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Identification of CLEC10A as a human lectin for pancreatic ductal adenocarcinoma

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The mechanism by which glycans in pancreatic ductal adenocarcinoma (PDAC) interact with human endogenous lectins in the tumor microenvironment remains largely unknown. This study aimed to identify endogenous lectins that recognize and bind to pancreatic ductal adenocarcinomas. The reactivity of 43 human endogenous lectins belonging to the Galectin, Siglec, and C-type lectin families in PDAC cell lines and clinical PDAC samples was analyzed using flow cytometry and immunostaining of tissues. C-type lectin domain family 10 member A (CLEC10A), a C-type lectin, was a candidate endogenous lectin with high reactivity in some pancreatic cancer cells. CLEC10A lectin bound in approximately 60% of 113 clinical pancreatic cancer tissue sections. Immunohistochemistry with anti-CLEC10A antibody showed that CLEC10A was mainly expressed in CD163-positive monocytic cells. Of the 57 patients (out of 113) who achieved R0 surgical resection at stage II/III, those with high CLEC10A expression on macrophages and CLEC10A ligand expression on PDAC cells had significantly shorter overall survival. CLEC10A is a human lectin receptor for pancreatic ductal adenocarcinoma. The coexistence of CLEC10A-expressing cells in pancreatic cancer tissues and CLEC10A ligands on pancreatic cancer cells indicates poor prognosis.

Keywords CLEC10A protein, Endogenous lectin, Pancreatic cancer, Tumor microenvironment

Diverse physiological activities and in vivo reactions are mediated by well-characterized enzymes and antibodies; however, the importance of lectin–glycan interactions have been highlighted only recently. The outermost layer of all cells consists of a complex glycocalyx mesh¹, which is involved in cell–cell communication, maintenance of internal stability, and protection from contact damage. Cancers are no exception, and cancer-specific changes in glycans, such as sialylation, fucosylation, truncation of O-glycans, and branching of N-glycans, are known to occur constantly in cancer cells, acting as biomarkers of cancer progression and a mechanism of invasion and immune evasion².

Lectins are proteins capable of binding specifically to glycans and an important class of endogenous lectins expressed on immune cells mediate non-self reactions³⁻⁵. HumanLectome (https://unilectin.unige.ch/humanLectome/), a database of human lectins, currently contains more than 100 types of endogenous human lectins, including major families consisting of Galectins, Siglecs, and C-type lectins. These moieties have been reported to promote multiple aspects of tumor-host interactions, such as metastasis or induction of anti-tumor immunity^{3,6,7}.

Previously, we have reported the presence of glycans in pancreatic cancer (the most intractable solid cancer) and discussed the diagnostic and therapeutic applications developed utilizing glycan–lectin reactions. Using high-density lectin microarray analysis, we demonstrated that the rBC2LCN lectin from *Burkholderia cenocepacia* strongly binds to the fucosylated glycan epitopes of H-1/3/4-type glycans expressed on the surface of pancreatic cancer cells⁸. We extended the research on lectins as drug carriers for therapy^{8,9} and probes for imaging diagnostics¹⁰. One of the biggest drawbacks of using the rBC2LCN lectin in human diagnosis and/or therapy is that proteins of non-human origin may precipitate severe immunoreactions. Therefore, we focused on human endogenous lectins, which may contribute to cancer progression in vivo and thus act as alternative drug carrier candidates. Similar to the rBC2LCN lectin, additional unreported endogenous lectins that recognize glycans expressed on the surface of pancreatic cancer cells may exist.

