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# Significant Impact of Homologous Recombination DNA Repair Gene Polymorphisms on Pancreatic Cancer Survival

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## Abstract

Genetic variation in DNA repair may affect the clinical response to cytotoxic therapies. We investigated the effect of 6 SNPs of the *RecQ1, RAD54L, XRCC2,* and *XRCC3* genes on overall survival of 378 patients with pancreatic adenocarcinoma who were treated at UT M.D. Anderson Caner Center during February 1999 to October 2004 and were followed up to October 2005. Genotypes were determined using the MassCode method. Survival was determined from pathological diagnosis to death. Patients who were alive at the last follow-up evaluation were censored at that time. Kaplan-Meier plot, log-rank test and Cox regression were used to compare overall survival by genotypes.

A significant impact on survival of all patients was observed for *RecQ1* and *RAD54L* genes. The median survival time was 19.2, 14.7, and 13.2 months for the *RecQ1* 159 AA, AC and CC and 16.4, 13.3 and 10.3 months for *RAD54L* 157 CC, CT and TT genotypes, respectively. A significantly reduced survival was associated with the variant alleles of *XRCC2* R188H and *XRCC3* A17893G in subgroup analysis. When the 4 genes were analyzed in combination, an increasing number of adverse alleles were associated with a significantly decreased survival. Subgroup analyses have shown that the genotype effect on survival was present among patients without metastatic disease or among patients who receive radiotherapy. These observations suggest that polymorphisms of genes involved in the repair of DNA double strand breaks significantly affect the clinical outcome of patients with pancreatic cancer.

### Keywords

DNA repair; homologous recombination; single nucleotide polymorphism; pancreatic cancer; survival

## INTRODUCTION

DNA repair has been termed a double-edged sword because decreased DNA repair may increase the risk of developing cancer but improve survival in patients already diagnosed with cancer when treated with DNA damaging agents. Previous studies have shown that single nucleotide polymorphisms (SNPs) of nucleotide excision repair genes affected clinical outcome in patients treated with platinum-based therapy (1–5). Studies on the predictive and prognostic role of other DNA repair genes and clinical outcome are limited.

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Pancreatic cancer is usually diagnosed at a late stage of the disease and surgical intervention is not an option for the majority of patients. Pancreatic tumors are highly aggressive and resistant to most treatment. Gemcitabine (GEM) and radiotherapy (XRT) are the current main therapeutic modalities for advanced pancreatic cancer (6,7). However, other than stage it is not clear what factors influence the clinical response to such treatment.

GEM is a radiosensitizer. Unlike other radiosensitizers, which either increases radiation-induced DNA damage or decreases the rate of DNA repair, GEM neither increases double-strand breaks nor decreases the rate of their repair (8). GEM inhibits DNA synthesis via a process called masked chain termination. After insertion of the drug into the DNA strand, one or more nucleotide base pairs incorporate normally before the DNA chain elongation is terminated. This process locks GEM into the DNA strand and prevents proofreading enzymes from detecting, excising, and repairing the DNA lesion (9). Little is known about DNA repair pathways that may alter cytotoxicity or radiosensitivity with GEM. Studies in Chinese hamster ovary cell lines deficient in base excision repair, nucleotide excision repair, homologous recombination repair and non homologous end joining showed similar sensitivity to GEM as their parental repair proficient cells, indicating that these pathways are most likely incapable of modulating the cytotoxicity of GEM (10). However, another study has shown that GEM induces radiosensitization in base excision repair-deficient cells but not in homologous recombination repair deficient cells (11). GEM also induces substantial enhancement of the cytotoxic effect of mitomycin C in homologous recombination repair proficient cells but not in homologous recombination repair deficient cells (11). These observations suggest that homologous recombination repair may play an important role in GEM-mediated cell killing.

For tumor cells, the most important factor influencing radiosensitivity is the expression of oncogenes and tumor suppressor genes (12). Other factors that may also alter radiosensitivity include cell cycle regulation, detoxification and stress response, and DNA repair (13). Double-strand breaks in DNA are the most common type of radiation lesions that lead to mammalian cell death. Homologous recombination repair is a major pathway of double strand breaks repair in all eukaryotes (14) and GEM-mediated radiosensitization seems to depend on the homologous recombination repair mechanism (11). To test the hypothesis that genetic variations in homologous recombination repair affect clinical response to GEM/XRT therapy and in turn the prognosis of pancreatic cancer, we have selected 6 common SNPs of four homologous recombination repair genes, i.e. *RecQ1, RAD54L*, X-ray repair complementing (*XRCC) 2* and *XRCC3* and examined their association with overall survival of patients with pancreatic cancer.

RecQ1 is a member of the RecQ DNA helicase family, which is involved in recombination and in various types of DNA repair, including mismatch repair, NER and direct repair (15). Three of the five human *RecQ* genes, i.e *BLM, WRN*, and *RecQ4*, have been associated with genetic disorders of Bloom, Werner and Rothmund-Thomson syndromes, respectively, all of which are characterized by predisposition to malignancies and chromosome instability (16). RecQ helicases play multiple roles in DNA recombination and repair, S phase checkpoint, and telomere maintenance, thus are considered the caretakers of the genome and tumor suppressors (17,18). The biological significance of the remaining two RecQ genes, *RecQ1* and *RecQ5*, remains unknown. It has been suggested that *RecQ1* and *RecQ5* genes maybe indispensable for cell viability and may contribute to cancer susceptibility in general (17,18). *RecQ1* gene is located on chromosome 12p12, the same region harboring the *K-ras* gene. RecQ1 is known to be able to unwind a diverse set of DNA substrates (19,20), to catalyze efficient strand annealing between complementary single-stranded DNA molecules (20) and to interact with several important DNA repair factors required for DNA mismatch repair (21). Thus, it is possible that this gene plays a role in genetic predisposition to cancer and in response to cytotoxic therapy. We examined two the most prevalent SNPs of this gene, i.e. *RecQ1* 3′-UTR A157C (rs#13035)

and IVS3 A-86G (rs#4987276) in this study. Neither of the two polymorphisms has been previously been studied in association with disease risk or functional significance.

