Diagnostic Pathology

RESEARCH

Open Access

Long-chain fatty acyl CoA synthetase 4 expression in pancreatic cancer: a marker for malignant lesions and prognostic indicator for recurrence

Daiki Uchihara[1,2*], Shohei Shimajiri[1], Yoshikazu Harada[1], Keiichiro Kumamoto[1,2], Shinji Oe[2], Koichiro Miyagawa[2], Koichi Nakamura[1,2], Eisuke Katafuchi[1], Fariza Nuratdinova[1], Yuichi Honma[2], Michihiko Shibata[2], Masaru Harada[2] and Toshiyuki Nakayama[1]

Abstract

Background Long-chain fatty acyl CoA synthetase 4 (ACSL4) is crucial for lipid metabolism, primarily catalyzing the formation of 12–20 carbon chain fatty acids. ACSL4 is upregulated in various cancers and linked to aggressive behavior and poor survival. A bioinformatics study showing ACSL4 upregulation in pancreatic cancer. However, utility for actual pathological diagnosis and clinical significance in pancreatic ductal adenocarcinoma (PDAC) and intraductal papillary mucinous neoplasm (IPMN) are unexplored. This study aimed to investigate ACSL4 expression in PDAC and IPMN, and evaluate its clinical implications.

Methods We examined ACSL4 expression using immunohistochemistry in 165 patients with PDAC and IPMN. Differences in ACSL4 expression between malignant and benign lesions were evaluated using the Pearson χ2 test. The association between ACSL4 expression, pathological parameters, and survival was assessed through Kaplan-Meier and Cox regression analyses in 96 patients with invasive cancer.

Results Compared to normal pancreatic ducts, low-grade pancreatic intraepithelial neoplasm, and intraductal papillary mucinous adenoma (IPMA) (3.3%, 3.4%, and 2.7%, respectively), ACSL4 expression was significantly higher in invasive PDAC, noninvasive intraductal papillary mucinous carcinoma (IPMC), and invasive IPMC (77%, 86.7%, and 93.9%, respectively). In invasive cancers, low ACSL4 expression was associated with a higher frequency of lymphovascular invasion and recurrence and shorter disease-free survival ($P=0.006$). Additionally, low ACSL4 expression was an independent prognostic factor for shorter disease-free survival in multivariable Cox regression analysis (HR = 2.409, 95% CI: 1.121–5.180, $P=0.024$).

Conclusion ACSL4 expression helps differentiate cancerous from precancerous lesions in pancreatic cancer, but low expression is linked to a higher frequency of lymphovascular invasion and shorter disease-free survival in invasive

*Correspondence:
Daiki Uchihara
uchihara-daiki1127@med.uoeh-u.ac.jp

Full list of author information is available at the end of the article

cases. Due to the limited sample size and broad confidence intervals, the findings of this study should be interpreted with caution and require validation in larger, independent cohorts.

Keywords ACSL4, Pancreatic ductal adenocarcinoma, Intraductal papillary mucinous neoplasm, Diagnosis, Prognosis

Introduction

Pancreatic cancer is one of the most aggressive solid malignancies, with a 5-year overall survival rate of 11.2% [1]. The poor prognosis is due to challenges in early detection, difficulty achieving complete surgical resection, and the development of chemotherapy resistance through various mechanism [2]. Therefore, pathological diagnosis of post-resection is important.

In the pathological diagnosis of pancreatic cancer, it can be challenging to differentiate between pancreatic ductal adenocarcinoma (PDAC), pancreatic intraepithelial neoplasm (PanIN), intraductal papillary mucinous carcinoma (IPMC), and adenoma (IPMA). Immunohistochemical markers that distinguish malignant from benign tumors would be valuable. Maspin and insulin-like growth factor II messenger ribonucleic acid-binding protein 3 (IMP3) are highly expressed in malignant tumors but not in benign ones [3]. However, they are occasionally expressed in low-grade PanIN and adenoma [4]. No immunohistochemical staining has been reported that can clearly distinguish these tumors.

Long-chain fatty acyl-CoA synthetase 4 (ACSL4) is mainly located in the endoplasmic reticulum, mitochondria, plasma membranes, and peroxisomes of the adrenal gland, ovary, testis, and brain. ACSL4 can function as either a tumor suppressor or promoter, depending on the cancer type and tissue environment [5]. High ACSL4 expression has been found in liver, ovarian, prostate, and quadruple-negative breast cancers, where it correlates with tumor proliferation, migration, and invasion. In contrast, ACSL4 acts as a tumor suppressor, inhibiting cell proliferation and migration in gastric cancer [6]. Bioinformatics analysis has identified ACSL4 as significantly upregulated in pancreatic cancer [7]. However, its role in pancreatic cancer remains unclear.

The aim of this study was to evaluate ACSL4 expression in surgically resected specimens using immunohistochemical staining. We assessed the usefulness of ACSL4 in distinguishing pancreatic cancer from benign lesions. Furthermore, we analyzed the relationship between ACSL4 expression, clinicopathological characteristics, and pancreatic cancer prognosis.

Methods

Study design

This retrospective, single-center study was approved by our institution's ethics committee (protocol no. CR24-016) and conducted in accordance with the Helsinki Declaration. Written informed consent was obtained from all participants, and an opt-out option was provided, allowing the participants to be notified and permitting the publication of research information on our website.

Patient selection

We retrospectively collected data from 165 patients with pancreatic tumors who underwent surgical resection at the University of Occupational and Environmental Health Hospital between January 2009 and December 2023. Only cases involving IPMN and PDAC were included. The patient consort diagram is shown in Fig. 1. The cohort consisted of 30 patients with IPMA, 15 with noninvasive IPMC, 87 with invasive PDAC, and 33 with invasive IPMC. To evaluate the expression of ACSL4 immunostaining in detail, we extracted cases that overlap, such as normal lesions and low-grade PanIN, such as IPMA and IPMC, such as low-grade PanIN and PDAC (Supplementary Data 1). We selected 90 cases of normal pancreatic ducts on the same slides as PDAC and IPMC, 44 cases of IPMA on the same slides as IPMC, and 29 cases of low-grade PanIN on the same slides as PDAC. In total, we evaluated ACSL4 immunostaining in 90 normal pancreatic duct cases, 29 low-grade PanIN cases, 87 PDAC cases, 74 IPMA cases, 15 noninvasive IPMC cases, and 33 invasive IPMC cases (Fig. 1).

Next, 135 pancreatic cancer cases (87 PDAC, 15 noninvasive IPMC, and 33 invasive IPMC) were classified into two categories based on the degree of differentiation: well or moderately differentiated vs. poorly differentiated (Supplementary Fig. 1). Pancreatic cancer often contains a mix of well-formed glands alongside individual cells and clusters, showing both well- and poorly differentiated areas. In such cases, the tumor is categorized based on the poorest degree of differentiation found in a significant portion of the carcinoma.

We also analyzed the correlation between ACSL4 expression and clinicopathological characteristics in 96 resected pancreatic tumors with invasive cancer (Supplementary Fig. 2). Since early recurrence may be associated with whether complete resection was achieved or the presence of potential metastasis [8], cases of early recurrence within 6 months were excluded. Additionally, cases of IPMA and IPMC with noninvasive or minimally invasive components comprising less than 50% were excluded due to their clearly better prognosis [9].

