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Single Nucleotide Polymorphisms of Gemcitabine Metabolic Genes and Pancreatic Cancer Survival and Drug Toxicity

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Abstract

Purpose—To demonstrate whether single nucleotide polymorphisms (SNPs) of drug metabolic genes were associated with toxicity of gemcitabine-based chemoradiotherapy and overall survival (OS) of patients with pancreatic cancer.

Experimental Design—We evaluated 17 SNPs of the *CDA, dCK, DCTP, RRM1, hCNT1, hCNT2, hCNT3*, and *hENT1* genes in 154 patients with potentially resectable pancreatic adenocarcinoma who were enrolled in clinical trials at The University of Texas M. D. Anderson Cancer Center (Houston, TX) from February 1999 to January 2006, with follow-up until April 2009. Patients received neoadjuvant concurrent gemcitabine and radiation therapy with or without gemcitabine-cisplatin induction therapy. The association of genotypes with toxicity or OS was tested, respectively, by logistic regression and Cox regression analysis.

Results—None of the 17 SNPs, individually, had a significant association with OS. A combined genotype effect of *CDA* A-76C, *dCK* C-1205T, *DCTD* T-47C, *hCNT3* C-69T, *hENT1* T-549C and *hENT1* C913T on OS was observed. Patients carrying 0–1 (n=43), 2–3 (n=77) or 4–6 (n=30) variant alleles had median survival time of 31.5, 21.4 and 17.5 months, respectively. The hazard ratio of dying (95% CI) was 1.71 (1.06–2.76) and 3.16 (1.77–5.63) for patients carrying 2–3 or 4–6 at-risk genotypes (P=0.028 and P<0.001), respectively, after adjusting for clinical predictors. *CDA* C111T, *dCK* C-1205T, *dCK* A9846G and *hCNT3* A25G, individually and jointly, had a significant association with nuetropenia toxicity.

Conclusions—These observations suggest that polymorphic variations of drug metabolic genes were associated with toxicity of gemcitabine-based therapy and OS of patients with resectable pancreatic cancer.

Keywords

gemcitabine metabolic genes; nucleotide transporter genes; single nucleotide polymorphism; pancreatic cancer

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Statement of Translational Relevance
This study demonstrated associations of polymorphic variants of gemcitabine metabolic genes and nucleotide transporter genes with toxicity and overall survival of 154 patients with resectable pancreatic cancer treated with preoperative gemcitabine-based chemoradiotherapy. This information might be helpful for treatment selection and dose management in future “individualized” cancer therapy.

Introduction

Gemcitabine (2′,2′-difluoro 2′-deoxycytidine) is the standard first-line agent for treatment of pancreatic cancer. However, 75% of patients do not benefit from this therapy (1), and other than stage, it is not clear what factors predict clinical response to gemcitabine. A major dose limiting side effect of gemcitabine is hematological toxicity such as neutropenia and thrombocytopenia, which often result in dose reduction or longer intervals between gemcitabine administrations. However, there is no available biomarker that predicts the toxicity of gemcitabine.

Gemcitabine is a nucleoside analogue and a prodrug that requires cellular uptake and intracellular phosphorylation (2) (Fig. 1). Five of the nucleotide transporters found in humans—human concentrative nucleotide transporter (hCNT) 1–3 (aka solute carrier family 28 A1–A3); and human equilibrative nucleotide transporter (hENT) 1 and 2 (solute carrier family 29)—appear to be responsible for cellular uptake of gemcitabine (2). Once inside the cell, gemcitabine is phosphorylated by deoxycytidine kinase (dCK) to its monophosphate form. This first stage of phosphorylation is the rate-limiting step for further phosphorylation to the active triphosphate form and, thus, is essential for the activation of gemcitabine (3). The active diphosphate metabolite of gemcitabine inhibits DNA synthesis indirectly through the inhibition of ribonucleotide reductase (RR) (4). Inhibition of RR by gemcitabine blocks the de novo DNA synthesis pathway and decreases the intracellular concentrations of normal deoxynucleotide triphosphate pools. Gemcitabine is inactivated primarily by deoxycytidine deaminase (CDA)-mediated conversion to difluorodeoxyuridine.

Previous studies in cell lines and in patients have associated gemcitabine resistance to decreased expression of the activation enzyme (5–8), increased degradation (9), decreased nucleoside transport of drug into cells (10–12), and increased expression of RRM1 (10). Over expression of hENT1 and RRM1/2 in tumors has been significantly correlated to survival in pancreatic adenocarcinoma treated with gemcitabine (11–14).

It is theorized that an association exists between the activity of these proteins and the polymorphic variation of genes coding for the proteins (14,15). Few clinical studies have shown a positive association between *CDA* SNPs and drug toxicity (16,17). The current study tested the hypothesis that genetic variations in gemcitabine transport and metabolism, as well as in the drug's target, may affect the clinical response, hematological toxicity, and overall outcome of pancreatic cancer patients treated with gemcitabine. We tested this hypothesis in a relatively homogeneous population of 154 patients with potentially resectable pancreatic cancer who had undergone neoadjuvant gemcitabine -based chemotherapy plus radiation therapy.

