Human equilibrative nucleoside transporter 1 and Notch3 can predict gemcitabine effects in patients with unresectable pancreatic cancer

A running title: Predictive genes of gemcitabine effect in pancreatic cancer

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A conflict of interest statement

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Author contributions

Kawakami H managed the patients, performed the endoscopic examination; Eto K and Kawakami H designed the research and provided discussion; Eto K, Takasawa A, and Fukuoka M performed tissue preparation and analyzed the data; Eto K, Kawakami H analyzed the data; Kuwatani M, Kudo T, Kawahata S, and Abe Y analyzed the data and provided clinical advice; Matsuno Y diagnosed the case of pathology; Eto K and Kawakami H collected the data and wrote the paper; Asaka M and Sakamoto N supervised the research; all authors approved the final manuscript for publication.

ABSTRACT

OBJECTIVES: Pancreatic ductal carcinoma (PDC) is one of the most lethal human carcinomas. Expression patterns of some genes may predict gemcitabine (GEM) treatment efficacy. We examined predictive indicators of survival in GEM-treated patients by quantifying the expression of several genes in pre-treatment endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) samples from patients with PDC.

METHODS: The expressions of hENT1, dCK, RRM1, RRM2 and Notch3 in EUS-FNA tissue samples from 71 patients with unresectable PDC were quantified using real-time reverse transcription-polymerase chain reactions and examined for correlations with GEM sensitivity. **RESULTS**: The log-rank test detected no significant differences in overall survival between GEM-treated patients with low and high mRNA levels of all genes examined. However, low Notch3 mRNA expression was significantly associated with longer overall survival in a multivariate analysis for survival (P = 0.0094). High hENT1 expression level was significantly associated with a longer time to progression (P = 0.039). Interaction tests for GEM administration and hENT1 or Notch3 mRNA expression were statistically significant (P = 0.0054 and P = 0.0047, respectively). **CONCLUSIONS**: hENT1 and Notch3 mRNA expression in EUS-FNA specimens are key predictive biomarkers of GEM effect and GEM sensitivity in patients

with unresectable PDC.

Keywords: EUS-FNA; hENT1; Notch3; unresectable pancreatic cancer.

Abbreviations

PDC, Pancreatic ductal carcinoma

GEM, gemcitabine

EUS-FNA, endoscopic ultrasound-guided fine-needle aspiration

US, abdominal ultrasound

CT, computed tomography

AC, adjuvant chemotherapy

OS, overall survival

hENT1, human equilibrative nucleoside transporter 1

dCK, deoxycitidine kinase

RRM1, ribonucleoside reductase 1

RRM2, ribonucleoside reductase 2

mRNA, messenger ribonucleic acid

qRT-PCR, quantitative real-time reverse transcription-polymerase chain reaction

TTP, time to progression

HRs, hazard ratios

CIs, confidence intervals

UICC, Unio Internationalis Contra Cancrum

EMT, epithelial–mesenchymal transition

TTP, time to progression

Introduction

Pancreatic ductal carcinoma (PDC) is one of the most lethal human cancers. PDCs are usually unresectable (80–90%) at the time of diagnosis, despite recent progress in imaging modalities. Gemcitabine (GEM) has been the standard first-line chemotherapy agent for unresectable PDC (Burris *et al*., 1997). Only 10–20% of patients with PDC are candidates for curative resection (Matsuno *et al*., 2004). Even if curative resection is performed, the postoperative 5-year survival rate is only 15–25% because of a high rate of recurrence (Wagner *et al*., 2004). Recently, two randomised clinical phase III trials of adjuvant chemotherapy (AC) for PDC showed significant increases in overall survival (OS) and disease-free survival (DFS) (Neoptolemos *et al*., 2004; Oettle *et al*., 2007). Therefore, adjuvant chemotherapy is important for patients with PDC. If GEM could be appropriately and selectively administered to patients with GEM sensitivity based on the expression of genes in the tumour, maximal chemotherapy efficacy could be achieved without subjecting GEM-resistant patients to unnecessary side effects.

