Excision repair cross-complementing group 2 upregulation is a potential predictive biomarker for oral squamous cell carcinoma recurrence

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Abstract. Oral cancer is the fourth most common type of cancer among males in Taiwan, and the prognosis for patients with advanced-stage oral squamous cell carcinoma (OSCC) remains poor. The present study investigated the prognostic value of three DNA repair genes, namely excision repair cross-complementing group 1 (ERCC1), ERCC2 and X-ray repair cross-complementing group 1 (XRCC1) in OSCC. The protein expression levels of XRCC1, ERCC1 and ERCC2 in oral cell lines were analyzed via western blotting and immunohistochemistry using samples from 98 patients with biopsy-proven OSCC, while the χ^2 test was used to analyze the clinicopathological association. Kaplan-Meier estimates were used to determine the prognostic value of XRCC1, ERCC1 and ERCC2 for overall survival, and the log-rank test was used to evaluate the significance of differences. Multivariate analysis revealed a positive association between ERCC2 expression and OSCC recurrence (19.64-fold; 95% CI, 5.00-77.1; P<0.001). In addition, the high protein expression levels of XRCC1, ERCC1 and ERCC2 were associated with poor disease-free and overall survival rates. Therefore, the present study suggested that high ERCC2 expression may be a risk factor for OSCC recurrence.

Introduction

The oral cavity is the most prevalent area of malignancy in the head and neck region, with the GLOBOCAN 2020 data estimating the annual incidence and mortality as ~377,713 new cases and 177,757 deaths, respectively, for lip and oral cavity cancer, which is the fourth most common type of cancer among males in Taiwan (1-3). Squamous cell carcinoma constitutes the most commonly seen histological type in patients with oral cavity cancer (4). Radical surgery with or without adjuvant chemo-radiotherapy is part of the primary management for patients with oral squamous cell carcinoma (OSCC), and radical surgery has proven to be valuable in loco-regional disease control (5). Although 80-90% of early OSCC cases are cured, the prognosis for patients with advanced-stage OSCC remains poor (6,7). For patients who have undergone standard management, OSCC recurrence varies between 18 and 76%, and recurrence has been identified as the major cause of poor survival rates (8-11). Previous studies have indicated that the median time to recurrence is 7.5 months after therapy, with 86% of recurrences occurring among 24 months (12-14).

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In Taiwan, cisplatin is the mainstay of chemotherapy for locally advanced oral cancer treatment. Its crucial cytotoxic activity is due to the formation of DNA adducts, which result in inter-strand and intra-strand cross-linking (15,16). These DNA cross-links are identified and eliminated by the nucleotide excision repair (NER) pathway protecting the integrity of the genome (17,18). Tumor resistance to this platinum complex seems to be multifactorial, with the NER pathway serving a crucial role (19). NER is a stepwise procedure of recognition, incision, excision, DNA synthesis and ligation, which is executed by a multienzyme complex (20,21). Previous studies have investigated the association between gene expression and the effects of various chemotherapeutic agents in cancer (22-24), such as excision repair cross-complementing group 1 (ERCC1) being identified as a marker for resistance to cisplatin in non-small-cell lung cancer. Platinum resistance has been attributed to enhanced repair of DNA damage via the NER pathway, which consists of X-ray cross-complementing 1 (XRCC1), ERCC1 and ERCC2 (25-28).

Concurrent chemo-radiotherapy is regularly used for locally advanced oral cancer treatment. DNA single-strand breaks may take place directly from the damage to deoxyribose, or indirectly as the ordinary intermediates of DNA base excision repair (29). Since single-strand breaks are provoked by endogenous reactive molecules, such as reactive oxygen species, these injuries create a sustained threat to genetic integrity (29). In mammalian cells, the XRCC1 protein plays a leading part in the repair of single-strand breaks via its capability to interact with multiple enzymatic complexes of restoration (29). Hypersensitivity to oxidative stress, ionizing radiation and alkylating agents has been observed in cells lacking XRCC1 (29). A previous study has indicated that high expression of both XRCC1 and ERCC1 is significantly associated with radioresistant laryngeal carcinoma (30).

XRCC1, ERCC1 and ERCC2 are well-reported DNA repair proteins, implying their involvement in resistance to chemo-radiotherapy; however, to the best of our knowledge, no studies have explored the association of these NER pathway-associated proteins with recurrence and prognosis in patients with OSCC. Therefore, the current study hypothesized that high protein expression levels of XRCC1, ERCC1 and ERCC2 may lead to treatment resistance and recurrence or poor clinical outcomes in patients with OSCC.

Materials and methods

Cell culture. Human oral keratinocytes (HOK; ScienCell Research Laboratories, Inc.) were incubated with Oral Keratinocyte Medium (ScienCell Research Laboratories, Inc.) in plates pre-coated with 2 μg/cm^2 poly-L-lysine. DOK oral precancerous cells were grown in DMEM supplemented with 10% FBS (both Thermo Fisher Scientific, Inc.), penicillin (100 U/ml), streptomycin (100 μg/ml), glutamine (2 mM) and hydrocortisone (5 μg/ml). Oral cancer SAS, OECM1, HSC-3 and Cal-27 cells were grown in Eagle's Minimum Essential Medium (Thermo Fisher Scientific, Inc.) with glutamine (2 mM) and FBS (10%), while human malignant glioma U87MG cells (American Type Culture Collection; glioblastoma of unknown origin) were grown in DMEM supplemented with 10% FBS. All cells were incubated in 5% CO_2 at 37˚C.

