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Effects of base excision repair gene polymorphisms on pancreatic cancer survival

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Abstract

To explore the association between single nucleotide polymorphisms of DNA repair genes and overall survival of patients with pancreatic cancer, we conducted a study in 378 cases of pancreatic adenocarcinoma who were treated at The University of Texas M. D. Anderson Cancer Center between February 1999 and October 2004 and were followed up to April 2006. Genotypes were determined using genomic DNA and the MassCode method. Overall survival was analyzed using the Kaplan–Meier plot, log-rank test and Cox regression. We observed a strong effect of the *POLB* A165G and T2133C genotypes on overall survival. The median survival time (MST) was 35.7 months for patients carrying at least 1 of the 2 homozygous variant *POLB* GG or CC genotypes, compared with 14.8 months for those carrying the AA/AG or TT/TC genotypes ($p = 0.02$, log rank test). The homozygous variants of *hOGG1* G2657A, *APEX1* D148E and *XRCC1* R194W polymorphisms all showed a weak but significant effect on overall survival as demonstrated by either log rank test or multivariate COX regression after adjusting for other potential confounders. In combined genotype analysis, a predominant effect of the *POLB* homozygous variants on survival was observed. When *POLB* was not included in the model, a slightly better survival was observed among those carrying none of the adverse genotypes than those carrying at least one of the adverse genotypes. These observations suggest that polymorphisms of base excision repair genes significantly affect the clinical outcome of patients with pancreatic cancer. These observations need to be confirmed in a larger study of homogenous patient population.

Keywords

base excision repair; single nucleotide polymorphism; pancreatic cancer; survival

Pancreatic cancer is a rapidly fatal malignancy. The prognosis is extremely poor because this disease is usually diagnosed at a late stage and the tumors are highly aggressive and resistant to most treatments.¹ Resistance to therapy leads to large individual variation in response to cytotoxic cancer therapies. To improve treatment efficacy, novel strategies are needed to help clinicians select the most suitable treatment regimen for each patient. Recent developments in pharmacogenomics and gene profiling have provided such opportunities.² Several studies have shown that genetic variations in DNA repair and drug metabolism significantly affect the clinical outcome of patients receiving cytotoxic therapies.^{3–7}

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Gemcitabine and radiotherapy are currently the main therapeutic modalities for advanced pancreatic cancer.⁸ However, it is not clear what factors influence the clinical response to such treatment. Gemcitabine is a potent radiosensitizer.⁹ Little is known about DNA repair pathways that may alter cytotoxicity or radio-sensitivity with gemcitabine. On the other hand, it is well known that oxidative DNA damage and resulting DNA strand breaks are the most common type of radiation lesions that lead to mammalian cell death.¹⁰ The base excision repair (BER) pathway and the DNA strand break repair pathway are the major repair systems that contribute to the processing of oxidative lesions.^{11,12} In our previous studies we have shown a significant effect of DNA homologous recombination gene polymorphisms on overall survival of patients with pancreatic cancer.^{13,14} The current study focuses on the BER pathway.

Two subpathways of BER have been characterized by *in vitro* methods and classified according to the length of the repair patch: the short patch pathway repairs single nucleotides and the long patch pathway repairs lesions of 2–10 nucleotides.¹⁵ Short patch repair is the primary pathway for the repair of oxidative DNA damages, but long patch repair may occur when components of short patch repair are saturated or missing. The short patch repair process requires a number of enzymes: a DNA glycosylase, such as 8-oxo-guanine DNA glycosylase (hOGG1), removes the damaged base (e.g., 8-oxoguanine); apurinic/apyrimidinic endonuclease I (APEX1) recognizes and cleaves the resulting abasic (AP) site, introducing a single strand break; X-ray repair cross complementing protein 1 (XRCC1) is a scaffold protein that brings polymerase β (POLB) and ligase III (LIG3) together at the site of repair. DNA POLB adds a single nucleotide to the 3'-end of the nicked AP site and removes the resulting 5'-deoxyribose phosphate group, and a DNA LIG3-XRCC1 complex seals the generated nick.¹⁶

Polymorphisms of DNA repair genes may be capable of serving as a genetic marker for individual susceptibility to cytotoxic cancer therapy because of the role of DNA repair in protecting cells from DNA damage-mediated death. To test the hypothesis that genetic variations in BER affect clinical response to chemoradiotherapy and thus patient survival, we examined 9 single nucleotide polymorphisms of 6 major genes (*hOGG1*, *APEX1*, *POLB*, *XRCC1*, *LIG3* and *LIG4*) that are involved in each step of the short patch BER pathway in 378 patients with a diagnosis of pancreatic ductal adenocarcinomas and who were treated at The University of Texas M. D. Anderson Cancer Center between 1999 and 2004. Overall survival was compared between patients with different genotypes.