RAD54L belongs to the DEAD-like helicase superfamily (22) and it plays a role in homologous recombination repair of DNA double-strand breaks (23). The binding of this protein to double-strand DNA induces a DNA topological change, which is thought to facilitate homologous DNA paring, and stimulate DNA recombination (24). RAD54L gene is located at chromosome 1p32. A synonymous C157T (a.k.a. 2290C/T, A730A, rs#1048771) polymorphism of the *RAD54L* gene has been associated with increased risk for meningioma (25). Whether this gene contributes to cellular radiosensitivity is unknown.

XRCC2 and XRCC3 are members of the RecA/Rad51-related protein family that participate in HRR (26,27). XRCC2 is a core component in the homologous recombination repair pathway, and it forms a heterodimer with Rad51-like protein (28). High levels of aneuploidy, chromosome rearrangement, and haploinsufficiency have been observed in *XRCC2* gene knockout cells (29). XRCC3 has a remarkable, diverse set of functions and acts both early and late in the HRR pathway (30). A rare microsatellite polymorphism in this gene was found to be associated with clinical radiosensitivity in cancer patients (31). *XRCC2* and *XRCC3* gene is located on chromosome 7q36.1 and 14q32.3, respectively. We examined 3 polymorphisms of the two genes, i.e. *XRCC2* R188H (exon 3 G442A, rs#3218536), *XRCC3* A17893G (IVS7-14, rs#1799796), and *XRCC3* T241M (Exon 8 C-53T, rs#861539). The *XRCC2* R188H variant allele has been associated with reduced cell survival after mitomycin-C exposure (32). The *XRCC3* A17893G polymorphism resides in the intron region, and the functional significance of this SNP has not been investigated. The *XRCC3* T241M variant allele has been found to have a mild effect on cell survival after radiation exposure (33).

## MATERIALS AND METHODS

### Patient Recruitment

A total of 378 patients with pathologically confirmed pancreatic ductal adenocarcinoma of all stages were recruited prospectively at the University of Texas M.D. Anderson Cancer Center (Houston, TX) between January 1999 and October 2004. These individuals participated in a larger ongoing molecular epidemiologic study in which demographic (age, sex, and race) and risk factor information (smoking status, medical history, family history of cancer, and exposures) were collected by personal interview and a blood sample was collected for genotyping at the time of enrollment. The study was approved by the Institutional Review Board of M.D. Anderson.

For this analysis, all 378 patients were treated at M.D. Anderson between January 1999 and October 2004 and were followed up to October 20, 2005. We chose October 2004 as the last month of eligibility in order to have at least 12 months of follow-up for all patients. We chose patients treated at our institute, because information about patients treated elsewhere was often sparse, and sometimes patients treated outside were not given standard treatments or observed in standard fashions.

Clinical information was collected by reviewing the medical records of consenting patients. This included date of pathological diagnosis, treatment received before evaluation at M. D. Anderson, clinical tumor stage (localized, locally advanced, and metastatic), performance status at first visit to M.D. Anderson, serum carbohydrate antigen 19-9 (CA19-9) values (unit/ml) at diagnosis, surgical procedure and date, and pre- and postoperative chemotherapy regimen and radiation, date of death or last follow up.

### DNA Extraction and Genotyping

Whole blood was collected in heparinized tubes from patients at the time of enrollment into an on-going molecular epidemiology study examining pancreatic cancer risk. DNA was extracted from peripheral lymphocytes using the Qiagen DNA Isolation kit (Qiagen, Valencia, CA). Polymorphisms were detected using the Masscode™ technique by 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 no call rate was 6% for *RecQ1* A159C and <4% for the remaining 5 polymorphisms.

### Survival Measurements

Our primary end point was overall survival from the time of pathologic diagnosis to date of death or last follow up for all patients. Patients who were not deceased were censored at the last date they were known to be alive based on the date of last contact. Median follow-up time was computed among censored observations only. The minimum follow up time is 12 months and the maximum follow up time is 60 months. Dates of death were obtained and crosschecked using at least one of the following three methods: Social Security Death Index, inpatient medical records and the M. D. Anderson tumor registry. Date of death was obtained most often through the Social Security Death Index, but in unusual instances in which the patient's date of death was not reported there (either due to death within 3 months of the data collection or some other reason), date of death was obtained through at least one of the other sources listed. This date was verified by inpatient records, and/or confirmation with the patient's primary care physician and/or family.

