ERCC1 Expression in Non-Small Cell Lung and Esophageal Cancer

Küçük Hücre Dışı Akciğer Kanseri ve Özofagus Kanserinde ERCC1 Ekspresyonu

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Abstract

Objective: “Excision Repair Cross-Complementation Group 1” (ERCC1) enzyme is a vitally important basic protein required for DNA repair. Recent studies suggest that ERCC1 is involved in resistance to antracycline-based chemotherapy regimens. In this study, we aimed to analyze ERCC1 expression in lung and esophageal cancer patients. We also aimed to investigate the possible correlation between the ERCC1 expression levels and patient demographic information.

Materials and Methods: Twenty esophageal and 20 non-small cell lung cancer (NSCLC) patients diagnosed between December 2009 and December 2010, via either endoscopic, bronchoscopic or tru-cut biopsy, were included in this study. The ERCC1 expression levels were analyzed by the reverse transcriptase-polymerase chain reaction (RT-PCR) method in RNA samples extracted from pathological biopsy specimens. The patient demographic information was also recorded.

Results: There was no significant correlation between the ERCC1 expression level and demographic parameters, including the tumor, node, metastatis (TNM) staging, World Health Organization (WHO) grading, age, gender, hemoglobin level and albumin level, in the patient groups. The mean ERCC1 expression levels in the NSCLC and esophageal cancer patients were 0.71±0.85 and 0.62±0.78, respectively. The ERCC1 expression level was elevated in 15% of each patient group.

Conclusion: Analysis of the ERCC1 expression in NSCLC and esophageal cancer patients prior to chemotherapy would be useful for personalized chemotherapy regimens and would provide more accurate prognostic information for the patient.

Key Words: ERCC1 expression, lung cancer, esophageal cancer

Introduction

Excision repair cross-complementation group 1 (ERCC1) is an essential enzyme and one of the key proteins in deoxyribonucleic acid (DNA) repair. ERCC1 breaks the 5’ end of the damaged DNA strand and constitutes the rate-limiting step in this process. In some studies, a statistically significant increase in survival was observed in the groups with low ERCC1 expression that were treated with platin-based chemotherapy. As a result of these studies, ERCC1 overexpression was suggested as an indicator of resistance against platin-based chemotherapies. Some cancers exhibit ERCC1
overexpression, as was shown with immunohistochemistry or RT-PCR [1, 2].

There are a limited number of studies on the ERCC1 expression levels in lung and esophageal cancer and the effect of chemotherapy on survival. The aim of this study is to analyze the ERCC1 expression levels in newly diagnosed lung and esophageal cancer cases that have not been treated with chemotherapy and to investigate the correlation between the expression data and the demographic information in the cases. Informed consent was collected for all patients prior to their participation in the study, and the study was proved by the local ethics committee.

Materials and Methods

The informed consent form was taken from all participating patients. The ERCC1 gene expression analysis was performed on paraffin-embedded tissues from cases that were histopathologically diagnosed with non-small cell lung cancer (NSCLC) or esophageal cancer using reverse transcriptase polymerase chain-reaction (RT-PCR), which was performed by an expert medical biologist. Demographic information at the time of diagnosis, performance scores and clinical phases of the cases that were diagnosed with lung or esophageal cancer were recorded.

Quantification of the ERCC1 gene expression mRNA isolation from the tissue samples

Formalin-fixed, paraffin-embedded tissue samples were cut with a microtome and 1020 sheets with a 5-micrometer thickness were placed into 1.5 mL Eppendorf tubes. For deparaffinization, 1 mL of xylene was added and the samples were incubated at 56°C for 15 minutes. Then, the samples were centrifuged for 5 minutes and the supernatant (upper phase) was discarded. This step was repeated 4 times. Then, the samples were washed with 1 mL of 100%, 80%, 60% and 40% of ethanol, respectively. A high pure RNA tissue isolation kit (Roche, Mannheim, Germany) was used to extract the mRNA from the melted tissues. Five hundred microliters of 100% ethanol was added onto the melted tissues and the resulting mixture was transferred to spin-columns. The mRNA values were defined by PCR and do not have units. The mRNA from the melted tissues and the resulting mixture was transferred to spin-columns. The columns were centrifuged for 30 seconds at 13,000 rpm and the filter-through was discarded. Ninety microliters of DNase I solution were added, and the samples were incubated for 15 minutes at room temperature (RT). The samples were centrifuged for 15 seconds at 8,000 rpm and the filter-through was discarded. Afterwards, 500 µL of washing solution I, 500 µL of washing solution II and 300 µL of washing solution II were added onto the columns in the given order. A 15 second centrifugation at 8,000 rpm was performed in between the washing steps. Finally, 100 µL of elution buffer was added, the columns were centrifuged for 1 minute at 8,000 rpm and the mRNA samples were recovered.

