

Expression of liver-intestine cadherin and its possible interaction with galectin-3 in ductal adenocarcinoma of the pancreas

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Liver-intestine (LI) cadherin represents a novel type of cadherin within the cadherin superfamily, and is distinguished from other cadherins by specific structural and functional features. Among normal tissues, LI-cadherin is known to be expressed in the intestinal mucosa, while its expression in cancerous tissue has not been investigated to date, except in gastric carcinoma. In the present study we investigated LI-cadherin expression immunohistochemically using our newly established monoclonal antibody in a large set ($n=102$) of tumor specimens from patients with ductal adenocarcinoma of the pancreas, and correlated the findings with the patients' survival. LI-cadherin expression was seen focally in normal pancreatic ducts. In carcinoma, well-differentiated carcinoma cases strongly expressed LI-cadherin, whereas less differentiated areas and poorly differentiated carcinoma cases expressed less or were negative. Kaplan-Meier analysis for all patients demonstrated that high LI-cadherin expression ($>25\%$ of cells stained positive) correlated with good survival ($P<0.001$). Cox regression analyses demonstrated that LI-cadherin expression was one of the strongest predictors of outcome, independent of all other variables, and low LI-cadherin expression correlated with tumor de-differentiation and advanced stage. Furthermore, galectin-3 was identified as being coimmunoprecipitated with LI-cadherin and this interaction was inhibited by lactose in a dose-dependent manner, but not by sucrose. Because galectin-3 has been observed to show a similar expression pattern to LI-cadherin in ductal adenocarcinoma of the pancreas, expression of LI-cadherin and this interaction could have some role in ductal adenocarcinoma of the pancreas. (Cancer Sci 2003; 94: 425–430)

Pancreatic carcinoma is one of the most lethal malignancies worldwide, and its incidence has been increasing in industrialized countries.^{1,2} Most patients with pancreatic carcinoma are diagnosed at an advanced stage because of the aggressiveness of this disease and the lack of early symptoms, and the 5-year survival rate is only 10–26% even after curative surgical resection.^{3–5} A number of studies have been done to date to reveal the pathological and biological prognostic factors in pancreatic carcinoma,⁶ but the precise reason for the biological aggressiveness of this disease has not yet been elucidated.

Cadherins are a family of Ca^{2+} -dependent homotypic cell-cell adhesion molecules that are involved in the maintenance of tissue structure and morphogenesis.^{7,8} It is known that all members of this family share common structural features, with some exceptions, having an amino-terminal extracellular region characterized by a unique domain called the cadherin repeat, a single transmembrane domain, and a cytoplasmic domain at the carboxyl terminus.⁹ A large number of cadherin superfamily members have been identified to date, and are expressed in different tissues of a variety of multicellular organisms. There is increasing evidence that cadherin-mediated cell adhesion addi-

tionally plays a crucial role in carcinoma cell behavior.^{10,11}

LI-cadherin, which is also called human peptide transporter-1 (HPT-1), is a structurally unique member of the cadherin superfamily.^{12,13} Whereas the so-called classic cadherins, such as E-, N- and P-cadherin, have five cadherin repeats within the extracellular domain, LI-cadherin consists of seven cadherin repeats. LI-cadherin has only 20 amino acids in the cytoplasmic domain, although classic cadherins have a highly conserved cytoplasmic domain which consists of 150 to 160 amino acids. The expression of LI-cadherin differs by species. In the rat, LI-cadherin is expressed in the liver and intestinal epithelial cells, while in humans and the mouse, LI-cadherin is expressed in the intestinal epithelial cells, but not in the liver.¹⁴ Although there is only one study with respect to expression of LI-cadherin in gastric carcinomas,¹⁵ to the best of our knowledge the clinicopathologic significance of its expression in ductal adenocarcinoma of the pancreas has not previously been investigated.

LI-cadherin is also known to possess biological functions distinct from classic cadherin, in addition to the differences in its structure as described above, e.g., the adhesive function of LI-cadherin is independent of any interaction with cytoplasmic components, although E-cadherin lacking the cytoplasmic domain is known to be unable to mediate strong cell-cell adhesion.^{16,17} Indeed, no binding partners have been identified for LI-cadherin, to date.

In this study, we raised monoclonal antibodies (mAbs) against a pancreatic cancerous tissue and one of these antibodies was an anti-LI-cadherin antibody. Using this antibody, we initially immunohistochemically investigated LI-cadherin expression in tumor specimens from a large series of patients ($n=102$) with ductal adenocarcinoma of the pancreas, and attempted to determine correlations between the extent of its expression and clinico-pathological parameters. To better understand the biological function of LI-cadherin, we further explored the molecules which associate preferentially with LI-cadherin in pancreatic carcinoma cells, and one of these molecules was identified as galectin-3.

Materials and Methods

Production of monoclonal antibody. Male mice (C.B-17/Icr Crj-scid/scid) with severe combined immunodeficiency were immunized with a pancreatic carcinoma surgical specimen by a rejection method, and hybridomas were produced as described previously.¹⁸ A hybridoma clone producing mAb, D-4 (IgG1, k), was selected on the basis of immunohistochemical reactivity with pancreatic carcinoma tissue.