Materials and Methods

Patient recruitment and data collection

The study involved 154 patients who, at the time of diagnosis, had potentially resectable adenocarcinoma of the head of the pancreas and were enrolled in one of two phase II clinical trials (ID98-020 or ID01–341) of preoperative (neoadjuvant) combined chemotherapy-radiation therapy at The University of Texas M. D. Anderson Cancer Center (Houston, TX) conducted sequentially from February 1999 to January 2006 and were observed through April 2009. These 154 patients represented the subset of patients enrolled in these clinical trials who had a DNA sample available. The study was approved by the institutional review board of M.D. Anderson Cancer Center. Patients in the ID98-020 trial (n = 70) received gemcitabine-based chemoradiotherapy consisting of weekly gemcitabine (400mg/m^2) for 4 weeks and radiation (30 Gy in 10 fractions) for 2 weeks. Patients in the ID01–341 trial (n = 84) received induction therapy of gemcitabine (750 mg/m^2/d) and cisplatin (30mg/m^2/d) every 2 weeks for

4 weeks and radiation (30 Gy in 10 fractions) for 2 weeks with weekly gemcitabine. The same eligibility criteria for patient recruitment had been applied in both trials, and no significant difference in any clinical feature was observed between the two patient populations (18,19).

Tumor response to therapy was evaluated by computed tomography (CT) before and after completion of the preoperative chemoradiation, and defined according to the RECIST criteria (Response Evaluation Criteria in Solid Tumors) as partial response (PR), stable disease (SD) or progressive disease (PD). Among patients with resected tumor, tumor response to preoperative treatment was histologically evaluated for percentage of viable tumor cells on resected tumor as previously described (20). Toxicity was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 3.0. Curative resection was defined by achievement of margin-negative resection. Treatment received after tumor recurrence was not considered in this study. Overall survival was calculated from the date of diagnosis to the date of death or date of last follow-up.

DNA extraction and genotyping

We selected 17 SNPs of the *CDA*, *dCK*, *RRM1*, deoxycytidylate deaminase (*DCTD)*, *hCNT1*, *hCNT2*, *hCNT3*, and *hENT1* genes in this exploratory investigation according to the following criteria: 1) The minor allele frequency of the SNP is greater than 10% among Caucasians; 2) coding SNPs including nonsynonymous and synonymous SNPs; and 3) SNPs that have been associated with cancer risk or clinical outcome in previous investigations. The genes, nucleotide substitutions, function (such as encoding amino acid changes), reference SNP identification numbers, and reported allele frequencies of the 17 SNPs evaluated in this study are summarized in Table 1.

DNA was extracted from peripheral blood lymphocytes of 127 patients and from paraffin sections of normal adjacent tissues of 27 patients with resected tumors (20 from the ID98-020 trial) using Qiagen DNA isolation kits (Valencia, CA). Normal and tumor tissues are expected to have the same genotype for these germline common polymorphic sequence variants. Taqman 5′ nuclease assay was performed to determine all genetic variants using the ABI Prism 7900HT Sequence Detection System, and SDS 2.3 software (Applied Biosystems).

Approximately 5% of the samples were analyzed in duplicate, and discrepancies were seen in less than 1% of the total samples. Samples with discordant results were genotyped repeatedly and consistent results from at least two analyses were included in the final data analysis. The clinical information on each patient was unknown to the individual who performed the genotyping assay.

Statistical methods

The genotype distribution was tested for Hardy-Weinberg equilibrium using the goodness-of-fit χ^2 test. Haplotype was inferred from the genotype data using the SNPAlyze software (version 4.1, DYNACOM Co., Ltd. Japan). The median follow-up time was computed with censored observations only, whereas the median survival time (MST) was calculated using data from all patients. Risk of dying was estimated by hazard ratios and 95% confidence intervals (CIs) in Cox proportional hazard models. Factors associated with tumor response to treatment or severe (grade 3–4) neutropenia toxicity was analyzed by logistic regression models. All clinical factors were modeled independently without additional variables in the model. Factors with $P<0.05$ in the univariate model were put into the initial multivariate model, and backward selection was then applied until all variables were statistically significant ($P<0.05$). These significant clinical factors were adjusted in all the regression models for genotype analyses. All statistical testing was conductedwith SPSS software, version 17.0 (SPSS, Chicago, IL), and statistical significance was defined as $P \leq 0.05$.

We estimated the false-positive report probability (FPRP) for the observed statistically significant associations using the methods described by Wacholder et al (21). FPRP is the probability of no true association between a genetic variant and a phenotype given a statistically significant finding. It depends not only on the observed P value but also on both the prior probability that the association between the genetic variant and the phenotype is real and the statistical power of the test. In the current study, we set the OR and HR values of 2.0 to 4.0 as a likely threshold value. The prior probability employed was 0.25 for all SNPs. The FPRP value for noteworthiness was set at 0.2, which indicates any finding with a FPRP *P* value <0.2 is noteworthy.

