의학박사 학위논문

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이 논문을 의학박사 학위논문으로 제출함

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Abstract

hENT1 as a predictive biomarker for a gemcitabine response in biliary tract cancer

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Background: Gemcitabine is one of the main chemotherapeutic agents for biliary tract cancer (BTC). Expression of human equilibrative nucleoside transporter 1 (hENT1) is considered as a potential predictive biomarker for a gemcitabine response in several cancers. This study aimed to investigate the association between hENT1 expression and the effects of gemcitabine on BTC cell lines and on patients with advanced BTC receiving gemcitabine-based chemotherapy.

Methods: Four BTC cell lines, HuCCT1, SNU-478, SNU-1079, and SNU-1196, were tested in this study. mRNA and protein

expression levels of hENT1 were measured by quantitative reverse-transcription polymerase chain reaction and western blotting, respectively. Cell viability after gemcitabine treatment was measured in a chemosensitivity assay. For clinical assessment, 40 patients with unresectable or recurrent BTC who were treated with gemcitabine (1000 mg/m2) and cisplatin (25 mg/m2) between June 2012 and May 2014 were enrolled.

Results: Among the four cell lines, SNU1196 showed the highest mRNA and protein levels of hENT1. Expression of hENT1 had a linear correlation with the log value of the half-maximal inhibitory concentration of gemcitabine. During incubation with gemcitabine, pretreatment with hENT1-specific small interfering RNA (siRNA) resulted in higher cell viability than that in samples pretreated with control siRNA. In a clinical evaluation, there were 22 intrahepatic cholangiocarcinoma, five as perihilar cholangiocarcinoma, 11 as distal bile duct cancer, and two as gallbladder cancers and 15 high-hENT1 and 25 low-hENT1 patients. The median progression-free survival was 24 and 11 weeks among patients with strong and weak intratumoral hENT1 immunohistochemical staining (P = 0.05),

and the median overall survival was 52 and 26 weeks (P = 0.15), respectively. Among 22 patients with intrahepatic cholangiocarcinoma, the median PFS was 22 and 7 weeks (P = 0.08) and the median OS was 60 and 21 weeks (P = 0.04) in the high- and low-hENT1 groups, respectively.

Conclusion: The current study showed that increased hENT1 expression is associated with a stronger toxic effect of gemcitabine on BTC cell lines. The clinical outcomes suggested that increased intratumoral hENT1 immunohistochemical staining is a possible biomarker predicting better therapeutic effects of gemcitabine on patients with advanced BTC. Further studies are necessary to determine the precise role of hENT1 in BTC.

Keywords: Cancer, Biliary Tract; Equilibrative Nucleoside Transporter 1; Gemcitabine; Biomarker

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BTC: biliary tract cancer

DBC: distal bile duct cancer

FOLFIRI, irinotecan with 5-fluorouracil and folinic acid

GBC: gallbladder cancer

GP: gemcitabine plus cisplatin

hCNT1: human concentrative nucleoside transporters 1

hCNT3: human concentrative nucleoside transporters 3

hENT1: human equilibrative nucleoside transporter 1

iFAM, infusional 5-fluorouracil, doxorubicin, and mitomycin C

IC: intrahepatic cholangiocarcinoma

OS: overall survival

PC: perihilar cholangiocarcinoma

PFS: progression-free survival

RT-PCR: reverse-transcription polymerase chain reaction

siRNA: small interfering RNA

TBS: Tris-buffered saline

TBST: Tris-buffered saline containing 0.1% Tween

XP, capecitabine and cisplatin

Introduction

Biliary tract cancer (BTC) is a malignant tumor that originates in the biliary tract including the intra- and extrahepatic bile duct and gallbladder. BTC has a poor prognosis because it is diagnosed at the advanced stage in many cases or frequently recurred even after curative resection (1, 2). Thus, most patients with advanced BTC have to depend on palliative systemic chemotherapy. A gemcitabine plus cisplatin (GP) regimen was recently accepted as first-line chemotherapy (3). Nonetheless, the efficacy of GP is different among individuals. A predictive biomarker for a GP response in BTC is necessary because of the medical cost and adverse effect related with this regimen.

Gemcitabine is an analog of cytidine and an important chemotherapeutic agent for various malignancies, including BTC. Just as other nucleoside analogs, it is a prodrug that requires cellular uptake and intracellular phosphorylation. Human equilibrative nucleoside transporter 1 (hENT1) and human concentrative nucleoside transporters 1 and 3 (hCNT1 and -3)

are important for the transport of it into the cell. Inside the cell, gemcitabine is metabolized to active gemcitabine diphosphate and triphosphate by deoxycytidine kinase (4, 5). During this metabolic process, active metabolites inhibit ribonucleotide reductase subunits 1 and 2, whose expression is associated with gemcitabine resistance (6). Gemcitabine is mainly inactivated by cytidine deaminase (7).

Recently, intratumoral hENT1 has been reported as a candidate predictive biomarker for gemcitabine therapy responses in various cancers (8–11). Nevertheless, its predictive value as a biomarker in BTC, particularly in patients at an unresectable stage, is unclear (12–14).

The current study aimed to investigate the association between hENT1 expression and the effects of gemcitabine both on BTC cell lines and on patients with advanced BTC who receive a GP regimen.

Methods

1. *In vitro* experiments

BTC cell lines and chemicals

Four BTC cell lines were analyzed in this study. The HuCCT1 cell line (intrahepatic cholangiocarcinoma origin) was purchased from the RIKEN BioResource Center (Ibaraki, Japan). The SNU-478 (ampulla of Vater adenocarcinoma origin), SNU-1079 (intrahepatic cholangiocarcinoma origin), and SNU-1196 (extrahepatic cholangiocarcinoma origin) cell lines were acquired from the Korean Cell Line Bank (Seoul, Korea). All the cell lines were cultured in the RPMI 1640 medium (Gibco, Grand Island, NY, USA) supplemented with 10% of fetal bovine serum (Gibco) and 1% of a penicillin/streptomycin solution (Gibco) and were maintained at 37° C and 5% atmospheric CO2. Gemcitabine was provided by Lilly Korea (Seoul, Korea) for *in vitro* experiments. The company was not involved in anything related to the study.