Follow up

Follow-up duration was defined as the time from pancreatic tumor surgery until death or the last visit in March

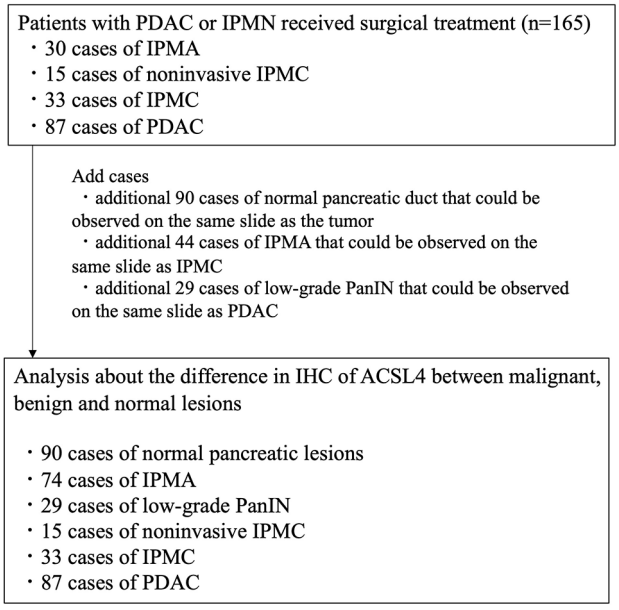

Fig. 1 Consort diagram of patients in this study showing analysis of ACSL4 immunohistochemistry differences between malignant, benign, and normal lesions

2024. Overall survival time was calculated from the surgery date to death, while disease-free survival time was the interval from the surgery date to the first documented recurrence. Recurrence was defined as the presence of locoregional disease (i.e., recurrence in the pancreatic remnant, peripancreatic tissue, or lymph node metastases) or metastasis (i.e., liver metastases, lung metastases, or peritoneal carcinomatosis) detected by radiologic imaging techniques.

Pathologic examination

Samples from 165 pancreatic tumors were stained with hematoxylin and eosin (H&E) and evaluated histopathologically by two certified pathologists from the Japanese Medical Specialty Board (SS and TN). They analyzed the histological findings without knowledge of the patients' clinical outcomes. The pathologic features of the pancreatic specimens, including the pathological diagnosis, tumor differentiation, tumor size, primary tumor stage, lymph node metastasis, lymphatic invasion, vascular invasion, neuroinvasion, and margin status, were carefully reevaluated.

Immunohistochemistry

Immunohistochemistry labeling was performed at the immunohistochemistry laboratory of the Department of Pathology. Tumor tissues were fixed in 10% neutral formalin and embedded in paraffin. Section (4 μm thick) were cut, mounted on glass slides, deparaffinized in xylene, and rehydrated in a graded ethanol series. Heat-induced antigen retrieval was performed by immersing the sections in a high pH buffer solution (pH 9.0) at 97℃ for 40 min. The deparaffinized and rehydrated 4 μm sections were then incubated in 3% H_2O_2 for 5 min to block endogenous peroxidase activity. Afterward, the sections were incubated for 60 min at room temperature with a mouse monoclonal antibody against ACSL4 (sc-235230, 1:500, Santa Cruz Biotechnology, CA, USA) and then incubated with peroxidase-conjugated secondary antibodies (Simple Stain MAX-PO Kit, Nichirei, Tokyo, Japan). A diaminobenzidine kit (Histofine, Nichirei, Japan) was used to detect immunoreactions, and the sections were counterstained with Meyer's hematoxylin. The labeling was performed using an automated immunostaining system (Histostainer36A, Nichirei, Japan). Immunostained sections were then dehydrated in ethanol and cleared in xylene.

Interpretation of the staining

Whole slide imaging was performed using a Hamamatsu NanoZoomer 2.0-HT (C9600-13) (Hamamatsu Photonics, K.K., Japan). Images were reviewed with the NanoZoomer Digital Pathology (NDP) viewer software (NDP view.2) (U12388-01) (Hamamatsu Photonics, K.K., Japan). We selected five random fields from PDAC, IPMC, IPMA, low-grade PanIN, or normal pancreatic duct in serial sections using the annotation system of NDP view.2 (0.015–0.025 mm^2 per field, magnification ×400). These fields were clearly diagnosable on H&E slides. ACSL4 expression levels in the cytoplasm were assessed by identifying the population and intensity of ACSL4-positive cells, referencing previous reports [10]. Levels were classified as 0–50% and 51–100%, with staining intensity graded as negative, weak, or strong. The results were categorized into three groups, as shown in Fig. 2: grade 1 (weak or negative with 0–50%); grade 2 (weak with 51–100% or strong with 0–50%); and grade 3 (strong with 51–100%). If three out of five fields were graded as 3, the case was classified as high ACSL4 expression. If three out of five fields were graded as 1 or 2, the case was classified as low ACSL4 expression. ACSL4 expression was assessed by two certified pathologists from the Japanese Medical Specialty Board (SS and TN) without knowledge of the patients' clinical outcomes.

Statistical analysis

Statistical analyses were conducted using SPSS 25 software (IBM, Chicago, IL, USA). ACSL4 expression in IPMC and PDAC was compared with low-grade PanIN, IPMA, and normal pancreatic ducts. The Pearson χ^2 test or Fisher's exact test was used to compare categorical variables between the two groups.

Survival curves were calculated using the Kaplan–Meier method, and differences between the curves were analyzed using the log-rank test. The association of

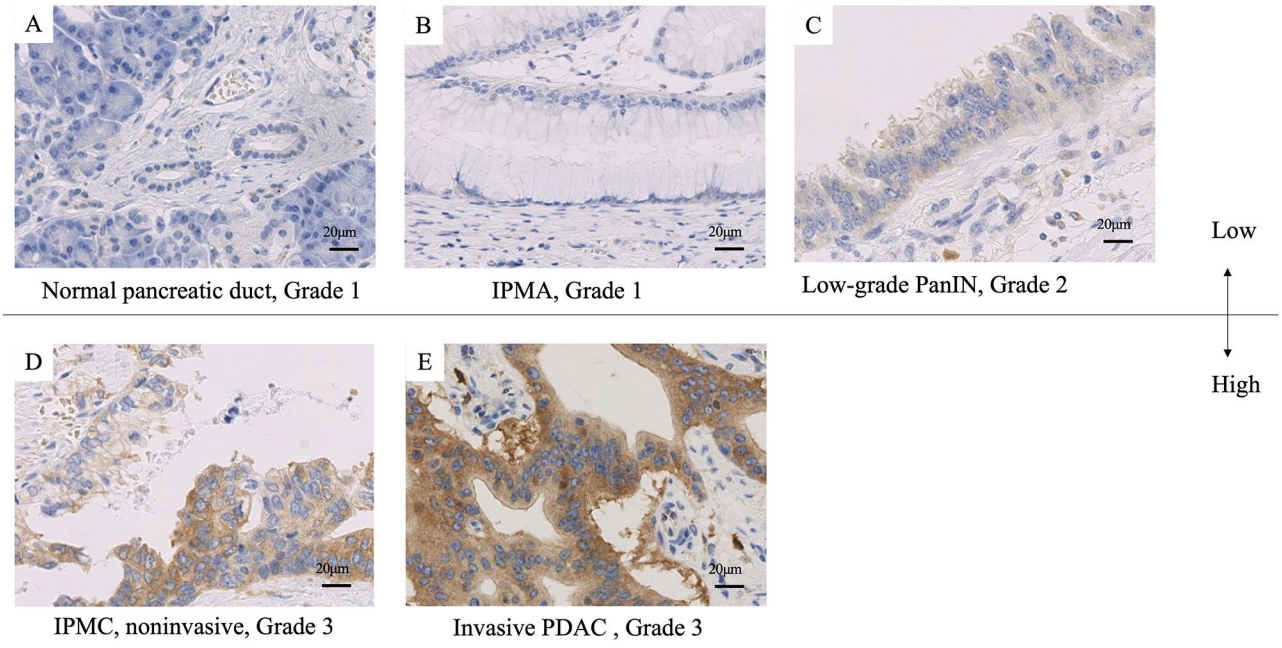

Fig. 2 ACSL4 expression via immunohistochemical staining (Magnification, x400. Normal pancreatic duct (**A**) and IPMA (**B**) show negative cytoplasmic ACSL4 expression (Grade 1). Low-grade PanIN (**C**) shows weak expression in 51–100% or strong expression in 0–50% of the cytoplasm (Grade 2). IPMC (**D**) and PDAC (**E**) show strong expression in 51–100% of the cytoplasm (Grade 3)

