Original Article

SOX2 amplification and *chromosome 3* gain significantly impact prognosis in esophageal squamous cell carcinoma

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Background: We aimed to investigate the prevalence and prognostic role of *Sex determining region Y-box 2* (*SOX2*) amplification and expression in surgically resected esophageal squamous cell carcinoma (ESCC).

Methods: We evaluated 450 ESCC samples using fluorescence in-situ hybridization and immunohistochemistry for *SOX2* gene amplification and protein expression, respectively. The relationships of gene status with various clinicopathological characteristics and patient survival were statistically analyzed.

Results: *SOX2* amplifications and *chromosome 3* gain were observed in 4.4% and 12.9% of patients with ESCC. *SOX2* amplification was associated with later clinical stage, and *chromosome 3* gain was associated with earlier clinical stage (P=0.025). Low and high SOX2 expression were found in 28.9% and 24.7% of cases, respectively. SOX2 expression was significantly associated with gene copy number variation (P=0.007). *SOX2* amplification was associated with a significantly shorter disease-free survival (DFS) or overall survival (OS). However, *chromosome 3* gain was associated with a significantly longer DFS or OS (P<0.001). Multivariate analysis using the Cox proportional hazard model indicated that *SOX2* amplification was an independently poorer prognostic factor (DFS, P<0.001, HR 2.638, 95% CI, 1.581–4.403; OS, P<0.001, HR 2.608, 95% CI, 1.562–4.355), along with pathology tumor-node-metastasis (pTNM) stage, whereas *chromosome 3* gain was an independently better prognostic factor (DFS, P=0.003, HR 0.486, 95% CI, 0.300–0.789; OS, P=0.003, HR 0.474, 95% CI, 0.289–0.779) for ESCC.

Conclusions: This is the first study wherein *SOX2* amplification and *chromosome 3* gain in a large cohort of ESCC were evaluated. *SOX2* amplification is an independently poorer prognostic factor, whereas *chromosome 3* gain is an independently favorable prognostic factor. Our results suggest that *SOX2* amplification and *chromosome 3* gain are potential biomarkers related to tumor progression and risk stratification in ESCC.

Keywords: *SOX2* amplification; *chromosome 3* gain; esophageal squamous cell carcinoma (ESCC); prognosis

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Introduction

Esophageal cancer (EC) is the leading cause of cancer mortality in China, where it was responsible for over 193,000 deaths in 2014 (1). Approximately 90% of the new EC cases diagnosed each year are esophageal squamous cell carcinoma (ESCC). Despite the optimization of surgery, radiotherapy, and cytotoxic chemotherapy, the survival of patients with advanced ESCC remains poor, with an age-standardized 5-year relative survival rate of only 30% (2). Recent advances in genetic profiling have led to more refined molecular classifications of specific tumor entities (3-5). To determine the optimal therapeutic approach for a given patient with ESCC, prognostic biomarkers that would predict outcomes more accurately than existing ones are urgently needed. Gene copy number alterations are frequently found in human epithelial malignancies and often play an essential role in tumor development (5-7). The detection of these alterations may allow the development of novel diagnostic, prognostic, and predictive biomarkers, and promote the use of effective therapeutic regimens.

Sex determining region Y-box 2 (*SOX2*) is a highly conserved, single exon gene that is located at 3q26.33 and encodes for a 317-amino acid protein. SOX2 contains a high mobility group (HMG) DNA-binding domain and belongs to the SOX family of transcription factors, which are essential for preserving the pluripotency of embryonic stem cells and self-renewal capacity of tissue-specific adult stem cells (8). *SOX2* has been found to be recurrently amplified in squamous cell carcinomas (SCC) of different organ sites, such as the head and neck (9), lung (10) and anus (11), and it is considered as a lineage-survival oncogene. In lung SCC (LSCC), *SOX2* amplification is frequently observed and associated with favorable prognosis (10). In head and neck SCC (HNSCC), *SOX2* activation is associated with improved prognosis (9). In sinonasal SCC (SSCC), *SOX2* amplification is an independent indicator of disease recurrence (12).

