ERCC1 and XRCC1 single nucleotide polymorphisms can guide treatment decision in patients with metastatic non-small cell lung cancer

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Abstract

Results from studies in several cancers on single nucleotide polymorphisms (SNPs) suggest that DNA repair capacity may have prognostic implication for disease recurrence, survival, and responses to treatment. This study aimed to evaluate the potential prognostic value of SNPs as biomarkers in patients with metastatic non-small cell lung cancer (mNSCLC) treated with platinum. Analysis of SNPs from peripheral blood cells was performed by polymerase chain reaction. Excision repair cross-complementing group 1 (ERCC1)-Asn118Asn, excision repair cross-complementing group 2 (ERCC2)-Lys751Gln, X-ray repair cross-complementing group 1 (XRCC1)-Arg399Gln, and tumor protein 53 (TP53)-Arg72Pro polymorphisms were evaluated in conjunction with clinical and pathological parameters, and survival. The median progression-free survival (PFS) and overall survival (OS) of 145 patients were 5.1 months and 30.9 months, respectively. In the univariate analysis ERCC1 genotype, XRCC1 genotype, and Eastern Cooperative Oncology Group Performance Status (ECOG-PS) were significant parameters for OS. In the multivariate analysis ERCC1 genotype, XRCC1 genotype, and ECOG-PS retained their significance. The median OS was 45.2 months for the ERCC1 normal (CC) and heterozygote (CT) genotypes, and 25.5 months for the ERCC1 mutant (TT) genotype. The median OS was 31.4 months for the XRCC1 normal (AA) and heterozygote (AG) genotypes, and 23.1 months for the XRCC1 mutant (GG) genotype. The median OS was 30.7 months for ECOG-PS ≤ 1 and 10.2 months for ECOG-PS ≥ 2. ERCC1 and XRCC1 genotypes, and ECOG-PS independently predicted OS in mNSCLC patients. Additional studies are needed for the further evaluation of potential prognostic SNPs in mNSCLC.

Keywords: Biomarker, lung cancer, platinum, single nucleotide polymorphism, survival

Introduction

Lung cancer is the second most common cancer and is the main cause of cancer death among both men and women. Although lung cancer is a molecularly heterogeneous group of diseases and new treatment methods are available, the 5-year survival rate in advanced disease is still 6% [1,2].

The treatment of lung cancer remains challenging. Platinum, the main component of cytotoxic chemotherapy (CT) used in the treatment of lung cancer, damages the DNA and causes the death of tumor cells [3]. However, platinum response rates are less than 30% in patients with metastatic non-small cell lung cancer (mNSCLC) [4]. The efficacy of platinum regimens is limited due to drug resistance. A thorough understanding of the mechanisms of resistance and the identification of new biomarkers for more accurate prognostic and predictive assessment is necessary for personalized cancer treatment and may emphasize the possibility of patient-tailored platinum-based CT for mNSCLC [5,6].

Accumulating results from the studies on single nucleotide polymorphisms (SNPs) in several cancers as well as NSCLC suggest that DNA repair capacity may have prognostic implication for disease recurrence, survival, and responses to platinum-based CT, but the results are controversial [7-9].

In this study, we aimed to evaluate the potential prognostic value of SNPs consisting of excision repair cross-complementing group 1 (ERCC1), excision repair cross-complementing group 2 (ERCC2), X-ray repair cross-complementing group 1 (XRCC1) and tumor
protein 53 (TP53) in mNSCLC patients treated with platinum-based chemotherapy.

Materials and Methods

This was a multicenter, cross-sectional, and retrospective study. In this study, we recruited 145 patients with mNSCLC who were treated with platinum at the Medical Oncology Departments of Necmettin Erbakan University, Ankara University, Akdeniz University, and Dicle University between April 2010 and July 2012. Data on age, gender, comorbidities, pathological diagnosis, previous treatments such as neoadjuvant and/or adjuvant chemotherapy, date of disease progression and date of death of patients were recorded. Patients were followed up every three months after completion of treatment to evaluate survival.

