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# Adipophilin expression is an indicator of poor prognosis in patients with pancreatic ductal adenocarcinoma: An immunohistochemical analysis

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ABSTRACT

*Objective:* Adipophilin is a lipid droplet-associated protein, and its expression has been correlated with aggressive clinical behavior in some types of carcinomas, though its role in pancreatic ductal adenocarcinoma (PDAC) has not been clarified. This study aimed to evaluate the role of adipophilin in PDAC.
*Methods:* By immunohistochemical staining using tissue microarrays, we analyzed the expression profiles of adipophilin in 181 consecutive PDAC patients who underwent macroscopic margin-negative resection from January 2008 to December 2015. Overall survival (OS) and recurrence-free survival (RFS) were compared based on adipophilin expression, and the risk factors for OS, RFS, and early recurrence (within 6 months) were analyzed.
*Results:* Of the 181 evaluated patients, 51 (28.2%) were positive for adipophilin expression. A histopathological grade of 3 ($p = 0.0012$), higher CA19-9 level ($p = 0.0016$), and R1 status ($p = 0.028$) were significantly associated with adipophilin-positive patients who had significantly poor OS and RFS compared to those associated with adipophilin-negative patients ($p = 0.0007$ and $p = 0.0022$, respectively). They also showed a significantly higher incidence of early recurrence ($p = 0.030$), based on multivariate analyses.
*Conclusions:* Adipophilin is a potential independent prognostic marker for PDAC.


## Introduction

Adipophilin is a lipid droplet-associated protein present on the surface of lipid droplets within the cytoplasm [1]. It has been reported to play an important role in the formation and maintenance of lipid droplets [1]. Recently, with the availability of an effective antibody against adipophilin, visualization of intracytoplasmic lipid droplets on formalin-fixed and paraffin-embedded tissue sections has become possible. Since then, the expression profiles of adipophilin in some tumor types have been examined. The anti-adipophilin antibody has also been used to analyze metabolic dysregulation in organs such as the liver [2].

It has been well recognized that lipogenic metabolism may be altered and up-regulated in various types of tumors [3]. Adipophilin expression in different benign and malignant tumors has, therefore, been analyzed [3–11]. Previous studies have demonstrated an association between adipophilin expression and aggressive clinical behavior in some types of carcinomas, including lung adenocarcinomas [6], colorectal cancers [9], and clear cell renal cell carcinomas [10]. It has also been shown to be associated with the malignant potential of tumors such as cutaneous melanocytic [5] and gastric epithelial neoplasms [7]. Pancreatic ductal adenocarcinoma (PDAC) is one of the highly aggressive carcinomas and is a leading cause of cancer-related deaths. Adipophilin expression in PDACs has not yet been analyzed. In the present study, we, therefore, aimed to examine the adipophilin expression profiles in PDACs using tissue microarrays and evaluate its relationship with the clinicopathological parameters in PDAC.

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## Materials and methods

### Patient selection

We enrolled 213 consecutive patients with PDAC who underwent surgical resection from January 2008 to December 2015 at the Kansai Medical University Hospital. Patients who died of other causes or complications (19 patients) were excluded from the study. Patients who were out of clinical follow-up within 24 months after surgery (13 patients) were also excluded because these patients were visiting a different hospital after surgery, and the information regarding recurrence or survival was not available. Finally, 181 patients who had R0 or R1 resection were included in this study.

This study was conducted in accordance with the Declaration of Helsinki, and the study protocol was approved by the Institutional Review Board of the Kansai Medical University Hospital (protocol no. 2017108 and 2017306).