¹Department of Gastrointestinal and Hepato-Biliary-Pancreatic Surgery, Faculty of Medicine, University of Tsukuba, Tsukuba, Japan. ²Cellular and Molecular Biotechnology Research Institute, National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan. [⊠]email: h-tateno@aist.go.jp; tatoda@md.tsukuba.ac.jp Tumor tissues consist of cancer cells, extracellular matrix, immune cells, fibroblasts, and vascular endothelial cells, collectively known as the tumor microenvironment (TME)¹¹. Glycans expressed on pancreatic cancer cells play an essential role in tumor progression by interacting with lectins expressed on immune cells, such as Siglec-9 and DC-SIGN, to form an immunosuppressive TME^{12,13}. However, the diverse nature of cancer-specific glycan alterations, along with the existence of many potential human endogenous lectins as receptors for these altered glycans, has rendered understanding the mechanism by which pancreatic cancer glycans interact with human endogenous lectins in the TME difficult.

Here, we analyzed the activity of 43 endogenous lectins in pancreatic ductal adenocarcinomas (PDACs). We identified CLEC10A (C-type lectin domain family 10 member A), a C-type lectin, as a candidate endogenous lectin with high reactivity in some pancreatic cancer cells. We also revealed that in PDAC patients, high CLEC10A expression in the TME and CLEC10A ligand expression in PDAC cells resulted in significantly shorter overall survival, suggesting that it is indicative of poor prognosis.

Materials and methods

Cell lines

Six human pancreatic cancer cell lines, Capan-1 (American Type Culture Collection [ATCC], Manassas, VA, USA; HTB-79), AsPC-1 (ATCC, CRL-1682), BxPC-3 (ATCC, CRL-1687), PANC-1 (ATCC, CRL-1469), MIA PaCa-2 (ATCC, CRL-1420), and SUIT-2 (Japanese Collection of Research Bioresources Cell Bank, Osaka, Japan, JCRB1094), and a normal human pancreatic duct cell line, hTERT-HPNE (ATCC, CRL-4023), were used in this study. Capan-1 cells were cultured in Iscove's modified Dulbecco's medium (FUJIFILM Wako Pure Chemical Corp., Osaka, Japan; Cat# 098-06465) supplemented with 20% fetal bovine serum (Thermo Fisher Scientific, Waltham, MA, USA; Cat# 12483- 020) and 1% penicillin-streptomycin (Thermo Fisher Scientific; Cat# 15140-122). AsPC-1 cells, BxPC-3 cells, and SUIT-2 cells were cultured in Roswell Park Memorial Institute-1640 medium (FUJIFILM Wako Pure Chemical Corp.; Cat# 189-02025) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. The PANC-1 cells were cultured in Dulbecco's modified Eagle's medium (FUJIFILM Wako Pure Chemical Corp.; Cat# 044-29765) supplemented with 10% fetal bovine serum and 1% penicillinstreptomycin. The MIA PaCa-2 cells were cultured in Dulbecco's modified Eagle's medium (FUJIFILM Wako Pure Chemical Corp.; Cat# 044-29765) supplemented with 10% fetal bovine serum, 2.5% horse serum, and 1% penicillin-streptomycin. The hTERT-HPNE cells were cultured in 75% glucose-free Dulbecco's modified Eagle's medium (FUJIFILM Wako Pure Chemical Corp.; Cat# 042-32255) and 25% M3 base medium (Incell Corp., San Antonio, TX, USA; Cat# M300F-500) supplemented with 5% fetal bovine serum, 10 ng/mL human recombinant epidermal growth factor (R&D Systems, Minneapolis, MN, USA; Cat# PRD236), 5.5 mM D-glucose (FUJIFILM Wako Pure Chemical Corp.; Cat# 079-05511), and 750 ng/mL puromycin (InvivoGen; Cat# ant- pr). All cells were incubated at 37 °C and 5% CO₂.

Clinical samples

Fresh human pancreatic cancer tissue samples were obtained throughout the course of care. Written informed consent was obtained from all patients and approval from the Tsukuba Clinical Research and Development Organization Institutional Review Board (T-CReDO protocol number: H28-90) (Tsukuba, Japan) was obtained before the clinical specimens were used for research. This study was performed in line with the principles of the Declaration of Helsinki.

