pancreatic ductal adenocarcinoma is involved in migration and invasion of cancer cells

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**Abstract.** The cell surface protein Transmembrane 4 L6 family member 1 (TM4SF1) has been detected in various tumors, and its expression on tumor cells is implicated in cancer cell metastasis and patient prognosis. The role of TM4SF1 in malignant tumors remains poorly understood, particularly in pancreatic cancer. We performed immunohistochemical staining to analyze the expression of TM4SF1 in resected pancreatic tissues and investigated the correlation between TM4SF1 expression and prognosis. The function of TM4SF1 in the invasion and migration of pancreatic cancer cells was analyzed *in vitro* using an RNA interference technique. In pancreatic cancer tissues, TM4SF1 expression was detected in cancer cells, and patients with high tumor levels of TM4SF1 showed longer survival times than those with low TM4SF1 levels (P=0.0332). *In vitro*, reduced TM4SF1 expression enhanced the migration (P<0.05) and invasion (P<0.05) of pancreatic cancer cells partially via decreased E-cadherin expression. TM4SF1 protein levels were also reduced after TGF-β1-induced epithelial-mesenchymal transition (EMT). TM4SF1 expression is associated with better prognosis in pancreatic cancer. Loss of TM4SF1 contributes to the invasion and migration of pancreatic cancer cells.

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## Introduction

Pancreatic cancer is one of the most deadly malignancies. In contrast to the stable or declining trends for most other cancer types, incidence rates are increasing for cancers of the pancreas (1). Despite the use of multimodality treatment, the overall 5-year survival rate for pancreatic cancer is only six percent because of its high recurrence rate (1). Invasion and metastasis are the major causes of the poor prognosis of pancreatic cancer (2). The coordination of intercellular adhesion, cell-matrix interaction, matrix degradation, and cell migration is essential for cancer cell invasion and metastasis, and a diverse variety of transmembrane proteins regulate these cellular events (3-5). Notably, proteins of the tetraspanin superfamily, such as Tspan8 (6), CD82 (7), CD9 (8), and CD151 (9) are important for the regulation of tumor cell motility and the interaction between tumor cells and their microenvironment. Therefore, tetraspanins may represent promising diagnostic or prognostic markers and therapeutic targets for tumor progression (10,11).

TM4SF1 is a member of the tetraspanin superfamily (12). Tetraspanins are characterized by four transmembrane domains delimiting two extracytoplasmic regions of unequal size (13). Elevated expression of TM4SF1 has been reported in several tumor types, and is implicated in cancer cell migration and patient outcome, but it adopts different roles that are dependent upon tumor type (14-17). In addition, TM4SF1 is important for endothelial cell migration and tumor angiogenesis (18,19), although the underlying mechanism of its function is not clear, especially in pancreatic cancer.

In the present study, we investigated the role of TM4SF1 in pancreatic ductal adenocarcinoma (PDAC). TM4SF1 expression in PDAC tissues was evaluated by immunohistochemistry, and the correlation between clinicopathological characteristics and TM4SF1 expression was assessed to determine its prognostic significance in PDAC. To determine the biomolecular functions of TM4SF1 in pancreatic cancer cells, we investi-

gated changes in cell migration and invasion *in vitro* using a siRNA knockdown approach.

## Materials and methods

*Cell lines*. The following six pancreatic cancer cell lines were used: KP-2, SUIT-2 (Dr H. Iguchi, National Shikoku Cancer Center, Matsuyama, Japan), MIA PaCa-2 (Japanese Cancer Resource Bank, Tokyo, Japan), Capan-1, Capan-2 and Hs766T (American Type Culture Collection, Manassas, VI, USA). The human immortalized pancreatic ductal epithelial cell line (HPDE6-E6E7 clone 6) was kindly provided by Dr Ming-Sound Tsao (University of Toronto, Toronto, Canada). All cells were maintained as previously described (20).

*Pancreatic tissues*. We analyzed TM4SF1 expression in 74 tissue samples obtained from patients who underwent pancreatic resection for pancreatic cancer at our institution between January 2000 and August 2009. We also obtained normal pancreatic tissue samples from intact pancreas resected for bile duct cancer as control tissues. Survival was measured from the time of pancreatic resection, with death as the end-point. Overall survival and disease-free survival analyses were performed in February 2012. The median observation time for overall survival and disease-free survival was 19 months (range 2-137 months) and 11 months (range 1-137 months), respectively. Forty-nine patients died during the follow-up. All surviving patients were censored. Histological diagnosis of specimens was in accordance with the criteria of the updated World Health Organization classification (21). Tumor stage was assessed according to the UICC classification, 7th edition (22). The clinicopathological characteristics of all patients are summarized in Table I. The study was approved by the Ethics Committee of Kyushu University and conducted according to the Ethical Guidelines for Human Genome/Gene Research enacted by the Japanese Government and the Helsinki Declaration.

