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dCK Expression and Gene Polymorphism With Gemcitabine Chemosensitivity in Patients With Pancreatic Ductal Adenocarcinoma

A Strobe-Compliant Observational Study

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Abstract: The aim of this study was to investigate the relationship of deoxycytidine kinase (dCK) protein expression and gene single-nucleotide polymorphisms to gemcitabine chemosensitivity in patients with pancreatic ductal adenocarcinoma (PDAC).

In total, 54 patients with resectable PDAC, who received postoperative gemcitabine-based therapy, were enrolled in this study, from January 2011 to April 2013. The dCK protein expression was measured retrospectively by immunohistochemistry. Furthermore, 5 single-nucleotide polymorphisms (C1205T, A9846G, A70G, C356G, and C364T) of the dCK gene were detected in PDAC cells by PCR amplification and sequencing.

The dCK protein expression was found to be negatively correlated with age ($P = 0.006$), but correlated positively with overall survival (OS) ($P = 0.000$) and disease-free survival (DFS) ($P = 0.003$). The A9846G AA genotype in the dCK gene was significantly associated with reduced mortality compared with AG and GG genotypes. The OS and DFS were longer in patients with the A9846G AA genotype than the AG and GG genotypes. In univariate and multivariate analyses, we found that the dCK protein expression and A9846G genotype were significant predictors of both OS and DFS.

Our study suggests that the dCK protein expression and A9846G genotype may act as prognostic biomarkers in identifying patients who are likely to benefit from postoperative gemcitabine therapy in PDAC.

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Abbreviations: dCK = deoxycytidine kinase, DFS = disease-free survival, OS = overall survival, PBS = phosphate-buffered saline, PDAC = pancreatic ductal adenocarcinoma, SNPs = single-nucleotide polymorphisms.

INTRODUCTION

Management of pancreatic ductal adenocarcinoma (PDAC) continues to be a diagnostic and therapeutic challenge. At diagnosis, only 20% patients have resectable tumor. However, 30% patients have a locally advanced tumor and 50% present with distant metastasis.[1] Currently, it has become the fourth cause of cancer death in the western countries.[2] Previously, the 5-year overall survival (OS) rate in patients with this disease was estimated to be approximately 5%.[3] With radical resection, it was still thought to be less than 20%.[4] Postoperative adjuvant chemotherapy, based on 2′,2′-difluoro 2′-deoxycytidine (gemcitabine), has been shown to prolong the OS in these patients.[5,6] However, the variation in clinical response as a result of gemcitabine chemoresistance has paved way for argument for the importance of individualized chemotherapy in patients with pancreatic cancer.[7]

Deoxycytidine kinase (dCK) plays an important role in the process of gemcitabine activation and is regarded as a rate-limiting kinase in gemcitabine metabolization.[8] Some studies have suggested that the expression of dCK gene and protein is closely associated with the gemcitabine chemosensitivity in patients with pancreatic cancer.[9–11] Furthermore, studies have also found single-nucleotide polymorphisms (SNPs) in the dCK gene to be closely associated with the gemcitabine chemosensitivity in these patients.[12,13]

However, in these studies, the gemcitabine was either given as a combination therapy with another chemotherapeutic agent or in combination with radiotherapy, and hence, efficacy of gemcitabine on its own was not evaluated. In this study, we investigated the dCK protein expression by immunohistochemistry in patients with resected PDAC who underwent postoperative gemcitabine chemotherapy alone. In addition, 5 SNPs of the dCK gene were also investigated. The relationship of dCK protein expression and SNPs to clinicopathological factors and outcomes was analyzed.

METHODS

Patients and Samples

Pancreatic tissues were collected retrospectively from patients with PDAC who had undergone radical (R0 resection)

pancreaticoduodenectomy or distal pancreatectomy with definite pathological diagnosis at West China Hospital of Sichuan University between January 2011 and April 2013. Patients who had neoadjuvant chemo or radiotherapy or postoperative radiochemotherapy, who were lost to follow-up or died in the perioperative period, were excluded from this study. In total, 54 patients, who completed postoperative gemcitabine treatment, were included. This was started within 8 weeks postresection. Gemcitabine (intravenous infusion of 1000 mg/m^2) was given on days 1, 8, and 15 of each cycle, to be repeated every 4 weeks. This lasted for 6 months. All procedures, involving human participants, were in accordance with the ethical standards of the institutional and national research committee, and with the 1964 Helsinki declaration and its later amendments. Written informed consent was obtained from these patients before their inclusion in the project. The study was approved by the institutional review board of West China Hospital of Sichuan University.