cDNA synthesis

Step 1; 6 µL of nuclease-free water, 2 µL of random hexamer primers, 5 µL of mRNA were added for a total of 13 µL-Incubated at 65 °C for 10 minutes.

Step 2; 4 µL of transcription reverse transcriptase reaction solution, 0.5 µL of transcription reverse transcriptase, 2 µL of deoxynucleotide mixture, 0.5 µL of RNase inhibitor for a total of 7 µL-Incubated at RT for 10 minutes.

PCR protocol for cDNA: 10 minutes at 25°C, 1 hour at 50°C, 5 minutes at 85°C

13 µL+7 µL=20 µL (PCR products from Step 1 and 2 were used in the RT-PCR).

The relative cDNA amounts for ERCC1 and the reference gene (beta-actin) were calculated with a fluorescence-based real-time method (Roche LC480 Light Cycler). The primers and probe sequences are presented below. The first primer is the forward PCR primer, the second is the reverse PCR primer and the third is the TaqMan probe.

For ERCC1 (Roche, Mannheim, Germany, Cat No; 03003248001, version 13.0), the PCR reaction mixture contained 600 nm of forward and reverse primers; 200 nm of probe; 2.5 units of Fast Start Taq polymerase; 200 micromoles of dATP, dCTP and dGTP each; 400 micromoles of dUTP; 5.5 millimoles of MgCl$_2$ and TaqMan buffer solution containing a reference dye in a total volume of 25 uL. The RT-PCR conditions consisted of 46 cycles of 10 seconds at 50°C, 10 minutes at 95°C, 15 seconds at 95°C and 1 minute at 60°C.

After RT-PCR, comparisons were made according to the differences in the CT (cycle threshold) values of the target and reference genes. The results were compared with the reference gene, a-actin. ERCC1 was detected in the range of 0.01-2.9. The mRNA values were defined by PCR and do not have units. A cut-off value of 2.0E2, which is similar to other studies, was used to divide the groups into low and high expression levels.

Statistical Analysis

The patient data were analyzed with Statistical Package for the Social Sciences (SPSS) software (Version 11.5, IBM, CA, USA). Student’s t test was used to compare the age parameter in two groups, which had a normal distribution. The Chi-square test was used for categorical parameters. The correlation between the ERCC1 expression levels was determined with Spearman correlation. Kaplan-Meier analysis was used to determine the relationship between the ERCC1 expression and patient survival. The Log rank test was used to determine whether the survival time was different between the groups with low and high ERCC1 expression. P values lower than 0.05 were accepted as statistically significant.
Ten out of the 20 cases with esophageal cancer were males and 10 were females. The mean age was 60.5 (between ages 41 and 80). The mean hemoglobin, leukocyte, thrombocyte, erythrocyte sedimentation rate (ESR), alanine amino transpherase (ALT), and creatinine levels were as follows; 13.2 gr/dL, 9100x10^3/mL, 277,000x10^6/mL, 39 mm/h, 15.6 IU/mL, and 0.9 mg/dL, respectively. Histopathologically, 13 cases (65%) were squamous cell carcinoma, 6 cases (30%) were adenocarcinoma and 1 case (5%) was signet ring cell carcinoma. According to the TNM staging system, 3 cases (15%) were stage II, 4 cases (20%) were stage III and the remaining 13 cases (65%) were stage IV. The demographical properties of the esophageal cancer cases are presented in Table 1.