Recently, with the availability of single-nucleotide polymorphism array and array comparative genomic hybridization, *SOX2* was found to be amplified in 10% ESCC cases (13), which was a little less than 15–18% in other reports on ESCC (14,15). Few studies have explored the association between *SOX2* amplification and patients' prognosis. Some studies, wherein the clinical significance of SOX2 expression in ESCC was evaluated, had controversial conclusions. A study from Norway showed that SOX2 expression correlated with higher histological grade and poorer clinical survival (16). A later Japanese study showed the negative expression of SOX2 appeared to be an independently poor prognostic factor (17). Therefore, further studies are required to validate the prognostic role of SOX2 in patients with ESCC.

In the present study, we aimed to investigate the prevalence of *SOX2* gene copy number changes in a large cohort of surgically resected ESCC patients using fluorescence in situ hybridization (FISH) analysis, and determine whether copy number alterations in selected genes affect patient outcomes. Furthermore, we examined whether gene amplification is associated with increased SOX2 protein expression in ESCC.

We present the following article in accordance with the REMARK reporting checklist (available at http://dx.doi.org/10.21037/atm-20-1290).

Methods

Patients and samples

This retrospective study consisted of 450 ESCC patients who had undergone esophagectomy without neoadjuvant treatment at the Department of Thoracic Surgery, Zhongshan Hospital, Fudan University, China, between 2007 and 2010. The patients who exhibited disease progression within three months after surgery were excluded from further analysis. Clinical information including patient prognoses was obtained from stored medical and imaging records. Patients were followed up with a clinical examination every 3 months for the first year, every 6 months for the second year, and every 6–12 months thereafter. Histology slides were reviewed in terms of differentiation, depth of invasion, vessel and nerve invasion, and lymph node metastasis. Tumor stages were defined based on the 8th edition of the American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) pTNM-staging system for ESCC.

Informed consent forms were obtained from all patients, and the study was conducted in accordance with the approved ethical standards of Zhongshan Hospital (B2016-135), which conforms to the provisions of the Helsinki Declaration (as revised in 2013).

Tissue microarray (TMA) construction

TMA construction was performed as previously described (18). Hematoxylin and eosin (HE) sections were evaluated and

confirmed by two pathologists (DJ and YH), who validated diagnosis and marked normal tissue and carcinoma tissue as target areas for TMA construction. The regions of interest (2 mm wide and 6 mm long) were obtained from the corresponding donor blocks. Two to three representative cores of viable tissue from each tumor were included. Additional control cores of normal esophageal tissue were incorporated. Then donor tissues were then manually planted into the recipient block one by one according to the corresponding locations indicated by letters and numbers. The planting surface was aggregated on the aggregation instrument. Subsequently, the recipient block with a transparent box was incubated at 4 ℃ for 10 min until the paraffin could be easily separated from the transparent box. TMA recipient blocks were collected and sectioned on a routine microtome machine for further IHC and FISH staining.

Fluorescence in-situ hybridization assay (FISH)

To assess the *SOX2* amplification status at the chromosomal level in TMA slides, we applied the same 2-color FISH assay as previously described by Wilbertz *et al.* (10). Briefly, a *SOX2* target probe (red fluorescent signal) spanning the locus 3q26.33 and a green centromeric probe on chromosome 3 (Empire Genomics, New York, USA) were selected for hybridization. Only the nuclei that displayed green reference signals were included for determination of the *SOX2* copy number status. All TMA slides were analyzed by two independent evaluators (J.H. and Q.S.) under an oil immersion objective using a fluorescence microscope (BX43; Olympus, Tokyo, Japan) equipped with a microscope digital camera (DP73; Olympus). In each case, we assessed at least 50 tumor cell nuclei.

