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Association of Multi-Drug Resistance Gene Polymorphisms with Pancreatic Cancer Outcome

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Abstract

BACKGROUND—The purpose of this study was to identify single nucleotide polymorphisms (SNPs) of multi-drug resistance genes that are associated with clinical outcome in patients with potentially resectable pancreatic adenocarcinoma who were treated with preoperative gemcitabine-based chemoradiotherapy at M.D. Anderson Cancer Center.

METHODS—We selected 8 SNPs of 7 drug resistance genes; *MDR1* (ABCB1), *MRP1-5* (ABCC1-5), and *BCRP* (ABCG2), which have been reported to be important in mediating drug resistance. Genotype was determined by the Taqman method. The associations of genotype with tumor response to therapy and overall survival (OS) were evaluated using log-rank test, Cox regression, and logistic regression models.

RESULTS—*MRP5* A-2G AA genotype showed significant association with OS (log-rank *P* =. 010). The hazard ratio (95% confidence interval) was 1.65 (1.11-2.45) after adjusting for clinical predictors. The *MRP2* G40A GG genotype had a weak association with reduced OS (log-rank *P* =. 097). A combined effect of the two genotypes on OS was observed. Patients with none of the adverse genotypes had a median survival time (MST) of 34.0 months, and those with 1-2 deleterious alleles had a significantly lower MST of 20.7 months, respectively (log-rank *P* =.006). *MRP2* G40A GG genotype was also significantly associated with poor histological response to chemoradiotherapy (*P* =.028).

CONCLUSIONS—These observations suggest a potential role of polymorphic variants of drug resistance genes to predict a therapeutic efficacy and survival of patients with potentially resectable pancreatic cancer.

Keywords

drug resistance gene; single nucleotide polymorphism; gemcitabine; pancreatic cancer; survival

Introduction

Pancreatic cancer is one of the most aggressive human cancers with a 5-year survival rate of less than 5%,[1] and highly resistant to most therapies. Gemcitabine is the current standard of care for the chemotherapy of pancreatic cancer but its efficacy is limited. Demonstrating the determinant of gemcitabine resistance in pancreatic cancer has great clinical implications.

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Drug resistance, a major cause of treatment failure in oncology,[2] is consisted of several processes, e.g. increased drug efflux and decreased accumulation of drugs in the cell.[3] Efflux transporters of the ATP-binding cassette (ABC) family such as ABCB1 (multidrug resistance 1, MDR1), the ABCC (multidrug resistance-associated protein, MRP) family, and ABCG2 (breast cancer resistance protein, BCRP) have been identified as major determinants of chemoresistance in tumor cells.[4] Because of their ability to regulate the intracellular concentration and tissue distribution of xenobiotics and their metabolites, ABC transporters are potentially important players in drug response.[5]

The MDR, MRP, and BCRP family has eleven, thirteen, and five (or six) members, respectively.[5] The *MDR1* gene products P-glycoprotein is a membrane protein that functions as an ATP-dependent exporter of drugs from cells. Some studies demonstrated a high rate of MDR1 expression in pancreatic cancer tissue and cell lines[6,7] and other studies reported that MDR1 overexpression is associated with sensitivity to gemcitabine.[8,9] MRP (ABCC) subfamily members differ in substrate specificity, tissue distribution, and cellular localization.[10] MRP1, MRP2, and MRP3 transport lipophilic compounds conjugated with glutathione, glucuronate or sulfate. MRP4 and MRP5 transport nucleotide analogues and cyclic nucleotides. In addition to endogenous compounds, MRPs are involved in exporting of a variety of organic anions of xenobiotics and are important in conferring resistance to cytotoxic and antiviral drugs.[10] It has been shown that *MRP1- MRP5* mRNAs were overexpressed in several pancreas cancer cell lines.[3]

Many reports of SNPs in ABC transporter family,[10-15] the impact of polymorphisms on pharmacokinetics and pharmacodynamics for gemcitabine still remains to be defined. We hypothesized that genetic variation in drug resistance genes is associated with the clinical response and overall prognosis 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 chemoradiation with or without cisplatin induction therapy. We evaluated eight coding region SNPs with minor allele frequencies >0.10 of the *MDR1*, *MRP1-5*, and *BCRP* genes in this study.