multiple prognostic factors with disease-free survival was assessed using univariate and multivariate Cox proportional hazard model analyses. The multivariate analysis included factors on the basis of clinical relevance previously reported to contribute to recurrence. Early recurrence, defined as recurrence within the first 6 months after surgery, is a characteristic of pancreatic cancer [11]. Several perioperative predictors of early recurrence, such as tumor size, lymph node metastasis, serum CA19-9 levels, symptom duration, tumor differentiation, and the absence of adjuvant therapy, have been identified as indicators of high-risk patients [11–14]. Pancreatic nerve plexus invasion has been identified as a risk factor for positive margin resection and poor prognosis [15, 16].

Differences were considered statistically significant at P values of < 0.05.

Results

Expression of ACSL4 in pancreatic tumors

We analyzed the differences in immunohistochemistry staining of ACSL4 among malignant, benign, and normal pancreatic ducts, as shown in Fig. 3A and Supplementary Data 1. Each experiment included at least two slides of normal duodenal and colonic tissue sections as negative and positive controls, respectively (Supplementary Fig. 3).

Normal pancreatic ducts, low-grade PanIN, and IPMA showed minimal reactivity (3.3%, 3.4%, and 2.7%, respectively). ACSL4 expression was noted in rare, small foci with acinar to ductal metaplasia (ADM) in chronic pancreatitis secondary to PDAC. Invasive PDAC, noninvasive IPMC, and invasive IPMC exhibited significantly higher ACSL4 expression compared to normal pancreatic ducts, low-grade PanIN, and IPMA, with rates of 77%, 86.7%, and 93.9%, respectively. ACSL4 expression was significantly higher in invasive PDAC than in low-grade PanIN and normal pancreatic duct ($P < 0.001$, respectively). Additionally, ACSL4 expression was significantly higher in both invasive and noninvasive IPMC than in IPMA ($P < 0.001$ for both).

The relationship between ACSL4 immunostaining and the differentiation of pancreatic cancer is shown in Fig. 3B. Poorly differentiated pancreatic cancer showed a lower positive rate of ACSL4 immunostaining compared to well or moderately differentiated tumors (55.6% vs. 84.9%, $P = 0.024$).

In specimens where both IPMA and IPMC were present, staining patterns were distinctly different, with IPMA showing negative staining and IPMC showing positive staining (Supplementary Fig. 4). The same distinction was observed between low-grade PanIN and PDAC (Fig. 4). Micropapillary, solid nest, or vacuolated cell patterns, which are characteristic of poorly differentiated pancreatic cancer, also exhibited a lower positive rate of ACSL4 immunostaining (Supplementary Fig. 5).

Correlation of ACSL4 expression with clinicopathologic parameters

The relationships between ACSL4 expression and clinicopathological parameters in patients with invasive

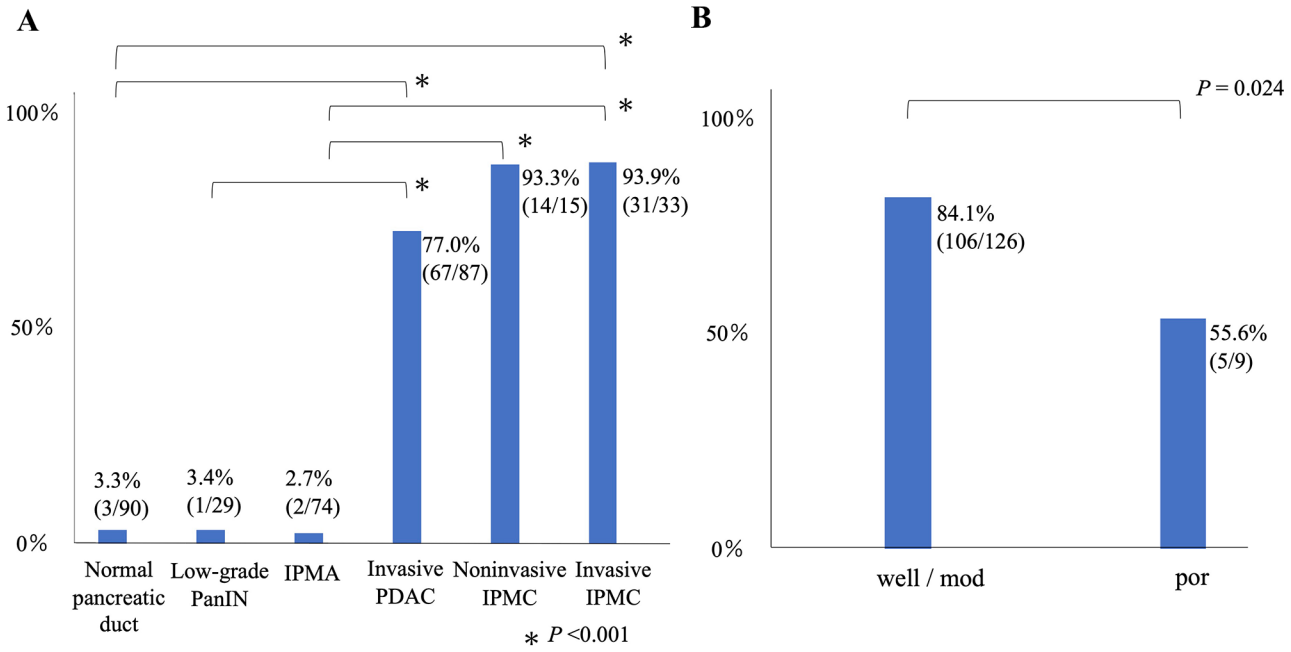

Fig. 3 ACSL4 expression in normal pancreatic ducts, low-grade PanIN, IPMA, invasive PDAC, noninvasive IPMC, and invasive IPMC. ACSL4 expression was significantly higher in malignant lesions compared to benign lesions (**A**). ACSL4 expression was significantly higher in invasive PDAC than in low-grade PanIN and normal pancreatic duct ($P < 0.001$, respectively). ACSL4 expression was significantly higher in invasive IPMC than in IPMA ($P < 0.001$). ACSL4 expression was significantly higher in noninvasive IPMC than in IPMA ($P < 0.001$). ACSL4 expression was higher in cases of well or moderately differentiated tumors compared to those of poorly differentiated tumors (**B**, $P = 0.024$)

