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Serum Carbohydrate Antigen 19-9 and Metabolite Hypotaurine Are Predictive Markers for Early Recurrence of Pancreatic Ductal Adenocarcinoma

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Objective: A significant number of patients experience early recurrence after surgical resection for pancreatic ductal adenocarcinoma (PDAC), negating the benefit of surgery. The present study conducted clinicopathologic and metabolomic analyses to explore the factors associated with the early recurrence of PDAC.

Materials and Methods: Patients who underwent pancreatectomy for PDAC at Kagawa University Hospital between 2011 and 2020 were enrolled. Tissue samples of PDAC and nonneoplastic pancreas were collected and frozen immediately after resection. Charged metabolites were quantified by capillary electrophoresis-mass spectrometry. Patients who relapsed within 1 year were defined as the early recurrence group.

Results: Frozen tumor tissue and nonneoplastic pancreas were collected from 79 patients. The clinicopathologic analysis identified 11 predictive factors, including preoperative carbohydrate antigen 19-9 levels. The metabolomic analysis revealed that only hypotaurine was a significant risk factor for early recurrence. A multivariate analysis, including clinical and metabolic factors, showed that carbohydrate antigen 19-9 and hypotaurine were independent risk factors for early recurrence ($P = 0.045$ and $P = 0.049$, respectively). The recurrence-free survival rate 1 year after surgery with both risk factors was only 25%.

Conclusions: Our results suggested that tumor hypotaurine is a potential metabolite associated with early recurrence. Carbohydrate antigen 19-9 and hypotaurine showed a vital utility for predicting early recurrence.

Key Words: capillary electrophoresis-mass spectrometry, hypotaurine, metabolomics, PDAC

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Pancreatic ductal adenocarcinoma (PDAC) has a poor prognosis, even if surgical resection is performed.[1,2] The main reason for the poor prognosis of PDAC is the high recurrence rate, as approximately 80% of patients experience recurrence, and more than 50% of such cases occur within 12 months after surgical resection.[3,4] Various preoperative predictors of early recurrence (ER, within 12 months after surgery) have been reported, such as tumor size,[4,5] preoperative lymph node metastasis,[4,5] the preoperative serum carbohydrate antigen 19-9 (CA19-9) value,[2,4,6] the duration of symptoms,[7] the modified Glasgow Prognostic Score,[8] the Charlson age-comorbidity index,[4] and tumor differentiation.[4]

For several years, metabolomics has been used to understand cancer biology. Metabolomics directly reflects the biological behavior of cancer compared with the other *omics* technologies, such as genomics, transcriptomics, and proteomics.[9] Therefore, investigating cancer metabolomes may uncover biomarkers for diagnosing, prognosis, and evaluating the treatment effect on cancer. Several metabolomic studies explored metabolic pancreas cancer biomarkers associated with the recurrence and survival after pancreatectomy.[10,11] However, few studies have focused on clinicopathologic and metabolic factors comprehensively. There are also few reports of metabolomic analyses using cancer tissue and normal tissue of human surgical specimens.

Although preoperative and postoperative treatment has improved the outcome of PDAC recently, the disease still has a high recurrence rate, as described previously. Chemotherapy instead of invasive surgery might be suitable in some cases. Therefore, identifying the risk factors associated with the ER of PDAC to find patients who should undergo surgery would improve the prognostic outcome of PDAC.

This study explored risk factors associated with the ER of PDAC after pancreatectomy using clinicopathologic and metabolomic analyses to aid in selecting treatment for PDAC.

MATERIAL AND METHODS

Patient and Tissue Samples

The study cohort consisted of 160 patients who underwent pancreatectomy for PDAC at Kagawa University Hospital between September 2011 and August 2020. Tissue samples of PDAC and nonneoplastic pancreas were collected and frozen immediately after resection and preserved at −80°C. Optimal frozen tissue samples from tumors and nonneoplastic pancreas for metabolomics were collected from 79 patients during the period. The included patients were those with less than 12 months of follow-up for whom neither recurrence nor death had occurred. There is no standard period for ER in pancreatic cancer, and 6 months or 1 year is often used as the standard. In this study, given the recent trend toward improved prognosis with multidisciplinary treatment, we set the cutoff at 1 year. In addition, as a surgeon, we

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also hope that patients who have undergone highly invasive surgery will live as long as possible, at least 1 year without recurrence. Among these 79 patients, 30 (38%) experienced ER within 1 year after pancreatectomy (ER group), and 49 (62%) did not (nonearly recurrence [NER] group). The study was conducted following the Declaration of Helsinki and approved by the institutional review board of Kagawa University (no. 2019-157).