Cell cultures and reagents. The human pancreatic carcinoma cell

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lines AsPC-1 and PANC-1 were obtained from the American Type Culture Collection (Rockville, MD). They were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μ g/ml streptomycin in a humidified atmosphere of 5% CO₂ at 37°C.

The goat polyclonal antibody against LI-cadherin (pAb anti-LI-cadherin) was purchased from Santa Cruz Biotechnology, Inc. (C-17; Santa Cruz, CA). MAb galectin-3 was obtained as described previously.¹⁹ Normal mouse IgG and normal goat IgG were purchased from Sigma Chemical Co. (St. Louis, MO).

Immunoprecipitation and immunoblotting. For immunoprecipitation, cells were lysed using an ice-cold lysis buffer [150 mM NaCl, 1% Triton X-100, 10 mM Hepes (pH 7.4)] containing a protease inhibitor cocktail (Roche Molecular Biochemicals, Mannheim, Germany) on ice for 30 min. Lysates were cleared by centrifugation (15 000 *g*, 30 min) and the soluble fractions were precleared with 50 μ l of protein-G Sepharose (Pharmacia Biotechnologies, Uppsala, Sweden) (50% slurry) for 2 h. After the addition of the indicated antibodies, samples were incubated overnight at 4°C. Immunocomplexes were precipitated by incubating the samples for 3 h with 50 μ l of protein G Sepharose. Sepharose beads were washed 5 times with a lysis buffer and were heat-denatured in an equal volume of 2× sodium dodecyl sulfate (SDS) sample buffer containing 25 mM dithiothreitol (Sigma Chemical Co.). Samples were electrophoresed on SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, MA). After blocking, the filters were reacted with primary antibodies followed by horseradish peroxidase-conjugated anti-mouse IgG+IgM (IBL, Fujioka) or anti-goat IgG (Santa Cruz Biotechnology, Inc.). Peroxidase-labeled bands were visualized using an enhanced chemiluminescence detection system (Amersham International, Buckinghamshire, UK) according to the manufacturer's instructions.

To ascertain the possible association of LI-cadherin with galectin-3, immunoprecipitation was carried by adding 2 μ g of mAb D-4 in the presence of 0, 1, 10, 100 mM lactose or 100 mM sucrose at 4°C for 2 h. Then, immunoprecipitates were washed five times with a lysis buffer. Immunoprecipitates with SDS sample buffer were heat-denatured and processed for immunoblotting as described above.

Protein sequencing. Protein immunoprecipitated with mAb D-4 was subjected to 7.5% SDS-PAGE and subsequently transferred onto a PVDF membrane. Protein was revealed by Coomassie blue staining [25% methanol, 7.5% acetic acid, and 0.25% brilliant blue R-250 (Sigma Chemical Co.)] for 3 min and subsequent destaining (40% methanol, 10% acetic acid) until bands became visible. The 120-kDa band of the antigen was cut out and sequenced (TaKaRa Shuzo Co., Ltd., Shiga). The protein, which co-immunoprecipitated with mAb D-4 was visualized by SYPRO Ruby protein gel staining (Bio-Rad Laboratories, Hercules, CA) and its band was excised from the gel and subjected to liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis (Promega Co., Ltd., Tokyo).

Patients and tissue specimens. Formalin-fixed, paraffin-embedded tumor specimens were obtained from a series of 102 patients with ductal adenocarcinoma of the pancreas, who had undergone surgical resection at the National Cancer Center Hospital, Japan, between 1990 and 1999. There were 62 men (61%) and 40 women (39%). The mean age was 62.0 years (range, 45 to 82 years). Eight patients underwent total pancreatectomy, 28 patients underwent distal pancreatectomy, and 66 patients underwent pancreaticoduodenectomy, of which 14 involved pylorus-preserving pancreaticoduodenectomy. Intraoperative radiotherapy was given in 77 cases. The specimens were classified by their TNM stage according to the Union Internationale Contre le Cancer (UICC),²⁰ and by the other clinico-pathologi-

cal parameters (histologic differentiation, venous invasion, lymphatic invasion, and perineural invasion) according to the Japan Pancreas Society classification.²¹ The median duration of follow-up was 654 days (range, 79 to 2685 days). Patients with pancreatic tumors of a special type, such as mucinous cystadenocarcinoma, intraductal papillary-mucinous adenocarcinoma, and adenosquamous carcinoma, were excluded. Three patients who died in the immediate postoperative period were also excluded.

Immunohistochemistry. Sections (5 μ m thick) of formalin-fixed, paraffin-embedded tissues were deparaffinized with xylene, treated with 0.3% hydrogen peroxide in methanol, immersed in 10 mM citrate buffer (pH 6.0), heated to 120°C in an autoclave for 10 min, and then allowed to cool at room temperature for 30 min. The sections were preincubated in 2% normal porcine serum in phosphate-buffered saline, incubated with the purified mAb D-4 (1.2 μ g/ml) at 4°C overnight, washed with phosphate-buffered saline, and incubated with the biotinylated secondary antibody (Vector Laboratories, Burlingame, CA) for 30 min at room temperature. Subsequently, they were incubated with the avidin-biotin-peroxidase complex using a Vectastain ABC kit (Vector Laboratories) for 30 min. For visualization of the antigen, sections were immersed in 0.05% diaminobenzidine tetrahydrochloride solution containing 0.01% hydrogen peroxide, and counter-stained with hematoxylin. Sections of formalin-fixed, paraffin-embedded human intestinal epithelial cells known to express LI-cadherin were used as a positive control. Negative control staining of every specimen, which was performed using the same class of mouse immunoglobulin instead of the primary antibody, yielded negative results.