Recent investigations using cell lines or surgical specimens have revealed that the expression of several genes may be predictors of GEM efficacy in GEM-treatment patients. Such GEM efficacy predictor genes include human equilibrative nucleoside transporter 1 (hENT1), the major mediator of GEM uptake in human cells (Farrell *et al*.,

2009); GEM-metabolism-related enzymes such as deoxycitidine kinase (dCK) (Maréchal *et al*., 2010)]; GEM resistance-related enzymes such as ribonucleoside reductase 1 (RRM1) (Nakahira *et al*., 2007), ribonucleoside reductase 2 (RRM2) (Itoi *et al*., 2007) and Notch3 (Yao *et al*., 2010), which is related to GEM-induced caspase-mediated apoptosis. Ashida *et al*. (2009) and Itoi *et al*. (2007) demonstrated that levels of expression of these genes correlated with GEM sensitivity in patients with unresectable PDC. The aim of this study was to determine a predictive indicator of survival and GEM sensitivity in GEM-treated patients with unresectable PDC by examining gene expression in pre-treatment tissue biopsy samples obtained by endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA).

Methods

Patients

The study included 185 consecutive patients in whom pancreatic masses had been identified by abdominal ultrasound (US) or computed tomography (CT) and who underwent EUS-FNA at Hokkaido Hospital between October 2007 and September 2010. Subjects were excluded if they had an extrapancreatic mass, tumour histology other than ductal adenocarcinoma or preoperative evidence of resectable PDC. Finally, the

analysed population comprised a consecutive series of 71 patients (Figure 1).

EUS-FNA procedure

EUS was performed using an oblique forward-viewing electronic linear scanning video echoendoscope equipped with an elevator and a 3.7-mm-diameter working channel (GF-UCT240-AL5; Olympus Co., Ltd., Tokyo, Japan). The echoendoscope was connected to a processor with a colour Doppler function (SSD-5500; Aloka Co., Ltd., Tokyo, Japan). EUS-FNA was performed before treatment, as described previously (Itoi *et al*., 2005). Briefly, the lesions were identified using B-mode imaging. The absence of vessels in the target area was confirmed with the colour Doppler mode. After determination of an adequate angle to the tumour, an aspiration needle was introduced into the lesion. While suction was applied through the catheter connected to the needle using a 20-mL syringe, the needle was moved back and forth 10–20 times within the tumour. Negative pressure was released before the needle was removed from the lesion. To obtain sufficient tissue for RNA extraction and pathological diagnosis, several biopsy specimens were collected from each tumour by EUS-FNA using 22-gauge aspiration needles (ECHOTIP ULTRA; Cook Japan, Tokyo, Japan). A cytologist immediately examined the specimens for cancer cells using part of

the obtained tissue. We performed an additional one to two punctures after conventional diagnostic puncture to obtain adequate tissue for RNA extraction.

mRNA extraction

Tissue and blood collected from the obtained specimens were examined for confirmation of carcinoma cells by an on-site cytologist. The remaining tissue was instantly transferred to a 1.5-mL micro test tube (Eppendorf, Saxony, Germany) and frozen at -80°C until use. The test tube that was used to inactivate the RNase was rinsed with 0.1 N NaOH/1 mM EDTA and diethylpyrocarbonate (DEPC) and was stored at room temperature. Tissue samples were crushed in a mortar and placed on ice for RNA detection.Total RNA was isolated using the TRIzol Reagent method. Total RNA concentration was determined by spectrophotometer (NanoDrop2000c; Thermo, Tokyo, Japan), and 1 μg total RNA was reverse transcribed using a Transcript First Strand cDNA Synthesis kit (Roche Diagnostics K.K., Tokyo, Japan). Quantification of the target cDNA and an internal reference gene (β2-microglobulin, β2M) was conducted by quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR).

qRT-PCR

We designed specific primers using Primer-BLAST (NCBI, http://www.ncbi.nlm.nih.gov/tools/primer-blast/). The following primers were used for real-time PCR: dCK, forward primer, 5′-TCAAGCCACTCCAGAGACATGCTT-3′; reverse primer, 5′-TGTCCTATGCAGGAGCCAGCTTTCA-3′; hENT1, forward primer, 5′-GGCCCAAGAAAGTGAAGCCA-3′; reverse primer, 5′-ACCACTCAGGATCACCCCTG -3′; RRM1, forward primer, 5′-TCAAGGTGGGAACAAGCGTC-3′; reverse primer, 5′-CGCTGCTCTTCCTTTCCTGT-3′; RRM2, forward primer, 5′-ACGGAGCCGAAAACTAAAGCAGCT-3′; reverse primer, 5′-AGAGTCCACCTCCTCGGCG-3′; Notch3, forward primer, 5′-TCCAGATTCTCATCCGAAACCGCT-3′; reverse primer, 5′-GGGTCTCCTCCTTGCTATCCTGCAT-3′. qRT-PCR was performed using a Rotor-Gene Q (QIAGEN, Hilden, Germany) for 40 cycles at 95°C for 5 s and 60°C for 10 s using a SYBR Green PCR Master Mix (QIAGEN), according to the manufacturer's instructions. Quantification was performed using the relative standard curve method. The standard curve was created automatically by Rotor-Gene Q by plotting the threshold cycle (Ct) against each input amount (containing 107, 106, 105 104, 103, 102, and 101 copies) of standard plasmid DNA. The standard plasmid DNA was created by