Material and methods

Patient

A total of 378 patients with pathologically confirmed pancreatic ductal adenocarcinoma of all stages were recruited prospectively at 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 the M. D. Anderson Cancer Center.

For this analysis, all 378 patients were treated at M. D. Anderson between January 1999 and October 2004 and were followed up to April 30, 2006. The minimum follow-up time was 18 months. 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 fashion.

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, pre- and postoperative chemotherapy regimen and radiation and 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. DNA was extracted from peripheral lymphocytes using the Qiagen DNA Isolation kit (Qiagen, Valencia, CA). Polymorphisms were detected using the Masscode™ technique¹⁷ by BioServe Biotechnologies (Laurel, MD). This mass spectrometry-based method uses standard PCR amplification of the region containing the polymorphism followed by hybridization with oligonucleotide primers that have been modified with photocleavable linkers attached to tags of variable composition (cleavable mass spectrometry tags) such that each allele-specific oligonucleotide primer is of different mass. For homozygotes, the mass spectrometer detects a single allele-specific tag, and for heterozygotes it detects 2 tags. In our previous study of other genes,¹⁸ we have observed a high concordance of data obtained from this method with those obtained from the Taqman and the PCR-RFLP methods. The reference numbers, gene locus, chromosome location, nucleotide change, amino acid change and minor allele frequency of the 9 SNPs examined in this study are described in Table I. The *XRCC1*, *APEX1*, *hOGG1* and *LIG4* SNPs were selected based on previous knowledge on the functional significance and their association with cancer risk or patient survival. The *POLB* and *LIG3* SNPs were selected for their higher allele frequency (>10%) because there is no report on the functional significance of these SNPs. About 10% of the samples were analyzed in duplicates, and discrepancies were seen in less than 0.1% of the samples. Those with discordant results from 2 analyses were excluded from the final data analysis. The no call rate (samples without informative genotyping data) was 5.4% for *hOGG1* C315G and *POLB* A165G and less than 4% for the remaining 7 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 and maximum follow-up times were 18 and 90 months, respectively. Dates of death were obtained and crosschecked using at least 1 of the following 3 methods: Social Security Death Index, inpatient medical records and the M. D. Anderson tumor registry.

Statistical methods

The distribution of genotypes was compared by racial groups as well as by tumor stage using Pearson χ^2 tests. Tests for Hardy–Weinberg equilibrium were conducted by goodness-of-fit χ^2 test to compare the observed genotype frequencies with the expected genotype frequencies with 1 degree of freedom. Linkage disequilibrium of the 2 polymorphic variants of the *hOGG1*, *POLB* and *XRCC1* genes was measured by using the SNPalyze software (Dynacom, Mobarra, 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. Because the wild-type and heterozygous variant genotypes of *hOGG1*, *APEX1* and *POLB* had comparable median survival times, these 2 groups were collapsed into 1 and was used as the reference in comparison with the homozygous variant genotype. Hazard ratio and 95% confidence interval were estimated using multivariate Cox proportional regression models. The multivariate models

included all factors that showed a significant association with overall survival in log rank test, *i.e.*, stage, surgery, performance status, CA19-9 level and cytotoxic treatment. Race was also included in the model because of its association with genotype distribution. All statistical testing was conducted using the SPSS software version 12.0 (SPSS, Chicago, IL). *p* values of less than 0.05 were considered as statistically significant.

Results

Patient characteristics and overall survival

The demographic and clinical characteristics of the patients are described in Table II. The MST was 16.0 months (95% CI: 14–17). The median follow-up time was 34 months (95% CI: 30–39) for the living patients. Age, race, sex and cigarette smoking did not show any significant effect on overall survival (Table II). Patients with a history of diabetes lived 3 months shorter than those without diabetes ($p = 0.08$, log rank test). Disease stage, serum CA19-9 levels and performance status at diagnosis, and whether tumor resection was achieved were all significant predictors of survival in this patient population (Table II). During the entire disease process, 84.4% of the patients received gemcitabine, 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 the patients received investigational therapy, such as bevacizumab, 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: chemotherapy alone, 5FU-based chemoradiation (5FU/XRT) and gemcitabine-based chemoradiation (GEM/XRT). The 11 patients who did not receive any cytotoxic therapy were excluded from the treatment subgroup analyses. Patients who were treated with GEM/XRT did better than those who were treated with other chemotherapies (Table II). Among the 121 patients who had their tumors resected, 19 (15.7%) did not receive neoadjuvant therapy and there is no significant difference in overall survival between these patients by neo-adjuvant or adjuvant therapy modality (data not shown).