### Statistical Methods

The distribution of genotypes was compared by racial groups as well as by tumor stage using Pearson $\chi^2$ tests. Tests for Hardy-Weinberg equilibrium were conducted by goodness-of-fit $\chi^2$ test to compare the observed genotype frequencies with the expected genotype frequencies with 1 degree of freedom. Linkage disequilibrium of the two polymorphic variants of the *RecQ1* and *XRCC3* genes was measured by using the SNPAlyze software (Dynacom Inc., Japan). The association between overall survival and each SNP was estimated using the method of Kaplan and Meier and assessed using the log-rank test. The combined genotype effect on survival was examined by the number of at-risk alleles. Hazard ratio and 95% confidence interval was estimated using both univariate and multivariate Cox proportional regression models. The multivariate models included all factors that showed a significant association with overall survival in univariate analysis. All statistical testing was conducted using the SPSS software version 12.0 (SPSS, Cary, NC).

## RESULTS

### Patient characteristics and overall survival (Table 1)

The median age of the 378 patients in this study was 63 years (range, 38 to 89 years); 13.2% were younger than 50, 27.5% were 51 to 60 years of age, 33.9% were 61 to 70, and 25.47% were older than 70. Caucasians, Hispanics, and African Americans constituted 87.8%, 5.8% and 5.0% of the study population, respectively. The male to female ratio was 1.2:1. There were 271 (72%) deaths among the 378 cases. The Median survival time (MST) is 15.4 months. The median follow-up time was 24.2 months for the living patients. Age, race, sex, cigarette smoking and history of diabetes did not show any significant effect on overall survival.

At diagnosis, there were 107 localized resectable tumors, 185 locally advanced (including 65 potentially or borderline resectable tumors) and 86 metastatic tumors. The MST was 24.5, 15.4

and 9.8 months for patients with localized, locally advanced and metastatic disease, respectively ($P<0.001$, log rank test). As expected, both performance status and serum CA19-9 levels at diagnosis were significant predictors of survival in this patient population. Patients with ECOG performance status grade 0 and 1 had better survival than those with grade 2 and 3. Patients with a CA19-9 value above 1000 u/ml had at least 6 months shorter survival than those with a value below 1000. There were 143 patients received tumor resection surgery and these patients survived much longer than those did not receive tumor resection. In this patient series, 6.2%, 3.8%, and 6.2% patients received chemotherapy, radiotherapy and tumor resection surgery before their first visit to M. D. Anderson. During the entire disease process, 29 (7.7%) patients received GEM alone; 53 (14%) received GEM-based chemoradiation; 86 (22.8%) received GEM-based chemoradiation plus other type of chemotherapy; 33 (8.7%) received 5 fluorouracil (5FU)-based chemoradiation; 72 (19%) received 5FU-based chemoradiation plus other type of chemotherapy; 95 (25.1%) received combined chemotherapy without radiation; and 11 (2.9%) received no cytotoxic therapy. Overall, 84.4% of the patients received GEM, 70.4% received radiation to the tumor, 53.4% received cisplatin (or oxaliplatin) and 43.1% received 5FU (or capecitabine). In addition, about 20.6% of patients received investigational therapy, such as bevacuzumab, celecoxib, α-interferon and others. Because of the heterogeneity of the patient population and the small number of patients, treatment regimens during the entire disease process were collapsed into 3 groups, i.e. chemotherapy alone, 5-FU-based chemoradiation (5FU/XRT) and GEM-based chemoradiation (GEM/XRT). The 11 patients who did not receive any cytotoxic therapy were excluded from the treatment subgroup analyses. Patients received GEM/XRT or 5FU/XRT did significantly better than those received chemotherapy alone.

## Genotype and survival (Table 2)

The 6 SNPs were successfully amplified in 93.6% to 98.2% of the patients. No homozygous AA genotype was detected for the *RecQ1* A-86G SNP. Genotype frequencies for all six SNPs were found to be in Hardy-Weinberg equilibrium ($\chi^2 = 0.21–2.9$, Ps>0.1). There was a significant difference in the racial distribution of the *RAD54L* and *XRCC3* T241M genotypes. For *RAD54L* African Americans had a higher frequency (94.7%) of the CC wild type than Caucasians (77.2%) and Hispanics (81.0%) ($P = 0.03$, $\chi^2$ test). The frequency of the *XRCC3* GG wild type was 68.4% in African Americans compared to 36.3% in Caucasians and 42.9% in Hispanics ($P = 0.046$). There were no significant differences in the genotype distributions by age, sex, disease stage and surgical status (data not shown). The two SNPs of the *RecQ1* gene are in linkage disequilibrium with a D' value of 0.90 ($P = 0.02$). The two SNPs of he *XRCC3* gene are in complete linkage disequilibrium and the D' value is 0.99 ($P<0.001$).

A significant effect of the *RecQ1* A159C and *RAD54L* C157T genotype on overall survival was observed. MST was 19.2, 14.7, and 13.2 months for the *RecQ1* A157C AA, AC and CC genotypes, and 16.4, 13.3 and 10.3 months for *RAD54L* CC, CT and TT genotypes, respectively. The *RecQ1* 159CC, and *RAD54L* 157 TT genotypes remained as significant predictors for survival in Cox proportional regression models with adjustment of all other significant clinical predictors. The *XRCC2* R188H AA genotype was associated with a significantly shorter survival than the GG/GA genotype, but the risk estimate was not precise (hazard ratio=10.75, 95% CI 2.58–44.7) because of the extremely low frequency of the AA genotype (n=2 only). Neither of the two *XRCC3* gene polymorphisms showed any significant impact on survival.

