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LMO2 Is a Novel Predictive Marker for a Better Prognosis in Pancreatic Cancer[1]

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Abstract

PURPOSE: LIM domain only 2 (LMO2) has been identified as a novel oncogene associated with carcinogenesis and better prognosis in several malignant tumors. We investigate the involvement of LMO2 in pancreatic cancer. *EXPERIMENTAL DESIGN:* We evaluated LMO2 expression in cultured cells, bulk tissues, and microdissected cells from pancreatic cancers by quantitative reverse transcription–polymerase chain reaction and immunohistochemistry. *RESULTS:* Of 164 pancreatic cancers, 98 (60%) were positive for LMO2 expression. LMO2 was more frequently detected in high-grade pancreatic intraepithelial neoplasia (PanIN) lesions (PanIN-2 and -3) than in low-grade PanIN lesions (PanIN-1A and -1B; $P < .001$) and was not detected in normal pancreatic ductal epithelium. The LMO2 messenger RNA levels were significantly higher in invasive ductal carcinoma cells than in normal pancreatic cells as evaluated by quantitative reverse transcription–polymerase chain reaction analyses of microdissected cells ($P = .036$). We also found higher incidence of LMO2 expression in histologic grade G1/G2 cancers than in grade G3 cancers ($P < .001$). The median survival time of LMO2-positive patients was significantly longer than that of LMO2-negative patients ($P < .001$), and multivariate analyses revealed that high LMO2 expression was an independent predictor of longer survival (risk ratio, 0.432, $P < .001$). Even among patients with a positive operative margin, LMO2-positive patients had a significant survival benefit compared with LMO2-negative patients. We further performed a large cohort study ($n = 113$) to examine the LMO2 messenger RNA levels in formalin-fixed paraffin-embedded samples and found similar results. *CONCLUSIONS:* LMO2 is a promising marker for predicting a better prognosis in pancreatic cancer.

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Introduction

Pancreatic cancer is the fourth leading cause of cancer-related death in Western countries and has the lowest patient survival rate of any solid cancer [1–3]. Recently, although the cancer death rates of most malignancies have decreased owing to improvements in early detection and treatment, the overall 5-year survival of patients with pancreatic cancer has only slightly increased from 3% to 5% [1] because of difficulties in the diagnosis of pancreatic cancer at early stages. Surgical resection is the only curative treatment of pancreatic cancer, and the survival rate for patients with a negative operative margin status (R0) is significantly higher than that for patients with positive operative margin status (R1 and R2) [4]. However, some patients with a positive operative margin survive longer than those with a negative operative margin, and a more aggressive surgical approach may be justified for patients with a probability of such a response after resection,

Abbreviations: LMO2, LIM domain only 2; qRT-PCR, quantitative reverse transcription–polymerase chain reaction; PanIN, pancreatic intraepithelial neoplasia; IDC, invasive ductal carcinoma; FFPE, formalin-fixed paraffin-embedded; DLBCL, diffuse large B-cell lymphoma; PIN, prostatic intraepithelial neoplasia

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even if the tumor is large and locally invasive. Conversely, the operation could be avoided if no surgical benefit can be predicted preoperatively. Therefore, we would like to identify a novel marker for predicting the prognosis of each patient.

The LIM domain only (LMO) proteins comprise one of the LIM domain-containing protein families and possess only two tandem LIM domains. These proteins act as adaptors for the assembly of large multiprotein complexes and play critical roles in both normal development and oncogenesis [5,6]. There are four members of the LMO family (*LMO1*, *LMO2*, *LMO3*, and *LMO4*), which have been recently identified and reported to be oncogenes [7–10]. LMO2 was also detected in several hematolymphoid neoplasias [11] and correlated with a good prognosis in diffuse large B-cell lymphoma (DLBCL) [12]. Recently, LMO2 was reported to play an important role in prostate cancer progression, and its expression was associated with the grade of prostatic intraepithelial neoplasia (PIN), the premalignant lesion of prostate cancer. These data suggest that LMO2 is associated with carcinogenesis and prognosis in several malignancies.

Similar to PIN, pancreatic intraepithelial neoplasia (PanIN) has been reported to be a premalignant lesion for conventional pancreatic cancer [13], and the gradual accumulation of molecular abnormalities supports this progression model. However, the involvement of LMO2 in pancreatic cancer remains to be investigated. Therefore, identifying the involvement of LMO2 in pancreatic cancer may be helpful toward understanding the mechanism of pancreatic carcinogenesis and progression and contribute to the detection of a biomarker for the diagnosis of pancreatic cancer or selection of therapy based on the features of individual tumors.

In the present study, we analyzed LMO2 expression in a large cohort of patients with pancreatic cancer. We focused on its prognostic and clinicopathological features using immunohistochemical staining and evaluation of messenger RNA (mRNA) extracted from formalin-fixed paraffin-embedded (FFPE) samples. We also examined its expression in PanIN lesions to investigate the involvement of LMO2 in pancreatic carcinogenesis. Our data suggest that LMO2 is associated with a better prognosis in pancreatic cancer.