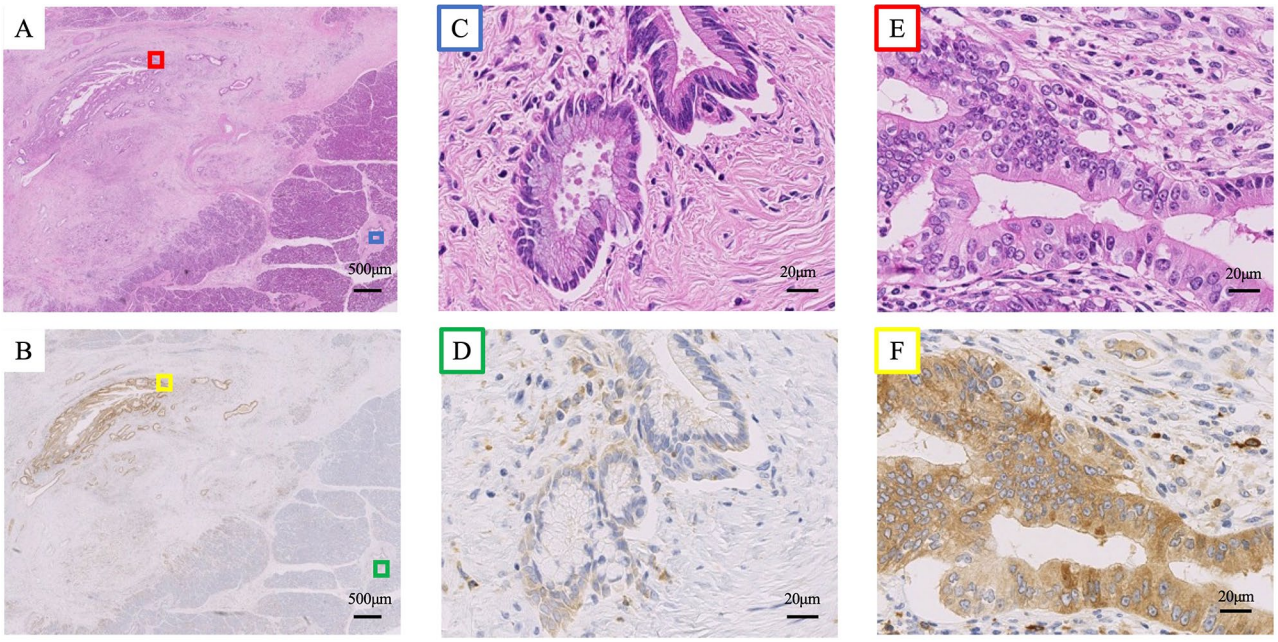

Fig. 4 In this slide, both low-grade PanIN, and invasive ductal carcinoma are present in the context of pancreatic ductal carcinoma (**A**, H&E, magnification x100; **B**, ACSL4, magnification x100). The low-grade PanIN exhibits ACSL4 low expression, graded as 1 or 2 (**C**, **H&E**, magnification x400; **D**, ACSL4, magnification x400). In contrast, invasive ductal carcinoma exhibit high ACSL4 expression (**E**, **H&E**, magnification x400; **F**, ACSL4, magnification x400). Even when low-grade PanIN and invasive PDAC were found in the same specimen, staining differences were typically clear, with low-grade PanIN being negative and invasive PDAC being positive

pancreatic cancers are presented in Table 1. A Judgement of high or low expression was made for each 96 cases based on the method (2.6).

The mean age of the patients was 71.4 years (range: 33–89 years), with 57 (59.4%) males and 37 (40.6%) females. The mean tumor size was 25.2 mm (range: 1–80 mm). Among the cases, 67 were invasive PDAC, and 29 were invasive IPMC. Low ACSL4 expression was significantly associated with lymphatic and vascular invasion ($P = 0.034$ and 0.014, respectively). However, ACSL4 expression did not correlate with other parameters. Notably, low ACSL4 expression was linked to a higher rate of recurrence ($P = 0.027$).

Table 1 Correlation of ACSL4 expression with clinicopathologic parameters

		ACSL4				
Characterstics	**No. of patients**	**High**	**Low**	**Odds Ratio**	**(95%CI)**	**p value**
Age						
71.4 years (range:33–89 years)		71.8	69			0.292
Sex				0.901	(0.271–2.990)	0.864
Male	57	49	8			
Female	39	34	5			
Location				1.351	(0.408–4.476)	0.622
Pbt	43	38	5			
Ph	53	45	8			
Histology				2.836	(0.489–16.439)	0.227
Well ~ mod	90	78	11			
Por	6	5	2			
Size						
25.2 (1–80 mm)		25	26.5			0.663
pT				1.459	(0.169–12.581)	0.729
T1	10	9	1			
≧T2	86	74	12			
LN metastasis				0.967	(0.299–3.124)	0.744
Negative	51	43	8			
Positive	45	39	6			
Lymphatic invasion				1.213	(1.092–1.348)	0.034
Negative	21	22	0			
Positive	75	61	13			
Vascular invasion				9.191	(1.142–73.991)	0.014
Negative	39	36	1			
Positive	57	47	12			
Neuro invasion				5.474	(0.676–44.346)	0.078
Negative	27	26	1			
Positive	69	57	12			
Margin				1.775	(0.426–7.402)	0.426
Negative	81	71	10			
Positive	15	12	3			
Neoadjuvant chemotherapy				0.622	(0.158–2.447)	0.494
No	66	56	10			
Yes	30	27	3			
Adjuvant chemotherapy				1.202	(0.303–4.775)	0.793
No	25	22	3			
Yes	71	61	10			
CA19-9				0.894	(0.276–2.890)	0.851
<37	42	36	6			
≧37	54	47	7			
Recurrence				5.116	(1.068–24.517)	0.027
Absent	43	40	2			
Present	53	43	11			

Correlation of clinicopathologic parameters and ACSL4 expression with survival

The median follow-up period for the entire cohort was 65.0 months (range: 6.2–170.3 months). Among the patients, 53 (55.2%) developed recurrence or metastasis, and 37 (38.5%) had died. The median overall survival and disease-free survival were 65.0 and 25.2 months, respectively. The 5-year overall survival and disease-free survival rates were 54.9% and 34.0%, respectively. No significant differences in overall survival or disease-free survival were observed between PDAC and IPMC (Supplementary Fig. 6). In patients with low ACSL4 expression, there was no association with overall survival (log-rank, $P = 0.503$), but differences in disease-free survival were noted (log-rank, $P = 0.006$) (Fig. 5). The results of this analysis did not change significantly even when cases of recurrence within 6 months and cases of IPMC with noninvasive or minimally invasive components comprising less than 50% (Supplementary Fig. 7). Supplementary Fig. 8A shows the histogram of the follow-up duration. The graph's peak was slightly skewed to the left, suggesting that the follow-up duration was not normally distributed. Therefore, we compared the follow-up duration between the high ACSL4 expression and the low ACSL4 expression groups using Mann-Whitney U test (Supplementary Fig. 8B). There were no significant differences between the two groups ($P = 0.672$), suggesting that the effect of survival bias was small.

The results of the univariate and multivariate Cox regression analyses are presented in Table 2. In the multivariable Cox regression analysis, low ACSL4 expression was identified as an independent prognostic factor for disease-free survival in pancreatic cancer (Hazard ratio (HR) = 2.409, 95% Confidence interval (CI): 1.121–5.180, $P = 0.024$).

The correlation between ACSL4 expression and recurrence patterns is detailed in Table 3. Low ACSL4 expression was associated with a higher incidence of distant metastases compared to local recurrence ($P = 0.026$, HR = 1.393, 95% CI = 1.144–1.696). However, no correlation was found among the recurrence patterns involving the liver, lung, and peritoneum.