### Histopathological analyses

Surgically resected specimens were formalin-fixed and sectioned. All sections were paraffin-embedded, stained with hematoxylin and eosin, and examined histopathologically. The histopathological grading (Grades 1, 2, and 3) was based on the recent World Health Organization Classification 2010 [12], which is based on mucin production, mitoses, and nuclear features as well as the degree of glandular formation. If there were different grading components within the same tumor, the highest grade was adopted. Neoplastic cells with a clear cytoplasm have been reported to have positive immunoreactivity for adipophilin [4,10]. According to these findings, we also evaluated the presence of clear neoplastic cells in the sections. The above-mentioned histopathological features were independently evaluated by more than two pathologists who were blinded to the clinical outcomes. Disagreements were resolved by reassessment using a multi-headed microscope.

All cases were staged according to the 8th Union for International Cancer Control TNM Classification [13].

### Tissue microarrays

The most morphologically representative carcinoma regions were selected on the hematoxylin and eosin-stained slides, and two tissue cores (2 mm in diameter) were punched out from the paraffin-embedded blocks for each patient. These tissue cores were then arrayed in a paraffin block.

### Immunohistochemistry

Immunohistochemical analyses were performed using an autostainer (Discovery, Roche Diagnostics, Basel, Switzerland) according to the manufacturer's instructions. An anti-adipophilin mouse monoclonal primary antibody (AP125, Progen Biotechnik, Heidelberg, Germany) was used to detect adipophilin expression. The sebaceous glands of the skin were used as an outer positive control. Adipophilin expression was considered to be positive when more than 5% of the neoplastic cells showed granular and/or globular cytoplasmic expression, as previously reported [6]. Immunohistochemical stainings were also independently evaluated by two pathologists who were blinded to the clinical outcomes. We defined an adipophilin-positive case as when one or more cores from the same patient showed immunoreactivity.

### Statistical analysis

JMP Start Statistics version 14 (Statistical Discovery Software; SAS Institute, Cary, NC, USA) was used to perform the statistical analyses. The Chi-square test was used for comparison between the adipophilin- positive and -negative groups. All patients have been followed up for at least 2 years, and the last follow-up date was March 16, 2018. The overall survival (OS) and recurrence-free survival (RFS) rates were evaluated using the Kaplan-Meier method. OS and recurrence-free times were calculated from the day of surgery in patients with or without neoadjuvant therapy. The log-rank tests were performed to compare the two groups. A p-value of $<0.05$ was considered to be significant. The continuous variables (tumor diameters and preoperative CA19-9 levels) were binarized by the cutoff values based on the ROC curve. Univariable and multivariable survival analyses were performed with the Cox proportional hazards model and were expressed as hazard ratios. The risk factors for early recurrence (within 6 months) were determined by logistic regression analysis and were expressed as odds ratios. Significant factors identified by univariate analyses were further evaluated by multivariate logistic regression analyses to identify significant independent factors for early recurrence.

## Results

### Clinicopathological features

Table 1 summarizes the clinicopathological features of the patients. Of all the included patients, 74 were women, and 107 were men. The age of the patients at the time of surgery ranged from 36 to 86 years (median: 68 years). The median follow-up period was 48 months (range: 25–115 months). While 122 (67.4%) patients died of the disease, 126 (69.6%) of them had a relapse.

In 117 cases, the tumor was located in the head of the pancreas, while in 64 of them, it was in the body or tail. Based on the National Comprehensive Cancer Network (NCCN) resectability status, 134 of the cases were classified as resectable (R), 43 as borderline resectable (BR), and 4 as unresectable (UR). Based on the residual tumor grading, 150 patients were classified as R0 and 31 as R1. The tumors were staged as pT1b, pT1c, pT2, pT3, and pT4 in 1, 10, 105, 63 and 2 patients, respectively. Lymph node metastasis was seen in 133 (73.5%) patients. The patients were staged as IA (5 patients), IB (36 patients), IIA (7 patients), IIB (69 patients), III (63 patients), and IV (1 patient). Preoperative neoadjuvant chemotherapy was given to 69 (38.1%) patients.