Table I. Clinicopathological characteristics of patients with PDAC (N=74).

| | |
|---|---|
| Median age | 66.55 (range 36–86 years) |
| Gender (male/female) | 46 (62.2%)/28 (37.8%) |
| pT category | |
| T1 | 2 (2.7%) |
| T2 | 2 (2.7%) |
| T3 | 68 (91.9%) |
| T4 | 2 (2.7%) |
| pN category | |
| pN0 | 16 (21.6%) |
| pN1 | 58 (78.4%) |
| UICC stage | |
| I | 3 (4.1%) |
| II | 68 (91.8%) |
| III | 2 (2.7%) |
| IV | 1 (1.4%) |
| Residual tumor category | |
| R0 | 52 (70.3%) |
| R1 | 22 (29.7%) |
| Histologic grade | |
| Grade 1 | 13 (17.6%) |
| Grade 2 | 35 (47.3%) |
| Grade 3 | 26 (35.1%) |
| Vascular invasion | |
| Negative | 20 (27.3%) |
| Positive | 54 (72.7%) |
| Perineural invasion | |
| Negative | 6 (8.2%) |
| Positive | 68 (91.8%) |
| Lymphatic invasion | |
| Negative | 17 (23%) |
| Positive | 57 (77%) |

*Quantitative reverse transcription polymerase chain reaction (qRT-PCR)*. One-step real-time qRT-PCR using gene-specific primers was performed as described previously (23). Primer sequences were as follows: TM4SF1 forward 5'-ATTGGAATTGCAGGATCTGG-3' and reverse 5'-GCCGAGGGAATCAAGACATA-3'; 18S rRNA forward 5'-GTAACCCGTTGAACCCCATT-3' and reverse 5'-CCATCCAATCGGTAGTAGCG-3'. BLAST searches were performed to ensure the specificity of all primers. Primers were purchased from Sigma Genosys (Tokyo, Japan).

*Immunohistochemical procedures and evaluation*. Immunohistochemistry was performed using a Histofine SAB-PO kit (Nichirei, Tokyo, Japan) (24). Sections were incubated with anti-TM4SF1 antibody (HPA002823; Sigma-Aldrich, Tokyo, Japan) overnight at 4˚C. Carcinoma cells were identified according to morphology and counted in at least 20 fields per section at x200 magnification. The distribution of TM4SF1 staining was evaluated as the percentage of stained cells, and was scored as follows: 0, no staining or <10%; 1, 11-25%; 2, 26-50%; 3, 51-75%; or 4, 76-100%. Cells were also scored for staining intensity, which was scored as 0, no staining; 1, weak; 2, moderate; or 3, strong. The multiplication product (from 0-12) from these two scores was used to assign patients into one of two groups according to TM4SF1 expression, where a score of 0-4 represented low expression and a score of 6-12 represented high expression. All slides were evaluated independently by two investigators who were unaware of the clinical features of each case.

*Silencing of TM4SF1 using small interfering RNAs (siRNAs)*. Gene silencing was carried out using siRNA (Qiagen, MA, USA) directed against human TM4SF1. Sequences were as follows: siRNA-1 (sense, 5'-ggaccacuaugucuugauutt-3'; antisense, 5'-aaucaagacauaguggucctt-3'); siRNA-2 (sense, 5'-cgaugacugggcaagaagatt-3'; antisense, 5'-ucuucuugcccagucaucgta-3'). Qiagen all-star siRNA was used as a negative control. Transfections were performed as described previously (25).

All cells were used in subsequent experiments 48 h after transfection.

*Matrigel invasion and migration assays*. Migration and invasion of cultured cancer cells were assessed by counting the number of cells migrating or invading through uncoated or Matrigel-coated transwell chambers (BD Biosciences, Franklin Lakes, NJ, USA) as described previously (20,26). Cells were maintained in 10% FBS/DMEM during these assays. Cells were transfected with siRNAs 48 h prior to experimentation. Migration was determined after a 24-h period, and invasiveness was determined after a 48-h period.