## Subgroup analysis by stage and surgery (Table 3)

Because disease stage and curative surgery are known to be the most significant predictors of survival, we have separated the patients into 3 groups in the subgroup analysis, i.e. patients with localized and locally advanced tumor and achieved tumor resection (Surgical group,

n=143), patients with localized and locally advanced tumors did not achieve tumor resection (Non-surgical group, n=149), and patients with metastatic tumors (Metastatic group, n=86). The MST for the surgical, non-surgical and metastatic groups was 29.8, 11.7 and 9.8 months, respectively ($P$ <0.001). The effect of *RecQ1* and *RAD54L* gene on survival was clearly seen in patients with localized disease, i.e. the combined surgical and non-surgical groups (Fig. 1) but was completely absent in patients with metastatic disease (data not shown). The *XRCC2* R188H variant allele showed a significant association with poorer survival in non-surgical patients only. When the *RecQ1, RAD54L* and *XRCC2* SNPs were analyzed in combination, a significantly reduced survival was associated with an increasing number of adverse alleles in both surgical and non-surgical patients.

### Subgroup analysis by treatment (Table 4)

Among patients with localized disease (the surgical and non-surgical groups), there are 45 patients who did not receive radiotherapy during the entire course of the disease. There are 138 and 101 patients received GEM-based or 5FU-based chemoradiation, respectively. The MST was 13.7, 23.4, 18.7 months for patients received chemotherapy alone, GEM/XRT or 5FU/XRT, respectively ($P$=0.003). No significant genotype effect was observed in patients received chemotherapy alone. The GEM group consisted more surgical patients (55.8% versus 47.5%, $P$ = 0.21, $\chi^2$ test) and less locally advanced disease (54.37% versus 70.3%, $P$ = 0.01, $\chi^2$ test) than the 5FU group. The significant effects of *RecQ1* and *RAD54L* gene on survival was observed among patients received GEM-based chemoradiation and the effect of *XRCC2* and *XRCC3* genes, however, was observed in patients received 5FU-based chemoradiation only. When the four genes were analyzed in combination, a significantly decreased survival was observed as the number of adverse alleles increased in both GEM/XRT and 5FU/XRT groups.

## DISCUSSION

In this study, we evaluated the effect of 6 SNPs of 4 homologous recombination repair genes, i.e. *RecQ1, RAD54L, XRCC2*, and *XRCC3*, on overall survival of patients with pancreatic adenocarcinoma. We demonstrated that the variant alleles of these genes, independently or jointly, are associated with significantly decreased overall survival. The genotype effect was present in patients with localized disease or patients received radiotherapy but absent in patients with metastatic disease or patients did not receive radiotherapy. These observations for the first time demonstrated the significant impact of homologous recombination repair genes on cancer patient survival.

Among the 4 genes examined in the current study, the *RecQ1* and *RAD54L* genes showed a significant impact on survival when all 378 patients were included in the analysis. The homozygous variant allele of either gene conferred an average 6 months shorter survival compared to the wild type. The frequency of the homozygous variant allele was 19.2% and 1.9% for the *RecQ1* A159C and *RAD54L* C157T, respectively. Both variant alleles remained as significant predictor for survival after adjusting for all known significant clinical predictors. These data support an important role of the *RecQ1* and *RAD54L* genes in pancreatic tumor progression or response to clinical therapy. *RecQ1* belongs to the DNA helicase *RecQ* gene family including the Werner, Bloom, and Rothmund-Thomson causative genes *WRN, BLM* and *RecQ4*. Previous studies have shown that under *BLM*-impaired conditions, RecQ1 acted as a backup mechanism for the helicase function in cell viability (34). Recent studies have also shown that RecQ1 may play a role in DNA strand break repair and mismatch repair (21,22). The *RecQ1* A159C SNP is located on the 3′-untranslated region of the gene and it 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 the translation efficiency and mRNA stability of the gene. If the variant allele confers a higher activity of the RecQ1 protein, it may repair

the therapy-related DNA damages more efficiently and in turn a poorer clinical response. RAD54L is also a DNA helicase and it is known to play a role in repairing DNA double strand breaks (35,36). *RAD54L* has been proposed as a candidate tumor suppressor gene in tumors bearing a non-random deletion of chromosome 1p32 (37,38,25). The *RAD54L* C157T SNP has previously been associated with increased risk of meningiomas (17). The current study found that the *RAD54L* C157T variant allele was associated with a significantly poorer survival in patients treated with chemoradiation. This effect was perhaps directly related to the repair efficiency of radiation-induced DNA double strand breaks. However, 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. The same possibility that the RecQ1 A159C SNP is in linkage disequilibrium with other SNPs of the gene or with other genes cannot be excluded. While haplotype analysis and in vitro experiments will be needed to clarify the exact role of these two SNPs in response to genotoxic stress, the association between these two polymorphic variants and cancer patient survival should be verified in other patient populations. If confirmed, it may have significant impact on the clinical management of cancer patients.

*XRCC2* and *XRCC3* are Rad51 paralogs that are expressed in mitotically growing cells and are thought to play mediating roles in homologous recombination repair, although their precise functions remain unclear. Loss of either XRCC2 or XRCC3 was sufficient to sensitize cells to agents that induce DNA double strand breaks (39). The current study observed a poorer survival of patients carrying the heterozygous and homozygous variant alleles of *XRCC2* R188H or the homozygous mutant allele of *XRCC3* 17893, which suggest that these mutant alleles may affect the repair efficiency of radiation induced DNA double strand breaks and in turn clinical outcome. Since most of the significant associations with survival was observed in subgroup analysis and the study power is limited by the small sample size, these observations need to be interpreted with caution.