Results

Patient characteristics and clinical predictors

The patients' characteristics and clinical features of their tumors have previously been described in details (Table 2) (22). The median age of the 154 patients in this study was 63 years (range, 38–84 years). There are 96 male and 58 female patients. Non-Hispanic whites consisted 86% of the patients. One hundred and sixteen patients had the primary tumor surgically resected after preoperative treatment and pathologic evaluation of the surgical specimens demonstrated a microscopically positive margin (R1 resection) in 9 of the 116. There were 117 deaths (76%) among 154 cases. The median follow-up time was 49.9 months for the patients who were still alive. The MST of the 154 patients was 21.7 months (95% CI, 17.7 to 25.6). Information on tumor grade, lymph node metastasis, and tumor response to treatment by histological evaluation was not available in patients with unresected tumors. The factors which were significantly associated with overall survival time in log-rank test included diabetes status, tumor size, serum CA19-9 level at diagnosis, tumor response by CT evaluation, curative resection, tumor grade, lymph node metastasis, and the two clinical protocols for the preoperative treatment (Table 2). In the multivariable Cox regression model, diabetes status, serum CA19-9 at diagnosis, and curative resection remained as significant predictor for OS (*P*=0.003, 0.020, and <0.001, respectively).

Genotype frequency and association with OS

The 17 genotypes of interest were successfully amplified in 95–100% of the samples. Genotype frequencies of the 17 SNPs were found to be in Hardy-Weinberg equilibrium (χ^2 = 0.009 3.684; *Ps*=0.055–0.924) except *RRM1* A33G (χ^2 = 14.294; *P*=0.0002), *RRM1* C-27A (χ^2 = 15.112; *P*=0.0001), and *hCNT1* A-16G (χ^2 = 9.070; *P*=0.0026). No significant racial differences in genotype frequency were observed (data not shown). The two or three SNPs each of the *dCK*, *RRM1*, *hCNT2*, *hCNT3* and *hENT1* (IVS12 -201A>G and IVS2 -549T>C) genes were in linkage disequilibrium ($|D'|$>0.5, $P < 0.01$).

The genotype frequencies and their associations with OS are shown in Table 3. None of the 17 SNPs showed significant association with OS by log rank test. Six SNPs (*CDA* A-76C, *dCK* C-1205T, *DCTD* T-47C, *hCNT3* C-69T, *hENT1* T-549C and *hENT1* C913T) showed weak associations with OS (Cox regression *P*<0.20). When these 6 SNPs were analyzed in combination, a gene-dosage effect on OS was observed. As the number of at-risk alleles increased the OS decreased (Fig. 2). Patients carrying 0–1 (n=43), 2–3 (n=77) or 4–6 (n=30) variant alleles had MST of 31.5, 21.4 and 17.5 months, respectively. The HR of dying (95% CI) was 1.71 (1.06–2.76) and 3.16 (1.77–5.63) for patients carrying 2–3 or 4–5 at-risk genotypes (*P*=0.028 and <0.001), respectively, after adjusting for clinical predictors. The FPRP was 0.102 and 0.005 for patients carrying 2–3 or 4–6 at-risk genotypes, respectively, indicating noteworthiness.

Haplotype association with OS

The *CDA* C111T and A-76C TA haplotype was significantly associated with increased risk and the *hENT1* A-201G, T-549C and C913T ACT haplotype was significantly associated with reduced risk of death compared to the most common haplotype of each gene respectively (*P*s<0.05, Table 4). No other haplotype showed significant association with OS.

Genotype association with tumor response to treatment

Tumor size was the only clinical factor that was significantly associated with tumor response to therapy by histological evaluation. None of the 17 SNPs showed significant association with tumor response to therapy by radiological evaluation (data not shown). Four SNPs, i.e. *dCK* C-1205T, *dCK* A9846G, hCNT3 C-69T, and *hCNT3* A25G had significant associations with tumor response by histological evaluation (Table 5). For example, 26.2% of the *dCK* 1205 TT carriers versus 46.4% of the CT/CC carriers had a poor response to preoperative chemoradiotherapy, i.e. >50% tumor cells were viable in resected tumor. Patients carrying 3 or more of the 4 at risk genotypes had a 5.77-fold higher risk of poor response to therapy after adjusting for tumor size (95% CI, 2.23–14.9, *P*<0.001). The FPRP was 0.058 for patients carrying 3–4 at-risk genotypes, indicating noteworthiness.

Genotype association with toxicity

None of the clinical factors was predictive for severe nuetropenia. The *CDA* C111T, *dCK* C-1205T, *dCK* A9846G and *hCNT3* A25G genotype individually and in combination were significantly associated with toxicity (Table 6). For example, 40.9% of the *CDA* 111 CT/TT carriers versus 24.6% of the CC carriers had grade 3–4 neutropenia (*P*=0.037). Patients carrying 3 or 4 variant alleles compared to those carrying 0–2 variant allele had a significantly higher risk for grade 3–4 neutropenia (OR: 3.57, 95% CI: 1.38–9.22, *P*=0.009; OR: 5.88, 95% CI: 2.10–16.5, *P*=0.001, respectively). The FPRP was 0.182 and 0.102 for patients carrying 3 or 4 at-risk genotypes, respectively, indicating noteworthiness. No significant association of toxicity with the remaining SNPs was noticed (data not shown).