Metabolomics Analyses

We performed metabolomics analyses for tissue samples from the 79 included patients. Metabolites were measured by capillary electrophoresis–mass spectrometry (CE-MS) at the Institute for Advanced Biosciences, Keio University.[12–14] Capillary electrophoresis–mass spectrometry can identify and quantify intermediate metabolites in primary pathways related to cancer metabolisms, such as glycolysis, tricarboxylic cycle, pentose phosphate, amino acid, nucleic acid, urea cycle, polyamine, and glutathione pathways.

Frozen tissue samples (2–42 mg each) were homogenized entirely by a cell disrupter (Shakemaster Neo; BMS, Tokyo, Japan) at 1500 rpm for 5 to 20 minutes after adding 500 μL of methanol containing 20 μmol/L methionine sulfone as the internal standard. A total of 500 μL of chloroform and 200 μL of Milli-Q water were then added. Subsequently, the solution was centrifuged at 4600*g* for 15 minutes at 4°C. The 300-μL upper aqueous layer was centrifugally filtered through a 5-kDa cutoff filter to remove proteins. The solution was centrifuged at 9100*g* for 3 minutes at 4°C. Next, the solution was centrifugally concentrated for 1.5 to 4 hours at 40°C, and the filtrate was lyophilized and dissolved in 50 μL of Milli-Q water containing 200 μL of 3-aminopyrrolidine and 200 μL of trimesate before CE-MS analyses.

Cationic and anionic compounds were analyzed using CE time-of-flight mass spectrometry, which can detect ion peaks in the 50 to 1000 *m/z* range. The used instruments and their parameters were described elsewhere.[12–14] Raw data were processed using MasterHands (Keio University, Tsuruoka, Japan). Briefly, all possible peaks were detected and integrated. Each metabolite's peak area was divided by internal standards' peak area to calculate a relative area for eliminating MS sensitivity fluctuation. The mixture of standard compounds, including the same internal standards, was measured before the sample measurement. The absolute concentration of each metabolite was calculated based on the relative area of corresponding peaks between samples and the standard mixture.

Follow-up

Patient follow-up occurred at the outpatient clinic of our institution. The follow-up examinations were performed every 3 months for 1 year and every 6 months after that until the disease progressed. Enhanced computed tomography of the chest, abdomen, and pelvis was performed every 6 months. If necessary, we moved the examination date forward or added magnetic resonance imaging or ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET).

Statistical Analyses

The clinicopathological factors and metabolic analysis findings of patients in the ER and NER groups were compared. The clinicopathological factors were compared using Student *t* test and Fisher exact test. Metabolic statistical analyses and pathway analyses were performed by using the MetaboAnalyst (v. 5.0, https://www.metaboabalyst.ca/, accessed on November 3, 2022). Heatmap was depicted using MeV TM4 (ver. 4.9.0, https://mev.tm4.org/).

Factors significant in the univariate analysis and metabolic statistical analysis were entered into multivariate analysis. $P < 0.05$ was considered statistically significant. The recurrence-free survival (RFS) was defined as the time interval between the operation and the recurrence date. The survival was calculated using the Kaplan-Meier method and compared using the log-rank test between the groups. The univariate analysis of the clinicopathological factors, multivariate analysis, and Kaplan-Meier methods was performed using the JMP statistical software program, version 15 (SAS Institute, Cary, NC).

RESULTS

Patients' Clinicopathological Factors

Patients' clinicopathological factors used in this study are summarized in Table 1. The table contains patient data, including age and sex, and clinical data, including the perioperative factors and pathological data. The resectability status was assessed according to the National Comprehensive Cancer Network guideline 2020 version 1.[15] This series included 60 patients with R-PDAC, 11 with BR-PV PDAC, 5 with BR-A PDAC, and 3 with UR-LA PDAC.

Preoperative therapy was given to 61 patients. For R- and BR-PV PDAC, we have administered neoadjuvant chemoradiotherapy (NACRT) since October 2013. Eighteen patients received short-term NACRT with S-1 from October 2013 to May 2016 (Phase II trial, University Hospital Medical Information Network Clinical Trials Registry [UMIN-CTR] number 000026438), and 22 patients received long-term NACRT with S-1 from June 2016 to December 2019 (Phase II trial, UMIN-CTR number 000035232). In these clinical trials, hypofractionated external-beam radiotherapy (30 Gy in 10 fractions for short-term NACRT, 50 Gy in 25 fractions for long-term NACRT) with concurrent S-1 (60 mg/m^2) was administered 5 days