Among patients with metastatic disease, none of the genotypes or any other factors significantly affected survival. Individuals with metastatic disease may already have too many genetic alterations driving tumor progression or treatment resistance, so that any subtle effect of genotypes to alter DNA repair capacity is overwhelmed.

Although our data could not definitely discriminate whether the genetic effect on survival was mediated through response to treatment or by affecting tumor aggressiveness, some preliminary observations suggest that the effect of these genotypes was probably related to radiation responsiveness. For example, the distribution of the SNPs was not significantly different by disease stage and the genetic effects on overall survival were observed in patients treated with radiotherapy but not in patients that did not receive radiotherapy. Whether the survival differences by genotypes truly reflect a radiation-related outcome may be best tested through studies that employ prospective study designs. If proven, modulation of these enzymes may serve as therapeutic targets to increase tumor cell radiosensitivity. The current study did not find evidence to support a specific role of any of these tested genes in GEM response. Although both *RecQ1* and *RAD54L* genes seemed to have a stronger effect on survival in patients received GEM-based therapy than those received 5FU-based therapy. Because of the former group had more early stage patients and surgical patients than the later group, the current observations cannot be accurately interpreted. Whether *RecQ1* and *RAD54L* play a specific role in the repair of GEM induced DNA damage need to be tested in a clinical trial with well-defined patient population.

The current study observed a significantly reduced survival associated with an increasing number of adverse genotypes. Because DNA repair is a complex process, it takes many proteins

to act in concert to maintain cell viability and genome integrity. Each single SNP of the low penetrance genes may have a weak effect at the functional level but the combined effect of several genes may have significant clinical value in predicting response to therapy and prognosis. The strength of this study is a large sample size and adequate statistical power. The limitation of the study is the heterogeneity of the patients, which make the data interpretation more difficult. Some subgroup analyses were conducted to overcome this problem but the small sample size in each group may increase the chance of false positive result. It is important to confirm these observations in other patient populations. 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 (40).

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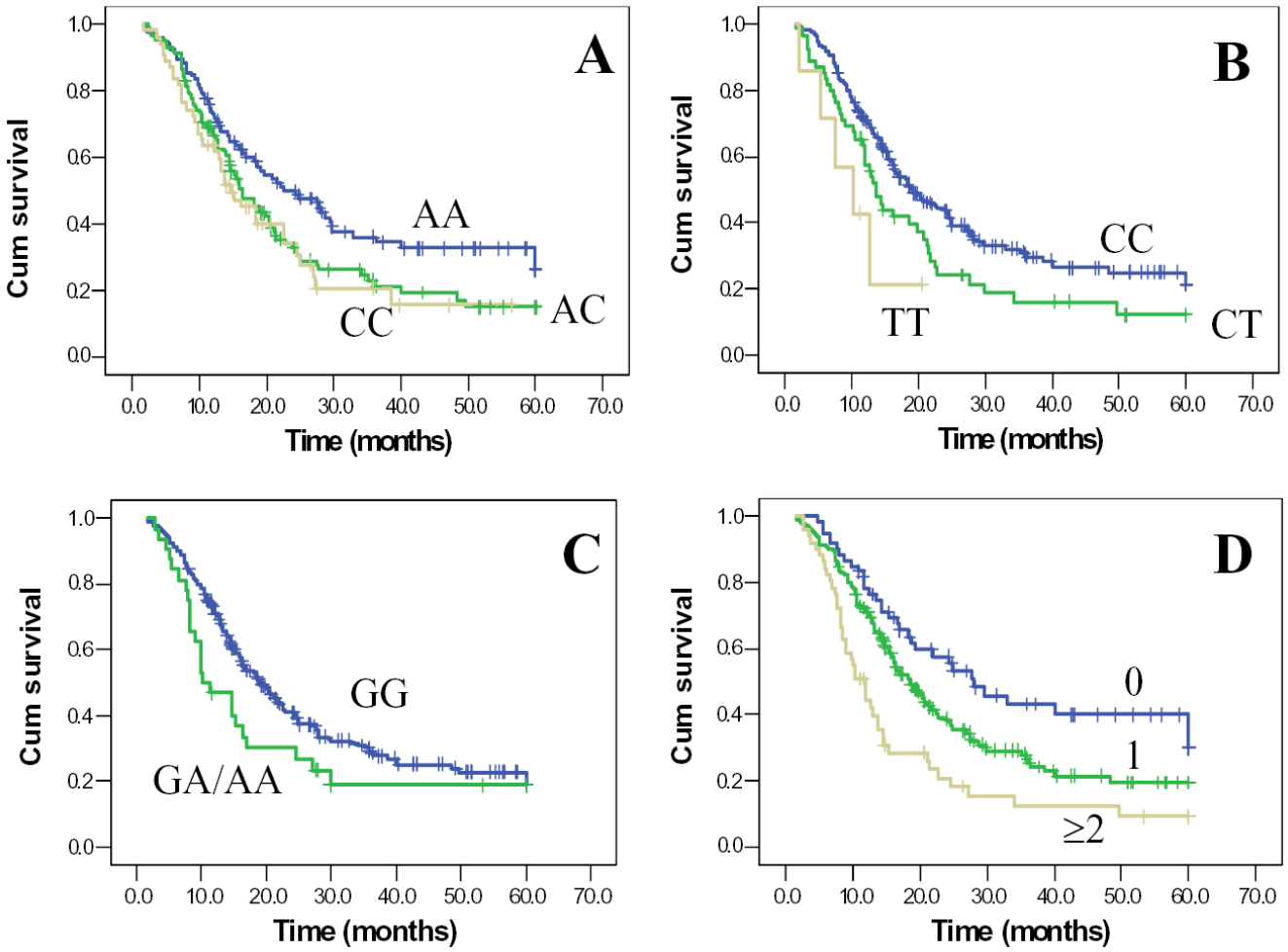