Based on the histopathology, 42 (23.2%), 115 (63.5%), and 24 (13.3%) patients had Grade 1, 2, and 3, respectively. Clear neoplastic cells were present in 44 (24.3%) patients (Fig. 1).

### Adipophilin expression

No adipophilin-positive cells were observed in the non-neoplastic pancreatic tissue. Adipophilin expression was observed in 51 (28.2%) patients, and as shown in Fig. 2, subnuclear globular staining was noted in most cases. Table 1 summarizes the relationship between adipophilin expression and the clinicopathological parameters. Adipophilin expression was significantly associated with a histopathological grade of 3 ($p = 0.0012$) and had higher preoperative CA19-9 levels ($p = 0.0016$). Although adipophilin expression showed no correlation with tumor diameter ($p = 0.052$), pathological tumor stage ($p = 0.23$), and presence of lymph node metastasis ($p = 0.57$), the adipophilin-positive patients had a significantly higher incidence of R1 resection than the adipophilin-negative patients (27.5% vs. 13.1%, respectively, $p = 0.028$). Moreover,

**Table 1**
Correlation between clinicopathological characteristics and adipophilin expression.

| | Adipophilin-positive (n = 51) (%) | Adipophilin-negative (n = 130) (%) | *P-value* |
|---|---|---|---|
| Age (year) | 67.5 ± 10.9 | 68.2 ± 9.2 | *0.69* |
| Gender | | | |
| Male | 26 (51.0) | 81 (62.3) | |
| Female | 25 (49.0) | 49 (37.7) | *0.16* |
| Location | | | |
| Head | 35 (68.6) | 82 (63.1) | |
| Body and tail | 16 (31.4) | 48 (36.9) | *0.48* |
| Body mass index | | | |
| <25 | 43 (84.3) | 114 (87.7) | |
| ≧25 | 8 (15.7) | 16 (12.3) | *0.55* |
| CA19-9 (U/mL) | 731.0 ± 1565.4 | 252.1 ± 436.5 | ***0.0016*** |
| Albumin (g/dL) | 3.77 ± 0.52 | 3.88 ± 0.53 | *0.2* |
| NCCN resectability status | | | |
| R | 38 (74.5) | 96 (73.8) | |
| BR or UR | 13 (25.5) | 34 (26.2) | *0.58* |
| Tumor diameter (mm) | 38.9 ± 15.5 | 34.5 ± 12.6 | *0.052* |
| Histopathological type | | | |
| Grade 1 | 5 (9.8) | 37 (28.5) | |
| Grade 2 | 33 (64.7) | 82 (63.1) | |
| Grade 3 | 13 (25.5) | 11 (8.5) | ***0.0012*** |
| T category [a] | | | |
| 1a | 0 (0) | 0 (0) | |
| 1b | 0 (0) | 1 (0.8) | |
| 1c | 0 (0) | 10 (7.7) | |
| 2 | 31 (60.8) | 74 (56.9) | |
| 3 | 20 (39.2) | 43 (33.1) | |
| 4 | 0 (0) | 2 (1.5) | *0.23* |
| Clear cells | | | |
| Present | 11 (21.6) | 33 (25.4) | |
| Absent | 40 (78.4) | 97 (74.6) | *0.59* |
| pStage[b] | | | |
| Ia | 0 (0) | 5 (3.8) | |
| Ib | 10 (19.6) | 26 (20.0) | |
| IIa | 2 (3.9) | 5 (3.8) | |
| IIb | 19 (37.3) | 50 (38.5) | |
| III | 20 (39.2) | 43 (33.1) | |
| IV | 0 (0) | 1 (0.8) | *0.74* |
| Lymph node metastasis | | | |
| Positive | 39 (76.5) | 94 (72.3) | |
| Negative | 12 (23.5) | 36 (27.7) | *0.57* |
| Neoadjuvant chemotherapy | | | |
| Present | 16 (31.4) | 53 (40.8) | |
| Absent | 35 (68.6) | 77 (59.2) | *0.24* |
| Adjuvant chemotherapy | | | |
| Present | 44 (86.3) | 110 (84.6) | |
| Absent | 7 (13.7) | 20 (15.4) | *0.77* |
| Operation procedures | | | |
| Pancreaticoduodenectomy | 32 (62.7) | 86 (66.2) | |
| Distal pancreatectomy | 15 (29.4) | 41 (31.5) | |
| Total pancreatectomy | 4 (7.8) | 3 (2.3) | *0.22* |
| Combined resection | | | |
| Present | 22 (43.1) | 71 (54.6) | |
| Absent | 29 (56.9) | 59 (45.4) | *0.16* |
| Hospital stays (day) | 16.9 ± 13.8 | 17.5 ± 15.7 | *0.82* |
| Bleeding (mL) | 1069.2 ± 920.4 | 1108.3 ± 771.9 | *0.77* |
| Resection status | | | |
| R0 | 37 (72.5) | 113 (86.9) | |
| R1 | 14 (27.5) | 17 (13.1) | ***0.028*** |

