Human equilibrative nucleoside transporter-1 (hENT1) and ribonucleotide reductase regulatory subunit M1 (RRM1) expression; do they have survival impact to pancreatic cancer?

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Backgrounds/Aims: Gemcitabine is still one of adjuvant options in chemotherapeutic agent for pancreatic ductal adenocarcinoma (PDAC). Integral membrane transporter protein and intracellular enzymes including human equilibrative nucleoside transporter 1 (hENT1), deoxycytidine kinase (dCK), ribonucleotide reductase (RR) M1, and M2 are known as important factors for chemosensitivity of gemcitabine. We aimed to investigate the correlation between these key molecules and 5-year actual survival in PDAC patients. **Methods:** The expression of intratumoral hENT1, dCK, RRM1, and RRM2 was assessed immunohistochemically in 160 PDAC patients underwent surgical resection. Association between clininopathologic factors, immunohistochemical results, and overall survival were analyzed. **Results:** Adjuvant chemotherapy including concurrent chemoradiotherapy was not associated with overall survival (HR, 0.92; 95% CI, 0.65-1.31; p=0.658). High hENT1 expression group did not show statistical survival difference, compared with all others (HR, 1.16; 95% CI, 0.82-1.65, p=0.396). Gemcitabine therapy and high hENT1 group was compared with all other patients, and no difference in overall survival was identified (HR, 0.99; 95% CI, 0.68-1.42; p=0.940). And, gemcitabine therapy and high hENT1 group did not differ statistically from gemcitabine therapy and low hENT1 expression (HR, 0.92; 95% CI, 0.55-1.56; p=0.764). The intensity of dCK, RRM1, and RRM2 expression was not associated with overall survival (p=0.413, p=0.138 and p=0.061) in univariate analysis. **Conclusions:** The expression of hENT1, dCK, RRM1 and RRM2 may not be associated with overall survival for patients with pancreatic cancer on gemcitabine adjuvant therapy. These proteins and other factors that may interact with or confound these results should be investigated in the near future. **(Ann Hepatobiliary Pancreat Surg 2020;24:127-136)**

Key Words: Pancreatic ductal adenocarcinoma; Adjuvant chemotherapy; Gemcitabine; hENT1; Overall survival; Surgery

INTRODUCTION

Despite surgical outcome has improved over the past few decades, pancreatic ductal adenocarcinoma (PDAC) remains one of the most lethal malignancies. After introduction of effective chemotherapeutic agents, the role of chemotherapy in both the adjuvant and neoadjuvant settings, becomes more important than ever. Gemcitabine (2',2'-difluorodeoxycytidine) is a standard chemotherapeutic agent in the treatment of both localized and metastatic PDACs in Korea. However, when administered in un-

selected patient populations, gemcitabine only showed a modest benefit in terms of survival. Therefore, better identification of patients who would benefit from administration of gemticibine is required.[1]

As part of efforts to identify predictive biomarkers that forecast the likely response to gemcitabine of PDAC, several studies have investigated the relationship between the chemosensitivity of gemcitabine and integral membrane transporter proteins including human equilibrative nucleoside transporter 1 (hENT1).[2,3] It was demonstrated that the deficiency in hENT1 was highly associated with the re-

sistant effect of gemcitabine.[4,5] As a prodrug, intracellular gemcitabine must be phosphorylated by deoxycytidine kinase (dCK) to its mononucleotide in the rate-limiting step of its cellular anabolism.[6] Gemcitabine monophosphate then converts to its active forms, gemcitabine diphosphate and gemcitabine triphosphate.[7] The cytotoxicity of gemcitabine is due to its blocking de novo DNA synthesis through inhibition of ribonucleotide reductase, which blocks production of the deoxyribonucleotide precursors required for DNA synthesis.[6] Ribonucleotide reductase (RR) is a dimeric enzyme composed of a regulatory subunit M1 and a catalytic subunit M2.

While, hENT1, dCK, and RRM1 are important determinants of gemcitabine activity, about the prognostic and predictive values of immunohistochemical assessment of hENT1, dCK, and RRM1 in pancreatic cancer remain unclear, especially in Korean populations.[8-10] In the present study, we aimed to investigate the correlation between key molecules (hENT1, dCK, RRM1 and RRM2) and 5-year actual survival in patients with PDAC.