TABLE 1. Clinicopathological Factors of 79 Patients

	All (N = 79)
Preoperative variables	
Age, y*	73 (51–90)
Male/female	39/40
BMI, kg/m^2*	22.1 (15.0–39.0)
CA19-9, U/mL*	89 (0–22,068)
SUV in FDG-PET*	4.62 (0–19.02)
Tumor location (head/body-tail)	46/33
Resectability (R/BR/UR)	60/16/3
Preoperative therapy (yes/no)	61/18
Intraoperative variables	
Operation time, min*	437 (223–816)
Blood loss, mL*	778 (73–3533)
Postoperative variables	
Complication (CD3)	17
Postoperative CA19-9 normalization (yes/no)	60/19
Adjuvant therapy (yes/no)	62/17
Pathological variables	
Tumor size, cm*	2.5 (0–7.5)
S (positive/negative)	43/36
RP (positive/negative)	56/23
PV (positive/negative)	24/55
A (positive/negative)	6/73
PL (positive/negative)	31/48
LN metastasis (positive/negative)	41/38
Resection status (R0/R1)	74/5

*Median (range).

TABLE 2. Univariate Analysis of the Clinical Factors Associated With Recurrence Within 1 Year After Pancreatectomy for PDAC

		ER (n = 30)*	NER (n = 49)†	P
Preoperative variables				
Age‡	≧67	26 (87%)	35 (71%)	0.168
Sex	Male	16 (53%)	23 (47%)	0.647
BMI, kg/m^2‡	≧23.3	5 (17%)	18 (37%)	0.075
CA19-9, U/mL‡	≧262	17 (57%)	13 (27%)	0.009
SUV in FDG-PET‡	≧5.40	16 (55%)	12 (21%)	0.026
Tumor location	head	17 (57%)	29 (59%)	1.000
Resectability	R	25 (83%)	35 (71%)	0.285
Preoperative therapy	No	8 (24%)	10 (20%)	0.388
Intraoperative variables				
Operation time, min‡	≧507	11 (37%)	14 (29%)	0.028
Blood loss, mL‡	≧1642	8 (27%)	3 (6%)	0.017
Postoperative variables				
Complication	≧CD3	8 (27%)	9 (18%)	0.572
Postoperative CA19-9 normalization	No	13 (43%)	6 (7%)	<0.001
Adjuvant therapy	No	12 (40%)	5 (10%)	0.004
Pathological variables				
Tumor size, cm‡	≧3.0	18 (60%)	14 (31%)	0.009
S	Positive	22 (70%)	21 (43%)	0.019
RP	Positive	27 (90%)	29 (59%)	0.008
PV	Positive	14 (48%)	10 (21%)	0.041
A	Positive	4 (14%)	2 (3%)	0.207
PL	Positive	16 (53%)	15 (35%)	0.099
LN metastasis	Positive	23 (70%)	18 (44%)	0.002
Resection status	R1	3 (10%)	2 (4%)	0.362

*Recurrence within 1 year after surgery.

†Recurrence at more than 1 year after surgery or no recurrence.

‡The cutoff values of age, BMI, CA19-9, SUV in FDG-PET, operation time, blood loss, and tumor size were set by the ROC curve.

per week for 2 (short-term NACRT) or 5 (long-term NACRT) weeks before pancreatectomy. We previously reported the efficacy and safety of short-term NACRT[16] and long-term NACRT.[17] Thirteen patients received NACRT with S-1 and gemcitabine from January 2020 to the present (Phase II trial, UMIN-CTR number 000038585). In this clinical test, hypofractionated external-beam radiotherapy (30 Gy in 10 fractions) with concurrent S-1 (60 mg/m^2) and gemcitabine (1000 mg/m^2) was administered 5 days per week for 2 weeks before pancreatectomy. For BR-A PDAC, 4 patients underwent FOLFIRINOX, and 1 underwent gemcitabine and nab-paclitaxel. For UR-LA PDAC patients, 3 patients underwent external-beam radiotherapy (54 Gy in 27 fractions) with concurrent 5-fluorouracil (250 mg/m^2) given by continuous infusion.[18]

Postoperative factors influencing morbidity according to the Clavien-Dindo classification were examined.[19] Carbohydrate antigen 19-9 normalization was defined in cases with a high preoperative level (≥37 U/mL) showing a decrease (<37 U/mL) at the first postoperative outpatient visit. Adjuvant therapy was performed for 62 patients. The patients received gemcitabine, referring to the results of the CONKO-001 trial[20] from 2006 to 2012, or S-1, referring to the results of the JASPAC01 trial since 2013,[21] according to the recommended protocols.