Materials and Methods

Clinical Samples

A total of 164 patients with pancreatic ductal adenocarcinomas underwent surgical resection at the Department of Surgery and Oncology, Kyushu University Hospital (Fukuoka, Japan) and its affiliated hospitals. The patients consisted of 103 men and 61 women with a median age of 66 years (range, 36-86 years). The median duration of follow-up was 14 months (range, 1-101 months). We also analyzed 41 patients with unresectable pancreatic cancer due to local invasion or distant metastasis. All tumors were staged according to the TNM classification system of the International Union against Cancer [14]. Histologic grading of the tumors and diagnosis of PanIN lesions were performed according to the World Health Organization's classification system [15]. Other pathological variables (lymphatic invasion, vascular invasion, and perineural invasion) were based on the Japan Pancreas Society's classification [16]. Patients with mucinous cystadenocarcinoma or intraductal papillary mucinous carcinoma were excluded from the study. Surgical specimens were fixed in 10% formalin and embedded in paraffin. The paraffin-embedded samples were serially sectioned at 4-μm thickness, mounted on slides, and stained with hematoxylin and eosin for histologic analysis. For quantitative reverse transcription–polymerase chain reaction (qRT-PCR) studies, 22 fresh-frozen samples were obtained from cancerous lesions of resected pancreases from patients with primary pancreatic invasive ductal carcinoma (IDC) and 7 normal tissue samples were taken from intact pancreatic tissue resected for bile duct cancer or a pancreatic endocrine tumor. The tissue samples were embedded in OCT compound (Sakura, Tokyo, Japan) as soon as possible after resection and stored at −80°C until analysis. This study was performed in accordance with the principles embodied in the Declaration of Helsinki. The study was also 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.

Cell Lines and Primary Cultures of Pancreatic Epithelial Cells

Normal human pancreatic epithelial cells were obtained from Cell Systems (Kirkland, WA) and maintained in CS-C medium containing 10% fetal calf serum according to the instructions of the supplier. Eleven pancreatic cancer cell lines, namely, ASPC-1, KP-1N, KP-2, KP-3, PANC-1, SUIT-2 (provided by Dr. H. Iguchi, National Shikoku Cancer Center, Matsuyama, Japan), MIA-PaCa2 (Japanese Cancer Resource Bank, Tokyo, Japan), Capan-1, Capan-2, CFPAC-1, and SW1990 (American Type Culture Collection, Manassas, VA), were used. A human pancreatic ductal epithelial cell line (HPDE6-E6E7 clone 6) immortalized by transduction with the *E6/E7* genes of human papillomavirus 16 was kindly provided by Dr. Ming-Sound Tsao (University of Toronto, Toronto, Ontario, Canada). Cells were maintained as described previously [17,18].

Immunohistochemical Procedures and Evaluation

Sections were cut at 4-μm thickness from paraffin-embedded material, deparaffinized in xylene, and rehydrated through a graded ethanol series. Endogenous peroxidase activity was blocked by incubating with 3% hydrogen peroxide in methanol for 30 minutes. Antigen retrieval was achieved by microwaving the sections in citrate buffer at pH 6.0. A Histofine SAB-PO kit (Nichirei, Tokyo, Japan) was used for immunohistochemical labeling. Each section was exposed to 10% nonimmunized rabbit serum for 10 minutes to block nonspecific binding of the antibodies, followed by incubation with a goat polyclonal anti-LMO2 primary antibody (AF2726; R&D Systems, Minneapolis, MN; 1:100 dilution) at 4°C overnight. The sections were then sequentially incubated with a biotinylated anti–goat immunoglobulin solution for 20 minutes followed by peroxidase-labeled streptavidin for 20 minutes. The reaction products were visualized using 3,3′-diaminobenzidine as a chromogen followed by nuclear counterstaining with hematoxylin. In the present study, cytoplasmic and nuclear immunoreactivities were detected in the tumor cells. The proportion of LMO2-positive cells was evaluated using the following scale according to the percentage of LMO2-positive tumor cells: negative, 0; less than 10%, 1+; 10% to 50%, 2+; greater than 50%, 3+. The LMO2 expression in tumor cells was defined as positive when 10% of the tumor cells or greater were stained (scores 2+ and 3+) and negative when less than 10% of the tumor cells were stained (scores 0 and 1+). All slides were evaluated independently by three investigators (K.N., Y.M., and A.H.) without any knowledge of the clinical features of each case.

RNA Isolation from Microdissected and FFPE Samples

Frozen tissue samples were cut into 5-μm-thick sections. One section from each sample was stained with hematoxylin and eosin for

histologic examination. Invasive ductal carcinoma cells from 11 lesions, PanIN-2 cells from 2 lesions, and normal pancreatic ductal epithelial cells from 5 lesions were isolated selectively using a laser microdissection and pressure catapulting system (PALM Microlaser Technologies, Bernried, Germany) in accordance with the manufacturer's protocols. Similar numbers of cells were isolated from sections of IDC lesions, PanIN lesions, and normal ductal epithelium. More than 500 cells could be obtained from each IDC section, whereas 3 to 10 sections were needed to isolate sufficient normal ductal epithelial cells and PanIN cells owing to the lower numbers of cells per section. After the microdissection, total RNA was extracted from the selected cells and subjected to qRT-PCR for quantification of LMO2 as described previously [19].

