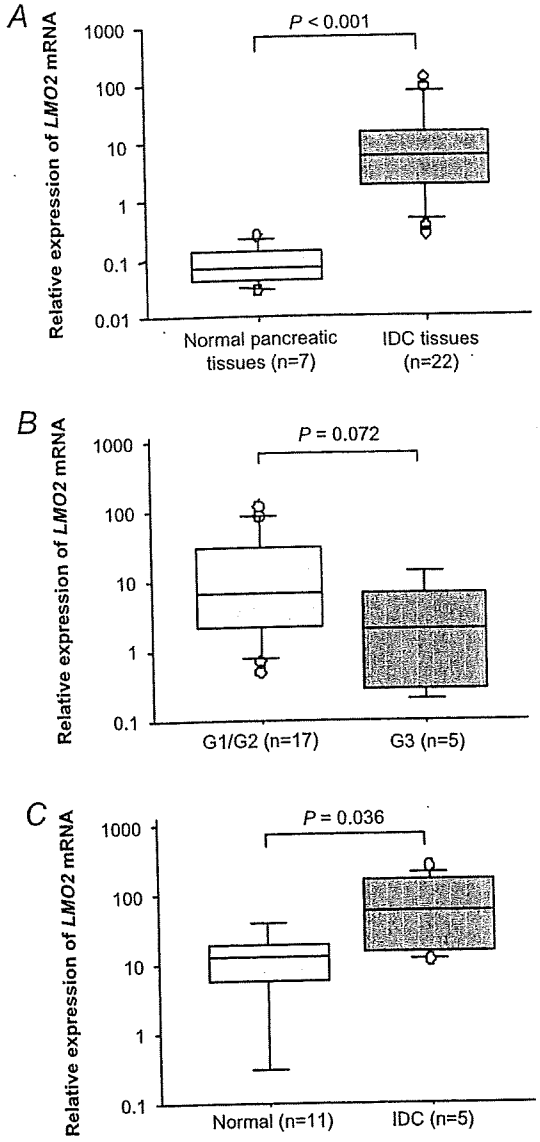

Figure 2. Relative expression levels of *LMO2* mRNA in bulk tissues and microdissected cells. Total RNA extracted from frozen bulk tissues and microdissected cells was subjected to qRT-PCR to measure the expression of *LMO2* mRNA. *18S rRNA* was used as a reference gene. The top and bottom horizontal 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

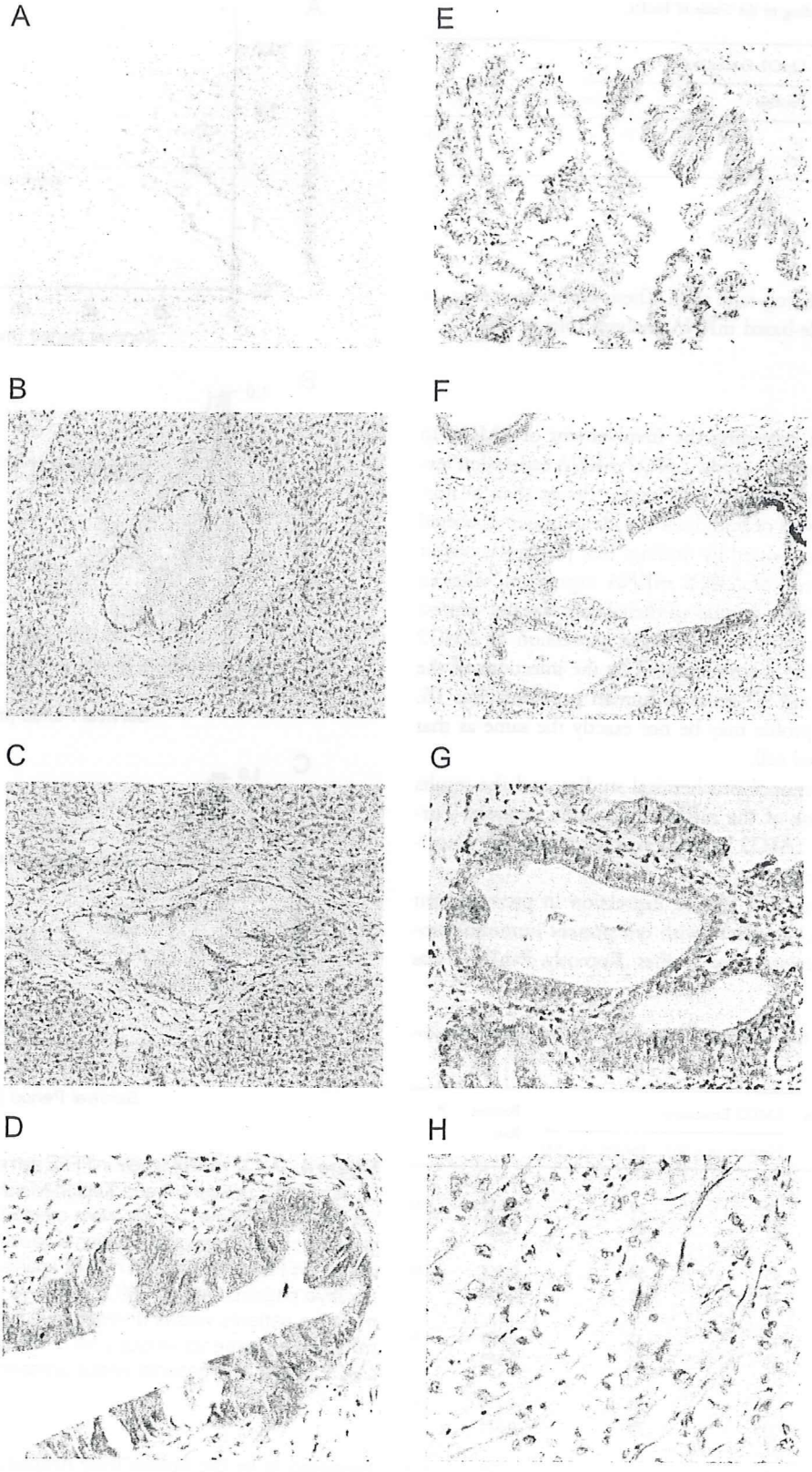

Figure 3. Representative microphotographs of LMO2 expression in pancreatic tissues. (A–C) LMO2 expression is not detected in the normal pancreatic ductal epithelium (A), PanIN-1A (B), and PanIN-1B (C) lesions. (D–F) A moderate to high expression is detected in PanIN-2 (D), PanIN-3 (E), and IDC (F) lesions. (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	

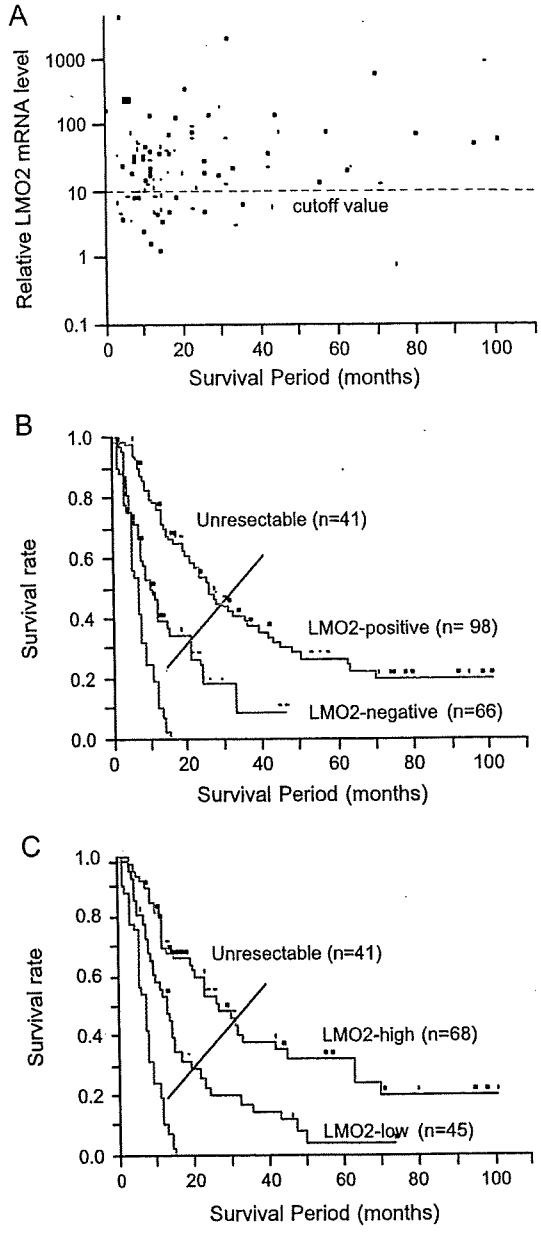

Figure 4. *LMO2* mRNA levels in FFPE samples derived from 113 cases of pancreatic cancer (A) and Kaplan-Meier survival curves for the patients. (B, C) Survival curves were created for LMO2-positive, LMO2-negative, and unresectable patients based on immunohistochemistry (B) and FFPE sample-based mRNA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