Total-RNA isolation, cDNA synthesis, and quantitative reverse-

transcription polymerase chain reaction (RT-PCR)

Total-RNA was isolated from the cells using the RNeasy Plus Mini Kit (Qiagen, Hilden, Germany), and cDNA was synthesized with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). The primer sets for RT-PCR analysis of *hENT1* and *GAPDH* were as follows:

hENT1, 5′-CAGGCAAAGAGGAATCTGGA-3′ and 5′-GGCCCAACCAGTCAAAGATA-3′;

GAPDH, 5′-TTCACCACCATGGAGAAGGC-3′ and 5′-GGCATGGACTGTGGTCATGA-3′.

The primers were designed by means of published sequence data from GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and a BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi/). The Power SYBR Green PCR Master Mix (Applied Biosystems) served as the reaction mixture, in a 20 μL volume in each reaction capillary. The mRNA expression of the gene under study was normalized to the corresponding expression of *GAPDH*.

Western blot analysis

Cells were washed rapidly with ice-cold PBS and lysed in RIPA buffer (Cell Signaling Technology, Danvers, MA, USA) containing 1 mM of the protease inhibitor PMSF (Cell Signaling Technology) and the Protease Inhibitor Cocktail (Sigma-Aldrich, St. Louis, MO, USA). The cell extracts were centrifuged at 13,000 rpm for 20 min. The total-protein concentration in the supernatant was measured with the BCA reagent (Pierce, Rockford, IL, USA). For each sample, equal amounts of total protein were denatured and separated by sodium dodecyl sulfate 10% polyacrylamide gel electrophoresis and then transferred onto a nitrocellulose membrane. The membranes were blocked for 1 h in 5% skimmed milk powder solution composed of Tris-buffered saline (TBS) containing 0.1% Tween (TBST). Membranes were incubated overnight at 4° C with primary antibodies against hENT1 (Abcam, Cambridge, UK) and β-actin (Cell Signaling Technology). After three washes with TBST, the membranes were incubated with the appropriate horseradish peroxidase-conjugated secondary antibodies for 2 h (Cell Signaling Technology).

Bound antibodies were detected by means of the ECL reagent (Amersham Pharmacia Biotech, Buckinghamshire, UK) and an X-ray film (AGFA, Mortsel, Belgium).

RNA interference and a cell viability assay

Cells were cultivated in culture plates to 60-70% confluence. After a wash with PBS, the cells were treated with the Lipofectamine RNAiMAX reagent (Invitrogen, Carlsbad, CA, USA) mixed with hENT1-targeting small interfering RNA (siRNA; Assay ID s4694; Ambion, Foster City, CA, US) or nontargeting control siRNA (catalog No. AM4620; Ambion) at a final amount of 10 nmol in Opti-MEM (Gibco). At 72 h after siRNA treatment, cells were harvested to examine silencing efficiency and the effect of the hENT1 knockdown.

These hENT1 knockdown cells and control cells were seeded at 3,000-5,000 per well in 96-well plates. After overnight incubation, the cells were treated with gemcitabine (0-100 μM) for 72 h. Cell viability was determined using the Cell Titer-Glo Luminescent Cell Viability Assay Kit (Promega, Madison, WI, USA). Luminescence was measured on a LMAXII 384

microplate reader (Molecular Devices, Sunnyvale, CA, USA). Half-maximal inhibitory concentrations (IC_{50}) were calculated in the SigmaPlot software.

2. Clinical validation

Patients

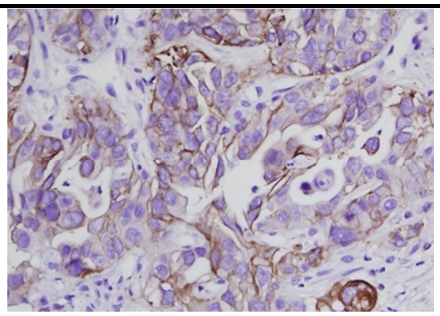
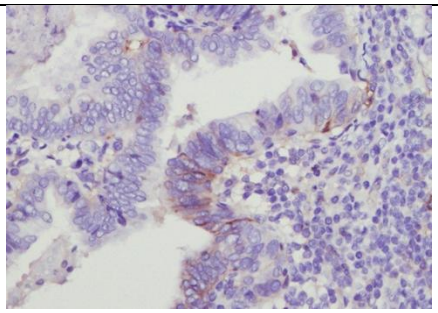
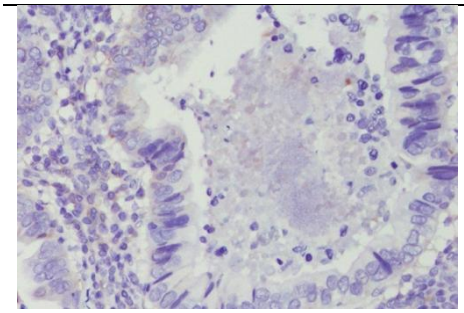
Between June 2012 and May 2014, 193 patients with BTC who received gemcitabine-based therapy were identified via electronic medical records at Seoul National University Bundang Hospital. Among them, 153 patients were excluded due to the following: gemcitabine as adjuvant therapy, single gemcitabine or combination with other agents as palliative therapy, a dose different from that in the current first-line regimen (gemcitabine 1,000 mg/m2 and cisplatin 25 mg/m2) (3), or a lack of formalin-fixed paraffin-embedded block samples for immunohistochemistry. Eventually, 40 patients were enrolled, and their medical records were retrospectively reviewed.

hENT1 immunohistochemistry

Immunohistochemical analysis of hENT1 was performed on 4-μm-thick unstained sections of formalin-fixed paraffin-embedded BTC specimens on an automated Ventana BenchMark XT system with an anti-hENT1 SP120 rabbit monoclonal antibody (Ventana Medical Systems, Inc., Tucson, AZ, USA) as previously reported (15). Immunohistochemical staining was done from 9 surgical specimens and 31 biopsy samples.