For analysis of FFPE samples, all paraffin blocks were cut into 5-μm-thick sections. Macrodissection was performed using a safety blade to enrich the neoplastic cell population, and 3 to 10 sections were used for RNA extraction. Total RNA was extracted using an RNeasy FFPE Kit (Qiagen, Tokyo, Japan) with DNase I treatment according to the manufacturer's instructions.

Quantitative Assessment of LMO2 *mRNA Levels by One-step qRT-PCR*

Total RNA was extracted from bulk tissues using an RNeasy Mini Kit (Qiagen) and from pellets of cultured cells using a High Pure RNA Kit (Roche Diagnostics, Mannheim, Germany) with DNase I treatment (Roche Diagnostics) according to the corresponding manufacturer's instructions. We designed specific primers (*LMO2*: forward, 5′-CACCTGGAATGTTTCAAATGC-3′ and reverse, 5′-TCCTGTTCGCACACTATGTCA-3′; *18S rRNA*: forward, 5′-GTAACCCGTTGAACCCCATT-3′ and reverse, 5′-CCATCCAATCGGTAGTAGCG-3′) and performed BLAST searches to ensure the specificity of each primer. The extracts were analyzed by qRT-PCR using a QuantiTect SYBR Green RT-PCR Kit (Qiagen) and a Chrom4 Real-time PCR Detection System (Bio-Rad Laboratories, Hercules, CA). Each reaction mixture was first incubated at 50°C for 30 minutes to allow reverse transcription, in which first-strand complementary DNA was synthesized by priming total RNA with the same gene-specific primer (reverse). PCR was initiated by incubation at 95°C for 15 minutes to activate the polymerase, followed by 40 cycles of 94°C for 15 seconds, 58°C for 30 seconds, and 72°C for 30 seconds. Each primer set used in the present study produced a single prominent band of the expected size after electrophoresis. Each sample was analyzed twice, and any sample showing more than 10% deviation in the qRT-PCR values was tested a third time. The level of mRNA expression in each sample was calculated by reference to a standard curve generated using total RNA from the PANC-1 human pancreatic cancer cell line. Expression of *LMO2* mRNA was normalized by that of *18S rRNA* mRNA. Cutoff point selection for the *LMO2* mRNA was carried out by searching for a cut point yielding the smallest log-rank *P* value and divided to the high and low levels.

Statistical Analysis

All calculations were carried out using JMP 7.0.1 software (SAS Institute, Cary, NC). Data were analyzed by the Mann-Whitney *U* test if comparisons involved two groups because a normal distribution was not obtained. Survival curves were calculated by the Kaplan-Meier method, and differences between curves were analyzed by the log-rank test. The rates of positive LMO2 expression for clinicopathological variables were compared using the χ^2 test. We also conducted univariate and multivariate analyses of the prognostic factors with a survival analysis using the Cox proportional hazards model. All differences were considered to be statistically significant if $P < .05$.

Results

LMO2 *mRNA Expression Levels in Cultured Pancreatic Cancer Cells*

We investigated the levels of LMO2 mRNA expression in cultures of 11 different pancreatic cancer cell lines and in cultures of primary normal pancreatic ductal epithelial cells and HPDE cells. As shown in Figure 1, all 11 pancreatic cancer cell lines and the HPDE cells expressed LMO2 mRNA. However, the primary normal pancreatic ductal epithelial cells did not express LMO2 mRNA.

Quantitative Analyses of LMO2 *mRNA Expression Levels in IDC and Normal Pancreatic Tissues*

We measured the *LMO2* mRNA levels in 22 IDC bulk tissues and 7 normal pancreatic tissues. The *LMO2* mRNA expression levels were normalized by the *18S rRNA* mRNA expression levels as a reference gene. All the IDC bulk tissues expressed *LMO2* mRNA. However, normal pancreatic tissues expressed no or significantly lower levels of *LMO2* than IDC tissues (Figure 2*A*; $P < .001$). There were no significant associations between the *LMO2* mRNA levels and clinicopathological features such as tumor size, tumor stage, venous invasion, and differentiation (data not shown). However, the median *LMO2* mRNA level in G1/G2 pancreatic cancer tissues was higher than that in G3 pancreatic cancer tissues, although the difference did not reach statistical significance (Figure 2*B*; $P = .072$).

