Excision Repair Cross-Complementation Group 1 Polymorphism Predicts Overall Survival in Advanced Non-Small Cell Lung Cancer Patients Treated With Platinum-Based Chemotherapy

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ABSTRACT

DNA repair is a critical mechanism of resistance to platinum-based chemotherapy. Excision repair cross-complementation group 1 (ERCC1) is the lead enzyme in the nucleotide excision repair process. Increased ERCC1 mRNA levels are related directly to platinum resistance in various cancers. We examined the association between two polymorphisms of ERCC1, codon 118 C/T and C8092A, which are associated with altered ERCC1 mRNA levels and mRNA stability, and overall survival (OS) in 128 advanced non-small cell lung cancer patients treated with platinum-based chemotherapy. The two polymorphisms were in linkage disequilibrium. There was a statistically significant association between the C8092A polymorphism and OS (P = 0.006, by log-rank test), with median survival times of 22.3 (C/C) and 16.1 (C/T), respectively. No statistically significant association was found for the codon 118 C/T polymorphism and OS (P = 0.41, by log-rank test), with median survival times of 19.9 (T/T), 16.1 (C/T), and 13.3 (C/C) months, respectively. In conclusion, the ERCC1 C8092A polymorphism may be a useful predictor of OS in advanced non-small cell lung cancer patients treated with platinum-based chemotherapy.

INTRODUCTION

Platinum compounds have become part of the mainstay chemotherapy treatment for advanced non-small cell lung cancer (NSCLC). However, patient response rates to platinum-based regimens remain very low (1). One critical mechanism of resistance to platinum drugs is efficient DNA repair capacity. Nucleotide excision repair (NER) is a major cellular defense mechanism against the cytotoxic effects of platinum-based chemotherapeutic agents (2). Excision repair cross-complementation group 1 (ERCC1), a highly conserved enzyme, is the lead component of the NER process and is specific to the NER pathway; its absence is incompatible with life (3). Increased ERCC1 mRNA levels are related directly to clinical resistance to platinum-based chemotherapy in human gastric, ovarian, cervical, colorectal, and NSCLCs (4–10).

Two common polymorphisms of ERCC1, codon 118 C/T and C8092A, have been reported (11–15). The codon 118 C/T polymorphism is associated with differential mRNA levels (11, 12) and has been found to be associated with shorter overall survival (OS) for advanced colorectal cancer patients treated with platinum-based chemotherapy (13). The C8092A polymorphism, located in the 3′-untranslated region of the gene, may affect ERCC1 mRNA stability and has been associated with the risk of adult-onset glioma (14). The roles of the two ERCC1 polymorphisms in the survival of NSCLC patients have not been evaluated. In this study, we investigated the association between these two ERCC1 polymorphisms and OS in advanced NSCLC patients who were treated with platinum-based chemotherapy.

MATERIALS AND METHODS

Study Population. This study was approved by the Human Subjects Committee of Massachusetts General Hospital (MGH) and Harvard School of Public Health, Boston, MA. The study population is derived from a large ongoing molecular epidemiological study that began in 1992 and now has greater than 1000 NSCLC patients recruited at MGH. Eligible patients were over 18 years old with histologically confirmed NSCLC. Before 1997, enrollment was restricted initially to individuals with operable lung cancer, explaining the large difference between the sample size for the underlying epidemiological study and the number of advanced NSCLC cases (42% of all patients). More than 85% of eligible patients were recruited in this cohort. In this study, we first identified 480 patients who had advanced NSCLC (stages IIIA-IV). Of these, 251 were chosen...
for this study because they were recruited after 1992 but no later than 1999, ensuring a follow-up time of at least 3 years. Among these 251 patients, there were 128 NSCLC patients whose histological diagnoses were confirmed at MGH, who were treated at MGH with platinum agents (cisplatin or carboplatin), either first- or second-line, and who had complete outpatient records available to us. The patients who were not included in this subset were either treated at outside facilities (such that methods of treatment could not be verified and may not have been standard), or did not have available MGH outpatient charts. The demographics of the subjects not included in the analysis were similar to those of the included subjects.