Tissue microarray

Specimens from patients who underwent pancreatectomy without preoperative treatment at the University of Tsukuba Hospital in 2020 were analyzed using microarray. From the formalin-fixed paraffin-embedded (FFPE) surgical excision specimens, the area that best represented the histopathological characteristics of the tumor was punched out in a 3-mm diameter area and 12 samples from cancer tissue were collected in addition to the 12 samples of normal pancreatic tissue collected from the explanted samples in the same manner (24 samples per single block for the tissue microarray). The tissue microarray was prepared using Tsukuba Pathological Analysis Support Service.

Selecting individual tissue sections

Among the patients who underwent pancreatectomy at the University of Tsukuba Hospital between 2010 and 2021, 113 who underwent prior resection without preoperative treatment were included in the analysis. For analysis, blocks near the maximally divided surface of the tumor were selected from FFPE surgical resection specimens.

Production of recombinant human lectins

The galectins were prepared as follows. Briefly, genes of carbohydrate recognition domains of galectins cloned into pET vectors (11a, 27b, or 29b) were overexpressed in the *Escherichia coli* BL21-CodonPlus (DE3)-RIL strain under the control of isopropyl- β -d-thiogalactopyranoside (Thermo Fisher Scientific) at appropriate temperatures. The galectins were purified using affinity chromatography with lactose-immobilized Sepharose 4B-CL (GE Healthcare, Chicago, IL, USA). The carbohydrate recognition domain or extracellular domain of C-type lectins and Siglecs was cloned into the pSecTag2 vector or AvT-EK-Fc-pcDNA5FRT containing a human IgG1 Fc region, transiently expressed in HEK293T cells, and purified using Protein G-Sepharose 4 Fast Flow (GE Healthcare). The purified lectins were analyzed using sodium dodecyl sulfate–polyacrylamide gel electrophoresis and western blotting. The protein concentration was calculated using the bicinchoninic acid protein assay kit (Thermo Fisher Scientific).

Flow cytometry

Galectin staining was performed using phosphate buffered saline supplemented with 1% bovine serum albumin (BSA) (Sigma-Aldrich; Cat# A3059). Staining with Siglec-Fc and CLEC-Fc was performed in Hank's balanced salt solution (FUJIFILM Wako Pure Chemical Corp.; Cat# 084-08965) containing calcium and magnesium ions with 1% BSA and 25 mM HEPES (Thermo Fisher Scientific; Cat# 15630-080). Cells (1×10^5) were incubated with phycoerythrin (PE)-labeled galectin (10 µg/mL) or lectin-Fc (10 µg/mL) for 60 min on ice. Cells allowed to react with lectin-Fc were then incubated with PE-labeled anti-human Fc antibody (Jackson ImmunoResearch, West Grove, PA, USA; Cat# 109-115-098) for 60 min on ice, followed by analysis using CytoFLEX (Beckman Coulter, Brea, CA, USA; Cat# B53016) and FlowJo_v10.8.1 (FlowJo LLC, Ashland, OR, USA). For galectin, cells incubated with PE-labeled BSA were used as a negative control, and for lectin-Fc, cells incubated with PE-labeled anti-Fc antibody only were used as the negative control.

Lectin staining

FFPE tissue was cut into 3-µm sections, deparaffinized, and autoclaved in citrate buffer (pH 6.0) for antigen activation. Endogenous peroxidases were inactivated using 3% hydrogen peroxide (FUJIFILM Wako Pure Chemical Corp.; Cat# 085-04056) in ultrapure water before blocking with 1% BSA in Tris-buffered saline (Tris-buffered saline [TBS] containing 1 mM CaCl₂, 1 mM MgCl₂, 1% Tween 20). Horseradish peroxidase (HRP)-conjugated Galectin-4 C (1 µg/mL) and Galectin-8 C (1 µg/mL) were added to the tissues and incubated for 60 min at room temperature. CLEC10A-Fc (2 µg/mL) was added to HRP-conjugated goat anti-human Fc antibody (Jackson ImmunoResearch; Cat# 109-035-008), incubated for 60 min at room temperature, and then blocked using 10% goat serum (Nichirei Biosciences, Tokyo, Japan; Cat# 426042) for 10 min. Tissues were visualized using 3,3'-diaminobenzidine (DAB) (Nichirei Biosciences; Cat# 425011) and counterstained using hematoxylin. Images were captured using a BZX-710 microscope (Keyence, Osaka, Japan). The stainability of the FFPE sections of clinical pancreatic cancer tissue with lectin was scored according to the percentage of positive cells, regardless of the staining intensity, as follows: 0 (0%), 1+ (< 1%), 2+ (1-24%), 3+ (25-49%), 4+ (50-74%), or 5+ (75-100%). For this study, a score of 1 +or higher was considered positive.