Data Collection and Follow-up

Clinicopathological and treatment data were obtained for each patient from the medical records in West China Hospital, Sichuan University. Patients were followed up till the end of December 2013, either in the form of telephonic conversation or as outpatient clinic appointment. The median follow-up time was 21 months (8–36 months). The OS was calculated from the date of curative-intent radical resection to the date of death or last follow-up. Furthermore, the disease-free survival (DFS) time was calculated from the date of curative-intent radical resection to the date of first recurrence or last follow-up.

Immunohistochemical Staining Method

The formalin-fixed paraffin-embedded tissue samples of the tumor, cut into sections of 4 μm, were mounted on salinized slides. The sections were deparaffinized in xylene and were subsequently rehydrated through a graded series of ethanol/water additions. Antigen retrieval was accomplished using pH 6.0 sodium citrate buffer (0.01 M) and microwave heating for 10 minutes at 95°C. After cooling, the sections were incubated with a primary antibody at 4°C overnight with a 1:300 dilution (polyclonal rabbit anti-dCK; Gentex, CA). The Power Vision 6000 immunohistochemistry detection reagent (ZSJQ Biotechnology, Beijing, China) was used as a second antibody by incubating for 1 hour at 37°C, and 3,3′-diaminobenzidine was used as a chromogen. Hematoxylin was used as a nuclear counterstaining agent. A negative control was used in each run in which the antibody was replaced by an equal volume of phosphate-buffered saline (PBS).

TABLE 1. SNPs Evaluated in This Study

Location	Variant Nueleotide	Sequence Change	SNP ID
Intron 2	C1205T	GAAA (C/T)ATGA	rs4694362
Intron 2	A9846G	GCCT (A/G)TAGT	rs12648166
Exon 3	A70G	GAAA (A/G)TCTC	rs66878317
Exon 3	C356G	GATG (C/G)AGAG	rs66472932
Exon 3	C364T	GAAA (C/T)CTGT	rs67437265

SNP = single-nucleotide polymorphism.

Evaluation of dCK Staining

The positive expression of dCK protein, represented as light yellow or brown yellow staining, is expressed in pancreatic cancer cells, acinar cell, islet cells, and lymphocytes. The positive signal is mainly in the cytoplasm and partially visible in the nucleus. Cytoplasmic and nuclear stainings were scored separately. The dCK protein expression was evaluated using the cytoplasm in PDAC cells. The quantitative scoring using light microscopy was performed by 3 independent pathologists who were blinded to the clinicopathological and follow-up data. The dCK protein staining was scored on a scale of 0 to 2 (no staining = 0, weakly positive staining = 1, strong positive staining = 2). The percentage of PDAC cells stained at each intensity level was recorded for each specimen. A final score was determined by multiplying the intensity score and the percentage of the positive cells in the specimen, which was in the range of 0 to 200.[14]

Detection SNPs of dCK

According to the National Center for Biotechnology Information (NCBI), 5 SNPs in the dCK gene which were closely associated with dCK protein activity or expression or gemcitabine chemosensitivity[12,13] were chosen. The location of these SNPs in the dCK gene is shown in Table 1.

PCR Amplification and Sequencing of dCK

Paraffin PDAC specimens were cut into 10 to 20-micron thick slices. Then, the genomic DNA was extracted from tissues using a DNeasy kit (OMEGA). All primer sequences were

TABLE 2. Primer Sequences for Amplifying the dCK Gene

Location		Primer Sequences	Amplicon Size
C-1205T	Forward	5′-CATTATGGTCAGTAGGCTAGGTTC-3′	327 bp
	Reverse	5′-TCAAGCAGTCTTCCCACCTC-3′	
A9846G	Forward	5′-TGGCTCAGGTGATCCTCTCT-3′	403 bp
	Reverse	5′-ACAGGCATCATAAGCATTCC-3′	
A70G	Forward	5′-GCAGGTCAGGATCTGGCTTA-3′	290 bp
	Reverse	5′-CGAGTTGCTGCAGAGAGATG-3′	
C356G	Forward	5′-CTCAGAAAAATGGTGGGAATG-3′	216 bp
	Reverse	5′-CTTCAAATGGCCACGTACAA-3′	
C364T	Forward	5′-TGAGAAACCTGAACGATGGTC-3′	256 bp
	Reverse	5′-CCCAGCTCAGCCATTCATTA-3′	

dCK = deoxycytidine kinase.

designed by the Beijing GENEWIZ Company (Table 2). The PCR amplification and sequencing of dCK were undertaken using single primers. The BECKMAN-DU 640 uv/visible spectrophotometer (Beckman) was used to assess quality of the extracted DNA. Usually, an OD260/280 ratio of 1.7 to 1.9 is considered satisfactory. Thermocycling was performed as follows: 1 cycle of denaturation at 94°C for 3 minutes; 30 cycles of 30 seconds at 94°C, 30 seconds at 55°C, and 45 seconds at 72°C; and a final extension step of 6 minutes at 72°C. The PCR-amplified products were purified using QIA quick columns (Qiagen, Germany) and automated sequencing was done using nested primers and ABI 3730 XL (Applied Biosysterns). Sequence analysis was performed using sequencher version 4.0.5 software (Gene Codes, Ann Arbor, MI). The primers were synthesized by Beijing GENEWIZ Company.