**ERCC1 Genotyping.** Whole blood was collected from patients at the time of enrollment, and DNA was extracted from these samples using the Puregene DNA Isolation kit (Genta Systems; Minneapolis, MN). The ERCC1 codon 118 polymorphism was detected using the PCR-RFLP method, with the published primer sequences of 5′-gagagtctacttggagac-3′ and 5′-gaggtgcaagaaggggga-3′ (13). The 199bp PCR products were then digested overnight by HpyCH4 enzyme (New England BioLabs, Beverly, MA) and led to C/C (21, 116 bp), C/T (21, 116, 137 bp), and T/T (137 bp) genotypes, with the control band of 62 bp for each genotype. For quality control purposes, genotyping on 10% random samples was repeated using the previously reported BsuDI enzyme (New England BioLabs) digestion with the PCR product (13). There was 100% agreement of the genotype results between these two methods.

The ERCC1 C8092A polymorphism was also detected using the PCR-RFLP method, with the primer sequences of 5′-tgagccatcagccatacag-3′ and 5′-cttagtctcatcaattc-3′ modified from published results (13–15). The 255 bp PCR products were then digested overnight by MboII enzyme (New England BioLabs) and led to C/C (158 bp), C/A (41, 117, 158 bp), and A/A (41, 117 bp) genotypes, with control bands of 6 and 91 bp for each genotype. For quality control purposes, 10% random samples were genotyped using an alternative pair of PCR primers, with the sequences of 5′-cagacagtgccatccagag-3′ and 5′-gggacctcatccttc-3′, and a PCR product of 161 bp. MboII enzyme digestion led to differentiating C/C (161bp), C/A (60, 101, 161 bp), and A/A (60, 101 bp) genotypes. There was 100% agreement between these two methods.

**Survival Measurements.** OS was the end point in this analysis. Survival time was calculated from the date of diagnosis to the date of last follow-up or death from any cause. Dates of death were obtained and cross-checked using at least one of the following four methods: (a) Social Security Death Index, (b) inpatient and outpatient medical records, (c) MGH tumor registry, and (d) confirmation with the patient’s primary care physician and/or family. Patients who were not deceased were censored at the last date they were known to be alive based on the date of last contact. This date was verified by methods (b) and/or (d) as described above.

**Statistical Analysis.** Demographic and clinical information was compared across genotype, using Pearson χ² tests (for categorical variables) and one-way ANOVA (for continuous variables), where appropriate. Linkage disequilibrium between the two polymorphisms was tested using Fisher’s exact test (16). The association between the genetic polymorphisms and OS was estimated using the method of Kaplan and Meier and assessed using the log-rank test. Median follow-up time was computed among censored observations only. Cox proportional hazards models were also used to adjust for clinical stage, performance status, and treatment, and genotype groups were treated as indicator variables. Because of the small number of patients with A/A genotype for the ERCC1 C8092A polymorphism, we combined the C/A and A/A genotype groups in the analysis, as suggested in other studies (13–15). All statistical testing was two-sided and used SAS software version 8 (Cary, NC). P values < 0.05 were considered statistically significant.

**RESULTS**

**Patient, Treatment, and Follow-up Characteristics.** Adenocarcinoma, squamous cell carcinoma, and large cell carcinoma represented 51%, 20%, and 18% of the total 128 NSCLC patients, respectively, and 11% were of mixed histological subtype or had more than one primary tumor. A majority of patients received radiation along with chemotherapy (66%). Most (91%) of the stage III patients received radiation therapy, whereas only 24% of the stage IV patients were radiated as part of the primary treatment, largely for palliation of symptoms caused by large or obstructing lesions. The median follow-up time was 65.3 months (range 4.9–121.5 months), with a median survival time of 16.1 months. Detailed demographic, treatment, and follow-up characteristics are shown in Table 1.

**Genotype Information.** For the ERCC1 codon 118 polymorphism, the frequencies of T/T, C/T, and C/C genotypes were 40%, 39%, and 21%, respectively. Although the T/T genotype generates the less commonly associated triplet codon sequence encoding the amino acid and has been termed the “variant” by convention, the T/T genotype has higher frequencies in several studies (12, 13, 17) and is used as a reference group in all our analyses. For the C8092A polymorphism, the frequencies of C/C, C/A, and A/A genotypes were 53%, 41%, and 6%, respectively. Genotype frequencies of the two polymorphisms were comparable with those reported in previous studies (13–15, 17). The two polymorphisms were in linkage disequilibrium (P < 0.0001, by Fisher’s exact test), and genotype concordances in our study between the two polymorphisms were 72% (i.e., the A allele of C8092A polymorphism correlated with the C allele of codon 118 C/T polymorphism). There were no statistically significant differences in the genotype frequencies of the two polymorphisms in different subgroups of age, gender, clinical stages, performance status, treatment, or death status (P > 0.05, by Fisher’s exact test).