Immunohistochemistry

FFPE tissue was cut into 3-µm sections and deparaffinized before heating to 97 °C for 20 min in Target Retrieval solution, high pH (Agilent, Santa Clara, CA, USA; Cat# K8000), for heat activation of antigens, washed in wash buffer (Agilent; Cat# K8000), and loaded onto an Agilent Autostainer Link48. Sections were blocked using peroxidase blocking solution (Agilent; Cat# K8000) for 5 min, washed in wash buffer, and incubated with mouse anti-human CLEC10A monoclonal antibody (1:500, clone OTI2B10, OriGene, Rockville, MD, USA; Cat# TA810180) for 20 min. The sections were washed in wash buffer, incubated with the secondary antibody, Envision FLEX/HRP (Agilent; Cat# K8000), for 20 min, and visualized using DAB and DAB plus chromogen solution (Agilent; Cat# K8000). Images were captured using a BZX-710 microscope (Keyence). Staining intensity was scored according to the number of fields of view admitting positive cells at 20× magnification as follows: 0, 0 fields of view; 1+, 1–2 fields of view; and 2+, 3 or more fields of view.

For immunofluorescence staining, FFPE tissue was deparaffinized and then heated at 121 °C for 20 min in Tris–EDTA buffer (pH 9.0) for heat-induced antigen activation. The sections were then subjected to endogenous peroxidase inactivation with 3% hydrogen peroxide in ultrapure water before blocking with 1% BSA in TBS (containing 1 mM CaCl₂, 1 mM MgCl₂, 1% Tween 20). Samples were pre-incubated with mouse antihuman CLEC10A monoclonal antibody (1:500, clone OTI2B10, OriGene; Cat# TA810180) and Alexa Fluor 488-conjugated rabbit anti-CD163 monoclonal antibody (1:500, clone EPR19518, Abcam, Cambridge, UK; Cat# AB313665) at room temperature for 30 min. Samples were incubated with Alexa Fluor 594-conjugated goat anti-mouse IgG (H + L) polyclonal antibody (1:500, Thermo Fisher Scientific; Cat#A-11005) for 30 min at room temperature. The slides were mounted with 4',6-diamidino-2-phenylindole (Thermo Fisher Scientific; Cat# P36941) and images were captured using a BZX-710 microscope (Keyence).

Statistical analysis

GraphPad Prism 10 (GraphPad Software, San Diego, CA, USA) was used for data analysis. Positive lectin staining was analyzed using Fisher's exact test. A log-rank test was used for survival analysis. *P* values less than 0.05 were considered significant.

Results

Screening of human endogenous lectin and pancreatic cancer cell binding

To screen for human endogenous lectins that recognize and bind to pancreatic cancer cells, we selected 43 lectins belonging to three representative lectin families (galectin, Siglec, and C-type lectin) that are expressed on immune cells and exhibit glycan-binding activity. To identify endogenous lectins that react in a cancer-specific manner, the reactivity of lectins with the histologically highly differentiated pancreatic cancer cell line, Capan-1, poorly differentiated pancreatic cancer cell line, AsPC-1, and normal pancreatic duct cell line, hTERT-HPNE, was analyzed using flow cytometry. The difference between the median fluorescence intensity (MFI) of each reacting lectin and the MFI of the control was termed Δ MFI and displayed on a heat map (Fig. 1a and Table S1). Lectins that met the criteria of MFI greater than 10⁴ when reacting with Capan-1 or AsPC-1 cell lines and less than 10⁴ when reacting with hTERT-HPNE were screened. Galectin-4 C-terminal domain (Galectin-4 C), Galectin-8 C-terminal domain (Galectin-8 C), and CLEC10A-Fc reacted more with Capan-1 than with hTERT-HPNE. Siglec-9-Fc showed high reactivity with the normal pancreatic duct cell line. The other lectins showed little or high reactivity with all the cell lines. Three lectins, Galectin-4 C, Galectin-8 C, and CLEC10A-Fc, were identified as candidates for microarray staining (Fig. 1b).



