Int. J. Radiation Oncology Biol. Phys., Vol. 80, No. 3, pp. 655–660, 2011

0360-3016/$–see front matter

ELSEVIER

doi:10.1016/j.ijrobp.2010.02.061

CLINICAL INVESTIGATION **Head and Neck**

EXPRESSION OF EXCISION REPAIR CROSS-COMPLEMENTATION GROUP 1 AS PREDICTIVE MARKER FOR NASOPHARYNGEAL CANCER TREATED WITH CONCURRENT CHEMORADIOTHERAPY

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Purpose: Cisplatin-based concurrent chemoradiotherapy is the standard treatment of nasopharyngeal cancer. The expression of excision repair cross-complementation group 1 (ERCC1) has been reported to be associated with resistance to platinum-based chemotherapy. We evaluated whether ERCC1 expression could predict the treatment response and survival outcome of patients with locally advanced nasopharyngeal cancer who were treated with cisplatin-based concurrent chemoradiotherapy.

Methods and Materials: Immunohistochemistry was used to examine the expression of ERCC1 in nasopharyngeal tumor tissue. Patients were categorized into either a resistant or sensitive group depending on their treatment response outcome. A total of 77 patients were assessed in the present study.

Results: The resistant and sensitive groups included 25 and 52 patients, respectively. ERCC1 expression was positive in the tumor tissue for 39 of the 77 patients (51%). Significantly more ERCC1-negative tumors were in the sensitive group than in the resistant group ($p = .035$). In terms of survival outcome, univariate analysis determined that patients with ERCC1-negative tumors had longer disease-free survival ($p = .076$) and overall survival ($p = .013$) than patients with ERCC1-positive tumors. Multivariate analysis determined that negative ERCC expression in tumors was an independent predictor for prolonged overall survival (hazard ratio, 0.14; 95% confidence interval, 0.03–0.71).

Conclusion: These results suggest that ERCC1 expression might be a useful predictive marker in patients with locally advanced nasopharyngeal cancer who are under consideration for cisplatin-based concurrent chemoradiotherapy.

Excision repair cross-complementation group 1, ERCC1, nasopharyngeal cancer, cisplatin, concurrent chemoradiotherapy, predictive factor.

INTRODUCTION

The current standard treatment of advanced nasopharyngeal cancer (NPC) is concurrent chemoradiotherapy (CCRT) with or without adjuvant cisplatin-based chemotherapy (1–3). However, the benefit of CCRT is hindered by the increased occurrence of therapy-associated complications. Therefore, identifying molecular markers that can predict which patients will benefit from CCRT is important for the appropriate management of patients with advanced NPC.

Cisplatin is the most important chemotherapy used to treat locally advanced NPC, and it is administered as a part of CCRT. Its cytotoxicity is attributed to the formation of DNA adducts, which cause inter- and intrastrand cross-linking, thereby inhibiting DNA replication. These cisplatin-induced DNA adducts are removed by the nucleotide excision repair pathway. The excision repair cross-complementation group 1 (ERCC1) plays a rate-limiting role in the nucleotide excision repair pathway, and its increased expression has been associated with resistance to cisplatin-based chemotherapy (4–9). ERCC1 is also involved in the repair of DNA strand breaks caused by RT (10–12), and overexpression of ERCC1 has been shown to be significantly correlated with resistance to RT alone or CCRT in multiple tumor types (13–19).

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Conflict of interest: none

Received Jan 8, 2010, and in revised form Feb 20, 2010. Accepted for publication Feb 23, 2010.

The purpose of the present study was to evaluate whether the ERCC1 expression status could predict the treatment response and survival outcome of patients with locally advanced NPC who underwent cisplatin-based CCRT.

METHODS AND MATERIALS

Patients and treatment

A total of 77 consecutive patients, who were histologically confirmed to have locally advanced NPC and were treated with CCRT between 1995 and 2008 at Samsung Medical Center (Seoul, Korea), were assessed in the present study. Their performance status was Eastern Cooperative Oncology Group 0–1. All 77 patients had adequate bone marrow, liver, and renal function. None of the patients had received previous RT or chemotherapy.

The external bean RT technique administered to patients was a three-dimensional conventional RT method. The primary tumor site and adjacent tissues were treated with 70 Gy in 35 fractions for 7 weeks using a shrinking-field technique. For all patients, three cycles of concurrent cisplatin were administered during Weeks 1, 4, and 7 of RT. Subsequently, three cycles of adjuvant chemotherapy, consisting of a combination of cisplatin and 5-fluorouracil, were administered between Weeks 11 and 19. RT or chemotherapy was delayed or discontinued in the event of patient refusal, physician decision, or unacceptable toxicities, including severe sepsis or renal impairment.

