ERCC1 Expression Is a Predictor of Survival in Resected Patients With Non-small Cell Lung Cancer*

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Study objectives: Proteins of the nucleotide excision repair pathway repair DNA damage. The excision repair cross-complementing (ERCC) gene family reduces damage to DNA by nucleotide excision and repair. Impaired nuclear excision repair could lead to increased genomic instability that in turn could lead to a more malignant phenotypic behavior of tumors. We therefore evaluated the effect of intratumoral ERCC1 expression on survival in non-small cell lung cancer (NSCLC) patients who underwent surgical resection for cure.

Design: Resected tumor and the corresponding normal lung specimens from 51 patients with NSCLC who underwent surgical resection were immediately frozen in liquid nitrogen. Total RNA was extracted, reverse transcribed, and amplified with intron-spanning primers. Quantitation for ERCC1 was done using the Taqman procedure, and gene expression was normalized using 18SrRNA expression as internal reference with ERCC1 levels expressed a unit-less ratio.

Results: Tumoral ERCC1 expression ranged from 4.96 to 2,008, with a median value of 54.76. Using an ERCC1 value of 50 to dichotomize the cohort, there was a statistically significant difference in median survival for patients with ERCC1 expression > 50 (94.6 months) compared to < 50 (35.5 months) [p = 0.01]. Multivariate analysis revealed that high ERCC1 expression independently predicted for longer survival. There were no significant correlations between ERCC1 expression in tumor tissue and normal lung.

Conclusions: We conclude that resected NSCLC patients with high ERCC1 expression (> 50) have a better survival when compared to patients with low ERCC1 expression (< 50). We postulate that an intact DNA repair mechanism may reduce the accumulation of genetic aberrations that are thought to contribute to a tumors malignant potential and therefore the risk of relapse after definitive treatment. Future adjuvant and neoadjuvant chemotherapy trials in NSCLC could stratify patients according to their ERCC1 expression levels. (CHEST 2005; 127:978–983)

Key words: adenocarcinoma; non-small cell lung cancer and prognosis; nuclear excision repair genes; squamous cell carcinoma

Abbreviation: NSCLC = non-small cell lung cancer

It is estimated that lung cancer was diagnosed in 171,900 people in the United States in 2003, resulting in 157,200 deaths.1 Non-small-cell lung cancers (NSCLCs) accounted for 85% of cases, and are predominantly adenocarcinomas, squamous cell carcinomas, or large cell carcinomas.2 Even though 5-year survival rates have improved from 8% in the early 1960s to 15% in the early 1990s, there is still considerable room for improvement.3 In early clinical stages, surgical resection alone offers poor long-term survival. For pathologic stages IA, IB, IIA, and IIB, 5-year survivals are approximately 70%, 60%, 55%, and 40%, respectively.4 When the tumor has spread to the ipsilateral mediastinal lymph nodes (N2 disease), 5-year survival is 13%; when contralateral lymph nodes (N3 disease, stage IIIB) are involved, it is only 5%. Most of the recurrences are distant, highlighting the fundamentally systemic nature of the disease.5 Adjuvant chemotherapy, radiation, or both in resectable NSCLC have shown only a marginal benefit.6–8

A better understanding of the biology of NSCLC...
could enable us to predict for recurrence and may also be useful to select the therapeutic intervention with optimal impact on recurrence, survival, and quality of life. Proteins of the nucleotide excision repair pathway are thought to repair DNA damage caused by platinum agents. The excision repair cross-complementing (ERCC) gene family reduces damage to DNA by nucleotide excision and repair. Modified nucleotides together with adjacent nucleotides are removed from the damaged strand during the first step (excision), which is followed by synthesis of an intact strand through DNA polmerase activity (repair synthesis). The ERCC1 gene encodes a protein of 297 amino acids that is considered to function in a complex with ERCC11, XPF, and ERCC4. This complex may be required in both recombinatorial repair and nucleotide excision repair. Impaired nuclear excision repair could lead to increased genomic instability that in turn could lead to a more malignant phenotypic behavior of tumors. The predominant type of genome instability in cancer is structural aberration of chromosomes (i.e., deletions, translocations, and insertions). These are thought to arise as a result of impaired repair of DNA double-strand breaks by homologous recombination and nonhomologous end joining. We evaluated the effect of ERCC1 expression on overall survival in 51 patients with stage IA to IIB NSCLC who underwent putatively curative surgical resection.

