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Single Nucleotide Polymorphisms of RecQ1, RAD54L and ATM Genes Are Associated With Reduced Survival of Pancreatic Cancer

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Abstract

PURPOSE—Our goal was to determine whether single nucleotide polymorphisms (SNPs), in DNA repair genes influence the clinical outcome of pancreatic cancer.

PATIENTS AND METHODS—We evaluated 13 SNPs of eight DNA damage response and repair genes in 92 patients with potentially resectable pancreatic adenocarcinoma. All patients were treated with neoadjuvant concurrent gemcitabine and radiotherapy with or without a component of induction gemcitabine/cisplatin at the M.D. Anderson Cancer Center during February 1999 to August 2004 and followed up to August 2005. Response to the pretreatment was assessed by evaluating time to tumor progression and overall survival. Kaplan-Meier plot, log-rank test and Cox regression were used to compare survival of patients according to genotype.

RESULTS—The *RecQ1* A159C, *RAD54L* C157T, *XRCC1* R194W, and *ATM* T-77C genotypes had a significant effect on the overall survival with a log rank *P* values of 0.001, 0.004, 0.001 and 0.02, respectively. A strong combined effect of the four genotypes was observed. Patients with none of the adverse genotypes had a mean survival time of 62.1 months, and those with 1, 2, or ≥ 3 at-risk alleles had median survival times of 27.5, 14.4, and 9.9 months, respectively ($P < 0.001$, log rank test). There is a significant interaction between the *RecQ1* gene and other genotypes. All four genes except *XRCC1* remained as independent predictors of survival in multivariate Cox regression models adjusted for other clinical predictors.

CONCLUSION—These observations support the hypothesis that polymorphic variants of DNA repair genes affects clinical prognosis of patient with pancreatic cancer.

INTRODUCTION

Pancreatic cancer is a rapidly fatal disease with a 5-year survival $< 5\%$ (1). Surgical resection offers the only potentially curative treatment. The 5-year survival rate for patients after tumor resection is 10% for node-positive and 30% for node-negative resections. Other known prognostic factors for surgical patients include tumor size, tumor grade, and tumor stage (2). The poor prognosis of pancreatic cancer is due to both its metastasis-prone and therapy-

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resistant nature, which is partially explained by the frequent genetic and epigenetic alterations described in this tumor (3). On the other hand, host variations in DNA repair or drug metabolism, may also influence clinical response to therapy and overall survival of the patients (4). Prognostic markers are needed to stratify patients on protocols and for use in clinical practice. Predictive markers identifying response to cytotoxic therapy would further potentially provide a means of individualizing treatment and could lead to a better understanding of resistance mechanisms of both standard and novel treatment strategies

Gemcitabine is a standard of care in pancreatic cancer, offering a slightly better overall survival than 5-fluorouracil for patients with locally advanced and metastatic tumors (5). Little is known about DNA repair pathways that may alter cytotoxicity or radiosensitivity of gemcitabine. Previous studies have shown that gemcitabine induced radiosensitization and cytotoxic effect of mitomycin C were absent in homologous recombination repair (HRR)-deficient cells, suggesting that HRR may play an important role in gemcitabine-mediated cell killing (6,7). HRR is also involved in the repair of DNA double-strand breaks (DSBs), which are the most common type of radiation lesions that lead to mammalian cell death (8,9).

Previous clinical studies have shown that individual variation in DNA repair capacity conferred by single nucleotide polymorphisms (SNPs) affects the clinical response to platinum-based cancer therapy and overall survival of patients (10-13). Because gemcitabine and radiation-induced DNA damage is most likely repaired through the BER and HRR pathways, we hypothesized that genetic variations in these pathways may affect sensitivity to gemcitabine and radiotherapy, thus overall prognosis. We tested this hypothesis in a relatively homogeneous population, i.e. 92 patients with potentially resectable pancreatic adenocarcinoma who had undergone neoadjuvant gemcitabine-based chemoradiation. We evaluated eight DNA repair genes, i.e. *RecQ1*, *RAD54L*, *ATM*, *XRCC1*, *XRCC2*, *XRCC3*, *LIG3*, and *LIG4*. We selected 13 SNPs of the eight genes on the basis of their known allele frequencies, functional significance or previous reports of association with cancer risk or clinical outcome.