[a] T1a, tumor 0.5 cm or less; T1b, tumor greater than 0.5 cm less than 1 cm; T1c, tumor greater than 1 cm but no more than 2 cm; T2, tumor more than 2 cm but no more than 4 cm; T3, tumor more than 4 cm; T4, tumor involves coeliac axis, superior mesenteric artery, and/or common hepatic artery (reference 13).
[b] Stage IA, T1N0M0; Stage IB, T2N0M0; Stage IIA, T3N0M0, Stage IIB, T1-3N1M0; Stage III, T1-4N2M0 or T4AnyNM0; Stage IV, AnyTAnyNM1 (reference 13).

adipophilin expression was not associated with the presence of clear cells ($p = 0.59$).

*Prognostic significance of adipophilin expression*

Fig. 3 compares the survival curves of the adipophilin-positive and -negative patients. The OS and RFS in the adipophilin-positive patients were significantly worse compared to those in the adipophilin-negative patients ($p = 0.0007$ and $p = 0.0022$, respectively). While the median survival time for the adipophilin-negative patients was 31.5 months, it was 16 months for the adipophilin-positive patients.

Univariate Cox regression analyses showed that adipophilin expression, preoperative albumin level (<3.8 g/dL), CA19-9 level (>186 U/mL), NCCN resectability status of BR or UR, larger tumor size (>32 mm), pathological stages of III or IV, resection status of R1,

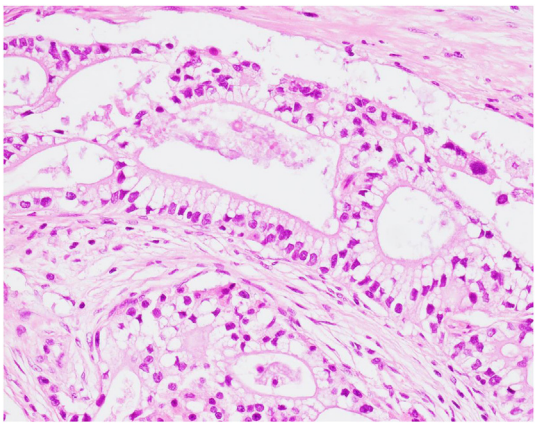


**Fig. 1.** Histopathological features of pancreatic ductal adenocarcinoma with clear cells (H&E, x 400).

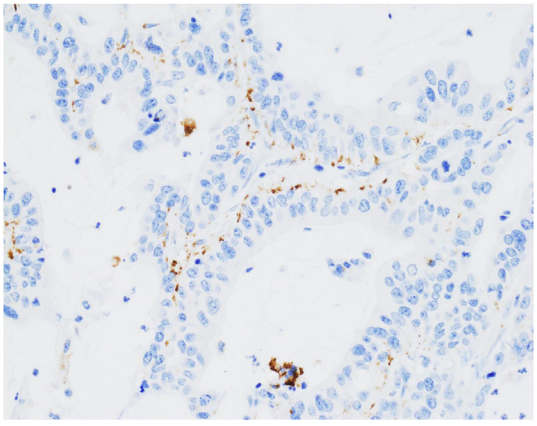


**Fig. 2.** Typical immunohistochemical staining of adipophilin in pancreatic ductal adenocarcinoma. Subnuclear globular staining is noted (x 400).