MATERIALS AND METHODS

Study population

This case-control study was conducted in 200 consecutive and unselected patients with pathologically proven PDAC who underwent curative intent surgery from June 2003 through May 2012 at the Department of Surgery, Seoul National University Bundang Hospital, Korea. Tissue microarrays (TMAs) were made from archived tumor specimen, and 35 patients were excluded because of absence of tumor specimen. After excluding five more patients whose survival status could not be identified as of August 2019, 160 patients were finally enrolled in this study.

Data collection

Demographic, clinical, and pathological data were collected by an independent reviewer who was masked from the biomarker assessment results. The database was constructed with sex, age at surgery, tumor site, type of operation, tumor stage according to the 8th edition of American Joint Committee on Cancer (AJCC) staging, histologic grade, maximal tumor size, adjuvant therapy, chemotherapeutic regimen, recurrence, disease-free survival, and 5-year actual survival.

Operative procedure

All type of pancreatectomy were included, such as conventional pancreaticoduodenectomy, pylorus-preserving pancreaticoduodenectomy, distal pancreatectomy, total pancreatectomy, and other types of parenchymal-sparing pancreatectomy. Depends on tumor location, types of pancreatectomy were decided. All patients underwent more than standard lymphadenectomy. When preoperative imaging studies showed invasion into the portal vein, the attending surgeon decided whether to perform portal vein resection. Intraoperative frozen section examination was performed regularly on proximal or distal pancreatic resection margin.

Biomarker assessment

An independent pathologist masked to clinicopathologic data and survival outcome of patients (E. Shin) reviewed hematoxylin and eosin-stained slides of each patient. Tissue microarrays (TMAs) were made by 2 mm sized core from formalin-fixed, paraffin-embedded tumor specimens.[11] Immunohistochemical staining for hENT1 and dCK was performed using Anti-hENT1 rabbit polyclonal antibody (PAB2255, 1:120, Abnova, Taipei, Taiwan) and anti-dCK mouse monoclonal antibody (LS-C172855, 1:50, LSBio, Seattle, WA, USA).[12,13]

RRM1 expression was evaluated with a polyclonal rabbit antibody against human RRM1 (ab81085, 1:100, Abcam, Cambridge, UK) and RRM2 with polyconal goat antibody against human RRM2 (SC-10846, 1:250, Santa Cruz Biotechnology, Dellas, TX, USA). 4-μm-thick sections were cut from each of the paraffin tissue blocks, mounted on positively charged slides. Immunohistochemical staining for hENT1, dCK and RRM2 was performed using Leica Bond-max autostainer (Leica Biosystem, Vista, CA). After undergoing heat epitope retrieval for 20 minutes in Epitomic retrieval solution 2 (pH 9.0) in the autostainer, the samples were incubated with individual primary antibodies for 15 minutes. Then sides were incubated with polymer for 8 minutes and subsequently treated with DAB substrate for 10 minutes. Treatment with Harris hematoxylin for 1 minute was performed for counterstain. Immunohistochemical staining for RRM2 was manually done. Briefly, antigen was retrieved by treating pH 9.0 Tris-EDTA buffer at 100℃ for

20 minutes. Endogenous peroxidase activity was blocked using 3% hydrogen peroxide for 6 minutes. For protein blocking, slides were incutaed in 5% normal horse serum for 30 minutes at room temperature. The primary antibody and the antibody binding were detected using an avidin-biotin complex reagent (Vector Lab., Burlingame, CA, USA) for 30 minutes and DAB substrate kit (Vector Lab., Burlingame, CA, USA) for 2 minutes.

The results of immunohistochemical staining was evaluated at ×200 magnificaion under a light microscope. The intensity staining was semi-quantitavely scored as scored as follows: grade 0, not stained; grade 1, equivocally stained; grade 2, weakly stained; grade 3, moderately stained; and grade 4, stained strongly. Islet cells, plasma and stromal cells were used as internal control. Additionally, the percentage of immune-reactive cells was assessed. For evaluation of intratumoral hENT1 expression, if grade 4 staining was observed in the neoplasms, the sample was considered to have high hENT1 expression, and if grade 0 to 3 staining was observed, the sample was considered to have low hENT1 expression.[14]

Survival

All patients were followed regularly in outpatient clinics with blood tests or imaging studies, including computed tomography (CT) every 3 to 6 months. Evaluation of recurrence was based on imaging studies. Final survival was assessed in at Aug. 2019 when all patients had achieved more than 5 years of follow-up. Survival information was collected by reviewing electrical medical records or telephone interview. For all patients, postoperative survival time, survival status, and recurrence status were reviewed and recorded.