Materials and Methods

Fifty-one patients with stage IA to IIB NSCLC, who underwent complete surgical resection with curative intent between February 1991 and January 2001, were included in the study, which was approved by the University of South Florida Institutional Review Board. Forty-five patients received no adjuvant or neoadjuvant radiation or chemotherapy. Five patients received postoperative adjuvant radiation, and one patient received postoperative adjuvant combined radiation and chemotherapy.

Tissue specimens from these patients were collected, grossly viewed and dissected by a pathologist, and frozen within 20 min in liquid nitrogen. Total RNA was extracted with a reagent (Trizol; Invitrogen; Carlsbad, CA) and quantified (GeneQuant; Pharmacia Biotech; Piscataway, NJ). Complementary DNA was prepared by reverse transcription (Superscript II; Invitrogen) of 2 μg of RNA and amplified with intron-spanning primers that generated a product of 67 base-pairs using the Taqman procedure and an ABI Prism sequence analysis system (Perkin Elmer; Foster City, CA). The Fam/Tamra ERCC1 probe was obtained from Eurogentec (Philadelphia, PA). The primer and probe concentrations were 10 pmol/L, and the polymerase chain reaction conditions were with two hold steps (50°C for 2 min, 95°C for 10 min) followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Reactions were set up in triplicate for each sample, and ERCC1 expressions were normalized to human 18S rRNA expression (#4310993E; ABI; Foster City, CA).

Statistical Analysis

Statistical comparisons of groups defined by demographic and disease characteristics with respect to ERCC1 levels were based on nonparametric methods: the Wilcoxon rank-sum test (two groups) and the Kruskal-Wallis Test (more than two groups). The decision to use these methods was based on the nonnormality of the ERCC1 data (Shapiro-Wilk statistic). Survival curves were produced and median survival times were estimated using the method of Kaplan and Meier. Comparisons of the survival curves of groups defined by ERCC1 level were based on the log-rank test. The prognostic significance of ERCC1 after adjustment for other prognostic factors was assessed using Cox proportional hazards regression. All statistical analyses were performed using statistical software (version 8.2; SAS Institute; Cary, NC). Statistical significance was based on a two-sided significance level of 0.05.

Results

The details of the patient characteristics are given in Table 1. All patients underwent either a lobectomy or pneumonectomy with mediastinal lymph node sampling. There were 37 male and 14 female patients (median age, 67 years; range, 25 to 81 years). The histologic types included 26 adenocarcinomas, 22 squamous cell carcinomas, and 3 large cell carcinomas. Six of the 51 patients were never-smokers. There were 11 patients with stage IA, 19 patients with stage IB, 2 patients with stage IIA, 11 patients with IIB, 5 patients with stage IIIA, 2 patients with stage IIIB (both with T4 primary lesions), and 1 patient with stage IV (T2N0M1, by virtue of a nodule in the adjacent lobe of the lung).

Overall, tumoral ERCC1 expression ranged from

<table>
<thead>
<tr>
<th>Variables</th>
<th>Patients, No.</th>
<th>Median ERCC1</th>
<th>p Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>51</td>
<td>54.78</td>
<td>0.10</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>37</td>
<td>64.56</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>14</td>
<td>41.22</td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td></td>
<td></td>
<td>0.18</td>
</tr>
<tr>
<td>≤ 67</td>
<td>26</td>
<td>41.22</td>
<td></td>
</tr>
<tr>
<td>&gt; 67</td>
<td>25</td>
<td>81.42</td>
<td></td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td>0.19</td>
</tr>
<tr>
<td>Never-smoker</td>
<td>6</td>
<td>224.78</td>
<td></td>
</tr>
<tr>
<td>Current or ex-smoker</td>
<td>45</td>
<td>54.78</td>
<td></td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
<td>0.04</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>26</td>
<td>100.40</td>
<td></td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>22</td>
<td>26.70</td>
<td></td>
</tr>
<tr>
<td>Large cell carcinoma</td>
<td>3</td>
<td>64.56</td>
<td></td>
</tr>
<tr>
<td>Pathologic stage</td>
<td></td>
<td></td>
<td>0.56</td>
</tr>
<tr>
<td>I</td>
<td>30</td>
<td>81.17</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>13</td>
<td>35.59</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>7</td>
<td>38.07</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>1</td>
<td>231.94</td>
<td></td>
</tr>
</tbody>
</table>