Grading of hENT1 staining was performed by an experienced pancreaticobiliary pathologist (HK) as follows: 2+, membranous staining in more than 50% of tumor cells; 1+, membranous staining in 5-50% of tumor cells; 0, no hENT1 staining or staining in <5% of tumor cells. Grade 0 or 1+ was regarded as low hENT1 expression, and grade 2+ was considered high hENT1 expression according to another study (Table 1) (15).

Table 1. Grading of hENT1 staining

Microscopic imaging (X400)	Grade of membranous staining	Expression level category
	2+ (in more than 50% of tumor cells)	High
	1+ (in 5– 50% of tumor cells)	Low
	0 (in less than 5% of tumor cells)	

3. Statistics

The *in vitro* experimental results shown are representative of three or more independent experiments. Pearson's χ^2 test or Fisher's exact test was conducted to determine the differences between categorical variables. Continuous variables were compared by the Mann–Whitney U test. Kaplan–Meier analysis was carried out to generate survival curves and calculate the median survival periods, which were compared by the log rank test. A two-sided P value less than 0.05 indicated statistical significance. All statistical analyses were performed in GraphPad Prism 7.0 (GraphPad Software, La Jolla, CA, USA) and in R software v3.4.3 (The R Development Core Team).

Results

1. Baseline mRNA and protein expression of hENT1

The baseline mRNA expression of *hENT1* in each cell line was measured in comparison to that in HuCCT1 cells by quantitative RT-PCR (Figure 1). Similarly, baseline levels of the hENT1 protein in each cell line were measured relative to HuCCT1 cells by western blotting (Figure 2). Among the four cell lines, SNU1196 showed the highest mRNA and protein levels of hENT1, whereas HuCCT1 had the lowest levels. The expression pattern of *hENT1* mRNA was similar to that of the hENT1 protein among the four cell lines.

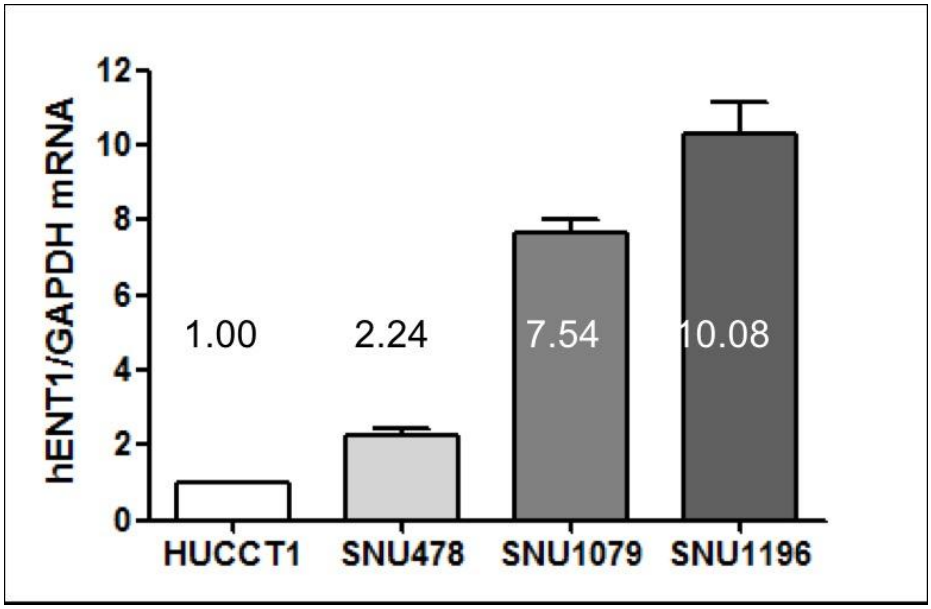


Figure 1. Expression of *hENT1* mRNA. Baseline levels of mRNA expression of hENT1 were measured relative to those of HuCCT1 cells by quantitative RT-PCR. SNU1196 showed the highest mRNA expression of *hENT1*.

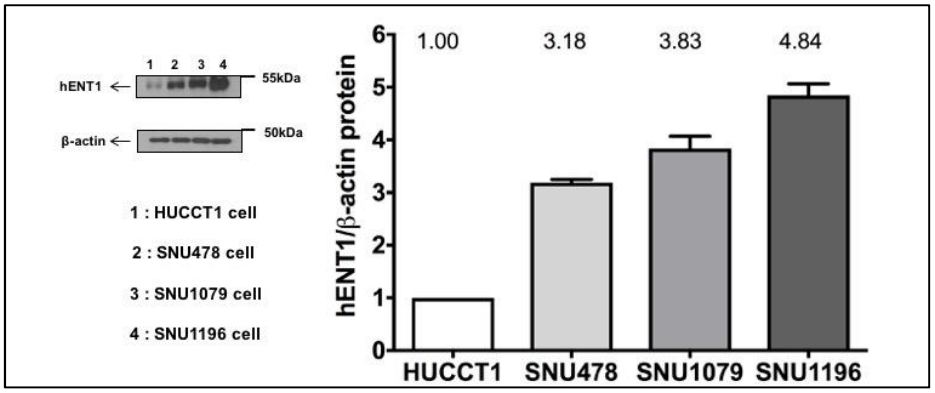


Figure 2. Expression of the hENT1 protein. Baseline levels of protein expression of hENT1 were measured relative to those of HuCCT1 cells by western blotting. SNU1196 showed the highest protein level of hENT1.