Quantitative Analyses of LMO2 *mRNA Expression Levels in Microdissected IDC and Normal Ductal Epithelial Cells*

As shown in Figure 2C, the LMO2 mRNA expression levels were significantly higher in IDC cells than in normal ductal epithelial cells ($P = .036$). This trend was consistent with the results of our bulk tissue analyses. We also analyzed the LMO2 mRNA levels in two PanIN-1B lesions and found that the mRNA levels were similar to

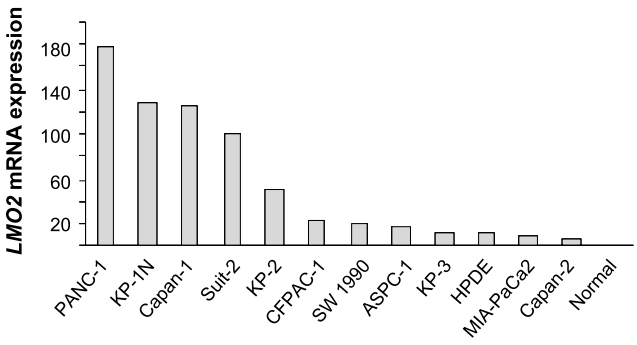

Figure 1. *LMO2* mRNA expression levels in 11 pancreatic cancer cell lines. The expression of *LMO2* mRNA was normalized by that of *18S RNA* mRNA. Values are expressed relative to 1.00 for expression in SUIT-2 cells. All 11 pancreatic cancer cells express *LMO2* mRNA, although the expression levels in the MIA-PaCa2 and Capan-2 cell lines are lower than those in HPDE cells.

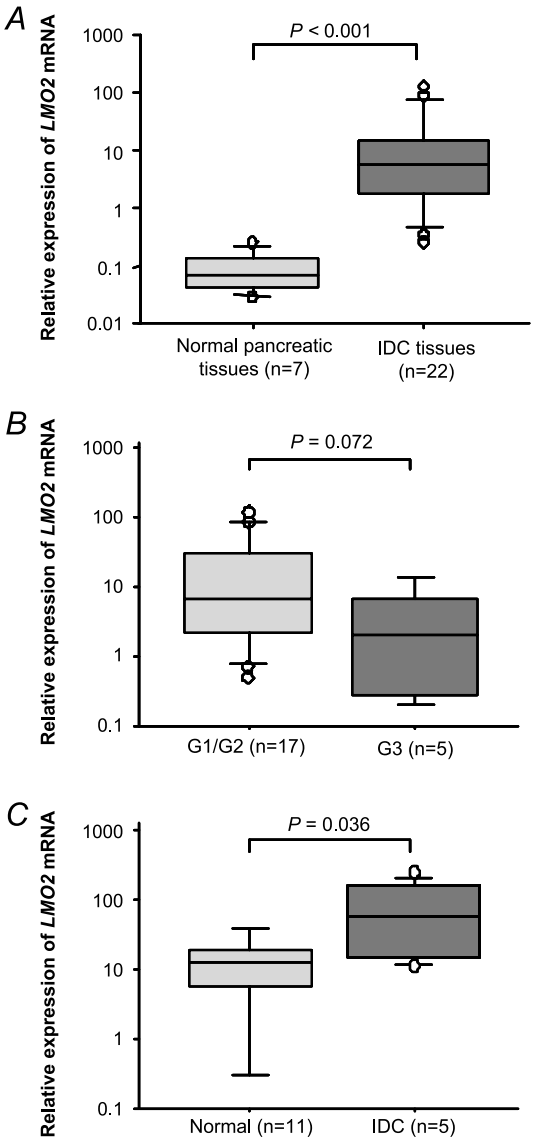

Figure 2. Relative expression levels of *LMO2* mRNA in bulk tissues and microdissected cells. Total RNA extracted from frozen bulk tissues and microdissected cells was subjected to qRT-PCR to measure the expression of *LMO2* mRNA. *18S rRNA* was used as a reference gene. The top and bottom horizontal lines indicate the 75th and 25th percentiles, respectively. The center horizontal lines represent the sample medians. The vertical lines drawn from the boxes extend to the 10th and 90th percentiles. (A) Relative *LMO2* mRNA expression levels in IDC and normal pancreatic tissues. (B) Relative *LMO2* mRNA expression levels in bulk pancreatic cancer tissues with G1/G2 and G3 histologic grades. (C) Relative *LMO2* mRNA expression levels in microdissected cells.

those in normal ductal epithelial cells. In a case with PanIN-1B and IDC lesions in the same section, high *LMO2* mRNA expression was detected in the IDC lesions but not in the PanIN-1B lesions (data not shown).