PATIENTS AND METHODS

Patient Recruitment and Data Collection

The study involves 92 patients who, at the time of diagnosis, had potentially resectable adenocarcinoma of the head of the pancreas and had not received any treatment for pancreatic cancer. All patients were enrolled in one of two phase II clinical trials (ID98-020 and ID01-341) of pre-operative (neo-adjuvant) chemoradiotherapy at The University of Texas M.D. Anderson Cancer Center (M.D. Anderson) conducted sequentially from February 1999 to August 2004 and were followed up to August 31, 2005. These 92 patients represent the subset of patients enrolled in these clinical trials who consented for blood donation. Patients in the ID98-020 trial (n=43) had received gemcitabine-based chemoradiotherapy consisting of weekly gemcitabine (400 mg/m²) for 4 weeks and radiation (30 Gy in 10 fractions) for 2 weeks. Patients in the ID01-341 trial (n=49) had received induction therapy of gemcitabine (750 mg/m²/day) and Cisplatin (30 mg/m²/day) every 2 weeks for 4 weeks followed by chemoradiotherapy with weekly gemcitabine (400 mg/m²) for 4 weeks and radiation (30 Gy in 10 fractions) for 2 weeks. Clinical information was collected from the medical records with the patients' consent. Clinical stage was defined based on the initial computed tomography imaging and defined as stated in the AJCC Cancer Staging Manual using the TNM staging system (14). Pathologic stage was determined after surgery, in those patients who underwent a successful resection of their primary tumor, and also used the TNM staging system (14). Tumor progression was defined as local tumor progression, tumor recurrence, metastasis and death due to the disease. Patients without tumor progression at the end of the follow-up were censored. The pre-operative treatment effect was evaluated histologically in resected tumors according to previously published criteria (15), i.e. tumors with >90% viable carcinoma cells were defined as treatment

effect grade I, 51-90% as grade IIA, 10-50% as grade IIB, and <10% as grade III. Post-surgical treatment or treatment received after tumor recurrence were not considered in this study.

DNA Extraction and Genotyping

Whole blood was collected from patients at the time of enrollment and DNA was extracted from peripheral lymphocytes using a DNA Isolation kit (Qiagen, Valencia, CA). Polymorphisms were detected using the Masscode technique (BioServe, Laurel, MD). About 10% of the samples were analyzed in duplicate, and discrepancies were seen in less than 0.1% of the samples. Those with discordant results from two analyses were excluded from the final data analysis. The genes, chromosome locations, nucleotide substitutions, amino acid changes, reference SNP identification numbers and reported allele frequencies of the 13 SNPs evaluated in this study are summarized in Table 1.

Survival Measurements

Dates of death were obtained and crosschecked using at least one of the following sources: the Social Security Death Index, inpatient medical records, or the M. D. Anderson Cancer Center tumor registry. Time to progression and overall survival time was calculated from the date the patient had enrolled in the trial to the date tumor progression was first recorded, or the date of death or last follow-up, respectively. When patients with tumor resection were analyzed as a separate group, overall survival time was calculated from the date of tumor resection to date of death or last follow up.

Statistical Methods

The distribution of genotypes was tested for Hardy-Weinberg equilibrium with the goodness-of-fit χ^2 test. The association between overall survival time and time to disease progression with genotypes was estimated using the method of Kaplan and Meier and assessed using the log-rank test. Median follow-up time was computed with censored observations only. Median survival time (MST) was calculated, and mean survival time was presented when MST could not be calculated. Hazard ratios and 95% confidence intervals (CIs) were estimated using univariate or multivariate Cox proportional hazard models. Known or potential clinical prognostic factors such as tumor size, tumor grade, tumor stage, the effects of preoperative treatment on tumors, as well as serum CA19-9 values at diagnosis were included in the multivariate model when appropriate. Gene-gene interactions were determined by generating a cross-product term and tested by using the likelihood ratio test. All statistical testing was conducted with SPSS software, version 12.0 (SPSS, Chicago, IL), and statistical significance was defined as $P \leq 0.05$.

RESULTS

Patient Characteristics and Survival Analysis

The median age of the 92 patients was 65 years (range, 38 to 83 years). The patient characteristics and clinical features of the tumor are summarized in Table 2. The median (range) time interval from the initial pathological diagnosis to enrollment in the trial was 16 (1 to 91) days. Of the 92 cases, 62 had their primary tumor surgically resected after pre-operative treatment; the remaining 30 patients were not resected due to disease progression found at restaging after preoperative therapy (23 patients) or at the time of operation (7 patients). All 62 patients who underwent pancreatic resection had a grossly complete resection, however, pathologic evaluation of the surgical specimens demonstrated a microscopically positive margin (R1 resection) in 7 (11.3%) of the 62 patients.