The pretreatment evaluation included a review of the patient's history, physical examination, performance status, chest X-ray, complete blood count, blood chemistry, fiberoptic nasopharyngoscopy, and computed tomography or magnetic resonance imaging of the head and neck. Patients were evaluated by computed tomography or magnetic resonance imaging of the head and neck and nasopharyngoscopy every 3 months for 2 years and then every 6 months thereafter. The clinical response to treatment was assessed according to the World Health Organization criteria. A complete response was defined as no residual disease found by nasopharyngoscopy and computed tomography or magnetic resonance imaging. The institutional review boards approved all experiments, according to legal regulations.

Immunohistochemistry for ERCC1

Formalin-fixed paraffin-embedded tissue blocks were sectioned at a 4-μm thickness. The tissue sections were deparaffinized in xylene and then rehydrated in serially graded alcohol. ERCC1 antigen retrieval consisted of heating in 10 mM citrate buffer at pH 6.0 with microwaves (15 min, 700 W) and cooling to room temperature for 20 min. After washing in Tris-buffered saline, the slides were preincubated in 5% normal blocking solution (goat serum) for 10 min to reduce nonspecific binding. The slides were incubated overnight with a mouse monoclonal antibody against ERCC1 (8F1, Neomarkers, Fremont, CA) at a 1:200 dilution in a humidified chamber at room temperature. The primary antibody was visualized using an avidin-biotin complex system (Dako, Carpinteria, CA). The slides were washed in Tris-buffered saline. The specific biotinylated goat anti-mouse IgG was diluted to 1:100, and the slides were incubated with this solution for 20 min at room temperature. The specimens were washed again in Tris-buffered saline and incubated for 10 min in a solution of streptavidin-avidin-biotin complex horseradish peroxidase at a dilution of 1:100. Color development was achieved by incubating tissues with 3,3′-diaminobenzidine tetrahydrochloride (Immunotech, Marseille, France). Finally, the sections were counterstained with Mayer hematoxylin. Negative controls were processed as described in this paragraph without inclusion of the primary antibody.

Evaluation of ERCC1 expression

Two pathologists blindly and independently evaluated the ERCC1 staining of the tissue specimens under a light microscope. The nuclear staining intensity within the tumor tissue was graded on a scale of 0–3, with a higher number indicating greater intensity using the adjacent nonmalignant cells as a reference (intensity 2). Discordant cases were reviewed by another pathologist. The percentage of positive tumor nuclei was calculated for each specimen, and a proportional score was assigned (0 if 0%, 0.1 if 1–9%, 0.5 if 10–49%, and 1.0 if $\geq$50%). Next, this proportional score was multiplied by the staining intensity of the nuclei to obtain a final semiquantitative H-score. ERCC1-positive tumors were defined those with greater than the median value, which was calculated from all the H-scores.

Statistical analysis

First, the predictive value of the ERCC1 status was assessed by analyzing the relationship between the ERCC1 expression status and the response to CCRT. We categorized patients into two response groups: the sensitive group and the resistant group. The sensitive group included those patients who achieved a complete response and remained in remission throughout the follow-up period. The resistant group included patients who had persistent disease after treatment or developed a relapse after remission. The relationship between ERCC1 expression status and the response groups was analyzed using chi-square tests.

The relationship between the survival outcome and ERCC1 expression status was also evaluated. Overall survival was calculated from the first day of CCRT until the date of death or the date of the most recent documented follow-up visit. In the analysis of disease-free survival (DFS), patients were considered to have an event if they developed a relapse after treatment completion. Patients who had persistent disease after treatment or patients who died of intercurrent causes were not considered to be disease free. The starting point for DFS was the first day of treatment, and the terminating point was the date when a relapse first occurred or, in the case of persistent disease and other causes of death, the date of the first follow-up and death, respectively. Patients who presented with no evidence of disease after treatment were censored at the date of last follow-up.

Survival curves were generated using the Kaplan-Meier method, and the log-rank test was used to compare the survival curves according to the clinicopathological characteristics, including ERCC1 status. All reported p values are two-sided, and $p \leq .05$ was considered statistically significant.

RESULTS

Patient characteristics

The median patient age was 49 years (range, 17–79), and 81% were men. Table 1 lists the baseline patient characteristics. Undifferentiated carcinoma of the nasopharyngeal type (UCNT), which included nonkeratinizing carcinoma and undifferentiated carcinoma, contributed to 66% of all NPC cases. Of the 77 patients who entered the study, 32 (42%) were treated with adjuvant chemotherapy after CCRT completion; the other 45 patients were only treated with CCRT. The median dose of administered cisplatin was 360