Analysis of the Clinicopathological Factors

Table 2 shows the results of the univariate analysis of the clinicopathological factors possibly influencing ER within 1 year after pancreatectomy for PDAC among the 79 patients. The receiver operating characteristic (ROC) curves demonstrated cutoff values of 67 years, 23.3 kg/m^2, 262 U/mL, 5.40, 507 minutes, 1642 mL, and 3.0 cm for the age, body mass index, preoperative CA19-9 level, standardized uptake value (SUV) on FDG-PET, operation time, blood loss, and tumor size, respectively, using recurrence within 1 year postoperatively for a cutoff determination. The areas under the curve were 0.513, 0.581, 0.686, 0.629, 0.563, 0.562, and 0.666, respectively. On comparing the clinicopathological factors between the ER group (n = 30) and NER group (n = 49), the ER group had a significantly higher preoperative CA19-9 level and SUV on FDG-PET, longer operation time, more blood loss, more frequent reductions in CA19-9 values to normal after the operation, more frequent receipt of adjuvant therapy, larger tumors, more frequent lymph node metastasis, and higher rates of histological positivity for S, RP, and PV factor than the NER group (P = 0.009, 0.026, 0.028, 0.017, <0.001, 0.004, 0.002, 0.009, 0.008, and 0.041, respectively).

Metabolomic Analyses

We performed CE-MS for samples of nonplastic and PDAC tissue in 79 patients. Capillary electrophoresis–mass spectrometry detected 211 metabolites; 90 metabolites frequently detected in more than 50% of the samples were used for the subsequent analyses. A heatmap (Fig. 1) shows the overview of the metabolite concentrations. There seemed to be metabolic changes between PDAC and nonneoplastic tissues, and we divided the samples into ER and NER groups. Figure 2A shows the volcano plot of the

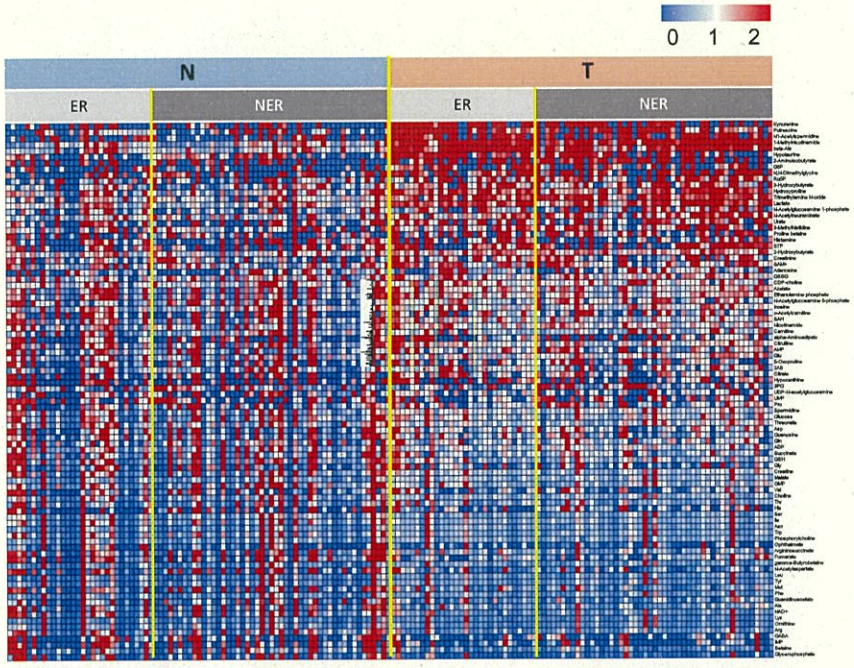

FIGURE 1. Heatmap of metabolite levels in paired nonneoplastic tissue (N) and PDAC tissue (T) of 79 patients with PDAC. Each group was divided into the ER and NER groups. In total, 90 metabolites frequently detected (>50% of the samples) are visualized. Each metabolite concentration was divided by the average of the normal tissue to assign the colors to each grid. Red and blue indicate relatively high and low, respectively.

differences in the metabolite concentrations (tumor tissue level minus the nonneoplastic tissue). Only hypotaurine showed significant differences between ER and NER. Figure 2B depicts the pathway-level difference, and only taurine and hypotaurine metabolites showed significant differences between ER and NER. Supplementary Figure 1, http://links.lww.com/MPA/B84, shows 10 metabolites showing different trends ($P < 0.3$) between ER and NER in volcano plots, box plots, and ROC curves.

Multivariate Analyses

Table 3 shows the results of the multivariate analysis of the clinicopathological factors and metabolomic factors found to have significant differences ($P < 0.05$) in the univariate analysis. In this multivariate analysis, we selected the preoperative CA19-9 level, SUV on FDG-PET, and tumor size as clinicopathological factors. We sought to identify ER factors detectable before surgery to allow doctors to choose the best treatment in the future. The ROC curve demonstrated that the cutoff value of hypotaurine was 61.12 nmol/mg, and the area under the curve was 0.704. The ER group had a significantly higher preoperative CA19-9 level (odds ratio [OR], 3.16; 95% confidence interval [CI], 1.03–9.74; $P = 0.045$) and hypotaurine concentration (OR, 3.11; 95% CI, 1.01–9.63; $P = 0.049$) than the NER group.

