ERCC1 Expression and Outcomes in Head and Neck Cancer Treated with Concurrent Cisplatin and Radiation

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Abstract. *Background: Overexpression of excision repair cross complementing group 1 (ERCC1), a DNA repair enzyme, is associated with resistance to cisplatin. Materials and Methods: Tissues from 73 patients with squamous cell carcinoma of the head and neck (HNSCC) who received concurrent cisplatin and radiation was analyzed immunohistochemically to determine if ERCC1 expression predicted for survival and response. Expression was scored as follows: 0=0% tumor nuclei positive, 1+=<50%, 2+=50-75% and 3+=>75%. Results: ERCC1 expression was 0 in 0%, 1+ (14%), 2+ (42%) and 3+ (44%). In uni- and multivariate analyses, 3+ ERCC1 expression was not a significant predictor of survival or response. Median survival for the ERCC1 3+ patients was 2.9 years versus 2.1 years for the ERCC1 <3+ group (p=0.44). Conclusion: In this retrospective review of HNSCC patients receiving concurrent cisplatin and radiation, ERCC1 expression was not a significant predictor of survival or response.*

Squamous cell carcinoma of the head and neck (HNSCC) is expected to account for 3% of newly diagnosed cancer cases in the United States in 2010 with 49,260 new cases and 11,480 anticipated deaths (1). Locally advanced disease (American Joint Committee on Cancer/Union for International Cancer Control, stages III-IVB) comprises 60% of new diagnoses. In the United States, the usual treatment for locally advanced or unresectable head and neck cancer is concurrent chemotherapy and radiation, typically with cisplatin (Bristol Myers Squibb, Princeton, NJ, USA). Cisplatin interacts with DNA to form intra-strand crosslink DNA adducts that trigger a series of intracellular events which eventually lead to cell death (2). DNA intra-strand crosslinks are repaired by the nucleotide excision repair (NER) pathway in cells (2). The NER pathway processes mutations induced by ultraviolet light and DNA adducts formed by cisplatin. Germline mutations in *NER* genes cause xeroderma pigmentosum, a condition characterized by sensitivity to ultraviolet light and resultant predisposition to development of malignancies. Excision repair cross complementing-group 1 (ERCC1) plays a critical role in NER, as it dimerizes with xeroderma pigmentosum complementation group F, and this complex is required for the successful excision of damaged DNA (3).

Pre-clinical data suggest that increased *ERCC1* mRNA expression levels or ERCC1 protein expression levels correlate with cisplatin resistance in human cancer in ovarian, cervical, colon, testis, and lung cancer cell lines (2). High levels of ERCC1 are associated with an increased rate of NER and reduced sensitivity to cisplatin, whereas cancer cells with low levels of ERCC1 are more sensitive to platinum.

In a subgroup analysis of a randomized trial of cisplatin-based chemotherapy *versus* observation after surgery for early-stage non-small cell lung cancer, patients with ERCC1 tumor expression that was greater than the median for the entire group did not benefit from cisplatin, while those patients with ERCC1 tumor expression less than the median did have a significant survival benefit with cisplatin-based treatment (4). In addition, four retrospective studies in HNSCC have evaluated ERCC1 as a predictive marker for response and survival with platinum-based chemotherapy with conflicting results (5-8). The present study is the largest to examine the impact of ERCC1 expression on tumor response and survival in patients with locally advanced HNSCC receiving definitive concurrent cisplatin and radiation.

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Key Words: Head and neck neoplasms, ERCC1 protein, cisplatin, DNA repair, radiation.

Materials and Methods

We performed a retrospective analysis of 73 consecutive HNSCC patients treated with curative intent concurrent cisplatin and radiation at the Dallas VA Medical Center (Dallas, TX, USA) from 2000-2006. All patients had histologically confirmed squamous cell carcinoma. The chemotherapy regimens consisted of cisplatin with or without 5-fluorouracil, and patients were included if they received at least one dose of cisplatin. Those treated with carboplatin were excluded. We included patients with AJCC/UICC stage II-IVB disease, performance status of 0-2, and an adequate biopsy specimen for the ERCC1 analysis. Tumor response was based on the first computed tomography scan performed following completion of concurrent therapy and was assessed using standard RECIST criteria.