There were 57 deaths (62.0%) among the 92 cases. The median follow-up time was 33 months for the living patients. The MST of the 92 patients was 20.3 months (95% CI: 14.7–25.9). The tumor size (>1 cm), tumor with poor differentiation, serum level of CA19-9 at diagnosis >1000 units/ml, tumor not resected, and node positive resection (Stage IIB) were significantly associated with reduced overall survival (Table 2).

Genotype Frequency and Effects on Overall Survival

The 13 SNPs were successfully amplified in 91% to 99% of the patients. Genotype frequencies of all 13 SNPs were found to be in Hardy-Weinberg equilibrium ($\chi^2 = 0.03$ – 1.22 , $P > 0.1$) except *RecQ1* A159 ($\chi^2 = 4.22$, $P = 0.04$). There was a significant difference in the ethnic distribution of the *ATM* E126D SNP, with a higher frequency of the variant allele in Hispanics (20%) and blacks (33%) than in whites (1%) ($P = 0.02$, Fisher exact test). The genotype frequencies and the MST by each genotype are shown in Table 3. Of the 13 SNPs evaluated, four showed a significant effect on overall survival. The strongest genetic effect on survival was observed for the *RecQ1* A159C, with MSTs of 18.9 and 13.1 months for the AC and CC genotypes, respectively, compared with a mean survival time of 46.9 months for the AA wild-type (P value of the log-rank test [P_{LR}] = 0.004). At the end of the study, about 62% of the AA genotype carriers were still alive, compared with 25% of the AC and 26% of the CC genotype carriers (Fig. 1). The *RAD54L* C157T, *ATM* T-77C and *XRCC1* R194W genotypes also showed significant effects on survival (Table 2 and Fig. 1). Other SNPs, such as the *XRCC2* R188H and *ATM* D126E genotypes, had a mild effect on survival, but the differences did not reach the level of statistical significance. Neither of the two *XRCC3* SNPs nor the *LIG3* and *LIG4* genotypes had any significant effects on overall survival.

Combined Genotype Effects

A strong gene-dosage effect was observed when the *RecQ1* 159 AC/CC, *RAD54L* 157 CT/TT, *XRCC1* 199W, and *ATM*-77TC genotypes were analyzed in combination. Patients with none of the adverse genotypes had a mean survival time of 62.1 months, whereas those with one, two, or more at-risk alleles had MSTs of 27.5, 14.4, and 9.9 months, respectively ($P_{LR} < 0.001$) (Table 3 and Fig. 2). Further analysis revealed a significant interaction between the *RecQ1* A159C genotype and the remaining three SNPs. A strong protective effect of the *RecQ1* 159AA genotype was observed. In the presence of the AA genotype, the overall survival time was 62.1 (mean), 40.3 (mean), and 10.3 (median) months for those with zero, one, or two adverse genotypes of the *RAD54L*, *ATM*, and *XRCC1* SNPs, respectively ($P_{LR} < 0.001$). Among patients with the *RecQ1* 159 AC/CC genotypes, however, the MSTs were 20.3, 14.6, and 9.9 months, respectively, if they also had zero, one, or two adverse genotypes of the other three genes ($P_{LR} = 0.17$). The likelihood ratio P value for the statistical interaction between *RecQ1* and the number of other adverse genotypes was 0.003.

Genotype Effects on Two-Year Survival and Time to Tumor Progression

Two-year survival was evaluated among 72 patients and the survival rate was 39%. *RecQ1* 159 AC/CC and *ATM* -77 TC genotypes were significantly associated with a reduced two year survival rate, 21% and 15%, respectively (Table 4). The odds ratio (95% CI) of dying was 7.5 (2.5–22.9) and 6.4 (1.9–22.1) for the two adverse genotypes, respectively. Patients with two or more adverse genotypes of the *RecQ1*, *RAD54L*, *ATM* and *XRCC1* genes had a 2-year survival rate of 8% only (OR: 20.7, 95% CI: 4.1–105, $P < 0.001$). The *RecQ1* A159C and *ATM* T-77C variant genotypes were also significantly associated with a shorter time to disease progression (Table 4).