Survival Analyses

Survival analyses were performed using the Kaplan-Meier method for the 2 factors that showed significant differences in the multivariate analysis (Fig. 3). The median follow-up for all

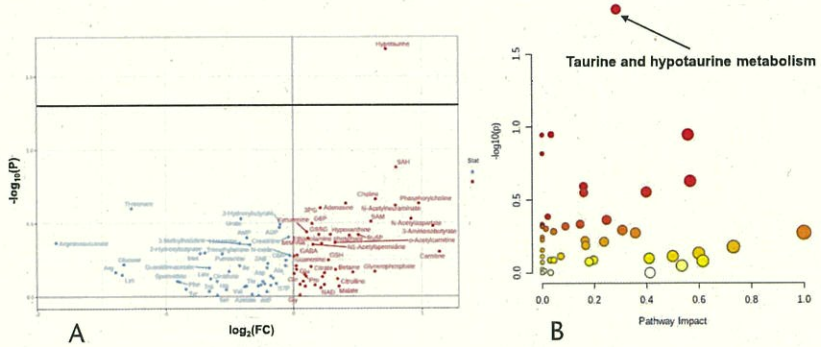

FIGURE 2. The differences in the metabolomics profiles between ER and NER. A, Volcano plots of the difference of the metabolite concentration calculated by T-N. The *X* axis indicates the $\log_2$-fold change of ER/NER. *Y* axis indicates $-\log_{10}$(*P* value). The Mann-Whitney test calculates *P* value. The metabolites above horizontal line ($Y = 1.3$) indicate a significant difference ($P < 0.05$). B, Pathway analysis using KEGG pathway categories. *X* and *Y* axes indicate the pathway impact and $-\log_{10}$(*P* value) of each pathway. KEGG indicates Kyoto Encyclopedia of Genes and Genomes.

TABLE 3. Multivariate Analysis of the Clinical and Metabolic Factors Affecting Recurrence Within 1 Year After Pancreatectomy for PDAC

		ER (n = 30)*	NER (n = 49)†	OR	95% CI	P‡
CA19-9, U/mL§	≧262	17 (57%)	13 (27%)	3.16	1.03–9.74	0.045
SUV in FDG-PET§	≧5.40	16 (55%)	12 (21%)	2.18	0.72–6.61	0.169
Tumor size, cm§	≧3.0	18 (60%)	14 (31%)	2.58	0.87–7.65	0.087
Hypotaurine, nmol/mg§	≧61.12	21 (70%)	21 (43%)	3.11	1.01–9.63	0.049

*Recurrence within 1 year after surgery.

†Recurrence at more than 1 year after surgery or no recurrence.

‡Logistic regression analysis.

§The cutoff value of CA19-9, SUV in FDG-PET, tumor size, and hypotaurine were determined by the ROC curves.

79 patients was 22 (range, 0–116) months. The median RFS was shorter in patients with a high CA19-9 level (≧262 U/mL) than in those with a low CA19-9 level (<262 U/mL) ($P = 0.095$; Fig. 3A). The median RFS was also significantly shorter in patients with a high hypotaurine concentration (≧61.12 nmol/mg) than in those with low hypotaurine concentration (<61.12 nmol/mg) ($P = 0.021$; Fig. 3B). We also analyzed the relationship between the number of positive values of these 2 risk factors of ER and the recurrence rate (Fig. 3C). The 1-year freedom from recurrence rates for patients with 0, 1, and 2 risk factors were 77.4%, 60.1%, and 25.0%, respectively. Patients with both risk factors had a significantly higher rate of ER than those with no and one risk factor (OR, 14.25; 95% CI, 2.99–68.02; $P < 0.001$ and OR, 5.57; 95% CI, 1.51–20.54; $P = 0.010$, respectively). The sensitivity and specificity of the 2 predictors were 63.2% and 91.8%, respectively.

Table 4 shows the results of the univariate analysis of the clinicopathological factors associated with the hypotaurine concentration in PDAC. On comparing the clinicopathological factors between the high hypotaurine concentration group (≧61.12 nmol/mg, n = 42) and low hypotaurine concentration group (<61.12 nmol/mg, n = 37), the high hypotaurine concentration group had a significantly higher SUV on FDG-PET. More patients underwent preoperative therapy than the low hypotaurine level group ($P = 0.030$ and 0.030). The hypotaurine concentration did not show a significant difference from the CA19-9 level. Table 5 shows the results of the univariate analysis of the clinicopathological factors associated with the CA19-9 level in PDAC. On comparing the clinicopathological factors between the high CA19-9 group (≧262 U/mL, n = 30) and low CA19-9 level group (<262 U/mL, n = 49), the high CA19-9 level group had a significantly more positive RP, PL, and LN metastasis than the low CA19-9 level group ($P = 0.037$, 0.034 and <0.001, respectively).