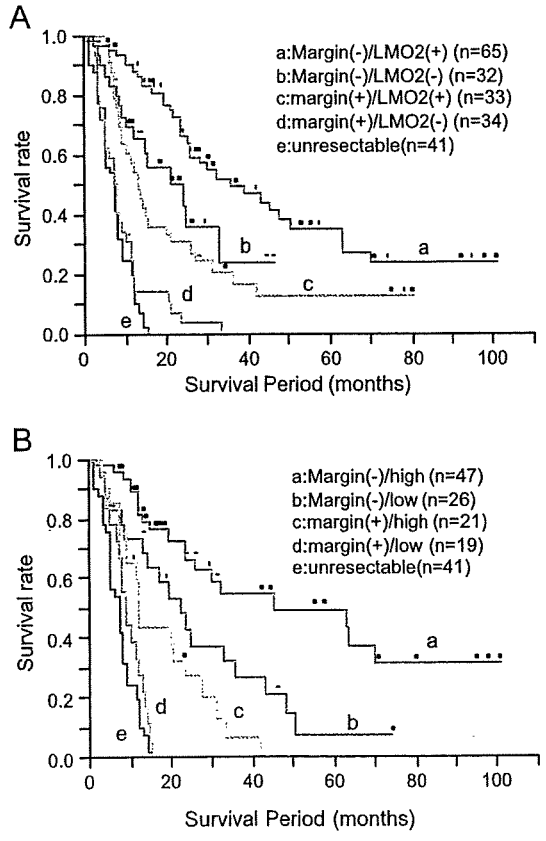

Figure 5. Kaplan-Meier survival curves for patients with positive/negative operative margins with and without LMO2 expression. Survival curves were created based on immunohistochemistry (A) and FFPE sample-based mRNA analyses (B). (A) $P = .250$, margin(+)/LMO2(+) patients *versus* margin(−)/LMO2(−) 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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SURGEON AT WORK

Spleen and gastrosplenic ligament preserving distal pancreatectomy under a minimum incision approach assisted by laparoscopy

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Abstract
Background As a modification of hand-assisted laparoscopic pancreatectomy, we devised a method of spleen and gastrosplenic ligament preserving distal pancreatectomy, in which pancreatic resection is performed under direct vision extracorporeally.
Methods The distal pancreas and spleen are pulled out of the peritoneal cavity through the minilaparotomy at the epigastrium following hand-assisted laparoscopic dissection of the distal pancreas. Spleen-preserving pancreatectomy is performed safely under direct vision. The gastrosplenic ligament is also preserved to prevent splenic volvulus after the operation. The transected main pancreatic duct is doubly ligated, and the transected pancreatic stump is sewn manually. The preserved spleen and splenic vessels are placed back in the peritoneal cavity after resection.
Results In the current study ($n = 3$), overall morbidity rate, including splenic volvulus and pancreatic fistula, was 0%.
Conclusion Preservation of the gastrosplenic ligament and extracorporeal preparation of the transected pancreatic stump under direct vision are useful measures in spleen-preserving distal pancreatectomy under a minimum incision approach assisted by laparoscopy.

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Keywords Laparoscopic pancreatectomy · Hand-assisted laparoscopic pancreatectomy · Spleen-preserving distal pancreatectomy · Pancreatic fistula · Minimum incision approach

Introduction

The advantages of laparoscopic surgery are obvious and the technique has been extended to pancreatic and splenic operations. Since 1994, various laparoscopic pancreatectomies, including pancreatoduodenectomy [1], enucleation [2, 3], and distal pancreatectomy [2, 4], have been performed. Nowadays, laparoscopic splenectomy, can be conducted safely even for splenomegaly due to portal hypertension [5]. Benign or low-grade malignant tumors of the pancreatic body or tail are a good indication for laparoscopic resection. Laparoscopic pancreatectomy, however, is still technically rather difficult because of the retroperitoneal position of the pancreas and the complex anatomical relationship between the pancreas and surrounding vessels. Thus, hand-assisted laparoscopic pancreatectomy is gaining recognition as a new and feasible technique that introduces a surgeon's hand into the abdominal cavity during laparoscopic surgery [6–8]. Closure of the residual pancreatic stump can be achieved through the minilaparotomy for hand assistance at the epigastrium [7, 8]. In addition, the dissected distal pancreas and spleen can also be pulled out of the peritoneal cavity through the minilaparotomy. Hence, as a modification of hand-assisted laparoscopic pancreatectomy, we devised a method of spleen and gastrosplenic ligament preserving limited pancreatectomy in which pancreatic resection is performed extracorporeally, after pulling out the distal pancreas and spleen.

Surgical technique

An 8-cm minilaparotomy incision is made in the middle upper abdomen. For obese patients, 10-cm laparotomy is better. An abdominal wall disc for hand assistance is placed at the site of the minilaparotomy. An ultrasonography probe can be inserted through this site for intrapancreatic imaging. Two trocars are then put in place. After abdominal access is established, the gastrocolic omentum is divided, and the splenic flexure of the colon is mobilized. The short gastric and left gastroepiploic vessels are not divided to prevent splenic volvulus after the operation. Retrosplenic Gerota's fascia is transected on the surface of the left kidney (Fig. 1a). Then, the posterior plane of Gerota's fascia is dissected in a lateral to medial direction, allowing the distal pancreas and spleen to be detached from retroperitoneum.

The distal pancreas and spleen are then pulled out of the peritoneal cavity through the minilaparotomy at the epigastrium for hand assistance (Fig. 2c). Spleen and gastrosplenic ligament preserving pancreatectomy is performed under direct vision. The advantage of this extracorporeal procedure is the safety and certainty in dissection of the splenic vessels (Fig. 1b) and preparation of the pancreatic stump. The transected main pancreatic duct is doubly ligated, and the transected pancreatic stump is sewn manually. The preserved spleen and splenic vessels are placed back in the peritoneal cavity after resection (Fig. 2d).

Outcome

From February 2007 through December 2008, three patients (two with intraductal papillary mucinous neoplasm

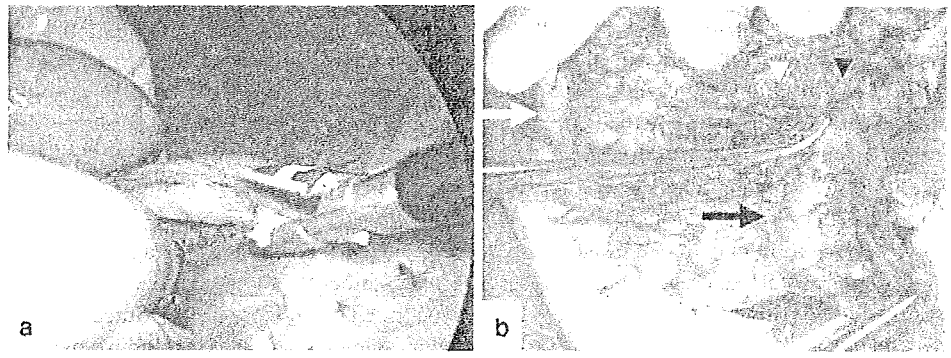

Fig. 1 Procedures. **a** Transection of retrosplenic Gerota's fascia on the surface of the left kidney under hand-assisted laparoscopic procedure, **b** dissection of the distal pancreas (*black arrow*) from the splenic artery (*white arrow head*) and vein (*black arrow head*), *white arrow* spleen