*Wilcoxon rank-sum test (two sided).
4.96 to 2,008 (median, 54.76) [since ERCC1 expression is expressed as a ratio, normalized, to 18SrRNA expression, it is unitless]. For the 37 men and 14 women, the median ERCC1 levels were 64.56 and 41.91, respectively (p = 0.10). The median age for the entire cohort was 67 years, and the median values of ERCC1 for those ≤ 67 years old and > 67 years old were 41.22 and 81.42, respectively (p = 0.18). For the 45 smokers, the median ERCC1 value was 54.78 as compared to 224.8 for the 6 never-smokers. This difference was not statistically significant (p = 0.19), possibly secondary to the small number of patients who were never-smokers.

The median values of tumoral ERCC1 for the 26 adenocarcinomas, 22 squamous cell carcinomas, and 3 large cell carcinomas were 100.4, 26.70, and 64.56, respectively. The difference in ERCC1 expression between adenocarcinomas and squamous cell carcinomas were statistically significant (p = 0.04). Squamous cell carcinomas had the lowest ERCC1 expression, which may explain the relative sensitivity of squamous cell carcinomas arising from different primary sites to platinum-based therapy, *i.e.*, head and neck, cervical, esophageal, anal, and NSCLCs to platinum-based chemotherapy. Adenocarcinomas, on the contrary, had the highest ERCC1 expression, and this may explain the relative resistance of adenocarcinomas to platinum-based chemotherapy. An analysis of ERCC1 expression by pathologic tumor stage revealed median values of 81.17 for the 30 stage I patients, 35.59 for the 13 stage II patients, and 38.07 for the 7 stage III patients. These differences were not statistically significant (p = 0.56). We also compared the ERCC1 expression of lung tumors with the ERCC1 expression of adjacent normal lung and found no statistically significant correlation (p = 0.094). Hence there were no significant correlations between ERCC1 expression in tumor tissue and normal lung (Fig 1).

We dichotomized the patient cohort based on tumoral ERCC1 expression using 50 as the cut-off value (this was rounded off from a median of 54.76 for convenience). As shown in Figure 2, there was a statistically significant difference (p = 0.01, two-sided log-rank test) in median survival for patients with ERCC1 expression > 50 (94.6 months) compared to < 50 (35.5 months). Furthermore, when we split the entire cohort into three groups based on ERCC1 expression < 30, 30 to 100, and > 100, we again found a statistically significant (p = 0.03, two-sided log-rank test) difference in survival. The median survivals were 94.6 months for the > 100 group, 62.1 months for the 30 to 100 group, and 35.5 months for the < 30 group (Fig 3). Multivariate analyses confirmed that ERCC1 expression of > 50 was an independent and significant predictor of favorable outcome (Table 2). The same conclusion

![Figure 1](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/22023/ on 04/06/2017)

*Figure 1.* No correlation was found between ERCC1 expression of lung cancers and adjacent normal lung (p = 0.094).
was reached by univariate analysis (p = 0.018; hazard ratio, 0.337; 95% confidence interval for hazard ratio, 0.137 to 0.830).

**Discussion**

For patients with NSCLC, tumor stage has been the most powerful and widely accepted parameter predictive of survival, with p < 0.05 in pair-wise within the broader stages I to IV. Many prognostic molecular markers have been described for patients with lung NSCLC, but none are currently being used in treatment decision making. Most notably, these include mutations of proto-oncogenes and tumor suppressor genes, measures of genome instability, evidence for autocrine/paracrine growth loops, expression of proteins involved in cell cycle progression and apoptosis pathways, expression of extracellular matrix proteinases and inhibitors, and metabolic activity by fluorodeoxyglucose-positron emission tomography. The future utility of many of these promising prognostic markers is presently being evaluated in prospective studies.

In the present study, we evaluated the effect of intratumoral ERCC1 expression on survival in resected patients with NSCLC. ERCC1 belongs to a family of nuclear excision repair genes, and it encodes a protein that functions in concert with other members of the repair complex to ensure genomic integrity through repair of structural aberrations and chemical nucleotide alterations. We found that increased expression of ERCC1 is a significant and independent predictor of improved survival in resected patients with NSCLC. Additionally, we also...
looked at ERCC1 expression of tumors and correlated this with the ERCC1 expression of adjacent normal lung and found the correlation to be statistically insignificant (p = 0.094). We believe that it is the intratumoral ERCC1 that is involved in tumor DNA repair and consequently influences tumor behavior. Adjacent normal lung ERCC1 expression is, therefore, in our opinion irrelevant.