Immunostaining for ERCC1. Tissue was immunostained for ERCC1 using ERCC-1, clone 8F antibody, 1:100 dilution (Cat#ab2356; Abcam, Cambridge, MA, USA) diluted in ChemMate buffer (Ventana Medical Systems, Tucson, AZ, USA) on a BenchMarkXT automated immunostainer using the UltraVIEW system with horseradish peroxidase and diaminobenzidine (DAB) chromogen (Ventana Medical Systems) per the standard protocol of the University of Texas Southwestern Medical Center Diagnostic Immunohistochemistry Laboratory.

ERCC1 scoring was determined by two pathologists who were blinded to clinical data. ERCC1 stain scoring was as follows: 0=0% of tumor nuclei were positive, 1+=less than 50% of tumor nuclei positive, 2+=50-75% nuclei positive and 3+=75-100% nuclei positive.

Statistical analysis. Patient characteristics were described by age (dichotomized as ≤60 years and >60 years), stage, tumor site, tumor differentiation, and performance status, and the reported *p*-values were calculated with Fisher's exact tests. The association between overall survival and age, stage, site, tumor differentiation, and ERCC1 expression was explored by a multivariate Cox regression model. All reported *p*-values are two-sided. All statistical analyses were performed using SAS 9.2 for Windows (SAS Institute Inc., Cary, NC, USA). Graphs were generated by GraphPad Prism 5.01 for Windows (GraphPad Software Inc., La Jolla, CA, USA).

Results

Patient and tumor characteristics. Patient characteristics are presented in Table I. The median age of the patients was 59 years (range 41-80 years), and 99% were men. Seven percent had AJCC/UICC stage II disease, 19% had stage III, and 74% had stage IV. The most common sites were the oropharynx (56%) and larynx (23%), followed by the hypopharynx (14%), oral cavity (4%), and nasopharynx (3%). Most patients had an ECOG performance status (PS) of 0 (25%) or 1 (45%), with 22% having an unknown performance status, and 8% having a PS of 2. The majority of tumors were moderately differentiated (60%); 4% were well differentiated, 15% were poorly differentiated, and in 21% differentiation was not specified. ERCC1 expression was 0 in 0 % of tumors, 1+ in 14%, 2+ in 42%, and 3+ in 44%. As shown in Table I, there were no significant differences in the characteristics of those patients with tumors with ERCC1 staining of 3+ and those with ERCC1 staining <3+.

Table I. *Patient characteristics*.

Characteristic	Number (%)	ERCC1		*P*-value
	All Patients (N=73)	≤3+ (N=41)	3+ (N=32)	
Age (years)				0.47
≤60	43 (59)	26 (63)	17 (53)	
>60	30 (41)	15 (37)	15 (47)	
Stage				0.79
II, III	19 (26)	10 (24)	9 (28)	
IV	53 (74)	31 (76)	23 (72)	
Site				0.08
Oral cavity	3 (04)	1 (02)	2 (06)	
Oropharynx	41 (56)	20 (49)	21 (66)	
Hypopharynx	10 (14)	8 (20)	2 (06)	
Larynx	17 (23)	12 (29)	5 (16)	
Nasopharynx	2 (03)	0 (00)	2 (06)	
Tumor differentiation				0.37
Well	3 (04)	2 (05)	1 (03)	
Moderate	44 (60)	21 (51)	23 (72)	
Poor	11 (15)	8 (20)	3 (09)	
Not specified	15 (21)	10 (24)	5 (16)	
Performance Status				0.48
0	18 (25)	9 (12)	9 (12)	
1	33 (45)	17 (23)	16 (22)	
2	6 (08)	5 (07)	1 (01)	
Unknown	16 (22)	10 (14)	5 (08)	

ERCC1, Excision repair cross-complementation group 1.