**Fig. 1.**

Survival plot for pooled surgical and non-surgical patients (patients without metastatic disease) by *RecQ1* A159C (*A*), *RAD54L* C157T (*B*), *XRCC2* R188H (*C*) and combined genotype of these 3 SNPs (*D*). The number of 0, 1 and ≥2 in panel D indicates number of alleles that are associated with reduced survivals, i.e. *RecQ1 AC/CC, RAD54L CT/TT, XRCC2 GA/AA* alleles. For example, "0" indicates having none of these alleles, "1" indicates having at least one of the 3 alleles, and " ≥2" indicates having 2 or 3 of these alleles. The survival plot shows the more adverse alleles one have, the shorter survival. *P* values from log rank test, hazard ratios and 95% confidence intervals are shown in Table 3.

## Table 1

Patient characteristics and overall survival

| Variable | No. of Patients | No. of Deaths | MST (months)* | Hazard ratio (95% CI)† | *P* |
|---|---|---|---|---|---|
| Age | | | | | |
| ≤50 | 50 | 34 | 20.3 | 1.0 | |
| 51–60 | 104 | 74 | 14.6 | 1.07(0.72–1.61) | 0.73 |
| 61–70 | 128 | 91 | 14.5 | 1.04 (0.70–1.54) | 0.85 |
| >70 | 96 | 72 | 16.5 | 1.15 (0.77–1.73) | 0.50 |
| Sex | | | | | |
| Male | 207 | 150 | 14.4 | 1.0 | |
| Female | 171 | 121 | 17.0 | 0.83 (0.66–1.06) | 0.14 |
| Race | | | | | |
| White | 332 | 237 | 15.8 | 1.0 | |
| Hispanics | 22 | 17 | 12.8 | 1.22 (0.75–2.00) | 0.43 |
| African American | 19 | 12 | 18.1 | 0.85 (0.47–1.52) | 0.58 |
| Other | 5 | 5 | 16.5 | 1.62 (0.67–3.94) | 0.29 |
| Smoking | | | | | |
| Never | 141 | 102 | 16.4 | 1.0 | |
| Ever | 237 | 169 | 14.7 | 1.05 (0.81–1.33) | 0.72 |
| Diabetes | | | | | |
| No | 290 | 203 | 16.3 | 1.0 | |
| Yes | 88 | 68 | 13.3 | 1.24 (0.94–1.64) | 0.12 |
| Stage | | | | | |
| Localized | 107 | 61 | 24.5 | 1.0 | |
| Locally Advanced | 185 | 133 | 15.4 | 1.73 (1.28–2.35) | <0.001 |
| Metastatic | 86 | 77 | 9.8 | 3.32 (2.35–4.69) | <0.001 |
| Performance Status | | | | | |
| 0 | 59 | 42 | 15.8 | 1.0 | |
| 1 | 270 | 185 | 16.4 | 0.99 (0.71–1.40) | 0.99 |
| 2/3 | 47 | 44 | 11.5 | 1.85 (1.21–2.83) | 0.005 |
| Serum CA 19–9 (u/ml) | | | | | |
| ≤47 | 85 | 58 | 19.2 | 1.0 | |
| 48–1000 | 189 | 123 | 17.3 | 1.08 (0.79–1.47) | 0.65 |
| 1001–3000 | 48 | 38 | 11.2 | 1.66 (1.10–2.50) | 0.02 |
| >3000 | 55 | 51 | 9.7 | 2.46 (1.68–3.60) | <0.001 |
| Surgical | | | | | |
| No | 235 | 201 | 10.7 | 1.0 | |
| Yes | 143 | 70 | 29.8 | 0.26 (0.20–0.35) | <0.001 |
| Cytotoxic Treatment | | | | | |
| Chemotherapy | 123 | 110 | 11.0 | 1.0 | |
| GEM/XRT | 139 | 87 | 21.7 | 0.41 (0.31–0.54) | <0.001 |
| 5FU/XRT | 105 | 64 | 19.2 | 0.47 (0.35–0.65) | <0.001 |

*Median survival time (months)

†Hazard ratio (95% confidence interval) from univariate Cox proportional regression.