The power was 0.80, confirming that the result was statistically significant.

Discussion

ACSL is a key enzyme involved in lipid metabolism in vivo, mainly catalyzing the formation of fatty acids with carbon chain lengths of 12 to 20. It is mainly localized in the outer mitochondrial membrane, peroxisomal membrane, and endoplasmic reticulum membranes, playing various roles in lipid metabolism. In mammals, there are five ACSL isoenzymes (ACSL1, ACSL3, ACSL4, ACSL5, and ACSL6), each with specific tissue localization and distinct functions.

There are various roles of polyunsaturated fatty acids converted to CoA esters (ACSL4- catalyzed PUFA acyl-CoAs). (1) These provide energy to cells through b-fatty acid oxidation [17]. (2) These synthesize phospholipids and form part of cell membranes and intracellular organelle membranes [18]. (3) These participate in the regulation of steroidogenesis and eicosanoid biosynthesis [19]. (4) These cause lipid peroxidation and induce ferroptosis [20].

A bioinformatics analysis identified ACSL4 as a significantly upregulated gene in pancreatic cancer [7]. However, its characterization and clinical applicability

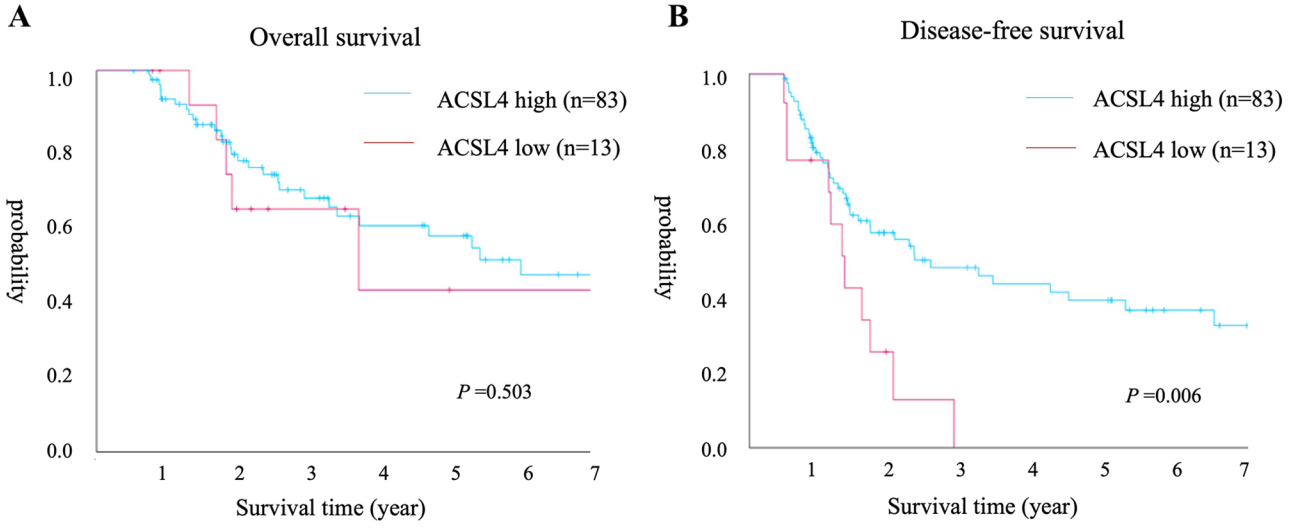

Fig. 5 Kaplan-Meier curves showing overall survival (**A**) and disease-free survival (**B**) stratified by ACSL4 expression in patients with invasive PDAC/IPMC. The red lines represent low ACSL4 expression, the blue lines represent high ACSL4 expression. Low ACSL4 expression is associated with shorter disease-free survival ($P = 0.006$)

Table 2 Univariate and multivariate Cox regression analyses about the potential prognostic factors for disease-free survival in patients with invasive PDAC and IPMC

			Univariate analysis			Multivariate analysis		
Characteristics		**n**	**HR**	**(95%CI)**	**p**	**HR**	**(95%CI)**	**p**
ACSL4 expression								
	High	82	1					
	Low	**13**	**2.533**	**(1.283–5.004)**	**0.007**	**2.409**	**(1.121–5.180)**	**0.024**
Age								
	< 70	**30**	**1**					
	≧ 70	66	1.032	(0.589–1.808)	0.913			
Sex								
	Male	57	1					
	Female	39	0.891	(0.679–1.170)	0.405			
Location								
	Pbt	43	1					
	Ph	53	1	(0.765–1.307)	0.999	0.684	(0.370–1.265)	0.226
Histology								
	Well ~ mod	90	1					
	Por	6	0.714	(0.257–1.984)	0.518	0.478	(0.152–1.503)	0.207
pT								
	T1	10	1					
	≧T2	86	0.916	(0.599–1.402)	0.687			
Size (mm)								
	< 30	64	1					
	≧ 30	32	1.674	(0.963–2.912)	0.068	1.543	(0.811–2.937)	0.187
LN metastasis								
	-	51	1					
	+	45	1.4	(0.819–2.392)	0.216	0.992	(0.528–1.862)	0.979
Lymphatic invasion								
	-	21	1					
	+	75	2.333	(1.100-4.949)	0.027	1.577	(0.563–4.416)	0.408
Vascular invasion								
	-	39	1					
	+	57	2.156	(1.199–3.878)	0.01	1.902	(0.843–4.292)	0.119
Neuro invasion								
	-	27	1					
	+	69	1.235	(0.672–2.272)	0.496	0.562	(0.262–1.207)	0.341
Margins								
	-	81	1					
	+	15	1.388	(0.697–2.762)	0.349	0.946	(0.431–2.076)	0.89
Neoadjuvant chemotherapy								
	-	66	1					
	+	30	0.829	(0.450–1.529)	0.548			
Adjuvant chemotherapy								
	-	25	1					
	+	71	1.12	(0.589–2.129)	0.73	1.484	(0.751–2.930)	0.256
CA19-9								
	< 37	42	1					
	≧ 37	54	1.712	(0.987–2.972)	0.056	1.655	(0.898–3.050)	0.107

in immunostaining remain unclear. In this study, we hypothesized that ACSL4 expression would be higher in malignant lesions than in benign lesions, reflecting increased lipid metabolism. Many cancer cells upregulate fatty acid synthesis to provide fatty acids for membrane formation, which is essential for proliferation [21, 22]. The usefulness of ACSL4 for differentiating between benign and malignant tissues has been reported in hepatocellular carcinoma (HCC), where ACSL4 expression was significantly greater than in all other tumors,

Table 3 Distribution of recurrence pattern among ACSL4 expression. Low ACSL4 expression was associated with a higher incidence of distant metastases compared to local recurrence ($P=0.026$)

	ACSL4		HR	(95%CI)	*P* values
Recurrence pattern	High	low	1.393	(1.144–1.696)	0.026
Local	14	0			
Distant metastasis	28	11			
Liver	13	4			
Lung	9	5			
Peritoneum	5	2			
Other	1	0			

distinguishing HCC from normal liver tissue with a sensitivity of 93.8% and specificity of 93.6% [23].