Immunohistochemical Patterns of LMO2 Expression in IDC and PanIN Lesions

Immunohistochemical staining for LMO2 was performed on pancreatic tissues. LMO2 expression was detected in IDC and PanIN lesions. However, LMO2 expression was not detected in normal pancreatic ductal epithelium (Figure 3*A*). LMO2 expression was evaluated in 164 IDC lesions and 30 PanIN lesions. It was detected in different grades of PanIN and IDC lesions as follows: PanIN-1A, 0% (0/9); PanIN-1B, 33% (3/9); PanIN-2, 80% (4/5); PanIN-3, 86% (6/7); IDC, 60% (98/164). LMO2 expression was significantly higher in high-grade PanIN lesions (PanIN-2 and -3) than in low-grade PanIN lesions (PanIN-1A and -1B; Table 1; $P < .001$). LMO2 expression was negative or very weak in PanIN-1A (Figure 3*B*) and PanIN-1B (Figure 3*C*) lesions but was moderate to high in PanIN-2 ($n = 5$; Figure 3*D*) and PanIN-3 ($n = 7$; Figure 3*E*) lesions. Among 164 cases of IDC, 98 (60%) were positive for LMO2 expression in the cytoplasm and nucleus of the carcinoma cells (Figure 3*F*). The relationships between LMO2 expression and various clinicopathological variables are summarized in Table 2. No significant relationships were found between LMO2 expression and age, sex, lymphatic invasion, lymph node metastasis, and depth of invasion. However, LMO2 expression had significant inverse associations with venous invasion ($P = .023$) and histologic grade ($P < .001$). A significantly higher proportion of tumors with a histologic grade of G1 or G2 (Figure 3*G*; 86/120, 72%) was LMO2-positive compared with tumors with a histologic grade of G3 (Figure 3*H*; 12/44, 27%, $P < .001$; Table 2).

Outcomes after Surgery and Prognostic Factors

We measured the *LMO2* mRNA levels in FFPE samples derived from 113 cases of pancreatic cancer (Figure 4*A*) and constructed survival curves based on both immunohistochemical staining and mRNA expression (Figure 4, *B* and *C*). Among the 164 patients with pancreatic cancer, the survival rates of patients with LMO2-positive cancer were significantly higher than those of patients with LMO2-negative cancer (Figure 4*B*; $P < .001$, log-rank test). Univariate analyses for overall survival identified LMO2 expression ($P < .001$), lymph node metastasis ($P < .001$), lymphatic invasion ($P < .001$), venous invasion ($P < .001$), and histologic grade ($P = .002$) as significant prognostic predictors. Age, sex, and depth of invasion had no prognostic value. Multivariate analyses of the same set of patients were performed for LMO2 expression and clinicopathological predictors of survival time. The results revealed that LMO2 expression was an independent favorable prognostic factor (Table 3; risk ratio, 0.432; 95% confidence interval (CI), 0.281-0.665; $P < .001$).

In accordance with the immunohistochemistry-based curves, the survival rates of patients with high levels of *LMO2* mRNA expression were significantly higher than those of patients with low levels of *LMO2* mRNA expression (Figure 4*C*; $P < .001$, log-rank test). We also analyzed *LMO2* mRNA normalized by β-actin and showed the same result (data not shown).

Comparisons between LMO2-Positive and LMO2-Negative Cases among Positive Operative Margin Cases

Among cases with a positive operative margin, the survival rates of LMO2-positive patients were significantly higher than those of LMO2-negative patients (Figure 5*A*; $P < .001$, log-rank test). Furthermore, the margin-positive/LMO2-positive group did not show any significant difference in survival rate compared with the margin-negative/LMO2-negative group (Figure 5*A*; $P = .250$, log-rank test), and the margin-positive/LMO2-negative group also did not show any significant difference in survival rate compared with the unresectable