The LI-cadherin expression of the tumor was evaluated according to the proportion of positively stained cells. When $\geq 25\%$ of the carcinoma cells were positively stained, the case was classified as positive (+), with other cases being classified as negative (−). All the hematoxylin and eosin-stained and immunohistochemistry slides were assessed by two independent observers who had no knowledge of the patients' clinical information. To confirm the quality of all sections, we immunohistochemically examined all samples using an antibody for cytokeratin (KL-1; Immunotech, Marseille, France), as described previously (data not shown).¹⁹

Statistical analysis. Statistical analyses were performed using the computer software Stat View-J 5.0 (Abacus Concepts, Berkeley, CA). Expression of LI-cadherin was assessed for associations with various clinico-pathological parameters using the χ^2 test. Survival rates were calculated by the Kaplan-Meier method. The difference between the survival curves was ana-

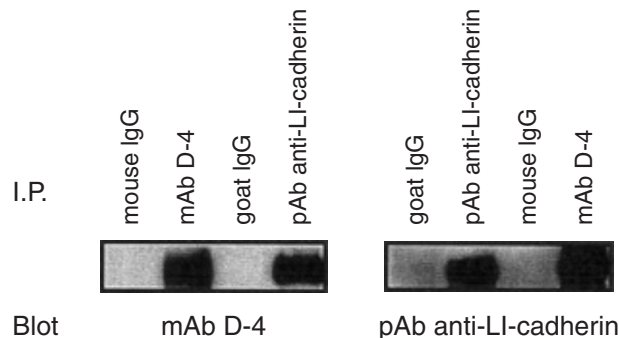


Fig. 1. MAb D-4 recognizes a 120-kDa protein, LI-cadherin. Lysates from AsPC-1 cells were immunoprecipitated with either mAb D-4 or pAb anti-LI-cadherin, resolved on 7.5% SDS-PAGE, and transferred to a membrane. Membranes were then probed with either pAb anti-LI-cadherin or mAb D-4 antibody. Immunoprecipitates with normal mouse and goat IgGs were used as controls for antibody reactivity.

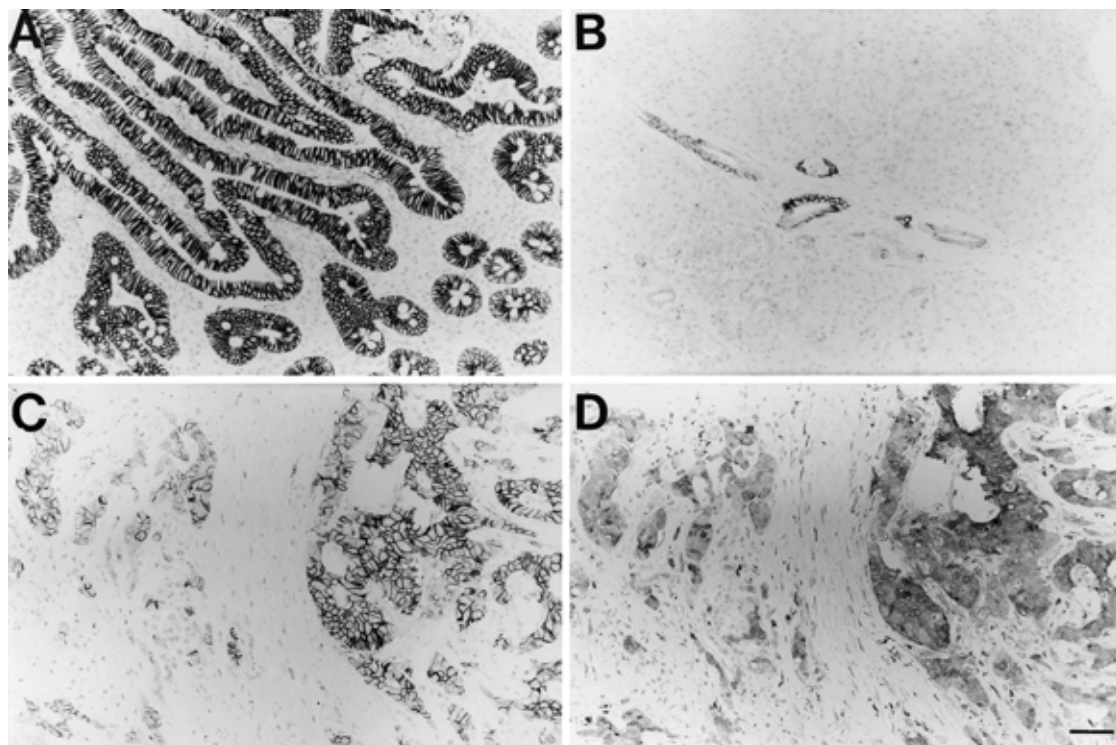


Fig. 2. Immunohistochemical expression of LI-cadherin and galectin-3 in normal and cancerous tissues. LI-cadherin is present strongly in the basolateral membrane in normal duodenal epithelial cells (A), and focally and weakly in normal pancreatic ducts (B). In pancreatic ductal adenocarcinoma (C and D: consecutive sections), LI-cadherin is expressed strongly in well-differentiated cancer nests, but less in invading poorly differentiated cancer nests (C). The expression pattern of galectin-3 (D) is observed to be similar to that of LI-cadherin. The scale bar indicates 100 μ m.