Statistical analysis

Statistical analysis was conducted using R 3.5.1. (R Foundation for Statistical Computing, Vienna, Austria). Categorical variables were reported as frequencies, and continuous variables were reported as median with range or mean with standard deviation, as appropriate. Categorical variables or proportions were compared using the chi-square test with continuity correction or Fisher's exact test. Continuous variables were compared using Student's t-test if normally distributed and the Wilcoxon rank-sum test otherwise. Survival curves were obtained using the Kaplan-Meier method. Log rank test and Cox proportional hazards models were used to compare overall survival. Tests of significance were undertaken at the two-tailed alpha level of 0.05.

RESULTS

Patients demographics and clinical data

Of the 160 patients, 93 (58.1%) were males and 67 (41.9%) were females; median age was 65 years (range, 40-89). Pancreatoduodenectomy, distal pancreatectomy, and total pancreatectomy were performed for 96 (60.0%), 54 (33.8%), and 6 (3.8%) patients, respectively. The proportion of patients with preoperative diabetes was significantly different between the two hENT1 intensity groups, with 30 (47.6%) and 28 (29.5%) patients, respectively (p=0.021). Pancreatic neoplasm were confined to the head and body/tail of the pancreas in 98 patients (62.5%) and 58 patients (36.3%), respectively. One hundred thirty seven patients (85.6%) underwent R0 resection.

According to TNM classification, 1 (0.6%), 3 (1.9%), and 150 (93.8%) patients had T1, T2, and T3 neoplasms, respectively; 97 patients (60.6%) had lymph node metastases. No patients had stage IA disease, whereas 3 (1.9%) patients had stage IB, 58 (36.3%) patients had stage IIA, 92 (57.5%) patients had stage IIB, and 7 (4.4%) patients had stage IV disease (Table 1).

Of 160 patients, 93 (58.1%) patients received adjuvant therapy. Among 93 patients, 70 received gemcitabine based adjuvant therapy. The proportion receiving adjuvant chemotherapy was not different between groups, with 46 (69.7%) and 47 (56.0%) patients, respectively (p=0.121). There was no significant difference with gemcitabine therapy (26 (41.3%) and 44 (45.4%) patients, p=0.729). High intratumoral expression of hENT1 was observed in 97 (60.6%) patients, and high hENT1 expression with gemcitabine therapy was identified in 44 (27.5%) patients.

DCK and RRM1 expression showed correlation with hENT1 expression, but, there was no significant correlation between RRM2 expression and hENT1 expression (Table 1).

Biomarker assessment and survival

Table 2 shows the results of analyses of predictive ef-

Table 1. Comparison of clinicopathologic factors based on the intensity of intratumoral hENT1 expression