Genotype and survival

The 9 SNPs were successfully amplified in 94.6–98.4% of the patients. There was a significant difference in the racial distribution of 6 SNPs (Table III). Genotype frequencies for all 9 SNPs were found to be in Hardy–Weinberg equilibrium ($\chi^2 = 0.53$ – 2.56 , all $p > 0.1$). The *hOGG1* G2657A, *POLB* A165G and *POLB* T2133C genotypes were not in Hardy–Weinberg equilibrium ($\chi^2 = 4.87$, 8.87 and 14.88, respectively) when data from all study subjects were analyzed together but all were in Hardy–Weinberg equilibrium ($\chi^2 = 1.21$ – 2.22 , all $p > 0.1$) when data were analyzed among non-Hispanic whites only. There were no significant differences in the genotype distributions by age, sex, disease stage or surgical status (data not shown). The 2 SNPs of the *hOGG1*, *POLB* and *XRCC1* genes were all in linkage disequilibrium with *D'* values of -0.78 , 0.91 and -0.59 , respectively (all $p < 0.001$).

A significantly reduced survival was associated with the homozygous variant alleles of the *hOGG1* G2657A, *APEX1* D148E and *XRCC1* R194W SNPs (Table IV). The MST was 4–7 months shorter for the *hOGG1* homozygous variant genotype than for the wild-type and heterozygous genotype (Fig. 1). The *hOGG1* 2657 AA but not the 326 GG genotype was a significant independent predictor of survival after adjusting for race and all other significant clinical factors (Table IV). When both SNPs of the *hOGG1* gene were analyzed in combination, carriage of at least 1 of the homozygous variant genotypes was associated with a significantly reduced survival with MST of 11.5 vs. 16.0 months ($p = 0.017$, log rank test). Both homozygous variants of the *APEX1* D148E and *XRCC1* R194W SNPs remained as significant predictors of survival after adjusting for race and other significant clinical predictors (Table IV).

On the other hand, a significant protective effect of the *POLB* homozygous variant genotype on overall survival was observed. The MST was 35.7 months for those carrying at least 1 of the 2 homozygous variant *POLB* 165 GG and 2133 CC genotypes, compared with a MST of 14.8 months for those carrying the 165 AA/ AG and 2133 TT/TC genotypes ($p = 0.006$). Both individual SNPs and the combined genotype of the 2 *POLB* SNPs remained as significant independent predictors for survival after adjusting for race and significant clinical factors in Cox regression models (Table IV). The *XRCC1* Q399R, *LIG3* and *LIG4* genotypes did not show any significant effect on overall survival of pancreatic cancer patients in this study (data not shown).

The effect of *APEX1*, *XRCC1* and combined *hOGG1* or *POLB* genotypes on survival was also confirmed when the analysis was conducted among non-Hispanic whites only. The adjusted hazard ratio (95% CI) was 1.37 (1.01–1.83, $p = 0.038$) for *APEX1* GG and 5.08 (1.56–16.5, $p = 0.007$) for *XRCC1* R194W TT genotypes and was 1.83 (1.03–3.25, $p = 0.038$) for those carrying any 1 of the 2 homozygous *hOGG1* variants and 2.58 (1.21–5.50) for those with any 1 of the 2 homozygous *POLB* variants ($p = 0.014$).