*TGF-β1 treatment*. SUIT-2 and Capan-2 cells were cultured in 6-well plates ($1x10^5$ cells/well) in 1% FBS/DMEM with or without 10 ng/ml recombinant human TGF-β1 (R&D, Oxon, UK) for 24 and 48 h (27). At each time-point, total RNA was extracted to evaluate TM4SF1 mRNA expression by qRT-PCR. To evaluate TM4SF1 protein levels, cells were seeded in 90-mm dishes at a density of $2x10^5$ cells/dish and cultured in 1% FBS/DMEM with or without 10 ng/ml TGF-β1 prior to cell lysis.

*Western blot analysis*. Western blotting was performed as described previously (28). Antibodies used in this study were as follows: anti-TM4SF1 (sc-103267; Santa Cruz, CA, USA), anti-E-cadherin (no. 3195), anti-vimentin (no. 5741), anti-N-cadherin (no. 4061), anti-claudin-1 (no. 4933) (Cell Signaling Technology, Danvers, MA, USA) and anti-β actin (ab8227; Abcam, Cambridge, MA, USA).

*Statistical analysis*. A $\chi^2$ test was used to analyze the correlation between TM4SF1 expression and clinicopathological characteristics. Survival analysis was performed using Kaplan-Meier analysis and curves were compared using the log-rank test. For *in vitro* experiments, values are expressed as means ± standard deviation. Comparison between two groups was performed using the Student's t-test. Statistical significance was defined as $P<0.05$. All statistical analyses were performed using JMP 9.0.2 software (SAS Institute, NC, USA).

## Results

*Pancreatic cancer cells express TM4SF1*. We first sought to determine the level of TM4SF1 expression in PDAC and normal pancreatic tissues. In normal pancreatic tissues, weak-to-no staining was detected in pancreatic ductal epithelial cells and TM4SF1 expression was not observed in acinar cells or stroma. Blood vessels consistently stained positive for TM4SF1, and were used as a positive control (Fig. 1A-a and -c). In tumor tissues, strong luminal membrane surface staining and a weak-to-moderate cytoplasmic staining was detected in cancer cells (Fig. 1A-b). In addition to tumor tissue samples where moderate staining was observed, there were also tumor tissue samples where little-to-no staining was observed (Fig. 1A-c).

*Prognostic significance of TM4SF1 expression*. Survival analysis was performed for patients with PDAC. The high TM4SF1 expression group showed better survival (P=0.0332; Fig. 1B) than the low expression group, and there was an obvious difference in the disease-free time between the groups (P=0.0409; Fig. 1B). Median survival was 30 months for the high expression group and 17 months for the low expression group (Table II). Next, we performed multivariate analysis based on the Cox proportional hazard model on all parameters that were found to be significant in the univariate analyses. Overall survival was significantly dependent on residual tumor (R1) status (relative risk = 2.732, P=0.0017), vascular invasion (relative risk = 2.461, P=0.0101) and low TM4SF1 expression (relative risk = 1.987, P=0.0196) (Table III).

Table II. Univariate survival analysis of conventional prognostic factors and TM4SF1 expression in PDAC patient resections (N=74).

| Characteristics | No. of cases | Median survival time (months) | 5-year survival rate | P-value |
|---|---|---|---|---|
| TM4SF1 expression | | | | 0.0332 |
| High | 35 | 30 | 40.8 | |
| Low | 39 | 17 | 13.2 | |
| Age (years) | | | | 0.3099 |
| <65 | 27 | 23 | 38.0 | |
| ≥65 | 47 | 23 | 17.7 | |
| UICC stage | | | | 0.0774 |
| I/IIA | 15 | 60 | 44.1 | |
| IIB/III/IV | 59 | 19 | 18.6 | |
| Histological grade | | | | 0.4285 |
| Grade 1/grade 2 | 48 | 23 | 30.8 | |
| Grade 3 | 26 | 19 | 14.2 | |
| Residual tumor | | | | 0.0001 |
| R0 | 52 | 30 | 36.8 | |
| R1 | 22 | 11 | 2.8 | |
| Vascular invasion | | | | 0.0079 |
| Negative | 20 | 60 | 53.1 | |
| Positive | 54 | 17 | 14.0 | |
| Perineural invasion | | | | 0.1458 |
| Negative | 6 | 90 | 60.3 | |
| Positive | 68 | 19 | 23.8 | |
| Lymphatic invasion | | | | 0.0667 |
| Negative | 17 | 60 | 46.5 | |
| Positive | 57 | 19 | 21.0 | |