Western blotting. Total cell lysates were extracted using RIPA buffer (Thermo Fisher Scientific, Inc.) and protein concentrations were detected using the BCA protein assay (Bio-Rad Laboratories, Inc.). Protein lysates (20 μg/lane) were separated via 10% SDS-PAGE and transferred to a PVDF membrane, which was then blocked for 1 h at room temperature with 5% non-fat milk in TBS-0.1% Tween-20 (TBST). Subsequently, the membrane was incubated overnight at 4˚C with primary antibodies including XRCC1 monoclonal antibody (1:1,000; cat. no. GTX83411; GeneTex, Inc.), ERCC1 monoclonal antibody (1:1,000; cat. no. GTX22356; GeneTex, Inc.), ERCC2 polyclonal antibody (1:1,000; cat. no. GTX105357; GeneTex, Inc.) and β-actin antibody (1:10,000; cat. no. GTX629630; GeneTex, Inc.). After washing three times with TBST for 10 min, the membrane was incubated with HRP-conjugated secondary antibody (1:5,000; cat. no. GTX213110-01; GeneTex, Inc.) for 2 h at room temperature. The immunoblots were visualized using Chemiluminescence Reagent Plus (PerkinElmer, Inc.) and quantified using Image Lab™ software version 5.1 (Bio-Rad Laboratories, Inc.).

Oncomine™ platform. To study the mRNA expression levels of XRCC1, ERCC1 and ERCC2 in oral cancer and normal oral tissues, Oncomine™ [Estilo Head-Neck: XRCC1160033_s_at (31), Peng Head-Neck: XRCC13864445 (32), Cromer Head-Neck: ERCC11902_at (33), Ginos Head-Neck: ERCC1203720_s_at (34), Peng Head-Neck: ERCC13865378 (32), Peng Head-Neck: ERCC23865301 (32), Ginos Head-Neck: ERCC2213468_at (34), Cromer Head-Neck: ERCC241095_at (33)], an integrated cancer microarray database and web-based data-mining platform (35), was used.

Patients. Between September 2002 and December 2011, a total of 98 patients with OSCC (92 men and 6 women) were enrolled from the Department of Oral and Maxillofacial Surgery of Kaohsiung Medical University Hospital (Kaohsiung, Taiwan), with a median follow-up time of 40 months (range, 2.4-137.4 months). G*Power (version 3.1.9.4; https://ps-power-and-sample-size-calculation.software.informer.com/3.1/), a freely available windows application software, was used for sample size and power estimation, with α=0.05 and estimated effect size w=0.46. A total of 98 patients were recruited in the present study to achieve sufficient power of ≥90%. The current study was approved by the Institutional Review Board of Kaohsiung Medical University Hospital (approval no. 20140158) and patient informed consent was waived by the Institutional Review Board due to the retrospective nature of the study. OSCC pathology was determined by two pathologists independently, and the final diagnoses were made using clinical and histological data. Patients without previous history of any treatment for oral cancer were included. Patients who were <18 or >80 years old were excluded. Baseline characteristic data included patient age, sex, tumor location, grade, tumor size, lymph node metastases and tumor stage; additionally, substance use, such as alcohol consumption, betel nut chewing or cigarette smoking, and adjunct treatment details were recorded (Table I). The mean age of the study group was 51.4 years and the median age was 51 years (age range, 31-76 years). Clinical staging of the patients was determined using the TNM staging system according to the 1992

criteria of the American Joint Committee on Cancer/Union for International Cancer Control (36). The primary tumor locations were buccal mucosa (77.6%) and tongue (22.4%). All of the patients received surgery as primary treatment, and some patients received adjuvant treatment, such as radiotherapy and chemotherapy. A total of 62 patients received adjuvant chemotherapy, with the chemotherapy regimen consisting of cisplatin or carboplatin with or without the addition of 5-fluorouracil or paclitaxel. A total of 45 patients received adjuvant intensity-modulated radiotherapy, and the scheduled doses were given once per day, 5 days per week. Postoperative patients received the planned course of adjuvant radiotherapy of 60-66 Gy in 2-Gy fractions to the post-operative high-risk region.

Survival endpoints and recurrence. Follow-up data was retrieved and updated from case records obtained from the medical records department until October 2014. Any patient not followed up within the last six months was contacted by phone to determine their current health status. Primary endpoints were disease-free survival (DFS) and overall survival (OS), with DFS being measured from the date of surgery to the date of locoregional recurrence, distant metastases or death from any cause, while OS was measured from the date of surgery to the date of death from any cause. Postoperative recurrence was defined as a lesion that exhibited postoperative regrowth at the same site after confirmation of healing of the surgical wounds.

Immunohistochemical (IHC) analysis. To determine the expression levels of XRCC1, ERCC1 and ERCC2 in OSCC tissues by IHC staining, the tissues were fixed in 10% neutral buffered formalin for 48 h at room temperature to prepare paraffin-embedded tumor tissue blocks for IHC sections (4-mm-thick). For the normal control, the oral mucosa tissue from a delinked patient with fibroma was used after Institutional Review Board approval (approval no. KMUH-IRB-20140158). Sections were deparaffinized and rehydrated following standard methods. Briefly, the sections were deparaffinized with xylene for 5 min for three times and rehydrated in graded ethanol (80-100%) for 5 min. A microwave antigen retrieval procedure was performed for 20 min in citrate buffer (pH 6.0), and 3% hydrogen peroxide was used to block non-specific peroxidase reactions at room temperature for 10 min. After washing twice with TBS, non-specific blocking was performed with Protein Block (Novolink Polymer Detection System; Leica Microsystems, Inc.) for 5 min at room temperature and washed twice with TBS. Following washing with TBS, sections were incubated with the aforementioned XRCC1 monoclonal antibody (1:100), ERCC1 monoclonal antibody (1:200) and ERCC2 polyclonal antibody (1:100) at 4˚C overnight. The sections were subsequently incubated with the Post Primary rabbit anti-mouse IgG antibody (Novolink Polymer Detection System; Leica Microsystems, Inc.) for 30 min at room temperature as secondary antibody application. The staining intensity of tumor tissues was determined as score 0 (negative), score 1 (weak), score 2 (moderate) and score 3 (strong) for antigens present in the cytoplasm and nucleus of cells using a light microscope (magnification, x200), determined separately by two independent pathologists.