Combined genotype effect

Because of *hOGG1*, *APEX1*, *XRCC1* and *POLB* are all involved in the same DNA repair pathway; we explored the effect of combined genotypes on overall survival of the patients. We observed that patients carrying none of the adverse genotypes (*hOGG1* 2657 AA or 326 GG, *APEX1* GG, *XRCC1* 194TT and *POLB* 165AG/GG or 2133 TC/CC) had a much better survival (mean survival time of 52.7 months) than those carrying 1, 2 or 3 adverse genotypes (MST of 16.0, 12.8 and 15.3 months, respectively) (Fig. 2, upper panel). This protective effect was mainly attributable to the *POLB* variant alleles. When *POLB* was not included in the analysis, carriage of 0, 1 or ≥ 2 adverse genotypes of the other 3 genes did not show a significant difference in the overall survival (data not shown). When *hOGG1*, *APEX1*, *XRCC1* and *LIG4* were all included in the combined genotype analysis, carriage of none of the adverse genotype had a MST of 18.1 months compared to a MST of 14.5, 13.3 and 15.3 months for those carrying 1, 2 or 3 adverse genotypes, respectively (Fig. 2, lower panel).

Genotype effect by treatment

Stratified analysis by treatment or surgical status did not reveal any significant differential effect of the *hOGG1*, *APEX1*, *XRCC1* and *POLB* genes because of the relatively low frequency of the homozygous variant alleles or reduced power associated with the small sample size in each subgroup. The combined genotype of these 4 genes showed similar effect on overall survival of patients who had their tumor resected or not resected, $p = 0.04$ and 0.001 , respectively, by log rank test. The combined genotype effect on survival was highly significant ($p = 0.005$, log rank test) among patients who received radiation but not significant for patients who did not receive radiation ($p = 0.11$). The combined genotype effect was at borderline significance ($p = 0.05$) for patients who received gemcitabine/radiation but not significant for those received 5 FU/radiation ($p = 0.11$).

Discussion

The current study evaluated the effect of 9 SNPs of 6 major BER genes (*hOGG1*, *APEX1*, *POLB*, *XRCC1*, *LIG3* and *LIG4*) on overall survival of patients with pancreatic adenocarcinoma. We demonstrated that the homozygous variant genotypes of the *hOGG1*, *APEX1* and *XRCC1* R194W were associated with significantly decreased overall survival and that the homozygous variant genotype of *POLB* was associated with a significantly increased overall survival. These observations demonstrated for the first time the significant effect of BER genes on pancreatic cancer patient survival.

Among the 6 genes examined in this study, *POLB* homozygous variants showed a strong protective effect on patient survival. The 2 homozygous variant genotypes were associated with a doubling of survival time compared with the wild-type and heterozygous variant genotypes. Although the frequencies of these homozygous alleles are low (2.8% and 2.4%), and the possibility that these observations were by chance alone cannot be excluded, such effect is consistent with the drug-resistant nature conferred by overexpression of DNA POLB. *POLB* is expressed at a constant low level throughout the cell cycle¹⁹ and is inducible by some genotoxic treatments.²⁰ The primary function of *POLB* is thought to be in the processing of damaged bases via the BER pathway, thereby protecting cells from DNA damage-induced cytotoxicity.^{21,22} At the transcriptional level, *POLB* is overexpressed in many cancer cells.²³ Overexpression of *POLB* has been associated with an increased chromosome instability and tumorigenesis as well as with resistance to many DNA-damaging agents, including chemotherapeutic drugs.^{24–26} In fact, inhibition of *POLB* expression has recently been used as a novel approach to increase sensitivity to cisplatin-based cancer therapy.²⁷ The 2 SNPs examined in the current study are both localized in the intron region (intron 10 and intron 2), and the functional significance of these SNPs has not been explored. It is tempting to speculate that the homozygous variants may confer a lower expression of *POLB* and thus an increased sensitivity to cyto-toxic treatment and an improved survival. This hypothesis needs to be tested in experimental systems. It is also possible that these 2 SNPs are in linkage disequilibrium with other functionally significant SNPs in the same gene; therefore haplotype analysis is required to identify the responsible SNPs. These homozygous variants of the 2 SNPs were observed in a much higher frequency in African Americans than in Caucasians and they were absent among Hispanics. Regardless of the mechanism underlying the observed associations, our observations suggest that *POLB* is an important gene influencing the overall survival of patients with pancreatic cancer, especially among African Americans.

hOGG1 is a DNA repair enzyme that excises 7,8-dihydro-8-oxo-guanine (8oxoG), an oxidative DNA lesion, from DNA. Because 8oxoG is a lesion leading to a high degree of DNA mispairing, decreased *hOGG1* expression level could lead to a higher frequency of mutation and could possibly increase the cancer risk of an individual under oxidative stress. There is some epidemiologic evidence supporting an association between the risk of several types of human cancers and the *hOGG1* genotype.²⁸ Although some functional studies suggest reduced repair function with variant alleles in *hOGG1*, the evidence is generally inconclusive.²⁸ No study has yet been reported on the role of *hOGG1* in the clinical outcome of cancer patients. In the current study, we observed a significantly reduced overall survival for patients carrying the homozygous variant genotypes of this gene. This observation would support a better DNA repair associated with the variant alleles and thus a poor response to therapy. Indeed, some epidemiological studies have shown a reduced cancer risk associated with the variant alleles of the *hOGG1* gene.²⁸ Because of the inconclusive observations on this gene in epidemiological studies and the uncertain functional effects of the variant *hOGG1* genotypes, our observations need to be confirmed in other studies as well.

