RESEARCH ARTICLE

ERCC1 Expression Can Predict Response to Platinum-Based Induction Chemotherapy in Head and Neck Cancer Cases

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Abstract

To investigate whether excision repair cross complementing-group1 (ERCC1) expression status could serve as a bio-predictor of response to platinum-based induction chemotherapy for head and neck cancers (HNCs) patients with a diagnosis of epithelial HNC were studied retrospectively. Paraffin embedded tumor samples of the patients were analyzed by reverse transcription-polymerase chain reaction (RT-PCR) to determine ERCC1 expression status and its correlation with response to platinum-based induction chemotherapy was investigated. Of 44 included patients, 33 were male (75%) and 11 were female (25%) with a mean age of 53 years. Some 36% of patients whose tumor samples had high ERCC1 expression showed no response to induction chemotherapy. The value for patients with low ERCC1 expression was 9% and the difference was statistically significant (p=0.03). The ERCC1 expression state did not significantly vary between patient groups according to sex, age, primary tumor site, and tumor and node stage. Our study indicates that ERCC1 expression status detected by RT-PCR might serve as a bio-predictor of response to platinum-based induction chemotherapy for epithelial HNCs.

Keywords: ERCC1 - induction chemotherapy – response - head and neck cancer

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Introduction

Worldwide, head and neck cancer is the seventh most common malignancy and also a major cause of morbidity and mortality (Ferlay et al., 2010; Alvarenga et al., 2008). Squamous cell carcinoma (SCC) represents the most common histologic subtype of cancers originating from this anatomic region (Ragin et al., 2007; Carey et al., 2015). Unfortunately, most head and neck cancer patients present with loco-regionally advanced disease (Jun et al., 2008) and despite improvements in treatment techniques, the five-year overall survival of such patients is still poor (Jemal et al., 2010). Currently, the standard non-surgical approach for loco-regionally advanced head and neck squamous cell carcinoma (HNSCC) is cisplatin-based concurrent chemoradiation (Bauman et al., 2013). Another approach used by many oncologists in this setting is adding induction chemotherapy to definitive local treatment. Induction cisplatin-based chemotherapy induces response rates of 80% to 90% and can potentially reduce distant metastasis rate in loco-regionally advanwced HNSCC (Brockstein et al., 2004; Argiris, 2005). Cisplatin (cis-diamminedichloroplatinum(II)) performs its cytotoxic effect by formation of either intra-strand or inter-strand DNA adducts (Gossage and Madhusudan, 2007). In normal cells, these cisplatin–induced DNA damages are repaired by the nucleotide excision repair (NER) pathway. Excision repair cross complementing-group1 (ERCC1) enzyme is a key protein in NER pathway and its increased expression correlates with resistance to cisplatin-based chemotherapy (Jun et al., 2008; Zamble et al., 1996). There is some clinical evidence suggesting that ERCC1 status (ERCC1 mRNA expression, ERCC1 protein expression, and ERCC1polymorphisms) is associated with platinum-based therapy efficacy in some kinds of cancers (Vilmar and Sorensen, 2009; Bohanes et al., 2011; Langer, 2012). In a recent meta-analysis, ERCC1 protein expression status detected by immunohistochemical methods significantly correlated with response to platinum-based chemotherapy in ovarian cancers (Li et al., 2013). Another meta-analysis evaluated non-small cell lung cancer patients treated with platinum-based chemoradiation showed that both low tumoral mRNA and protein levels were associated with a better response rate and overall patient survival (Chen et al., 2010). In head and neck cancers, the available studies have mixed results. Of six studies evaluating the relation between ERCC1 status and outcomes of head and neck cancer patients, three showed positive (Jun et al., 2008; Fountzilas et al., 2009; Handra-Luca et al., 2007) and another three were with negative results (Fountzilas et al., 2009; Koh et al., 2009; Hayes et al., 2011).