direct cloning using a TA cloning vector and the PCR product generated using the specific primers described above and checked by sequencing. The correlation coefficient determined by linear regression (r) for each standard curve was greater than 0.990. The relative amount of each unknown sample was calculated by linear regression analysis from the respective standard curve. A relative target-gene expression value for β2M was used as an internal reference gene.

Target mRNA

Expressions of dCK, hENT1, RRM1, RRM2, and Notch3 were examined as genetic predictive markers associated with GEM transport and metabolism.

Statistical analyses

The primary endpoint was survival in GEM-treated patients with unresectable PDC according to the expression levels of the examined genes. The cut-off for analysis of survival was April 30, 2011. The secondary endpoint was time to progression (TTP) in the patients. Survival and TTP curves were estimated using the Kaplan-Meier technique. Differences between the survival curves and those between TTP curves were assessed using the log-rank test. The Cox proportional hazard regression model was used for

multivariate analyses of survival and for estimating hazard ratios (HRs) with 95% confidence intervals (CIs). The χ^2 test was used to compare proportions. Statistical analyses were performed after dichotomising subgroups as follows: hENT1 low *versus* high, dCK low *versus* high, RRM1 low *versus* high, RRM2 low *versus* high, and Notch3 low *versus* high. The thresholds were determined by the median of the mRNA expression in each of the 71 patients.

A value of $P < 0.05$ was considered to indicate statistical significance. All statistical analyses were performed using JMP ver. 9.0 software (SAS Institute, Gary, NC, USA).

This study was carried out in accordance with the Institutional Review Board guidelines (Hokkaido University Hospital, clinical research approval number 010-0152), and written informed consent was obtained from all patients.

Results

Patient characteristics

The clinical characteristics of the 56 patients who received GEM-based chemotherapy (GEM population) and the 15 who did not (non-GEM population) are shown in Table 1. There was no significant difference between the groups for all clinical characteristics.

mRNA expression

Total RNA was successfully extracted from all specimens from the patients. The mean RNA concentration was 124 ± 85 ng/μL (mean ± SD) (range 13.2–478.8). The mean dCK, hENT1, RRM1, RRM2, and Notch3 mRNA levels relative to the β2M internal reference gene were 63 ± 79 (range 0–546), 590 ± 620 (5–3,178), 576 ± 3,973 (0.3–41,508), 757 ± 2,195 (5–13,286), 242 ± 629 (0–4,490), respectively.

Association between overall survival and mRNA expression levels in patients treated with GEM

Patients with low Notch3 mRNA levels tended to have a better prognosis than those with high Notch3 mRNA level (low *vs*. high = 23.6 *vs*. 19.3 months, $P = 0.068$). However, there were no tendencies and no significant differences in overall survival between patients with low and high mRNA levels of hENT1 (low *vs*. high = 23.6 *vs*. 20 months, $P = 0.302$), dCK (low *vs*. high = 23.6 *vs*. 20 months, $P = 0.930$), RRM1 (low *vs*. high = 27.7 *vs*. 19.3 months, $P = 0.215$), and RRM2 (low *vs*. high = 20 *vs*. 27.7 months, $P = 0.520$) (Figure 2).

Association between time to progression and mRNA expression levels in the GEM-treated population

The patients with high hENT1 (low *vs*. high = 15 *vs*. 21 months, $P = 0.051$) or low Notch3 (low *vs*. high = 31 *vs*. 21 months, $P = 0.051$) mRNA levels had longer TTP than the patients with low hENT1 or high Noch3 mRNA levels. In contrast, there were no differences in TTP between patients with low and high mRNA levels of dCK ($P = 0.162$), RRM1 ($P = 0.200$) and RRM2 ($P = 0.225$) (Figure 3).