Table I. Clinicopathological characteristics of 98 patients with oral squamous cell carcinoma.

Characteristics	N (%)
Age, years	
<50	47 (48.0)
≥50	51 (52.0)
Sex	
Male	92 (93.9)
Female	6 (6.1)
Cigarette smoking	
No	11 (11.1)
Yes	87 (88.9)
Betel quid chewing	
No	30 (30.6)
Yes	68 (69.4)
Alcohol consumption	
No	35 (35.7)
Yes	63 (34.3)
Tumor location	
Buccal mucosa	76 (77.6)
Tongue	22 (22.4)
Grade	
Grade 0	86 (87.8)
Grade I+II+III	12 (12.2)
Tumor size	
T1	22 (22.4)
T2	30 (30.6)
T3	6 (6.1)
T4	40 (40.8)
Lymph node metastases	
N0	50 (51.0)
N1	30 (30.6)
N2	18 (18.4)
Tumor stage	
I	18 (18.4)
II	17 (17.3)
III	18 (18.4)
IV	45 (45.9)
RT	
No	53 (54.1)
Yes	45 (45.9)
CT	
No	36 (36.7)
Yes	62 (63.3)
Recurrence	
No	29 (29.6)
Yes	
Pre-CT/RT recurrence	22 (22.4)
Post-CT/RT recurrence	47 (48.0)

RT, radiotherapy; CT, chemotherapy.

Statistical analysis. Statistical analyses were performed using SPSS 19.0 (IBM Corp.). Descriptive statistics were used and compared using independent t-test, χ^2 test or Fisher's exact test. Survival analyses were evaluated using the Kaplan-Meier method with the log-rank test, while the Cox proportional-hazards model was used for multivariate analysis. Furthermore, hazard ratios (HRs) and 95% CIs were calculated by multivariable Cox regression models and used to investigate the association between clinicopathological characteristics and survival. DFS was defined as the time after surgery during which the patient survived with no sign of recurrence. OS was defined as the time elapsed between surgery and death. All P-values were two-sided, with P<0.05 considered to indicate a statistically significant difference.

Results

Expression profiles of XRCC1, ERCC1 and ERCC2 in oral cancer cell lines. Since DNA repair proteins are essential for oral cancer cells in response to chemo-radiotherapy, the expression levels of XRCC1, ERCC1 and ERCC2 in HOK normal oral epithelial cells, DOK oral pre-cancer cells and oral cancer SAS, OECM1, HSC-3 and Cal-27 cell lines were first examined using western blotting (Fig. 1), with U87MG glioblastoma cells being included as a positive control for XRCC1, ERCC1 and ERCC2 proteins (37). The results revealed that the expression levels of XRCC1 and ERCC1 were weakly detected in all oral cancer cells, while ERCC2 expression was detected in all cell lines (Fig. 1). Notably, HOK cells had lower ERCC2 protein expression compared with DOK cells and the oral cancer cell lines.

Association of XRCC1, ERCC1 and ERCC2 expression in OSCC tissues with clinicopathological characteristics. In the present study, 98 patients with OSCC (92 men and 6 women) were included. The clinicopathological characteristics of these patients, including age, sex, tumor location, grade, tumor size, lymph node metastases, tumor stage, radiotherapy, chemotherapy, smoking habit and recurrence, are shown in Table I. XRCC1, ERCC1 and ERCC2 expression in oral cancer tissues, as well as in normal oral mucosa tissue of a patient with fibroma, was analyzed using an online database and IHC analysis. The Oncomine database revealed that the mRNA expression levels of XRCC1, ERCC1 and ERCC2 were significantly increased in oral cancer tissues compared with in normal epithelial tissues (Fig. S1), except in the Peng Head-Neck XRCC1-3864445 dataset, where there was no significant difference, but there was still a trend toward XRCC1 elevation in oral cancer tissues (P=0.061; Fig. S1A). Notably, IHC staining without addition of the primary antibody was used as a negative control for XRCC1, ERCC1 and ERCC2 staining (Fig. S2). According to the staining intensity in tumor tissues, the expression levels of the three proteins were categorized into four scores (0-3). XRCC1 and ERCC1 proteins were predominantly stained in the nuclei, while ERCC2 protein was predominantly stained in the cytoplasm of oral cancer tissues (Fig. 2). To analyze the association between the protein expression levels of the three proteins in OSCC tissues and clinicopathological characteristics, the expression levels of the three proteins were further divided into high expression (score 3) and low expression (score 0-2) groups. High expression groups of XRCC1
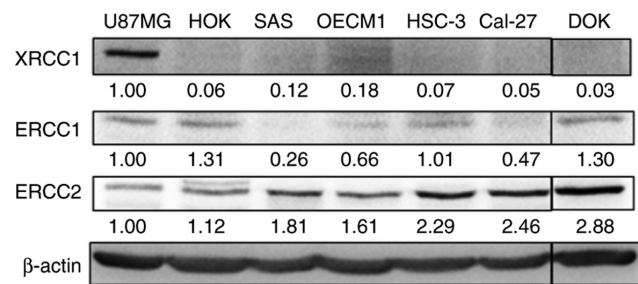

Figure 1. XRCC1, ERCC1 and ERCC2 protein expression in primary oral epithelial cells, oral pre-cancer cells and various oral cancer cells. The cell lysates extracted from HOK primary oral epithelial cells, DOK oral pre-cancer cells and oral cancer cell lines, including SAS, OECM1, HSC-3 and Cal-27, underwent western blotting for detecting the expression levels of XRCC1, ERCC1 and ERCC2. Glioblastoma U87MG cell line served as a positive control. The numbers correspond to the grey density values of a single replicate. XRCC1, X-ray repair cross-complementing group 1; ERCC1/2, excision repair cross-complementing group 1/2.