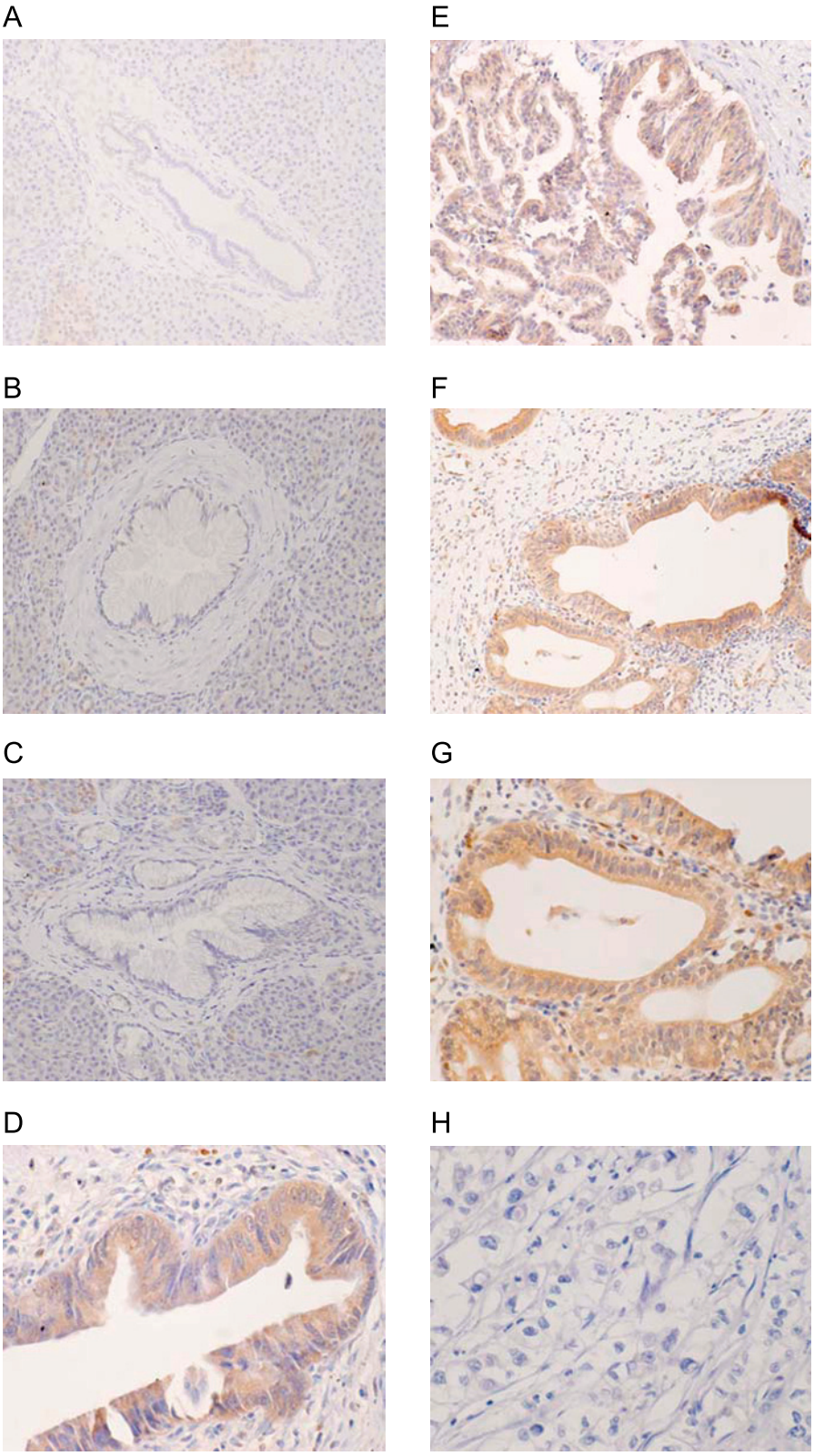

Figure 3. Representative microphotographs of LMO2 expression in pancreatic tissues. (A–C) LMO2 expression is not detected in the normal pancreatic ductal epithelium (A), PanIN-1A (B), and PanIN-1B (C) lesions. (D–F) A moderate to high expression is detected in PanIN-2 (D), PanIN-3 (E), and IDC (F) lesions. (G, H) LMO2 expression in lesions according to histologic differences. A well-differentiated adenocarcinoma (G) and a poorly differentiated adenocarcinoma (H) are shown.

Table 1. LMO2-Positive Ratio According to the Grade of PanIN.

	LMO2 Expression		*P*
	Positive	Negative	
Low-grade PanIN*	3	15	<.001
High-grade PanIN†	10	2	

*PanIN-1A and PanIN-1B.
†PanIN-2 and PanIN-3.

group (Figure 5*A*; $P = .226$, log-rank test). These data were consistent with those of FFPE sample-based mRNA analyses (Figure 5*B*).

Discussion

This is the first report regarding the involvement of LMO2 in pancreatic cancer. In the present study, *LMO2* mRNA expression levels were significantly higher in pancreatic cancer tissues than in normal tissues or cells in analyses of both bulk tissues and microdissected cells. These results were supported by findings that pancreatic cancer cell lines showed high levels of *LMO2* mRNA expression, whereas primary cultures of pancreatic normal epithelial cells did not express *LMO2* mRNA. HPDE cells showed a slight expression of *LMO2* mRNA because this cell line is immortalized by the infections of the retrovirus containing *E6* and *E7* genes of human papillomavirus 16. Therefore, its expression profile may be not exactly the same as that in normal pancreatic ductal cell.

We also performed immunohistochemical studies, and the results were consistent with those of the mRNA expression analyses. Furthermore, we found that LMO2 expression was significantly associated with a better prognosis.

Ma et al. [20] demonstrated LMO2 expression in premalignant lesions in prostate tissues, consistent with our present immunohistochemical and microdissection-based studies. Recently, PanIN-2 was suggested to be the earliest truly neoplastic lesion in the progression of pancreatic carcinogenesis, rather than PanIN-1B [21]. The frequency of LMO2 expression in IDC group was lower than that in high-grade PanIN group, which was possibly due to the low expression rates of LMO2 in G3 pancreatic tissues. In the present study, we observed accentuated expression of LMO2 in PanIN-2 lesions. However, LMO2 expression was also detected in 30% of PanIN-1B lesions, and it may therefore be difficult to use LMO2 as a clear marker to distinguish between PanIN-2 and PanIN-1B lesions.

Table 2. Relation between LMO2 Expression and Clinicopathological Characteristics in Pancreatic Cancer.