lyzed by means of the log-rank test. To assess the correlation between survival time and multiple clinico-pathological parameters, multivariate analyses were performed using the Cox proportional hazards regression model. Differences were considered significant when the *P* value was less than 0.05.

Results

Characterization of mAb D-4. The mAb D-4 showed strong reactivity to pancreatic carcinoma tissues and also other types of carcinoma tissues, such as colon carcinoma and gastric carcinoma (data not shown). To purify the D-4 antigen, we performed immunoprecipitation experiments. We chose the pancreatic cancer cell line AsPC-1 as an antigen source because it strongly expressed the D-4 antigen. Precipitated antigen was separated on SDS-PAGE, blotted onto a PVDF membrane, and detected by Coomassie blue staining as a 120-kDa protein. Amino acid sequence analysis revealed the sequence IDHVT-GEIFSVA. This amino acid sequence corresponds to the human LI-cadherin amino acid sequence at residues 612–623. AsPC-1 cell lysates were immunoprecipitated with pAb anti-LI-cadherin, and the resulting immunoprecipitates were reacted with mAb D-4 on immunoblots, and vice versa (Fig. 1). The mAb D-4 had the same immunoreactivity in the specimens of this study as did pAb anti-LI-cadherin (data not shown). These analyses showed clearly that mAb D-4 reacted specifically with LI-cadherin.

LI-cadherin expression in normal tissues and ductal adenocarcinoma of the pancreas. LI-cadherin immunoreactivity was observed in normal tissues and ductal adenocarcinoma of the pancreas. In agreement with previous reports,^{13, 15)} LI-cadherin expression was seen in normal intestinal epithelial cells (Fig. 2A) and pancreatic ducts (Fig. 2B); however, LI-cadherin expression of the latter was only seen focally and weakly in about half of the

Table 1. Relationship between LI-cadherin expression and clinico-pathological parameters

	LI-cadherin expression		<i>P</i> value
	(+)	(–)	
Gender			
Male	23	40	0.84
Female	15	24	
Age (years)			
<65	19	33	0.88
≥65	19	31	
Tumor size (cm)			
<3.6	16	31	0.54
≥3.6	22	33	
Histologic differentiation			
Well	24	14	<0.001
Moderately, poorly	14	50	
Venous invasion			
v0, 1	25	38	0.52
v2, 3	13	26	
Lymphatic invasion			
ly0, 1	24	32	0.20
ly2, 3	14	32	
Perineural invasion			
ne0, 1	19	30	0.76
ne2, 3	19	34	
pTNM stage			
I, II, III	23	24	0.02
IVa, IVb	15	40	

cases, as compared with the former. Also, in other normal tissues (brain, esophagus, stomach, lung, liver, kidney, adrenal gland and skin), LI-cadherin expression was not seen, as re-

ported previously (data not shown).^{13, 15)} Immunoreactivity was present in the basolateral plasma membrane, but not the apical membrane.

In carcinoma tissues, LI-cadherin expression was noted in 84 of 102 carcinoma cases (82%). Most of the cases of well-differentiated carcinoma with tight cell-cell adhesion expressed LI-cadherin strongly, but in the less differentiated areas, LI-cadherin expression seemed to be reduced (Fig. 2C). Expression of LI-cadherin was evaluated according to our criteria (see "Materials and Methods"). Thirty-eight cases (37%) were classified as positive, whereas the remaining 64 cases (63%) were classi-

fied as negative.

Relationship of LI-cadherin expression with clinico-pathological parameters. Table 1 shows the relationship between LI-cadherin expression and clinico-pathological parameters. Among 38 well-differentiated carcinomas, 24 (63%) cases were positive, whereas 14 (22%) cases were positive among 64 moderately to poorly differentiated carcinomas. Similarly, among 47 cases in stage pI, pII, pIII, 23 (49%) were positive, whereas 15 (23%) cases were positive among 55 cases in stage pIVa, pIVb. Thus, the extent of LI-cadherin staining decreased with histologic de-differentiation and advanced pTNM stage, and these findings were statistically significant ($P<0.001$ and $P=0.02$, respectively).