Discussion

In this study, we observed a significant association of combined genotype of gemcitabine metabolic genes *CDA*, *dCK, and DCTD* as well as transporter genes *hCNT3 and hENT1* with overall survival of patients with resectable pancreatic cancer and treated with preoperative gemcitabine-based chemoradiotherapy. We also observed a significant association of *dCK* and *hCNT3* gene variants with tumor response to therapy and drug toxicity. These data support the hypothesis that genetic variations in gemcitabine metabolism affect the clinical outcome of pancreatic cancer patients receiving gemcitabine-based chemotherapy.

CDA, an enzyme involved in the pyrimidine salvage pathway, is the major gemcitabine inactivation enzyme. Three main SNPs were identified in the *CDA* gene: C111T (T145T), A-76C (K27Q), and G208A (14,23,24). *CDA* A-76C (K27Q) has previously been reported to result in a moderate decrease in activity with gemcitabine (25). The *CDA* 208AA homozygote allele and related haplotype have been associated with severe drug toxicity in Japanese cancer patients treated with gemcitabine plus cisplatin (16,17). However, *CDA* G208A was not detected in Caucasians (26). Although the *CDA* A-76C (K27Q) and G208A SNPs have been associated with drug toxicity, no previous study has shown an association with patient survival. In the current study, the *CDA* A-76C (K27Q) variant C allele was associated with a better overall survival suggesting reduced enzyme activity conferred by this allele resulted in a higher level of drug availability. In consistency, the *CDA* C111T and A-76C TA haplotype was significantly associated with increased risk of death. The *CDA* C111T (T145T) T allele showed a significant association with gemcitabine toxicity, although the functional significance of this

SNP is not clear. DCTD is another gemcitabine degradation enzyme. The *DCTD* gene polymorphic variants, including the nonsynonymous A172G, have not been associated with clinical response to gemcitabine in previous studies (15). We observed a weak association of the *DCTD* T-47C (V116V) SNP with OS in our patient population. These observations were consistent with previous reports, which support a role of CDA genotype in affecting gemcitabine toxicity and clinical outcome of patients receiving gemcitabine.

dCK plays a key role in the activation of gemcitabine, and its activity has been correlated with gemcitabine sensitivity and clinical outcomes in several studies (27–29). One study reported that the haplotype containing *dCK* C-360G and C-201T was correlated with the clinical outcomes of cancer patients treated with Ara-C (30). In our study, we found a significant difference in toxicity and tumor response to therapy but not in OS associated with the *dCK* C-1205T *and dCK* A9846G SNPs, both are located in the intron region. It is not clear whether these SNPs are directly responsible for gemcitabine sensitivity or whether they are in linkage disequilibrium with other SNPs or other genes. Alternatively, it is conceivable that their functional difference may be mediated by affecting RNA splicing. Further haplotype analysis of the *dCK* gene is required to answer these questions.

The activity of nucleotide transporters is expected to play a role in gemcitabine cytotoxicity and efficacy (31). In the current study, we observed a marginally significant association of the *hCNT3* C-69T (L461L), *hENT1* C-549T and *hENT1* C913T genotype and a significant association of *hENT1* A-201G, T-549C and C913T ACT haplotype with overall survival. Further more, *hCNT3* genotypes were associated with drug toxicity and tumor response to therapy. The major route for transporting gemcitabine is hENT1 and, to a lesser extent, hCNT1 and hCNT3. A previous study reported that the *hENT1* promoter region haplotype containing the C1345G, G1050A, and G706C SNPs might influence gene expression (32). Two studies have explored the *hENT1* haplotype and no functional significance was reported (33,34). Considering that hENT1 expression has been associated with pancreatic cancer survival (35), it would be important to demonstrate the genotype and phenotype association to determine whether the genotype may be used as a surrogate for tumors.

A recent study in pancreatic adenocarcinoma cell lines showed that the ratio of the expression level of *hENT1*, *dCK*, *RRM1*, and *RRM2* genes was correlated to acquired gemcitabine chemoresistance (36). However, tissue samples are not available for most pancreatic cancer patients and it is possible that some SNPs might alter substrate specificity, resulting in altered function without increased levels of mRNA and protein (37). Thus, if confirmed in other patient populations, the genotype information might be useful in stratifying patients on protocol and in predicting response and toxicity.

In summary, we observed significant associations of gemcitabine metabolic genes on the toxicity and tumor response to gemcitabine-based preoperative therapy and overall survival of patients with resectable pancreatic cancer. Even though the effect of individual common SNPs may be trivial, the combined genotype effects are remarkable. Genetic profiling of patients may provide the fundamental information required for future "individualized" therapy.