Variable	No. Cases	LMO2 Expression		Positive Rate	*P*
		Negative ($n = 66$)	Positive ($n = 98$)		
Age (years)					
<59	49	19	30	0.612	.802
>60	115	47	68	0.591	
Sex					
Male	103	45	58	0.563	.240
Female	61	21	40	0.656	
Lymph node metastasis					
Negative	52	17	35	0.673	.176
Positive	112	49	63	0.563	
Lymphatic invasion					
Negative	31	9	22	0.710	.151
Positive	133	57	76	0.571	
Venous invasion					
Negative	51	14	37	0.725	.023
Positive	113	52	61	0.540	
Histologic grading					
G1	40	8	32	0.800	<.001
G2	80	26	54	0.675	
G3	44	32	12	0.273	
Depth of invasion					
T1	8	3	5	0.625	.316
T2	9	4	5	0.556	
T3	142	55	87	0.613	
T4	5	4	1	0.200	

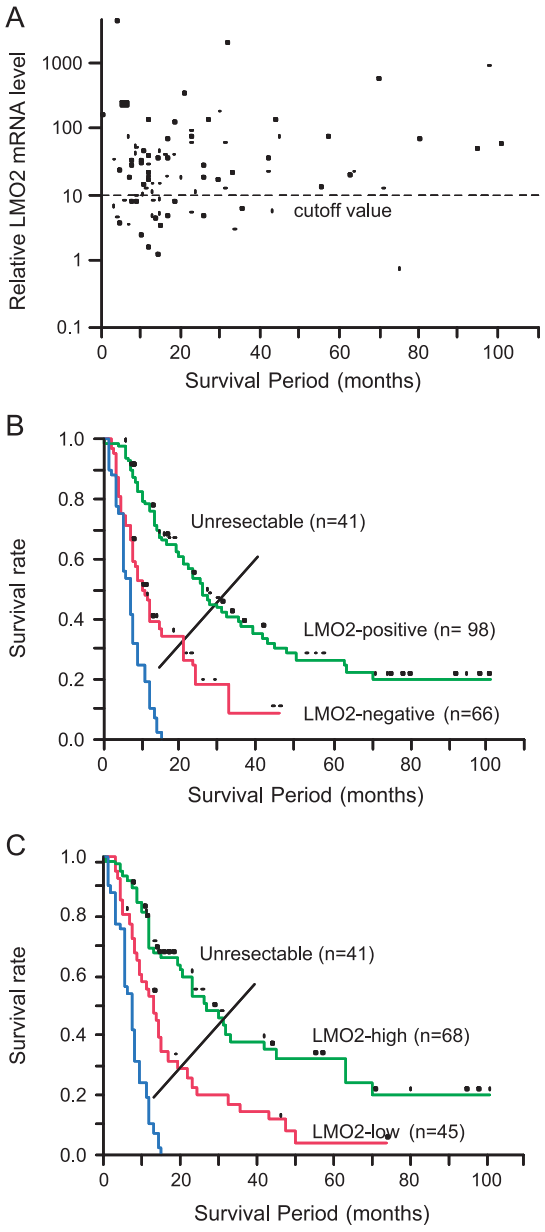

Figure 4. *LMO2* mRNA levels in FFPE samples derived from 113 cases of pancreatic cancer (A) and Kaplan-Meier survival curves for the patients. (B, C) Survival curves were created for LMO2-positive, LMO2-negative, and unresectable patients based on immunohistochemistry (B) and FFPE sample-based mRNA analyses (C). (B) $P < .001$, LMO2-positive patients *versus* LMO2-negative patients. $P < .001$, LMO2-negative patients *versus* unresectable patients. (C) $P < .001$, *LMO2* mRNA high patients *versus LMO2* mRNA low patients. $P < .001$, *LMO2* mRNA low patients *versus* unresectable patients.

Table 3. Prognostic Factors in Cox Proportional Hazards Model.

Variable	Univariate			Multivariate		
	Risk Ratio	95% CI	*P*	Risk Ratio	95% CI	*P*
Age (years)						
>60/<59	1.129	0.748-1.749	.572			.435
Sex						
Male/female	0.965	0.658-1.432	.859			.648
Depth of invasion						
T3, T4/T1, T2	1.470	0.805-3.012	.223			.900
Lymph node metastasis						
Positive/negative	1.996	1.316-3.105	.001			.059
Lymphatic invasion						
Positive/negative	2.719	1.553-5.228	<.001			.110
Venous invasion						
Positive/negative	2.705	1.747-4.340	<.001	1.943	1.174-3.328	.009
Histologic grading*						
G3/G1, G2	1.762	1.152-2.643	.010			.123
LMO2						
Positive/negative	0.398	0.267-0.596	<.001	0.432	0.281-0.665	<.001

*G1 and G2 were grouped for survival analysis.