(P=0.020), ERCC1 (P=0.006) or ERCC2 (P<0.001) were significantly associated with OSCC recurrence (Tables II-IV). Furthermore, univariate and multivariate analyses were used to explore OSCC recurrence predictors in patients with OSCC. In univariate analysis, lymph node metastases (N1+N2), high XRCC1 expression, high ERCC1 expression and high ERCC2 expression were significantly associated with OSCC recurrence (P=0.006, P=0.020, P=0.006 and P<0.001, respectively; Table V). In multivariate analysis, lymph node metastases (N1+N2) (2.38-fold; 95% CI, 1.02-5.58; P=0.045) and high ERCC2 expression (4.84-fold; 95% CI, 2.56-9.16; P<0.001) were significantly associated with increased risk of OSCC recurrence (Table V).

Univariate and multivariate analyses were also used to explore OSCC survival predictors in patients with OSCC. In univariate analysis, tumor size, lymph node metastases (N1+N2), tumor stage, high XRCC1 expression, high ERCC1 expression and high ERCC2 expression were significantly associated with a worse survival in patients with OSCC (P=0.001, P=0.005, P=0.001, P=0.002, P=0.014 and P<0.001, respectively; Table VI). In multivariate analysis, tumor location (4.11-fold; 95% CI, 1.392-12.14; P=0.01) and high ERCC2 expression (15.55-fold; 95% CI, 4.34-55.67; P<0.001) were significantly associated with increased risk of death (Table VI).

The association between the expression levels of the three proteins and DFS or OS rates in patients with OSCC was analyzed using the Kaplan-Meier method. Significantly decreased DFS rates were observed in patients with high expression levels of XRCC1, ERCC1 and ERCC2 (P=0.020, P=0.040 and P<0.001, respectively), as well as significantly decreased OS rates in patients with high expression levels of XRCC1, ERCC1 and ERCC2 (P=0.002, P=0.014 and P<0.001, respectively), as determined using the log-rank test (Fig. 3). The findings of the survival analysis indicating that high ERCC2 expression indicated a poor prognosis were consistent with the results of the multivariate analysis.

Discussion

In the present study, the association between the expression levels of NER pathway-associated genes and the recurrence
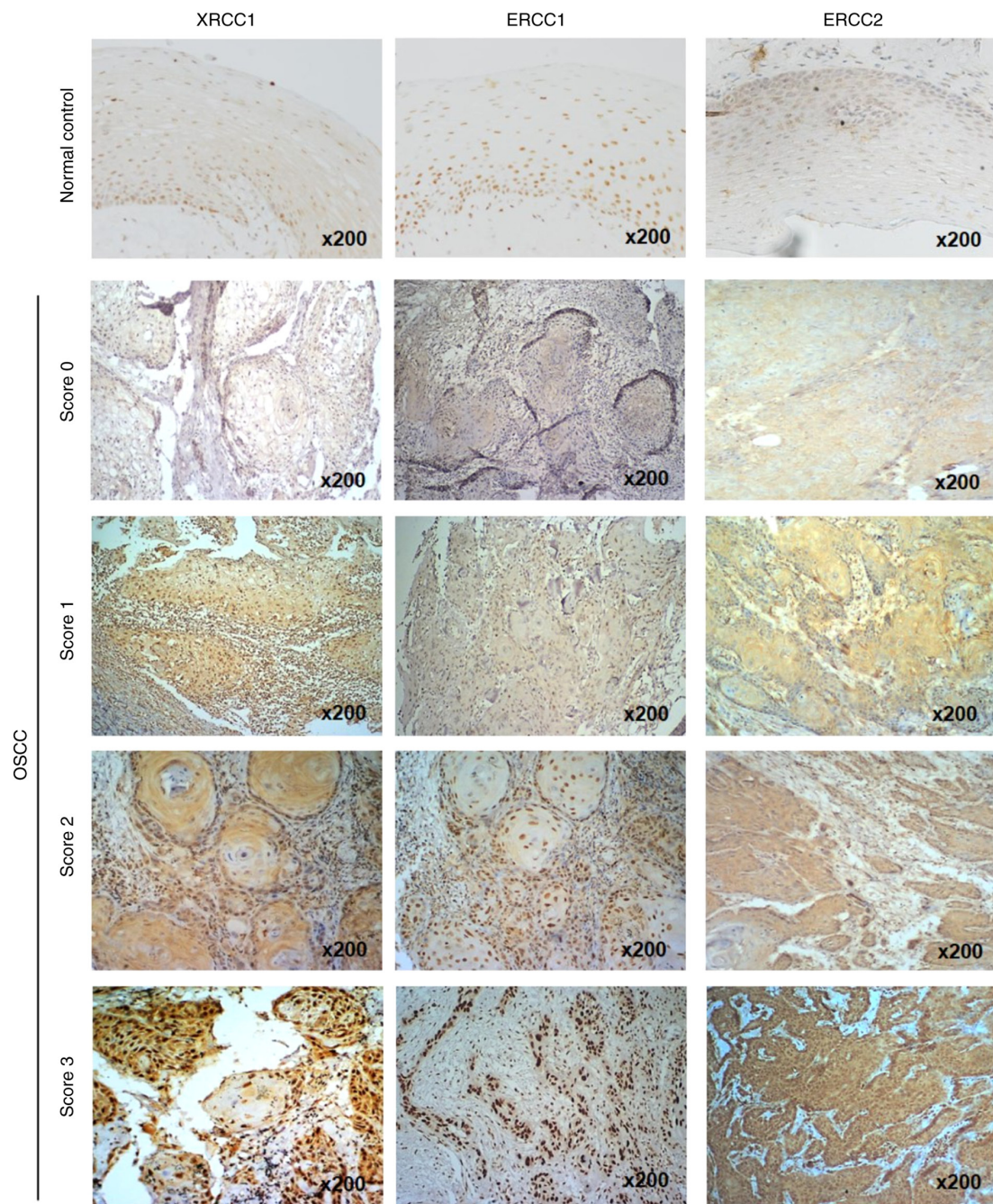
Figure 2. Immunohistochemical staining of XRCC1, ERCC1 and ERCC2 expression in OSCC tissues. ERCC1, ERCC2 or XRCC1 expression in oral normal tissues and cancer tissues, determined by immunohistochemistry, was divided into 4 categories according to the staining intensity: Score 0, no staining; score 1, weak staining; score 2, moderate staining; and score 3, strong staining. Original magnification, x200. XRCC1, X-ray repair cross-complementing group 1; ERCC1/2, excision repair cross-complementing group 1/2; OSCC, oral squamous cell carcinoma.