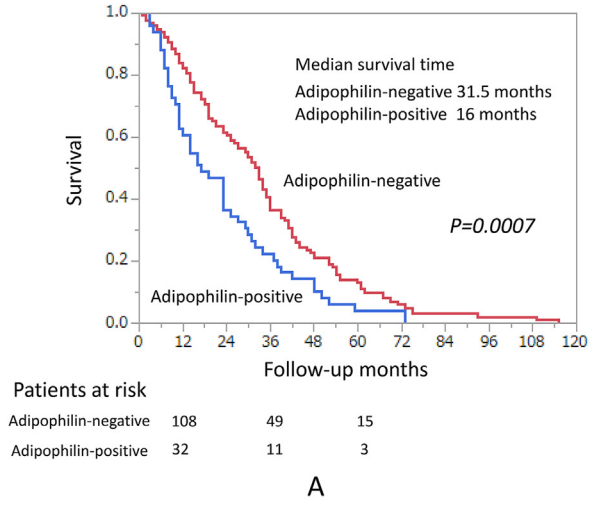
and presence of adjuvant chemotherapy were all significant poor prognostic factors (Table 2). Multivariate analyses clearly demonstrated that adipophilin expression was a significant and independent poor prognostic factor for OS (hazard ratio 1.64; 95% confidence interval 1.14–2.34; $p = 0.0084$) and early recurrence (odds ratio 2.43; 95% confidence interval 1.09–5.43; $p = 0.030$) (Table 3) in patients with PDAC. Additionally, the NCCN resectability status of BR or UR (hazard ratio 1.64; 95% confidence interval 1.13–2.34; $p = 0.0088$), higher preoperative CA19-9 levels (hazard ratio 1.48; 95% confidence interval 1.05–2.07; $p = 0.026$), and adjuvant chemotherapy (hazard ratio 0.45; 95% confidence interval 0.29–0.73; $p = 0.0018$) were also significant and independent poor prognostic factors.

## Discussion

In the present study, we demonstrate that 28.2% of the patients with PDAC expressed adipophilin. We also showed that the adipophilin expression in patients who underwent R0 or R1 resection was a significant and independent poor prognostic factor for OS ($p = 0.0084$) and early recurrence ($p = 0.030$), based on multivariate analyses.

Some previous reports have shown that adipophilin expression is related to poor prognosis in lung adenocarcinomas [6], colorectal carcinomas [9], and clear cell renal cell carcinomas [10]. However, to the best of our knowledge, this is the first study on adipophilin expression and its prognostic significance in PDAC. Fujimoto et al. have reported that the 5-year overall and disease-free survivals in patients with adipophilin-positive lung adenocarcinoma were significantly worse compared to those in the adipophilin-negative patients [6]. Our results are in line with these previous reports and suggest that adipophilin could be a useful prognostic marker in some kinds of carcinomas, including PDAC.