Statistical Analysis

The data are represented as the number (n) and percentage (%) or mean ± standard deviation. The genotype distribution was tested for Hardy–Weinberg equilibrium using the goodness-of-fit chi-square test. The correlation between dCK protein expression, clinicopathological factors, and genotype was tested by chi-square test or Fisher exact test. The Kaplan–Meier curves were used to plot DFS and OS, and the log-rank test was used to compare them. A Cox proportional-hazards multivariate model was used to corroborate the association between clinicopathologic factors and dCK expression or dCK SNPs related to the efficacy of adjuvant chemotherapy in terms of DFS and OS. Multivariate analyses used a step-down procedure based on the likelihood ratio test. The variables of statistical significance during univariate analysis were included in a follow-up multivariate analysis. Data were analyzed using the SPSS software package (version 17.0; SPSS, Inc., Chicago, IL). Statistical significance was prespecified at $P < 0.05$.

RESULTS

dCK Immunostaining

Fifty out of 54 (92.6%) resected PDAC samples had the positive dCK protein expression. The dCK protein expression was mainly localized in the cytoplasm, and partially visible in the nucleus. Nuclear staining was observed in 26 of 54 samples (48.1%) and was restricted exclusively to carcinoma cells that exhibited high cytoplasmic intensity staining. In addition, dCK protein expression was found in the acinar cells, islet cells, and lymphocytes. We only considered cytoplasmic immunostaining for statistical analysis. We dichotomized dCK cytoplasmic staining basing on the median staining score according to literature.[14] By using this criterion and a cut-off score of 130, patients were divided into 2 groups: low dCK expression, with a staining score <130 (Figure 1A), and high dCK expression, with a staining score ≥130 (Figure 1B).

Correlation Between dCK Expression and Clinicopathologic Factors

In total, there were 39 males and 15 females. The median age of these patients was 58 years (range 24–79years). Among these tumors, 4 were found to be highly differentiated, whereas 35 cases presented with moderate and 15 with poor differentiation. Lymph node metastasis was found in 26 patients. In addition, there were 9, 38, and 7 patients in stage I, II, and III, respectively. The correlation between dCK expression and clinicopathologic features is shown in Table 3. Based on the observed staining, we noticed that low dCK expression was negatively associated with age ($P = 0.006$). However, no significant correlation was found between the dCK protein expression and other factors, such as sex, tumor location, tumor size, carbohydrate antigen 19–9 (CA19–9) level, pathologic stage, T stage, Tumor Node Metastasis stage, and lymph node metastasis ($P > 0.05$).

Correlation Between dCK Expression and Patient Outcomes

Overall, the median OS and DFS were 16 months (range 8–35 months) and 14 months, respectively. At the last follow-up, 34 patients had died as result of disease recurrence, whereas 20 were still alive. A significant difference in OS (27 months in high and 14 months in low-expression group; $P = 0.000$) (Figure 2A) and DFS (21 months in high and 11 months in low-expression group; $P = 0.003$) (Figure 2B) was noted between high and low dCK expression groups.

dCK Single-Nucleotide Polymorphisms

The 5′-untranslated region and some coding region of dCK were sequenced in all 54 samples from patients with PDAC. In total, 5 SNPs in the dCK gene (C1205T, A9846G, A70G, C356G, and C364T) were investigated. The genotype variation of the A70G, C356G, and C364T SNPs in the dCK gene was not found. However, in C1205T SNP, 3 genotypes, including the wild genotype CC, hybrid genotype CT, and mutant genotype TT, were found. Additionally, the wild genotype AA, hybrid genotype AG, and mutant genotype GG were found in the A9846G SNP (Figure 3).