Two red and two green signals in a cell were observed in a wild-type nucleus. A sample was considered amplified if *SOX2* amplification was observed in at least 30% of the nuclei. Amplification status was defined according to the criteria established by Maier *et al.* (19). *SOX2* amplification was defined as four red target signals exceeding the number of green signals. Three or more green reference signals were defined as *chromosome 3* gain.

Immunohistochemistry (IHC)

The IHC was performed on a Bond Max autostainer (Leica Microsystems, Wetzlar, Germany) according to the manufacturers' instructions using SOX2 rabbit monoclonal antibody (clone SP76, dilution 1:300, Genetech, China). Normal IgG from the same species of primary antibody diluted to match the concentration of the primary antibody was used as the negative control.

For SOX2, only nuclear staining was considered specific. A Histo-score (H-score; range, 0–300) was calculated by multiplying the intensity score (0= negative; 1= weak; 2= intermediate; 3= strong) and the fraction score (percentage of positive tumor cells; range, 0–100) (20). All stained slides were scored independently by two investigators (HW and YH), who were blinded for clinical outcome. In cases of disagreement between the two investigators, slides were simultaneously reviewed by both investigators until a consensus was reached.

Statistical analysis

The results of FISH and IHC were analyzed in subgroups and compared with clinical parameters, histologic subtypes, and grade using χ^2 or Fisher exact test, whichever was appropriate. Disease-free survival (DFS) was defined as the time between the date of surgery and the date of recurrence, death, or latest follow-up. Overall survival (OS) was defined as the interval between the date of surgery and the documented date of ESCC-associated death or latest follow-up. Survival curves were constructed according to the Kaplan-Meier method and compared using the log-rank test. Associations between clinicopathological factors and DFS/OS were estimated according to odds ratios (ORs) and 95% CIs. The factors with a P value <0.05 for either OS or DFS that met the proportional hazards criteria in the univariate analysis, were included in stepwise multivariate Cox regression.

For all tests, statistical significance was defined as P<0.05. Statistical analyses were performed using IBM SPSS (SPSS Inc., Chicago, IL), version 20.0.

Results

Patient characteristics

The clinical and pathological characteristics of the 450 ESCC patients are summarized in *Table 1*. The mean age was 61.4 years (range, 34-83 years). Among the patients, 364 (80.9%) were male and 171 (38.0%) were smokers (ever or now smoked). The mean tumor size was 3.4 cm (range, 0.3–10 cm). In terms of anatomic site, 4.7% of tumors were located in the upper esophagus, whereas 46.0% and

Table 1 Patient characteristics

Characteristic	Number	%
Sex		
Female	86	19.1
Male	364	80.9
Age		
<60	186	41.3
≥60	264	58.7
Smoking		
No	279	62
Yes	171	38
Tumor size		
<3.4	257	57.1
≥3.4	193	42.9
Tumor site		
Upper	21	4.7
Middle	207	46
Lower	202	44.9
Differentiation		
Well	18	4
Middle	267	59.3
Poor	165	36.7
Vessel invasion		
No	353	78.4
Yes	97	21.6
Nerve invasion		
No	304	67.6
Yes	146	32.4
pT		
T1	47	10.4
T2	100	22.2
T3	303	67.3
pN		
N0	255	56.7
N1	122	27.1
N2	73	16.2
pTNM stage		
IB	42	9.3
IIA	199	44.2
IIB	21	4.7
IIIA	24	5.3
IIIB	164	36.4
Adjuvant therapy		
No	361	80.2
Yes	89	19.8

44.9% of tumors were located in the middle and lower esophagus, respectively. Among these tumors, 18 (4.0%) were histologically graded as well differentiated (Grade I), 267 (59.3%) were moderately differentiated (Grade II), and 165 (36.7%) were poorly differentiated (Grade III). Vessel and nerve invasions were identified in 97 (21.6%) and 146 (32.4%) tumors, respectively. Lymph node metastasis was observed in 43.3% (195/450) of patients. Invasive depths were also evaluated, 13 (2.9%) cases were confined to the mucosal layer, 34 (7.6%) in submucosal layer, 100 (22.2%) in muscular layer, and 303 (67.3%) beyond muscular layer. Pathological stages of all the tumors were evaluated according to the 8th edition pTNM classification; stage IB was identified in 9.3% of tumors, stage IIA in 44.2%, stage IIB in 4.7%, stage IIIA in 5.3%, and stage IIIB in 36.4%.