Variables	Overall	hENT1 intensity		p-value
		0 to 3 (n=63)	4 (n=97)	
Age (mean (SD), years)	64.61 (9.8)	66.30 (9.1)	63.50 (10.1)	0.077
Sex				
Male	93 (58.1)	42 (66.7)	51 (52.1)	0.126
Female	67 (41.9)	21 (33.3)	46 (47.9)	
Body Mass Index (mean, kg/m^2)	21.76 (3.33)	21.70 (3.00)	21.80 (3.55)	0.848
Diabetes, preoperative				
Yes	58 (36.7)	30 (47.6)	28 (29.5)	0.021
No	100 (63.3)	33 (52.4)	67 (70.5)	
Preoperative CA19-9 (U/ml)				
<37	46 (29.5)	15 (24.6)	31 (32.6)	0.322
≥37, <300	56 (35.9)	22 (36.1)	34 (35.8)	
≥300, <600	18 (11.5)	5 (8.2)	13 (13.7)	
≥600, <900	7 (4.5)	4 (6.6)	3 (3.2)	
≥900	29 (18.6)	15 (24.6)	14 (14.7)	
Postoperative CA19-9 (U/ml)				
<37	82 (54.7)	31 (50.0)	51 (58.0)	0.732
≥37, <300	49 (32.7)	21 (33.9)	28 (31.8)	
≥300, <600	9 (6.0)	5 (8.1)	4 (4.5)	
≥600, <900	3 (2.0)	1 (1.6)	2 (2.3)	
≥900	7 (4.7)	4 (6.5)	3 (3.4)	
Location of tumor				
Head	98 (62.0)	40 (62.5)	58 (60.4)	0.502
Body	20 (12.7)	6 (9.4)	14 (14.6)	
Body and tail	8 (5.1)	2 (3.1)	6 (6.3)	
Tail	30 (19.0)	14 (21.9)	16 (16.7)	
Whole pancreas	4 (2.5)	2 (3.1)	2 (2.1)	
Type of operation				
Pancreaticoduodenectomy including pylorus-preserving	96 (60.0)	37 (58.7)	59 (60.8)	0.818
Distal pancreatectomy	54 (33.8)	21 (33.3)	33 (34.0)	
Total pancreatectomy	6 (3.8)	2 (3.2)	4 (4.1)	
Others	4 (2.5)	3 (4.8)	1 (1.0)	
R0 resection				
Yes	137 (85.6)	54 (84.4)	83 (86.5)	0.780
No	23 (14.4)	10 (15.6)	13 (13.5)	
T stage				
T1	1 (0.6)	0 (0.0)	1 (1.0)	0.593
T2	3 (1.9)	2 (3.2)	1 (1.0)	
T3	150 (93.8)	58 (92.1)	92 (94.8)	
T4	6 (3.8)	3 (4.8)	3 (3.1)	
N stage				
N0	62 (38.8)	20 (31.7)	42 (43.3)	0.227
N1	97 (60.6)	43 (68.3)	54 (55.7)	
Nx	1 (0.6)	0 (0.0)	1 (1.0)	
M stage				
M0	155 (96.9)	63 (100.0)	92 (94.8)	0.172
M1	5 (3.1)	0 (0.0)	5 (5.2)	
Stage, AJCC 8th				
IB	3 (1.9)	2 (3.2)	1 (1.0)	0.154
IIA	58 (36.2)	17 (27.0)	41 (42.3)	
IIB	92 (57.5)	42 (66.7)	50 (51.5)	
IV	7 (4.4)	2 (3.2)	5 (5.2)	

Table 1. Continued

| Variables | Overall | hENT1 intensity | | *p*-value |
		0 to 3 (n=63)	4 (n=97)	
Adjuvant therapy				
Yes	93 (58.1)	46 (69.7)	47 (56.0)	0.121
Chemotherapy, only	61	27	34	
CCRT	32	19	13	
No	57 (41.9)	20 (30.3)	37 (44.0)	
Gemcitabine adjuvant therapy				
Yes	70 (43.8)	26 (41.3)	44 (45.4)	0.729
No	90 (56.2)	37 (58.7)	53 (54.6)	
hENT1 (intensity)				
0	5 (3.1)	5 (7.9)		
1	9 (5.6)	9 (14.3)		
2	10 (6.2)	10 (15.9)		
3	39 (24.4)	39 (61.9)		
4	97 (60.6)		97 (100.0)	
DCK (intensity)				
0	48 (30.6)	33 (53.2)	15 (15.8)	<0.001
1	0 (0.0)	0 (0.0)	0 (0.0)	
2	39 (24.8)	19 (30.6)	20 (21.1)	
3	64 (40.8)	10 (16.1)	54 (56.8)	
4	6 (3.8)	0 (0.0)	6 (6.3)	
RRM1 (intensity)				
0	25 (16.1)	21 (33.3)	4 (4.3)	<0.001
1	15 (9.7)	7 (11.1)	8 (8.7)	
2	66 (42.6)	25 (39.7)	41 (44.6)	
3	43 (27.7)	8 (12.7)	35 (38.0)	
4	6 (3.9)	2 (3.2)	4 (4.3)	
RRM2 (intensity)				
0	16 (10.3)	8 (12.7)	8 (8.7)	0.150
1	0 (0.0)	0 (0.0)	0 (0.0)	
2	7 (4.5)	1 (1.6)	6 (6.5)	
3	62 (40.0)	30 (47.6)	32 (34.8)	
4	70 (45.2)	24 (38.1)	46 (50.0)	
hENT1 high+gemcitabine	44 (27.5)	0 (0.0)	44 (45.4)	<0.001
Others	116 (72.5)	63 (100.0)	53 (54.6)	