Genotype Distribution and Hardy–Weinberg Balance Test

In C1205T SNP, gene frequencies in CC, CT, and TT were 14.8%, 37.0%, and 48.1%, respectively. In addition, C and

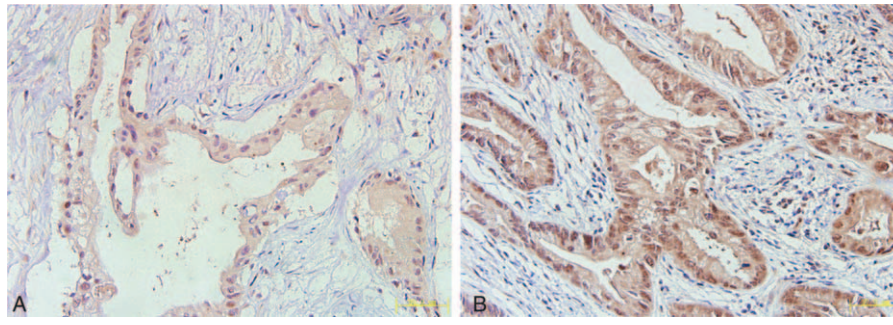

FIGURE 1. Immunohistochemical staining of dCK protein expression. A, Low dCK expression (×400); B, high dCK expression (×400). dCK = deoxycytidine kinase.

TABLE 3. Correlation Between dCK Protein Expression and Clinicopathological Factors

Variables		n	High Expression, n (%)	Low Expression, n (%)	*P*
Age	>60	24	7 (29.2%)	17 (70.8%)	0.006
	≤60	30	20 (66.7%)	10 (33.3%)	
Sex	Male	39	19 (48.7%)	20 (51.3%)	0.761
	Female	15	8 (53.3%)	7 (46.7%)	
Tumor location	Head	41	21 (51.2%)	20 (48.8%)	0.705
	Body and tail	13	6 (46.2%)	7 (53.8%)	
Tumor size	≥2.5 cm	41	20 (48.8%)	21 (51.2%)	0.750
	<2.5 cm	13	7 (53.8%)	6 (46.2%)	
CA19–9	≥1000 U/mL	36	19 (52.8%)	17 (47.2%)	0.564
	<1000 U/mL	18	8 (44.4%)	10 (54.6%)	
Differentiation	Low	15	7 (46.7%)	8 (53.3%)	0.761
	High and moderate	39	20 (51.3%)	19 (48.7%)	
T stage	T1–T2	11	6 (54.5%)	5 (45.5%)	0.841
	T3–T4	43	21 (48.8%)	22 (51.2%)	
TNM stage	I–II	47	24 (51.1%)	23 (48.9%)	0.685
	III–IV	7	3 (42.9%)	4 (57.1%)	
Lymph node metastasis	Yes	26	13 (50%)	13 (50%)	1.000
	No	28	14 (50%)	14 (50%)	

P value when expression levels were compared using the chi-square test or Fisher exact test.
CA19–9 = carbohydrate antigen 19–9, dCK = deoxycytidine kinase, TNM = tumor, node, and metastasis.

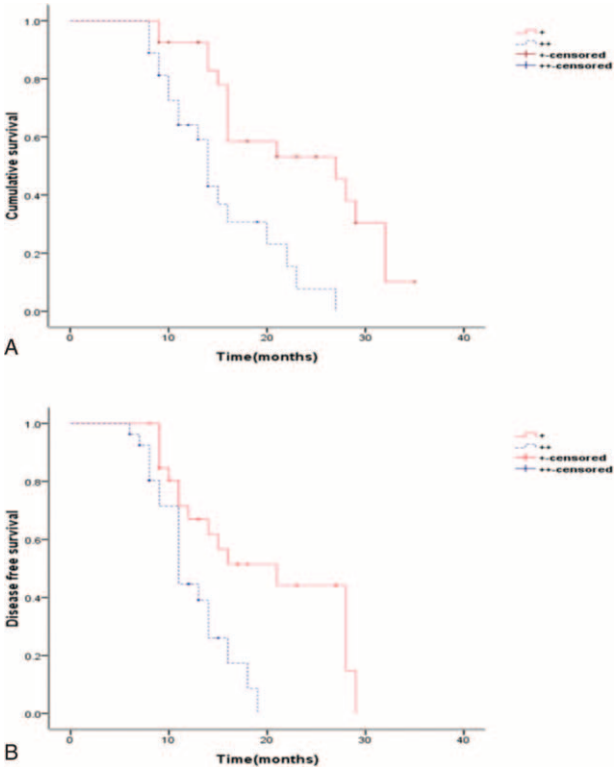

FIGURE 2. dCK expression and patients survival. A, Overall survival in patients with high and low expression of dCK protein (+ high expression, ++ low expression). B, Disease-free survival in patients with high and low expression of dCK protein (+ high expression, ++ low expression). dCK = deoxycytidine kinase.

T-allele frequencies were 33.3% and 66.7%, respectively. The test conformed the genetic balance according to the Hardy–Weinberg equilibrium ($\chi^2 = 1.5$, $P = 0.22$). As for the A9846G SNP, gene frequencies in AA, AG, and GG were 16.7%, 51.9%, and 31.5%, respectively. The A and G-allele frequencies were found to be 42.6% and 57.4%, respectively. The test also confirmed the genetic balance according to the Hardy–Weinberg equilibrium ($\chi^2 = 0.196$, $P = 0.66$). Therefore, samples had good representation and reached genetic equilibrium.