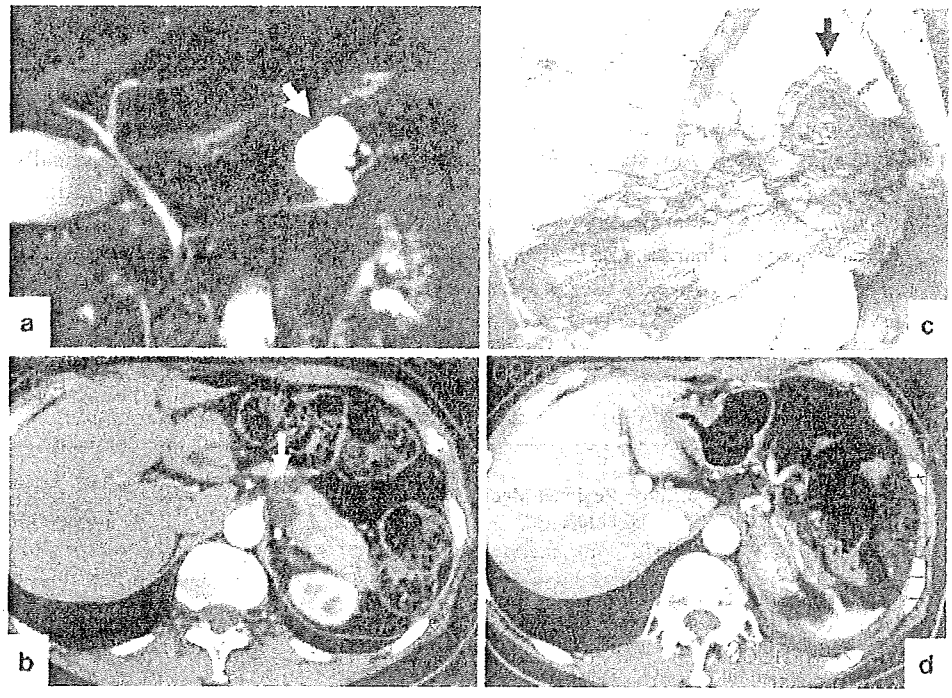

Fig. 2 A case of branched type intraductal papillary mucinous neoplasm who underwent spleen and gastrosplenic ligament preserving pancreatectomy. **a** MRCP, **b** CT before operation, **c** the dissected distal pancreas and tumor, **d** CT 1 week after operation, *arrow* tumor

and one with benign insulinoma) have been operated on following this technique. Among these cases, there was 0% morbidity including splenic volvulus and pancreatic fistula.

Discussion

Open pancreatic surgery requires a relatively large incision for a small lesion, and therefore the potential benefits of the laparoscopic approach are substantial. The most common indications for laparoscopic pancreatic resection are presumed benign pancreatic diseases, such as insulinoma or localized neuroendocrine neoplasms and branch type intraductal papillary mucinous neoplasms. The most common indication for laparoscopic pancreatic resection appears to be enucleations and distal pancreatectomy.

The successful management of the pancreatic stump remains the challenge of this procedure. In some laparoscopic enucleation studies, the rate for low volume pancreatic fistula is reported to be high [9]. This complication does not create an important problem as long as the main duct is not injured. Even though self-limiting, the pancreatic fistula formation rate remains high after either laparoscopic enucleation or resection. Pancreatic fistula after distal pancreatectomy has been a concern for decades, even in the era of laparoscopic pancreatectomy. Patterson et al. collected data from the literature on morbidity after open and laparoscopic pancreatic resections, and found that the rate of pancreatic fistula ranged from 20 to 33% after laparoscopic pancreatectomy and from 5 to 23% after open pancreatectomy [10]. The way in which the surgeon approaches the pancreatic transection seems to be important. Ninety-seven percent of the patients underwent laparoscopic transection of the pancreas by use of a stapling technique [9]. Closing the pancreatic stump with interrupted mattress sutures and selectively ligating the pancreatic duct, the usual practice in open surgery, are more difficult to replicate laproscopically. This factor could explain the high rate of pancreas-related complications. Hand-sewn parenchymal closure and duct ligation are an advantage of this extracorporeal pancreatic resection, to prevent pancreatic juice leakage, compared with the procedure done by laparoscopy only. We could safely and securely handle the pancreatic duct and fine branches of the splenic vessels under direct vision.

Distal pancreatectomy with preservation of the spleen was first reported in 1988 [11]. The advantage of preserving the spleen is obvious; it reduces the risk of postoperative severe inflammation and peripheral blood count aberration. Preserving the spleen has been a major procedure in distal pancreatectomy. Warshow reported a case of splenic abscess that occurred after sacrificing the splenic artery and vein [11]. Kimura et al. reported five patients successfully treated with splenic vessel-preserving distal pancreatectomy to maintain the blood supply to the spleen and to avoid splenic necrosis and abscess [12]. Spleen-preserving pancreatectomy has recently been shown to have a risk of complication comparable to that of standard pancreatectomy where the spleen is removed. Nevertheless, spleen-preserving pancreatectomy remains an uncommon and technically demanding operation, due to the difficulty of dissecting the distal pancreas from the splenic vessels. An advantage of our procedure is the safety it provides in dissecting the distal pancreas from the splenic vessels. The displacement of the spleen with the inherent risk of torsion or hemorrhage is another disadvantage of spleen-preserving pancreatectomy. If spleen-preserving pancreatectomy is performed, the spleen is often free in the abdomen, where it is prone to torsion or trauma. Various techniques have been described to reposition the spleen (splenopexy). Appu et al. [13] report a novel technique for splenic repositioning and fixation, using a peritoneal pocket. We experienced one case of splenic bleeding due to venous congestion after spleen-preserving pancreatic tail resection using Appu's splenopexy. Since that experience we have chosen to preserve the gastrosplenic ligament.

This approach is suitable for the very distal lesion of the pancreas. However, if the posterior plane of Gerota's fascia is dissected, this method could be applied to more proximal lesions as shown in Fig. 2. For obese patients, because pulling the distal pancreas and spleen out through a small laparotomy is difficult, a 10 cm incision is preferable. This procedure is applicable only for lesions in the pancreatic body and tail. For the benign head lesions, another approach should be conducted [14].

Preservation of the gastrosplenic ligament and extracorporeal preparation of the transected pancreatic stump and splenic vessels under direct vision are useful measures in spleen-preserving distal pancreatectomy under a minimal incision approach assisted by laparoscopy.

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Whole Stomach and Spleen Preserving Total Pancreatectomy: A New Surgical Technique for Pancreatic Cancer

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SUMMARY

Total pancreatectomy has been used to treat both benign and malignant diseases of the pancreas. The procedure of total pancreatectomy for invasive pancreatic cancer usually includes distal gastrectomy and splenectomy to prevent ischemic changes due to decreased blood supply. In this report, it was introduced a new technique of total pancreatectomy for invasive pancreatic cancer preserving both the whole stomach and spleen. The patient was a 61 year old man. Preoperative computed tomography (CT) showed a mass of tumor, measuring 23×18×25mm, located in the pancreatic head. It was tried, initially to perform pylorus-preserving pancreatoduodenectomy (PPPD). Repeated frozen section examination of the pancreatic stumps, however, revealed persistent cancer infiltration to the distal pancreas. Therefore, we altered the planned PPPD to total pancreatectomy preserving the whole stomach and spleen with severing both the splenic artery and vein at their origins. The postoperative course was uneventful. Enhanced CT following surgery showed sufficient blood supply to the whole stomach and spleen without any congestive changes of blood flow. This method is considered safe and useful for patients with both benign and malignant disease of the pancreas.