hENT1, human equilibrative nucleoside transporter 1; dCK, deoxycytidine kinase; RRM1, ribonucleotide reductase regulatory subunit M1; RRM2, ribonucleotide reductase regulatory subunit M2; AJCC, American Joint Committee on Cancer

fects of various factors including immunohistochemical test results on overall survival. Age, sex, presence of preoperative diabetes and preoperative CA 19-9 level was not associated with overall survival. However, preoperative body mass index (BMI) and postoperative CA 19-9 level over 900 U/ml displayed significant interactive effects with overall survival (HR, 0.93; 95% confidence interval (CI), 0.88-0.98; *p*=0.007 and HR, 5.22; 95% CI, 2.33-11.68; *p*<0.001). Tumor location, operation type, and extent of resection were not associated with survival. AJCC staging including T and N staging shows statisti-

cally significant interaction with overall survival.

Adjuvant chemotherapy including concurrent chemoradiotherapy was not associated with overall survival (HR, 0.92; 95% CI, 0.65-1.31; *p*=0.658). The intensity of hENT1 expression itself was not significantly associated with actual overall survival (Table 2, Fig. 1). After grouping high hENT1 expression and others, there was no statistical survival difference (HR, 1.16; 95% CI, 0.82-1.65, *p*=0.396). To assess interactive effects of gemcitabine therapy, the group with gemcitabine therapy and high hENT1 expression was compared with all other patients,

Table 2. Univariate overall survival analysis for 160 patients

Variables	Univariate			
	HR	95% CI		*p*-value
Age	1.002	0.984	1.020	0.848
Sex	0.891	0.632	1.256	0.510
BMI	0.927	0.878	0.980	0.007
Preoperative diabetes	1.010	0.705	1.447	0.957
Preoperative CA 19-9 (reference, <37 U/ml)				0.475
≥37, <300	1.272	0.828	1.952	0.272
≥300, <600	1.183	0.657	2.130	0.576
≥600, <900	1.736	0.731	4.120	0.211
≥900	1.529	0.923	2.531	0.099
Postoperative CA 19-9 (reference, <37 U/ml)				0.008
≥37, <300	1.440	0.983	2.110	0.061
≥300, <600	1.568	0.751	3.272	0.231
≥600, <900	1.555	0.485	4.985	0.458
≥900	5.222	2.335	11.680	<0.001
Location of tumor (reference, head)				0.884
Body	0.970	0.407	2.308	0.944
Body and tail	0.799	0.485	1.317	0.379
Tail	1.893	0.251	14.273	0.536
Whole pancreas	0.591	0.079	4.427	0.609
Type of operation (reference, pancreaticoduodenectomy)				0.235
Distal pancreatectomy	2.454	0.563	10.694	0.232
Total pancreatectomy	0.578	0.313	1.067	0.080
Others	0.389	0.113	1.345	0.136
Extent of resection	1.013	0.531	1.934	0.968
T stage				0.025
T2	0.038	0.002	0.618	0.022
T3	0.159	0.021	1.174	0.071
T4	0.408	0.048	3.476	0.412
N stage				0.017
N1	1.421	1.004	2.009	0.047
Nx	27.920	3.388	230.051	0.002
AJCC 8th stage (reference, stage IB)				0.032
IIA	3.668	0.506	26.581	0.198
IIB	4.690	0.651	33.764	0.125
IV	9.351	1.146	76.266	0.037
Adjuvant chemotherapy	0.923	0.649	1.314	0.658
Gemcitabine adjuvant therapy	1.006	0.719	1.409	0.971
hENT1 (reference, intensity 0)				0.398
1	0.347	0.105	1.149	0.083
2	0.647	0.217	1.935	0.436
3	0.629	0.244	1.622	0.338
4	0.698	0.283	1.722	0.435
dCK (reference, intensity 0)				0.413
2	1.253	0.795	1.976	0.331
3	0.956	0.635	1.437	0.828
4	0.612	0.218	1.714	0.350
RRM1 (reference, intensity 0)				0.138
1	0.793	0.387	1.626	0.527
2	1.337	0.803	2.225	0.264
3	0.907	0.523	1.572	0.728
4	0.565	0.193	1.656	0.298