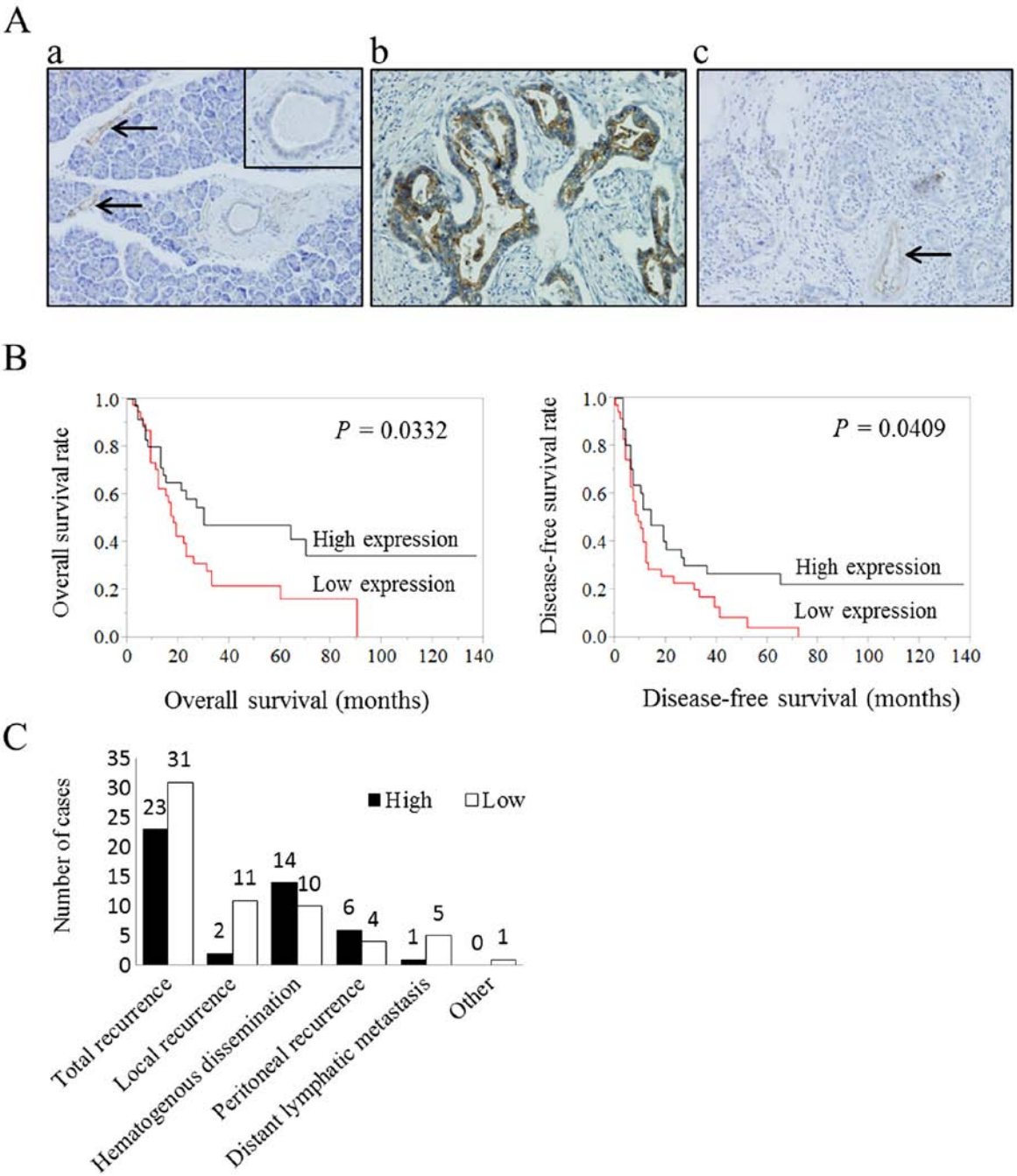


Figure 1. TM4SF1 expression correlates with good-outcome in PDAC. (A) Immunohistochemical analysis of TM4SF1 in human PDAC specimens. (a) A normal pancreatic specimen showing weak-to-no staining in pancreatic ductal epithelial cells (inset). (b) A PDAC specimen demonstrating high TM4SF1 expression in pancreatic cancer cells. (c) A PDAC specimen demonstrating low TM4SF1 expression in pancreatic cancer cells (arrows indicate blood vessels; original magnification, x200) (B) Kaplan-Meier survival analysis of TM4SF1 expression in PDAC cancer cells. Low expression of TM4SF1 was associated with shorter overall survival (left) and disease-free survival (right). (C) Total recurrence and site of first recurrence for each group.