KEY WORDS: Total pancreatectomy; Pancreatic cancer; Preservation of the spleen; Preservation of the stomach

ABBREVIATIONS: Computed Tomography (CT); Gastroduodenal Artery (GDA); Left Epigastric Artery (LEGA); Left Gastric Artery (LGA); Pancreatoduodenectomy (PPPD); Right Epigastric Artery (REGA); Right Gastric Artery (RGA); Splenic Artery (SpA); Splenic Vvein (SpV)

INTRODUCTION

Total pancreatectomy for invasive pancreatic cancer usually includes distal gastrectomy and splenectomy to prevent ischemic changes due to decreased blood supply. Most reports of spleen preservation in pancreatic resection relate to distal pancreatectomy (1, 2, 3).[3] In this report, we introduce a new technique of total pancreatectomy for invasive pancreatic cancer preserving both the whole stomach and spleen.

CASE REPORT

A 61-year-old man was admitted to Tochigi Cancer Center Hospital with a complaint of epigastralgia. Laboratory results on admission were as follows : leukocyte count, 6380cells/mm^3 ; hemoglobin, 13.3g/dl; platelet count, 269000cells/mm^3; asparate aminotransferase, 15IU/L; alanine aminotransferase, 17IU/L; alkaline phosphatase, 303IU/L; carbohydrate antigen, 17U/ml; FBS 114mg/dl and HbA1c 5.3%. Laboratory tests for tumor markers revealed a carcinoembryonic antigen value of 1.7ng/ml, and a carbohydrate antigen 19-9 value of 61.5U/ml.

Ultrasonography revealed a hypoechoic mass of tumor in the head of the pancreas, measuring 23×18×25mm. The main pancreatic duct distal to the tumor was dilated to about 10mm in diameter. Helical dynamic CT revealed a poorly enhanced tumor with pooling of contrast medium in the delayed phase. There were no signs of distant and regional lymph node metastases. Endoscopic retrograde pancreatography showed the main pancreatic duct was interrupted 2 cm distal to the papilla vater. With a preoperative diagnosis of cancer of the pancreatic head, PPPD was planned.

At laparotomy, there was no evidence of peritoneal dissemination or liver metastasis. Following mobilization of the duodenum and pancreatic head, the right gastroepiproic and right gastric vessels were severed at the pylorus. The duodenum was divided 2cm distal to the pylorus. The pancreas was then divided at the left side of the portal vein. The cut edge of the proximal pancreas was submitted to frozen section examination and found to have cancer cells. Three additional cut surfaces of the distal pancreas were also found to have cancer cells by frozen section examination. Hence, we abandoned preservation of the distal pancreas and decided to perform total pancreatectomy. The splenic artery was divided at its origin and the splenic vein was divided at the confluence with the superior mesenteric vein. The distal portion of the splenic artery and vein were severed at the hilum of the spleen without retraction of the spleen from the retroperitoneum in order not to sacrifice retroperitoneal drainage veins of the spleen. The left gastric vein had already been cut during dissection of the

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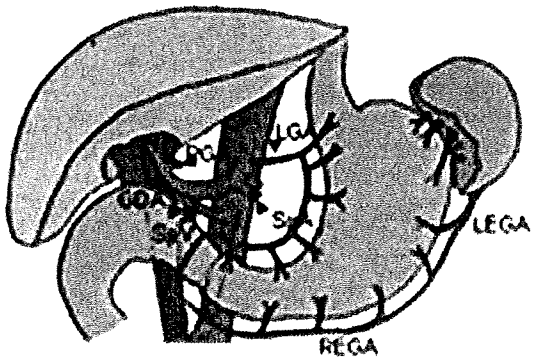

FIGURE 1
Removed part of the duodenum and total pancreas, splenic artery and vein. LGA: left gastric artery, RGA: right gastric artery, GDA: gastroduodenal artery, SpV: splenic vein, SpA: splenic artery, LEGA: left epigastric artery, REGA: right epigastric artery

lymph nodes around the common hepatic artery. The short gastric vessels were carefully preserved. Then, the blood supply to the whole stomach and spleen was only via the left gastric artery and their venous drainage was via the esophagus and retroperitoneum (**Figure 1**).

After confirming neither ischemia nor venous congestion of the whole stomach with preserved 1cm-long duodenum and spleen, gastrointestinal continuity was restored by end-to-end duodenojejunostomy and end-to-side choledochojejunostomy.

The postoperative course of this patient was uneventful. After 6 months from surgery, enhanced CT showed sufficient blood supply to the stomach and spleen without splenomegaly (**Figure 2**). Endoscopic examination revealed neither esophageal nor gastric varices. The blood sugar level was well controlled with routine self injection of insulin and hypoglycemic attack rarely occurred. This patient was able to maintain a good quality of life and accomplish planned adjuvant chemoradiotherapy. He died of liver metastasis 3 years after surgery without local recurrence.

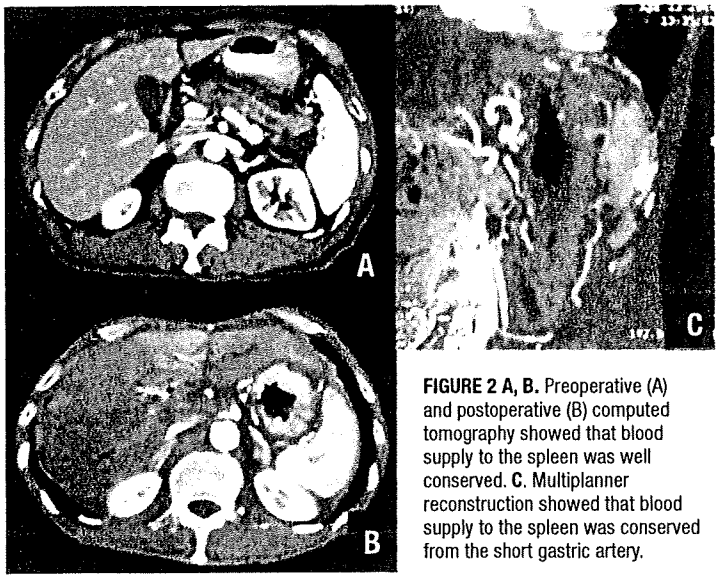

FIGURE 2 A, B. Preoperative (A) and postoperative (B) computed tomography showed that blood supply to the spleen was well conserved. **C.** Multiplanner reconstruction showed that blood supply to the spleen was conserved from the short gastric artery.

DISCUSSION

The significance of preservation of the spleen has been widely recognized, because it became apparent that severe inflammation often occurs after splenectomy in both children and adults. (1,4-6). Another advantage of spleen preservation is that a peripheral blood count keeps normal lesion, especially in the white cell or platelet counts (1,2). Furthermore, individuals splenectomized in conjunction with surgery for non-malignant conditions of adjacent organs had significant increases of lung and ovarian cancers (7). The spleen, potentially having both metabolic and immunologic functions, is worthy of being preserved if safely achieved (8). Most reports of spleen preservation in pancreatic resection relate to distal pancreatectomy (1, 2, 3), and several different variations have been described. Warshaw *et al.* (2) first reported on the feasibility of distal pancreatectomy with spleen preservation. The spleen was preserved with the short gastric vessels, while the splenic artery and vein were divided 2cm distal from the splenic hilum so as to preserve the left gastroepiploic artery and vein (2). White *et al.* (9) also documented that splenic preservation was feasible with intact short gastric and gastroepiploic vessels. Kimura *et al* (1) performed distal pancreatectomy with preservation of the splenic artery and vein, which aimed to secure blood flow of the spleen.

During total pancreatectomy for pancreatic cancer, the distal portion of the stomach is usually removed en bloc because of its possible ischemic change. In this case, the whole stomach was successfully preserved without any postoperative complications. It is well known that patients undergoing distal gastrectomy often suffer from poorly-controlled glycemia, in particular from hypoglycemia (10). Therefore, it seems more difficult to keep well-controlled glycemic conditions after total pancreatectomy when distal gastrectomy is combined. In this case, preservation of the whole stomach undoubtedly contributed to maintenance of well-controlled blood sugar level and quality of life of this patient.