2. The chemosensitivity assay

Among the four BTC cell lines, sensitivity to gemcitabine was measured as IC_{50}. This parameter was the highest in HuCCT1 cells and the lowest in SNU1196 cells (Table 2 and Figure 3). In the analysis of correlation between gemcitabine chemosensitivity and basal expression of *hENT1* mRNA, mRNA expression manifested a clear linear correlation with the log value of IC_{50} (Figure 4).

Table 2. Half-maximal inhibitory concentration (IC_{50}) of gemcitabine in 4 biliary tract cancer cell lines

	HuCCT1	SNU478	SNU1079	SNU1196
IC_{50} (nM) (mean ± SD)	11682.5 ± 632.3	3516.4 ± 928.9	4093.5 ± 202.3	1498.9 ± 24.1

IC_{50}, half-maximal inhibitory concentration; SD, standard deviation

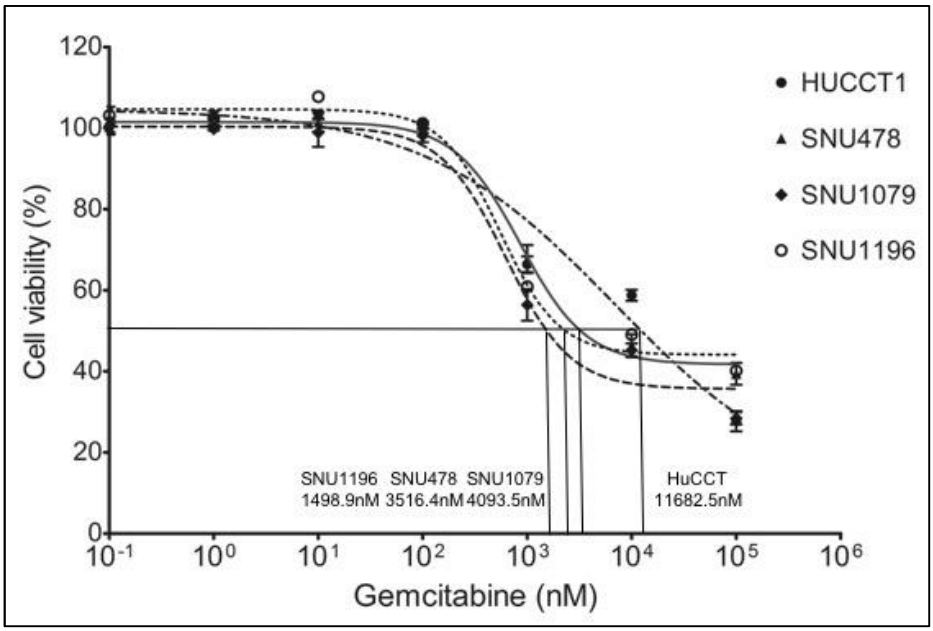


Figure 3. Cell viability after gemcitabine treatment in four biliary tract cancer (BTC) cell lines. IC_{50} was the highest in HuCCT1 cells and the lowest in SNU1196 cells.

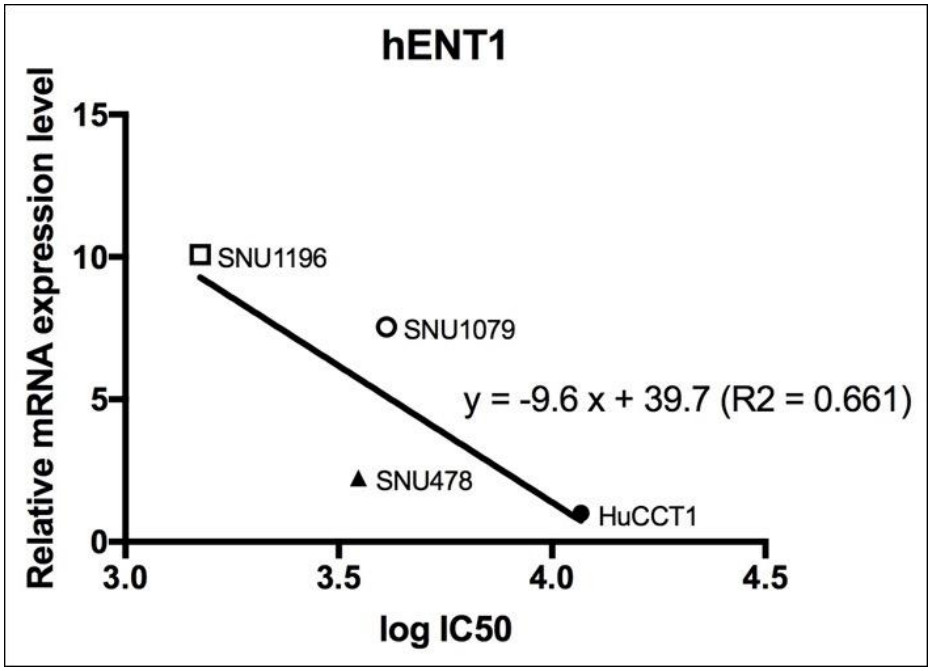


Figure 4. Correlation between gemcitabine chemosensitivity and basal expression of *hENT1* mRNA. Expression of *hENT1* mRNA showed a clear linear correlation with the log value of the half-maximal inhibitory concentration (IC_{50}).

3. Inhibition of cancer cell viability by hENT1 siRNA during gemcitabine treatment

Pretreatment with hENT1 siRNA was conducted to assess the influence of hENT1 on the toxic effects of gemcitabine. In all BTC cell lines, viability after hENT1 siRNA treatment was higher than that after control siRNA treatment (Figure 5). Cancer cell proliferation at 144 h after treatment was significantly higher after hENT1 siRNA treatment than after control siRNA treatment (Figure 6). There was no difference in the inhibition of cell proliferation among the BTC cell lines.