We conducted the present study to investigate whether ERCC1 mRNA expression status in tumor cells could serve as a bio-predictor of response to induction platinum-based chemotherapy for head and neck cancer .

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Materials and Methods

We performed a retrospective analysis of 44 non-metastatic epithelial head and neck cancer patients treated with induction platinum-based chemotherapy at our clinical oncology center (Jorjani Cancer Center, Emam Hossein Hospital, Tehran, Iran) from 2010 to 2014. The study inclusion criteria were as follows: (a) patients of any age with the biopsy proven diagnosis of primary epithelial head and neck cancer, (b) the primary disease was measurable by physical examination or imaging studies, and (c) the patient had not undergone definitive surgical treatment. Each patient received one of the following induction chemotherapy regimens: TPF (docetaxel plus cisplatin plus 5-fluorouracil for two cycles with three-week intervals); PF (cisplatin plus 5-fluorouracil for two cycles with three-week intervals) and TC (weekly placlitaxel plus carboplatin for six cycles).

Table 1. The Patient, Tumor and Treatment Characteristics

Age (years)	Number	%
< 50	15.0	34.0%
>= 50	29.0	66.0%
Sex		
Female	11.0	25.0%
Male	33.0	75.0%
Primary tumor site		
Hypopharynx	3.0	7.0%
Larynx	25.0	57.0%
Nasopharynx	13.0	29.5%
Oropharynx	1.0	2.0%
Tongue	2.0	4.50%
Primary tumor site		
Nasopharynx	13.0	29.50%
Non-Nasopharynx	31.0	70.50%
T-stage		
T1/T2	18.0	41.0%
T3/T4	26.0	59.0%
N-stage		
N-negative	7.0	16.0%
N-positive	37.0	84.0%
N-stage		
N0/N1	17.0	39.0%
N2/N3	27.0	61.0%
Induction chemotherapy regimen		
PF	25.0	57.0%
TPF	13.0	29.0%
TC	6.0	14.0%
Induction chemotherapy regimen		
PF/TPF	38.0	14.0%
wTC	6.0	86.0%
Response to treatment		
PR/CR	34.0	77.0%
SD/PD	10.0	23.0%

*T-stage, tumor stage; N-stage, lymph node stage; wTC, weekly paclitaxel plus carboplatin; TPF, docetaxel plus cisplatin plus 5-fluorouracil; PF, cisplatin plus 5-fluorouracil; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

Table 2. Correlation between ERCC1 Expression Status and Patient, Tumor and Response Characteristics

	ERCC1 Expression Status		P-value
	Low	High	
Response to treatment			
CR/PR	20.0 (91.0%)	14.0 (64.0%)	0.0
SD/PD	2.0 (9%)	8.0 (36.0%)	
Sex			
Female	4.0 (18.0%)	7.0(32.0%)	0.3
Male	18.0 (82.0%)	15.0 (68.0%)	
Age (years)			
< 50	5.0 (23.0%)	10.0 (45.0%)	0.1
>= 50	17.0 (77.0%)	12.0 (55.0%)	
Primary tumor site			
Nasopharynx	6.0 (27.0%)	7.0 (32.0%)	0.7
Non-Nasopharynx	16.0 (73%)	15.0 (68.0%)	
T-stage			
T1/T2	9.0 (41.0%)	9.0 (41.0%)	>0.9
T3/T4	13.0 (59.0%)	13.0 (59.0%)	
N-stage			
N0/N1	10.0 (45.5%)	7.0 (32.0%)	0.3
N2/N3	12.0 (54.5%)	15.0 (68.0%)	

*ERCC1, Excision Repair Cross Complementing-group1; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; T-stage, tumor stage; N-stage, lymph node stage.

Response Assessment

Tumor response was based on the first computed tomography (CT) scan or magnetic resonance imaging (MRI) performed following completion of induction chemotherapy and was assessed using Response Evaluation Criteria in Solid Tumors (RECIST 1.1 Published in January 2009) with four categories of complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). The patients then were categorized into response (CR plus PR) and no-response (SD plus PD) groups.