Correlation Between Genotype and Clinicopathologic Factors

In C1205T and A9846 SNPs, the genotype AA and CC were less frequent. Therefore, these were merged into 1 group. Similarly, AA and AG genotypes were merged into 1 group for ease of analysis. No statistically significant correlation was found between C1205T, A9846G genotypes, and clinical pathological factors ($P > 0.05$; Table 4).

Correlation Between C1205T/A9846G Genotype and dCK Expression

As is shown in Table 5, no difference in dCK expression was elucidated among different genotypes for C1205T site ($\chi^2 = 2.685$, $P = 0.261$). However, for A9846G site, statistically significant difference in dCK expression was noted among different genotypes ($\chi^2 = 12.025$, $P = 0.002$). The tendency was G/G<A/A + A/G or G/G<A/G<A/A.

Correlation Between Genotype and Patient Outcome

The C1205T haplotype showed no significant association with OS time (CC vs CT, $P = 0.200$; CT vs TT, $P = 0.510$; CC vs TT, $P = 0.096$; CC/CT vsTT, $P = 0.166$; Figure 4A and B).

FIGURE 3. Different genotypes in C1205T and A9846G SNPs of the dCK gene. A, Genotype 1205CC; B, genotype 1205CT; C, genotype 1205TT; D, genotype 9846AA; E, genotype 9846AG; F, genotype 9846GG. dCK = deoxycytidine kinase.

The median OS time was 32, 16, 15, and 6 months in patients with CC, CT, TT, and CC/CT genotype, respectively. Moreover, no significant association was noted between C1205T genotype and DFS (CC vs CT, $P = 0.345$; CT vs TT, $P = 0.283$; CC vs TT, $P = 0.654$; CC/CT vs TT, $P = 0.358$) (Figure 4C and D). The median DFS was 21, 16, 13, and 16 months in patients with CC, CT, TT, and CC/CT genotype, respectively. However, A9846G genotype significantly affected the OS (AA vs AG, $P = 0.020$; AG vs GG, $P = 0.030$; AA vs GG, $P = 0.002$; AA/AG vs GG, $P = 0.002$) (Figure 5A and B). The median OS time was 32, 16, 14, and 22 months in patients with AA, AG, GG, and AA/AG genotype, respectively. But it failed to show any association with DFS ($P = 0.024$; Figure 5C and D). The median DFS time was 21, 12, 13, and 16 months in patients with AA, AG, GG, and AA/AG genotype, respectively.

Univariate and Multivariate Analysis for OS and DFS

The results of univariate and multivariate analysis are summarized in Table 6. In univariate analysis, the OS was significantly associated with dCK protein expression ($P = 0.017$) and A9846G genotype ($P = 0.025$). DFS was also significantly associated with dCK protein expression ($P = 0.042$) and A9846G genotype ($P = 0.001$). A multivariate model was used to identify independent prognostic factors. The model included all histopathologic variables that had significant prognostic value in univariate analysis. For the OS, this analysis revealed that dCK protein expression ($P = 0.001$) and A9846G genotype ($P = 0.009$) were significant prognostic factors for patients with PDAC. As for the DFS, results pointed at dCK protein expression ($P = 0.006$)

TABLE 4. Correlation Between C1205T/A9846G Genotype and Clinicopathological Factors