Association between ERCC1 staining and survival. The median duration of follow-up for the entire group was 2.2 years, and the median survival was 2.8 years, with a 5-year overall survival (OS) rate of 41%. In univariate analysis, ERCC1 staining 3+ was not predictive of OS, with a hazard ratio (HR) of 0.80 (95% confidence interval, CI=0.44-1.43; p=0.44). In multivariate analysis, ERCC1 staining of 3+ was also not predictive of OS with a HR of 0.62 (95% CI=0.28-1.39; p=0.25) (Table II). In addition, in the multivariate model, age, stage, site, and tumor differentiation were not significant predictors of survival. Separating the groups into ERCC1 3+ *versus* <3+ revealed that ERCC1 3+ patients had worse median (2.1 years *vs*. 2.9 years; p=0.44) and 5-year survivals (38% *vs*. 44% (Figure 1). However, these differences were not statistically significant. A similar analysis comparing those patients with ERCC1 1+ staining with those with >1+ ERCC1 staining also failed to demonstrate significant survival differences between the groups (data not shown).

Association between ERCC1 staining and response. Objective tumor response data was available for 66 patients following completion of concurrent chemotherapy and radiation. Twenty-three patients (35%) had a complete response, 37 (56%) had a partial response, and 6 (9%) had

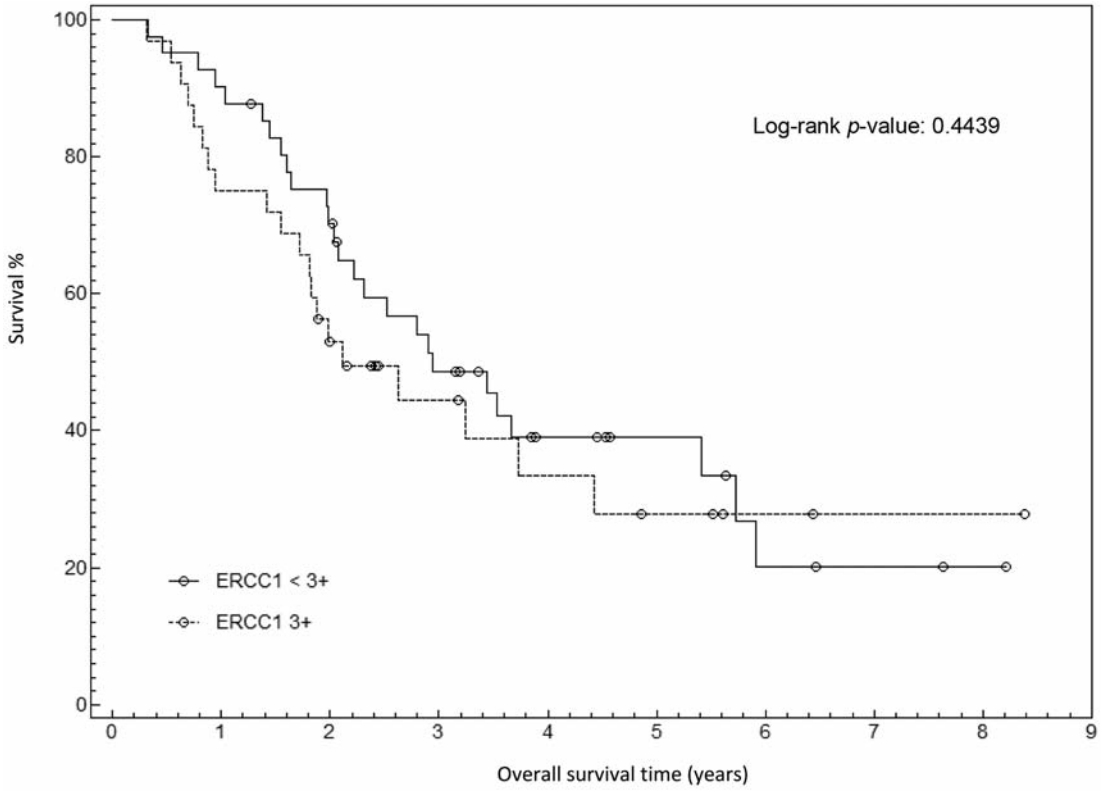

Figure 1. *Kaplan-Meier estimates of the probability of survival according to excision repair cross complementing group 1.*