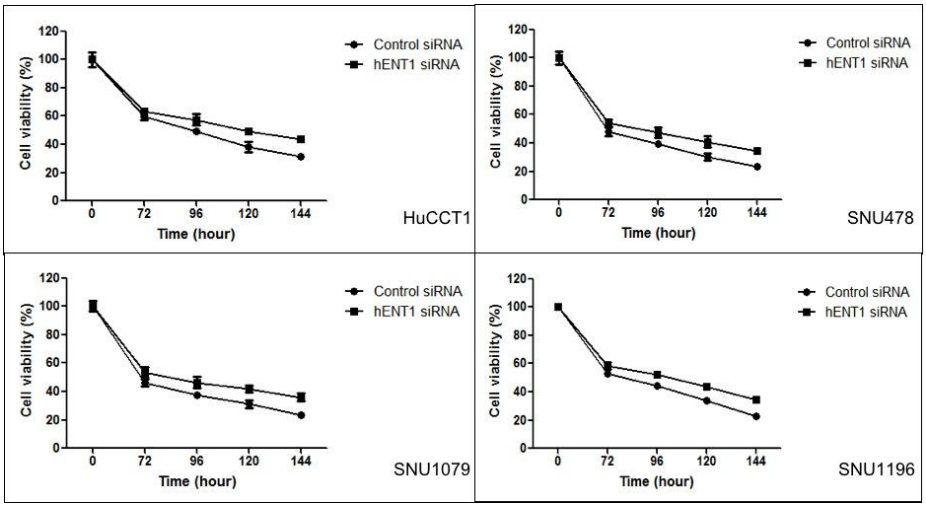


Figure 5. Effects of hENT1 siRNA on sensitivity of BTC cells to gemcitabine. Pretreatment with hENT1 siRNA was conducted to assess the effect of the hENT1 knockdown on gemcitabine sensitivity. During incubation with gemcitabine, in all cell lines, viability after hENT1 siRNA treatment was higher than that after control siRNA treatment.

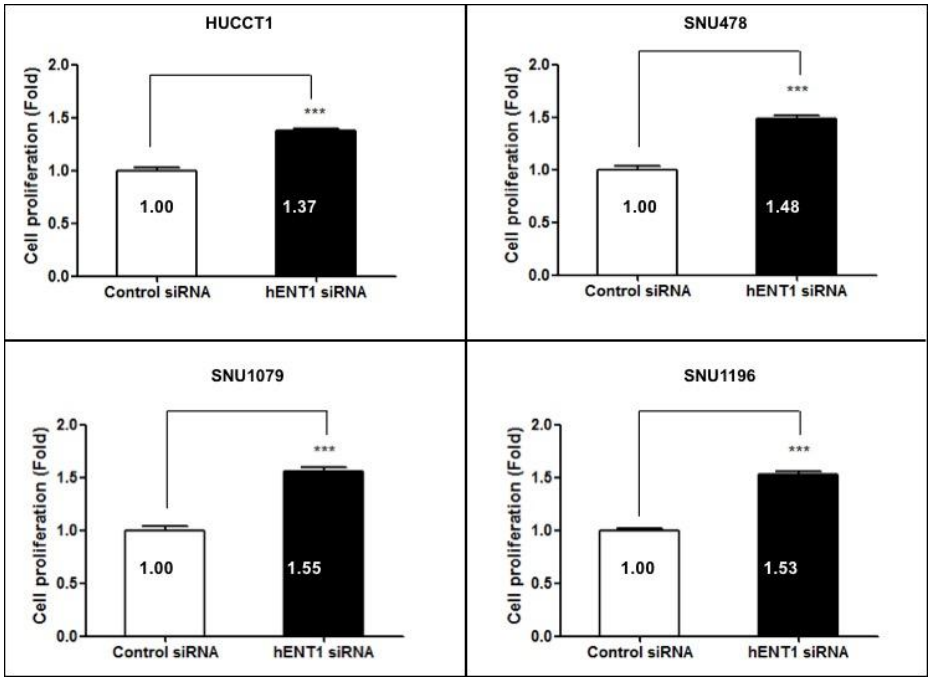


Figure 6. Effects of hENT1 siRNA on gemcitabine sensitivity. During incubation with gemcitabine, cell proliferation at 144 h after treatment with hENT1 siRNA was significantly higher than that after control siRNA treatment.

4. Clinical outcomes

According to hENT1 immunohistochemical staining grades, there were 15 patients with grade 2+ in the tumor, nine with grade 1+, and 16 with grade 0. As a result, there were 15 high-hENT1 and 25 low-hENT1 patients (Table 3). There were 24 men and 16 women with a median age of 62.5 years (43-80 years). As for tumor location, 22 BTCs were diagnosed as intrahepatic cholangiocarcinoma, five as perihilar cholangiocarcinoma, 11 as distal bile duct cancer, and two as gallbladder cancers without a significant difference in hENT1 expression among these types. Six patients underwent curative surgery, and five patients were treated with palliative surgery. Between initially unresectable and recurrent BTCs, there were no differences in progression-free survival (PFS; median 20.6 vs 12.9 weeks, P = 0.87) or overall survival (OS; median 51.5 vs 27.0 weeks, P = 0.71). Besides, there were no significant differences in clinicopathological factors such as cellular differentiation, median chemotherapy cycle, and CA 19-9 levels between the two groups. Eleven patients were administered second-line chemotherapy with various regimens

based on 5-fluorouracil after failure of the GP regimen as follows: 1 iFAM (infusional 5-fluorouracil, doxorubicin, and mitomycin-C), 3 XP (capecitabine and cisplatin), and 1 FOLFIRI (irinotecan with 5-fluorouracil) in high-hENT1; 2 iFAM and 4 XP in low-hENT1. Nevertheless, there was no difference in the number of patients receiving second-line chemotherapy.