Table III. Multivariate analysis of conventional prognostic factors and TM4SF1 expression in PDAC patients (N=74).

| Characteristics | Relative risk | 95% confidence interval | P-value |
|---|---|---|---|
| Low TM4SF1 expression | 1.987 | 1.116-3.632 | 0.0196 |
| R factor | 2.732 | 1.479-4.949 | 0.0017 |
| Vascular invasion | 2.461 | 1.226-5.503 | 0.0101 |

The relationship between TM4SF1 expression and clinicopathological factors of PDAC is shown in Table IV. There was no statistically significant correlation between TM4SF1 expression and clinicopathological factors possibly due to the relatively small size of the patient cohort, although it appears that the patients with low expression have a higher tumor grade and a more advanced clinical stage. Because there was an obvious difference in the disease-free time, we analyzed the site of first recurrence (Fig. 1C) and found that the locoregional recurrence rate in the low TM4SF1 expression

Table IV. Relationship between TM4SF1 expression and various clinicopathological factors in patients with PDAC (N=74).

| Characteristics | High expression group n=35 (47.3%) | Low expression group n=39 (52.7%) | P-value |
|---|---|---|---|
| Age (years) | | | 0.9115 |
| <65 | 13 (37.14) | 14 (35.90) | |
| ≥65 | 22 (62.86) | 25 (64.10) | |
| pT category | | | 0.9114 |
| pT1/pT2 | 2 (5.71) | 2 (5.13) | |
| pT3/pT4 | 33 (94.29) | 37 (94.87) | |
| pN Category | | | 0.4179 |
| pNo | 9 (25.71) | 7 (17.95) | |
| pN1 | 26 (74.29) | 32 (82.05) | |
| UICC stage | | | 0.6002 |
| I/IIA | 8 (22.86) | 7 (17.95) | |
| IIB/III/IV | 27 (77.14) | 32 (82.05) | |
| Histological grade | | | 0.5263 |
| Grade 1/grade 2 | 24 (68.57) | 24 (61.54) | |
| Grade 3 | 11 (31.43) | 15 (38.46) | |
| Residual tumor category | | | 0.8363 |
| R0 | 25 (71.43) | 27 (69.23) | |
| R1 | 10 (28.57) | 12 (30.77) | |
| Lymphatic invasion | | | 0.9821 |
| Negative | 8 (22.86) | 9 (23.08) | |
| Positive | 27 (77.14) | 30 (76.92) | |
| Vascular invasion | | | 0.8095 |
| Negative | 9 (25.71) | 11 (28.21) | |
| Positive | 26 (74.29) | 28 (71.79) | |
| Perineural invasion | | | 0.3189 |
| Negative | 4 (11.43) | 2 (5.13) | |
| Positive | 31 (88.57) | 37 (94.87) | |

Table V. Relationship between TM4SF1 expression and patterns of recurrence in patients with PDAC (N=74).

| Characteristics | High expression group n=35 (47.3%) | Low expression group n=39 (52.7%) | P-value |
|---|---|---|---|
| Total recurrence | | | 0.1823 |
| Negative | 12 (34.29) | 8 (20.51) | |
| Positive | 23 (65.71) | 31 (79.49) | |
| Local recurrence | | | 0.0079 |
| Negative | 33 (94.29) | 28 (71.79) | |
| Positive | 2 (5.71) | 11 (28.21) | |
| Hematogenous dissemination | | | 0.8363 |
| Negative | 21 (60.00) | 29 (74.36) | |
| Positive | 14 (40.00) | 10 (25.64) | |
| Peritoneal recurrence | | | 0.3866 |
| Negative | 29 (82.86) | 35 (89.74) | |
| Positive | 6 (17.14) | 4 (10.26) | |
| Distant lymphatic metastasis | | | 0.0647 |
| Negative | 34 (97.14) | 34 (87.18) | |
| Positive | 1 (2.86) | 5 (12.82) | |

group (28.21%) was higher than in the high expression group (5.71%; P=0.0079) although the total recurrence rates for the two patients groups were similar (Table V). The data suggest that tumors with low TM4SF1 expression undergo local spread more frequently than those with high expression, and this may contribute to the observed survival differences.