Fig. 1. Three lectins, Galectin-4 C, Galectin-8 C, and CLEC10A-Fc, were extracted as candidate human endogenous lectins that recognize pancreatic cancer cells. (**a**) Among the 43 lectins, pancreatic cancer-reactive lectins were screened using flow cytometry. The difference (Δ MFI) between the median fluorescence intensity (MFI) of each lectin and the MFI of the control is shown in the heat map. Red arrows indicate lectins that met the criteria of MFI greater than 10⁴ for the pancreatic cancer cell lines, Capan-1 or AsPC-1, and less than 10⁴ for the pancreatic ductal cell line, hTERT-HPNE. (**b**) Histograms of Galectin-4 C, Galectin-8 C, and CLEC10A-Fc. The gray-filled histogram indicates control cells stained with PE-labeled BSA (for galectins) or PE-labeled anti-Fc antibody (for C-type lectins) used as negative controls, and the cyan line is the histogram of cells incubated with PE-labeled galectins or C-type lectins followed by PE-labeled anti-Fc antibody.

Selection of CLEC10A using lectin staining on tissue microarrays

Next, lectin staining was performed on clinical pancreatic cancer samples using tissue microarrays to select a lectin with higher affinity for cancer cells than normal cells. Tissue microarrays of 11 normal pancreatic tissue samples 3 mm diameter (one sample was missing) and 12 pancreatic cancer tissue samples were aligned on a single block and stained with Galectin-4 C, Galectin-8 C, and CLEC10A-Fc. Galectin-4 C positivity was observed in eight of 11 (73%) normal pancreatic duct cell samples versus seven of 12 (58%) pancreatic cancer cell samples. Galectin-8 C positivity was observed in normal pancreatic duct cells of nine of 11 cases (82%), while pancreatic cancer cells were positive in 11 of 12 cases (92%), showing no differences in the positive ratios between normal duct cells and pancreatic cancer cells for either Galectin-4 C or Galectin-8 C. CLEC10A-Fc staining showed no reaction in normal pancreatic duct cells (zero of 11 cases [0%]) but positive reaction in five of 12 pancreatic cancer cell samples (42%) (Fig. 2a, b). Based on these results, we focused on CLEC10A, which had the highest ratio of positive cells between normal and cancer cells in the tested clinical samples.

Expression analysis of CLEC10A ligand in 113 clinical specimens

Considering the heterogeneity within the tumor samples, staining patterns featuring CLEC10A were confirmed in the tumor sections. Clinical pancreatic cancer tissues from 113 patients who had not undergone preoperative treatment (Table 1) were stained with CLEC10A-Fc, as in the tissue microarray, and graded into six levels (0–5+, where 1 + was deemed positive) based on the percentage of stained cells in the cell membranes of pancreatic cancer cells in the tissue sections (Fig. 3a). Of the 113 samples, 73 (65%) were positive, confirming that the CLEC10A ligand was expressed in clinical pancreatic cancer cells in approximately 60% of the analyzed cases (Fig. 3b). We also verified the reactivity of CLEC10A in other PDAC cell lines. We found that CLEC10A showed reactivity in BxPC-3, SUIT-2, PANC-1, and MIA PaCa-2, confirming that CLEC10A is a human lectin that is reactive in multiple PDAC cell lines with distinct properties (Fig. S1).