Table 3. Baseline characteristics of patients

	High hENT1 (n=15)	Low hENT1 (n=25)	*P*
Male (%)	8 (53.3)	16 (64.0)	0.51
Median age (range)	61 (51–70)	63 (43–80)	0.61
IC/PC/DBC/GBC	10/1/3/1	12/4/8/1	0.66
Unresectable/ Recurred patients	12/3	22/3	0.65
Cellular differentiation (Well/Moderate/ Poorly/NA)	0/6/3/6	1/17/3/4	0.54
Initial operation (number of patients)	Pancreatico-duodenectomy (1) Segmentectomy (2)	Pancreatico-duodenectomy (2) Extended left lobectomy (1)	

Median GP cycle (range)	6 (2−12)	3 (1−18)	0.10
Best Response Rate with GP chemotherapy (number of patients, %)	PR/SD/PD 0/11/4 (0/73.3/26.7)	PR/SD/PD 2/12/11 (8.0/48.0/44.0)	0.33*
Counts of patients with second line chemotherapy	5	6	0.17
Median pre−treatment CA 19−9 (range)	167.7 (6−20000)	740.0 (0.6−20000)	0.82
Pre−treatment CA 19−9 > 37 (%)	11 (73.3)	20 (80.0)	> 0.99

hENT1, human equilibrative nucleoside transporter 1; IC, intrahepatic cholangiocarcinoma; PC, perihilar cholangiocarcinoma; DBC, distal bile duct cancer; GBC, gallbladder carcinoma; NA, not applicable; GP, gemcitabine and cisplatin; PR, partial response; SD, stable disease; PD, progressive disease; CA 19-9, carbohydrate antigen 19-9; *, disease control rate (PR + SD vs. PD)

Survival analysis was performed to compare the hENT1 groups. PFS was 24 and 11 weeks in the high- and low-hENT1 groups, respectively (P = 0.05, Figure 7). The median OS was 52 and 26 weeks (P = 0.15, Figure 8), respectively. Among the patients with intrahepatic cholangiocarcinoma, the median PFS was 22 and 7 weeks (P = 0.08, Figure 9) and the median overall survival was 60 and 21 weeks (P = 0.04, Figure 10) in the high- and low-hENT1 groups, respectively. The median PFS of 11 patients with the second line chemotherapy was much shorter than those with the GP chemotherapy, however, there was no difference between the high- and low-hENT1 groups (48 and 49 days, P = 0.26, Figure 11)

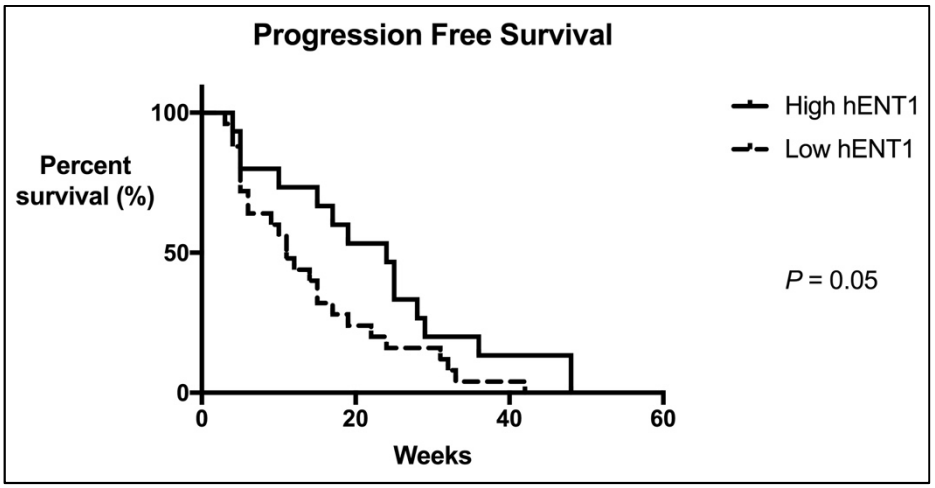


Figure 7. Median progression-free survival of patients administered gemcitabine and cisplatin combination chemotherapy. There was borderline significance of the difference in progression free survival between high- and low-hENT1 groups (24 and 11 weeks, P = 0.05).

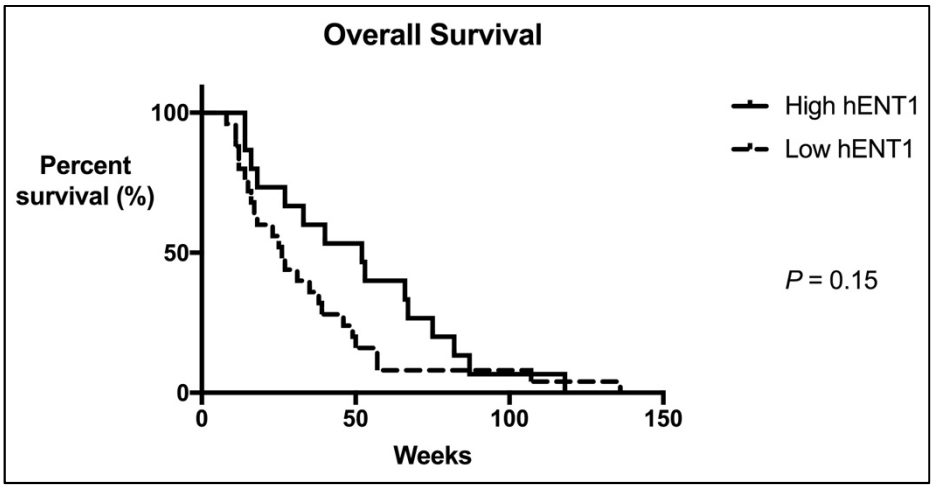


Figure 8. Median overall survival of patients who received gemcitabine and cisplatin combination chemotherapy. There was no significant difference in overall survival between high- and low-hENT1 groups (52 and 26 weeks, P = 0.15).