DISCUSSION

This study aimed to identify the factors associated with the ER of PDAC based on clinicopathologic and metabolomic analyses. We found that the preoperative CA19-9 level and hypotaurine were such risk factors.

As previously reported, the preoperative CA19-9 level is associated with survival after curative resection for PDAC.[2,4,6] The finding of the preoperative CA19-9 level as a risk factor for ER within 1 year after surgery varies among studies. This is because the patient background, such as resectability and preoperative treatment, differs among studies. However, a high preoperative CA19-9 level is a common risk factor, and the higher the CA19-9 level, the shorter the median overall survival.[6]

The metabolomics analysis showed that the ER group had a significantly higher hypotaurine concentration than the NER group. Regarding the metabolome pathway, taurine and hypotaurine metabolism were enhanced in the ER group compared with the NER group. To our knowledge, this is the first study showing a relationship between hypotaurine and PDAC. In glioma, tissue levels of hypotaurine strongly and positively correlate with glioma grade,[22,23] and hypotaurine leads to the activation of hypoxia signaling and increased glioma cell proliferation and invasion.[22] Taurine and hypotaurine metabolism (Supplementary Fig. 2, http://links.lww.com/MPA/B85) is associated with a poor prognosis in colon, kidney, and lung carcinoma.[24–26] For PDAC, some studies have reported that the metabolomics of taurine and the pathway of taurine and hypotaurine metabolism are related to the prognosis.[10,27]

Taurine and hypotaurine metabolism is suggested to function as a critical pathway in tumor development by modifying the indices of oxidative stress and membrane damage.[28,29] Hypotaurine is an intermediate in the taurine and hypotaurine pathway. Taurine is the metabolic end-product of methionine and cysteine and has many biological functions, such as bile salt conjugation, osmoregulation, membrane stabilization, calcium modulation, antioxidation, and immunomodulation.[30] Taurine and hypotaurine are closely related; hypotaurine differs from taurine only in the oxidation state of the sulfur center and the resulting change in the acidity of the molecule. It has been pointed out that hypotaurine is an

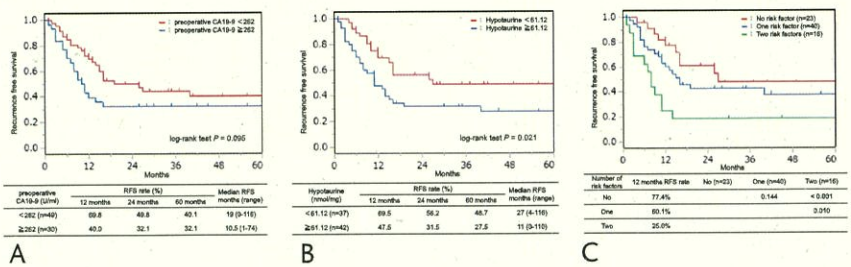

FIGURE 3. A, Kaplan-Meier survival curves from pancreatectomy to initial recurrence classified by level of CA19-9. B, Kaplan-Meier survival curves from pancreatectomy to initial recurrence classified by level of hypotaurine. C, Kaplan-Meier survival curves from pancreatectomy to initial recurrence for the number of risk factors.

TABLE 4. Univariate Analysis of the Clinicopathological Factors Associated With Hypotaurine Level of PDAC

		Hypotaurine ≧61.12 (n = 42)	Hypotaurine <61.12 (n = 37)	*P*
Preoperative variables				
Age*	≧67	36 (86%)	25 (68%)	0.065
Sex	Male	20 (48%)	19 (51%)	0.823
BMI, kg/m^{2}*	≧23.3	9 (21%)	14 (38%)	0.139
CA19-9, U/mL*	≧262	16 (38%)	14 (38%)	1.000
SUV in FDG-PET*	≧5.40	19 (51%)	9 (25%)	0.030
Tumor location	head	18 (43%)	15 (41%)	1.000
Resectability	R	33 (79%)	27 (73%)	0.610
Preoperative therapy	No	14 (33%)	4 (11%)	0.030
Intraoperative variables				
Operation time, min*	≧507	11 (26%)	7 (19%)	0.592
Blood loss, mL*	≧1642	8 (19%)	3 (8%)	0.203
Postoperative variables				
Complication	≧CD3	7 (17%)	10 (29%)	0.270
Postoperative CA19-9 normalization	No	9 (23%)	7 (19%)	0.785
Adjuvant therapy	No	13 (31%)	4 (11%)	0.053
Pathological variables				
Tumor size, cm*	≧3.0	19 (45%)	13 (35%)	0.491
S	Positive	25 (61%)	18 (50%)	0.366
RP	Positive	32 (78%)	24 (67%)	0.311
PV	Positive	16 (43%)	8 (23%)	0.083
A	Positive	4 (11%)	2 (6%)	0.676
PL	Positive	19 (48%)	12 (34%)	0.347
LN metastasis	Positive	24 (60%)	17 (49%)	0.359
Resection status	R1	1 (2%)	4 (11%)	0.180