and survival outcome in patients with OSCC was explored by examining the expression levels of XRCC1, ERCC1 and ERCC2 in a series of oral cavity epithelial cells, revealing that ERCC2, but not ERCC1 or XRCC1, exhibited a trend of positive association with the neoplastic development from normal epithelium to dysplasia and OSCC. However, some cancer cells expressed lower expression levels of XRCC1, ERCC1 and ERCC2 than HOK normal oral keratinocytes and DOK oral precancerous cells, and this requires further investigation. In addition, high expression levels of the three proteins in OSCC tissues were significantly associated with worse OS and DFS rates compared with low expression levels.

Notably, the multivariate analysis revealed that high ERCC2 expression was an independent prognostic marker for OSCC recurrence.

High ERCC1 expression in head and neck squamous cell carcinoma (HNSCC) has been extensively studied as a potential prognostic biomarker for the chemoradiotherapy response and survival prognosis of patients with HNSCC (38-42). Although the genetic variations of ERCC2 and XRCC1 have been reported to be associated with increased risk and worse clinical outcome of oral cancer (43-47), there is very little data on ERCC2 and XRCC1 expression in oral cancer. A systematic review and case-control study has revealed

Table II. Association between clinical characteristics of patients with oral squamous cell carcinoma and XRCC1 expression.

Characteristics	XRCC1 expression		P-value
	Low, n (%)	High, n (%)	
Age, years			
<50	13 (46.4)	34 (48.6)	0.850
≥50	15 (53.6)	36 (51.4)	
Sex			
Male	28 (100.0)	64 (91.4)	0.180[a]
Female	0 (0.0)	6 (8.6)	
Cigarette smoking			
No	1 (3.6)	10 (14.3)	0.170[a]
Yes	27 (96.4)	60 (85.7)	
Betel quid chewing			
No	7 (25.0)	23 (32.9)	0.450
Yes	21 (75.0)	47 (37.1)	
Alcohol consumption			
No	6 (21.4)	29 (41.4)	0.070
Yes	22 (78.6)	41 (58.6)	
Tumor location			
Buccal mucosa	24 (85.7)	52 (74.3)	0.290[a]
Tongue	4 (14.3)	18 (25.7)	
Grade			
Grade I	26 (92.9)	60 (85.7)	0.500[a]
Grade II+III	2 (7.1)	10 (14.3)	
Tumor size			
T1+T2	16 (57.1)	36 (51.4)	0.610
T3+T4	12 (42.9)	34 (48.6)	
Lymph node metastases			
N0	15 (53.6)	35 (50.0)	0.750
N1+N2	13 (46.4)	35 (50.0)	
Tumor stage			
I+II	11 (39.3)	24 (34.3)	0.640
III+IV	17 (60.7)	46 (65.7)	
Radiotherapy			
No	18 (64.3)	35 (50.0)	0.200
Yes	10 (35.7)	35 (50.0)	
Chemotherapy			
No	12 (42.9)	24 (34.3)	0.430
Yes	16 (57.1)	46 (65.7)	
Recurrence			
No	13 (46.4)	16 (22.9)	0.020
Yes	15 (53.6)	54 (77.1)	

[a]Analyzed using Fisher's exact test. XRCC1, X-ray repair cross-complementing group 1.

that ERCC2 expression is significantly increased in HNSCC tissues compared with in adjacent normal tissues, and that it is positively associated with tumor stage and grade (48). The present study indicated that ERCC1, ERCC2 and XRCC1 expression was associated with the survival rate of patients with OSCC. To the best of our knowledge, the current study is the first to suggest ERCC2 as a prognostic marker for OSCC recurrence.

The association between the synthesis of DNA repair proteins in tumor cells and the patient response to

Table III. Association between clinical characteristics of patients with oral squamous cell carcinoma and ERCC1 expression.