Ethical approval

The study was performed according to the Declaration of Helsinki and approved by the Ethics Committee of the Meram Medical School of Selcuk University and also performed with the financial support of the Scientific Research Project of Selcuk University (Project no.: 11102028).

Eligibility

Age between 18 to 80 years, having pathologically proven mNSCLC with radiologically measurable disease, being treated with platinum-based CT regimens (dose concerning the National Comprehensive Cancer Network guidelines for CT), allowing informed consent for genetic analysis were the eligibility criteria.

DNA analysis

A ten mL of a peripheral venous blood sample by the nursing staff was obtained from each patient for DNA analysis. All of these blood samples taken from participating centers were sent to Meram Medical Faculty Department of Genetics. High Pure Polymerase Chain Reaction (PCR) Template Preparation Kits (Roche Diagnostics, Mannheim, Germany) were used for DNA isolation. After DNA isolation each sample was stored for PCR analysis.

Polymerase chain reaction analysis

PCR analysis was performed with the Roche Light Cycler 480 II real-time PCR system (Roche Molecular Systems, Inc.). The polymorphisms of codon 118 (rs11615) of ERCC1, codon 751 (rs13181) of ERCC2, codon 399 (rs25487) of XRCC1, and codon 72 (rs1042522) of TP53 were genotyped. Each PCR reaction (20 µL) contained 200 ng of DNA template, 200 µM of dNTP, 1 unit of Taq DNA polymerase, and 200 µM of primers, as well as 1.5 mM of MgCl2. The PCR conditions were one cycle of 10 min at 95 ºC; 45 cycles of 10 s denaturation at 95 ºC; 10 s hybridization at 60 ºC for ERCC1, ERCC2, and XRCC1; or 58 ºC for TP53 and 15 s elongation at 72 ºC. Following PCR amplification, the results were classified as normal, heterozygote, or mutant with melting curve analysis. Genotyping was done blind, and a random 5% of the samples were repeated to validate the genotyping procedures. Two authors independently reviewed all genotyping results.

Statistical analysis

Primary statistical analysis has included descriptive statistics of the patients as age, gender, Eastern Cooperative Oncology Group Performance Status (ECOG-PS), histological subtypes, the status of neoadjuvant CT, subtypes of the surgical procedure, the status of adjuvant CT, first-line CT regimens for metastatic disease. All patients underwent overall survival (OS) and progression-free survival (PFS) analysis.

Statistical analysis was performed by using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA). Univariate and multivariate Cox regression analyses and Kaplan Meier survival curves subjected to log-rank testing were utilized for the survival analyses. Forward likelihood ratio was used for the multivariate selection process. A p-value <0.05 was required for statistical significance.

Results

Characteristics of patients

A total of 145 patients were enrolled in this study. There were 126 males and 19 females. The median age was 60 years (range 34-79). The ECOG-PS of 99 patients was 0-1, while the ECOG-PS of the remaining 46 patients was 2 or more. There were 64 cases of adenocarcinoma, 55 cases of squamous cell carcinoma, and 26 cases of the other histological subtypes. All patients received platinum-based CT as the first line for metastatic disease Demographic and clinical parameters of patients were shown in Table 1.