APEX1 acts in base excision repair to hydrolyze abasic sites arising from enzymatic removal of damaged purine and pyrimidine bases. It has a 3'-phosphodiesterase activity that excises de-oxyribose fragments and phosphate groups at the 3' terminus of DNA strand breaks caused by ionizing radiation, yielding a 3'-OH substrate for DNA repair synthesis. Nuclear expression of *APEX1* protein has been related to a better survival in resected non-small cell lung cancer.²⁹ However, overexpression of *APEX1* was related to resistance to bleomycin therapy in patients with testicular cancer.³⁰ Although the *APEX1* D148E (Asp148Glu) polymorphism does not result in reduced endonuclease activity,³¹ cells having the variant allele may have higher sensitivity to ionizing radiation.³² In this study, however, we observed a slightly reduced overall survival among patients carrying the homozygous variant allele, which does

not support a higher sensitivity to radiotherapy. The functional significance of this SNP and its role in the clinical response to cytotoxic therapy need further investigation.

XRCC1 acts as a scaffold protein that coordinates the enzymatic activities at different steps of the BER pathway by interacting with various repair enzymes such as APEX1, polynucleotide kinase, LIG3, DNA POLB, poly (ADP-ribose) polymerase and proliferating cell nuclear antigen.³³ The lack of coordination in the repair process could result in the persistence and exposure to the cellular milieu of potentially toxic DNA intermediates such as single strand breaks. The *XRCC1* Q399R (Gln399Arg) polymorphism has been shown to affect the clinical response to platinum-based cancer therapy,⁶ and the *XRCC1* R194W (Arg194Trp) SNP has not been evaluated as a predictor of clinical outcome. In the current study, we observed a significant effect of the *XRCC1* R194W but not the Q399R SNP on survival in pancreatic cancer patients. However, the frequency of the *XRCC1* R194W homozygote was extremely low (0.8%), our observation could be made by chance alone.

The strength of this study is its large sample size and adequate statistical power. The limitation of the study is the heterogeneity of the patients, which makes the data interpretation more difficult. Some subgroup analyses were conducted in an attempt to overcome this problem, but the small sample size in each subgroup may increase the chance of false positive results. The significant effects of combined genotype effects on overall survival among patients who received radiation but not in those without radiation were consistent with our previous observations in homologous recombination repair genes.¹⁴ It supports the hypothesis that genetic variation in the BER pathway would affect the repair of radiation-induced DNA damage and cell survival. However, patients who received no radiation were mostly with metastatic disease, therefore, the overwhelming genetic abnormalities in the tumors may confer resistance to most therapies and any subtle genetic effects can not be detected. It is therefore important to confirm these observations in other patient populations, preferably in prospectively designed trials where treatment regimens can be standardized and patient homogeneity adequately addressed.

In summary, the current study has demonstrated a statistically significant effect of hOGG1, APEX1, XRCC1 and DNA *POLB* gene polymorphisms on the overall survival of patients with pancreatic adenocarcinoma. Except for XRCC1, this is the first study to show that polymorphic variants of BER genes may have an important role in influencing the clinical outcome of patients with cancer. We have previously shown that DNA homologous recombination repair gene polymorphisms significantly affect the overall survival of pancreatic cancer patients.^{13, 14} Because DNA repair is a complex process, it takes many proteins acting in concert to maintain cell viability and genome integrity. Each individual 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. Genetic profiling of enzymes that are involved in drug metabolism, DNA repair or cellular response to cytotoxic agents may provide opportunities for the discovery of novel therapeutic targets and information that can direct the choice of therapy and predict the treatment tolerance, response and overall outcome.³⁴