We also demonstrated that ACSL4 is a potential novel biomarker for specifically identifying noninvasive IPMC and invasive pancreatic cancer while showing no significant expression in non-neoplastic pancreatic ducts or low-grade PanIN / IPMA. Several useful biomarkers have been reported previously to aid in the identification of pancreatic cancer. One such biomarker is insulin-like growth factor II messenger ribonucleic acid-binding protein 3 (IMP3), an oncofetal protein that is expressed during embryogenesis but nearly silenced in normal mature tissues. IMP3 is highly expressed in PDAC (72.3%) and IPMC (50%), although it can occasionally be found in low-grade PanIN and adenomas (20%) [3]. Maspin, a serine proteinase inhibitor, was first identified as a potential tumor suppressor due to its differential expression between normal mammary epithelial cells. It has been detected in PDAC (94%) and high-grade PanIN (78%) but is expressed at a lower rate in low-grade PanIN (48%) [4, 24]. A novel murine monoclonal antibody, mAb Das-1 (formerly known as $7E_{12}H_{12}$, IgM isotype), serves as a sensitive and highly specific biomarker for high-grade PanIN (58%), PDAC (74%) and IPMC (57–100%). However, Das-1 expression is less pronounced in IPMA (4.3–5.9%) and low-grade PanIN (0%) [25, 26]. Many of the biomarkers previously reported are often biased by immunostaining results. When a marker shows high expression in malignant lesions, there is still a notable incidence of high expression in low-grade PanIN and IPMA. Conversely, if a marker exhibits low expression in low-grade PanIN and IPMA, the expression rates in PDAC and IPMC tend to be similarly low. To date, no markers have been reported that can accurately distinguish between PDAC and low-grade PanIN, or IPMC and IPMA, while simultaneously identifying PDAC and IPMC with a high degree of accuracy (around 80%). Our findings suggest that ACSL4 expression could effectively differentiate malignant lesions from benign ones, including low-grade PanIN and IPMA. In some cases, resected or rapid intraoperative specimens of pancreatic cancer can be challenging to differentiate from malignancy due to the presence of low-grade PanIN or IPMA at the resection margins. Immunostaining of ACSL4 may be beneficial in these scenarios. Our study was exploratory in nature, and therefore, *P* values were not adjusted for multiple comparisons. However, these findings should be interpreted with caution, and further validation studies are needed. For the interpretation of immunostaining in the present study, multiple locations were randomly evaluated within a narrow range of 0.015–0.025 mm^2. This field of view corresponds to what can be observed at 400x magnification during actual pathological diagnoses. The focused evaluation approach may ultimately be applicable to specimens with minute volumes obtained through endoscopic ultrasound-guided fine-needle biopsy or peroral pancreatoscopy-guided biopsy.

Interestingly, poorly differentiated pancreatic cancer exhibited a lower positive rate of ACSL4 immunostaining compared to well or moderately differentiated tumors. This observation aligns with the finding that poorly differentiated pancreatic cancer is associated with a shorter disease-free survival after resection [27]. Surgical specimens of pancreatic cancer are highly heterogeneous, often containing predominantly well-differentiated regions alongside small proportions of poorly differentiated regions. ACSL4 is primarily active in the early stages of carcinogenesis and may be suppressed by other factors during tumor progression and metastasis. Therefore, in cases where ACSL4 expression is low in pancreatic cancer, careful diagnosis is essential to ensure that poorly differentiated cells are not overlooked locally.

The expression of ACSL4 and its clinical significance in patients with pancreatic cancer had not been thoroughly examined prior to this study. In the present research, we assessed ACSL4 expression through immunohistochemistry and investigated its prognostic significance alongside clinicopathologic parameters in 96 patients with pancreatic cancer who underwent surgical resection at our institution. While numerous studies have reported prognostic analyses of pancreatic cancer using immunostaining of various biomarkers, many of these investigations have included cases of early recurrence within 6 months [28–30]. Since early recurrence is often closely linked to whether complete resection was achieved and whether any potential metastases were present [8], the

clinical utility of these analyses, particularly for pancreatic cancer, raises questions. Unlike many other studies, cases of early recurrence within 6 months were excluded from the present study. Previous research has shown that ACSL4 exhibits both tumor-promoting and tumor-suppressing functions across various tumor types, making its role in pancreatic cancer particularly elusive. In gastric cancer and lung adenocarcinoma, ACSL4 has been identified as having a tumor-suppressive role by impairing cell growth and migration [6, 31]. Conversely, overexpression of ACSL4 has been associated with poor prognosis in estrogen receptor-negative breast cancer, colorectal cancer, HCC, and prostate cancer [32].

In the 13 cases with low ACSL4 expression, disease-free survival was significantly shorter compared to those with high expression. This study is the first to report that low ACSL4 expression is an independent risk factor for disease-free survival in pancreatic cancer.

The association of low ACSL4 expression with shorter disease-free survival may be influenced by multiple factors, though these interpretations warrant further validation. Several reasons may explain the association of low ACSL4 expression with shorter disease-free survival. First, pancreatic cancer cases with low ACSL4 expression may include a higher proportion of poorly differentiated carcinoma cells, such as those exhibiting vacuolated, micropapillary, or solid nest patterns. Even well or moderately differentiated cancers with low ACSL4 expression can contain these cell patterns, which may correlate with metastasis and recurrence. Second, ferroptosis may play a role in this outcome. Although we did not investigate the specific molecules regulating ACSL4 expression, some regulators have been reported to suppress ACSL4 [33]. In pancreatic cancer, proteins, such as protein tyrosine phosphatase mitochondrial 1, ADP ribosylation factors (ARF6), and microRNA-3173-5p, have been identified as upward regulators [34–37]. These regulators could lead to the downregulation of ACSL4 expression, resulting in decreased ferroptosis and promoted metastasis. Hypoxia and reduced tumor immunity may be also involved in low ACSL4 expression [38, 39]. Alternatively, knockdown of ACSL4 directly may be directly involved in differentiation, invasion, and migration., as the previous report of stomach cancer [6].

Immunostaining of ACSL4 in pancreatic cancer holds significant implications for both pathological diagnosis and treatment options. Detecting lymphovascular invasion in pancreatic cancer can be challenging due to fibrosis and infiltrating lymphocytes. Our research indicates that low ACSL4 expression is associated with lymphovascular invasion. Therefore, when diagnosing pancreatic cancer with low ACSL4 expression, it is crucial to carefully evaluate for lymphovascular invasion.

In Japan, post-operative follow-up for pancreatic cancer typically involves imaging studies and monitoring tumor markers, with adjuvant chemotherapy using oral fluoropyrimidine often administered for 6 months to a year [40]. In our study, the Kaplan–Meier curve for disease-free survival indicates a gradual divergence after one year, aligning with the typical endpoint for adjuvant chemotherapy. Given the association of low ACSL4 expression with poorer outcomes, extending the duration of adjuvant chemotherapy may be warranted for patients with this expression profile.

Our study had some limitations. First, it was a single-center study with a relatively small sample size and broad confidence intervals, which may affect the generalizability of the findings. Second, we did not investigate the relationship between ACSL4 and the stroma, which plays an important role in pancreatic cancer progression. Third, we included a mixture of PDAC and IPMC cases for prognostic analysis, although we excluded IPMC cases with noninvasive or small invasive components. Notably, the statistical analysis results were consistent only in cases of PDAC. Further studies with larger sample sizes are required to directly compare the sensitivity and specificity of ACSL4 with other biomarkers.

In conclusion, our study showed that ACSL4 expression in malignant lesions was higher than in benign lesions in pancreatic tumors. The cases of ACSL4 low expression in malignant lesions are associated with shorter disease-free survival, lymphovascular invasion, and recurrence of distant metastasis.