*The cutoff value of age, BMI, CA19-9, SUV in FDG-PET, operation time, blood loss, and tumor size were determined by the ROC curves.

indicator of oxidative stress and membrane damage, both of which are associated with cancer.[28] In addition, hypotaurine has been suggested to be a more reliable indicator of ER of PDAC than taurine. First, hypotaurine is much more reactive than taurine, reacting rapidly and efficiently with the hydroxy radical, hypochlorous acid, and other oxidants with rate constants 100 to 10,000 times that of taurine.[31–33] The reaction rate of hypotaurine with superoxide would depend on the superoxide concentration.[34] Second, hypotaurine is abundant in the cytoplasm and extracellular space and may function as an antioxidant. Effective enzymes for eliminating hydrogen peroxides, such as catalase and glutathione peroxidases, are abundant in the mitochondria, peroxisomes, and other subcellular locations.[34] Third, hypotaurine is less susceptible to errors in detection methods than taurine. For example, a simple method of identifying these metabolites is using LC with some detectors; however, LC alone cannot separate them. Peroxytaurine, composed of taurine and hydrogen peroxide, seems to elute similarly to taurine. Taurine concentration might be affected by the diet, such as seafood. Conversely, hypotaurine is not present in the more oxidized plasma compartment or received via the diet. Thus, the hypotaurine concentration is a better indicator of taurine synthesis than the taurine concentration.[35]

Our study performed a disease-free survival analysis using the Kaplan-Meier method for each risk factor and the number of risk factors (Fig. 3C). Previous studies have demonstrated the risk factors for ER of PDAC as only clinical factors or metabolic factors, but no study has considered them simultaneously. The 1-year freedom from recurrence rate was significantly worse (25%) than with one or neither risk factor (60% and 77%, respectively) under the presence of both risk factors. Correlation between CA19-9 level and metabolite concentration is shown as a correlation heatmap (Fig. 4). Two prominent clusters were observed. At the bottom right, most amino acids strongly correlated with each other. A large cluster was observed at the center, including various metabolites, such as adenosine metabolite (eg, ADP and AMP) and organic acids (eg, citrate, malate, and succinate). Both CA19-9 and hypotaurine were not included in these prominent clusters, and thus, they are independent factors, presumably leading to a significantly worse prognosis with 2 risk factors than one. In addition, hypotaurine concentration related to fewer clinicopathological factors than CA19-9 level, which are different (Tables 4, 5). Thus, they are not only independent factors but also each has its significance. Combining these 2 factors would result in a more accurate prediction. Furthermore, high hypotaurine concentration is helpful for patients diagnosed with CA19-9–negative PDAC. In this study, we did not check Lewis antigens. In CA19-9–negative patients (CA19-9 < 37), there were no significant differences in hypotaurine concentration ($P = 1.000$).

Several limitations associated with the present study warrant mention. We used tissue samples taken from surgical specimens and cannot say that the same results would be obtained using preoperative tissue. It would be ideal if endoscopic ultrasound–fine-needle aspiration tissue could be used, but this is currently difficult because of the small amount of tissue involved. A noninvasive and straightforward method should be developed that can alternatively assess the expression of hypotaurine in the tumor of PDAC. Immunostaining or polymerase chain reaction of endoscopic ultrasound–fine-needle aspiration tissue, liquid biopsy, and PET assessment are the potential approaches, which is the next challenge to address. The development of PET tracers that label hypotaurine may be a promising method. In addition, the tissue

TABLE 5. Univariate Analysis of the Clinicopathological Factors Associated With CA19-9 Level of PDAC