no response or progressive disease. No significant association between tumor response and ERCC1 expression was noted. As shown in Table III, in tumors with ERCC1 expression of 3+, the objective tumor response rate was 96% *versus* 87% in those with ERCC1 expression <3+ (p=0.23). A similar analysis evaluated those patients with complete response compared to those with less than a complete response. In tumors with ERCC1 expression of 3+, the complete response rate was 43% *versus* 29% in those with ERCC1 expression <3+ (p=0.3). Multivariate analyses for both response and complete response also failed to find a significant association with ERCC1 expression.

Discussion

Low expression of ERCC1 has been shown to correlate with improved tumor response and survival in patients with non-small cell lung cancer who have received cisplatin-based chemotherapy (4, 9). To our knowledge, there are four other retrospective studies reported in the literature regarding ERCC1 expression in HNSCC patients and response to cisplatin. A French retrospective review found that HNSCC patients with tumors expressing ERCC1 at low levels (defined as having an 'H-score' lower than the median score for the group) had a greater chance of benefiting from platinum chemotherapy (5). The 'H-score' is derived from the number of nuclei staining positively for ERCC1 and the intensity of that staining. Their cohort consisted of 96 patients with locally advanced cancer and all received induction

Table II. *Hazard ratios for overall survival.*

Characteristic	Hazard ratio (95% CI)	*P*-value
Age, years		0.35
≤60	Reference	
>60	1.4 (0.69-2.84)	
Stage		0.99
II, III	1.0 (0.43-2.34)	
IV	Reference	
Site		0.32
All other	1.52 (0.67-3.46)	
Oropharynx	Reference	
Tumor differentiation		0.28
Well	4.1 (0.73-22.96)	
Moderate	Reference	
Poor	1.32 (0.49-3.60)	
ERCC1		0.25
<3+	0.62 (0.28-1.39)	
3+	Reference	

ERCC1, Excision repair cross-complementation group 1; CI, confidence interval.

Table III. *ERCC1 expression and objective tumor response.*

	ERCC1		
	<3+ (N=38) N (%)	3+ (N=28) N (%)	*P*-value
Response			0.23
Complete	11 (29)	12 (43)	
Partial	22 (58)	15 (53)	
Stable disease	1 (03)	1 (04)	
Progressive disease	4 (10)	0 (00)	

ERCC1, Excision repair cross-complementation group 1.

chemotherapy with cisplatin and 5-fluorouracil. Patients with tumors that responded were subsequently treated with radiation. The 28 patients who had tumors expressing ERCC1 at lower levels had a 4-fold greater likelihood of objective response to chemotherapy compared to the 68 patients with high ERCC1 expression. The median survival times, although shorter for those patients with high ERCC1-expressing tumors compared to those with low ERCC1-expressing tumors, were not statistically different (28 *versus* 41 months). However, when the authors used a Cox proportional hazard model adjusted for age, tumor stage, tumor differentiation, and tumor localization, low ERCC1 expression was associated with a lower risk of cancer death (HR=0.42; 95% CI=0.20-0.90; p=0.04). Another retrospective study from Korea reviewed 45 HNSCC patients with AJCC/UICC stage III or IV disease. Thirty-three patients had tumors with high ERCC1 expression (defined as having an H-score greater than or equal to the median), while 12 patients had tumors with low expression of ERCC1. Those patients with low ERCC1 expression had significantly higher 3-year progression-free survival (83% *versus* 49%) and overall survival (92% *versus* 46%) rates (6). A third study analyzed archival tissue to determine if baseline ERCC1 levels were associated with prognosis or benefit from adjuvant treatment in patients with resectable HNSCC (7). Tissues from 109 patients were analyzed. Thirty-three patients were treated with surgery alone and 76 received adjuvant radiation or platinum based chemoradiation. Among the 76 patients who received adjuvant radiation/ chemoradiation, low ERCC1 expression was associated with a statistically significant improvement in survival. Conversely, Saada and colleagues looked for an association between ERCC1 expression and survival in 142 HNSCC patients who received platinum-based chemotherapy as either adjuvant therapy with radiation following surgery, as part of definitive concurrent chemoradiation, or as single modality treatment for metastatic disease and found that the patients with tumors exhibiting low ERCC1 expression had a non-significant trend towards a worse overall survival (8).