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Abbreviations

APEX1	apurinic/aprimidinic endonuclease 1
BER	base excision repair
95% CI	confidence interval
5FU	5 fluorouracil
GEM	gemcitabine

hOGG1	8-oxoguanine DNA glycosylase
LIG3	ligase III
LIG4	ligase IV
MST	median survival time
POLB	DNA polymerase β
XRCC1	X-ray repair cross complementing protein 1
XRT	radiotherapy

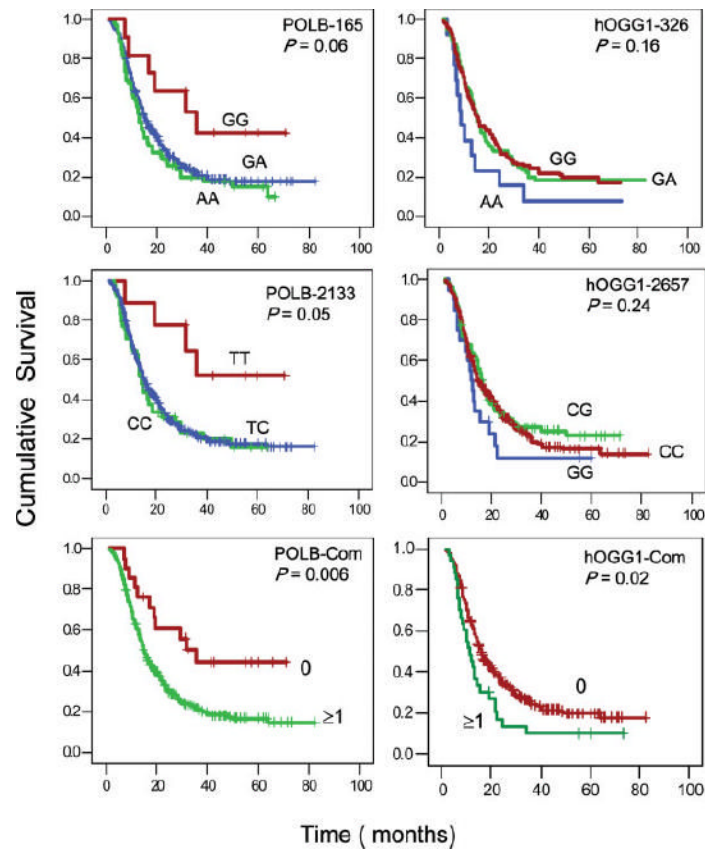


FIGURE 1.

Survival plots for all patients by genotype of *POLB* A165G (A), *POLB* T2133C (B), *hOGG1* G2657A (D) and *hOGG1* C326G (E). Panels C and F are combined genotypes of the 2 SNPs for *POLB* and *hOGG1*, respectively. The numbers 0 and ≥ 1 in panels C and F indicate the number of variant alleles that are associated with significantly increased or reduced survivals. For example, in panel C, 0 indicates having *POLB* 165 AA/AG and 2133 TT/TC genotypes and ≥ 1 indicates having at least 1 of the 2 variant alleles of 165 GG and 2133 CC. In panel F, 0 indicates having *hOGG1* 2657GG/ GA, and 326 CC/CG genotypes and ≥ 1 indicates having at least 1 of the 2 variant alleles of 2657AA and 326GG. *P* values from log rank test, hazard ratios, and 95% confidence intervals are shown in Table IV. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

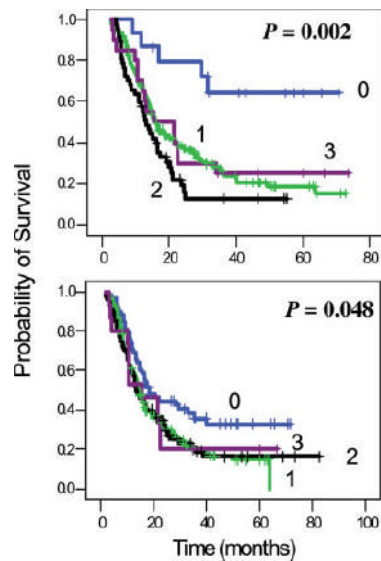


FIGURE 2.