Cox Regression Models

Finally, we conducted a multivariate analysis of the effects of genotype on survival using Cox proportional hazards models adjusted for other clinical factors. As shown in Table 5, *RecQ1* A159C, *RAD54L* C157T, and *ATM* T-77C genotypes remained significant independent predictors of survival among all patients regardless of their surgical status. The hazard ratios (95% CI) for the *RecQ1* 159 AC/CC, *RAD54L* 157 CT/TT, and *ATM*-77TC genotypes were 2.7 (1.3–5.8), 2.7 (1.3–5.4), and 2.0 (1.0–4.0), respectively, after adjusting for tumor size and CA19-9 level. Tumor grade was not included in this model or in the model for patients with non-resected tumors because information on tumor grade was missing from 28% of the 92 patients. Among patients who had undergone tumor resection, the *RecQ1* and *RAD54L* genotypes remained as significant independent predictors for survival after adjusting for other clinical factors (Table 5).

DISCUSSION

In this study, we evaluated the effect of 13 SNPs of eight DNA repair genes on the overall survival of patients with resectable pancreatic adenocarcinoma who had received preoperative gemcitabine-based chemoradiotherapy. We demonstrated that the *RecQ1* A159C variant genotype, both alone and in combination with other genes, was associated with significantly decreased overall survival in this patient population. The *RecQ1* 159 AA genotype displayed a strong protective effect and a significant interaction with other genotypes. Three SNPs of the *RAD54L*, *ATM*, and *XRCC1* genes were also found to significantly affect overall survival.

The *RecQ1* gene belongs to the DNA helicase family, which includes four additional members—*WRN*, *BLM*, *RecQ4*, and *RecQ5* (16). RecQ helicases are important tumor suppressors that are not only involved in DNA HRR but also in S phase checkpoint and telomere maintenance (17,18). Of the five human RecQ helicases identified, three are associated with genetic disorders characterized by an elevated incidence of cancer or premature aging: Werner, Bloom, and Rothmund-Thomson syndrome (19). Although the biological significance of the *WRN* and *BLM* helicases has been extensively investigated, less information is available concerning the functions of the other human RecQ helicases. *RecQ1* is known to be able to unwind a diverse set of DNA substrates (20,21), to catalyze efficient strand annealing between complementary single-stranded DNA molecules and to interact with several important factors required for DNA mismatch repair (22). It is possible that a deficient *RecQ1* gene may confer an aggressive tumor phenotype through rapid accumulation of genetic alterations and genomic instability. On the other hand, a deficient *RecQ1* function may confer a higher cellular sensitivity to genotoxic stress, thus a better clinical response to cytotoxic therapy. It is tempting to speculate that *RecQ1* may play a role in resolving gemcitabine-induced DNA replication arrest. Because the functional significance of the *RecQ1* SNPs investigated in the current study is currently unknown, the biologic interpretation of the data is difficult. The *RecQ1* A159C SNP is located on the 3'-untranslated region of the gene and exerted its effect in a dominant-inheritance mode, i.e., one variant allele is required to alter the chance of survival. It is possible that this SNP may affect translation efficiency and mRNA stability, or it may be in linkage disequilibrium with other SNPs of the same gene or other important genes located in the same chromosome region. Further investigations to confirm our observations in other patient populations as well as on the biological functions of this SNP and this gene are warranted.

RAD54L belongs to the DEAD-like helicase superfamily (19), and it plays a role in the HRR of DNA DSBs. *RAD54L* has been proposed as a candidate tumor suppressor gene in tumors with a nonrandom deletion of chromosome 1p32 (23–25). The *RAD54L* C157T SNP has previously been associated with increased risk of meningioma (25). The variant allele of this SNP was associated with a significantly reduced overall survival in the current study, which could be related to a better repair of DNA DSBs and thus poorer clinical response to therapy.

However, functionally, the C157T SNP is a silent polymorphism (Ala730Ala) that does not induce any amino acid change. We can only speculate that the observed effect on survival was related to linkage disequilibrium of this SNP with other SNPs of the same gene or with other genes on the same chromosome. A haplotype analysis will help to explain this observation.