In our retrospective study of HNSCC patients treated with concurrent cisplatin and radiation, we found no significant correlation between ERCC1 expression and tumor response or survival. The present study is unique in several ways. It is the first and largest study evaluating the predictive value of ERCC1 to evaluate solely HNSCC patients receiving concurrent platinum chemotherapy and radiation as definitive treatment. One possible hypothesis for the lack of utility of ERCC1 expression as a predictor of outcome in our cohort is that the concurrent use of both chemotherapy and radiation is able to overcome the relative resistance to platinum conferred by ERCC1 expression. Arguing against this are preclinical data suggesting that ERCC1 may play a role in DNA repair processes other than NER, such as recombination. Recombination is the method utilized by cells to repair double-strand DNA breaks, such as those induced by radiation. Joshi and colleagues reported that increased *ERCC1* mRNA expression was associated with reduced survival in patients with esophageal cancer receiving trimodal treatment with chemotherapy, radiation, and surgery (10). In addition, Nix *et al.* found that expression of ERCC1 correlated with resistance to radiation in laryngeal tumors (11). Additional prospective studies in patients receiving concurrent cisplatin and radiation are required to better define the likely complex role of ERCC1 and sensitivity to both treatment modalities in this group of patients.

A second unique feature of our study is the scoring system utilized to quantify ERCC1 expression. Most prior studies have utilized an 'H-score' which was derived in studies of non-small cell lung cancer patients and is based on the number of nuclei staining positively for ERCC1 and the intensity of that staining. Those with H-scores greater than the median for the cohort being studied are arbitrarily defined as being 'ERCC1-positive'. This method appears to effectively segregate patients with non-small cell lung cancer where the median percentage of cells that have nuclear staining is 24% and the median H-score is 1 (4). However, in the published studies of ERCC1 staining in HNSCC, >70% of tumors have ERCC1 expression with 80-92% of nuclei positive and the resultant median H-scores for the cohorts are 2-3. In these series, only 27-29% of patients were identified as having 'low ERCC1 expression' and therefore presumed sensitivity to cisplatin (5, 6). Given that clinical experience with cisplatin in HNSCC patients suggests it is effective in a significantly greater proportion of patients, it is logical to assume that factors other than ERCC1 play a role in cisplatin sensitivity in this group. In the present study, our scoring system identified 58% of patients as having 'low expression' of ERCC1 and 42% as having 'high expression'. However, despite the better segregation that was achieved, no significant association between ERCC1 expression and outcome was identified.

The present study has several limitations. It is retrospective and included only 73 patients. However, all patients receiving concurrent cisplatin and radiation at the institution were included and accurate long-term survival and follow-up data were available for all patients. In addition, response was assessed by retrospective review of computed tomography scan reports and patient notes rather than by review of the actual scans. Lastly, given the relatively small numbers of patients analyzed, no meaningful comment can be made on the predictive value of ERCC1 expression in various patient subsets, such as by tumor subsite (oral cavity, oropharynx, hypopharynx, larynx, nasopharynx).