Abbreviations

ACSL4	Long chain fatty acyl CoA synthetase 4
PDAC	Pancreatic ductal adenocarcinoma
IPMN	Intraductal papillary mucinous neoplasm
IPMA	Intraductal papillary mucinous adenoma
IPMC	Intraductal papillary mucinous carcinoma
PanIN	Pancreatic intraepithelial neoplasm
NDP	NanoZoomer Digital Pathology
HCC	Hepatocellular carcinoma
OR	Odds ratio
CI	Confidence interval
HR	Hazard ratio

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13000-025-01659-6.

Supplementary Material 1: Figure 1. Analysis of the differences in ACSL4 immunohistochemistry based on differentiation.

Supplementary Material 2: Figure 2. Analysis of clinicopathological characteristics of ACSL4 in invasive PDAC and IPMC.

Supplementary Material 3: Figure 3. Normal duodenum mucosa exhibits cytoplasmic expression of ACSL4 (A, ACSL4, magnification x400), whereas normal colonic mucosa is negative for ACSL4 (B, ACSL4, magnification x400).

Supplementary Material 4: Figure 4. In this slide of IPMC, both IPMA and invasive IPMC are present (A, H&E, magnification x100; B, ACSL4, magnification x100). IPMA exhibits low ACSL4 expression, graded as 1 or 2 (C, H&E,

magnification x400; D, ACSL4, magnification x400). Invasive IPMC exhibits high ACSL4 expression (E, H&E, magnification x400; F, ACSL4, magnification x400). Even when IPMA and IPMC were found in the same specimen, staining differences were generally clear, with IPMA being negative and IPMC being positive.

Supplementary Material 5: Figure.5. In vacuolated cell patterns, infiltrating glandular elements often form a cribriform pattern characterized by multiple vacuoles of varying sizes, separated by thin strands of cytoplasm. These vacuoles frequently contain necrotic debris mixed with neutrophils (A, H&E, magnification x400). Vacuolated cell patterns exhibit low ACSL4 expression, corresponding to Grade 2 (B, ACSL4, magnification x400). The micropapillary cell pattern is characterized by clusters of neoplastic cells forming micropapillary structures in empty spaces, often accompanied by neutrophils that create microabscesses (C, H&E, magnification x400). Micropapillary cell patterns exhibit low ACSL4 expression, indicating Grade 1 (D, ACSL4, magnification x400). Solid nest cell patterns are characterized by focal proliferation without clear glandular duct formation (E, H&E, magnification x400). These patterns also exhibit low ACSL4 expression, classified as Grade 1 (F, ACSL4, magnification x400).

Supplementary Material 6: Figure6. Kaplan–Meier curves showing overall survival (A) and disease-free survival (B) in patients with pancreatic cancer. The red lines represent patients with PDAC who received neoadjuvant therapy, the blue lines represent patients with PDAC who underwent upfront surgery, and the green lines represent patients with IPMC. No significant differences were observed in overall or disease-free survival among the three groups.

Supplementary Material 7: Figure7. Kaplan–Meier curves showing overall survival (A) and disease-free survival (B) in patients with pancreatic cancer, including cases of recurrence within 6 months and cases of IPMC with noninvasive or minimally invasive components comprising less than 50%. The red lines represent low ACSL4 expression, the blue lines represent high ACSL4 expression. Low ACSL4 expression is associated with shorter overall survival and disease-free survival ($P = 0.012$, < 0.001, respectively).

Supplementary Material 8: Figure8. the histogram showed the follow-up duration (A). The graph's peak was slightly skewed to the left, suggesting that the follow-up duration was not normally distributed. Therefore, we compared the follow-up duration between the high ACSL4 expression and the low ACSL4 expression groups using Mann-Whitney U test (B). There were no significant differences between the two groups ($P = 0.672$).

Supplementary Material 9

Acknowledgements
None.

Author contributions
SS, YH, KK, EK, FN, and TN analyzed and interpreted the pathological data. SO, KM, and KN collected and prepared the clinical data. YH and MS carried out statistical analysis. MH conducted the literature search and wrote the paper. All authors read and approved the final manuscript.

Funding
None.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This retrospective, single-center study was approved by our institution's ethics committee (protocol no. CR24-016) and conducted in accordance with the Helsinki Declaration. Written informed consent was obtained from all participants, and an opt-out option was provided, allowing the participants to be notified and permitting the publication of research information on our website.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Pathology, School of Medicine, University of Occupational and Environmental Health, Kitakyushu, Japan
[2]Third Department of Internal Medicine, School of Medicine, University of Occupational and Environmental Health, Kitakyushu, Japan

Received: 26 February 2025 / Accepted: 30 April 2025
Published online: 10 May 2025

References

1. Kolbeinsson HM, Chandana S, Wright GP, Chung M. Pancreatic cancer: A review of current treatment and novel therapies. J Invest Surg. 2023;36(1):2129884.
2. Wang S, Zheng Y, Yang F, Zhu L, Zhu XQ, Wang ZF, et al. The molecular biology of pancreatic adenocarcinoma: translational challenges and clinical perspectives. Signal Transduct Target Ther. 2021;6(1):249.
3. Senoo J, Mikata R, Kishimoto T, Hayashi M, Kusakabe Y, Yasui S, et al. Immunohistochemical analysis of IMP3 and p53 expression in endoscopic ultrasound-guided fine needle aspiration and resected specimens of pancreatic diseases. Pancreatology. 2018;18(2):176–83.
4. Cao D, Zhang Q, Wu LS, Salaria SN, Winter JW, Hruban RH, et al. Prognostic significance of Maspin in pancreatic ductal adenocarcinoma: tissue microarray analysis of 223 surgically resected cases. Mod Pathol. 2007;20(5):570–8.
5. Quan J, Bode AM, Luo X. ACSL family: the regulatory mechanisms and therapeutic implications in cancer. Eur J Pharmacol. 2021;909:174397.
6. Ye X, Zhang Y, Wang X, Li Y, Gao Y. Tumor-suppressive functions of long-chain acyl-CoA synthetase 4 in gastric cancer. IUBMB Life. 2016;68(4):320–7.
7. Bai R, Rebelo A, Kleeff J, Sunami Y. Identification of prognostic lipid droplet-associated genes in pancreatic cancer patients via bioinformatics analysis. Lipids Health Dis. 2021;20(1):58.
8. Chick RC, Gunderson AJ, Rahman S, Cloyd JM. Neoadjuvant immunotherapy for localized pancreatic cancer: challenges and early results. Cancers (Basel). 2023;15(15).
9. Kang MJ, Lee KB, Jang JY, Kwon W, Park JW, Chang YR, et al. Disease spectrum of intraductal papillary mucinous neoplasm with an associated invasive carcinoma invasive IPMN versus pancreatic ductal adenocarcinoma-associated IPMN. Pancreas. 2013;42(8):1267–74.
10. Oda Y, Aishima S, Shindo K, Fujino M, Mizuuchi Y, Hattori M, et al. SLC2A1/GLUT1 expression in mural nodules of intraductal papillary mucinous neoplasm of the pancreas. Hum Pathol. 2017;65:71–8.
11. Seelen LWF, van Floortje A, Brada LJH, Groot VP, Daamen LA, Walma MS, et al. Early recurrence after resection of locally advanced pancreatic Cancer following induction therapy: an international multicenter study. Ann Surg. 2023;278(1):118–26.
12. Groot VP, Gemenetzis G, Blair AB, Rivero-Soto RJ, Yu J, Javed AA, et al. Defining and predicting early recurrence in 957 patients with resected pancreatic ductal adenocarcinoma. Ann Surg. 2019;269(6):1154–62.
13. Imamura M, Nagayama M, Kyuno D, Ota S, Murakami T, Kimura A et al. Perioperative predictors of early recurrence for resectable and Borderline-Resectable pancreatic Cancer. Cancers (Basel). 2021;13(10).
14. Kim N, Han IW, Ryu Y, Hwang DW, Heo JS, Choi DW et al. Predictive nomogram for early recurrence after pancreatectomy in resectable pancreatic cancer: risk classification using preoperative clinicopathologic factors. Cancers (Basel). 2020;12(1).
15. Konishi T, Takano S, Furukawa K, Takayashiki T, Kuboki S, Suzuki D, et al. Impact of resection margin status on survival after operation for pancreatic head cancer with extrapancreatic nerve plexus invasion. J Surg Oncol. 2022;126(6):1038–47.
16. Kato K, Yamada S, Sugimoto H, Kanazumi N, Nomoto S, Takeda S, et al. Prognostic factors for survival after extended pancreatectomy for pancreatic head cancer: influence of resection margin status on survival. Pancreas. 2009;38(6):605–12.
17. Tuohetahuntila M, Spee B, Kruitwagen HS, Wubbolts R, Brouwers JF, van de Lest CH, et al. Role of long-chain acyl-CoA synthetase 4 in formation of