The *ATM* gene encodes a protein kinase that plays a key role in the detection and repair of DNA DSBs and in maintaining genome integrity (26,27). A previous study has shown that polymorphic variants of the *ATM* gene are associated with increased in vitro chromosomal radiosensitivity (28). Another study showed that the *ATM* -77 TC heterozygote was associated with a reduced radiosensitivity among patients with breast cancer compared with the TT homozygote (29). Interestingly, we observed significantly reduced survival time among TC heterozygote carriers relative to that among TT and CC homozygote carriers, which is consistent with a poor response to radiotherapy for the TC heterozygote. Because the T-77C SNP is located in an intron region, it is conceivable that its functional difference may be mediated by affecting RNA splicing. However, to date, no functional studies have been reported linking altered protein function or cellular phenotype with the presence of this SNP. Further analysis of the *ATM* haplotypes is underway.

The *XRCC1* protein plays an important role in BER (30,31). The *XRCC1* 194W allele has been previously associated with a reduced risk of cancer but has not been evaluated as a predictor of clinical outcome (32). In the current study, we observed an association between the 194W allele and a shorter overall survival time, even though this effect disappeared after other clinical and genetic factors had been adjusted for (Table 5). The Q399R genotype has been shown to affect clinical response to platinum-based cancer therapy (13), but we did not observe any significant effect of this SNP on clinical outcome in the current study. The weak effect or lack of effect of the *XRCC1* gene SNPs observed in the current study are consistent with those of a previous report that the BER pathway does not significantly affect the repair of gemcitabine-induced DNA damage and cell killing (6).

XRCC2 and *XRCC3* are *RAD51* paralogs that play mediating roles in homologous recombination. Ligase IV and ligase III are components of the NHEJ machinery required for DNA replication and repair (33,34). None of the SNPs of these genes had any significant effect on patient survival or other clinical parameters in the current study, suggesting a minor role of these genes in clinical response to gemcitabine-based chemoradiation in patients with resectable pancreatic cancer.

This study is limited by its small size and some of the observations maybe made by chance alone. However, the remarkable effect these genotypes have on the overall survival of this relatively homogeneous patient population and the gene dosage effects observed in this study warrant further confirmation in a larger study. If confirmed, such information may provide opportunities for discovery of novel therapeutic targets and genetic profiles that can direct the choice of therapy and predict the treatment tolerance, response, and overall outcome.

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Figure 1.

Overall survival curves by genotypes. P values of the log rank test were 0.02 and 0.004 for *RecQ1* AC or CC versus AA genotype; 0.16 and 0.002 for *RAD54L* CT or TT versus CC genotype; 0.04 and 0.001 for *XRCC1* CT or TT versus CC genotype; and 0.009 and 0.85 for *ATM* TC or CC versus TT genotype.

Figure 2.

Combined effect of *RecQ1* 159 AC/CC, *RAD54L* 157 CT/TT, *XRCC1* 194CT/TT, and *ATM*-77 TC on overall survival (upper panel). The number of 0–3 indicates number of adverse genotypes. $P(LR) = 0.03, 0.007, \text{ and } 0.001$ for patients carrying 1, 2 or 3 adverse genotypes compared with those having none. Combined effect of *RAD54L*, *XRCC1* and *ATM* genotypes in patients carrying the *RecQ1* 159 AA genotype (middle panel) or the *RecQ1* AC/CC genotypes (lower panel). Note the strong protective effect of *RecQ1* AA genotype on survival. The likelihood ratio P value for the statistical interaction between *RecQ1* and the number of other adverse genotypes was 0.0035.

Table 1

SNPs evaluated in this study

Gene	Chromosome	SNP	Nucleotide change	RS# ^a	Allele Frequency ^b
<i>RECQL</i>	12p12	3'UTR EX15+159 IVs3-86	A to C A to G	13035 4987216	0.28 0.02
<i>RAD54L</i>	1p32	A730A ex18+157	C to T	1048771	0.12
<i>ATM</i>	11q22-q23	IVS22-77 D1853N, Ex38+61 D126E, Ex6+47	T to C A to G A to T	664677 1801516 2234997	0.29 0.92 0.94
<i>XRCC1</i>	19q13.2	R194W Q399R	C to T A to G	1799782 25487	0.13 0.66
<i>XRCC2</i>	7q36.1	R188H	G to A	3218536	0.05
<i>XRCC3</i>	14q32.3	T241Met IVs7-14	C to T A to G	861539 1799796	0.22 0.29
<i>LIG3</i>	17q11.2-q12	IVs18-39	G to A	2074522	0.10
<i>LIG4</i>	13q33-q34	5'UTR Ex2+54, T9I	C to T	1805388	0.13

^aRS#: reference SNP identification number.^bAllele frequencies were all from the National Cancer Institute SNP500Cancer database.