Factors associated with overall survival of all patients with unresectable pancreatic cancer

Multivariate analysis for survival of all patients with unresectable PDC based on the Cox proportional hazard model was performed on all parameters described in Table 2. Survival was significantly associated with Notch3 expression levels (HRs, high vs. low = 1.00 vs. 0.0255, $P = 0.0094$) (Table 2). Although a significant difference was not observed, a tendency for the prognosis to be long was seen in patients with high hENT1 expression levels (HRs, high vs. low = 1.00 vs. 0.0333, P = 0.074). Interaction tests for GEM administration and hENT1 or Notch3 mRNA expression levels were statistically significant ($P = 0.0054$ and 0.0047, respectively). Furthermore, we examined predictors

of TTP in all patients. Multivariate analysis for TTP based on the Cox proportional hazard model was also performed on all parameters described in Table 3. A high hENT1 expression level was significantly associated with a long TTP (high vs. low = 1.00 vs. 29.9; $P = 0.039$) (Table 3).

Discussion

In this prospective study, we demonstrated that the expression levels of the hENT1 and Notch3 genes are promising predictive markers for GEM-responsiveness in patients with unresectable PDC. The possibility that Notch3 was a prognostic predictive factor was considered on the basis of the results shown in Table 2. Furthermore, the possibility that hENT1 was a predictive of GEM responsiveness was suggested by the results shown in Table 3. Interaction tests involving these two genes supported the possibility that they are predictive of GEM responsiveness.

hENT1 is a major GEM transporter that is overexpressed in pancreatic cancer cells (Garcia-Manteiga J *et al*., 2003). Expression of hENT1 mRNA in resected specimens from patients with pancreatic cancer is associated with long overall survival, disease-free survival, and time to disease progression (Giovannetti *et al*., 2006; Farrell *et al*., 2009; Raphael *et al*., 2012). Our results of multivariable analysis and interaction

testing are compatible with the findings of previous reports. However, due to the limitation represented by the small non-GEM-treated population, we could not compare Kaplan-Meier curves of the non-GEM population *vs*. those of the GEM-treated population.

Notch3 plays important roles in the control of cell extracellular interactions, such as spreading, migration, motility, and survival in pancreatic cancer cells (Dang *et al*., 2006). The Notch signalling pathway is involved in the acquisition of the epithelial–mesenchymal transition (EMT) phenotype related to invasion of pancreatic cancer cells, and down-regulation of Notch signalling is associated with decreased invasive behaviour of pancreatic cancer cells, followed by partial reversal of the EMT phenotype (Wang *et al*., 2009). In previous reports regarding resected PDC specimens, Notch3 was frequently overexpressed in PDC lesions compared with normal pancreatic ductal tissue (Doucas *et al*., 2008). Meanwhile, nuclear Notch3 expression was clinically correlated with a lower overall survival time of PDC patients (Doucas *et al*., 2008) and also other carcinomas such as ovarian carcinoma (Park *et al*., 2010). Additionally, it was reported that suppression of Notch3 expression decreased the average half maximal inhibitory concentration (IC50) of GEM in pancreatic cell lines (Yao *et al*., 2010). Thus, there may be at least two pathways for the GEM effect:

suppression of Notch3 expression by GEM, and amplification of the GEM effect through the suppression of Notch3.

To-date, there has been no report of the relationship between the effectiveness of GEM and Notch3 mRNA expression in the clinical course of PDC. For the first time, our results using pre-treatment EUS-FNA specimens suggest that Notch3 is a promising informative biomarker for predicting the effectiveness of GEM and GEM sensitivity in patients with unresectable PDC. Using Kaplan-Meier analysis of OS and TTP and multivariate analysis for OS, our expression data and clinical results might be used to address the results of patients with low Notch3 mRNA levels compared to patients with high levels. However, further study in more patients is needed to confirm this correlation.

EUS-FNA is widely used as a cytological and histological sample collection tool for pancreatic cancer (Khalid *et al*., 2006; Takahashi *et al*., 2005), and there have been some reports of oncogene analysis in pancreatic cancer using EUS-FNA samples (Ashida *et al*., 2009; Khalid *et al*., 2006; Tada *et al*., 2002; Buchholz *et al*., 2005). The reliability of tests based on tissue or cell extracts is often dependent on the relative abundance of the target cell population. Moreover, sampling errors or a large number of "contaminating cells" can lead to false-negative results (Fujita *et al*., 2008). This is

likely why Fujita *et al*. were unable to detect significant differences in mRNA levels among whole-cell pellet samples; however, they could distinguish higher and lower expression of each gene among neoplastic cell samples by microdissection (Fujita *et al*., 2010). In the present study, we could distinguish higher and lower expression of genes by selection of white tissue on site, although we used total RNA isolated from EUS-FNA tissue samples without microdissection. It may be important to trim white tissue for high-quality RNA analysis, although there is no evidence to support this. In addition, some reports suggest that flow cytometry and cytogenetic analysis or improvement of diagnostic accuracy using specimens of EUS-FNA could be achieved using a thick 19-G needle instead of a 21- or 25-G needle puncture (Yasuda *et al*., 2011). Therefore, further development of methods for sample collection and processing are required to improve wide genetic analysis of EUS-FNA specimens.