Variables		n	C1205T		P	A9846G		P
			CC + CT (28)	TT (26)		AA + AG (37)	GG (17)	
Age	>60	24	11 (45.8%)	13 (54.2%)	0.429	14 (58.3%)	10 (41.7%)	0.149
	≤60	30	17 (56.7%)	13 (43.3%)		23 (76.7%)	7 (23.3%)	
Sex	Male	39	20 (51.3%)	19 (48.7%)	0.893	26 (66.7%)	13 (33.3%)	0.884
	Female	15	8 (53.3%)	7 (46.7%)		11 (73.3%)	4 (26.7%)	
Tumor location	Head	41	21 (51.2%)	20 (48.8%)	0.869	29 (70.7%)	12 (29.3%)	0.534
	Body and tail	13	7 (53.8%)	6 (46.2%)		8 (61.5%)	5 (38.5%)	
Tumor size	≥2.5 cm	41	22 (53.7%)	19 (46.3%)	0.637	27 (65.9%)	14 (34.1%)	0.685
	<2.5 cm	13	6 (46.2%)	7 (53.8%)		10 (76.9%)	3 (23.1%)	
CA19–9	≥1000 U/mL	36	18 (50%)	18 (50%)	0.700	25 (69.4%)	11 (30.6%)	0.836
	<1000 U/mL	18	10 (55.6%)	8 (44.4%)		12 (66.7%)	6 (33.3%)	
Differentiation	Low	15	8 (53.3%)	7 (46.7%)	0.893	10 (66.7%)	5 (33.3%)	0.856
	High and moderate	39	20 (51.3%)	19 (48.7%)		27 (69.2%)	12 (30.8%)	
T stage	T1–T2	11	6 (54.5%)	5 (45.5%)	0.841	7 (63.6%)	4 (36.4%)	0.979
	T3–T4	43	22 (51.2%)	21 (48.8%)		30 (69.8%)	13 (30.2%)	
TNM stage	I–II	47	25 (53.2%)	22 (46.8%)	0.916	32 (68.1%)	15 (31.9%)	1.000
	III–IV	7	3 (42.9%)	4 (57.1%)		5 (71.4%)	2 (28.6%)	
Lymph node metastasis	Yes	26	14 (53.8%)	12 (46.2%)	0.777	17 (65.4%)	9 (34.6%)	0.633
	No	28	14 (50%)	14 (50%)		20 (71.4%)	8 (28.6%)	

P value when expression levels were compared using the χ^2 test or Fisher exact test.
CA19–9 = carbohydrate antigen 19–9, TNM = tumor node metastasis.

and A9846G genotype ($P = 0.0001$) to be the main prognostic factors.

DISCUSSION

It is very well established in literature that the expression level of various metabolic enzymes in the process of gemcitabine metabolism affects its chemosensitivity. dCK is an important rate-limiting kinase in the process of metabolism of gemcitabine, which can phosphorylate it into an active product in the cell. Some studies have found the elevated expression level of dCK gene and protein to be associated with increased chemosensitivity of gemcitabine in various malignant tumors.[9,15–17] In this study, we used immunohistochemical staining to investigate dCK protein expression in PDAC tissues, demonstrating a positive expression rate of dCK protein of 92.6%. However, this has been found to be rather variable in different malignancies. Hubeek et al,[18] while demonstrating a higher dCK protein expression in childhood acute myelogenous leukemia, found a lower expression in brain tumors. Van der Wilt et al[19] investigated the expression of dCK gene and protein in 7 tumor cell lines with different histological origin. They found a statistically significant difference in dCK protein expression in all these tumor cell lines. Sebastiani et al[10] investigated dCK protein expression in 44 patients with primary or metastatic infiltrating PDAC and found that positive expression rate was in the order of 91%. Our results are in line with these previous findings and further confirm that PDAC is a tumor with high dCK protein expression.

Location of dCK protein has also been a matter of great debate. Johansson et al[20] and Sebastiani et al[10] inferred, through their work, that dCK protein expression was only evident in the nucleus. However, other studies[11,21] hypothesized that dCK was usually located in the cell cytoplasm and was noted to be in the nucleus during phases of higher expression. This has also been scientifically confirmed, through various experimental processes, such as immunoblotting of cellular fractions, enzymatic activity studies, and kinetic isotope incorporation experiments.[22–25] Our study also concurs that dCK is a unique cytoplasmic enzyme in terms of its expression and location.

Although other studies have linked dCK expression to sex and tumor differentiation,[12,13] we did not find dCK expression to be associated with any clinicopathological features, other than age.

TABLE 5. Correlation Between C1205T/A9846G Genotype and dCK Expression

SNP	dCK Expression		χ^2	P
	High	Low		
dCK C1205T				
C/C	5	3	2.685	0.261*
C/T	12	8		
T/T	10	16		
C/C + C/T	17	11	2.670	0.102†
dCK A9846G				
A/A	9	0	12.025	0.002‡
A/G	13	15		
G/G	5	12		
A/A + A/G	22	15	4.207	0.040§

dCK = deoxycytidine kinase, SNP = single-nucleotide polymorphism.
* C/C vs C/T vs T/T.
† C/C + C/T vs T/T.
‡ A/A vs A/G vs G/G.
§ A/A+A/G vs G/G.