In patients with pancreatic cancer, total pancreatectomy is performed if a pancreatic head carcinoma reaches too close to the plane of a possible Whipple resection, or if a carcinoma of the body or tail of the pancreas reaches too far to the right, i.e. into the pancreatic head (10). Generally total pancreatectomy involves en bloc resection of the pancreas, duodenum, spleen, and distal half of the stomach, in view of blood supply and drainage of those organs. In the present case, total pancreatectomy was carried out preserving the spleen, and whole stomach with sacrificing the splenic artery and vein. The potential complications related to this operative procedure are ischemia or blood congestion of the stomach and spleen. Ischemia of the stomach will induce anastomotic leakage of duodenojejunostomy. Blood congestion of the stomach and spleen may provoke gastric varices and severe splenomegaly. During the 3 years of survival, these episodes were not observed in this patient. Pres-

ervation of the spleen without retraction from the retroperitoneum may contribute to development of venous drainage pathways of the stomach and spleen.

To evaluate the long-term outcome of this procedure, further accumulation of case numbers is necessary. However, this study suggests that this procedure is feasible and can be adopted for patients not only with invasive ductal carcinoma of the head of the pancreas, but also with pancreatic trauma, chronic or acute pancreatitis, benign or malignant tumor of the pancreas.

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Serine Protease Inhibitor Kazal Type 1 (SPINK1): Beyond the Trypsin Inhibitor

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Abstract: Serine protease inhibitor Kazal type 1 (SPINK1) was originally identified as a trypsin inhibitor in the pancreatic acinar cells in 1948. Recent studies showed an association of mutations in SPINK1 gene and hereditary chronic pancreatitis. Thus, a lack of SPINK1 may result in the premature conversion of trypsinogen into active trypsin in acinar cells, leading to pancreatitis. However, we found that mice deficient for Spink3, a mouse homologue of SPINK1, died after birth due to excessive autophagy (cellular self-digestion) in the pancreatic acinar cells, suggesting that Spink3 is involved in the regulation of autophagy. We further demonstrated that autophagy is involved in trypsinogen activation within the pancreatic acinar cells in experimentally induced pancreatitis. These results suggest that Spink3 has protective roles in pancreatitis by dual mechanisms, one as a trypsin inhibitor and a second as a suppressor of trypsinogen activation through negative regulation of autophagy. On the other hand, SPINK1 is structurally similar to epidermal growth factor (EGF), in terms of the number of amino acid residues and the presence of 3 intrachain disulfide bridges. In fact, Spink3 acts as a growth factor in various cell lines *in vitro*. To gain additional insight into the new function of Spink3 *in vivo*, we examined the expression pattern of Spink3 during development. We found that Spink3 was expressed in unexpected tissues such brain and mesonephric tubules. In this review, we summarize the old and new roles of SPINK1/Spink3 in trypsin inhibition, autophagy, and cell proliferation/differentiation.

Keywords: SPINK1, Spink3, PSTI, autophagy, EGF, EGFR, seminal vesicle.

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Abstract: Serine protease inhibitor Kazal type 1 (SPINK1) was originally identified as a trypsin inhibitor in the pancreatic acinar cells in 1948. Recent studies showed an association of mutations in SPINK1 gene and hereditary chronic pancreatitis. Thus, a lack of SPINK1 may result in the premature conversion of trypsinogen into active trypsin in acinar cells, leading to pancreatitis. However, we found that mice deficient for Spink3, a mouse homologue of SPINK1, died after birth due to excessive autophagy (cellular self-digestion) in the pancreatic acinar cells, suggesting that Spink3 is involved in the regulation of autophagy. We further demonstrated that autophagy is involved in trypsinogen activation within the pancreatic acinar cells in experimentally induced pancreatitis. These results suggest that Spink3 has protective roles in pancreatitis by dual mechanisms, one as a trypsin inhibitor and a second as a suppressor of trypsinogen activation through negative regulation of autophagy. On the other hand, SPINK1 is structurally similar to epidermal growth factor (EGF), in terms of the number of amino acid residues and the presence of 3 intrachain disulfide bridges. In fact, Spink3 acts as a growth factor in various cell lines *in vitro*. To gain additional insight into the new function of Spink3 *in vivo*, we examined the expression pattern of Spink3 during development. We found that Spink3 was expressed in unexpected tissues such brain and mesonephric tubules. In this review, we summarize the old and new roles of SPINK1/Spink3 in trypsin inhibition, autophagy, and cell proliferation/differentiation.

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There are two types of trypsin inhibitors in the pancreas, basic pancreatic trypsin inhibitor (BPTI), and pancreatic secretory trypsin inhibitor (PSTI) [1]. BPTI strongly inhibits trypsin, chymotrypsin, plasmin and kallikrein, but is not secreted into pancreatic juice. The human pancreas, however, does not have BPTI.

Kazal *et al.* purified bovine PSTI from a side fraction of a commercial insulin process in 1948 [2]. PSTI can be distinguished from the BPTI on the basis of its lack of inhibitory effect on chymotrypsin and pancreatic kallikrein [3]. The gene encoding human or mouse PSTI has been recently named the serine protease inhibitor, Kazal type 1 (SPINK1), or Spink3, respectively. The SPINK1 gene is located on chromosome 5 and is composed of four exons, spanning about 7.2 kb in length [4]. The SPINK1 gene encodes an mRNA of 237bp, which is translated to a 79 amino acid peptide including a 23 amino acid signal peptide. The secreted SPINK1 consists of 56 amino acids with three intramolecular disulfide bridges, and the molecular weight is estimated to be 6240 based on the amino acid composition. SPINK1, which binds rapidly to trypsin and inhibits its activity, is an important factor in the onset of pancreatitis [5].

1. SPINK1, WORKING AS A TRYPSIN INHIBITOR

Activation of Trypsinogen (Trypsin Production) and Inhibition of Trypsin Activity by SPINK1

Pancreatic digestive enzymes are stored as inactivated precursors (ie trypsin as trypsinogen) in pancreatic zymogen granules. Under normal conditions, activation is strictly controlled to prevent autodigestion of the pancreas (ie pancreatitis). However, in certain circumstances, excessive amounts of pancreatic trypsinogen are activated to trypsin (ectopic activation), activating other zymogens, and leading to autodigestion of the pancreas. Triggers for the activation of trypsinogen to trypsin in the pancreas include excessive pancreatic exocrine stimulation, reflux of bile or duodenal fluid, disturbance of pancreatic duct flow, and inflammation. Enterokinase is the most efficient activator, but other molecules that activate trypsinogen include trypsin (trypsin catalysed autoactivation), lysosomal enzyme cathepsin B and neutrophilic enzymes [5-8]. Also, activation of trypsin from trypsinogen can also occur without enzyme involvement (non-enzymatic autoactivation). Calcium inhibits the degradation (autolysis) of activated trypsin, whereas bile acids promote the autoactivation of trypsin. SPINK1 is synthesised in acinar cells of the pancreas and is thought to inhibit up to 20% of the trypsin activity in the pancreas by binding to its catalytic site. However, pancreatitis can ultimately develop, if pancreatic activation of trypsinogen is too high or the trypsin-binding ability of SPINK1 is too low [5].

Onset of Hereditary Chronic Pancreatitis by Cationic Trypsinogen and *SPINK1* Gene Mutations

Whitcomb *et al.* [6] determined the sequence of five exons of the cationic trypsinogen genes (protease serine 1; PRSS1) using genomic DNA from patients with hereditary chronic pancreatitis. They discovered a point mutation in exon 3 of the cationic trypsinogen gene (365GRA: R122H) that results in an amino acid substitution in the autolytic domain of trypsin. Thus, the mutation blocks autolysis and results in continuous trypsin activity.

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1573-4080/09 $55.00+.00

Because balance of intrapancreatic balance between trypsin and SPINK1 activity is important for pancreatitis, it is hypothesised that mutations in the *SPINK1* gene that affect SPINK1 binding with trypsin will contribute to the onset of pancreatitis. There have been many reports of mutations in the *SPINK1* genes in patients with pancreatitis, and several hypothetical roles of these mutant proteins in pancreatitis [9-14]. A typical example is a substitution of Asn (AGT) with Ser (AAT) at position 34 in exon 3 (N34S). SPINK1 mutations are also found in normal people, and both homozygotes and heterozygotes are attributable to the development of pancreatitis. On the basis of these facts, some investigators recognize the role of the SPINK1 mutation as a disease modifier. However, the frequency of the N34S mutation occurring in patients with pancreatitis was considerably higher than that in people without pancreatitis [15]. Also, the rate of association of pancreatitis in people with the homozygotic N34S mutation was shown to be high (98%; 49/50) [15]. This high rate of association of pancreatitis in people with the homozygotic N34S mutation suggests that this mutation may be a recessive inherited trait.