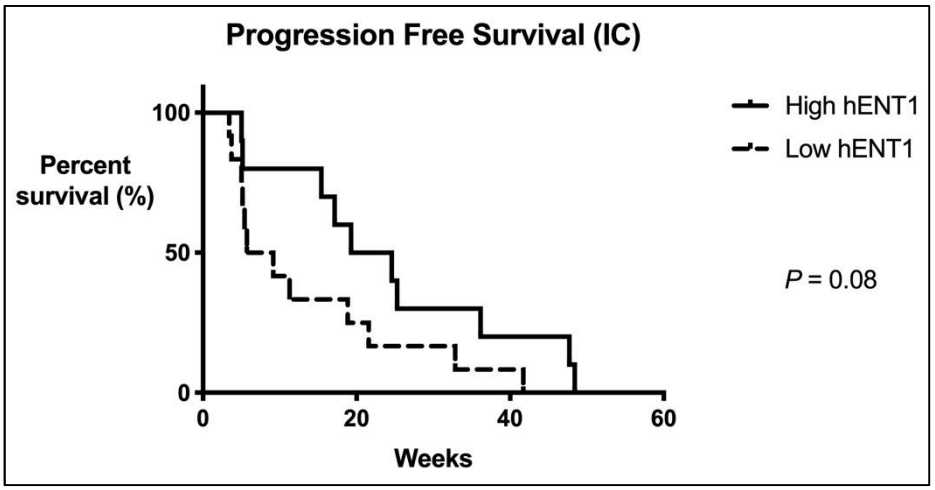


Figure 9. Median progression-free survival of patients with intrahepatic cholangiocarcinoma administered gemcitabine and cisplatin combination chemotherapy. There was no significance of the difference in progression free survival between high- and low-hENT1 groups (22 and 7 weeks, P = 0.08). IC, intrahepatic cholangiocarcinoma

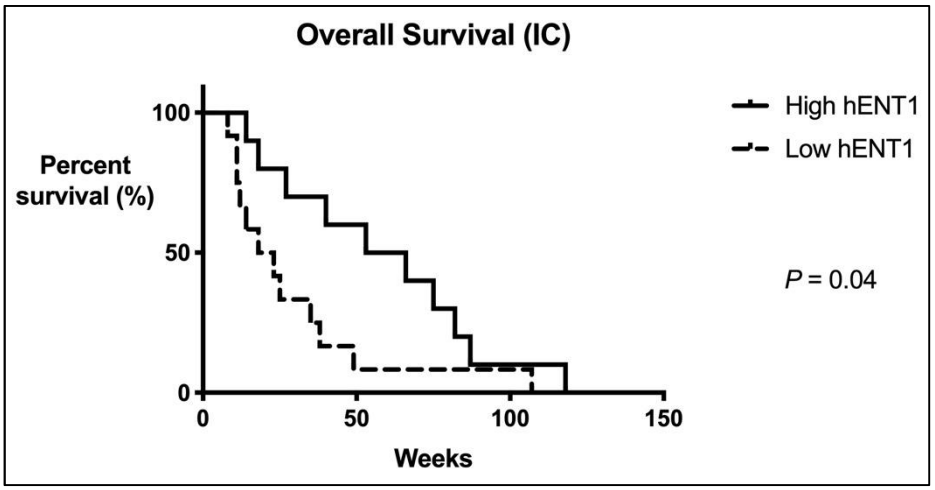


Figure 10. Median overall survival of patients with intrahepatic cholangiocarcinoma who received gemcitabine and cisplatin combination chemotherapy. There was a significant difference in overall survival between high- and low-hENT1 groups (60 and 21 weeks, P = 0.04). IC, intrahepatic cholangiocarcinoma

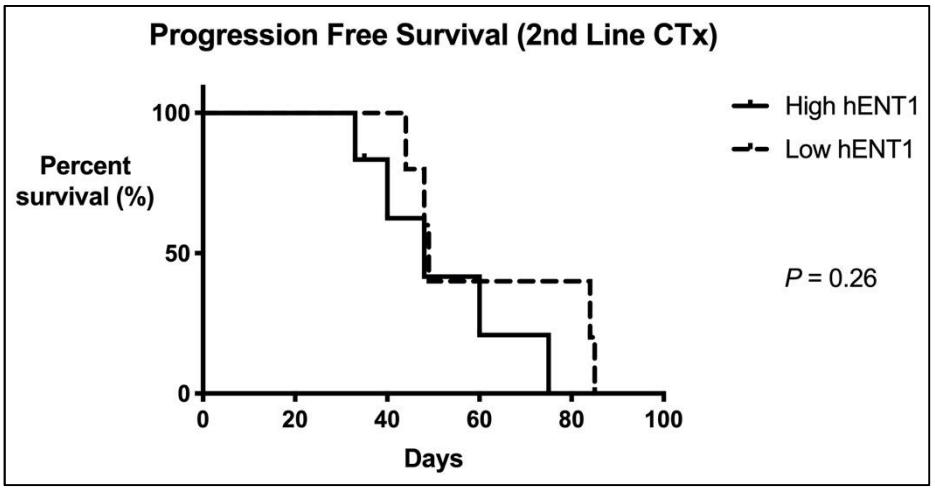


Figure 11. Eleven patients received the second line chemotherapy after progression with gemcitabine and cisplatin combination chemotherapy. There was no significant difference in progression free survival of the patients with the second line chemotherapy between high- and low-hENT1 groups (48 and 49 days, P = 0.26).

Discussion

Gemcitabine is one of the important chemotherapeutic agents widely used for many malignant tumors (8, 10, 11, 13). As a candidate cancer biomarker, particularly in pancreatic cancer, hENT1 has been examined because of its role as an intracellular transporter of gemcitabine (8, 9, 15–19). However, few studies on BTC have examined the combination of hENT1 and gemcitabine because of the low incidence of BTC compared to pancreatic cancer in western countries. Moreover, the results have been inconsistent because different investigators employed either palliative (12, 20) or adjuvant (14) settings with various regimens. Therefore, the patients with advanced BTC receiving the current first-line GP regimen as palliative chemotherapy were focused on in this study.