Fig. 2. CLEC10A is a human endogenous lectin with high affinity for pancreatic cancer cells. (a) Representative images of pancreatic cancerous and noncancerous tissues stained with three lectins (Galectin-4 C, Galectin-8 C, and CLEC10A-Fc). Scale bars, 10 μ m. The arrowhead (\checkmark) indicates the positive staining of the cell membrane. (b) Percentage of cases positive for lectin staining in pancreatic cancer cells (C) and pancreatic duct cells (N) in noncancerous tissue compared using Fisher's exact test (NS: not significant, **P* < 0.05).

Localization of CLEC10A-expressing monocytic cells in the TME

Tissue microarrays were immunostained with an anti-CLEC10A antibody to analyze CLEC10A-expressing immune cells in the TME. The presence of CLEC10A-expressing cells in the tumor stroma was confirmed in all the 12 aligned cancer tissue samples (Fig. 4a). Double-fluorescence staining of tissue microarrays with antibodies against CLEC10A and CD163 (markers for monocytic cells [monocytes/macrophages]) confirmed the co-expression of CLEC10A and CD163 in all 12 cancer samples (Fig. 4b).

Coexistence of CLEC10A-expressing monocytic cells in the TME and CLEC10A ligand on pancreatic cancer cells is reliably prognostic for poor stage II /III outcomes

Next, we investigated the relationship between the expression scores of the CLEC10A ligand and CLEC10A in the TME and linked them to prognostic outcomes. Among the 113 clinical pancreatic cancer samples stained with CLEC10A-Fc, those from 57 stage II/III radically resected cases that achieved R0 resection were immunostained with the anti-CLEC10A antibody. The staining of CLEC10A-expressing monocytic cells in the stroma was classified into three levels: 0 (none), 1+, or 2+ (high) (Fig. 5a). We predicted that an immunosuppressive TME would form in pancreatic cancer tissue due to the interaction between the CLEC10A ligand on pancreatic cancer cells and CLEC10A on monocytes. Therefore, we considered that samples with high expression of both would be "Category High" (Fig. 5b), and that they would have a high degree of malignancy and a poor prognosis. Conversely, from the perspective of interaction, we considered that if the expression of either was low, the interaction would also be poor, and we considered samples with low expression of both or either of them as "Category Low" (Fig. 5b). Comparison of the "Category Low" and the "Category High" revealed that

	CLEC10A staining	
	Positive (Score > 1+)	Negative (Score 0)
	<i>n</i> = 73	<i>n</i> = 40
Age (years)		
Median (IQR)	70 (64–76)	68 (64-72)
Gender, <i>n</i> (%)		
Male	40 (54.8)	22 (55.0)
Female	33 (45.2)	18 (45.0)
CA19-9	1	
Median (IQR)	75 (26–271)	215 (30-509)
CEA	1	
Median (IQR)	2.6 (1.9-4.1)	3.4 (2.6-5.1)
Procedure, n (%)		
Pancreaticoduodenectomy	68 (93.2)	37 (92.5)
Distal pancreatectomy	5 (6.8)	3 (7.5)
Differentiation, n (%)		
Well	21 (28.8)	7 (17.5)
Moderate	48 (65.8)	29 (72.5)
Poorly	3 (4.1)	4 (10.0)
Papillary	1 (1.4)	0 (0.0)
Tumor, <i>n</i> (%)	1	
T1	10 (13.7)	7 (17.5)
T2	45 (61.6)	28 (70.0)
T3	18 (24.7)	5 (12.5)
T4	0 (0.0)	0 (0.0)
Node, <i>n</i> (%)	1	I
N0	22 (30.1)	14 (35.0)
N1	31 (42.5)	13 (32.5)
N2	20 (27.4)	13 (32.5)
Metastasis, n (%)	1	1
M0	70 (95.9)	37 (92.5)
M1	3 (4.1)	3 (7.5)
Margin, <i>n</i> (%)	1	l
Negative	57 (78.1)	32 (80.0)
Positive	16 (21.9)	8 (20.0)
UICC 8th stage, n (%)	1	1
I	19 (26.0)	13 (32.5)
II	32 (43.8)	13 (32.5)
III	19 (26.0)	11 (27.5)
IV	3 (4.1)	3 (7.5)

Table 1. Characteristics of clinical pancreatic cancer tissues from 113 cases.

the "Category High" showed significantly shorter overall survival (Fig. 5c). Thus, the coexistence of CLEC10Aexpressing monocytic cells in the TME and CLEC10A ligands in pancreatic cancer cells is indicative of poor prognosis.