Table 1. Demographic and clinical parameters of patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>(n=145)</th>
<th>(100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), median (range)</td>
<td>60(34-79)</td>
<td></td>
</tr>
<tr>
<td>Gender, n (%)</td>
<td>Male</td>
<td>126(86.9)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>19(13.1)</td>
</tr>
<tr>
<td>ECOG-PS, n (%)</td>
<td>≤1</td>
<td>99(68.2)</td>
</tr>
<tr>
<td></td>
<td>≥2</td>
<td>46(31.8)</td>
</tr>
<tr>
<td>Histological subtype, n (%)</td>
<td>Adenocarcinoma</td>
<td>64(44.1)</td>
</tr>
<tr>
<td></td>
<td>Squamous cell carcinoma</td>
<td>55(37.9)</td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td>26(18.0)</td>
</tr>
<tr>
<td>Neoadjuvant CT, n (%)</td>
<td>Yes</td>
<td>13(9.0)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>136(91.0)</td>
</tr>
<tr>
<td>Surgery subtype (if done), n (%)</td>
<td>Lobectomy</td>
<td>30(20.7)</td>
</tr>
<tr>
<td></td>
<td>Pneumectomy</td>
<td>3(2.1)</td>
</tr>
<tr>
<td>Adjuvant CT, n (%)</td>
<td>Yes</td>
<td>23(15.9)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>122(84.1)</td>
</tr>
<tr>
<td>First line CT for metastatic disease, n (%)</td>
<td>Cisplatin+Gemcitabine</td>
<td>44(30.3)</td>
</tr>
<tr>
<td></td>
<td>Carboplatin+Paclitaxel</td>
<td>33(22.8)</td>
</tr>
<tr>
<td></td>
<td>Cisplatin+Docetaxel</td>
<td>21(14.5)</td>
</tr>
<tr>
<td></td>
<td>Other Platinum-based regimens</td>
<td>47(32.4)</td>
</tr>
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</table>
Gene polymorphisms
ERCC1 was 25.1% in CC, 52.8% in CT heterozygosity, and 22.1% in TT mutant alleles. ERCC2 was 23.1% in CC, 52.8% in CT heterozygosity, and 24.1% in TT mutant alleles. XRCC1 polymorphism was 34.4, 41.0, and 24.6% in AA, AG heterozygosity, and GG mutant alleles, respectively. TP53 was 14.4% in CC, 48.2% in CG heterozygosity, and 37.4% in GG mutant alleles.

Gene polymorphisms and survival outcomes
The median PFS and OS were 5.1 months (95% confidence interval (CI), 4.3-5.8) and 30.9 months (95% CI, 28.2-33.6), respectively.

In the univariate analysis for OS, ERCC1 genotype (hazard ratio (HR), 0.26; 95% CI, 0.12-0.57; p=0.001), XRCC1 genotype (HR, 0.37; 95% CI, 0.16-0.83; p=0.01), and ECOG-PS (HR, 5.25; 95% CI, 1.45-19.04; p=0.01) were significant parameters. Therefore, multivariate analyzes were performed, and ERCC1 genotype (HR, 0.36; 95% CI, 0.14-0.92; p=0.03), XRCC1 genotype (HR, 0.37; 95% CI, 0.14-0.98; p=0.04), and ECOG-PS (HR, 4.80; 95% CI, 1.22-18.87; p=0.02) retained their significance. In univariate analysis, there was no significant difference in PFS according to age, gender, number of metastatic organs, ERCC1, ERCC2, XRCC1, TP53, and ECOG-PS (Table 2).

The median OS was 45.2 months (95% CI, 25.3-65.0) for the ERCC1 normal (CC) and heterozygote (CT) genotypes, and 25.5 months (95% CI, 9.5-41.5) for the ERCC1 mutant (TT) genotype (p<0.01) (Figure 1).

The median OS was 31.4 months (95% CI, 20.1-42.7) for the XRCC1 normal (AA) and heterozygote (AG) genotypes, and 23.1 months (95% CI, 20.0-26.3) for the XRCC1 mutant (GG) genotype (p=0.01) (Figure 2).

The median OS was 30.7 months (95% CI, 26.4-35.1) for ECOG-PS ≤ 1 and 10.2 months (95% CI, 3.4-16.9) for ECOG-PS ≥ 2 (p<0.005) (Figure 3).

Table 1. The predictors of PFS and OS

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>OS (univariate)</th>
<th>PFS (univariate)</th>
<th>OS (multivariate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), median (range)</td>
<td>HR</td>
<td>p value</td>
<td>HR</td>
</tr>
<tr>
<td>Age</td>
<td>1.01</td>
<td>0.40</td>
<td>1.00</td>
</tr>
<tr>
<td>Gender</td>
<td>0.70</td>
<td>0.57</td>
<td>1.36</td>
</tr>
<tr>
<td>Number of metastatic organs</td>
<td>1.60</td>
<td>0.30</td>
<td>1.01</td>
</tr>
<tr>
<td>ERCC1 (Asn118Asn)</td>
<td>0.26</td>
<td>0.001*</td>
<td>0.84</td>
</tr>
<tr>
<td>ERCC2 (Lys751Gln)</td>
<td>0.88</td>
<td>0.80</td>
<td>0.79</td>
</tr>
<tr>
<td>XRCC1 (Arg399Gln)</td>
<td>0.37</td>
<td>0.01*</td>
<td>1.30</td>
</tr>
<tr>
<td>TP53 (Arg72Pro)</td>
<td>1.31</td>
<td>0.69</td>
<td>1.10</td>
</tr>
<tr>
<td>ECOG-PS</td>
<td>5.25</td>
<td>0.01*</td>
<td>3.49</td>
</tr>
</tbody>
</table>