The mechanism by which ERCC1 expression affects tumor behavior is postulated to be by the ability of ERCC1 to repair DNA damage in cells. The current model of carcinogenesis and tumor progression hypothesizes that progressive genetic damage accumulates in epithelial cells. Published reports also suggest that lung cancers with extensive genomic alterations as determined by DNA ploidy, microsatellite instability, and allele loss have a more malignant phenotype with increased growth rates and higher propensity for metastatic dissemination. Lung cancers display a spectrum of genetic alterations that ranges from few, yet biologically crucial, aberrations to extensive genomic damage. The extent of this damage is likely the result of the type and dosage of carcinogen exposure as well as the intrinsic ability of cells to repair this damage. Cells with extensive damage that have escaped from the physiologic proapoptotic surveillance may, overall, have a proliferative advantage and more malignant phenotypic behavior compared to cells with less extensive damage. The DNA damage repair gene measured in our investigation, ERCC1, may be representative of the intrinsic DNA damage-repair ability of the cell, and it may thus represent an intermediate biomarker of the extent of accumulated intratumoral DNA damage.

This hypothesis is supported by a recent publication on the potential role of DNA damage repair genes in lung carcinogenesis. In a pilot case-control study, Cheng et al measured the relative expression levels of five genes (ERCC1, XBP/ERCC3, XPG/ERCC5, CSB/ERCC6, and XPC) in mitogen-stimulated peripheral lymphocytes from 75 lung cancer patients and 95 control subjects. A 12 to 13% decrease in the baseline expression levels of XPG/ERCC5 and CSB/ERCC6 in cases compared to controls was observed. This difference was statistically significant (p < 0.01). There was also a dose-response relationship between reduced expression levels and increased lung cancer risk (trend test, p < 0.01). Enhanced ERCC1 expression, however, has also been shown to predict for cisplatin resistance and therefore decreased survival in gastric, ovarian, esophageal, and colorectal cancers, and NSCLC. These results are consistent with the role of ERCC1 in the repair of modified nucleotides, specifically increased removal of cis-platinum-induced DNA adducts. Hence, in patients with advanced cancers who undergo treatment with platinum-based chemotherapy, increased expression of ERCC1 results in efficient removal of platinum-induced DNA adducts and thus reduced treatment efficacy and survival.

In summary, the role of ERCC1 in the genesis, progression, and clinical management of lung cancer is becoming increasingly clear. The level of ERCC1 expression is predictive of survival as reported here. This may be secondary to the decreased accumulation of genomic alterations as a result of efficient DNA damage repair. However, elevated ERCC1 expression also reduced the benefit of platinum-based chemotherapy. After confirmation by a prospective trial, which is currently underway, these results could be considered in the future design of adjuvant and neoadjuvant chemotherapy trials. Since patients with relatively low ERCC1 expression have poor survival yet their tumors are relatively sensitive to platinum-based therapy, it is likely that this is the group of patients that would derive the most benefit from treatment with platinum-based chemotherapy in the neoadjuvant, adjuvant, and metastatic settings.

### Table 2—Multivariate Analysis of Prognostic Factors

<table>
<thead>
<tr>
<th>Prognostic Factors</th>
<th>β</th>
<th>p Value</th>
<th>Relative Hazard</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERCC1 &gt; 50</td>
<td>-0.08</td>
<td>0.971</td>
<td>0.931</td>
<td>0.816–1.067</td>
</tr>
<tr>
<td>Male gender</td>
<td>0.02</td>
<td>0.896</td>
<td>0.979</td>
<td>0.817–1.166</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>-0.05</td>
<td>0.990</td>
<td>0.950</td>
<td>0.828–1.086</td>
</tr>
<tr>
<td>Squamous cell</td>
<td>0.07</td>
<td>0.648</td>
<td>1.071</td>
<td>0.910–1.253</td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td>0.02</td>
<td>0.896</td>
<td>0.979</td>
<td>0.817–1.166</td>
</tr>
</tbody>
</table>

### References