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 pancreas head and had not received any treatment for pancreatic cancer. All patients were enrolled in one of two phase II clinical trials (ID98-020 or ID01-341) of preoperative combined chemoradiation therapy for pancreatic cancer at The University of Texas M.D. Anderson Cancer Center (Houston, TX) conducted sequentially from February 1999 to 2006.[16,17] These 154 patients represented the subset of 176 patients enrolled in these clinical trials who had DNA samples for genotyping. The study was approved by the institutional review board of M.D. Anderson Cancer Center. Patients in the ID98-020 trial (n = 70) had received gemcitabine-based chemoradiotherapy consisting of weekly gemcitabine (400 mg/m^2) for 4 weeks and radiation (30 Gy in 10 fractions) for 2 weeks. Patients in the ID01-341 trial (n = 84) had received induction therapy of gemcitabine (750 mg/m^2/d) and cisplatin (30 mg/m^2/d) every 2 weeks for 4 weeks, followed by weekly gemcitabine (400mg/m^2) for 4 weeks and radiation (30 Gy in 10 fractions) for 2 weeks. After chemoradiation, patients underwent pancreatoduodenectomy using Whipple procedure.

Clinical information was collected retrospectively from the patients' medical records. Dates of death were obtained and cross-checked using at least one of the following sources:

inpatient medical records, the M. D. Anderson tumor registry, and the Social Security Death Index (www.deathindexes.com/ssdi.html). OS times were calculated from the date of pathologic diagnosis to the date of death or last follow-up. Data for patients who were alive at the last follow-up evaluation were censored at that time. Serum CA19-9 levels were measured at the time of cancer diagnosis. Tumor size was estimated from measurements made by endoscopic ultrasonography (EUS) or computed tomography (CT) at the time of cancer diagnosis. Tumor differentiation was evaluated in most surgically resected tumors and a few biopsy samples. Tumor response to preoperative chemoradiotherapy was evaluated by CT before and after treatment and defined according to the Response Evaluation Criteria in Solid Tumors as partial response, stable disease, or progressive disease. The histological effect of preoperative chemoradiotherapy was evaluated in resected tumors according to previously published criteria,[18] i.e., tumors with >90% viable cancer cells were defined as treatment effect grade I, 51%–90% viable cells as grade IIA, 10%–50% viable cells as grade IIB, and <10% viable cells as grade III. Postsurgical treatment or treatment received after tumor recurrence was not considered in this study because of the minimal effect of these treatments on overall survival.

DNA Extraction and Genotyping

We selected 8 SNPs of the *MDR1*, *MRP1-5*, and *BCRP* genes according to the following criteria: 1) minor allele frequency of the SNP was greater than 10% among Caucasians, 2) coding SNPs including nonsynonymous or synonymous SNPs, and 3) SNPs that have been associated with cancer risk or clinical outcome in prior studies. Table 1 summarizes the genes, chromosome locations, nucleotide substitutions, amino acid changes, reference SNP identification numbers, and minor allele frequencies of the 8 SNPs evaluated in this study.

Whole blood was collected from patients at the time of clinical trial enrollment, and 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 DNA isolation kit (Qiagen, Valencia, CA). Polymorphisms were detected using the TaqMan genotyping assays provided by Applied Biosystems (Foster City, CA). In duplicate analysis, discrepancies were seen in less than 1% of total samples. Discordant results were resolved by further genotyping analysis.

Statistical Methods

The distribution of genotypes was tested for Hardy-Weinberg equilibrium using the goodness-of-fit Chi-square test. The association of clinical factors or genotypes with OS was evaluated using Kaplan-Meier method and the log-rank test. The median follow-up time was computed with censored observations only. Median survival time (MST) was calculated using data from all patients. The heterozygous and homozygous genotypes were combined in these analyses if the frequency of the homozygous mutant was low or if the homozygous and heterozygous genotypes had the same direction of effect on survival or tumor response. Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated using Cox regression models. Significant clinicopathologic factors on OS by log-rank test were included in the multivariate model when appropriate. Chi-square test and logistic regression was used to evaluate the association of genotypes with tumor response. All statistical testing was conducted with SPSS software, version 17.0 (SPSS, Chicago, IL), and statistical significance was defined as $P < .05$ and borderline significance as $P < .10$.

We estimated the false-positive report probability (FPRP) for the observed statistically significant associations using the methods described by Wacholder et al.[19] The prior probability employed was 0.25 and the FPRP value for noteworthiness was set at 0.2.