FS; Progression free survival, OS; Overall survival, HR; Hazard ratio, ERCC1: Excision repair cross-complementing group 1, ERCC2: Excision repair cross-complementing group 2, XRCC1: X-ray repair cross-complementing group 1, TP53: Tumor protein 53, ECOG-PS; Eastern Cooperative Oncology Group Performance Status *; statistically significant (p value<0.05)

Figure 1. The overall survival with respect to ERCC1 genotypes

Figure 2. The overall survival with respect to XRCC1 genotypes
Lys751Gln, have been identified in some exons of ERCC2 [16]. SNPs, which have been shown to induce amino acid changes such as Asp312Asn and helicase activity, participates in NER [15]. Also, ERCC2, where the gene product has ATP-dependent nucleotide excision repair (NER) pathway which consists of many polypeptides, including ERCC1 and ERCC2 are thought to be the cause of this critical problem [11,12]. Explaining the mechanisms of resistance and the identification of prognostic and predictive biomarkers are necessary for personalized treatment of mNSCLC.

Although many newer treatment methods that target mutations and rearrangements in oncogenes are available and have excellent clinical benefit and minimum toxicity, the majority of patients with mNSCLC are not appropriate for these drugs due to the lack of target mutations or rearrangements in these oncogenes [10]. Currently, platinum-based CT is still the primary treatment modality for most of the patients with mNSCLC. But, the platinum response rate is not high in patients with advanced NSCLC [4]. The efficacy of platinum regimens is limited due to drug resistance. Decreased accumulation of platinum, increased drug inactivation, enhancement of tumor cells’ tolerance to platinum-DNA adducts, and enhancement of the repair of the damaged DNA by base excision repair (BER) pathway which consists of many polypeptides, including ERCC1 and ERCC2 are thought to be the cause of this critical problem [11,12].

ERCC1 plays a critical role in the NER pathway and simplifies the excision of damaged DNA, and thus, can be used as a prognostic biomarker to reestablish the therapeutic sensitivity to platinum-based agents in chemoresistant patients [5,6,13,14]. Also, ERCC2, where the gene product has ATP-dependent helicase activity, participates in NER [15]. SNPs, which have been shown to induce amino acid changes such as Asp312Asn and Lys751Gln, have been identified in some exons of ERCC2 [16]. Several studies demonstrated the relationship between ERCC2 SNPs and platinum activity in many different types of cancer, but the relationship between ERCC2 levels and treatment results in patients treated with platinum-based CT is still controversial [17-19]. Kang et al. demonstrated that upregulation of ERCC1 in metastatic lymph nodes was a poor prognostic factor in N1 patients but not in N2 patients who underwent surgical resection followed by platinum-based adjuvant CT [20]. In several studies, it has been demonstrated that the suppression of ERCC1 can regenerate or increase platinum sensitivity and the low level of ERCC1 was associated with significantly improved OS in lung cancer patients treated with platinum-based CT [21-23]. Olaussen et al. demonstrated that ERCC1 expression is a possible marker for the effect of postoperative adjuvant CT in patients with resected tumors. Adjuvant platinum-based CT was reported to be beneficial for survival in ERCC1-negative patients, but no benefit was observed in ERCC1-positive patients [22]. In another study, Geredeli et al. evaluated ERCC1 and ERCC2 polymorphisms in surgically treated NSCLC patients, and an increase in relapse-free survival (RFS) was observed, but was not statistically significant [24]. Kalikaki et al. showed that ERCC1 polymorphic variants were independent prognostic factors for improved OS and ERCC1 genotype was significantly associated with response to platinum-based treatment in advanced NSCLC patients treated with platinum-based CT [25]. On the other hand, Yin et al. performed a meta-analysis in patients who received platinum-based treatment and demonstrated that the rate of ERCC1 and ERCC2 polymorphisms did not have a predictive or prognostic value for RFS and OS [26]. Besides all these, in this study, we evaluated the association between ERCC1 and ERCC2 polymorphisms and survival in mNSCLC treated with platinum-based CT. So here we showed that the median OS was longer for the ERCC1 normal and heterozygote genotypes, and shorter for the ERCC1 mutant genotype and it was statistically significant. These results confirmed the positive data related to ERCC1 in the literature and revealed that platinum-based CT may be more beneficial in patients with ERCC1 normal and heterozygote genotypes. And, for the first time, these results showed that ERCC1 had a prognostic role also in the Turkish cohort. However, unlike ERCC1, we demonstrated no significant difference between ERCC2 polymorphism and survival in mNSCLC.