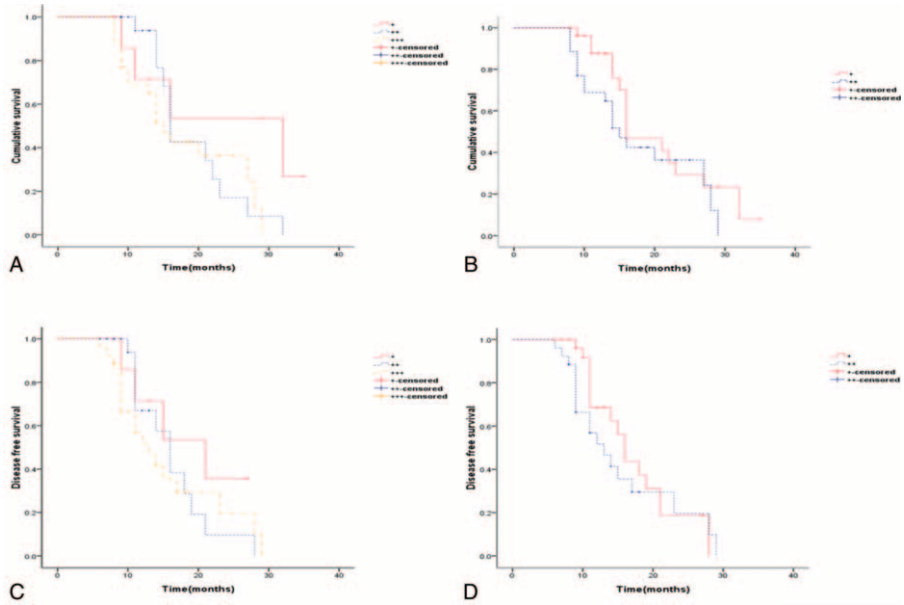

FIGURE 4. C1205T genotypes and patient survival. A, C1205T genotypes and overall survival (+ CC, ++ CT, +++TT); B, C1205T genotypes and overall survival (+ CC/CT, ++ TT); C, C1205T genotypes and disease-free survival (+ CC, ++ CT, +++TT); D, C1205T genotypes and disease-free survival (+ CC/CT, ++ TT).

We also found the high dCK protein expression to be beneficial for patients in terms of improved OS and DFS, indicating a possible role of dCK in predicting which patients will benefit from gemcitabine treatment. With 5 heterotopic transplantation tumor models, involving various types of malignancies, Kroep et al[26] showed that dCK protein expression was very closely linked to gemcitabine chemosensitivity. They also demonstrated, through association between expression level of dCK mRNA and protein expression, that transcriptional regulation in dCK gene and protein expression can be reliably used as a prognostic marker of response to gemcitabine.

By employing immunohistochemistry, another small retrospective study found high level of dCK protein expression to be associated with a higher 3-year OS in patients undergoing adjuvant combination chemoradiotherapy with gemcitabine.[27] They also categorically demonstrated that a low dCK expression was an independent risk factor for recurrence and mortality. Two recent retrospective studies have also signified

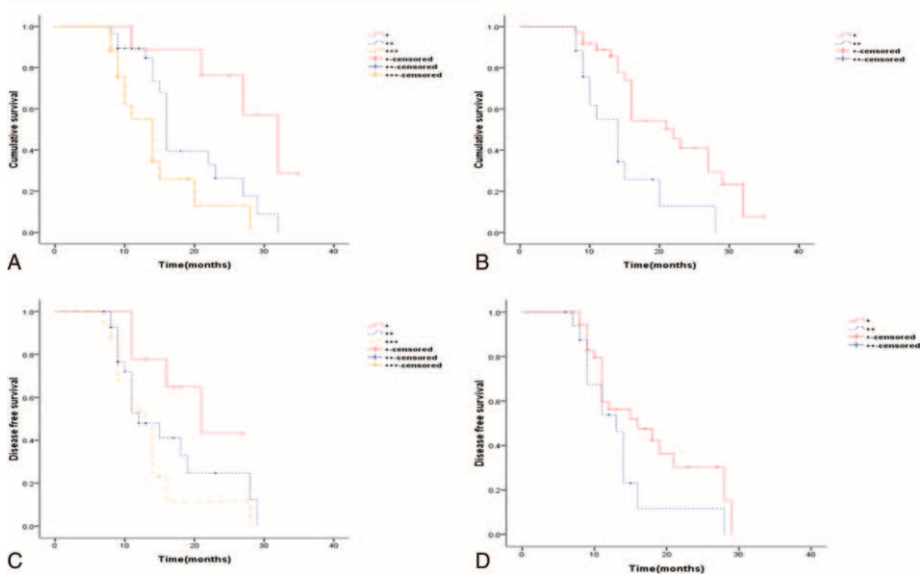

FIGURE 5. A9846G genotypes and patient survival. A, A9846G genotypes and overall survival (+ AA, ++ AG, +++ GG); B, A9846G genotypes and overall survival (+ AA/AG, ++ GG); C, A9846G genotypes and disease-free survival (+ AA, ++ AG, +++ GG); D, A9846G genotypes and disease-free survival (+ AA/AG, ++ GG).