Results

Patients Characteristics and Survival Analysis

The patients' characteristics and clinical features of their tumors are summarized in Table 2. The median age of the 154 patients in this study was 62.8 years (range, 38–80 years). There were 111 deaths (72%) among 154 cases, and the MST was 21.7 months (95% CI, 17.5 to 25.9). The median follow-up time was 63.1 months for the living patients at the end of July, 2008. After preoperative chemoradiotherapy, 116 patients underwent grossly completed surgical resection of the primary tumor. However, 7 resections among them turned out to be microscopically margin positive (R1 resection). The remaining 38 patients could not undergo resection because of disease progression. Diabetes, tumor size larger than 2.0 cm, a higher serum level of CA19-9 at diagnosis, progressive disease on tumor response, no resection, poor differentiation, lymph node metastasis, and ID01-341 trial were significantly associated with reduced OS (Table 2).

Genotype Frequency and Association with OS

Eight SNPs of interest were successfully amplified in 95.5%–100% of the samples. Genotype frequencies of all 8 SNPs were found to be in Hardy-Weinberg equilibrium (χ^2 = 0.0003–3.78; P >.05). No significant racial difference in genotype frequency was observed (data not shown).

The genotype frequencies, MSTs, and hazard ratios (95% CI) are shown in Table 3. Two SNPs, i.e. *MRP5* A-2G and M*RP2* G40A, showed a significant or borderline significant association with OS in log-rank test (P = .010 and .097, respectively). The HRs (95% CI) of *MRP5* A-2G and *MRP2* G40A was 1.65 (1.11-2.45) and 1.55 (1.02-2.36). The MSTs of *MRP5* A-2G AG/GG and AA genotype were 28.1 and 18.4 months, respectively. In addition, a combined genotype effect of *MRP5* A-2G and M*RP2* G40A were observed. As the number of at-risk alleles increased, the OS decreased; patients carrying 0 (n = 39) or 1-2 (n = 112) at-risk alleles had median OS times of 34.0 and 20.7 months (P = .006, Table 3), as well as 5-year survival rate of 41.3% and 20.0% in Kaplan-Meier plot (Fig. 1).

Multivariate analysis on OS

We performed multivariate analysis including both genotypes of *MRP2* G40A and *MRP5* A-2G by adjusting for significant clinical predictors for OS. In this analysis, *MRP*5 -2AA genotype remained as an independent predictor for reduced OS (HR = 1.56, 95% CI = 1.05-2.34, P = .029, Table 4). Because tumor resection was the strongest predictor for OS (HR = 7.56, 95% CI = 4.19-13.6, P = <.001) and 75% of the patients had tumor resection in this study, we further analyzed the genotype effect on survival among 116 patients with resected tumor only (Table 4). Both genotypes of *MRP2* G40A and *MRP5* A-2G were significant independent predictors for OS with the HR (95% CI) of 1.99 (1.15-3.45, P = .015) and 1.88 (1.16-3.06, P = .011), respectively. The FPRP for these findings was 0.078 and 0.053, respectively, indicating noteworthiness.

Genotype Effects on Response to Therapy

None of the genotypes was associated with tumor response in radiological evaluation (data not shown). However, the *MRP2* G40A GG genotype, which was associated with a reduced survival, had a significant association with poor response to therapy in histological evaluation of the resected tumor (P = .028, Table 5). Thirty (47.6%) of the *MRP2* 40 GG carriers compared with 13 (27.1%) of the AG/AA carriers had a grade I or IIA response, i.e. >50% of tumor cells were viable in the resected tumor. The odds ratio (95% CI) was 2.45 (1.09-5.48).

Discussion

In this study, we evaluated the association between 8 SNPs of drug resistance genes and clinical outcomes of patients with potentially resectable pancreatic adenocarcinoma. We demonstrated that the genotypes of *MRP5* A-2G and *MRP2* G40A had a significant association with OS. The *MRP2* G40A GG genotype carriers also had a poorer histological response to preoperative gemcitabine-based chemoradiotherapy. These observations support a role of drug resistance-associated genes in cellular sensitivity to gemcitabine-based therapy and, as the result, survival of pancreatic cancer patients.