**Survival Analyses.** Results of survival analyses are shown in Table 2 and Fig. 1. The associations between OS and the two ERCC1 polymorphisms were assessed first using the log-rank test. For the codon 118 polymorphism, there was no statistically significant difference in OS among different genotype groups (P = 0.41, by log-rank test; Fig. 1A). The MSTs of codon 118 C/C, C/T, and T/T genotypes were 13.3, 16.1, and 19.9 months, respectively. For the ERCC1 C8092A polymorphism, individuals carrying at least one A allele (i.e., C/A + A/A) had a worse OS when compared with those carrying the C/C genotype, with MSTs of 13.4 and 22.3 months, respectively (P = 0.006, by log-rank test, Fig. 1B). Even when we considered the C/A (MST, 13.4 months) and A/A (MST, 12.1 months)
genotypes separately, the log-rank test comparing all three groups was still significant ($P = 0.02$).

In the Cox proportional hazards models, we first incorporated both ERCC1 polymorphisms in the same model. Because there were no statistically significant associations between the codon 118 polymorphism and the risk of dying ($P = 0.42$ for the C/C genotype and $P = 0.47$ for the C/T genotype, when compared with the T/T genotype), the codon 118 polymorphism was removed from the model. In the final Cox proportional hazards model that included only the C8092A polymorphism, individuals carrying at least one A allele (C/A + A/A) had a statistically worse survival, with a hazard ratio of 1.5 [95% confidence interval (CI), 1.01–2.22] when compared with the C/C genotype group (Table 2). Considered separately, the hazard ratios were 1.58 (95% CI, 1.05–2.38; C/A versus C/C) and 1.1 (95% CI, 0.49–2.49; A/A versus C/C), although the latter comparison was based on only eight individuals with the A/A genotype.

In the analyses stratified by stage (Table 2), the association between ERCC1 C8092A polymorphism and OS appeared to be stronger in the stage III (both IIIA and IIIB) patients (hazard ratio $= 2.02$, 95% CI, 1.21–3.39, $P = 0.008$) than in the stage IV patients (hazard ratio $= 0.89$, 95% CI, 0.48–1.65, $P = 0.71$), which is consistent with the results of the log-rank tests. No statistically significant association was found for the codon 118 polymorphism and OS in either stage III or stage IV patients (Table 2).

**DISCUSSION**

Interindividual variability in drug efficacy has multiple sources. DNA repair systems are critical in correcting DNA damage induced by carcinogens; however, they also play an important role in repairing the cross-linking and oxidative damage caused by platinum drugs (18). The effect on clinical outcome can be difficult to predict based on the level of DNA repair. As an example, impaired DNA repair capacity may increase carcinogenesis and lead to more biologically aggressive tumors and decreased survival; on the other hand, decreased DNA repair may contribute to the persistence of functional platinum-DNA adducts that confer antitumor activity and impart more favorable prognoses.

DNA repair, especially NER, plays important roles in the defense of platinum-based drug-induced DNA damage, including the removal of DNA adducts (2, 19). ERCC1 is the lead enzyme in the NER process. High ERCC1 levels are associated with increased removal of platinum-induced DNA adducts and relative platinum resistance (20), and ERCC1-defective cells or knockout mice are highly sensitive to DNA cross-linking agents (21, 22). Increased ERCC1 mRNA levels have been related directly to clinical resistance to 5-fluorouracil and cisplatin in human gastric, ovarian, and cervical cancers (4–6). Higher intratumoral ERCC1 mRNA levels have been associated with poorer OS (but not response) in patients with metastatic colorectal cancer treated with 5-fluorouracil/oxaliplatin (7). Overexpression of ERCC1 also has been correlated with poorer survival in gemcitabine/cisplatin-treated NSCLC patients (8–10). Therefore, polymorphisms that may affect ERCC1 mRNA expression or stability may be used as surrogate prognostic and/or predictive markers of tumor behavior and cancer outcomes for patients treated with platinum agents.