Table 2. Continued

Variables	Univariate		
	HR	95% CI	p-value
RRM2 (reference, intensity 0)			0.048
2	0.475	0.155 1.458	0.193
3	0.951	0.517 1.749	0.872
4	1.375	0.757 2.499	0.296
hENT1 high	1.163	0.821 1.648	0.396
hENT1 high+gemcitabine	0.986	0.683 1.424	0.940

hENT1, human equilibrative nucleoside transporter 1; dCK, deoxycytidine kinase; RRM1, ribonucleotide reductase regulatory subunit M1; RRM2, ribonucleotide reductase regulatory subunit M2; AJCC, American Joint Committee on Cancer
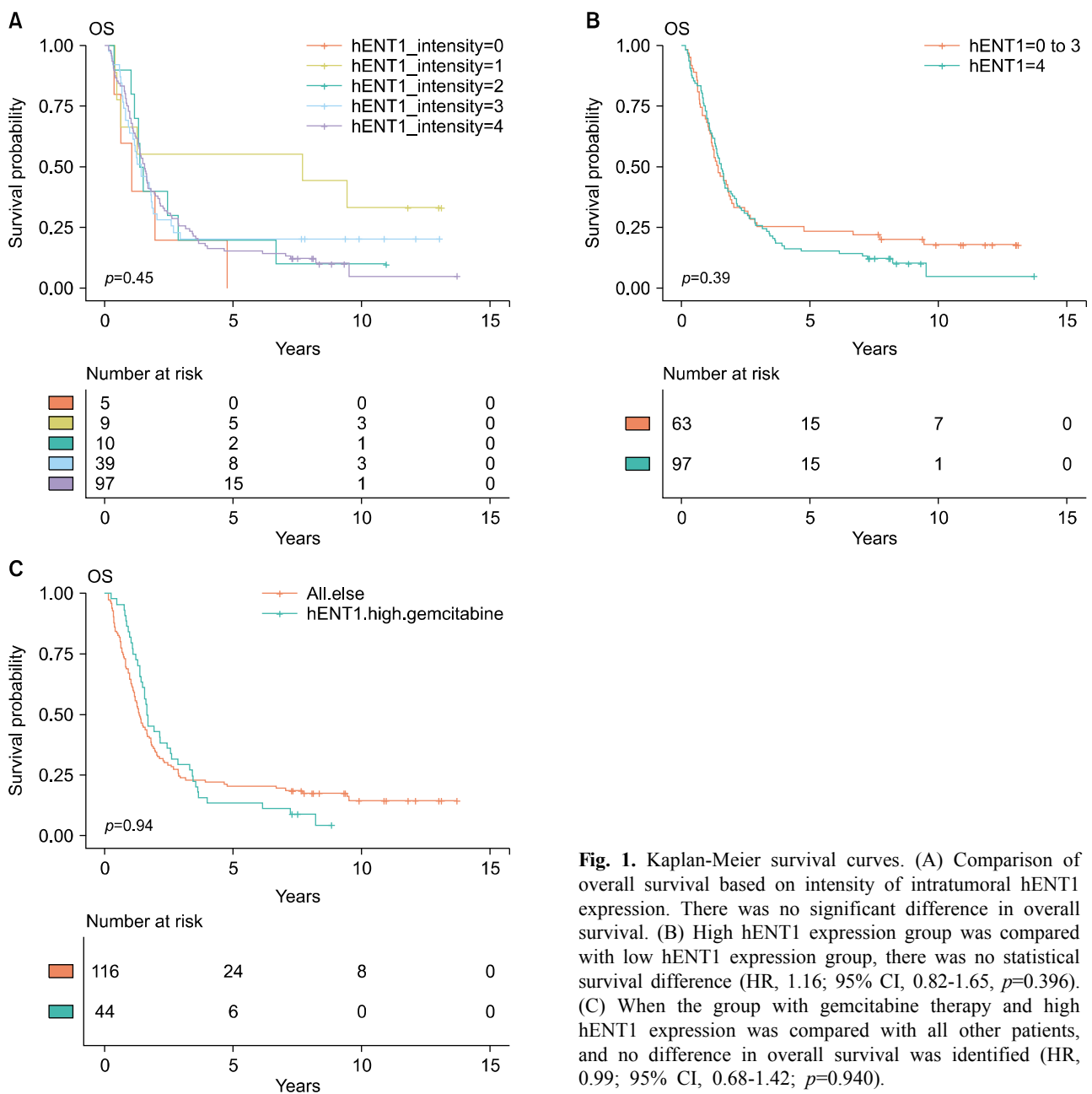
Fig. 1. Kaplan-Meier survival curves. (A) Comparison of overall survival based on intensity of intratumoral hENT1 expression. There was no significant difference in overall survival. (B) High hENT1 expression group was compared with low hENT1 expression group, there was no