RNA Extraction, Reverse Transcription and Quantitative Real Time RT-PCR (QRT-PCR) Assays

RNA was extracted from the cancerous tissues using the RNeasy FFPE kit (Qiagen, Germany) according to manufacturer's instructions. Concentration of total RNA was estimated by a nanodrop spectrophotometer (A and E, England), complementary DNA (cDNA) was synthesized using Super Script III Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA) and stored at -20°C until use. The mRNA expression levels of ERCC1 and beta-actin were measured by quantitative RT-PCR using SLAN Real-Time PCR Detection System (HONGSHI, Shanghai, China). The cycling conditions were as follows: 15 min of an

initial denaturation step at 95°C, followed by 40 cycles of 30 secs at 95°C, 30 secs at 60°C and 30 secs at 72°C. The following primers were used:

ERCC1, forward: CAATTTGCCCTTGACGATCTG; reverse: CCCTGTTTCTCTCTGTAGCTTCAA; and beta-actin, forward: CCTGGCACCCAGCACAAT; Reverse: GCCGATCCACACGGAGTACT. The expression of beta-actin was used as an internal control. The ERCC1 expression level was normalized to the beta-actin mRNA level using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). This study was approved by the local scientific and ethical committee.

Statistical Analysis

Statistical analysis was performed using the SPSS (statistical package for the social sciences, Chicago, IL) version 21.0. Associations between ERCC1 expression status and clinicopathological characteristics were assessed for statistical significance using Fisher Exact test. The level of significance was considered 0.05.

Results

Patient and Tumor Characteristics

Of 44 included patients, 33 were male (75%) and 11 were female (25%) with mean age of 53 years (23 to 79 years). For 13 patients (29.5%) the primary tumor site was nasopharynx and for 31 non-nasopharyngeal cases, larynx was the most common primary tumor site (57% of all patients). According to primary tumor and lymph node stage, most cases were T3/T4 (59%) and N2/N3 (61%) respectively. The patient, tumor and treatment characteristics are detailed in Table1.

Treatment's Efficacy

25 patients (57%) received PF and 13 patients (29%) received TPF induction chemotherapy regimen and for remaining 6 patients (14%) weekly paclitaxel plus carboplatin (wTC) was administered. The overall response rate (CR plus PR) was 77% in our patient population and in ten patients (23%) no objective responses were observed (SD plus PD group).

ERCC1 analysis

For all 44 patients, the median value of $2^{-\Delta\Delta CT}$ was 2.0 (0.0 to 396.2). The specimens with $2^{-\Delta\Delta CT}$ values lower and higher than 2.0 were categorized as low expressed and high expressed ERCC1 respectively. As shown in Table2, 4% of patients whose tumor samples had high ERCC1 expression showed no-response to induction chemotherapy. This value for patients with low ERCC1 expression was 9% and this difference was statistically significant (p=0.03). The ERCC1 expression states were not significantly different between patient groups according to sex, age, primary tumor site, tumor and node stage. The correlation between ERCC1 expression status and patient, tumor and response characteristics are detailed in Table2.

We analyzed the distribution of some variables other than ERCC1 expression status that could probably interfere with response to induction chemotherapy.