Sixteen out of 20 cases (80%) with NSCLC were males, 4 cases (20%) were females and the mean age was 61.4 (between ages 31-79). Twelve cases (60%) were squamous cell carcinoma and 8 cases (40%) were adenocarcinoma. According to the TNM staging system, 4 (20%) cases were stage I, 8 (40%) cases were stage II and the remaining 8 (40%) cases were stage III. The demographical properties of the NSCLC cases are presented in Table 2.

### Results

#### Quantitative results and the distribution of the ERCC1 gene measured by RT-PCR

ERCC1 was measured quantitatively in the tumor tissue of esophageal cancer cases and the ratio of ERCC1 to the reference gene ranged between 0.1 and 2.81. The mean value was calculated as 0.71±0.85. Three cases (15%) with esophageal cancer were positive for ERCC1 amplification and 17 cases (85%) were negative.

ERCC1 was measured quantitatively in the tumor tissue of the NSCLC cases and the ratio of ERCC1 to the reference gene ranged between 0.1 and 2.51. The mean value was calculated as 0.62±0.78. Three cases (15%) with NSCLC were positive for ERCC1 amplification and 17 cases (85%) were negative.

In the esophageal cancer cases, there was no statistically significant correlation between ERCC1 positivity and TNM staging (p=0.3), WHO classification (p=0.73), age (p=0.76), gender (p=0.61), albumin level (p=0.82), and Hb level (p=0.21). Although statistically insignificant, all 3 ERCC1 positive cases were squamous cell carcinoma. A statistically significant correlation between the tumor diameter and ERCC1 positivity was determined (p=0.039).

There was no statistically significant correlation between ERCC1 positivity and the tumor diameter (p=1), TNM staging (p=0.68), WHO grading (p=0.89), Hb level (p=0.15), erythrocyte sedimentation rate (p=0.5), albumin level (p=0.15) or patient age (p=0.54).

### Discussion

The nucleotide excision repair (NER) pathway plays a role in DNA repair by removing the DNA damage. The NER pathway is highly polymorphic and the roles of acquired and hereditary genetic defects in this pathway are being investigated [3]. ERCC1 is one of the most important DNA repair genes in the NER pathway.

In our study, the patients’ ERCC1 expression levels were determined using the RT-PCR method from RNA samples extracted from the paraffin blocks that were used in the pathological diagnosis. High ERCC1 expression was detected...
in 15% of the esophageal cancer cases and 15% of the NSCLC cases. There was no statistically significant correlation between the ERCC1 expression levels and TNM stage, WHO grade, age, gender, albumin or hemoglobin levels in the esophageal cancer and NSCLC patients (p>0.05). There was a statistically significant correlation between the tumor diameter and ERCC1 positivity, but this was only true for esophageal cancer cases.

In a previous study, ERCC1 expression and the effect of esophageal cancer on survival were investigated in 99 cases with esophageal cancer, which were surgically excised after neoadjuvant chemotherapy. The survival was significantly lower in patients with ERCC1>3.0 compared with patients with ERCC1<3.0. In addition, the relapse risk was two-fold higher for ERCC1>3.0. In that study, the gene expression was analyzed from biopsies that were taken for diagnostic purposes and that were associated with chemoresistance; ERCC1 expression could be employed as a useful parameter for the patients' response to therapy and their survival [4]. In another study of 76 late-stage gastric cancer cases, the cut-off value for ERCC1 was taken as 0.47 and higher ERCC1 expression was observed in 19.7% of the patients. The mean survival was significantly lower in the group with higher ERCC1 levels compared to the group with lower ERCC1 levels. In that study, the ERCC1 mRNA expression level was reported as an independent predictor for the survey [5]. Despite the higher cut-off value in our study, ERCC1 expression was found higher in 15% of the cases.

Immunohistochemical studies on ERCC1 expression ratios report that these ratios have promising predictive value in gastro-esophageal cancer patients who are treated with platin-based neoadjuvant chemotherapy [2].

The relationship between ERCC1 expression and cisplatin-induced DNA damage in different cancer types, including ovarian cancer, colorectal cancer, primary stomach cancer and esophageal cancer, has been demonstrated in different clinical and preclinical studies. There are reports that ERCC1 expression may have prognostic importance in NSCLC patients who have and have not undergone treatment [6].