Association between gene copy number variation and clinicopathological characteristics

Among 450 patients, 20 (4.4%) met the inclusion criteria (≥4 red target signals in no less than 30% tumor cells and less than three green signals), and were classified as cases with SOX2 amplification. Fifty-eight (12.9%) cases were defined as *chromosome 3* gain with three or more green signals, among which 28 (6.2%) cases also had ≥4 red *SOX2* signals in no less than 30% tumor cells. Low polysomy or disomy (82.7%) was observed in other specimens (*Figure 1*).

Table 2 reveals that *SOX2* amplification and *chromosome 3* gain were not significantly associated with sex (P=0.855), age (P=0.464), smoking (P=0.066), tumor size (P=0.410), tumor site (P=0.150), differentiation (P=0.620), vessel

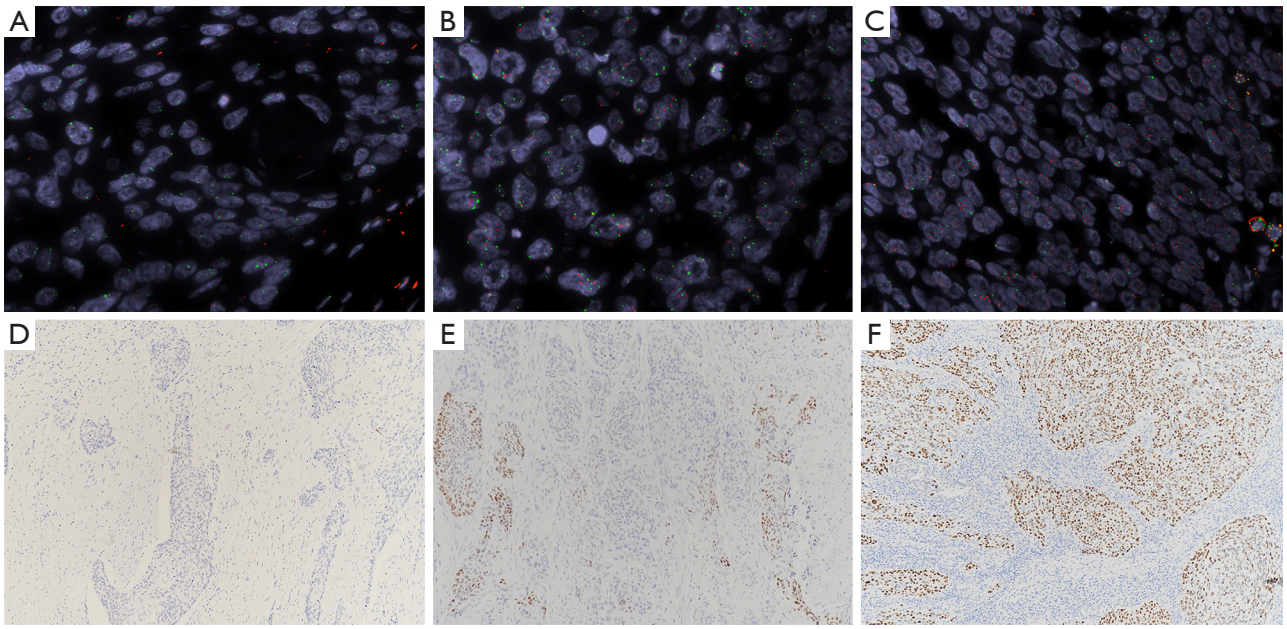

Figure 1 Gene copy number variation and SOX2 protein expression status in esophageal squamous cell carcinoma tissue assessed by fluorescence *in-situ* hybridization (1,000×) and immunohistochemistry (100×). (A) SOX2 disomy or low polysomy; (B) *chromosome 3* gain with three or more green signals; (C) SOX2 amplification; (D) negative SOX2 expression; (E) low SOX2 expression; (F) high SOX2 expression.