Prognostic significance of LI-cadherin expression. Kaplan-Meier survival curves demonstrated that patients whose tumors were LI-cadherin-positive had a significantly longer overall survival time than did those with LI-cadherin-negative tumors ($P<0.001$ by log-rank test, Fig. 3). In univariate analysis of overall survival, histologic differentiation ($P=0.01$; hazard ratio 1.87, 95% confidence interval (CI) 1.14–3.05), pTNM stage ($P<0.001$; hazard ratio 2.44, 95% CI 1.50–3.95) and the extent of LI-cadherin staining ($P<0.001$; hazard ratio 2.48, 95% CI 1.51–4.09) were all significant (Table 2). Multivariate analysis of overall survival revealed that the independent significant factors were the extent of LI-cadherin staining ($P=0.01$; hazard ratio 2.04, 95% CI 1.16–3.61) and the pTNM stage ($P=0.02$; hazard ratio 1.90, 95% CI 1.11–3.24) (Table 3).

Purification and identification of the 30-kDa protein coimmunoprecipitated with LI-cadherin. To explore the role of LI-cadherin in pancreatic carcinoma, we next attempted to isolate LI-cadherin-interacting proteins using immunoprecipitation experiments. A 30-kDa protein coimmunoprecipitated with mAb D-4 in AsPC-1 cells, but not in PANC-1 cells, a LI-cadherin negative cell line (Fig. 4). The purified 30-kDa protein was identified as ga-

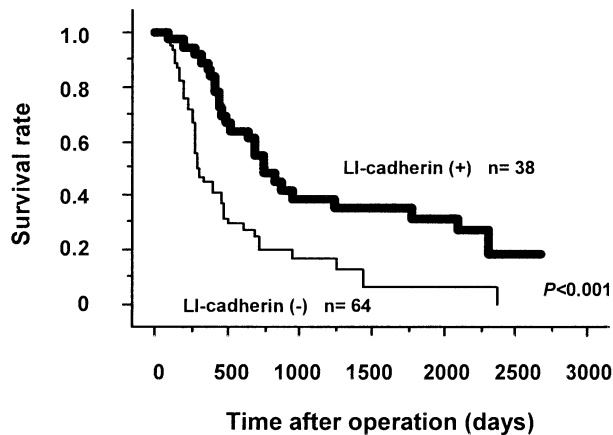


Fig. 3. Kaplan-Meier survival curves of 102 patients. The prognosis became significantly worse in the negative LI-cadherin expression group (thin line) compared with the positive LI-cadherin expression group (thick line; log-rank test, $P<0.001$).

Table 2. Univariate analysis of overall survival for LI-cadherin expression and clinico-pathological parameters

	Number of cases	Hazard ratio	95% CI	P value
Gender				
Male	63	1		
Female	39	1.07	0.66–1.72	0.79
Age (years)				
<65	52	1		
≥65	50	1.16	0.73–1.83	0.54
Tumor size (cm)				
<3.6	47	1		
≥3.6	55	1.20	0.76–1.90	0.43
Histologic differentiation				
Well	38	1		
Moderately, poorly	64	1.87	1.14–3.05	0.01
Venous invasion				
v0, 1	63	1		
v2, 3	39	1.60	1.00–2.57	0.05
Lymphatic invasion				
ly0, 1	56	1		
ly2, 3	46	1.49	0.94–2.36	0.09
Perineural invasion				
ne0, 1	49	1		
ne2, 3	53	1.20	0.75–1.91	0.45
pTNM stage				
I, II, III	47	1		
IVa, IVb	55	2.44	1.50–3.95	<0.001
LI-cadherin staining				
+	38	1		
–	64	2.48	1.51–4.09	<0.001

CI: confidence interval.

Table 3. Multivariate analysis of overall survival

	Number of cases	Hazard ratio	95% CI	P value
Gender				
Male	63	1		
Female	39	0.93	0.56–1.54	0.79
Age (years)				
<65	52	1		
≥65	50	1.29	0.80–2.08	0.30
Tumor size (cm)				
<3.6	47	1		
≥3.6	55	1.32	0.78–2.22	0.30
Histologic differentiation				
Well	38	1		
Moderately, poorly	64	1.34	0.76–2.35	0.31
Venous invasion				
v0, 1	63	1		
v2, 3	39	1.09	0.60–1.96	0.78
Lymphatic invasion				
ly0, 1	56	1		
ly2, 3	46	1.15	0.65–2.03	0.64
Perineural invasion				
ne0, 1	49	1		
ne2, 3	53	0.89	0.51–1.56	0.69
pTNM stage				
I, II, III	47	1		
IVa, IVb	55	1.90	1.11–3.24	0.02
LI-cadherin staining				
+	38	1		
–	64	2.04	1.16–3.61	0.01

CI: confidence interval.

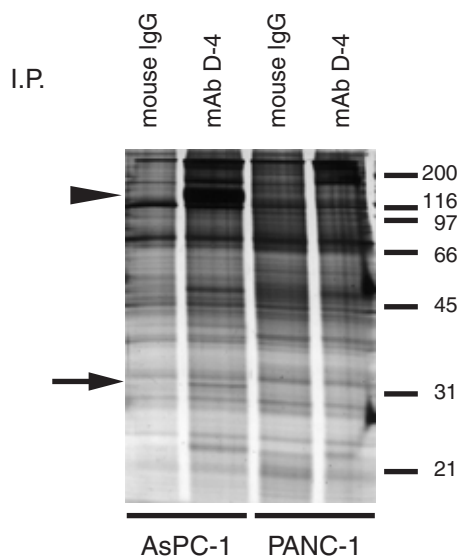


Fig. 4. Detection of protein coimmunoprecipitated with LI-cadherin. A nonreducing, silver-stained SDS-PAGE gel of protein immunoprecipitated with either normal mouse IgG or mAb D-4 is shown. A 30-kD protein (arrow) coimmunoprecipitated with mAb D-4 in AsPC-1 cells, but not in PANC-1 cells, a LI-cadherin-negative cell line. Molecular weight markers ($\times 10^{-3}$) are shown in the right margin. An arrowhead indicates the position of the LI-cadherin. These results are representative of three independent experiments.