The response rate to platin-based chemotherapies and to adjuvant therapies with gemcitabine, vinorelbine and taxanes in NSCLCs, for which a surgical operation is not possible, ranges between 30 to 60% [7, 8]. In addition to its key role in the NER pathway, the ERCC1 enzyme also catalyzes the removal of DNA fragments. The impact of ERCC1 overexpression on chemotherapy planning in NSCLC and on the survey has been investigated in recent years [9]. In our study, 15% of the NSCLC patients had ERCC1 positivity.

### Table 2. Demographic properties of the non-small cell lung cancer patients

| Subgroups               | n (max) | Percent (%) | Mean value (std) |
|-------------------------|---------|-------------|------------------|
| **Sex**                 |         |             |                  |
| Male                    | 16      | 80          |                  |
| Female                  | 4       | 20          |                  |
| **Stage**               |         |             |                  |
| Stage I                 | 4       | 20          |                  |
| Stage II                | 8       | 40          |                  |
| Stage III               | 8       | 40          |                  |
| **Histopathologic Diagnosis** |         |             |                  |
| Adenocancer             | 8       | 40          |                  |
| Squamous cell epithelial cancer | 12  | 60          |                  |
| **Presence of any risk factor** |         |             |                  |
| Present                 | 12      | 60          |                  |
| Absent                  | 8       | 40          |                  |
| **Age**                 | 31      | 79          | 61.4 (std±10.3)  |
| **Hemoglobin (gr/dL)**  | 10      | 17          | 13.3 (std±2.2)   |
| **Leukocyte count (x10^3/mL)** | 5500  | 16000       | 10560 (std±3560) |
| **Platelet count (x10^3/mL)** | 150.000 | 366.000 | 274.000 (std±66.000) |
| **Alaninamino trans**   | 8       | 60          | 25 (std±15.9)    |
| **Albumin**             | 2.7     | 4.5         | 3.81 (std±0.56)  |

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In a study of 34 NSCLC cases, a 7% higher ERCC1 expression was determined and a correlation between the ERCC1 expression and tumor cell type was reported [10]. In another study, ERCC1 expression was significantly higher in metastatic tissues compared with primary tumor tissues [11]. Because our expression analysis was carried out solely on tumor tissues, the ERCC1 expression might have been detected at lower levels than the actual levels.

In NSCLC cases, ERCC1 expression was significantly lower in squamous cell carcinoma than in adenocarcinoma. The differential ERCC1 expression between squamous cell carcinoma and adenocarcinoma may explain why squamous cell tumors respond well to platin-based chemotherapies, whereas adenocarcinomas show resistance to platin-based chemotherapies [12]. In our study, we could not identify a correlation between the tumor type and ERCC1 levels in NSCLC cases, which may be due to the low sample size of the subgroups.

A statistically significant correlation between ERCC1 expression and the age, histological type and pleural invasion was determined in a study of 783 NSCLC cases. Higher positivity of ERCC1 expression was determined in squamous cell carcinoma compared with adenocarcinoma; this was true for both patients >55 years of age, compared with younger patients, and patients with pleural effusion, compared with patients without pleural effusion. In the same study, when the ERCC1 levels of the control group that did not receive chemotherapy and the group that received chemotherapy were compared, the mean survival time was significantly higher for ERCC1 negative tumors in the group that received chemotherapy (14 months longer than the control group); the disease-free survival time after chemotherapy was also higher in that group [7]. In our study, there was no correlation between the age, tumor stage and ERCC1 expression. The differences in our results may be attributed to the different methods used to analyze the ERCC1 expression and to the varying sample sizes of the studies.

In conclusion, 15% ERCC1 positivity was observed in the esophageal cancer and NSCLC tissues used in our study. There are no available standardized treatment protocols for cases with esophageal cancer or NSCLC. Analysis of ERCC1 expression prior to treatment may guide the treatment according to the prognosis, response to treatment, relapse and overall survival. Analyzing the ERCC1 gene expression levels prior to chemotherapy will enable more accurate determination of the patient profile; the next step is a personalized treatment plan that will decrease the toxic effects of chemotherapy protocols for patients with low response rates and will improve our understanding of each patient’s prognosis.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Ataturk University Local Ethic Committee (Date 23.06.2009 Decision no: 207).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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