Table 2

Patients' characteristics and clinical features

Characteristics	No. of cases	(%)	No. of deaths	MST (months)	P (LR)
Age (years)					
≤50	12	(13)	6	20.3	0.85
51–60	23	(25)	16	16.1	
61–70	29	(32)	17	18.9	
>70	28	(30)	18	22.7	
Sex					
Male	52	(56)	33	16.5	0.38
Female	40	(44)	24	22.7	
Race					
White	83	(90)	52	20.3	0.43
Hispanics	5	(5)	3	18.2	
Black	3	(3)	1		
Other	1	(1)	1	10.7	
Diabetes					
No	69	(75)	40	22.5	0.20
Yes	23	(25)	17	16.9	
Smoking					
Never	27	(29)	15	24.5	0.46
Ever	65	(71)	42	18.4	
CA19-9 level (units/ml)					
≤47	22	(24)	12	24.5	0.05
48–500	44	(48)	23	21.7	
501–1000	10	(11)	8	23.9	
>1000	16	(17)	14	15.3	
Tumor size (cm)					
≤1	24	(26)	11	44.8 ^a	0.06
1.1–2.5	38	(41)	28	16.1	
>2.5	32	(34)	18	24.3	
Tumor resection					
Yes	55	(60)	31	28.7	<0.001
No	37	(40)	26	10.3	
Stage after surgery					
0 or I	8	(11)	3	49.1 ^a	0.006
IIA	20	(34)	7	56.3 ^a	
IIB (node positive)	34	(55)	20	18.9	
Clinical protocol					
GEM/XRT	44	(48)	29	22.5	0.321
GEM/Cisplatin/XRT	48	(52)	28	16.1	
Tumor grade (differentiation)					
Well	3	(4)	1	48.4 ^a	0.01
Moderate	44	(67)	22	27.8	
Poor	19	(29)	14	15.3	
Treatment effect (viable cells)					
GI (>90%)	4	(7)	3	18.2	0.34
G2A (50–90%)	17	(30)	9	24.5	
G2B (10–49%)	27	(47)	14	24.3	
G3 (<10%)	9	(16)	2	60.4 ^a	

Table 3

Overall survival by genotype

Genotype	No. of cases	No. of deaths	MST ^a (months)	HR (95% CI) ^b	P	Genotype	No. of cases	No. of deaths	MST (months)
RecQ1 159						ATM-D1853N			
AA	34	13	46.9 ^a	Reference		GG	74	45	18.4
AC	32	24	18.9	2.5 (1.3–5.0)	0.008	GA	16	11	23.9
CC	19	14	13.1	3.8 (1.8–8.4)	0.001	AA	1	0	
AC/CC	41	38	16.1	2.8 (1.5–5.4)	0.001				
<i>P</i> _(LR) ^c			0.001(0.001)			ATM-E126D			
RAD54L						TT	88	54	21.4
CC	72	38	23.9	Reference		TA	3	2	6.2
CT	17	11	14.6	1.6 (0.8–3.0)	0.16	LIG3			
TT	1	1	5.4			GG	73	45	20.3
CT/TT			14.6	1.8 (1.0–3.3)	0.06	GA	19	8	18.9
<i>P</i> _(LR) ^c			0.004 (0.06)			LIG4			
ATM C77T						CC	69	43	18.9
TT	33	17	27.8	Reference		CT	20	12	22.7
TC	36	25	14.4	2.2 (1.2–4.2)	0.01	TT	1	1	
CC	15	10	27.5	1.2 (0.7–3.8)	0.69				
TC vs TT/CC				2.1 (1.2–3.7)	0.002	XRCC1-399			
<i>P</i> _(LR) ^c			0.018 (0.006)			AA	29	16	24.5
XRCC1-194						AC	48	32	14.8
CC	72	39	22.7	Reference		CC	14	9	21.4
CT	13	9	12.0	1.8 (0.9–3.7)	0.08	XRCC2 R188H			
TT	1	1	5.4			GG	79	48	21.4
CT/TT			10.7	2.0 (1.0–3.9)	0.04	GA	10	7	14.6
<i>P</i> _(LR) ^c			0.001 (0.04)			XRCC3 17893			
Combined						AA	43	25	22.5
0	8	1	62.1 ^a	Reference ^d		AG	38	23	16.9
1	32	17	27.5	Reference ^d		GG	8	6	18.2
2	24	19	14.4	3.6(1.8–7.2)	<0.001	XRCC3 T241M			
3	11	9	9.9	4.5(2.0–10.0)	<0.001	GG	40	25	21.7
<i>P</i> _(LR)			<0.001			GA	37	24	16.1
RecQ1 –86						AA	14	8	27.8
GG	87	54	21.4						
GA	2	2	16.1						