(P=0.640) and nerve invasions (P=0.426), and pT (P=0.207). *SOX2* amplification was associated with later clinical stage, but *chromosome 3* gain was associated with earlier clinical stage (P=0.025). Similarly, *SOX2* amplification was associated with lymph node metastasis, however *chromosome 3* gain tended to be associated with no lymph node metastasis (P=0.038).

SOX2 expression and the clinicopathological characteristics

SOX2 protein expression was highly heterogeneous among the patients, with 53.6% tumor samples having 1–80% positive stained cells, and no staining was detected in 46.4% of the samples. The H-scores of the SOX2 positive tumors ranged from 1 to 160, with a median of 30. Therefore, an H-score higher than 30 was considered indicative of high SOX2 expression (n=111), an H-score of 30 or lower was considered indicative of low expression (n=130) and an H-score of 0 was considered indicative of negative expression (n=209) (*Figure 1*).

The clinicopathological characteristics of patients grouped according to SOX2 expression are listed in *Table 2*. SOX2 expression was significantly associated with copy number variation (P=0.007). We found no correlation of SOX2 expression with sex (P=0.772), age (P=0.333), smoking (P=0.261), tumor site (P=0.881), differentiation (P=0.315), vessel or nerve invasion (P=0.085 or 0.868), pT stage (P=0.590), pN stage (P=0.070), and pTNM stage (P=0.089).

Survival analyses

The 5-year DFS and disease-specific OS rates of patients were 46.7% and 47.2%, respectively, with a median follow-up period of 36 months (range, 4–102 months). There were 232 disease progression documented. The mean and median DFS were 58.0 and 40.0 months, respectively. A total of 231 patients (51.3%) died during the follow-up period, and 224 (49.8%) patients died of EC. The mean and median disease-specific OS were 61.4 and 47.0 months, respectively.

A significantly shorter DFS or OS in the *SOX2* amplification group, whereas a significantly longer DFS or OS in the *chromosome 3* gain group, than those in the group with low polysomy or disomy were observed (*Figure 2*). In detail, a significantly poorer prognosis was observed in 20 patients with *SOX2* amplification, whose median DFS or OS were 18.0 or 23.0 months, respectively, whereas a significantly better prognosis was observed in 59 patients with *chromosome 3* gain, whose median DFS or OS were no-reached, compared with 36.0 or 43.0 months in the group with low polysomy or disomy (P<0.001). The difference in

Table 2 Associations between SOX2 status and patient characteristics

Characteristic	Number	Gene copy number variation				SOX2 expression			
		Negative	Chromosome 3 gain	SOX2 Amp	P value	Negative	Low	High	P value
Sex					0.855				0.772
Female	86	71	12	3		40	27	19	
Male	364	301	46	17		169	103	92	
Age					0.464				0.333
<60	186	158	22	6		80	54	52	
≥60	264	214	36	14		129	76	59	
Smoking					0.066				0.261
No	279	223	44	12		138	76	65	
Yes	171	149	14	8		71	54	46	
Tumor Size					0.410				0.053
<3.4	257	212	36	9		107	83	67	
≥3.4	193	160	22	11		102	47	44	
Tumor site					0.150				0.881
Upper-middle	228	182	35	11		107	67	54	
Lower	202	175	19	8		93	57	52	
Differentiation					0.620				0.315
Well-middle	285	239	35	11		126	89	70	
Poor	165	133	23	9		83	41	41	
Vessel invasion					0.640				0.085
No	353	293	46	14		171	103	79	
Yes	97	79	12	6		38	27	32	
Nerve invasion					0.426				0.868
No	304	252	41	11		141	86	77	
Yes	146	120	17	9		68	44	34	
pT					0.207				0.590
T1–T2	147	126	18	3		72	43	32	
T3	303	246	40	17		137	87	79	
pN					0.038				0.070
N0	255	213	36	6		130	70	55	
N1–N3	195	159	22	14		79	60	56	
pTNM Stage					0.025				0.089
I–II	262	219	37	6		133	71	58	
III	188	153	21	14		76	59	53	
SOX2 expression					0.007				-
Negative	209	185	17	7		-	-	-	
Low	130	107	17	6		-	-	-	
High	111	80	24	7		-	-	-	