If GEM sensitivity could be predicted using specimens collected by EUS-FNA at the same time as pathological diagnosis, the appropriate anticancer agents such as oxaliplatin, irinotecan, fluorouracil, and leucovorin (FOLFIRINOX) could be selected (Conroy *et al*., 2011). Furthermore, based on the results of RNA analyses of EUS-FNA specimens, the appropriate preoperative neoadjuvant chemotherapy could be administered.

There are several limitations to our study. One is that the sample size and observation period were not sufficient. Another limitation is that this was a retrospective and single centre study. To address these issues, a large scale, multicentre prospective study is needed.

In conclusion, based on genetic analysis of EUS-FNA tissue samples, our data suggest that hENT1 and Notch3 mRNA expression levels are promising novel and informative biomarkers for predicting and monitoring Gem sensitivity in patients with unresectable PDC.

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TABLES

Table 1. Clinical characteristics of patients.

	GEM population (n = 56)	Non-GEM population (n = 15)	P value $\chi 2$ test
Median age in years (range)	69 (37-88)	68 (49-84)	
< 69 years	24 (43%)	8 (53%)	0.023
> 68 years	32 (57%)	7 (47%)	
Sex			
Male	26 (46.4%)	9 (60%)	0.349
Female	30 (53.6%)	6 (40%)	
Location			
Ph	24 (42.9%)	6 (40%)	0.842
Pb and Pt	32 (57.1%)	9 (60%)	
UICC TNM 7^{th} f-Stage			
Stage III	17 (30.4%)	3 (20%)	0.346
Stage IV	39 (69.6%)	12 (80%)	
Performance status			
0	49 (87.5%)	11 (73.3%)	0.201
1-3	7 (12.5%)	4 (26.7%)	
Comorbidities			
Some	39 (69.6%)	11 (73.3%)	0.779
None	17 (30.4%)	4 (26.7%)	
GEM efficacy			
CR	0 (0%)		
PR	1 (1.8%)		
SD	34 (60.7%)		
PD	21 (37.5%)		
No. of chemotherapy cycles, median (range)	4 (1-31)		

GEM, gemcitabine; Ph, head of the pancreas; Pb, body of the pancreas; Pt, tail of the pancreas; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

Table 2. Multivariate analysis of survival in patients with unresectable PDC.

Patient stratification	Multivariate analysis (survival)			
	n	HR	95% CI	*P* value
Age (years) < 69 / ≥ 69	32 / 39	1 / 3.83	0.44-68.0	0.229
Sex Female / Male	36 / 35	1 / 0.154	0.011-1.12	0.06
Location Ph / Pb & Pt	30 / 41	1 / 7.31	0.29-728	0.244
UICC TNM 7th f-Stage III / IV	21 / 50	1 / 0.0384	0.000347-0.734	0.0275
Performance status 0 / 1-3	65 / 11	1 / 0.000127	0-117429839	0.995
Comorbidities Some / None	50 / 21	1 / 0.0557	0.0007-0.682	0.020
GEM-treated Yes / No	56 / 15	1/ 0.209	0.00204-8.75	0.419
hENT1 High / Low	33 / 38	1 / 29.9	0.730-2918	0.074
dCK High / Low	38 / 33	1 / 4.098	0.371-61.8	0.236
RRM1 High / Low	37 / 34	1 / 0.515	0.0174-20.1	0.702
RRM2 High / Low	39 / 32	1 / 1.64	0.117-56.0	0.733
Notch3 High / Low	43 / 28	1 / 0.0255	0.0000483-0.503	0.0094

PDC, pancreatic ductal carcinoma; N, number; HR, hazard ratio; CI, confidence interval; Ph, head of the pancreas; Pb, body of the pancreas; Pt, tail of the pancreas; GEM, gemcitabine; hENT1, human equilibrative nucleoside transporter 1; dCK, deoxycitidine kinase; RRM1, ribonucleoside reductase 1; RRM2, ribonucleoside reductase 2

Table 3. Multivariate analysis of progression of disease in the GEM population.