^aMean survival time was given when MST could not be calculated;^bHazard ratio and 95% CI from univariate Cox regression;^c*P*_(LR) in parenthesis is from comparisons of the combined heterozygous and homozygous variants with the wild type;^{b,c}Data of log rank test and Cox regression were not shown for genotypes when *P* values were >0.1;^dPatients with 0 or 1 adverse allele were combined as the reference group.

Table 4
Two-year survival and time to progressions by genotype

Genotype	Dead N (%)	Two-year survival		<i>P</i>	No. of events	Progression MTP (months) ^a	<i>P</i> _(LR)
		Alive N (%)	OR (95% CI)				
All cases	44 (61)	28 (39)			68	12.1	
RecQ1-159							
AA	9 (33)	18 (67)	1.0		18	25.2	
AC/CC	30 (69)	8 (21)	7.5 (2.5–22.9)	0.001	44	10.3	0.002
RAD54L							
CC	31 (56)	24 (44)	1.0		53	13.4	
CT/TT	11 (63)	4 (27)	2.1 (0.6–7.5)	0.38	13	10.9	0.44
ATM-77							
TT/CC	18 (46)	21 (54)	1.0		34	14.0	
TC	22 (85)	4 (15)	6.4 (1.9–22.1)	0.005	28	8.7	0.03
XRCC1-194							
CC	31 (57)	23 (43)	1.0		52	11.9	
CT/TT	10 (83)	2 (17)	3.7 (0.7–18.6)	0.11	11	4.3	0.06
Combined							
0/1	11 (37)	19 (63)	1.0		26	15.6	
2/3	24 (92)	2 (8)	20.7 (4.1–105) <0.001	29	8.2	0.001	

^aMTP, median time to progression.

Table 5
Hazard ratios and their 95% CIs for overall survival in multivariate Cox regression models

Variable	All Patients (n=75)			Non-resected (n=26)			Resected (n=46) ^a		
	HR	(95% CI)	P	HR	(95% CI)	P	HR	(95% CI)	P
<i>RecQ1</i> (AA vs AC/CC)	3.0	(1.4–6.3)	0.004	6.0	(1.1–39.9)	0.012	14.8	(1.8–124)	0.013
<i>RAD54L</i> (CC vs CT/TT)	2.9	(1.4–6.0)	0.004	6.8	(1.5–24.3)	0.035	28.3	(2.5–318)	0.007
<i>ATM</i> 77 (TT/CC vs TC)	1.9	(1.0–3.8)	0.054	3.6	(1.2–11.2)	0.025	3.7	(1.1–12.6)	0.037
<i>XRCC1</i> (CC vs CT/TT)	1.8	(0.8–4.0)	0.136	1.1	(0.4–3.4)	0.876	3.1	(0.7–14.3)	0.146
Tumor Size (≤1 vs. >1)	1.2	(0.6–2.2)	0.585	0.4	(0.1–1.3)	0.127	0.7	(0.2–1.7)	0.386
CA19-9 level (<1000 vs. ≥1000)	2.3	(1.0–5.0)	0.040	2.8	(0.9–8.6)	0.072	3.0	(0.5–8.9)	0.339
Clinical protocol (1 vs. 2)	1.6	(0.9–3.1)	0.132	1.4	(0.4–3.4)	0.876	0.8	(0.3–2.3)	0.667
Stage after surgery (I/IIA vs. IIB)	NA			NA			10.4	(2.5–42.5)	0.001
Treatment Effect (G1/G2A vs. G2B/G3)	NA			NA			0.2	(0.1–0.6)	0.004
Differentiation (well/moderate vs. poor)	NA			NA			0.4	(0.04–6.3)	0.520

^a Overall survival time was calculated from the date of tumor resection to date of death or last follow-up.