Characteristics	ERCC1 expression		P-value
	Low, n (%)	High, n (%)	
Age, years			
<50	19 (47.5)	28 (48.3)	0.940
≥50	21 (52.5)	30 (51.7)	
Sex			
Male	36 (90.0)	56 (96.6)	0.220[a]
Female	4 (10.0)	2 (3.4)	
Smoking			
No	4 (10.0)	7 (12.1)	>0.999[a]
Yes	36 (90.0)	51 (87.9)	
Betel quid chewing			
No	11 (27.5)	19 (32.8)	0.580
Yes	29 (72.5)	39 (67.2)	
Alcohol consumption			
No	14 (35.0)	21 (36.2)	0.900
Yes	26 (65.0)	37 (63.8)	
Tumor location			
Buccal mucosa	32 (80.0)	44 (75.9)	0.630
Tongue	8 (20.0)	14 (24.1)	
Grade			
Grade I	35 (87.5)	51 (87.9)	>0.999
Grade II+III	5 (12.5)	7 (12.1)	
Tumor size			
T1+T2	22 (55.0)	30 (51.7)	0.750
T3+T4	18 (45.0)	28 (48.3)	
Lymph node metastases			
N0	22 (55.0)	28 (48.3)	0.510
N1+N2	18 (45.0)	30 (51.7	
Tumor stage			
I+II	15 (37.5)	20 (34.5)	0.760
III+IV	25 (62.5)	38 (65.5)	
Radiotherapy			
No	23 (57.5)	30 (51.7)	0.680
Yes	17 (42.5)	28 (48.3)	
Chemotherapy			
No	17 (42.5)	19 (32.8)	0.330
Yes	23 (57.5)	39 (67.2)	
Recurrence			
No	18 (45.0)	11 (19.0)	0.006
Yes	22 (55.0)	47 (81.0)	

[a]Analyzed using Fisher's exact test. ERCC1, excision repair cross-complementing group 1.

chemo-radiotherapy has been reported in different types of cancer. In patients with non-small cell lung cancer, low ERCC1 expression indicates an improved prognosis after treatment with multidrug chemotherapy (22). High XRCC1 and ERCC1 expression is associated with a poor prognosis in patients with HER2^{+} breast cancer (47), while high ERCC1 expression has been associated with resistance to platinum-based chemotherapy in patients with ovarian cancer (49). In addition, ectopic ERCC1 expression in ovarian cancer cells increases the resistance to cisplatin-mediated growth inhibition (50).

Table IV. Association between clinical characteristics of patients with oral squamous cell carcinoma and ERCC2 expression.

Characteristics	ERCC2 expression		P-value
	Low, n (%)	High, n (%)	
Age, years			
<50	21 (53.8)	26 (44.1)	0.340
≥50	18 (46.2)	33 (55.9)	
Sex			
Male	36 (92.3)	56 (94.9)	0.680[a]
Female	3 (7.7)	3 (5.1)	
Smoking			
No	5 (12.8)	6 (10.2)	0.680
Yes	34 (87.2)	53 (89.8)	
Betel quid chewing			
No	8 (20.5)	22 (37.3)	0.080
Yes	31 (79.5)	37 (62.7)	
Alcohol consumption			
No	12 (30.8)	23 (39.0)	0.410
Yes	27 (39.2)	36 (61.0)	
Tumor location			
Buccal mucosa	28 (71.8)	48 (81.4)	0.270
Tongue	11 (28.2)	11 (18.6)	
Grade			
Grade I	34 (87.2)	52 (88.1)	>0.999
Grade II+III	5 (12.8)	7 (11.9)	
Tumor size			
T1+T2	24 (61.5)	28 (47.5)	0.170
T3+T4	15 (38.5)	31 (52.5)	
Lymph node metastases			
N0	24 (61.5)	26 (44.1)	0.090
N1+N2	15 (38.5)	33 (55.9)	
Tumor stage			
I+II	18 (46.2)	17 (28.8)	0.080
III+IV	21 (53.8)	42 (71.2)	
Radiotherapy			
No	20 (51.3)	33 (55.9)	0.650
Yes	19 (48.7)	26 (44.1)	
Chemotherapy			
No	18 (46.2)	18 (30.5)	0.120
Yes	21 (53.8)	41 (69.5)	
Recurrence			
No	23 (59.0)	6 (10.2)	<0.001
Yes	16 (41.0)	53 (89.8)	

[a]Analyzed using Fisher's exact test. ERCC2, excision repair cross-complementing group 2.

NER capacity serves a major role in normal tissue tolerance and drug resistance; moreover, ERCC2 and ERCC1 act as rate-limiting enzymes in NER (51). During NER, ERCC2 participates in DNA unwinding, and this function may alter the platinum-based chemotherapy effect (52). In a previous study, the genotypes of XRCC1 rs1799782 and XRCC2 rs2040639 DNA repair genes were significantly associated with oral cancer in Taiwan (44). Several studies have suggested that cancer cells with upregulation of DNA repair proteins are more resistant to chemoradiotherapy (53-55). Therefore, according to the present study, further investigation on the mechanism of the biological process of ERCC2 may provide

Table V. Univariate and multivariate analyses of recurrence predictors in 98 patients with oral squamous cell carcinoma.