Many studies have attempted to determine the prognostic factors for PDAC [14]. CA19-9 levels, surgical margin status, tumor stage, and performance status have been shown to be strong prognostic factors for OS [14]. Moreover, a recent study showed that the NCCN resectability status was a useful prognostic indicator [15]. The results of our multivariate analyses showing that CA19-9 levels and NCCN resectability status are strong prognostic



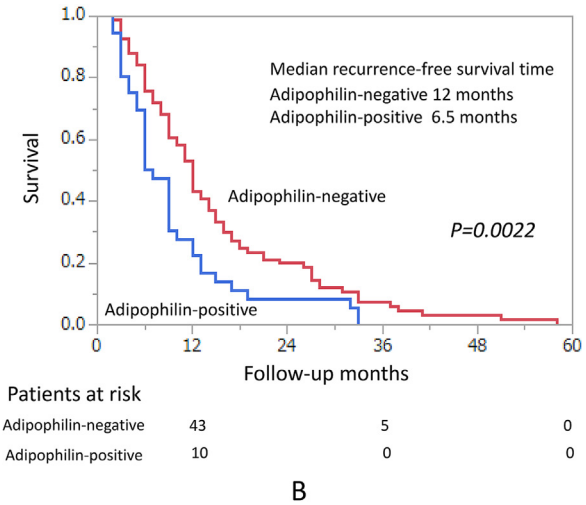




**Fig. 3.** The effect of adipophilin expression on the prognosis in pancreatic ductal adenocarcinoma (PDAC). (A) Overall survival curves for patients with adipophilin-positive (blue line) and adipophilin-negative (red line) ($P = 0.0007$) PDAC. (B) Recurrence-free survival curves for patients with adipophilin-positive (blue line) and adipophilin-negative (red line) ($P = 0.0022$) PDAC.

**Table 2**
Univariate and multivariate analyses for overall survival.

| Variables | Univariate | | | Multivariate | | |
|---|---|---|---|---|---|---|
| | Hazard ratio | 95% CI | *p value* | Hazard ratio | 95% CI | *p value* |
| Adipophilin (positive versus negative) | 1.68 | 1.19–2.33 | ***0.0036*** | 1.64 | 1.14–2.34 | ***0.0084*** |
| Age (≧75 versus <75) | 0.93 | 0.65–1.31 | *0.71* | | | |
| Gender (male versus female) | 1.13 | 0.82–1.53 | *0.44* | | | |
| Location (head versus body and tail) | 1.08 | 0.79–1.49 | *0.61* | | | |
| Body mass index (≧25 versus <25) | 0.97 | 0.61–1.48 | *0.91* | | | |
| CA19-9 (>186 versus ≦186 U/mL) | 1.63 | 1.17–2.23 | ***0.0037*** | 1.48 | 1.05–2.07 | ***0.026*** |
| Albumin (≧3.8 versus <3.8 g/dL) | 0.61 | 0.45–0.84 | ***0.0027*** | 0.78 | 0.56–1.10 | *0.164* |
| NCCN resectability status (BR/UR versus R) | 1.55 | 1.09–2.18 | ***0.015*** | 1.64 | 1.13–2.34 | ***0.0088*** |
| Tumor diameter (≧32 versus <32 mm) | 1.41 | 1.03–1.93 | ***0.028*** | 1.09 | 0.77–1.55 | *0.62* |
| Histopathological type (Grade 3 versus Grade 1/2)Histopathological type (Grade 3 versus Grade 1/2) | 1.45 | 0.91–2.22 | *0.11* | | | |
| Clear cells (present versus absent) | 1.21 | 0.83–1.71 | *0.3* | | | |
| T category (III/IV versus I/II) | 1.32 | 0.96–1.81 | *0.086* | | | |
| pStage (III/IV versus I/II) | 1.41 | 1.02–1.93 | ***0.037*** | 1.16 | 0.83–1.61 | *0.38* |
| Lymph node metastasis (present versus absent) | 1.4 | 0.99–2.03 | *0.051* | | | |
| Neoadjuvant chemotherapy (present versus absent) | 1.11 | 0.81–1.52 | *0.49* | | | |
| Combined resection (present versus absent) | 1.2 | 0.88–1.62 | *0.24* | | | |
| Resection status (R1 versus R0) | 1.85 | 1.20–2.75 | ***0.0059*** | 1.57 | 0.98–2.42 | *0.056* |
| Adjuvant therapy (present versus absent) | 0.47 | 0.31–0.73 | ***0.0016*** | 0.45 | 0.29–0.73 | ***0.0018*** |