Combined genotype effects on overall survival. The numbers 0–3 indicate the number of variant alleles that are associated with significantly reduced survivals. For example, 0 indicates having none of the *POLB* 165 AG/AA or 2133 TC/CC, *hOGG1* 2657AA or 326 GG, *APEX1* D148E GG and *XRCC1* R194W TT genotypes and 1 indicates having at least 1 of these variant alleles. *P* values are from log rank test. The hazard ratio (95% confidence interval) is 4.35 (1.74–10.9), 6.45 (2.55–16.4) and 4.25 (1.51–11.9) for patients carrying 1, 2 or 3 adverse genotypes, respectively, with adjustment of race and other clinical factors. Lower panel excluded the *POLB* gene but included *XRCC1* Q399R AG/GG and *LIG4* TT as the additional adverse genotypes. The adjusted hazard ratio (95% confidence interval) is 1.53 (1.05–2.24), 1.65 (1.08–2.52) and 1.60 (0.83–3.07), respectively, for those carrying 1, 2 or 3 adverse genotypes. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE I

SNPs EXAMINED IN THE STUDY

Gene	Chromosome	SNP	Amino acid	RS no. ¹	MAF ²
<i>hOGG1</i>	3p26.2	IVS7 + G2657A		293794	0.27
		3'UTR, Ex6-C315G	S326C	1052133	0.23
<i>APEX1</i>	14q11.2-q12	Ex5 + T5G	D148E	3136820	0.51
<i>POLB</i>	8p11.2	IVS10 + A165G		2272615	0.27
		IVS2-T2133C		2953993	0.78
<i>XRCC1</i>	19q13.2	Ex6-C22T	R194W	1799782	0.13
		Ex10-A4G	Q399R	25487	0.35
<i>LIG3</i>	17q11.2-q12	IVS18-G39A		2074522	0.10
<i>LIG4</i>	13q33-q34	5'UTR Ex2 + C54T	T9I	1805388	0.13

¹Reference SNP identification number.

²Minor allele frequency from the National Cancer Institute SNP500Cancer database.

TABLE II
PATIENT CHARACTERISTICS AND OVERALL SURVIVAL

Variable	No. of patients	No. of death	MST (months)*	<i>p</i> (log rank)
Age (years)				
≤50	50	39	18.4	
51–60	104	76	14.5	
61–70	128	97	14.4	
>70	96	75	16.4	0.97
Sex				
Male	207	161	14.4	
Female	171	126	16.5	0.11
Race				
White	332	252	15.4	
Hispanic	22	18	12.8	
African American	19	12	18.1	
Other	5	5	16.5	0.51
Smoking				
Never	141	108	16.4	
Ever	237	179	14.4	0.68
Diabetes				
No	290	215	16.0	
Yes	88	72	13.3	0.08
Stage				
Localized	107	65	24.5	
Locally advanced	185	143	15.3	
Metastatic	86	79	9.8	<0.001
Performance status				
0	59	43	15.3	
1	270	198	16.4	
2/3	47	46	11.5	0.0002
Serum CA 19-9 (U/ml)				
≤47	85	60	19.5	
48–1000	189	135	16.5	
1001–3000	48	40	11.7	
>3000	55	51	9.7	<0.001
Surgical				
No	235	210	10.7	
Yes	143	77	29.9	<0.001
Cytotoxic treatment				
Chemotherapy	123	113	11.0	
GEM/XRT	139	93	21.4	
5FU/XRT	105	71	18.6	<0.001

p-values were from log rank test for the overall comparison within each group.

* Median survival time.