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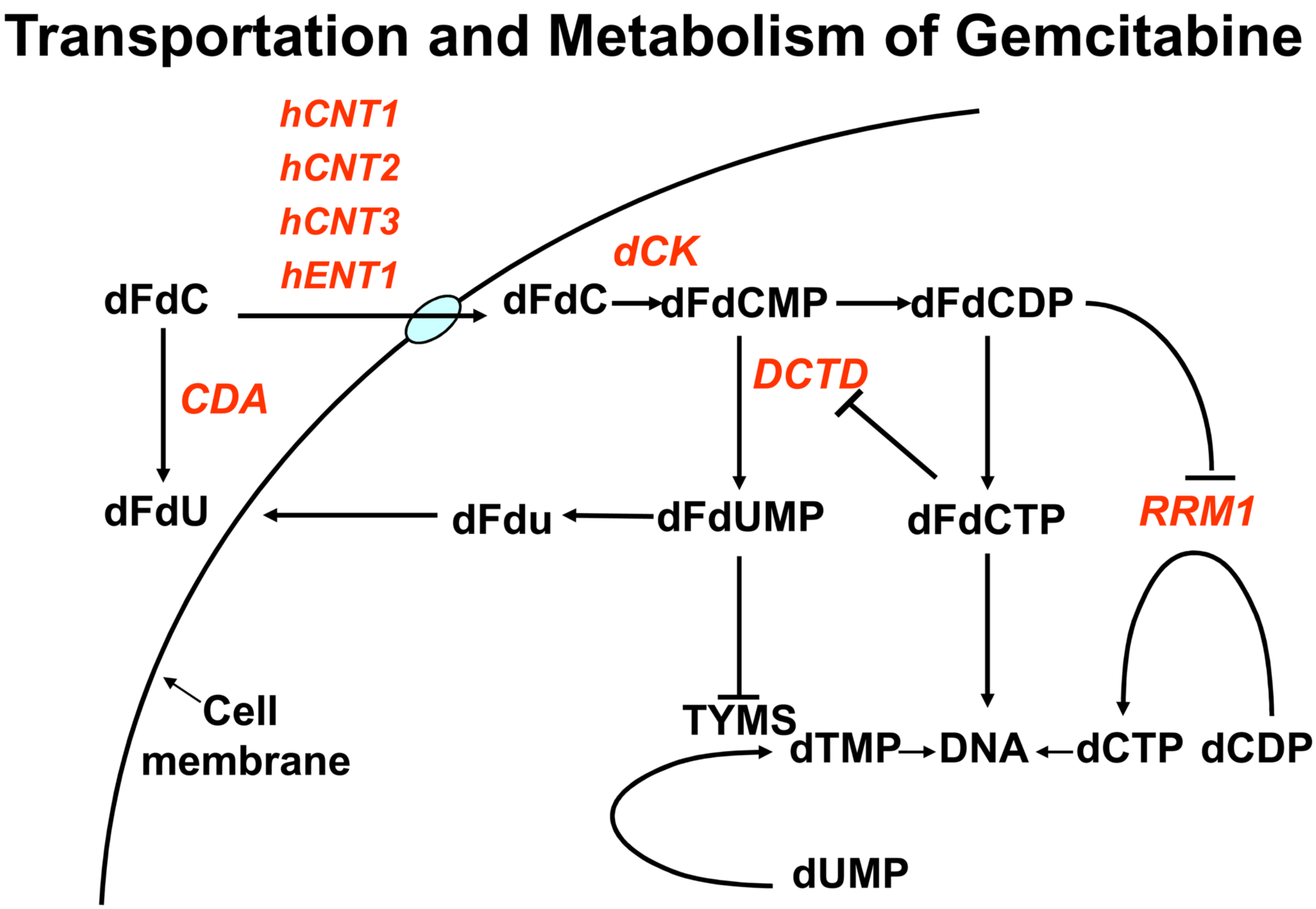

Fig. 1.

Schematic description of gemcitabine (dFdC) transportation and metabolism. The italic letters indicate genes that are examined in this study.