Analysis of the Mechanism of Onset of Pancreatitis Using Genetically Altered Mice

To analyse the importance of trypsinogen activation (trypsin production) and its regulation by SPINK1 in the onset of pancreatitis, we generated the *Spink3* gene knockout mice by gene targeting and analysed their phenotypes [16]. The pancreatic acinar cells in knockout mouse showed excessive autophagy and disappeared completely after birth, indicating that Spink3 molecules are important in maintaining the integrity of pancreatic acinar cells. Interestingly, Chera *et al.* reported that similar phenotypes parallel in the endodermal epithelial cells observed upon silencing of the *Kazal1* gene in hydra [17]. In hydra, the endodermal epithelial cells carry out the digestive function together with the gland cells that produce zymogens and express the evolutionarily conserved gene *SPINK1*. A progressive *Kazal1* silencing induced excessive autophagy in the cytoplasm of digestive cells, and dramatic disorganization followed by a massive death of gland cells. These data suggests that the SPINK1 activity is required to prevent excessive autophagy in food digestive systems.

2. SPINK1, WORKING AS AUTOPHAGY REGULATOR

Autophagy; Bulk Degradation System in the Cell

Most intracellular short-lived proteins are selectively degraded by the ubiquitin-proteasome pathway [18, 19], while most long-lived proteins are degraded in lysosomes [20]. The general mechanism to deliver cytoplasmic components to the lysosomes is called autophagy (Fig. 1). The best understood role of autophagy is cellular housekeeping, a function that extends beyond the simple removal of damaged or unwanted products [21-23]. In fact, along with other proteolytic systems, lysosomes participate in the continuous turnover of intracellular constituents. Not only soluble cytosolic proteins but also organelles, such as mitochondria and peroxisomes, can be removed by autophagy [24-26]. In addition to maintaining cellular homeostasis, there is growing evidence for the participation of autophagy in processes such as cellular differentiation, tissue remodeling, growth control, cell defense and adaptation to adverse environments [27-30].

SPINK1 Regulates Autophagy in Acute Pancreatitis?

Vacuoles are found in pancreatic acinar cells of human acute pancreatitis and rodent experimental pancreatitis, but its origin has not been well understood. The vacuoles we observed by histological examination correspond to autophagosomes using autophagosome specific probe, microtubule-associated protein 1 light chain 3 (LC3-II) (Fig. 1), which are the hallmark of autophagy and contain zymogen granules in which trypsinogen exists physiologically [31]. What is the role of autophagy in acute pancreatitis? Two theoretical mechanisms of trypsinogen activation in pancreatic acinar cells have been proposed. In the "colocalization

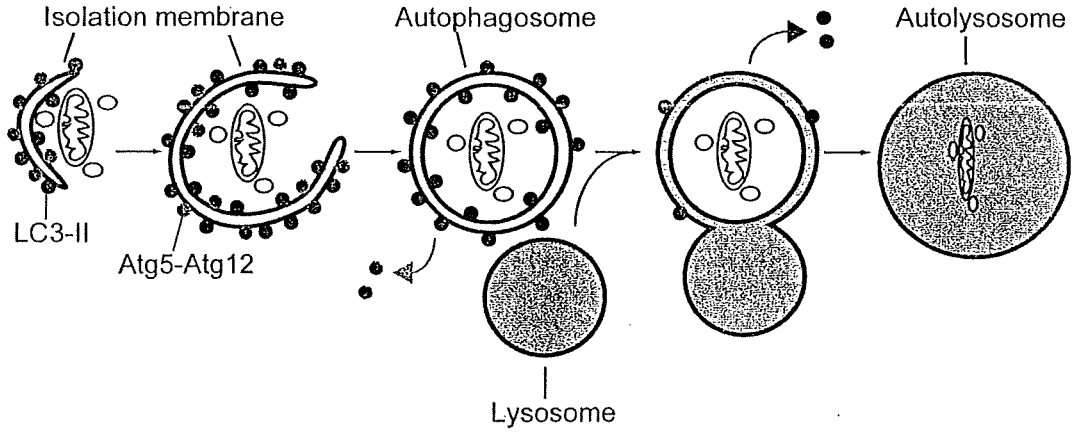

Fig. (1). Sheme of autophagy in mammalian cells. A portion of cytoplasm is enclosed by isolation membrane to form an autophagosome. Autophagosome fuses with lysosome to degrade the inside materials. The Atg12-Atg5 conjugate localized to the isolation membrane throughout its elongation process. LC3 is recruited to the membrane in the Atg5-dependent manner. Atg12-Atg5 dissociate from the membrane upon completion of autophagosome formation, while LC3 (-II) remains on the autophagosome membrane. Atg5 is required for elongation of the isolation membrane.

theory (cathepsin B theory)" [32-34], trypsinogen is activated by erroneous sorting of the lysosomal enzyme, cathepsin B. In the "autoactivation theory" [35], trypsinogen is transferred to an acidic environment in a subcellular compartment and activated by certain serine protease or independently of protease activity. We recently reported that conversion of trypsinogen to trypsin was greatly suppressed in autophagy-related gene 5 (*Atg5*; Fig. 1) deficient acinar cells, suggesting involvement of autophagy in trypsinogen activation [31]. We propose a third hypothetical mechanism of trypsinogen activation, autophagy theory [36]. Autophagy can provides both acidic and lysosomal hydrolase conditions. As SPINK1 recombinant protein both wild and mutated (N34S and R67C) have similar trypsin inhibition activity [37] (Fig. 2), it is possible that familial pancreatitis caused by mutation of the *SPINK1* gene is due to autophagy induction, but not to loss of binding to trypsin (Fig. 3).

3. SPINK1, WORKING AS A GROWTH FACTOR

Expression Pattern of *Spink3* During Mouse Embryonic Development

In human, SPINK1 is detected in many extrapancreatic tissues, including the stomach, colon, small intestine, liver, lung, kidney, and ovary [38-41]. To gain insight into its function, we analyzed the spatiotemporal expression profile of *Spink3*, using *in situ* hybridization and a *Spink3*$^{+/lacZ}$ knock-in mouse, in which *lacZ* was inserted into the *Spink3* locus [42]. *Spink3*^{lacZ} expression was first observed in the foregut, midgut, hindgut and the forebrain/midbrain junction region at 9.5 days post coitus (dpc). In the pancreas, *Spink3* mRNA was detected at 11.5 dpc, before formation of the typical shape of the exocrine structure of the pancreas. After differentiation of the intestinal tract, *Spink3*^{lacZ} expression was observed in the large intestine at 11.5 dpc, followed by expression in the small intestine at 13.5 dpc, before appearance of intestinal digestive enzymes. *Spink3* mRNA and *Spink3*^{lacZ} activity were also detected in other tissues, including the mesonephric tubules, urogenital ridge, genital swelling, and ductus epididymis. These data suggest that *Spink3* may play important roles in proliferation and/or differentiation of various cell types during development.