lectin-3 by LC-MS/MS which identified three tryptic peptides (MLITILGTVKPNANR, QSVFPFESGKPKF and VAVNDAHLLQYNHR). In fact, galectin-3 was detected in LI-cadherin immune complexes immunoprecipitated with pAb anti-LI-cadherin as well as mAb D-4 (Fig. 5). Moreover, using immunohistochemical experiments, we and others have revealed that galectin-3 was frequently expressed in well-differentiated carcinomas, but its expression was decreased in moderately and poorly differentiated carcinomas (Fig. 2D).^{19, 22} This result indicates that the expression pattern of galectin-3 is very similar to that of LI-cadherin in ductal adenocarcinoma of the pancreas (Fig. 2C), and this interaction is likely not only *in vitro*, but also *in vivo*.

Galectin-3 binds to LI-cadherin in a carbohydrate-dependent manner. Next, we tested whether the interaction between LI-cadherin and galectin-3 is mediated by the carbohydrate recognition domain of galectin-3; thus, the effect of lactose, a competitive disaccharide of galectin-3 carbohydrate recognition domain, on galectin-3 binding to LI-cadherin was assayed. In the presence of lactose, binding of galectin-3 to LI-cadherin was inhibited in a dose-dependent manner, but in the presence of sucrose, a noncompetitive disaccharide, their binding was not inhibited (Fig. 6).

Discussion

This is the first study to investigate the clinico-pathologic significance of LI-cadherin expression in ductal adenocarcinoma of the pancreas. Consistent with the findings of others on gastric carcinomas,¹⁵ we observed that LI-cadherin expression was reduced in less differentiated areas and poorly differentiated carcinomas exhibiting invasive growth, compared with well-differentiated carcinomas in pancreatic carcinoma. This observation is also in good agreement with some reports about E-cadherin,^{23, 24} and supports the previous reports that LI-cadherin is capable of mediating cell-cell adhesion as well as the classic cadherins.^{12, 17} In normal pancreatic ducts, we observed that LI-

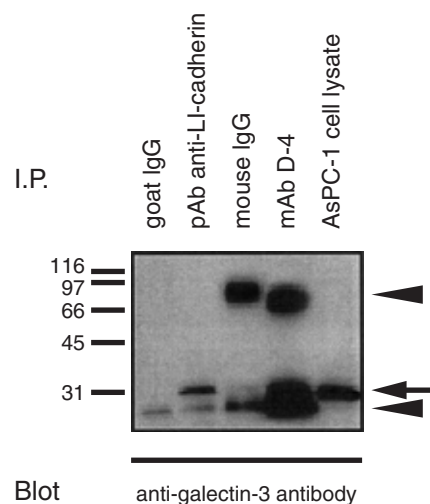


Fig. 5. Interaction of LI-cadherin with galectin-3. Galectin-3 was detected in LI-cadherin immune complexes precipitated either with pAb anti-LI-cadherin or mAb D-4, but not with control Abs. Molecular weight markers ($\times 10^{-3}$) are shown in the left margin. An arrow indicates the position of galectin-3. Arrowheads indicate the position of the immunoglobulin heavy and light chains. These results are representative of three independent experiments.

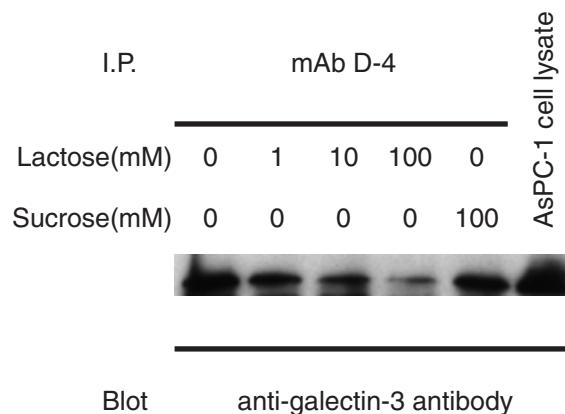


Fig. 6. LI-cadherin interacts with galectin-3 in a carbohydrate-dependent manner. Lysates from AsPC-1 cells were incubated with or without various concentrations of lactose (1, 10, 100 mM) and 100 mM sucrose, and immunoprecipitated with mAb D-4. Binding of galectin-3 to LI-cadherin was inhibited by lactose in a dose-dependent manner, but not by sucrose. These results are representative of three independent experiments.

cadherin was expressed focally and weakly in about half of the cases. On the other hand, E-cadherin was expressed uniformly in almost all the normal pancreatic ducts as reported previously (data not shown).²⁴ These observations suggest that in normal pancreatic ducts, LI-cadherin plays an auxiliary role in cell-cell adhesion.