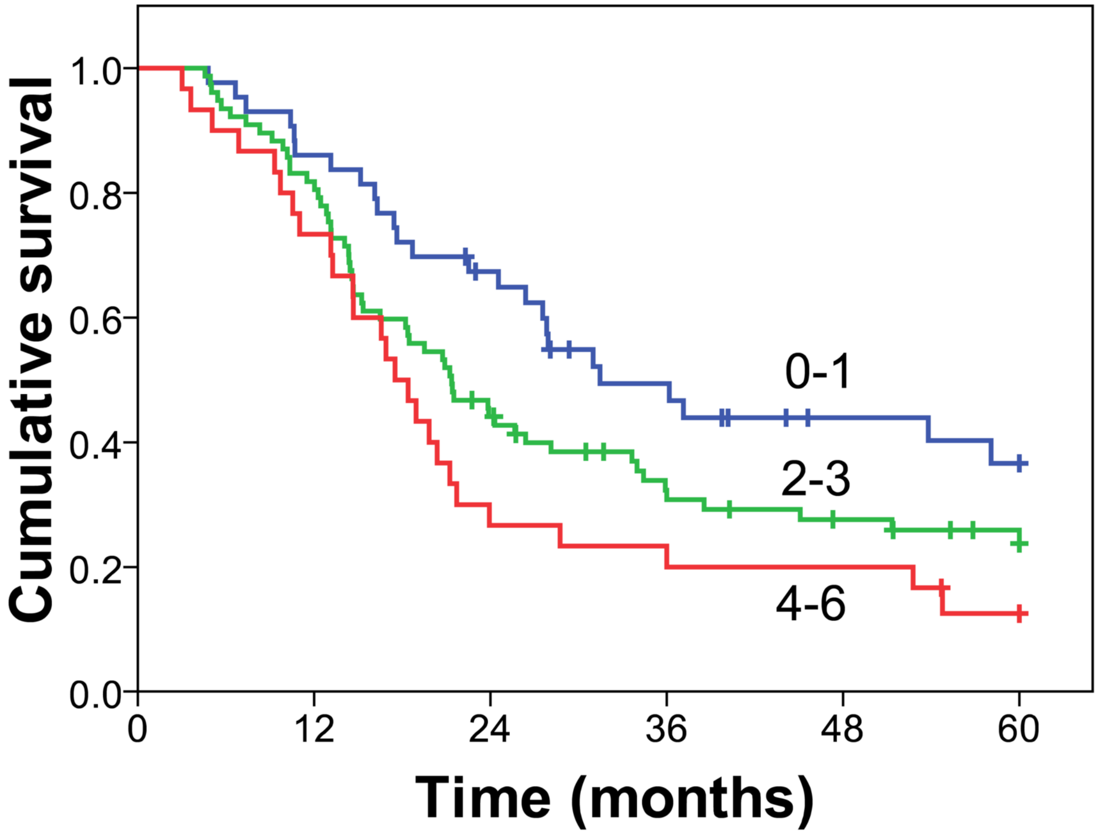

Fig. 2.
Combined genotype effect of *CDA* -76AA, *dCK* -1205TT, *DCTD* -47CT, *hCNT3* -69CT/TT, *hENT1* -549CT/TT, and *hENT1* 913CC on overall survival. The number of 0 to 6 indicates the number of deleterious genotypes associated with reduced survival.

Table 1

SNPs evaluated

Gene	**Chromosome**	**SNP**	**Reference SNP ID number**	**Minor allele frequency***
CDA	1p36.12b	Ex4 +111C>T, T145T	1048977	0.28
		Ex2 −76A>C, K27Q	2072671	0.44
dCK	4q13.3b	IVS6 −1205C>T	4694362	0.45
		IVS2 +9846A>G	12648166	0.43
RRM1	11q15.4d	Ex19 +42G>A, A744A	1042858	0.11
		Ex19 +33A>G, T741T	3177016	0.47
		Ex9 −27C>A, R284R	183484	0.48
DCTD	4q35.1b	Ex4 −47T>C, V116V	7663494	0.33
hCNT1	15q25.3a	Ex15 −16A>G, Q456Q	2242048	0.15
		Ex9 −9C>A, Q237K	8187758	0.19
hCNT2	15q21.1a	Ex4 −38C>A, S75R	1060896	0.29
		Ex2 −17C>T, P22L	11854484	0.34
hCNT3	9q21.32c	Ex14 −69C>T, L461L	7853758	0.15
		Ex5 +25A>G, T89T	7867504	0.39
hENT1	6p21.1b	IVS12 −201A>G	760370	0.35
		IVS2 −549T>C	324148	0.30
		IVS2 +913C>T	9394992	0.32

*Allele frequencies (Caucasian) were from the National Cancer Institute SNP500 cancer.

Table 2

Patient characteristics (n=154)

Variable	No. of patients	No. of deaths (%)	MST (months)	***P*** **(Log-rank)**
Age (years)				.098
≤50	17	16 (94.1)	18.5	
51–60	44	27 (61.4)	36.0	
61–70	60	41 (68.3)	21.5	
>70	33	27 (81.8)	21.2	
Sex				.369
Male	96	70 (72.9)	20.9	
Female	58	41 (70.7)	24.5	
Race				.704
White	133	97 (72.9)	23.9	
Hispanic	10	7 (70.0)	18.2	
African American	7	4 (57.1)	33.6	
Other	4	3 (75.0)	10.7	
Diabetes status				.017
Negative	109	74 (67.9)	27.6	
Positive	45	37 (82.2)	18.2	
Tumor size (cm)				.010
≤2	65	40 (61.5)	34.0	
>2	89	71 (79.8)	20.7	
CA19-9 (units/mL)				.001
≤47	40	21 (52.5)	52.8	
48–500	78	58 (74.4)	22.5	
501–1,000	14	12 (85.7)	18.4	
>1,000	22	20 (90.9)	15.3	
Tumor response in CT				< .001
PR/SD	126	85 (67.5)	27.8	
PD	27	25 (92.6)	10.3	
Curative resection				< .001
Yes	107	64 (59.8)	34.4	
No*	47	47 (100)	12.8	
Tumor grade				.017
Well-to-moderate	88	57 (64.8)	33.6	
Poor	32	25 (78.1)	19.8	
Lymph node metastasis				< .001
Negative	61	33 (54.1)	51.3	
Positive	55	40 (72.7)	26.4	
Treatment Effect†				.259
≤50%	70	43 (61.4)	27.9	
>50%	43	30 (69.8)	37.1	

Variable	**No. of patients**	**No. of deaths (%)**	**MST (months)**	***P* (Log-rank)**
Clinical Protocol				.026
GEM/XRT	70	49 (70.0)	28.1	
GEM/Cisplatin/XRT	84	62 (73.8)	18.4	

Abbreviations: MST, median survival time; PR, partial response; SD, stable disease; PD, progressive disease.