Patient stratification	Multivariate analysis (progression)			
	n	HR	95% CI	*P* value
Age (years) < 69 / ≥ 69	29 / 27	1 / 1.42	0.0427-54.1	0.836
Sex Female / Male	30 / 26	1 / 0.396	0.0121-5.62	0.501
Location Ph / Pb & Pt	24 / 32	1 / 1.35	0.0137-3848	0.908
UICC TNM 7th f-Stage III / IV	18 / 38	1 / 0.687	0.000491-50.7	0.873
Performance status 0 / 1-3	49 / 7	1 / 54.7	0.777-14312	0.064
Comorbidities Some / None	39 / 17	1 / 0.281	0.00541-3.94	0.374
TMs decline < 50% / ≥ 50%	32 / 24	1 / 0.906	0.0558-13.0	0.939
hENT1 High / Low	30 / 26	1 / 29.9	1.13-8605	0.039
dCK High / Low	30 / 26	1 / 0.199	0.00503-2.70	0.238
RRM1 High / Low	32 / 24	1 / 0.180	0.00187-3.53	0.278
RRM2 High / Low	35 / 21	1 / 4.63	0.155-641	0.393
Notch3 High / Low	38 / 18	1 / 0.233	0.000862-8.00	0.460

GEM, gemcitabine; N, number; HR, hazard ratio; CI, confidence interval; Ph, head of the pancreas; Pb, body of the pancreas; Pt, tail of the pancreas; TMs, tumour markers; hENT1, human equilibrative nucleoside transporter 1; dCK, deoxycitidine kinase; RRM1, ribonucleoside reductase 1; RRM2, ribonucleoside reductase 2.

FIGURE LEGENDS

Figure 1.

Flow diagram of the study participants.

Figure 2.

Kaplan-Meier curves of survival in the gemcitabine (GEM)-treated population according to each mRNA expression level.

Each mRNA expression level was assigned to high or low using the median as a threshold.

Figure 3.

Kaplan-Meier curves of progression-free survival in the GEM-treated population according to each mRNA expression level. Each mRNA expression level was assigned to high or low using the median as a threshold.

Figure 1.