Table 3. Distribution of Confounding Variables in Response to treatment groups

	Response to treatment		P value
	SD/PD	CR/PR	
Age (years)			
< 50	2.0 (20.0%)	13.0 (38.0%)	0.3
>=50	8.0(80.0%)	21.0 (62.0%)	
Sex			
Female	3.0 (30.0%)	8.0 (23.5%)	0.7
Male	7.0 (70.0%)	26.0 (76.5%)	
Primary tumor site			
Nasopharynx	0 (0%)	13.0 (38.0%)	0.0
Non-Nasopharynx	10.0 (100%)	21.0 (62.0%)	
T-stage			
T1/T2	3.0 (30.0%)	15.0 (44.0%)	0.5
T3/T4	7 .0(70.0%)	19.0(56.0%)	
N-stage			
N0/N1	4 .0(40.0%)	13.0 (38.0%)	>0.9
N2/N3	6 .0(60.0%)	21.0 (62.0%)	
Induction chemotherapy regimen			
PF	6.0 (60.0%)	19.0 (56.0%)	
TPF	3.0 (30.0%)	10.0 (29.0%)	0.7
TC	1.0 (10.0%)	5.0 (15.0%)	
Induction chemotherapy regimen			
TC	1.0 (10.0%)	5.0 (15.0%)	>0.9
PF/TPF	9.0 (90.0%)	29.0 (85.0%)	

*CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; T-stage, tumor stage; N-stage, lymph node stage; wTC, weekly paclitaxel plus carboplatin; TPF, docetaxel plus cisplatin plus 5-fluorouracil; PF, cisplatin plus 5-fluorouracil.

As shown in Table3, other than primary tumor site, distributions of these confounding variables in response and no-response groups were not significantly different.

Discussion

In the present study, patients' demographic characteristics like age and sex were similar to ones in other studies (both local and in other geographic regions of the world) (Ferlay et al., 2010; Alvarenga et al., 2008; Ragin et al., 2007). Nasopharyngeal carcinoma consisted about 30% of all primary sites in the resent study. For malignancies of nasopharynx are not eligible for surgery in most situations and are referred for non-surgical treatments, this is why nasopharyngeal cancers included considerable percentage of our study population. However, larynx was the most common non-nasopharyngeal primary site and this is in concordance with most other reports (Ferlay et al., 2010; Alvarenga et al., 2008; Ragin et al., 2007). Overall response rate (CR+PR) to platinum-based induction chemotherapy in the present study was 77% and this is also in accordance with some other studies on head and neck cancers that

revealed a range of 64% to 81% for response to induction chemotherapy (Hitt et al., 2013; Cohen et al., 2014; Zhong et al., 2012).

In our patients, ERCC1 expression status was demonstrated to be associated with response to platinum-based induction chemotherapy. Patients in poor response group compared with good response one had more often high ERCC1 expression states and this difference was statistically significant (P=0.03). In a cohort study of 107 patients who were treated by a cisplatin-based induction chemotherapy regimen for locally advanced head and neck squamous cell carcinoma, Handra-Luca et al showed that patients with tumors expressing ERCC1at lower levels had a 4-fold greater odds of benefiting from an objective response to chemotherapy (P = 0.01) compared with the group of patients with high ERCC1expression. Unlike our study, they assessed ERCC1 expression status by immunohistochemical methods (Handra-Luca et al., 2007). In the only available study using RT-PCR to determine ERCC1 expression in patients with locally advanced head and neck cancer, Fountzilas et al (2009) identified no association between high ERCC1 mRNA expression and complete response to treatment Small sample size of their study and different treatment modalities (radiation concurrent with cisplatin plus cetuximab in Fountzilas et al study versus induction chemotherapy in the present study) are two possible explanations for the discordance between their result and one observed in our patients. In the present study no association between ERCC1 expression status and different demographic and clinicopathologic characteristics like sex, age, primary tumor site and stage were detected (Table2). Primary tumor site (nasopharynx versus non-nasopharynx) could be a possible confounding variable interfering with response to treatment as all nasopharyngeal primary tumors showed objective response to induction chemotherapy (Table3).

To date, there is much evidence that shows prognostic and predictive role of ERCC1 expression in patients with different types of cancers undergoing platinum-based treatment. Our study proposed that ERCC1 expression status detected by RT-PCR might serve as a bio-predictor of response to platinum-based induction chemotherapy for head and neck cancers.

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