TABLE III
GENOTYPE FREQUENCIES BY RACE

Genotype	White <i>N</i> (%)	Hispanic <i>N</i> (%)	Black <i>N</i> (%)	<i>p</i> value (χ^2 test)
<i>APEX1</i> D148E				
TT	85 (26.8)	11 (52.4)	5 (26.3)	0.09
TG	155 (48.9)	9 (42.9)	9 (47.4)	
GG	77 (24.3)	1 (4.8)	5 (26.3)	
<i>hOGG1</i> -C326G				
CC	197 (62.9)	10 (45.5)	16 (88.9)	0.08
CG	104 (33.2)	11 (50.0)	2 (11.1)	
GG	12 (3.8)	1 (4.5)	0 (0)	
<i>hOGG1</i> -G2657A				
GG	219 (66.6)	18 (81.8)	11 (57.9)	0.07
GA	90 (28.9)	2 (9.1)	5 (26.3)	
AA	12 (4.6)	2 (9.1)	3 (15.8)	
<i>POLB</i> -A165G				
AA	268 (81.7)	17 (77.3)	8 (44.4)	0.00
AG	55 (16.8)	5 (22.7)	4 (22.2)	
GG	5 (1.5)	0 (0)	6 (33.3)	
<i>POLB</i> -T2133C				
TT	283 (87.9)	18 (81.8)	5 (26.3)	0.00
TC	36 (11.2)	4 (18.2)	8 (42.1)	
CC	3 (0.9)	0 (0)	6 (31.6)	
<i>XRCC1</i> -R194W				
CC	271 (86.6)	16 (76.2)	19 (100.0)	0.23
CT	39 (12.5)	5 (23.8)	0 (0)	
TT	3 (1.0)	0 (0)	0 (0)	
<i>XRCC1</i> -Q399R				
AA	103 (32.4)	6 (27.3)	11 (61.1)	0.06
AG	170 (53.5)	11 (50.0)	7 (38.9)	
GG	45 (14.2)	5 (22.7)	0 (0.0)	
<i>LIG3</i> G39A				
GG	258 (79.4)	21 (95.5)	17 (89.5)	0.35
GA	66 (20.3)	1 (4.5)	2 (10.5)	
AA	1 (0.3)	0 (0)	0 (0)	
<i>LIG4</i> C54T				
CC	225 (70.3)	17 (77.3)	13 (68.4)	0.81
CT	88 (27.5)	5 (22.7)	5 (26.3)	
TT	7 (2.2)	0 (0)	1 (5.3)	

Data from 5 study subjects of other ethnicities are not shown.

TABLE IV
GENOTYPE FREQUENCY AND OVERALL SURVIVAL

Genotype	No. of patients	No. of deaths	MST (months) ¹	<i>p</i> ^(LR)	Multivariate hazard ratio (95% CI) ²	<i>p</i> value
<i>hOGG1</i> -G2657A						
GG	253	93	14.8		1.0	
GA	102	4	16.2		1.04 (0.79–1.37)	0.765
AA	20	7	12.0	0.241	1.73 (1.05–2.85)	0.033
GG/GA vs. AA				0.049	1.35 (0.86–2.10)	0.189
<i>hOGG1</i> -C326G						
CC	225	165	15.8		1.0	
CG	119	91	15.4		0.88 (0.68–1.14)	0.318
GG	13	12	8.6	0.159	1.23 (0.68–2.23)	0.491
CC/CG vs. GG				0.058	1.30 (0.72–2.33)	0.387
<i>APEX1</i> D148E						
TT	101	77	14.4		1.0	
TG	178	128	16.5		0.94 (0.71–1.26)	0.686
GG	82	67	12.9	0.133	1.42 (1.02–1.98)	0.041
TT/TG vs. GG				0.096	1.47 (1.11–1.95)	0.007
<i>POLB</i> -A165G						
AA	297	223	15.4		1.0	
AG	65	54	13.1		1.24 (0.91–1.68)	0.172
GG	11	6	35.7	0.064	0.41 (0.17–0.95)	0.037
AA/AG vs. GG				0.039	0.39 (0.17–0.90)	0.027
<i>POLB</i> -T2133C						
TT	311	236	15.4		1.0	
TC	48	38	14.0		1.18 (0.83–1.67)	0.367
CC	9	4	48.4 ³	0.053	0.30 (0.11–0.85)	0.023
TT/TC vs. CC				0.017	0.29 (0.10–0.81)	0.019
<i>XRCC1</i> -R194W						
CC	307	230	14.7		1.0	
CT	48	37	16.5		0.91 (0.64–1.29)	0.590
TT	3	3	5.9	0.023	4.64 (1.43–15.0)	0.011
<i>XRCC1</i> -Q399R						
AA	123	90	16.3		1.0	
AG	189	150	14.7		1.18 (0.91–1.54)	0.229
GG	51	40	14.6	0.321	0.98 (0.67–1.43)	0.907
<i>LIG3</i> G39A						
GG	300	229	15.3		1.0	
GA	70	51	15.3		0.96 (0.71–1.32)	0.817
AA	1	1	22.5	0.956	0.87 (0.12–6.29)	0.886
<i>LIG4</i> C54T						
CC	257	196	14.7		1.0	
CT	100	76	16.9		0.86 (0.66–1.12)	0.270
TT	8	4	12.6	0.523	0.49 (0.18–1.34)	0.165

¹ MST, median survival time. The numbers of patients for each genotype do not add up to 378 because of the failure of genotyping assays in some patients.

² Multivariate Cox regression with adjustment of race, stage, surgery, performance status, CA19-9 level and cytotoxic treatment.

³ Mean survival time is presented because MST could not be calculated.