XRCC1 is another protein involving NER and a member of the base excision pathway in DNA repair paths. XRCC1 gene polymorphism is associated with suboptimal DNA repair, and as a result of this inadequate process, the tumors may become more biologically aggressive [16,27]. XRCC1 T-77C polymorphisms are associated with increased risk of NSCLC [28]. Kim et al. showed that non-mNSCLC patients with AA homozygous variants of XRCC1 gene polymorphism had better survival rates [29]. Besides, the polymorphisms of XRCC1 399 and XRCC3 241 have been reported to be a prognostic factor in survival [25,30,31]. However, Yao et al. examined XRCC1 polymorphism in advanced-stage NSCLC patients, but no statistically significant correlation was observed [32]. Also, Gurubhagavatula et al. demonstrated that XRCC1 variant alleles were associated with short-term survival in NSCLC patients treated with platinum-based CT [33]. Moreover, XRCC1 was found not to be associated with prognosis and survival in another study on T-77C polymorphism [7]. In this study, we also evaluated the relationship between XRCC1 polymorphism and survival in mNSCLC. So here we demonstrated that the median OS was shorter in XRCC1 mutant genotype and it was statistically significant. This result suggested that polymorphism of XRCC1 may play an essential role as a prognostic marker in the survival
of mNSCLC patients and should be considered as a predictive marker for treatment outcome of platinum-based CT.

As in lung cancer, the most commonly mutated gene in cancer is the TP53 tumor suppressor gene, which, as a result of these mutations, has acquired oncogenic properties to promote invasion, metastasis, proliferation and cell survival [34,35]. Many studies have demonstrated that TP53 mutations have a potential prognostic role for disease recurrence and shorter survival in non-mNSCLC [36-38]. Although Fukuyama et al. demonstrated that the NSCLC patients with TP53 mutations had a worse prognosis in early-stage cases, there was no statistically significant difference when advanced-stage cases or all patients were considered [39]. To the best of our knowledge, few studies in the literature investigated the relationship between TP53 polymorphism and prognosis and survival in patients with mNSCLC. In this study, we evaluated the relationship between TP53 polymorphism and survival in mNSCLC and found no statistically significant difference. This result is consistent with the current literature.

Performance status is one of the most critical criteria in the choice of treatment in patients with mNSCLC and is an independent predictor of poor prognosis in patients with advanced or mNSCLC receiving CT [40-42]. Also, we demonstrated that a good ECOG-PS was statistically significant on prolonged survival.

Conclusion

We showed that ERCC1 and XRCC1 gene polymorphisms and ECOG-PS are prognostic markers in survival and should be considered as predictive markers for the treatment decision of patients with mNSCLC. Our results may provide insight and guidance on prospective studies of the assessment of potential predictive and prognostic biomarkers in patients with mNSCLC.

Competing interests

The authors declare that they have no competing interest.

Financial Disclosure

The study was approved by the Ethical Board of the Meram Medical School of Selcuk University and performed with the financial support of the Scientific Research Project of Selcuk University (Project no: 11102028)

Ethical approval

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References