It has been shown that nucleotide analogs and cyclic nucleotides are substrates for MRP5 and MRP4,[20] and, particularly, gemcitabine was reported to be a typical substrate for MRP5 efflux pump.[21] One study showed a significant association between expression level of MRP5 and gemcitabine sensitivity in non-small cell lung cancer cell line.[22] Another study demonstrated that HEK293 cells overexpressing the human MRP5 protein are 2-fold more resistant to gemcitabine compared to vector control cells.[21] Because this study population received preoperative chemoradiotherapy, we did not think it was appropriate to examine the gene expression profiles in resected tumors. However, a previous study has shown that MRP5 mRNA level was significantly higher in pancreatic carcinoma tissue compared to normal pancreatic tissue,[3] suggesting that overexpression of MRP5 could contribute to drug resistance in pancreatic cancer. Although many SNPs of the *MRP5* gene have been reported, [23] the functional significance of the vast majority of these SNPs is still unclear.[24] The *MRP5* A-2G (Q382Q) is a synonymous SNP that does not produce altered coding sequences and amino acid substitution. However, a previous study has demonstrated that a synonymous SNP in the *MDR*1 gene results in a protein product with altered drug and inhibitor interactions.[25] Thus, the functional consequence by this SNP is warranted to be investigated. It is also possible that this SNP is in linkage disequilibrium with other functional SNPs of this gene or some important genes in this chromosome location. Further investigations including haplotype analysis will help to determine how this SNP is functionally associated with the gemcitabine sensitivity and survival in pancreatic cancer patients.

MRP2 has been reported to involve in exporting not only bilirubin and certain drug glucuronides but also anticancer drug (4). This includes cisplatin, doxorubicin, etoposide, and methotrexate.[26,27] We observed that *MRP2* G40A genotype has a relatively weak association with survival and tumor response to gemcitabine therapy in the entire study population. However, this association diminished when the 84 patients receiving cisplatin induction therapy was analyzed separately (data not shown). Gemcitabine is not thought to be a typical substrate of MRP2.[28] Therefore, MRP2 might play an indirect role in the sensitivity to gemcitabine and cisplatin combination therapy.

Although *MDR1* T-55C and *BCRP* C43A were reported to be functional SNPs that change their protein functions,[25,29] none of these SNPs and *MRP1*, *MRP3*, and *MRP4* gene SNPs examined in this study had any association with overall survival and tumor response to therapy, suggesting that these genes are probably not the most important determinants in cellular response to gemcitabine-based chemoradiotherapy. However, it is also possible that the limited number of SNPs selected for this study missed the most important functional variants of these genes. Further investigation is required to illustrate the role of these genes in clinical outcome of pancreatic cancer.

In the same study population, we have previously shown significant associations of SNPs of the DNA homologues recombination repair genes,[30]DNA damage response genes,[31] DNA mismatch repair genes,[32] and gemcitabine metabolic genes[33] with clinical outcome of pancreatic cancer. The current study has identified another important genetic factor that is

associated with drug sensitivity and patient survival. Our results need to be confirmed in other study populations. The ultimate goal of this research is to generate a genetic profile that can be used to predict response to preoperative gemcitabine-based therapy and to predict prognosis in patients with potentially resectable pancreatic cancer. If the genetic markers are established and validated, such markers may help with choice of therapy and patient stratification in future individualized cancer treatment.

Condensed Abstract

In this study, we demonstrated that polymorphic variants of drug resistance genes, MRP5 and MRP2, are associated with tumor response to gemcitabine-based chemoradiotherapy and overall survival in patients with potentially resectable pancreatic cancer. This information has the potential to help with treatment selection for individualized therapy of pancreatic cancer.

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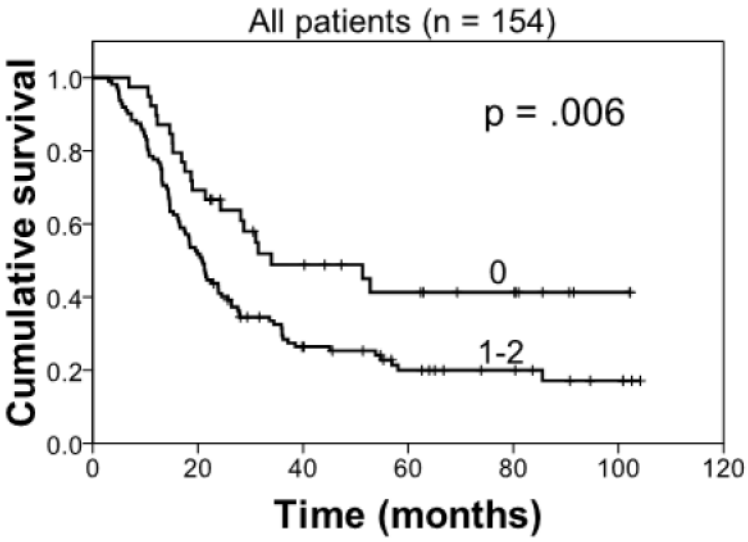