## Table 2

Genotype frequency and overall survival

| Genotype | No. of Patients | No. of Deaths | MST (months)* | Univariate Hazard ratio (95% CI) | *P* | Multivariate Hazard ratio (95% CI)† | *P* |
|---|---|---|---|---|---|---|---|
| *RecQ1* A159C | | | | | | | |
| AA | 129 | 84 | 19.2 | 1.0 | | 1.0 | |
| AC | 157 | 119 | 14.7 | 1.44 (1.08–1.92) | 0.010 | 1.22 (0.90–1.64) | 0.195 |
| CC | 68 | 50 | 13.2 | 1.60 (1.12–2.27) | 0.009 | 1.45 (1.01–2.10) | 0.045 |
| *P* (LR) | | | 0.011 | | | | |
| RecQ1 A-89G | | | | | | | |
| GG | 348 | 249 | 15.8 | 1.0 | | 1.0 | |
| GA | 16 | 14 | 13.7 | 1.24 (0.72–2.11) | 0.440 | 1.58 (0.91–2.73) | 0.106 |
| *P* (LR) | | | 0.440 | | | | |
| *RAD54L* C157T | | | | | | | |
| CC | 288 | 203 | 16.4 | 1.0 | | 1.0 | |
| CT | 74 | 57 | 13.3 | 1.31 (0.98–1.76) | 0.070 | 1.31 (0.97–1.76) | 0.080 |
| TT | 7 | 5 | 10.3 | 2.05 (0.84–4.99) | 0.120 | 3.30 (1.34–8.15) | 0.010 |
| *P* (LR) | | | 0.066 | | | | |
| *XRCC2* R188H | | | | | | | |
| GG | 319 | 226 | 16.4 | 1.0 | | 1.0 | |
| GA | 40 | 31 | 11.4 | 1.22 (0.84–1.78) | 0.290 | 1.41 (0.95–2.08) | 0.088 |
| AA | 2 | 2 | 5.1 | 10.75 (2.58–44.7) | 0.001 | 9.72 (2.30–41.1) | 0.002 |
| GA/AA | 42 | 33 | 11.4 | 1.29 (0.89–1.85) | 0.178 | 1.29 (0.89–1.87) | 0.183 |
| *P* (LR) | | | 0.0002 (0.176)‡ | | | | |
| *XRCC3* 17893 | | | | | | | |
| AA | 169 | 119 | 15.3 | 1.0 | | 1.0 | |
| AG | 165 | 118 | 16.3 | 1.04 (0.81–1.35) | 0.750 | 1.15 (0.88–1.50) | 0.304 |
| GG | 37 | 29 | 14.7 | 1.19 (0.79–1.78) | 0.410 | 1.35 (0.89–2.06) | 0.159 |
| *P* (LR) | | | 0.703 | | | | |
| *XRCC3* T241M | | | | | | | |
| CC | 143 | 105 | 16.5 | 1.0 | | 1.0 | |
| CT | 182 | 130 | 14.7 | 1.13 (0.87–1.46) | 0.370 | 1.05 (0.80–1.38) | 0.704 |
| TT | 42 | 28 | 18.9 | 0.83 (0.55–1.26) | 0.370 | 0.75 (0.88–2.08) | 0.191 |
| *P* (LR) | | | 0.298 | | | | |

*MST, median survival time. The numbers of patients for each genotype do not add up to 378 because of failure of genotyping assays in some patients.

†Multivariate Cox regression with adjustment of race, stage, surgery, performance status, CA19-9 level, and cytotoxic treatment.

‡*P* value in parenthesis was from log rank test of GG versus GA/AA genotype.

**Table 3**

enotypes on survival in patients with localized and locally advanced tumors*

| e | Surgical | | | | Non-surgical | | | | Surgical & Non-surgical | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. of Patients | No. of Deaths | MST (months) | Hazard ratio (95% CI) | No. of Patients | No. of Deaths | MST (months) | Hazard ratio (95% CI) | No. of Patients | No. of Deaths | MST (months) | Hazard ratio (95% CI) |
| 59 | | | | | | | | | | | | |
| | 53 | 22 | 60.0 | 1.0 | 51 | 40 | 13.3 | 1.0 | 104 | 62 | 24.4 | 1.0 |
| | 56 | 31 | 24.3 | 1.59 (0.88–2.86) | 58 | 50 | 11.9 | 1.13 (0.73–1.74) | 114 | 81 | 16.4 | 1.29 (0.91–1.82) |
| | 24 | 12 | 24.5 | 1.56 (0.72–3.36) | 31 | 26 | 9.7 | 1.38 (0.82–2.32) | 55 | 38 | 14.6 | 1.48 (0.97–2.25) |
| | | | 0.070 | | | | 0.278 | | | | 0.016 | |
| 157 | | | | | | | | | | | | |
| | 112 | 50 | 38.6 | 1.0 | 111 | 92 | 12.4 | 1.0 | 223 | 142 | 19.0 | 1.0 |
| | 24 | 16 | 21.7 | 1.69 (0.95–3.00) | 31 | 27 | 9.9 | 1.54 (0.99–2.39) | 55 | 43 | 13.7 | 1.57 (1.11–2.22) |
| | 4 | 2 | 10.3 | 4.98 (1.13–21.9) | 3 | 3 | 7.7 | 3.19 (0.96–10.5) | 7 | 5 | 10.3 | 3.67 (1.47–9.18) |
| | | | 0.055 | | | | 0.026 | | | | 0.009 | |
| R188H‡ | | | | | | | | | | | | |
| | 118 | 56 | 35.9 | 1.0 | 129 | 105 | 12.6 | 1.0 | 247 | 161 | 19.0 | 1.0 |
| | 17 | 10 | 24.5 | 1.10 (0.54–2.27) | 15 | 15 | 9.0 | 2.64 (1.47–4.74) | 32 | 25 | 10.1 | 1.99 (1.29–3.09) |
| | | | 0.268 | | | | 0.005 | | | | 0.052 | |
| H§ | | | | | | | | | | | | |
| | 27 | 7 | -‖ | 1.0 | 33 | 25 | 14.3 | 1.0 | 60 | 32 | 28.0 | 1.0 |
| | 95 | 50 | 27.5 | 2.82 (1.22–6.51) | 83 | 68 | 13.1 | 1.25 (0.79–1.99) | 178 | 118 | 18.5 | 1.61 (1.08–2.40) |
| | 19 | 12 | 21.4 | 3.92 (1.46–10.5) | 32 | 30 | 8.5 | 2.06 (1.19–3.57) | 51 | 42 | 11.8 | 2.52 (1.57–4.04) |
| | | | 0.004 | | | | 0.005 | | | | <0.001 | |