statistical survival difference (HR, 1.16; 95% CI, 0.82-1.65, p=0.396). (C) When the group with gemcitabine therapy and high hENT1 expression was compared with all other patients, and no difference in overall survival was identified (HR, 0.99; 95% CI, 0.68-1.42; p=0.940).
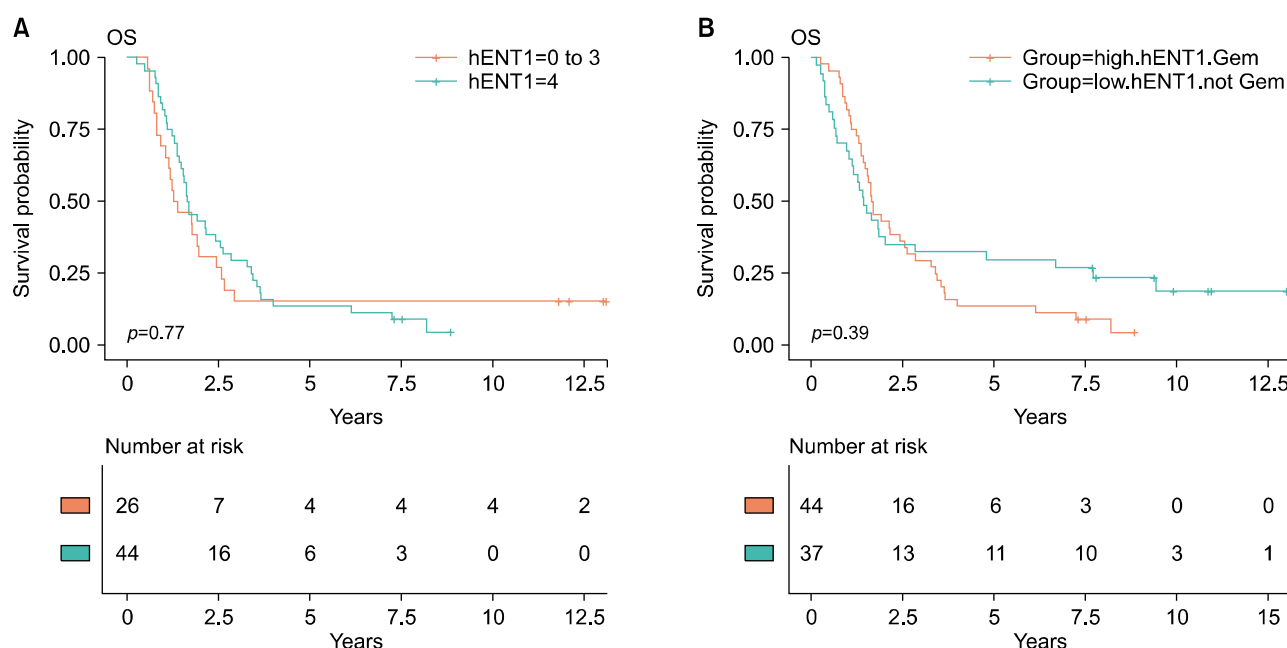
Fig. 2. Kaplan-Meier survival curves. The association between intratumoral hENT1 expression and overall survival according to adjuvant chemotherapeutic agent. (A) Overall survival between the intensity of hENT1 expression within patients with gemcitabine therapy (HR, 0.92; 95% CI, 0.55-1.56; *p*=0.764). (B) The comparison between gemcitabine with high hENT1 expression group and other chemotherapy with low hENT1 did not show significant survival difference (HR, 0.81; 95% CI, 0.49-1.32; *p*=0.394).