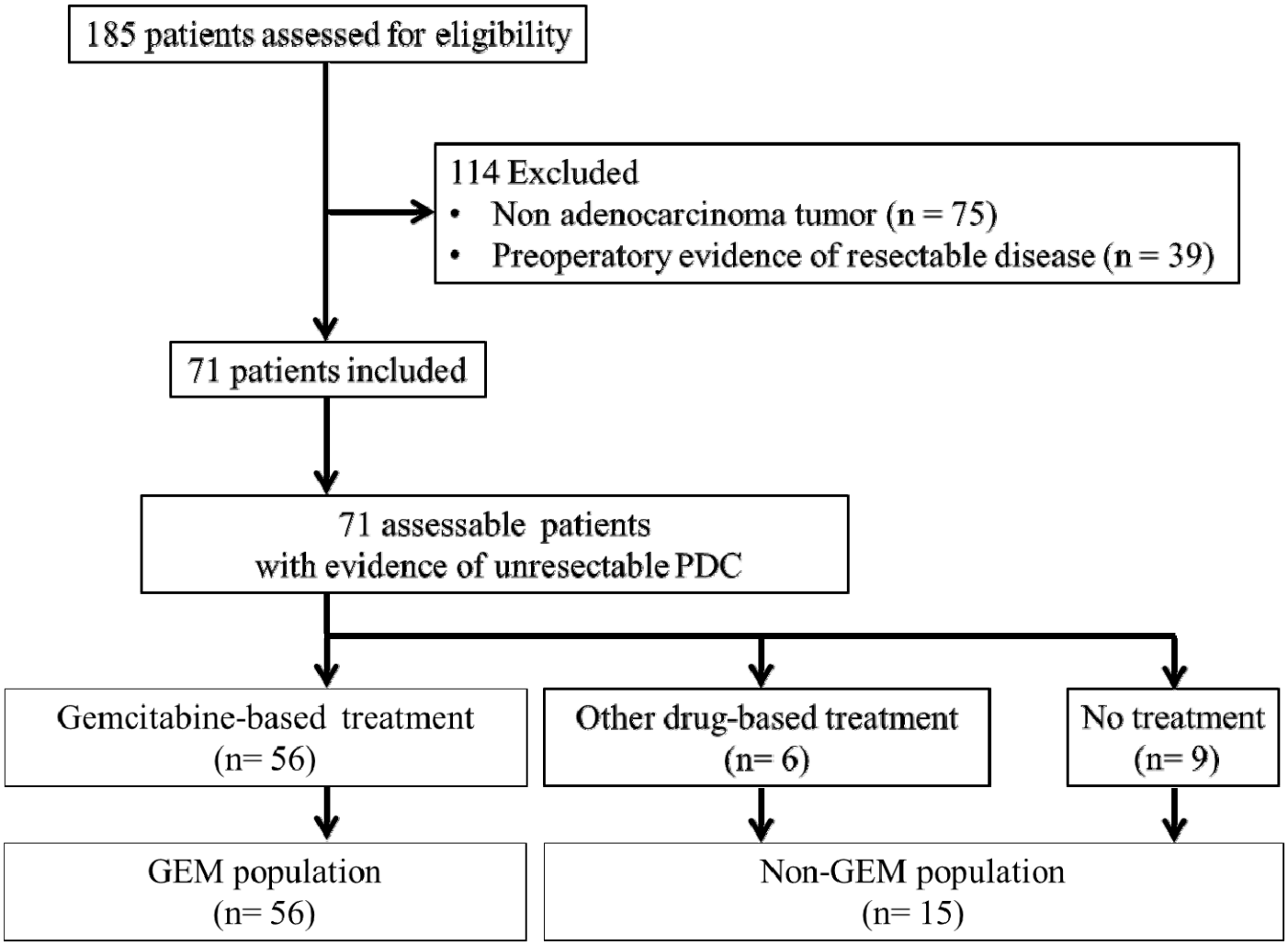
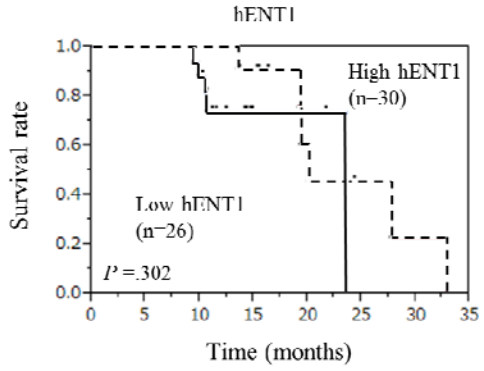
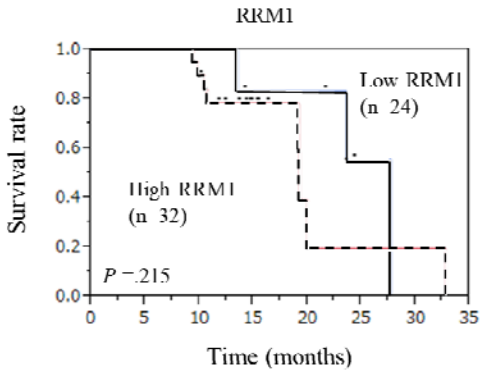
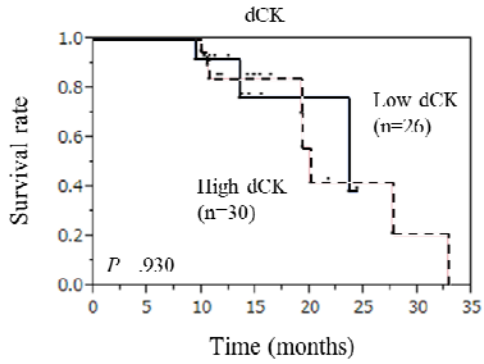
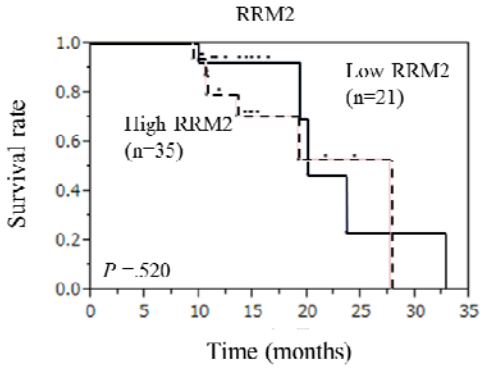

185 patients assessed for eligibility
114 Excluded
• Non adenocarcinoma tumor (n = 75)
• Preoperatory evidence of resectable disease (n = 39)
71 patients included
71 assessable patients
with evidence of unresectable PDC
Gemcitabine-based treatment
(n= 56)
Other drug-based treatment
(n= 6)
No treatment
(n= 9)
GEM population
(n= 56)
Non-GEM population
(n= 15)

Figure 2.


hENT1
High hENT1
(n=30)
Low hENT1
(n=26)
P =.302
Survival rate
Time (months)


RRM1
Low RRM1
(n 24)
High RRM1
(n 32)
P =.215
Survival rate
Time (months)


dCK
Low dCK
(n=26)
High dCK
(n=30)
P .930
Survival rate
Time (months)