SPINK1 and Cancers

Tumor-associated trypsin inhibitor (TATI) was initially isolated from the urine of a patient with ovarian cancer [43]. TATI is a peptide produced at high concentrations by mucinous ovarian tumors, but it is expressed by several other tu-

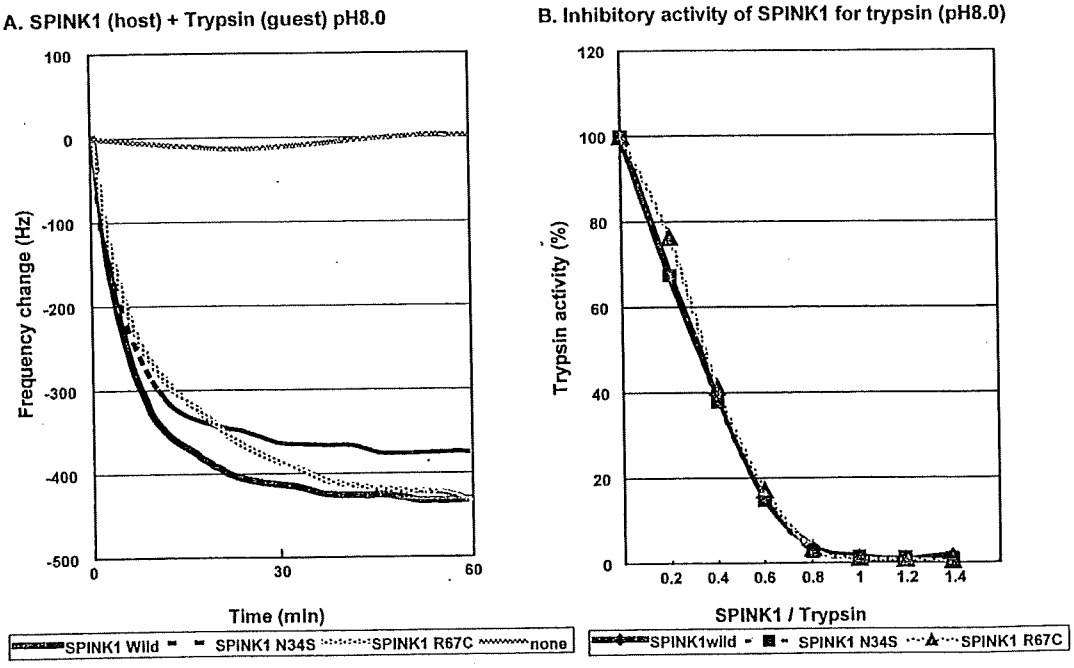

Fig. (2). (A) The binding affinity of trypsin with recombinant SPINK1 (wild, N34S, R67C) protein in comparison using a highly sensitive 27-MHz QCM (Initium, Tokyo, Japan) technique. In this method, qualitative and quantitative changes resulting from intermolecular interactions can be monitored and frequency (Hz) decreases in proportion to the change of molecular mass caused by binding of SPINK1, which are immobilized on the gold electrode surface, with trypsin. When trypsin was injected into the equilibrated solution containing SPINK1-immobilized sensor chip, the frequency decreased to about 400 Hz, suggesting that the binding affinity of SPINK1 N34S or R67C is almost same that of SPINK1 wild. (B) Trypsin inhibitory activity was determined from the residual trypsin activity after mixing bovine trypsin with recombinant SPINK1 (wild, N34S, R67C) protein (trypsin inhibitor [T.I.] / trypsin ratio ; 0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4) in 0.1 M Tris-HCl buffer (pH 8.0) containing 0.02M $CaCl_2$ and 0.01% Triton X- 100 at 37°C, using *N*-Benzoyl-l-arginine *p*-nitroanilide (L-BAPA) [8]. After 10 min of incubation at 37°C, the reaction was stopped by adding 0.5 ml of 30% acetic acid, and the absorbance at 410nm was measured. Residual trypsin activity decreased with the increase in the molar ratio of SPINK1 wild to trypsin. SPINK1 N34S or R67C showed almost the same inhibitory activity for trypsin as did SPINK1 wild. At each trypsin inhibitor/trypsin ratio of 1, each residual trypsin activity had almost disappeared.

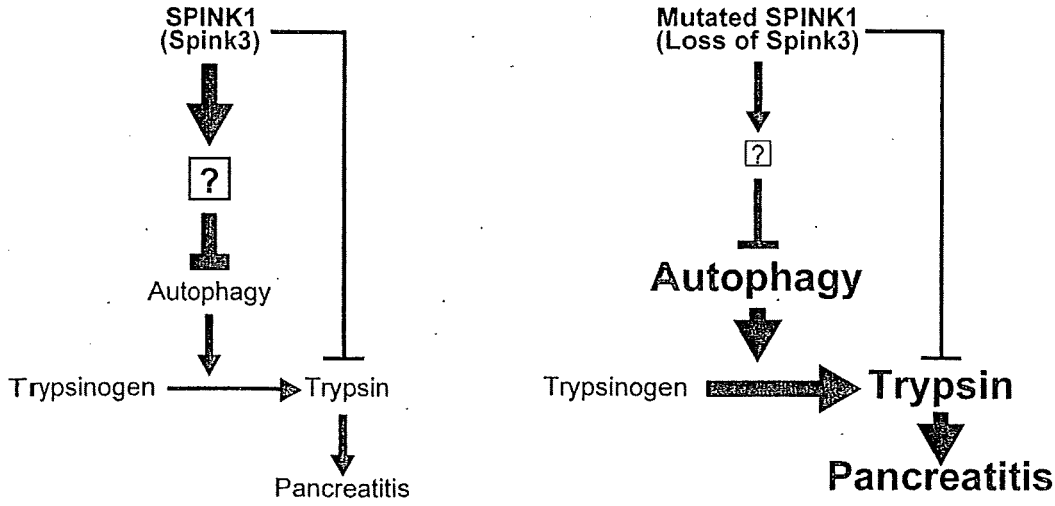

Fig. (3). Scheme of autophagy regulation by SPINK1 (Spink3). SPINK1 (Spink3) regulates both autophagy and activated trypsin in physiological condition. Mutated SPINK1 (loss of Spink3) can regulate activated trypsin, but not autophagy, which induces pancreatitis.

mors. TATI is identical to the SPINK1 [44]. Clinically, SPINK1 is most useful for monitoring of patients with mucinous ovarian cancer, but increased serum concentrations of SPINK1 may occur in many types of cancer [45]. In ovarian, bladder, and kidney cancer, SPINK1 is a marker of adverse prognosis. Tonouchi *et al.* reported that by the DNA microarray analysis, 13 differentially expressed genes were picked up, and quantitative RT-PCR reaction identified the SPINK1 as a candidate gene associated with early recurrence of intrahepatic cholangiocarcinoma after resection [46]. They described that the patients with higher levels of SPINK1 mRNA expression had significantly shorter recurrence-free survival. Recently, Tomlins *et al.* reported that an outlier-meta-analysis to identify SPINK1 outlier expression exclusively in a subset of ETS rearrangement-negative prostate cancers [47]. They demonstrated that SPINK1 outlier expression can be detected noninvasively in urine, and observed that SPINK1 outlier expression is an independent predictor of biochemical recurrence after resection.

SPINK1 Can Work as a Growth Factor *Via* Epidermal Growth Factor Receptor

Spink3 deficient pancreas shows no sign of regeneration of acinar cells [16], and SPINK1 silencing affects low budding rate in hydra [17], but its roles are not well known. There are some structural similarities between SPINK1 and the potent growth factor epidermal growth factor (EGF); both have similar numbers of amino-acid residues (56 and 53, respectively), molecular weights (about 6 kD), three intra-chain disulphide bridges (Fig. 4A) and limited sequence homology [1]. There is 50% gene sequence homology between SPINK1 and EGF [48]. In 1985, Ogawa *et al.* [49] reported that SPINK1 was mitogenic for human fibroblasts. Some studies support the concept that SPINK1 binds to the EGF receptor (EGFR). Rat monitor peptide (rat homologue of SPINK1) has been reported to compete with mouse EGF for binding to the receptors of Swiss 3T3 fibroblasts [50] and an EGF receptor-blocking antibody removed the promigratory effects of SPINK1 on human HT-29 cells [51]. However, the structures of the SPINK1 and EGF are so different (Fig. 4B), recently, we showed that SPINK1 binds to EGFR to activate its downstream signaling; resulting in proliferation of pancreatic and breast cancer cells (manuscript in submission). These data suggests that SPINK1 may act as growth factor *via* EGFR, as EGF families, in embryonic and cancer development.