We have found that LI-cadherin expression was associated with histologic differentiation and pTNM stage, which are important prognostic factors in pancreatic carcinoma.⁶ Moreover, the survival analysis showed that the prognosis for survival was significantly poorer in the LI-cadherin-negative tumors compared with the LI-cadherin-positive tumors, in both univariate and multivariate analyses. These observations suggest that LI-cadherin expression is a good predictor for the prognosis of patients with ductal adenocarcinoma of the pancreas.

In the present study, we found that galectin-3 was coimmu-

noprecipitated with LI-cadherin. Galectins are a family of proteins defined by having at least one characteristic carbohydrate recognition domain with an affinity for β -galactosides, and sharing certain conserved sequence elements.^{25–27} At least 10 galectins have now been identified in mammals, and galectin-3 has been the best characterized and studied among them. Galectin-3 is presumed to be involved in multiple biological processes, such as cell-cell and cell-matrix interactions, cell proliferation, differentiation, cell cycle regulation, angiogenesis, apoptosis resistance and metastasis.^{28–35} In pancreatic carcinoma, we have previously demonstrated that decreased expression of galectin-3 was associated with an advanced stage, tumor de-differentiation.¹⁹ In this study, we have found that the expression pattern of galectin-3 is very similar to that of LI-cadherin. Therefore, we speculate that LI-cadherin interacts with galectin-3 not only *in vitro*, but also *in vivo*, and this interaction may have some role in ductal adenocarcinoma of the pancreas.

Galectin-3 is known to interact with cell surface proteins, such as laminin, carcinoembryonic antigen, and $\alpha\beta 1$ integrin

via its carbohydrate recognition domain.^{28–30} We have demonstrated that the interaction between LI-cadherin and galectin-3 is mediated by the carbohydrate recognition domain. Thus, we speculate that galectin-3 binds to LI-cadherin on the cell surface of pancreatic carcinoma. Further study is necessary to understand the cellular changes induced by this interaction between LI-cadherin and galectin-3.

In conclusion, we have shown in this study that the expression of LI-cadherin could be useful as a marker for predicting the outcome of patients with resectable ductal adenocarcinoma of the pancreas. LI-cadherin might participate in the early stage of this disease through interacting with galectin-3.