*No resection (n=38) and margin positive resection (n=9).

†Percentage of viable cells by histological evaluation of resected tumor.

Table 3

Genotype and Overall Survival

Genotype	No. of Cases	No. of Deaths	MST ± SE (months)	*P* (log-rank)	* HR (95% CI)	*P*
CDA C111T (T145T)				.567		
TT	20	15	23.8 ± 2.1			
CT	68	46	27.8 ± 6.2			
CC	65	49	20.9 ± 1.2			
CT versus TT/CC					1.13 (0.76–1.69)	.544
CDA A-76C (K27Q)				.183		
CC	20	13	27.8 ± 12.6			
AC	60	39	27.6 ± 5.0			
AA	69	54	20.4 ± 1.5			
CC/AC versus AA					1.30 (0.88–1.93)	.193
dCK C-1205T				.449		
CC	22	15	28.7 ± 4.0			
CT	72	48	24.3 ± 5.7			
TT	57	45	20.7 ± 1.5			
CC/CT versus TT					1.44 (0.96–2.17)	.079
dCK A9846G				.583		
AA	24	16	31.5 ± 4.1			
AG	71	48	21.5 ± 3.6			
GG	55	43	20.9 ± 1.5			
AA versus AG/GG					1.33 (0.76–2.32)	.319
RRM1 G42A (A744A)				.069		
AA	126	88	23.9 ± 2.6			
AG	20	15	16.1 ± 3.1			
GG	3	3	14.1 ± 2.8			
AA versus GG/AG					1.32 (0.79–2.21)	.292
RRM1 A33G (T741T)				.908		
GG	49	34	26.4 ± 4.8			
AG	51	36	21.2 ± 3.1			
AA	48	35	22.5 ± 2.4			

Genotype	No. of Cases	No. of Deaths	MST ± SE (months)	*P* (log-rank)	* HR (95% CI)	*P*
GG versus AG/AA					1.18 (0.78–1.79)	.442
RRM1 C-27A (R284R)				.911		
CC	52	38	22.8 ± 3.8			
AC	52	37	20.7 ± 1.7			
AA	48	34	24.3 ± 4.1			
AA versus AC/CC					1.16 (0.77–1.76)	.471
DCTD T-47C (V116V)				.395		
CC	12	8	28.1 ± 10.8			
CT	52	39	17.5 ± 2.2			
TT	87	61	26.4 ± 2.0			
CC/TT versus CT					1.35 (0.90–2.01)	.148
hCNT1 A-16G (Q456Q)				.918		
GG	125	89	23.9 ± 2.8			
AG	21	16	21.5 ± 3.8			
AA	5	3	21.7 ± 18.2			
GG versus AA/AG					1.24 (0.74–2.08)	.425
hCNT1 C-9A (Q237K)				.942		
CC	88	65	23.9 ± 2.8			
AC	53	37	20.9 ± 3.2			
AA	10	6	13.1 ± 17.8			
CC versus AA/AC					1.16 (0.77–1.76)	.480
hCNT2 C-38A (S75R)				.636		
AA	56	39	24.5 ± 2.5			
AC	66	48	18.4 ± 1.7			
CC	29	21	23.8 ± 12.7			
AA/CC versus AC					1.04 (0.71–1.53)	.852
hCNT2 C-17T (P22L)				.282		
TT	46	31	28.1 ± 4.3			
CT	67	47	20.7 ± 4.3			
CC	39	31	18.7 ± 2.9			
TT/CT versus CC					1.23 (0.80–1.90)	.353

Genotype	No. of Cases	No. of Deaths	MST ± SE (months)	*P* (log-rank)	* HR (95% CI)	*P*
hCNT3 C-69T (L461L)				.565		
CC	111	77	24.3 ± 2.7			
CT	36	29	20.4 ± 3.7			
TT	6	4	16.9 ± 7.4			
CC versus CT/TT					1.39 (0.91–2.11)	.125
hCNT3 A25G (T89T)				.492		
AA	78	53	24.5 ± 2.8			
AG	53	42	19.8 ± 2.6			
GG	17	11	18.4 ± 6.8			
AA versus GG/AG					1.03 (0.70–1.52)	.883
hENT1 A-201G				.966		
AA	56	40	23.8 ± 5.1			
AG	65	44	20.9 ± 5.1			
GG	28	22	23.9 ± 2.8			
AA/GG versus AG					1.27 (0.84–1.91)	.258
hENT1 T-549C				.338		
CC	86	59	24.5 ± 3.2			
CT	57	44	18.9 ± 2.2			
TT	11	8	17.5 ± 11.0			
CC versus CT/TT					1.32 (0.91–1.92)	.142
hENT1 C913T				.175		
TT	8	5	26.4 ± 19.0			
CT	68	43	27.9 ± 7.0			
CC	74	59	21.2 ± 1.4			
CT/TT versus CC					1.44 (0.98–2.10)	.064
No. of at-risk genotypes†				.019		
0–1	43	25	31.5 ± 5.4		1.0	
2–3	77	56	21.4 ± 2.6		1.71 (1.06–2.76)	.028
4–6	30	26	17.5 ± 1.6		3.16 (1.77–5.63)	<.001

Abbreviations: MST, median survival time; SE, standard error.