4. SPINK1, WORKING AS A SPERM ACTIVITY REGULATION

Spink3 Binding Sites on Sperm Surface

Spink3 is strongly expressed in epitherial cells of seminal vesicle in male adult mice [42] (Fig. 5). Chen *et al.* reported that Spink3 in mouse seminal vesicle, binds to the surface of sperm, and suppresses of Ca^{2+} uptake by spermatozoa [52]. It is well recognized that mammalian sperm from epididymis should undergo some Ca^{2+}-dependent modifications before fertilization. In the reproductive tract, the Ca^{2+} concentration is sufficient to elevate intracellular Ca^{2+} in the induction of these cell modifications at any time earlier than the sperm-egg encounter. However, earlier modifications before reaching the oviduct would cause spermatozoa to become infertile. Thus, the calcium movement across the membrane of spermatozoa should be prohibited at ejaculation until the cells reach the oviduct. Spink3-sperm binding leading to the suppression of Ca^{2+} movement across the cell membrane sheds some light into the function of the Spink3-binding sites. Future study is needed to elucidate the role of Spink3 as regulator of sperm activity.

CONCLUSIONS

Although, SPINK1 are expressed in variety tissues and solid tumors, the roles of each tissues and tumors are not known well. As Spink3 null deficient mice died within two weeks after birth, we cannot analyze the function of Spink3 in extra-pancreatic tissues at adulthood. In addition, whether SPINK1 mutations can cause hereditary chronic pancreatitis

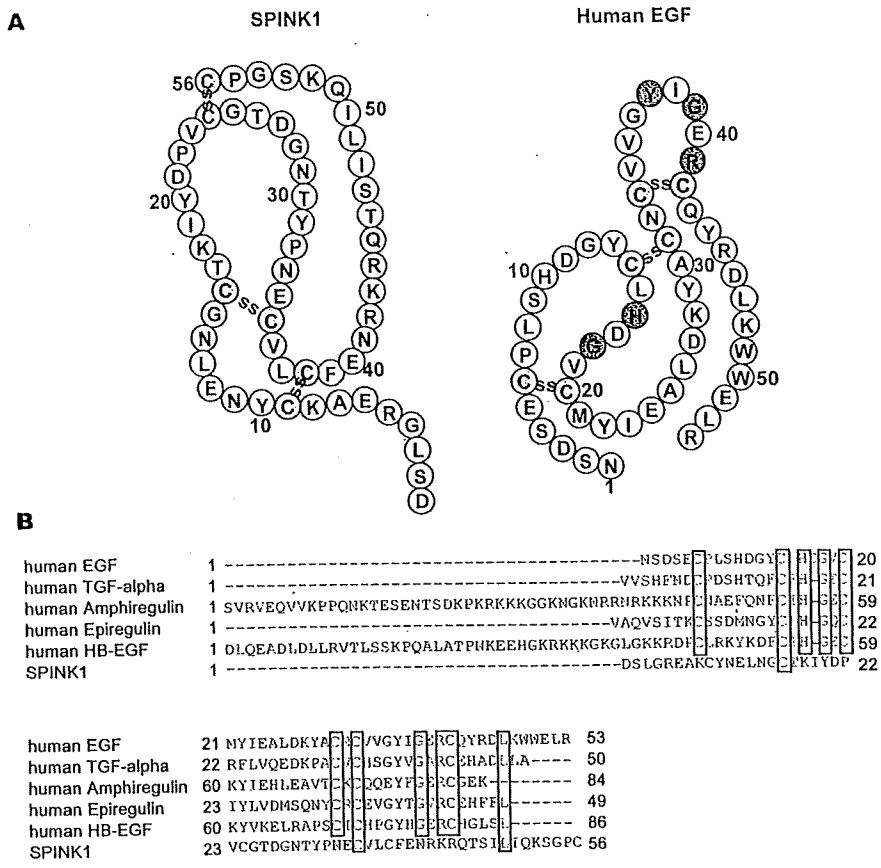

Fig. (4). (A) The comparison of SPINK1 and human EGF. Gray circles in human EGF indicate conserved in other EGF families. Cys residures (C) involved in the intrachain Cys-Cys disulphide bridge (SS). (B) The sequences of SPINK1 and EGF families. Amino acids identical in all structures are indicated by open boxes.

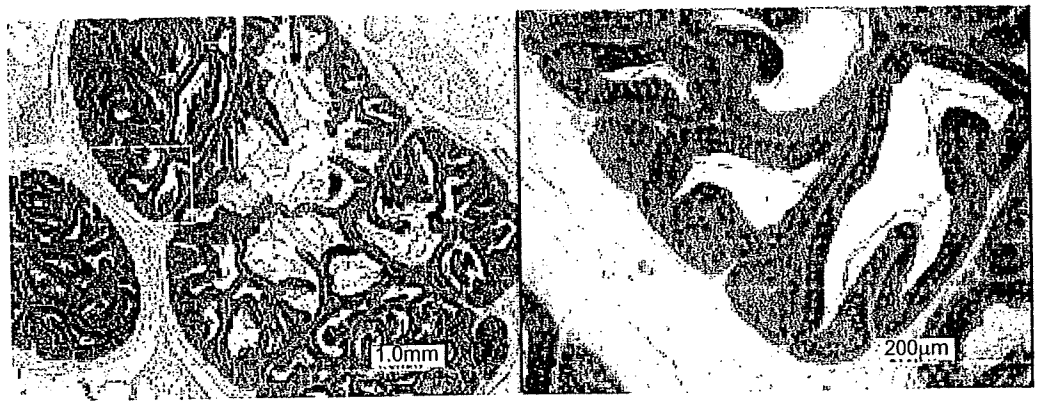

Fig. (5). Spink3 mRNA expression in mouse seminal vesicle. Spink3 strongly express epitherial cell in seminal vesicle (8 weeks old, C57BL/6J). Methods are previously described [42].

or not, is still unclear. In the next step, Spink3 conditional knockout models and human SPINK1 replacement models will be needed.

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ORIGINAL ARTICLE

Prospective randomized controlled study of gastric emptying assessed by ^{13}C-acetate breath test after pylorus-preserving pancreaticoduodenectomy: comparison between antecolic and vertical retrocolic duodenojejunostomy

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Abstract
Background/Purpose To examine whether vertical retrocolic duodenojejunostomy is superior to antecolic duodenojejunostomy with respect to gastric emptying in a prospective, randomized, controlled study of patients undergoing pylorus-preserving pancreaticoduodenectomy (PpPD).
Methods Thirty-five patients undergoing PpPD between March 2005 and July 2007 were enrolled in the study. All provided informed consent. During PpPD, the patients were randomly assigned to either the antecolic (antecolic group, $n = 17$) or vertical retrocolic route (vertical retrocolic group, $n = 18$) just before the reconstruction. Each patient ingested ^{13}C-acetate in a liquid meal before surgery and on postoperative day (POD) 30. Gastric emptying variables (Tmax, T1/2) were determined and compared between groups.
Results Clinical delayed gastric emptying, defined as an inability of patients to take in an appropriate amount of solid food orally by POD 14, was found in 1 of 17 patients (6%) in the antecolic group and in 4 of 18 patients (22%) in the vertical retrocolic group, but the difference was not significant ($P = 0.34$). Tmax and T1/2 on POD 30 were prolonged in both groups in comparison to preoperative levels, but no significant difference was found between the two groups. Follow-up examinations revealed that gastric emptying had recovered to the preoperative level by POD 30 in approximately 80% of the patients, regardless of the reconstruction route.
Conclusions Vertical retrocolic duodenojejunostomy does not seem to offer an advantage with respect to gastric emptying.

Keywords Gastric emptying · Pylorus-preserving pancreaticoduodenectomy · Antecolic duodenojejunostomy · Vertical retrocolic duodenojejunostomy

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Introduction

Pylorus-preserving pancreaticoduodenectomy (PpPD) is generally accepted as a standard operation for periampullary lesions. PpPD, in comparison to classic pancreaticoduodenectomy with hemigastrectomy, is reported to improve quality of life, nutritional status and weight gain without any difference in operative morbidity and mortality or in postoperative survival [1–4].

Delayed gastric emptying (DGE), however, is reported to be the most common and frustrating complication after PpPD. Despite the lack of a certain definition for DGE, the reported incidence varies from 20 to 60% [5–13]. DGE results in a prolonged hospital stay, which adds to hospital costs. Although DGE itself is not a fatal complication, minimizing DGE is important in patients undergoing PpPD.

Two reconstruction routes are used for duodenojejunostomy, the antecolic route and the retrocolic route. The reported incidence of DGE is >30% for the retrocolic route [12, 14, 15], whereas that for the antecolic route is <15%