T, median survival time. Hazard ratio was adjusted for race, performance status, CA19-9 values, stage, and cytotoxic treatment for all groups. Surgery was adjusted for the pooled surgical and urgical patients.

alues from log rank test.

GA and AA genotypes were combined because only one AA genotype was detected in these patients.

nber of variant alleles of the 3 SNPs listed in this table.

dian survival time could not be calculated.

**Table 4**

and survival by treatment in patients with localized and locally advanced tumors

| e | Chemotherapy Alone | | | | GEM/XRT | | | | 5FU/XRT | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. of Patients | No. of Deaths | MST (months) | Hazard ratio (95% CI) | No. of Patients | No. of Deaths | MST (months) | Hazard ratio (95% CI) | No. of Patients | No. of Deaths | MST (months) | Hazard ratio (95% CI) |
| 59 | | | | | | | | | | | | |
| | 18 | 14 | 13.3 | 1.0 | 48 | 24 | 28.7 | 1.0 | 37 | 23 | 22.5 | 1.0 |
| | 18 | 16 | 15.8 | 0.47 (0.22–1.12) | 49 | 36 | 19.9 | 1.57 (0.93–2.68) | 43 | 26 | 15.8 | 1.64 (0.89–3.03) |
| | 7 | 6 | 13.7 | 0.73 (0.24–4.22) | 32 | 21 | 15.3 | 1.73 (0.93–3.20) | 15 | 10 | 24.5 | 1.82 (0.84–3.92) |
| ) | | | 0.542 | | | | 0.028 | | | | 0.244 | |
| L 157 | | | | | | | | | | | | |
| | 35 | 29 | 14.3 | 1.0 | 109 | 67 | 22.7 | 1.0 | 72 | 40 | 20.3 | |
| | 10 | 9 | 12.6 | 1.10 (0.50–2.44) | 21 | 15 | 14.6 | 2.18 (1.22–3.88) | 24 | 19 | 13.7 | 1.56 (0.88–2.74) |
| | 0 | 0 | | | 3 | 3 | 10.3 | 4.29 (1.23–14.9) | 4 | 2 | 7.7 | 3.58 (0.80–16.0) |
| | | | 0.808 | | | | 0.007 | | | | 0.134 | |
| R188H | | | | | | | | | | | | |
| | 41 | 36 | 13.3 | 1.0 | 117 | 73 | 22.0 | 1.0 | 81 | 47 | 20.3 | 1.0 |
| | 3 | 2 | 9.8 | 1.71 (0.37–8.02) | 16 | 12 | 14.6 | 2.18 (1.15–4.12) | 13 | 11 | 9.0 | 2.45 (1.20–5.02) |
| ) | | | 0.927 | | | | 0.263 | | | | 0.012 | |
| *17893* | | | | | | | | | | | | |
| | 15 | 14 | 14.3 | 1.0 | 65 | 39 | 22.5 | 1.0 | 48 | 29 | 20.3 | 1.0 |
| | 23 | 19 | 13.7 | 1.43 (0.62–3.30) | 57 | 36 | 21.4 | 1.12 (0.69–1.82) | 44 | 26 | 19.2 | 1.25 (0.72–2.19) |
| | 7 | 5 | 11.8 | 0.93 (0.32–2.69) | 11 | 9 | 18.2 | 1.43 (0.63–3.25) | 9 | 7 | 10.6 | 4.12 (1.67–10.2) |
| ) | | | 0.932 | | | | 0.819 | | | | 0.032 | |
| ed | | | | | | | | | | | | |
| | 12 | 10 | 14.3 | 1.0 | 26 | 12 | 32.9 | 1.0 | 18 | 7 | 40.1 | 1.0 |
| | 18 | 16 | 13.7 | 0.79 (0.31–2.00) | 68 | 42 | 20.6 | 1.64 (0.85–3.19) | 44 | 28 | 18.6 | 2.94 (1.27–6.83) |
| | 6 | 5 | 12.6 | 0.92 (0.29–2.98) | 23 | 20 | 14.7 | 3.03 (1.46–6.29) | 24 | 16 | 10.3 | 4.36 (1.72–11.0) |
| | 4 | 3 | 11.8 | 0.55 (0.13–2.29) | 2 | 2 | 4.6 | 12.9 (2.66–62.5) | 3 | 3 | 7.4 | 25.4 (5.29–122) |
| | | | 0.974 | | | | 0.004 | | | | 0.0003 | |

zard ratio was adjusted for race, performance status, CA19-9 values, stage, and surgery.

values for log rank test.

e GA and AA genotypes were combined because only one AA genotype was present in these patients.

mber of variant alleles for the 4 SNPs listed in this table.