*Inhibition of TM4SF1 expression promotes migration and invasion of pancreatic cancer cells*. We also determined the level of TM4SF1 expression in a variety of pancreatic cancer cell lines, and the level of TM4SF1 mRNA expression was found to be higher in pancreatic cancer cell lines than in normal (HPDE) epithelial cells (Fig. 2A). Because local tumor invasion is more likely in patients with low TM4SF1 tumor expression, and because TM4SF1 has been reported to be involved in the migration of cancer cells (14,15), we next performed migration and invasion assays following knockdown of TM4SF1 in pancreatic cancer cells. Decreased TM4SF1 protein expression was confirmed by western blotting following transfection of cells with TM4SF1-specific siRNA (Fig. 2B). The invasion and migration of two cell lines studied, SUIT-2 and Capan-2, were enhanced following siRNA-mediated knockdown of TM4SF1 (Fig. 2C). These suggest that TM4SF1 has an inhibitory role in the invasion and migration of pancreatic cancer cells.

*E-cadherin expression is reduced in pancreatic cancer cells following knockdown of TM4SF1*. Because the process of cancer invasion requires epithelial tumor cells to undergo epithelial to mesenchymal transition (EMT) either transiently or stably (29), we examined the correlation between the expression of TM4SF1 and EMT markers *in vitro*. We found that E-cadherin expression was reduced after knockdown of TM4SF1, but there were no changes in the expression of other makers including vimentin, N-cadherin and claudin-1 (Fig. 3A). Changes in cell morphology, however, were not observed after knockdown of TM4SF1 (data not shown).