polyunsaturated lipid species in hepatic stellate cells. Biochim Biophys Acta. 2015;1851(2):220–30.
18. Golej DL, Askari B, Kramer F, Barnhart S, Vivekanandan-Giri A, Pennathur S, et al. Long-chain acyl-CoA synthetase 4 modulates prostaglandin E_2 release from human arterial smooth muscle cells. J Lipid Res. 2011;52(4):782–93.
19. Shimbara-Matsubayashi S, Kuwata H, Tanaka N, Kato M, Hara S. Analysis on the substrate specificity of Recombinant human Acyl-CoA synthetase ACSL4 variants. Biol Pharm Bull. 2019;42(5):850–5.
20. Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, et al. Ferroptosis: an iron-dependent form of nonapoptotic cell death. Cell. 2012;149(5):1060–72.
21. Bozza PT, Viola JP. Lipid droplets in inflammation and cancer. Prostaglandins Leukot Essent Fat Acids. 2010;82(4–6):243–50.
22. Tang Y, Zhou J, Hooi SC, Jiang YM, Lu GD. Fatty acid activation in carcinogenesis and cancer development: essential roles of long-chain acyl-CoA synthetases. Oncol Lett. 2018;16(2):1390–6.
23. Ndiaye H, Liu JY, Hall A, Minogue S, Morgan MY, Waugh MG. Immunohistochemical staining reveals differential expression of ACSL3 and ACSL4 in hepatocellular carcinoma and hepatic Gastrointestinal metastases. Biosci Rep. 2020;40(4).
24. Fukushi K, Yamamiya A, Tominaga K, Abe Y, Hoshi K, Nagashima K et al. Usefulness of adding Maspin staining to p53 staining for EUS-FNA specimens of pancreatic ductal adenocarcinoma. J Clin Med. 2022;11(20).
25. Das KK, Xiao H, Geng X, Fernandez-Del-Castillo C, Morales-Oyarvide V, Daglilar E, et al. mAb Das-1 is specific for high-risk and malignant intraductal papillary mucinous neoplasm (IPMN). Gut. 2014;63(10):1626–34.
26. Das KK, Brown JW, Fernandez Del-Castillo C, Huynh T, Mills JC, Matsuda Y, et al. mAb Das-1 identifies pancreatic ductal adenocarcinoma and high-grade pancreatic intraepithelial neoplasia with high accuracy. Hum Pathol. 2021;111:36–44.
27. Crippa S, Partelli S, Zamboni G, Barugola G, Capelli P, Inama M, et al. Poorly differentiated resectable pancreatic cancer: is upfront resection worthwhile? Surgery. 2012;152(3 Suppl 1):S112–9.
28. Adachi Y, Sato N, Oba T, Amaike T, Kudo Y, Kohi S, et al. Prognostic and functional role of hyaluronan-binding protein 1 in pancreatic ductal adenocarcinoma. Oncol Lett. 2022;24(1):222.
29. Koga A, Sato N, Kohi S, Yabuki K, Cheng XB, Hisaoka M, et al. KIAA1199/CEMIP/HYBID overexpression predicts poor prognosis in pancreatic ductal adenocarcinoma. Pancreatology. 2017;17(1):115–22.
30. Kudo Y, Kohi S, Hirata K, Goggins M, Sato N. Hyaluronan activated-metabolism phenotype (HAMP) in pancreatic ductal adenocarcinoma. Oncotarget. 2019;10(54):5592–604.
31. Zhang Y, Li S, Li F, Lv C, Yang QK. High-fat diet impairs ferroptosis and promotes cancer invasiveness via downregulating tumor suppressor ACSL4 in lung adenocarcinoma. Biol Direct. 2021;16(1):10.
32. Hou J, Jiang C, Wen X, Li C, Xiong S, Yue T, et al. ACSL4 as a potential target and biomarker for anticancer: from molecular mechanisms to clinical therapeutics. Front Pharmacol. 2022;13:949863.
33. Ding K, Liu C, Li L, Yang M, Jiang N, Luo S, et al. Acyl-CoA synthase ACSL4: an essential target in ferroptosis and fatty acid metabolism. Chin Med J (Engl). 2023;136(21):2521–37.
34. Yu WM, Liu X, Shen J, Jovanovic O, Pohl EE, Gerson SL, et al. Metabolic regulation by the mitochondrial phosphatase PTPMT1 is required for hematopoietic stem cell differentiation. Cell Stem Cell. 2013;12(1):62–74.
35. Huang XD, Xiao FJ, Guo YT, Sun Y, Zhang YK, Shi XJ. Protein tyrosine phosphatase 1 protects human pancreatic cancer from erastin-induced ferroptosis. Asian J Surg. 2022;45(11):2214–23.
36. Ye Z, Hu Q, Zhuo Q, Zhu Y, Fan G, Liu M, et al. Abrogation of ARF6 promotes RSL3-induced ferroptosis and mitigates gemcitabine resistance in pancreatic cancer cells. Am J Cancer Res. 2020;10(4):1182–93.
37. Qi R, Bai Y, Li K, Liu N, Xu Y, Dal E, et al. Cancer-associated fibroblasts suppress ferroptosis and induce gemcitabine resistance in pancreatic cancer cells by secreting exosome-derived ACSL4-targeting MiRNAs. Drug Resist Updat. 2023;68:100960.
38. Zheng S, Mo J, Zhang J, Chen Y. HIF–1α inhibits ferroptosis and promotes malignant progression in non–small cell lung cancer by activating the Hippo–YAP signalling pathway. Oncol Lett. 2023;25(3):90.
39. Liao P, Wang W, Wang W, Kryczek I, Li X, Bian Y, et al. CD8(+) T cells and fatty acids orchestrate tumor ferroptosis and immunity via ACSL4. Cancer Cell. 2022;40(4):365–e786.
40. Uesaka K, Boku N, Fukutomi A, Okamura Y, Konishi M, Matsumoto I, et al. Adjuvant chemotherapy of S-1 versus gemcitabine for resected pancreatic cancer: a phase 3, open-label, randomised, non-inferiority trial (JASPAC 01). Lancet. 2016;388(10041):248–57.

Publisher's note