Our results suggest that the **ERCC1 C8092A** polymorphism predicts OS for advanced NSCLC patients treated with platinum-based chemotherapy. The precise mechanism for the association of the **C8092A** polymorphism with NSCLC survival remains unclear, as there are no direct functional data available for this polymorphism. One hypothesis is that this polymorphism may be associated with mRNA stability (14). Previous studies have demonstrated an association between this polymorphism and cancer risks (14, 15), although no association with OS was found in advanced colorectal cancer patients treated with platinum-based chemotherapy (13).

The effect of **ERCC1 C8092A** polymorphism on survival was observed only in stage III patients. One possible reason is that stage IV patients may have too many somatic mutations driving tumor growth or treatment resistance, such that any subtle capacity of genotypes to alter DNA repair capacity is overwhelmed. Alternatively, the survival differences may reflect a radiation-related outcome, because most stage III individuals received radiation to the primary tumor, whereas only a minority of stage IV individuals was radiated as part of the
primary treatment. This latter explanation does not explain the common occurrence of relapsed metastatic disease outside the field of radiation.

We did not find a statistically significant association between the ERCC1 codon 118 polymorphism and OS in our advanced NSCLC patients. Park et al. (11) reported a trend toward higher intratumoral ERCC1 mRNA levels with an increasing number of T alleles in a study of 31 advanced colorectal cancer patients. This group also found that the ERCC1 codon 118 polymorphism was associated with shorter OS for advanced colorectal cancer patients treated with platinum-based chemotherapy (13). However, another study reported that ovarian cell lines containing the variant genotype were shown to have decreased ERCC1 mRNA induction after cisplatin exposure when compared with the wild-type cell line (12). Thus, the predictive role of the codon 118 polymorphism in platinum-treated patients warrants additional study.

The survival differences between different ERCC1 genotype groups are more pronounced in subjects after the first year (Fig. 1). One explanation is that subjects who died quickly (i.e., within 1 year) responded poorly to platinum treatment; therefore, the platinum treatment had only a modest effect on survival, and the effect of ERCC1 genotypes was minimal. However, subjects with more prolonged survivals may have had a higher response to platinum treatment. If so, platinum treatment may have had a greater impact on survival, leading to larger observed differences between different ERCC1 genotypes for these individuals. Treatment response should be assessed as an additional end point in prospective studies.

There are a number of limitations in this study. First, as a retrospective study, the only accurately measurable outcome was OS. Measuring response rate and time to progression is critical in understanding further the mechanism of the ERCC1 polymorphisms, and will help to distinguish whether ERCC1 polymorphisms are predictive of treatment response or prognostic by determining outcome. Second, as with any study of modest size, this one may lack some generalizability. Patients were selected based on advanced stage disease and receipt of

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Abbreviations: MST, median survival time; CI, confidence interval.
* Adjusted for stage, performance status, and treatment, and genotypes were treated as indicator variables.

Fig. 1 Kaplan-Meier curves of the ERCC1 codon 118 polymorphism (A, *P = 0.41*, by log-rank test) and the ERCC1 C8092A polymorphism, where C/A and A/A genotypes are combined (B, *P = 0.006*, by log-rank test). The graph presents 5 years of follow-up. Log-rank test was based on the full data.
platinum drugs, allowing us to address our question regarding the association among the ERCCI polymorphisms, platinum agents, and clinical outcomes. Third, because of the advanced stage of disease of the patients, and the potential that deficient DNA repair could result in higher toxicity, we could not distinguish between death from cancer and death from other causes such as toxicity, which may introduce bias in our results. However, most advanced NSCLC deaths are attributable to disease progression rather than treatment-related toxicities (23). Fourth, there are no clear functional data, especially in vivo functional data, for the ERCCI polymorphisms. Lastly, we did not include polymorphisms of the other DNA repair genes or platinum metabolizing genes in the analysis to avoid multiple comparisons and ensure enough statistical power.

To our knowledge, this is the first study demonstrating that the ERCCI C8092A polymorphism is associated with OS in advanced NSCLC patients treated with platinum-based chemotherapy. Our results suggest that polymorphisms of DNA repair genes may play an important role in the prognosis of advanced stage NSCLC patients. Larger sample size studies and in vivo functional studies are needed to confirm the results and identify the clear biological basis of these findings.

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