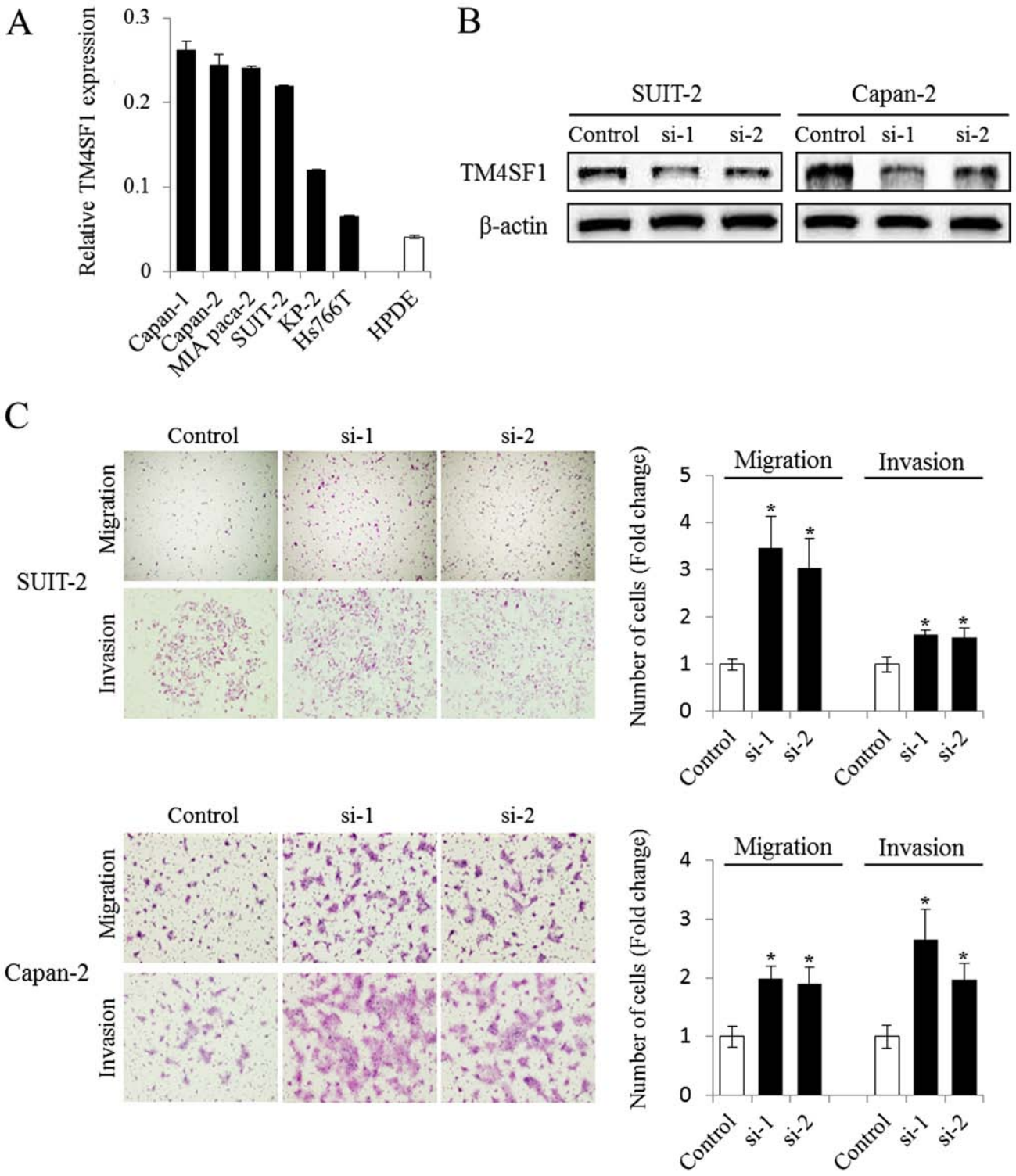


Figure 2. Knockdown of TM4SF1 increases the migration and invasion of pancreatic cancer cells. (A) The levels of TM4SF1 mRNA expression were analyzed by qRT-PCR and were normalized to 18S rRNA. (B) Western blotting showing TM4SF1 protein levels in SUIT-2 and Capan-2 pancreatic cancer cells 72 h following transfection with indicated siRNAs. (C) Evaluation of the migration and invasion of SUIT-2 and Capan-2 cells following transfection with control or TM4SF1-specific siRNAs. Representative photomicrographs are shown in the panels on the left-hand side (x100 magnification). Bar charts summarize the migration and invasion of cells in each siRNA treatment group. Bars represent mean cell counts ± SD and are normalized to the control siRNA group (fold change, $^{*}P<0.05$, compare with control).

*TGF-β1 negatively regulates TM4SF1 expression*. TGF-β1 is a major inducer of EMT in a number of cellular contexts (30), and plays a pivotal role in driving EMT in the pathogenesis of pancreatic cancer (27,31). To investigate whether TGF-β1 influences TM4SF1 expression, we performed additional *in vitro* analyses on pancreatic cancer cells treated with TGF-β1. Western blot analysis revealed that TGF-β1 treatment significantly downregulated E-cadherin expression and upregulated

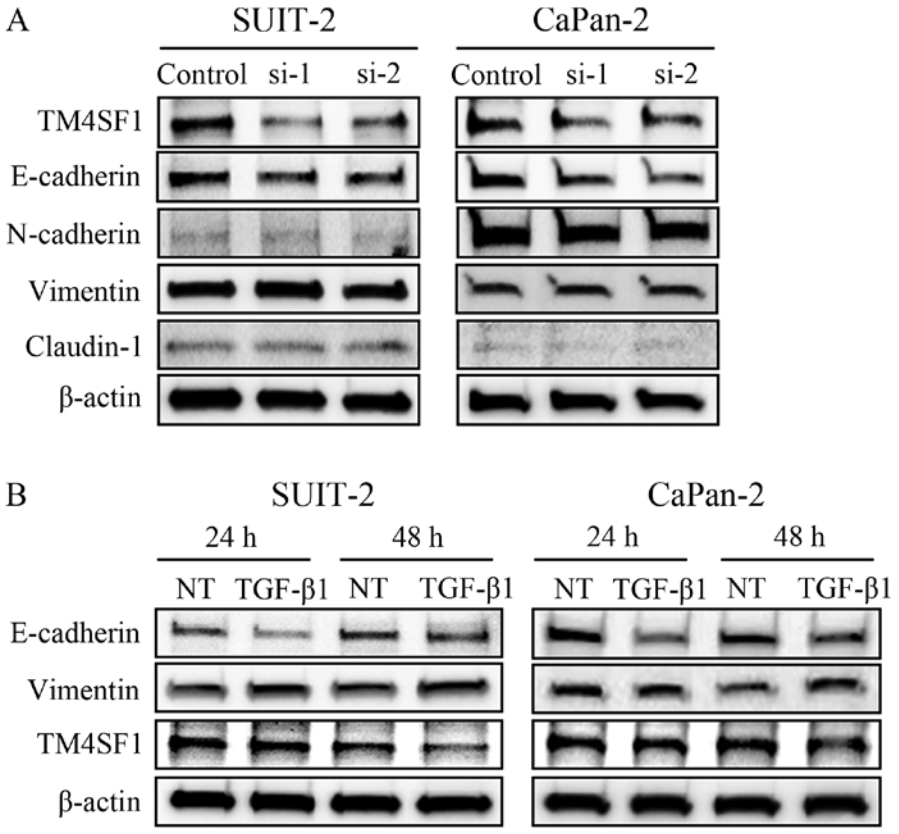


Figure 3. TM4SF1 expression influences E-cadherin, and is reduced upon TGF-β1 treatment. (A) E-cadherin expression was reduced 48 h after knockdown of TM4SF1. No detectable change in the expression of other EMT makers was observed following knockdown of TM4SF1. (B) Western blot analyses showed that E-cadherin expression was reduced, and that vimentin expression was increased in SUIT-2 and Capan-2 cells following treatment with TGF-β1. Reduced TM4SF1 protein expression was observed 48 h after treatment with TGF-β1 (NT, non-treated).