Figure 1.
Kaplan-Meier plot of overall survival by combined genotype effect of *MRP2* 40GG and *MRP5* -2AA. The numbers of 0–2 indicate the number of deleterious alleles associated with reduced survival. *P* value was from log-rank test.

Table 1

SNPs evaluated

Gene	Chromosome	SNP	Function	RS No.	Minor allele frequency observed*	Minor allele frequency reported†
MDR1	7q21.12a	Ex27 −55T>C	I1145I	1045642	0.49	0.47
MRP1	16p13.11a	Ex28 36G>A	S1219S	2239330	0.28	0.24
MRP2	10q24.2c	Ex10 40G>A	V417I	2273697	0.25	0.23
		Ex28 -16C>T	I1324I	3740066	0.35	0.35
MRP3	17q21.33b	Ex26 -13C>T	H1314H	2277624	0.24	0.17
MRP4	13q32.1a	Ex8 40A>G	R317R	2274406	0.39	0.37
MRP5	3q27.1b	Ex10 -2A>G	Q382Q	7636910	0.35	0.39
BCRP	4q22.1b	Ex5 43C>A	Q141K	2231142	0.12	0.10

Abbreviations: SNP, single-nucleotide polymorphism; RS No., reference SNP identification number.

*The data observed in current study.

†The reported minor allele frequency (Caucasian) was from SNP500 cancer database.

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Table 2

Patient characteristics and overall survival (n = 154)

Variable	No. of patients	No. of deaths	MST (months)	Log-Rank *p*	HR* (95% CI)	p
Age (years)				.098		
≤50	17	16	18.5		reference	
51-60	44	27	36.0		0.52 (0.28-0.97)	.039
61-70	60	41	21.5		0.64 (0.36-1.15)	.134
>70	33	27	21.2		0.89 (0.48-1.65)	.701
Sex				.369		
Male	96	70	20.9		reference	
Female	58	41	24.5		0.84 (0.57-1.23)	.370
Race				.704		
White	133	97	23.9		reference	
Hispanic	10	7	18.2		0.95 (0.44-2.06)	.902
African American	7	4	33.6		0.75 (0.27-2.03)	.565
Other	4	3	10.7		1.79 (0.56-5.70)	.322
Diabetes status				.017		
Negative	109	74	27.6		reference	
Positive	45	37	18.2		1.61 (1.08-2.40)	.019
Tumor size (cm)				.010		
≤2	65	40	34.0		reference	
>2	89	71	20.7		1.66 (1.13-2.45)	.011
CA19-9 (units/mL)				.001		
≤47	40	21	52.8		reference	
48-500	78	58	22.5		1.83 (1.11-3.01)	.018
501-1,000	14	12	18.4		3.03 (1.48-6.22)	.002
>1,000	22	20	15.3		2.90 (1.57-5.36)	.001
Tumor response				<.001		
PR/SD	126	85	27.8		reference	
PD	27	25	10.3		4.35 (2.74-6.91)	<.001
Tumor resection				<.001		

Variable	No. of patients	No. of deaths	MST (months)	Log-Rank *p*	HR* (95% CI)	p
Yes	116	73	34.0		reference	
No	38	38	10.5		9.28 (5.78-14.9)	<.001
Tumor grade				.017		
Well-to-moderate	88	57	33.6		reference	
Poor	32	25	19.8		1.76 (1.10-2.82)	.019
Lymph node metastasis				.009		
Negative	61	33	51.3		reference	
Positive	55	40	26.4		1.85 (1.16-2.94)	.010
Histological effect †				.259		
Grade I/IIA (>50%)	70	43	27.9		reference	
Grade IIB/III(≤50%)	43	30	37.1		1.31 (0.82-2.09)	.261
Clinical Protocol				.026		
ID98-020 (GEM/RT)	70	49	28.1		reference	
ID01-341 (GEM/Cisplatin/RT)	84	62	18.4		1.54 (1.05-2.26)	.028

Abbreviations: MST, median survival time; PR, partial response; SD, stable disease; PD, progressive disease; GEM, gemcitabine; RT, radiation therapy

*Crude hazard ratio.