Several key proteins in the metabolic pathway of gemcitabine may be considered as predictive biomarkers of a response to gemcitabine chemotherapy. Among these proteins, hENT1 was investigated in this study because its clinical value has been widely examined in pancreatic cancer (8, 9, 16, 19). Among the

four BTC cell lines, a linear correlation between the basal expression of *hENT1* mRNA and sensitivity to gemcitabine was detected. Unexpectedly, when the expression of hENT1 was knocked down, similar chemoresistance was found among the four cell lines, regardless of their different basal expression levels of *hENT1* mRNA. This result suggests that hENT1 acts as a threshold in the transport of gemcitabine; however, further studies are necessary to confirm this finding.

In this study, the correlates of intratumoral hENT1 expression point to its usefulness as a predictive biomarker in addition to the *in vitro* results. Although the clinical value of hENT1 in the transport of gemcitabine in various malignancies has been demonstrated by immunohistochemical staining elsewhere (9–11, 18, 19, 21, 22), there is no standardized method for discrimination among clinical outcomes. Therefore, he hENT1 immunostaining method recently accepted in pancreatic cancer research was adopted in this study (15) because of the similar clinical and genetic features of these malignancies. Then, a cutoff value was set to discriminate between high and low expression of hENT1 in relation to clinical outcomes. As a

result, hENT1 was found to be associated only with PFS, in agreement with other studies on BTC (12–14). Although there was no significant difference in OS (52 and 26 weeks, P = 0.15), borderline significance of the difference in PFS (24 and 11 weeks, P = 0.05) was observed between the high- and low-hENT1 groups. In addition, there was a significant difference of OS in patients with intrahepatic cholangiocarcinoma. These outcomes are consistent with the results of another study, which revealed better time to progression in high-hENT1 patients without clinical significance of OS in 31 patients (12). Despite low statistical power due to the small number of patients in this study, intratumoral hENT1 expression was found to be a predictive marker independent from other clinicopathological factors (14).

This study has several strengths. First, this study conducted both *in vitro* experiments and clinical analysis. Due to the limited number of studies on BTC cell lines, the results are important because the consistent experimental and clinical results were demonstrated. Second, all the patients in this study received the same chemotherapy regimen (gemcitabine

1000 mg/m2 and cisplatin 25 mg/m2) that was used in the ABC-2 trial (3). Finally, a direct correlation between hENT1 expression and sensitivity of BTC to gemcitabine was found, and the suppressive effects of hENT1 knockdown (via siRNA) on gemcitabine sensitivity of BTC cells were demonstrated.

This study has certain limitations. First, no *in vivo* experiments were conducted. Second, a type II error may be possible in survival analysis because of the limited number of patients following the standard GP regimen. Small sample sizes have limited the conclusions of previous BTC studies as well. Third, the ratio of the patients with high to low hENT1 staining in this study (15/25) was different from the whole patients with hENT1 staining between 2013 and 2017 (181/115) (P=0.004). Therefore, there is a possibility of selection bias.

Thus, the experimental results in this study suggest that higher hENT1 expression is associated with a stronger toxic effect of gemcitabine on BTC cell lines. The clinical outcomes implicated that increased hENT1 expression is a potential biomarker predicting a better response to gemcitabine among patients with advanced BTC. Consequently, it is possible to speculate on the

possible utility of this marker for identifying the patients who would not benefit from gemcitabine-based chemotherapy. Further studies are needed to determine the usefulness of hENT1 and to develop effective therapeutic strategies against BTC.

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요약 (국문초록)

배경: 젬시타빈은 담도계암 치료에서 가장 중요한 약제 중 하나이다. Human equilibrative nucleoside transporter 1 (hENT1)의 발현은 일부 암에서 젬시타빈 치료 반응을 예측하는 잠재적인 바이오마커로 여겨진다. 본 연구는 담도계암 세포주와 젬시타빈 기반 복합항암치료를 받은 진행성 담도계암 환자에서 hENT1 발현과 젬시타빈 효과 사이의 연관성을 평가하고자 한다.

방법: 담도계암 세포주 4종 (HuCCT1, SNU-478, SNU-1079, SNU-1196)에서 hENT1의 mRNA와 단백질 발현을 측정하였다. 젬시타빈 처치 후 세포 생존 능력은 항암제 감수성 분석으로 측정하였다. 임상적인 평가는 2012년 6월에서 2014년 5월 사이에 절제 불가능하거나 혹은 재발한 담도계암 환자 40명에서 젬시타빈 (1000mg/m2)과 시스플라틴 (25mg/m2)으로 치료받은 환자를 대상으로 하였다.

결과: 담관암 세포주 4종 중 SNU 1196이 가장 높은 mRNA와 단백질 발현을 보였다. hENT1 발현은 젬시타빈의 50% 최대 저해 농도 로그값과 선형 상관관계를 보였다. 항암제 주입 전 hENT1 특이 siRNA 전처치는 대조 siRNA 전처치에 비해 높은 세포 생존을 보여주었다. 임상적으로, 간내 담관암 22명, 간문부 담관암

5명, 원위부 담관암 11명, 담낭암 2명이 포함되었다. 종양 내 hENT1의 면역화학염색 발현이 강한 환자군과 약한 환자군에서 중간 무진행생존기간은 24주와 11주 (P=0.05)였고, 중간 전체생존기간은 52주와 26주 (P=0.15)였다. 22명의 간내 담관암 환자에서 중간 문진행생존기간은 22주와 7주 (P=0.08)였고, 중간 전체생존기간은 60주와 21주 (P=0.04)였다.

결론: 본 연구는 hENT1의 증가된 발현이 담도계암 세포주에서 젬시타빈의 강한 독성 효과와 연관되어 있음을 보여주었고, 임상 적으로 증가된 종양 내 hENT1의 면역화학염색이 진행성 담도계암 환자에서 젬시타빈의 더 나은 치료 효과를 예측하는 잠재적인 바이오마커임을 제시하였다.

주요어: 담도계암; equilibrative nucleoside transporter 1; 젬시타빈; 바이오마커

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