and no difference in overall survival was identified (HR, 0.99; 95% CI, 0.68-1.42; *p*=0.940). And, the group with gemcitabine therapy and high hENT1 expression was compared with gemcitabine therapy and low hENT1 expression, there was no statistical survival difference (HR, 0.92; 95% CI, 0.55-1.56; *p*=0.764) (Fig. 2A). The comparison between gemcitabine with high hENT1 expression group and other chemotherapy with low hENT1 did not show significant survival difference (Fig. 2B).

Similarly, the intensity of DCK, RRM1, and RRM2 expression was not associated with overall survival (*p*=0.413, *p*=0.138 and *p*=0.061) in univariate analysis (Table 2).

DISCUSSION

In this study, we investigated the expression of hENT1, DCK, RRM1 and RRM2 by immunohistochemical analysis to determine their prognostic value in patients with pancreatic cancer. The results showed no significant relationship between protein expression and overall survival. Intratumoral hENT1 expression itself was not a prognostic factor for survival, and high hENT1 expression with gemcitabine therapy was not associated with survival after

pancreatic cancer surgery. Moreover, expression of DCK, RRM1, and RRM2 had no correlation with survival.

Although combination chemotherapy with fluorouracil, leucovorin, irinotecan, and oxaliplatin (FOLFIRINOX) leads to longer overall survival than gemcitabine therapy, gemcitabine still plays an important role in systemic chemotherapy for pancreatic cancer.[15] Survival rate improves significantly with FOLFIRINOX, but gemcitabine remains an option because of the high incidence of toxic effects with FORFIRINOX. There are many reports about adjuvant gemcitabine therapy and prognostic factors. The majority of these reports showed that gatekeepers for intracellular uptake of gemcitabine (hENT1) and metabolic regulators of gemcitabine after intracellular entry (dCK, RRM1, and RRM2) are strongly associated with disease-free and overall survival in pancreatic cancer.[6,14,16-18]

Our results are not in line with the results of previous reports investigating the prognostic and predictive value of these proteins in pancreatic cancer. First, we analyzed the prognostic value of hENT1 itself, whether or not gemcitabine was used because most previous studies reported an association between hENT1 and survival without analyzing the survival impact of hENT1 expression alone.

The intensity of hENT1 expression did not show any correlation with survival, even in the high hENT1 expression group (p=0.398). We then investigated whether survival impact was enhanced when gemcitabine was used in patients with high hENT1. Survival of the group with high hENT1 and gemcitabine was not significantly different, compared with all other patients (HR, 0.986; 95% CI, 0.68-1.42; p=0.940). And, even in patients with gemcitabine adjuvant therapy, the survival of the group with high hENT1 did not show the statistical survival difference, compared with low hENT1 (HR, 0.92; 95% CI, 0.55-1.56; p=0.764). Expression of dCK also did not show any association with survival, unlike in previous studies.[6,14,17] According to known intracellular mechanism and previous reports, RRM1 and RRM2 show reverse correlation with the effect of hENT1. However, in our results, RRM1 and RRM2 were not associated with overall survival. Although RRM2 showed marginal significance with survival by Cox proportional hazard ratio, the association was not proven by Kaplan-Meier survival curve.

This study may have had different results than the majority of previous studies for two main reasons. The first is the actual length of follow-up. If follow-up is less than 5 years, then 5-year overall survival may be overestimated compared to actual survival. The second consideration is the size of the study population. Except for a few large scale studies, most previous studies were conducted with small study populations, which affects the association and interpretation of results.[6,17-19]

Our study had several limitations. First, this is a case-control study. A nonrandomized observational study should be interpreted with caution because of the potential for bias, especially selection bias. Second, a long study period would affect the outcome. A 10 year study period might coincide with major improvements in surgery, oncology, and general supportive care, which could mask the survival impact of gemcitabine related proteins. Third, inter-rater reliability was not measured. Measurement and selection biases should be avoided using all available methods.

Despite its limitations, this study suggests that expression of hENT1, dCK, RRM1 and RRM2 may not be associated with overall survival for patients with pancreatic cancer on gemcitabine adjuvant therapy. These proteins and other factors that may interact with or confound these results should be investigated in the near future. A well-designed cohort study with expression profiling of all available genes is required to create criteria regarding chemotherapeutic applications in patients with PDAC.

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CONFLICT OF INTEREST

The authors declare no competing interests in relation to this study.

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AUTHOR CONTRIBUTIONS

Conceptualization: DWH, YSY, JYC, HSH. Data curation: DWH, YSY, ES. Formal analysis: DWH, YSY. Methodology: DWH, JYC, HSH, ES. Project administration: DWH, YSY. Visualization: DWH, JYC, HSH. Writing - original draft: DWH, ES. Writing - review & editing: DWH, YSY.

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