	Recurrence		Univariate analysis	Multivariate analysis	
Characteristics	Yes	No	P-value	Hazard ratio (95% CI)	P-value
Age, years					
≥50	32 (46.4)	19 (65.5)	0.080	1.0	0.370
<50	37 (53.6)	10 (34.5)		1.28 (0.75-2.20)	
Sex					
Female	3 (4.3)	3 (10.3)	0.360	1.0	0.290
Male	66 (95.7)	26 (89.7)		0.48 (0.13-1.83)	
Smoking					
No	8 (11.6)	3 (10.3)	>0.999	1.0	0.400
Yes	61 (88.4)	26 (89.1)		0.64 (0.22-1.84)	
Betel quid chewing					
No	23 (33.3)	7 (24.1)	0.370	1.0	0.330
Yes	46 (66.7)	22 (75.9)		1.39 (0.72-2.71)	
Alcohol consumption					
No	27 (39.1)	8 (27.6)	0.370	1.0	0.960
Yes	42 (60.9)	21 (72.4)		0.98 (0.54-1.81)	
Tumor location					
Buccal mucosa	53 (76.8)	23 (79.3)	0.790	1.0	0.190
Tongue	16 (23.2)	6 (20.7)		0.64 (0.32-1.25)	
Grade					
Grade I	60 (87.0)	26 (89.7)	>0.999	1.0	0.680
Grade II+III	9 (13.0)	3 (10.3)		0.86 (0.40-1.81)	
Tumor size					
T1+T2	34 (49.3)	18 (62.1)	0.250	1.0	0.150
T3+T4	35 (50.7)	11 (37.9)		1.78 (0.81-3.88)	
Lymph node metastases					
N0	29 (42)	21 (72.4)	0.006	1.0	0.045
N1+N2	40 (58)	8 (27.6)		2.38 (1.02-5.58)	
Tumor stage					
I+II	21 (30.4)	14 (48.3)	0.090	1.0	0.460
III+IV	48 (69.6)	15 (51.7)		0.65 (0.20-1.71)	
XRCC1 expression					
Low	15 (21.7)	13 (44.8)	0.020	1.0	0.450
High	54 (78.3)	16 (55.2)		1.31 (0.65-2.66)	
ERCC1 expression					
Low	22 (31.9)	18 (62.1)	0.006	1.0	0.460
High	47 (68.1)	11 (37.9)		1.25 (0.69-2.25)	
ERCC2 expression					
Low	16 (23.2)	23 (79.3)	<0.001	1.0	<0.001
High	53 (76.8)	6 (20.7)		4.84 (2.56-9.16)	

XRCC1, X-ray repair cross-complementing group 1; ERCC1/2, excision repair cross-complementing group 1/2.

potential targets for pharmacological modulation that helps improve the efficiency of chemo-radiotherapy.

Cigarette smoking, alcohol consumption and betel quid chewing are well-known risk factors for oral cancer. Most oral cancer cases (~90%) in South-East Asia are associated with smoking (56), while the proportions of cases associated with alcohol drinking and betel quid chewing are 80 and 75%, respectively. These agents may act synergistically. A working group of the International Agency for Research on Cancer concluded that there was adequate evidence of an association between chewing betel quid together with tobacco use (chewing or smoking) as a combined risk factor (57). In areas

Table VI. Univariate and multivariate analysis of survival predictors in 98 patients with oral squamous cell carcinoma.

Characteristics	Death		Univariate analysis	Multivariate analysis	
	Yes	No	P-value	Hazard ratio (95% CI)	P-value
Age, years					
≥50	25 (54.3)	26 (50.0)	0.540	0.96 (0.48-19.2)	0.910
<50	21 (45.7)	26 (50.0)		1	
Sex					
Female	3 (6.5)	3 (5.8)	0.410	1	0.290
Male	43 (93.5)	49 (94.2)		0.43 (0.09-2.04)	
Smoking					
No	5 (10.9)	6 (11.5)	0.880	1	0.350
Yes	41 (89.1)	46 (88.5)		0.53 (0.14-2.00)	
Betel quid chewing					
No	19 (41.3)	15 (28.8)	0.270	1	0.790
Yes	27 (58.7)	37 (71.2)		1.11 (0.53-2.32)	
Alcohol consumption					
No	17 (37.0)	18 (34.6)	0.910	1	0.460
Yes	29 (63.0)	34 (65.4)		1.33 (0.62-2.86)	
Tumor location					
Buccal mucosa	39 (84.8)	37 (71.2)	0.060	1	0.010
Tongue	7 (15.2)	15 (28.8)		4.11(1.39–12.14)	
Grade					
Grade I	38 (82.6)	48 (92.3)	0.710	1	0.430
Grade II+III	8 (17.4)	4 (7.7)		0.71 (0.30-1.67)	
Tumor size					
T1+T2	17 (37.0)	35 (37.3)	0.001	1	0.430
T3+T4	29 (63.0)	17 (32.7)		1.55 (0.52-4.58)	
Lymph node metastases					
N0	29 (63.0)	19 (36.5)	0.005	1	0.560
N1+N2	17 (37.0)	33 (63.5)		1.31 (0.54-31.8)	
Tumor stage					
I+II	9 (19.6)	26 (50.0)	0.001	1	0.500
III+IV	37 (80.4)	26 (50.0)		1.70 (0.36-8.04)	
XRCC1 expression					
Low	5 (10.9)	23 (44.2)	0.002	1	0.100
High	41 (89.1)	29 (41.4)		2.65 (0.82-8.52)	
ERCC1 expression					
Low	10 (21.7)	30 (57.7)	0.014	1	0.900
High	36 (78.3)	22 (42.3)		1.06 (0.45-2.50)	
ERCC2 expression					
Low	3 (6.5)	36 (69.2)	<0.001	1	<0.001
High	43 (93.5)	16 (60.2)		15.55 (4.34-55.67)	

XRCC1, X-ray repair cross-complementing group 1; ERCC1/2, excision repair cross-complementing group 1/2.