CI, Confidence interval.
*T1a, tumor 0.5 cm or less; T1b, tumor greater than 0.5 cm less than 1 cm; T1c, tumor greater than 1 cm but no more than 2 cm; T2, tumor more than 2 cm but no more than 4 cm; T3, tumor more than 4 cm; T4, tumor involves coeliac axis, superior mesenteric artery, and/or common hepatic artery (reference 13).
**Stage IA, T1N0M0; Stage IB, T2N0M0; Stage IIA, T3N0M0, Stage IIB, T1-3N1M0; Stage III, T1-4N2M0 or T4AnyNM0; Stage IV, AnyTAnyNM1 (reference 13).

**Table 3**
Univariate and multivariate analyses for early recurrence (within 6 months).

| Variables | Univariate | | | Multivariate | | |
|---|---|---|---|---|---|---|
| | Odds ratio | 95% CI | *p value* | Odds ratio | 95% CI | *p value* |
| Adipophilin (positive versus negative) | 2.83 | 1.35–5.93 | ***0.0059*** | 2.43 | 1.09–5.43 | ***0.03*** |
| Age (≧75 versus <75) | 0.97 | 0.43–2.20 | *0.96* | | | |
| Gender (male versus female) | 0.76 | 0.37–1.55 | *0.45* | | | |
| Location (head versus body and tail) | 1.12 | 0.53–2.37 | *0.77* | | | |
| Body mass index (≧25 versus <25) | 0.29 | 0.07–1.31 | *0.11* | | | |
| CA19-9 (>186 versus ≦186 U/mL) | 2.97 | 1.43–6.16 | ***0.0033*** | 2.37 | 1.08–5.19 | ***0.03*** |
| Albumin (≧3.8 versus <3.8 g/dL) | 0.75 | 0.36–1.56 | *0.45* | | | |
| NCCN resectability status (BR/UR versus R) | 1.58 | 0.73–3.42 | *0.24* | | | |
| Tumor diameter (≧32 versus <32 mm) | 1.08 | 0.52–2.22 | *0.83* | | | |
| Histopathological type (Grade 3 versus Grade 1/2)Histopathological type (Grade 3 versus Grade 1/2) | 1.25 | 0.46–3.40 | *0.66* | | | |
| Clear cells (present versus absent) | 1.29 | 0.58–2.88 | *0.52* | | | |
| T category (3/4 versus 1/2) | 1.73 | 0.84–3.55 | *0.13* | | | |
| pStage (III/IV versus I/II) | 2.34 | 1.14–4.82 | ***0.021*** | 1.53 | 0.65–3.63 | *0.33* |
| Lymph node metastasis (present versus absent) | 2.95 | 1.16–9.06 | ***0.021*** | 1.87 | 0.59–5.92 | *0.28* |
| Neoadjuvant chemotherapy (present versus absent) | 1.33 | 0.65–2.74 | *0.43* | | | |
| Combined resection (present versus absent) | 1.69 | 0.82–3.49 | *0.15* | | | |
| Resection status (R1 versus R0) | 2.39 | 1.03–5.56 | ***0.042*** | 1.41 | 0.53–3.77 | *0.49* |
| Adjuvant therapy (present versus absent) | 0.39 | 0.16–0.95 | ***0.038*** | 0.4 | 0.15–1.09 | *0.074* |

CI, Confidence interval.
*T1a, tumor 0.5 cm or less; T1b, tumor greater than 0.5 cm less than 1 cm; T1c, tumor greater than 1 cm but no more than 2 cm; T2, tumor more than 2 cm but no more than 4 cm; T3, tumor more than 4 cm; T4, tumor involves coeliac axis, superior mesenteric artery, and/or common hepatic artery (reference 13).
**Stage IA, T1N0M0; Stage IB, T2N0M0; Stage IIA, T3N0M0, Stage IIB, T1-3N1M0; Stage III, T1-4N2M0 or T4AnyNM0; Stage IV, AnyTAnyNM1 (reference 13).