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

Table 3

Overall survival by genotype

Genotype	No. of Patients	No. of Deaths	MST ± SE (months)	Log-Rank *P*	HR* (95% CI)	P
MDR1 T-55C						
CC	45	32	23.8 ± 5.3			
CT	64	46	21.5 ± 3.3			
TT	43	31	21.7 ± 3.4	.982		
CC vs. TT/CT				.869	1.24 (0.81-1.90)	.317
MRP1 G36A						
GG	81	64	21.5 ± 2.4			
AG	60	38	23.9 ± 9.3			
AA	13	9	21.4 ± 6.1	.247		
AA/AG vs. GG				.136	1.03 (0.69-1.53)	.890
MRP2 G40A						
GG	86	67	21.2 ± 2.6			
AG	55	34	31.0 ± 7.1			
AA	10	7	17.5 ± 4.8	.172		
AG/AA vs. GG				.097	1.55 (1.02-2.36)	.040
MRP2 C-16T						
CC	63	44	23.9 ± 4.2			
CT	69	49	20.7 ± 4.0			
TT	19	15	22.5 ± 2.2	.962		
CC vs. TT/CT				.785	1.13 (0.75-1.69)	.557
MRP3 C-13T						
CC	89	67	21.4 ± 2.3			
CT	47	32	28.7 ± 7.0			
TT	13	8	23.9 ± 10.9	.307		
CT/TT vs. CC				.129	1.10 (0.72-1.67)	664
MRP4 A40G						
GG	54	34	26.4 ± 8.1			
AG	76	58	21.7 ± 2.4			

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Genotype	No. of Patients	No. of Deaths	MST ± SE (months)	Log-Rank *P*	HR* (95% CI)	P
AA	22	17	14.6 ± 2.6	.172		
GG/AG vs. AA				.158	1.29 (0.75-2.23)	357 .
MRP5 A-2G						
AA	68	54	18.4 ± 3.0			
AG	62	38	31.0 ± 4.2			
GG	22	17	20.7 ± 7.0	.017		
AG/GG vs. AA				.010	1.65 (1.11-2.45)	.013
BCRP C43A						
CC	116	83	23.9 ± 3.3			
AC	27	19	21.2 ± 0.8			
AA	4	3	20.4 ± 10.0	.960		
AA/AC vs. CC				.960	1.05 (0.65-1.72)	.834
No. of deleterious alleles†				.006		
0	39	21	34.0 ± 12.0		reference	
1-2	112	87	20.7 ± 1.5		2.18 (1.32-3.60)	.002

Abbreviations: MST, median survival time; SE, standard error; HR, Hazard ratio

*HR was adjusted for history of diabetes, tumor size, serum level of CA 19-9, tumor response, and tumor resection.

†*MRP2* 40GG and *MRP5* −2AA.

Table 4

Multivariate Cox regression analysis of genotypes on OS

Covariate	Patients			
	All (n = 154)		Resected (n = 116)	
	HR [*](95% CI)	P	HR [†](95% CI)	P
MRP2 G40A				
AG/AA	reference		reference	
GG	1.48 (0.97-2.27)	.068	1.99 (1.15-3.45)	.015
MRP5 A-2G				
AG/GG	reference		reference	
AA	1.56 (1.05-2.34)	.029	1.88 (1.16-3.06)	.011

[*] HR was adjusted for history of diabetes, tumor size, serum level of CA19-9, tumor response, tumor resection, and genotype.

[†] HR was adjusted for history of diabetes, tumor size, serum level of CA19-9, tumor response, and genotype.

Table 5

Association between Genotypes and histological effect of chemoradiotherapy

Variable	Histological effect						
	Grade IIB/III		Grade I/IIA				
	No.	(%)	No.	(%)	*P* (X^2)	OR* (95% CI)	P
MRP2 G40A					.028		
AG/AA	35	(72.9)	13	(27.1)		reference	
GG	33	(52.4)	30	(47.6)		2.45 (1.09-5048)	.030

*Crude odds ratio