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[*] HR was adjusted for history of diabetes, serum level of CA19-9, and curative resection.

[†] At risk genotypes: *CDA* -76AA, *dCK* -1205TT, *DCTD* -47CT, *hCNT3* -69CT/TT, *hENT1* -549CT/TT, and *hENT1* 913CC.

Table 4

Haplotype and Overall Survival

Haplotype	Frequency	* HR (95% CI)	*P*
CDA C111T/A-76C			
CA	0.45	Reference	
TA	0.21	1.49 (1.03–2.15)	.036
CC	0.19	1.03 (0.68–1.54)	.898
TC	0.15	0.71 (0.48–1.05)	.089
dCK C-1205T/A9846G			
TG	0.60	Reference	
CA	0.38	0.77 (0.58–1.03)	.081
Others	0.02	0.98 (0.45–2.12)	.959
RRM1 G42A/A33G/C-27A			
AGA	0.48	Reference	
AAC	0.41	1.18 (0.88–1.58)	.270
Others	0.11	1.33 (0.85–2.08)	.210
hCNT1 A-16G/C-9A			
GC	0.67	Reference	
GA	0.23	0.90 (0.64–1.25)	.518
Others	0.10	1.22 (0.77–1.93)	.394
hCNT2 C-38A/C-17T			
AT	0.50	Reference	
CC	0.39	0.96 (0.72–1.29)	.802
Others	0.11	1.04 (0.65–1.68)	.863
hCNT3 C-69T/A25G			
CA	0.67	Reference	
CG	0.17	0.87 (0.60–1.27)	.474
Others	0.12	1.17 (0.80–1.71)	.416
hENT1 A-201G/T-549C/C913T			
GCC	0.30	Reference	
ACC	0.22	0.81 (0.55–1.22)	.314
ATC	0.16	1.26 (0.85–1.88)	.243
ACT	0.15	0.65 (0.43–0.98)	.037
Others	0.17	1.04 (0.65–1.67)	.875
dCK C-1205/A9846G & *DCTD* T-47C			
TGT	0.45	Reference	
CAT	0.28	0.70 (0.49–1.01)	.054
TGC	0.15	1.34 (0.86–2.10)	.196
CAC	0.10	1.03 (0.68–1.54)	.895
Others	0.02	1.02 (0.47–2.22)	.954

* HR was adjusted for history of diabetes, serum level of CA19-9, and curative resection.

Table 5

Genotype and Tumor Response to Preoperative Treatment

Genotype	≤50%*	>50%*		
	N (%)	N (%)	† OR (95% CI)	*P* value
dCK C-1205T				
TT	31 (73.8)	11 (26.2)	1.0	
CT/CC	37 (53.6)	32 (46.4)	2.73 (1.15–6.45)	.022
dCK A9846G				
GG	31 (75.6)	10 (24.4)	1.0	
AG/AA	37 (53.6)	32 (46.4)	2.96 (1.23–7.13)	.015
hCNT3 A25G				
AA	42 (70.0)	18 (30.0)	1.0	
AG/GG	24 (49.0)	25 (51.0)	2.733 (1.21–6.17)	.016
hCNT3 C-69T				
CC	55 (68.8)	25 (31.2)	1.0	
CT/TT	14 (43.8)	18 (56.3)	3.08 (1.30–7.31)	.011
No. of at-risk genotypes				
0–2	52 (72.2)	20 (27.8)	1.0	
3–4	14 (38.9)	22 (61.1)	5.77 (2.23–14.9)	<.001

*Percentage of viable cells by histological evaluation of resected tumor.

†OR was adjusted for tumor size.

Table 6

Neutropenia toxicity and genotype

Genotype	Grade1–2 N (%)	Grade 3–4 N (%)	* OR (95% CI)	*P* value
CDA C111T				
CC	49 (75.4)	16 (24.6)	1.0	
CT/TT	52 (59.1)	36 (40.9)	2.12 (1.05–4.30)	.037
dCK C-1205T				
CC	19 (86.4)	3 (13.6)	1.0	
CT/TT	80 (62.0)	49 (38.0)	3.88 (1.09–13.8)	.036
dCK A9846G				
AA	20 (83.3)	4 (16.7)	1.0	
AG/GG	78 (61.9)	48 (38.1)	3.08 (0.99–9.55)	.052
hCNT3 A25G				
GG/AG	52 (74.3)	18 (25.7)	1.0	
AA	45 (57.7)	33 (42.3)	2.12 (1.05–4.26)	.035
No. of at-risk genotypes				
0–2	39 (84.8)	7 (15.2)	1.0	
3	39 (60.9)	25 (39.1)	3.57 (1.38–9.22)	.009
4	18 (48.6)	19 (51.4)	5.88 (2.10–16.5)	.001

*Crude odds ratio.