factors confirm these earlier findings. Novel prognostic markers that reflect the metabolic condition of the neoplastic cells such as the hypoxia-inducible factor (HIF) 1 alpha have also been reported in PDAC [16]. Our findings clearly demonstrate that adipophilin is an independent and strong prognostic factor for PDAC, probably reflecting the metabolic condition of the neoplastic cells.

Some previous reports have suggested that adipophilin expression indicates an up-regulation of lipid synthesis in the neoplastic cells [5,6]. Glycolysis has been established as the main energy-generating pathway in cancer cells, known as the Warburg effect [17]. Cancer cells have also been shown to contain high amounts of cytoplasmic lipids. The high rate of proliferation in cancer cells is mainly maintained by lipid catabolism [18,19], an effect that is pronounced under conditions of hypoxia [18,19]. Because PDAC cells survive under hypoxic conditions [20], adipophilin expression in these cells may reflect an up-regulation of lipid metabolism. Additional analyses are needed to clarify the molecular mechanisms underlying adipophilin expression in PDAC and its relationship with pathways that are activated by the hypoxic tumor microenvironment in PDAC.

In our study, clear neoplastic cells were occasionally found in PDACs, but we did not find an association between their presence and adipophilin expression, which is in confirmation with a

previous study on lung adenocarcinoma [6]. The clear cytoplasm in PDAC cells might be due to glycogen deposition. Moreover, in this study, most of the PDAC cases showed subnuclear globular staining for adipophilin, an expression pattern that has been observed in lung adenocarcinomas [6], gastric epithelial neoplasms [7], and colorectal adenomas [4].

There are some limitations to the present study. First, adipophilin expression was examined in the operative specimens. However, whether its expression in the pre-operative biopsy specimens can also be a prognostic marker has not been clarified. Additional studies are, therefore, needed to determine the correlation between the adipophilin expression in operative and biopsy specimens. Moreover, in the present study, tissue microarray cores of 2 mm diameter were obtained for each case, which could have led to heterogeneity, as suggested by another study [6]. Second, the present study included patients who had received neoadjuvant chemotherapy. Although there was no significant difference in the rate of neoadjuvant chemotherapy between the adipophilin-positive and -negative groups ($p = 0.24$), the possibility that chemotherapy could have changed the expression of adipophilin has been proposed and cannot be ruled out [2]. A large study is needed to clarify whether adipophilin is an independent prognostic factor for PDAC patients with and without neoadjuvant chemotherapy.

In conclusion, this study clearly demonstrates that adipophilin expression is an independent and strong prognostic factor for OS and early recurrence in patients with PDAC. Additional studies are needed to clarify the molecular mechanisms that lead to adipophilin expression and treatment strategies for adipophilin-positive PDAC patients.

## Additional information

**Ethics approval and consent to participate**: This study was conducted in accordance with the Declaration of Helsinki, and the study protocol was approved by the institutional review board of Kansai Medical University Hospital (protocol no. 2017108 and 2017306).

**Consent for publication**: Not concerned.

**Availability of data and materials**: All data analyzed and generated during the study are available in our database.

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**Conflict of Interest**: The authors declare no competing interests.

**Funding:** None.

**Authorship**: MI conceived and designed the work. YH, MI, and HR conducted TMA construction, and the immunohistochemical stainings and analyses. YH, HR, TY, HK, SH, SY, MK, YM, HY, and SS obtained the clinical data. KT and SS supervised this project. All authors discussed the results and contributed to preparing the draft of the manuscript and have approved the final version. All authors agreed to be accountable for all aspects of the work.

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