In our study, multivariate analyses clearly showed that LMO2 expression was associated with a better prognosis in pancreatic cancer, consistent with a previous report that LMO2 expression is related with prolonged survival in DLBCL [12]. Alizadeh et al. [22] reported that LMO2 was expressed in germinal center B-like DLBCL, a DLBCL subtype with a better prognosis than DLBCL. They suggested that LMO2 may play a role in inhibiting the differentiation of the B-cell lineage and is related with the DLBCL phenotype malignancy. The present immunohistochemical analyses revealed that LMO2 expression was significantly correlated with lower histologic grades in pancreatic cancer. Conversely, Ma et al. suggested LMO2 expression was related with aggressive behavior and distant metastasis in prostate cancer, although its relation with prognosis was not described. Therefore, the function of LMO2 and its relation with prognosis might be different in each type of tumor.

In the present study, LMO2 expression was associated with a better prognosis in pancreatic cancer and its expression also influenced the survival rate of patients with a positive operative margin. Surgical resection is the only curative treatment of managing pancreatic cancer, and a negative operative margin was found to be associated with a greater overall survival compared with a positive operative margin [4]. Therefore, complete resection (R0) should be considered for each operation. However, the surgical margins are positive (R1 or R2) in many cases [23], especially cases with borderline resectable tumors defined according to the National Comprehensive Cancer Network. The National Comprehensive Cancer Network also comments that a uniform consensus of resectability has not yet been defined and that approaches to patients with locally invasive cancers differ among individual institutions. Nevertheless, patients with a positive operative margin sometimes survive longer than expected. In our analysis, the survival rate of patients with LMO2 expression was significantly longer than that of patients without LMO2 expression, even when the surgical margin was positive. Furthermore, the survival rates of margin-positive/LMO2-positive patients were as high as those of margin-negative/LMO2-negative patients. These findings suggest the possibility that the surgical approach for patients with borderline resectable tumors could be individualized by the level of LMO2 expression. Patients with LMO2-negative expression may not achieve any benefit from surgical resection, and then other treatments, such as chemoradiation, should be given to reduce the operative morbidity.

We also analyzed *LMO2* mRNA levels normalized by both 18S and β-actin to confirm the immunohistochemistry-based analyses. There are few reports about pancreatic cancer involving FFPE sample-based mRNA expression analyses in large cohorts. Formalin-fixed paraffin-embedded samples are usually associated with large amounts of clinicopathological data. Therefore, analyzing FFPE samples may be helpful for identifying the characteristics of tumors. Moreover, we have already reported the mRNA expression levels of several genes in pancreatic juice in studies to identify novel biomarkers for preoperative diagnosis of pancreatic cancer [24]. Therefore, analyses of *LMO2* mRNA levels in pancreatic cancer may be useful for estimating the operative benefit in patients with borderline resectable tumors.

In conclusion, we analyzed LMO2 expression in a large cohort of patients with pancreatic cancer. Our results have revealed that LMO2 is correlated with the prognosis of patients after resection of pancreatic cancer.

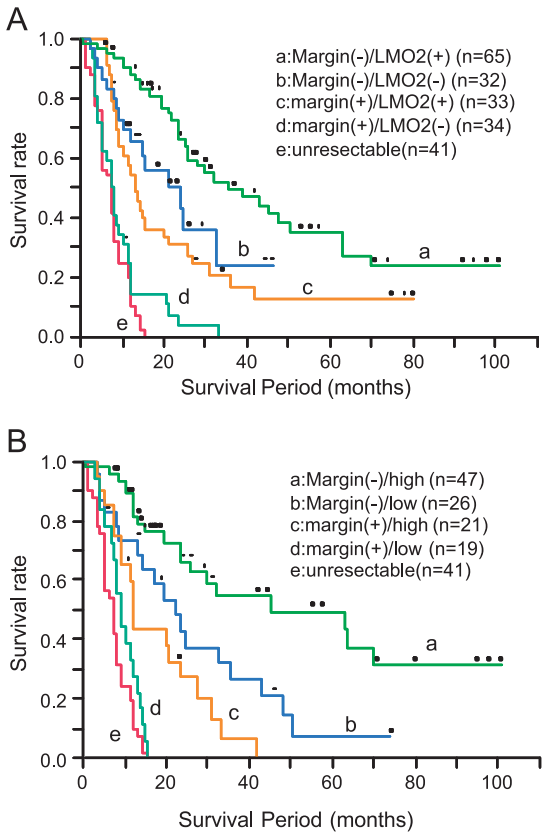

Figure 5. Kaplan-Meier survival curves for patients with positive/negative operative margins with and without LMO2 expression. Survival curves were created based on immunohistochemistry (A) and FFPE sample-based mRNA analyses (B). (A) $P = .250$, margin(+)/LMO2(+) patients *versus* margin(−)/LMO2(−) patients. $P < .001$, margin(+)/LMO2(+) patients *versus* margin(+)/LMO2(−) patients. $P = .226$, margin(+)/LMO2(−) patients *versus* unresectable patients. (B) $P = .071$, margin(+)/*LMO2* mRNA high patients *versus* margin(−)/*LMO2* mRNA low patients. $P = .011$, margin(+)/*LMO2* mRNA high patients *versus* margin(+)/*LMO2* mRNA low patients. $P = .116$, margin(+)/*LMO2* mRNA low patients *versus* unresectable patients.

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