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- Jemal A, Thomas A, Murray T, Thun M. Cancer statistics, 2002. *CA Cancer J Clin* 2002; **52**: 23–47.
- Statistics and Information Department, Minister's Secretariat. Vital statistics of Japan. Tokyo: Ministry of Health, Labour and Welfare; 1999. p. 284–99.
- Conlon KC, Klimstra DS, Brennan MF. Long-term survival after curative resection for pancreatic ductal adenocarcinoma. Clinicopathologic analysis of 5-year survivors. *Ann Surg* 1996; **223**: 273–9.
- Geer RJ, Brennan MF. Prognostic indicators for survival after resection of pancreatic adenocarcinoma. *Am J Surg* 1993; **165**: 68–72.
- Yeo CJ, Cameron JL, Lillemoe KD, Sitzmann JV, Hruban RH, Goodman SN, Dooley WC, Coleman J, Pitt HA. Pancreaticoduodenectomy for cancer of the head of the pancreas. 201 patients. *Ann Surg* 1995; **221**: 721–31.
- Roder JD, Ott K. Cancer of the pancreas. In: Gospodarowicz MK, Henson DE, Hutter RVP, O'Sullivan B, Sobin LH, Wittekind Ch, editors. Prognostic factors in cancer. 2nd ed. New York: Wiley-Liss; 2001. p. 333–47.
- Ranscht B. Cadherins and catenins: interactions and functions in embryonic development. *Curr Opin Cell Biol* 1994; **6**: 740–6.
- Takeichi M. Morphogenetic roles of classic cadherins. *Curr Opin Cell Biol* 1995; **7**: 619–27.
- Takeichi M. Cadherins: a molecular family important in selective cell-cell adhesion. *Annu Rev Biochem* 1990; **59**: 237–52.
- Takeichi M. Cadherins in cancer: implications for invasion and metastasis. *Curr Opin Cell Biol* 1993; **5**: 806–11.
- Hirohashi S. Inactivation of the E-cadherin-mediated cell adhesion system in human cancers. *Am J Pathol* 1998; **153**: 333–9.
- Berndorff D, Gessner R, Kreft B, Schnoy N, Lajous-Petter AM, Loch N, Reutter W, Hortsch M, Tauber R. Liver-intestine cadherin: molecular cloning and characterization of a novel Ca(2+)-dependent cell adhesion molecule expressed in liver and intestine. *J Cell Biol* 1994; **125**: 1353–69.
- Dantzig AH, Hoskins JA, Tabas LB, Bright S, Shepard RL, Jenkins IL, Duckworth DC, Sportsman JR, Mackensen D, Rostek PR Jr, Skatrud PL. Association of intestinal peptide transport with a protein related to the cadherin superfamily. *Science* 1994; **264**: 430–3.
- Gessner R, Tauber R. Intestinal cell adhesion molecules. Liver-intestine cadherin. *Ann NY Acad Sci* 2000; **915**: 136–43.
- Grotzinger C, Kneifel J, Patschan D, Schnoy N, Anagnostopoulos I, Faiss S, Tauber R, Wiedenmann B, Geßner R. LI-cadherin: a marker of gastric metaplasia and neoplasia. *Gut* 2001; **49**: 73–81.
- Nagafuchi A, Takeichi M. Cell binding function of E-cadherin is regulated by the cytoplasmic domain. *EMBO J* 1988; **7**: 3679–84.
- Kreft B, Berndorff D, Bottinger A, Finnemann S, Wedlich D, Hortsch M, Tauber R, Geßner R. LI-cadherin-mediated cell-cell adhesion does not require cytoplasmic interactions. *J Cell Biol* 1997; **136**: 1109–21.
- Watanabe M, Hirohashi S, Shimosato Y, Ino Y, Yamada T, Teshima S, Sekine T, Abe O. Carbohydrate antigen defined by a monoclonal antibody raised against a gastric cancer xenograft. *Jpn J Cancer Res* 1985; **76**: 43–52.
- Shimamura T, Sakamoto M, Ino Y, Shimada K, Kosuge T, Sato Y, Tanaka K, Sekihara H. Clinicopathological significance of galectin-3 expression in ductal adenocarcinoma of the pancreas. *Clin Cancer Res* 2002; **8**: 2570–5.
- Sobin LH, Wittekind Ch. TNM classification of malignant tumours. 5th ed. New York: Wiley-Liss; 1997.
- Japan Pancreas Society. Classification of pancreatic cancer. Tokyo: Kanehara Shuppan Co., Ltd.; 1996.
- Berberat PO, Friess H, Wang L, Zhu Z, Bley T, Frigeri L, Zimmermann A, Buchler MW. Comparative analysis of galectins in primary tumors and tumor metastasis in human pancreatic cancer. *J Histochem Cytochem* 2001; **49**: 539–49.
- Pignatelli M, Ansari TW, Gunter P, Liu D, Hirano S, Takeichi M, Kloppel G, Lemoine NR. Loss of membranous E-cadherin expression in pancreatic cancer: correlation with lymph node metastasis, high grade, and advanced stage. *J Pathol* 1994; **174**: 243–8.
- Gunji N, Oda T, Todoroki T, Kanazawa N, Kawamoto T, Yuzawa K, Scarpa A, Fukao K. Pancreatic carcinoma: correlation between E-cadherin and alpha-catenin expression status and liver metastasis. *Cancer* 1998; **82**: 1649–56.
- Barondes SH, Cooper DN, Gitt MA, Leffler H. Galectins. Structure and function of a large family of animal lectins. *J Biol Chem* 1994; **269**: 20807–10.
- Barondes SH, Castronovo V, Cooper DN, Cummings RD, Drickamer K, Feizi T, Gitt MA, Hirabayashi J, Hughes RC, Kasai K, Poirier F, Raz A, Rigby P, Rini JM, Wang JL. Galectins: a family of animal beta-galactoside-binding lectins. *Cell* 1994; **76**: 597–8.
- Kasai K, Hirabayashi J. Galectins: a family of animal lectins that decipher glycocodes. *J Biochem (Tokyo)* 1996; **119**: 1–8.
- Ochieng J, Leite-Browning ML, Warfield P. Regulation of cellular adhesion to extracellular matrix proteins by galectin-3. *Biochem Biophys Res Commun* 1998; **246**: 788–91.
- Ohannesian DW, Lotan D, Thomas P, Jessup JM, Fukuda M, Gabius HJ, Lotan R. Carcinoembryonic antigen and other glycoconjugates act as ligands for galectin-3 in human colon carcinoma cells. *Cancer Res* 1995; **55**: 2191–9.
- Matarrese P, Fusco O, Tinari N, Natoli C, Liu FT, Semeraro ML, Malorni W, Iacobelli S. Galectin-3 overexpression protects from apoptosis by improving cell adhesion properties. *Int J Cancer* 2000; **85**: 545–54.
- Kim HR, Lin HM, Biliran H, Raz A. Cell cycle arrest and inhibition of anoikis by galectin-3 in human breast epithelial cells. *Cancer Res* 1999; **59**: 4148–54.
- Inohara H, Raz A. Functional evidence that cell surface galectin-3 mediates homotypic cell adhesion. *Cancer Res* 1995; **55**: 3267–71.
- Akahami S, Nangia-Makker P, Inohara H, Kim HR, Raz A. Galectin-3: a novel antiapoptotic molecule with a functional BH1 (NWGR) domain of Bcl-2 family. *Cancer Res* 1997; **57**: 5272–6.
- Nangia-Makker P, Honjo Y, Sarvis R, Akahami S, Hogan V, Pienta KJ, Raz A. Galectin-3 induces endothelial cell morphogenesis and angiogenesis. *Am J Pathol* 2000; **156**: 899–909.
- Song YK, Billiar TR, Lee YJ. Role of galectin-3 in breast cancer metastasis: involvement of nitric oxide. *Am J Pathol* 2002; **160**: 1069–75.