Discussion

In this study, we evaluated the reactivity of 43 human endogenous lectins using flow cytometry in two pancreatic cancer cell lines (Capan-1 and AsPC-1) and a normal pancreatic duct cell line (hTERT-HPNE). Although Galectin-4 C, Galectin-8 C, and CLEC 10 A-Fc showed higher binding to a pancreatic cancer cell line (Capan-1) than to a normal pancreatic duct cell line (hTERT-HPNE), tissue microarray analysis revealed that only CLEC10A-Fc was highly specific for pancreatic cancer cells. When 113 clinical pancreatic cancer tissue samples were stained with CLEC10A-Fc, the signal was confirmed in approximately 60% of cases, indicating that CLEC10A ligands are frequently expressed in pancreatic cancer cells.

Glycan structures containing terminal GalNAc (Tn antigen and its derivative, sialylated Tn [sTn] antigen) have been identified as CLEC10A ligand glycans¹⁴. As truncated O-type glycans result from incomplete synthesis due to COSMC gene mutations, Tn and sTn are representative of terminal GalNAc structures and are characteristic of cancer-mediated modifications^{2,15}. In addition, Radhakrishnan et al.¹⁶ identified hypermethylation in the



CLEC10A ligand expression



Fig. 3. CLEC10A ligand is expressed in 60% of clinical pancreatic cancer cells. (**a**) Representative images of histochemically processed sections of clinical pancreatic cancer tissue analyzed for CLEC10A-Fc staining. Scale bars (Large window) 200 μ m, (Small window) 10 μ m. Staining intensity was scored according to the percentage of positive cells regardless of staining intensity as follows: 0, 0%; 1+, < 1%; 2+, 1–24; 3+, 25–49%; 4+, 50–74%; 5+, 75–100%. (**b**) A pie chart showing the percentage of each staining score in 113 processed sections of clinical pancreatic cancer tissue stained with CLEC10A-Fc.

promoter of *COSMC* in 38% patients with pancreatic cancer in a cohort, which is believed to be responsible for the truncation of O-glycans; overexpression of the resulting Tn and sTn antigens is associated with cancer progression and worse prognosis^{17,18}. Thus, the expression of CLEC10A ligands in pancreatic cancer cells may be a reliable indicator of disease progression.

CLEC10A is an immunosuppressive lectin receptor expressed on immature and M2 macrophages, as well as on immature dendritic cells¹⁹. Tumor-associated macrophages, an innate immune population that constitute a significant proportion of the TME, consist of a mixture of anti-tumor (M1) and tumor-promoting (M2) residents who have the opportunity to interact with transformed cells at high frequencies because of increased immune cell infiltration into tumor tissues²⁰. The majority of tumor-associated macrophages in pancreatic cancer are predominantly M2²¹, which play important roles in immunosuppression, epithelial-mesenchymal transition, and angiogenesis in the TME^{22,23}. In this context, tumor cell-M2 macrophage signaling mediated by CLEC10A and its binding counterpart may be pivotal for immune avoidance and signaling for tumor progression¹⁹. We found that CLEC10A was expressed on pancreatic cancer cells in the TME, may interact with CLEC10A-expressing monocytic cells and render the TME immunosuppressive. This phenomenon has precedence, as previous reports indicated the involvement of CLEC10A and its ligands in cancer progression and immunosuppression via their interactions in colorectal cancer²⁴, cervical cancer²⁵, glioblastoma²⁶, and ovarian cancer²⁷.