TABLE 6. Univariate and Multivariate Analysis For Overall and Disease-Free Survival

	Univariate Analysis				Multivariate Analysis			
	OS		DFS		OS		DFS	
Variables	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***
Age								
>60 (n = 24)	1.00		1.00					
≤60 (n = 30)	1.665 (0.710–3.903)	0.241	2.074 (0.790–5.445)	0.139				
Sex								
Male (n = 39)	1.00		1.00					
Female (n = 15)	2.067 (0.819–5.217)	0.124	0.656 (0.226–1.904)	0.438				
Tumor location								
Head (n = 41)	1.00		1.00					
Body and tail (n = 13)	0.991 (0.372–2.639)	0.986	0.557 (0.212–1.459)	0.233				
Tumor size								
≥2.5 cm (n = 41)	1.00		1.00					
<2.5 cm (n = 13)	0.520 (0.248–1.089)	0.083	1.966 (0.799–4.836)	0.141				
CA19–9								
≥1000 U/mL (n = 36)	1.00		1.00					
<1000 U/mL (n = 18)	1.642 (0.700–3.851)	0.255	0.722 (0.320–1.631)	0.433				
Differentiation								
Low (n = 15)	1.00		1.00					
High and moderate (n = 39)	1.283 (0.494–3.335)	0.609	0.355 (0.105–1.194)	0.094				
T stage								
T1–T2 (n = 11)	1.00		1.00				1.00	0.063
T3–T4 (n = 43)	1.354 (0.461–3.980)	0.582	3.601 (1.264–10.259)	0.016			2.333 (0.956–5.693)	
TNM stage								
I–II (n = 47)	1.00		1.00					
III–IV (n = 7)	0.324 (0.078–1.347)	0.121	1.186 (0.398–3.533)	0.760				
Lymph node metastasis								
Yes (n = 26)	1.00		1.00					
No (n = 28)	1.380 (0.405–4.709)	0.607	0.557 (0.191–1.624)	0.284				
dCK expression								
High (n = 27)	1.00		1.00		1.00		1.00	
Low (n = 27)	0.036 (0.002–0.553)	0.017	0.360 (0.135–0.962)	0.042	0.268 (0.123–0.585)	0.001	0.297 (0.125–0.707)	0.006
A9846G								
AA/AG (n = 37)	1.00		1.00		1.00		1.00	
GG (n = 17)	0.385 (0.167–0.889)	0.025	0.133 (0.042–0.419)	0.001	0.367 (0.172–0.780)	0.009	0.227 (0.095–0.540)	0.0001
C1205T								
CC/CT (n = 28)	1.00		1.00				1.00	
TT (n = 26)	3.943 (0.415–37.488)	0.232	0.587 (0.244–1.410)	0.234			0.480 (0.224–1.025)	0.058

CA19–9 = carbohydrate antigen 19–9, CI = confidence interval, dCK = deoxycytidine kinase, DFS = disease-free survival, OR = odds ratio, OS = overall survival, TNM = tumor node metastasis.

the fact that a preoperative high level of dCK protein is able to predict patient response to gemcitabine and is associated with an improved outcome for these patients in way of increased OS.[11,12]

Five SNPs of the dCK gene were investigated which are closely related to gemcitabine chemosensitivity. We found a statistically significant impact on OS and DFS with 3 different genotype carriers of A9846G, but not with C1205T. Moreover, the OS and DFS were higher in patients with AA genotype than AG and GG genotypes, denoting a prognostic role for this as a biomarker for gemcitabine chemosensitivity. This has also been shown in previous studies. Si et al[28] found that pancreatic cancer cell lines with the AG genotype were more sensitive to gemcitabine, as compared with the GG genotype. On the contrary, Tanaka et al[13] and Okazaki et al[12] found a relationship between granulocytopenia and C1205T genotype, and also noted that 1205TT genotype carriers were more likely to develop toxicity to chemoradiation as compared with their CC/CT counterparts. They also showed that neither A9846G nor C1205T was associated with improvement in OS and DFS.

In conclusion, our results suggest that dCK protein is widely expressed in PDAC cells. Patient age was found to be strongly associated with expression of dCK protein, which in turn correlated positively to OS in patients with PDAC. We also identified a close correlation between the A9846G genotype and gemcitabine chemosensitivity in patients with PDAC. Therefore, dCK protein can act as a predictive biomarker for response to gemcitabine in patients undergoing surgery for PDAC. The A9846G SNP may also be used as a biomarker to predict a good response to gemcitabine in PDAC. Our study certainly suffers from being retrospective in nature and having a smaller sample size, and therefore, larger, prospective, and multicenter studies should inform us of the value of dCK and polymorphisms in context of gemcitabine chemosensitivity in future.

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