In conclusion, the present results suggest that ERCC1 protein expression is not an optimal predictor of OS or tumor response with cisplatin-based concurrent chemoradiation in patients with HNSCC. Taken in conjunction with the other published results, it appears that a significant proportion of HNSCC patients identified as having 'high expression' of ERCC1 still respond to cisplatin, suggesting that resistance to platinum in HNSCC is complex and will involve other markers yet to be identified. Further prospective studies that include mandated tissue acquisition should be carried out to confirm the role of ERCC1 and other putative markers as predictors of response and outcome in these patients.

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References

1 Jemal A, Siegel R, Xu J and Ward E: Cancer Statistics, 2010. CA Cancer J Clin *60*: 277-300, 2010.

2 Gossage L and Madhusudan S: Current status of excision repair cross complementing-group 1 (ERCC1) in cancer. Cancer Treat Rev *33*: 565-577, 2007.

3 Martin LP, Hamilton TC and Schilder RJ: Platinum resistance: the role of DNA repair pathways. Clin Cancer Res *14*: 1291-1295, 2008.

4. Olaussen KA, Dunant A, Fouret P, Brambilla E, André F, Haddad V, Taranchon E, Filipits M, Pirker R, Popper HH, Stahel R, Sabatier L, Pignon JP, Tursz T, LeChevalier T, and Soria JC: DNA repair by ERCC1 in non-small-cell lung cancer and cisplatin-based adjuvant chemotherapy. N Engl J Med *355*: 983-991, 2006.

5 Handra-Luca A, Hernandez J, Mountzios G, Taranchon E, Lacau-St-Guily J, Soria JC and Fouret P: Excision repair cross complementation group 1 immunohistochemical expression predicts objective response and cancer-specific survival in patients treated by cisplatin-based induction chemotherapy for locally advanced head and neck squamous cell carcinoma. Clin Cancer Res *13*: 3855-3859, 2007.

6 Jun HJ, Ahn MJ, Kim HS, Yi SY, Han J, Lee SK, Ahn YC, Jeong HS, Son YI, Baek JH and Park K: ERCC1 expression as a predictive marker of squamous cell carcinoma of the head and neck treated with cisplatin-based concurrent chemoradiation. Br J Cancer *99*: 167-172, 2008.

7 Mehra R, Cai KQ, Zhu F, Weaver JE, Nicolaou N, Lango M, Ridge JA, Godwin AK and Burtness BA: ERCC1 is a predictive biomarker of survival in squamous cell carcinoma of the head and neck [abstract]. Abstract no. 2804. *In*: Proceedings of the 101st Annual Meeting of the American Association for Cancer Research 2010 April 17-21 Washington DC. Philadelphia (PA): AACR, 2010.

8 Saada E, Thariat J, Ettore F, Sudaka A, Chamorey E, Italiano A, Poissonet G, Bensadoun R, Dassonville O and Peyrade F: Correlation of global expression of ERCC1 in head and neck squamous cell carcinoma with prognosis [abstract]. J Clin Oncol *27(20 Suppl)*: e17016, 2009.

9 Cobo M, Isla D, Massuti B, Montes A, Sanchez JM, Provencio M, Vinolas N, Paz-Ares L, Lopez-Vivanco G, Munoz MA, Felip E, Alberola V, Camps C, Domine M, Sanchez JJ, Sanchez-Ronco M, Danenberg K, Taron M, Gandara D and Rosell R: Customizing cisplatin based on quantitative excision repair cross-complementing 1 mRNA expression: a phase III trial in non-small cell lung cancer. J Clin Oncol *25*: 2747-2754, 2007.

10 Joshi MB, Shirota Y, Danenberg KD, Conlon DH, Salonga DS, Herndon JE 2nd, Danenberg PV and Harpole DH Jr.: High gene expression of TS1, GSTP1, and *ERCC1* are risk factors for survival in patients treated with trimodality therapy for esophageal cancer. Clin Cancer Res *11*: 2215-2221, 2005.

11 Nix P, Greenman J, Stafford N and Cawkwell L: Expression of XRCC1 and ERCC1 proteins in radioresistant and radiosensitive laryngeal cancer. Cancer Ther *2*: 47-53, 2004.

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