RRM2
Low RRM2
(n=21)
High RRM2
(n=35)
P =.520
Survival rate
Time (months)

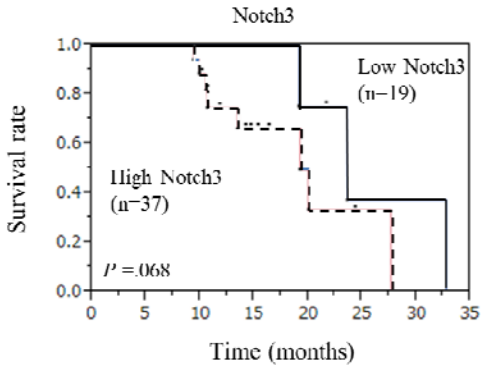
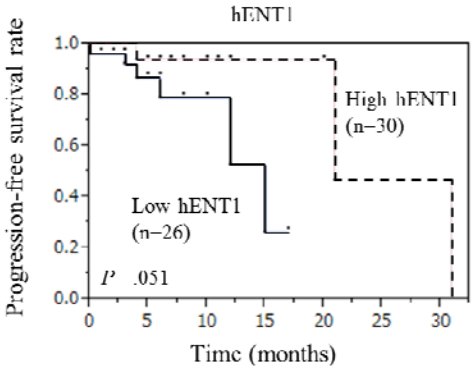
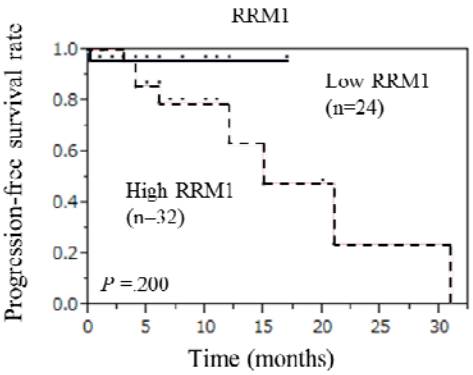
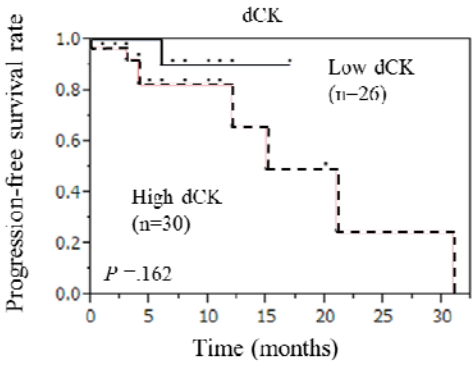
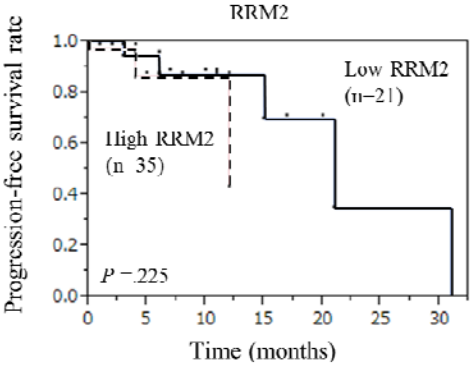

Notch3
Low Notch3
(n=19)
High Notch3
(n=37)
P =.068
Survival rate
Time (months)

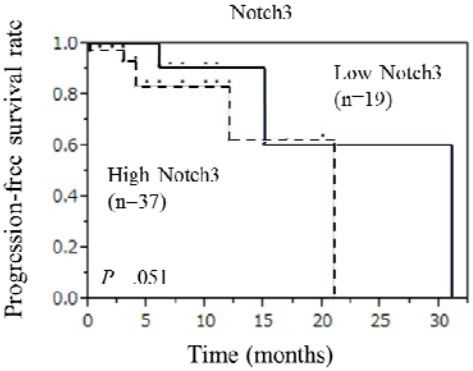
Figure 3.


hENT1
Progression-free survival rate
High hENT1
(n=30)
Low hENT1
(n=26)
P .051
Time (months)


RRM1
Progression-free survival rate
Low RRM1
(n=24)
High RRM1
(n=32)
P =.200
Time (months)


dCK
Progression-free survival rate
Low dCK
(n=26)
High dCK
(n=30)
P =.162
Time (months)


RRM2
Progression-free survival rate
Low RRM2
(n=21)
High RRM2
(n 35)
P =.225
Time (months)


Notch3
Progression-free survival rate
Low Notch3
(n=19)
High Notch3
(n=37)
P .051
Time (months)