		CA19-9 ≧262 U/mL (n = 30)	CA19-9 <262 U/mL (n = 49)	*P*
Preoperative variables				
Age*	≧67	25 (83%)	36 (74%)	0.411
Sex	Male	14 (47%)	25 (51%)	0.818
BMI, kg/m^{2*}	≧23.3	9 (30%)	14 (29%)	1.000
SUV in FDG-PET*	≧5.40	14 (52%)	14 (30%)	0.085
Tumor location	head	18 (60%)	28 (57%)	0.819
Resectability	R	22 (73%)	38 (78%)	0.788
Preoperative therapy	No	24 (80%)	37 (76%)	0.785
Intraoperative variables				
Operation time, min*	≧507	11 (37%)	7 (14%)	0.028
Blood loss, mL*	≧1642	5 (17%)	6 (12%)	0.740
Postoperative variables				
Complication	≧CD3	2 (7%)	15 (33%)	0.011
Postoperative CA19-9 normalization	No	19 (63%)	41 (89%)	0.010
Adjuvant therapy	No	22 (73%)	40 (82%)	0.410
Pathological variables				
Tumor size, cm*	≧3.0	15 (58%)	14 (33%)	0.077
S	Positive	20 (67%)	23 (49%)	0.161
RP	Positive	26 (87%)	30 (64%)	0.037
PV	Positive	10 (33%)	14 (33%)	1.000
A	Positive	3 (10%)	3 (7%)	0.685
PL	Positive	17 (57%)	14 (31%)	0.034
LN metastasis	Positive	24 (80%)	17 (38%)	<0.001
Resection status	R1	2 (7%)	3 (6%)	1.000

*The cutoff value of age, BMI, SUV in FDG-PET, operation time, blood loss, and tumor size were determined by ROC curves.

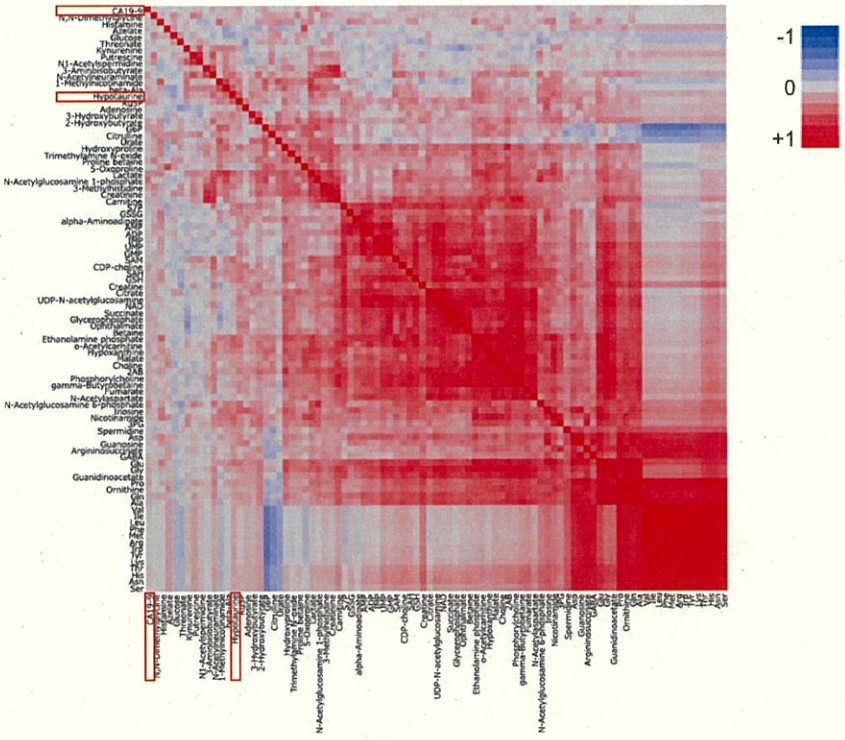

FIGURE 4. Heatmap to show Pearson correlations among metabolites and CA19-9. The difference of the concentration of T-N is used for metabolite levels. Red and blue indicate higher and lower correlations between the two features.

samples may have been affected by preoperative treatment. We also compared metabolites between the groups with NACRT and without NACRT. Hypotaurine, *N*-acetylneuraminate, SAM, N^1-acrtylspermidine, and glucose showed significant differences between the groups. The hypotaurine concentration of the group without NACRT is significantly higher than that with NACRT. The present study had no significant association between NACRT and ER. However, it was suggested that a reduction of hypotaurine concentration in the tumor is related to the effect of NACRT. We previously examined the relationship between NACRT and metabolites and reported that choline metabolism was involved in NACRT.[36] This different result is because the number of cases, observation period, and metabolite values used for the analysis differed from those used in the present study. There are several possibilities to affect the hypotaurine concentration in the tissue samples. For example, cysteamine dioxygenase (EC 1.13.11.19) is an enzyme that synthesizes hypotaurine from cysteamine. The cysteamine was not frequently detected in our data and was eliminated from the subsequent analyses. The evaluation of activities of such enzymes and higher sensitive metabolomic analyses would help understand their mechanism.

In conclusion, we found the preoperative CA19-9 level and hypotaurine to be risk factors for the ER of PDAC. Further elucidation for the role of hypotaurine on ER and developing a simple preoperative evaluation method of hypotaurine are the following priority issues.

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