In the present study, among the 57 patients with stage II/III pancreatic cancer who achieved R0 resection, high expression of both CLEC10A and its ligands correlated with significantly shorter overall survival than low

а



b

Pancreatic cancer tissue



Fig. 4. CLEC10A-expressing monocytic cells in pancreatic cancer stroma. (a) Left: Hematoxylin and eosin staining of representative pancreatic cancer tissue. Scale bars, 50 μ m. Right: Immunostaining with anti-CLEC10A antibody in representative pancreatic cancer tissue. Scale bars, 50 μ m. Cancer duct structures are surrounded by dotted lines (---); CLEC10A-expressing cells are indicated by arrowheads ($\mathbf{\nabla}$). (b) Immunofluorescence staining of CLEC10A (red), CD163 (green), and nuclei (blue) in pancreatic cancer tissue; merge: yellow. Scale bars, 50 μ m.

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expression of either CLEC10A or its ligands. Our results suggest that the binding of CLEC10A to GalNAc is involved in pancreatic cancer progression and immunosuppression, and that this tumor-specific pathway is an attractive new therapeutic target for cancer.

This study had some limitations. First, the analysis was limited to patients with surgically resectable pancreatic cancers. It should be noted that only approximately 20% cases of pancreatic cancer are resectable at the time of diagnosis, and that the expression of CLEC10A and its ligands in the remaining 80% of the cases is unknown. Second, this study only analyzed samples collected during a limited period during the natural progression of pancreatic cancer. Third, although this study revealed an association between the expression of CLEC10A and its ligand and poor prognosis, the formation of an immunosuppressive environment in the pancreatic cancer microenvironment is complex, and the multifactorial involvement of other endogenous lectins, tumor-associated fibroblasts, bone marrow-derived suppressor cells, and regulatory T cells cannot be ruled out. Finally,



Fig. 5. Appearance of CLEC10A-expressing monocytic cells and coexistence of CLEC10A ligand on pancreatic cancer cells are poor prognostic factors in stage II and III cases. (a) Representative images of formalin-fixed paraffin-embedded (FFPE) sections of clinical pancreatic cancer tissues stained with anti-CLEC10A antibody. Scale bars, 100 μ m. Staining intensity was scored according to the number of positive cells in the 20× field of view as follows: 0 (0 fields); 1+ (1–2 fields); 2+ (> 3 fields). (b) Classification based on the combination of CLEC10A and CLEC10A ligand expression intensity. (c) Kaplan–Meier curve comparing category Low (*n* = 23) with category High (*n* = 5). Log-rank test (**P* < 0.05) was used.

whether CLEC10A in monocytic cells interacts with CLEC10A ligands in cancer cells has not yet been verified. Therefore, the extent to which CLEC10A and its ligands contribute to the formation of an immunosuppressive environment remains unclear, and further clinical studies are required to delineate robust patterns of expression by cancer stage, age at diagnosis, and presence of comorbidities.

Conclusion

We identified CLEC10A as a human lectin receptor for PDAC. Furthermore, we showed the coexistence of CLEC10A-expressing monocytic cells in pancreatic cancer tissues and CLEC10A ligands in pancreatic cancer cells, which is suggestive of poor prognosis.

Data availability

The datasets generated and/or analyzed in the current study are available from the corresponding author upon reasonable request.

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Author contributions

All authors contributed to the conception and design of this study. S.T. collected and analyzed the data. The first draft of the manuscript was written by S.T., and all authors commented on previous versions of the manuscript.

H.T. and O.T. contributed to the interpretation of results and critically revised them for important intellectual content. All the authors have read and approved the final version of the manuscript.

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Declarations

Competing interests

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Ethics approval

The research protocol and all procedures were approved by the Institutional Review Board of the University of Tsukuba Hospital (IRB code: H28-90), Tsukuba, Japan. This study was performed in line with the principles of the Declaration of Helsinki.

Consent to participate

Pancreatic cancer tissue samples were obtained from patients who provided written informed consent.

Additional information

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