vimentin expression in both these cell lines. TM4SF1 protein levels were also reduced following treatment with TGF-β1 for 48 h (Fig. 3B).

## Discussion

TM4SF1 is a tumor-associated antigen that is detected widely in human carcinomas (32), and it represents a promising target for antibody-mediated immunotherapy (33). However, the exact role of TM4SF1 in cancer remains controversial. TM4SF1 is reported to be a prognostic marker in a number of malignant tumors. In lung (14) and colorectal cancer (34) patients, increased expression of TM4SF1 was shown to be significantly associated with early postoperative relapse and shorter survival. In contrast, our study revealed that decreased expression of TM4SF1 was significantly associated with shorter survival and early local spread in PDAC patients, suggesting that TM4SF1 plays an inhibitory role in pancreatic cancer relapse and tumor progression. These results are consistent with those of a previous study of TM4SF1 in mesothelioma patients (16). Furthermore, serial analysis of gene expression in breast cancer tissues revealed a negative correlation between TM4SF1 expression and progression of breast carcinomas (17). These data suggest that TM4SF1 may be a novel predictive marker of good prognosis in several tumors including PDAC, although the clinical significance of TM4SF1 expression varies depending on tumor type.

With respect to the cellular functions of TM4SF1, it has previously been reported that high expression of TM4SF1 increases the invasiveness of lung carcinoma cells (14) and the migration of prostate cancer cells (15). Our study demonstrates a contrasting function for TM4SF1 in pancreatic cancer cells as cancer cell motility was enhanced following inhibition of TM4SF1 expression, a finding which may account for the differences in local recurrence rate and prognosis among PDAC patients. Furthermore, we found that knockdown of TM4SF1 reduced E-cadherin expression in pancreatic cancer cells (although other EMT markers examined were unaffected) and that treatment with TGF-β1 downregulated TM4SF1 in these cells. E-cadherin is closely implicated in the motility of cancer cells (35), and loss of E-cadherin expression is associated with poor clinical outcome in several cancer types (36,37) including pancreatic cancer (38). Our findings therefore suggest a role for TM4SF1 in TGF-β1-induced EMT, and that TM4SF1 may alter the migration of pancreatic cancer cells partially via regulating E-cadherin expression. Targeting TM4SF1 expression may therefore potentially lead to the development of promising therapies for several types of tumor, including PDAC.

Previous reports indicate that tetraspanins, through protein-protein and protein-lipid interactions, recruit various transmembrane and cytoplasmic proteins to form tetraspanin-enriched microdomains (TERM), functional complexes involved in cell migration (39). Tetraspanins interact with a

variety of transmembrane proteins, participate in various TERM formations, and can perform different functions in a variety of cell types. For example, CD151, one of the tetraspanin members, regulates cell migration in a cell type-specific manner (40). The CD151-α6β4 complex is a major component of hemidesmosomes in skin keratinocytes, structures that oppose cell migration by stabilizing cell attachment to the extracellular matrix (41). However, in human cervical carcinoma cells, CD151 promotes tumor cell migration but does not appear to affect cell adhesion to matrix proteins (42). TM4SF1 is also associated with TERM, and influences cell motility via regulating the surface presentation and endocytosis of a number of components of these complexes (43). Therefore, we speculate that in diverse tissues or cell types, TM4SF1 may interact with different transmembrane proteins (such as E-cadherin) and influence cell migration by modulating the endocytosis and recycling of proteins associated with motility.

In conclusion, our results suggest that TM4SF1 plays an inhibitory role in the migration and invasion of pancreatic cancer cells. Further investigation is now needed to elucidate the mechanisms of interaction between TM4SF1 and either E-cadherin or TGF-β1 in the migration and invasion of pancreatic cancer cells. Furthermore, decreased expression of TM4SF1 is significantly associated with shorter survival in PDAC patients, suggesting that TM4SF1 is a promising prognostic biomarker in PDAC.

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