where the habit of betel quid chewing is widespread, these risk factors should be taken into consideration and require further investigation. However, the influence of smoking on clinical outcome of patients with oral cancer is in debate, since while smoking has a negative impact on survival, its effect is influenced by other confounding factors, such as treatment method, age, tumor size, smoking status or dose-response relationship (58-62). When patients stop smoking, survival benefits have been observed in several types of cancer, including head and neck cancer (59,63). In the current study, cigarette smoking, alcohol consumption and betel quid chewing were not associated with recurrence in univariate or multivariate
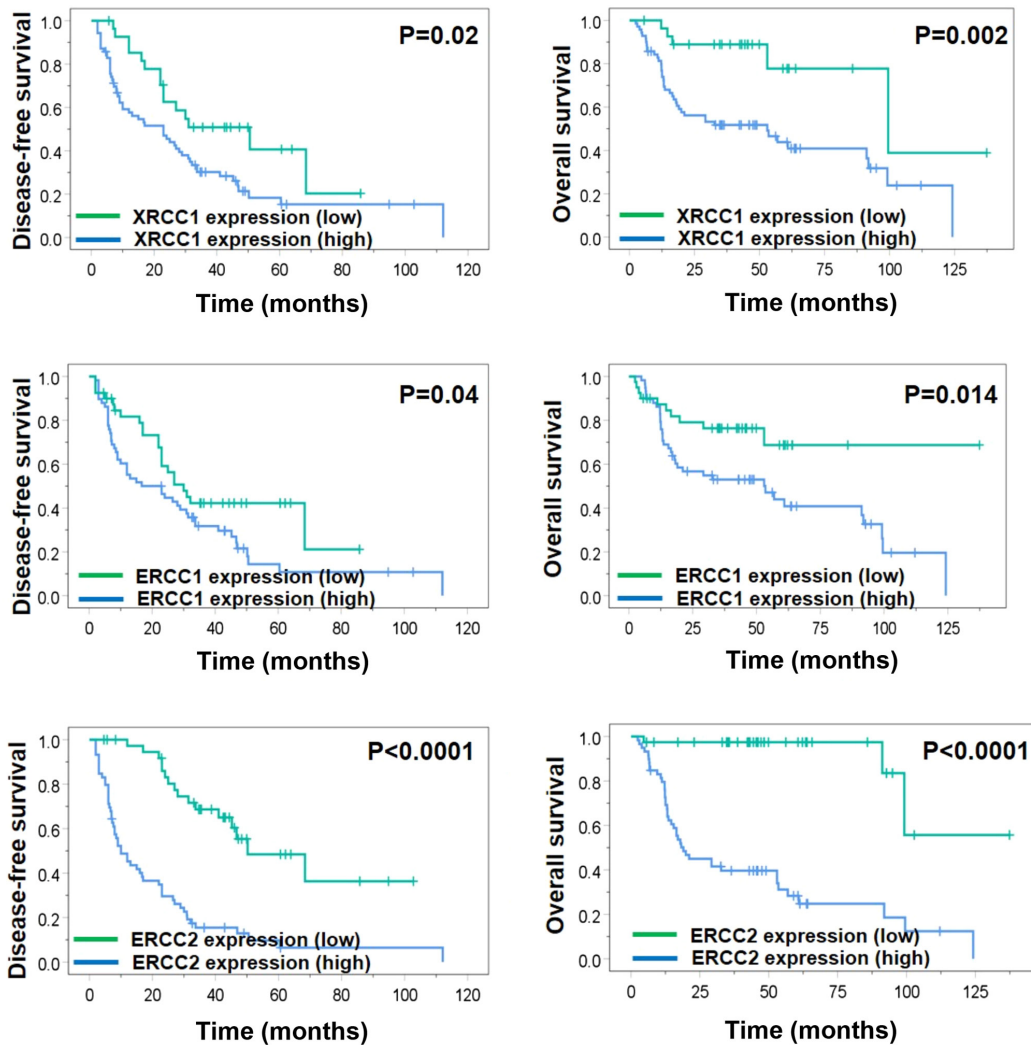
Figure 3. Disease-free survival and overall survival rates of patients with oral squamous cell carcinoma divided in low and high XRCC1, ERCC1 and ERCC2 expression groups. Survival curves were generated using the Kaplan-Meier method and the P-values were calculated using the log-rank test. XRCC1, X-ray repair cross-complementing group 1; ERCC1/2, excision repair cross-complementing group 1/2.

analysis, which may be due to fewer non-users. Further larger scale studies are required to solve this contradiction.

According to GLOBOCAN 2020, the ratio of oral cancer incidence in male and female is 2:1 in Asia and 2.5:1 worldwide; however, male is the predominant sex for oral cancer in Taiwan (2). According to the Taiwan cancer registry annual report 2017, the proportion of female patients with oral cancer was 10.7%, and females represented 6% of the total sample size in the present study, which is similar to the general oral cancer population in Taiwan (3).

The current study has some limitations. First, data regarding socioeconomic factors were not collected and analyzed, and second, data were from a single tertiary cancer care center; therefore, more extensive multi-center studies are required to reinforce the present findings. Third, the median follow-up time was 40 months, and a longer follow-up time is required for full surveillance of cancer recurrence. Lastly, reverse transcription-quantitative PCR may also be a useful approach to analyze mRNA expression.

In future studies, the mechanisms of ERCC1, ERCC2 and XRCC1 participating in OSCC carcinogenesis through the NER pathway should be analyzed both *in vitro* and *in vivo*,

and larger prospective studies to validate the possible role of ERCC2 in OSCC outcome prediction should be performed.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on request.

Authors' contributions

YYW, PTF, CWS, YKC, JJH, MYH and SSFY conceived and designed the experiments. YYW, YKC, MYH and SSFY performed the experiments. YYW, PTF, JYH, MYH and SSFY analyzed and discussed the data. YYW, PTF, MYH and SSFY contributed to reagents/materials/analysis tools. YYW, PTF, JYH, YKC, JJH, MYH and SSFY wrote the manuscript. MYH and SSFY are responsible for confirming the authenticity of the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Ethics approval was obtained from the Institutional Review Board of Kaohsiung Medical University Hospital (approval no. 20140158) and informed consent was waived due to the retrospective nature of the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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