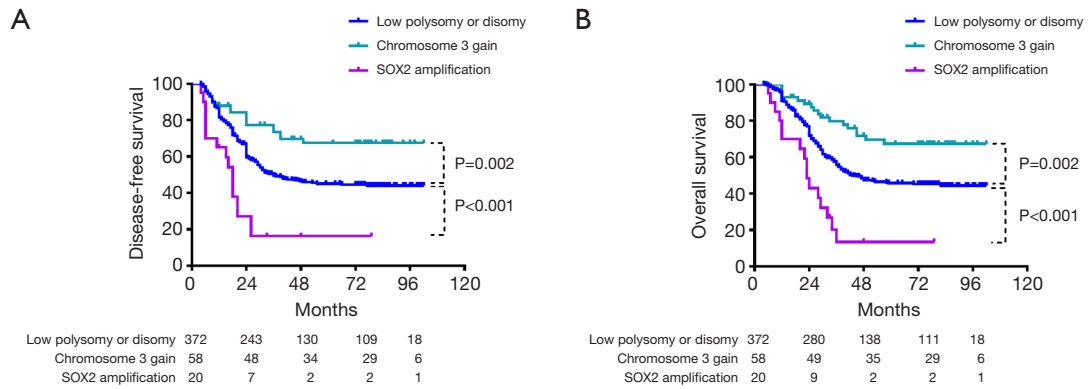

Figure 2 Kaplan-Meier survival curves for disease-free survival (DFS, A) and overall survival (OS, B). A significantly shorter DFS or OS was observed in the SOX2 amplification group, while a significantly longer DFS or OS was observed in the chromosome 3 gain group, compared with the group with low polysomy or disomy.

terms of patient 5-year survival between *SOX2* amplification and normal groups was deceased 28.8% for DFS and 32.3% for OS, while the difference between *chromosome 3* gain and normal groups was increased 22.4% for DFS and 21.6% for OS. SOX2 expression was not associated with DFS or OS (P=0.159 and 0.230). In addition, *SOX2* amplification or *chromosome 3* gain, vessel invasion, nerve invasion, and pTNM stage were associated with DFS and OS in univariate analysis. With variables that were found to be significant in the univariate analyses (P<0.05), a multivariate analysis using the cox proportional hazard model was performed. Multivariate analysis indicated that *chromosome 3* gain was an independently better prognostic factor (DFS, P=0.003, HR 0.486, 95% CI, 0.300–0.789; OS, P=0.003, HR 0.474, 95% CI, 0.289–0.779), while *SOX2* amplification was an independently poorer prognostic factor (DFS, P<0.001, HR 2.638, 95% CI, 1.581–4.403; OS, P<0.001, HR 2.608, 95% CI, 1.562–4.355), along with pTNM stage (*Table 3*).

Discussion

Identifying the copy number variation of target genes represents an important step toward understanding and potentially preventing the complex changes a cell undergoes during ESCC tumor development, which also facilitates the further outcome stratification and treatment decisions of these patients (5-7). The aim of our study was to elucidate whether *SOX2* amplification is a common event in ESCC and to determine whether SOX2 amplification is correlated with prognosis. FISH analysis was performed to detect *SOX2* copy number gains, followed by IHC SOX2 protein expression analysis. At present, there are no data available in literature on both *SOX2* amplification and SOX2 expression in large scale of ESCC patients.

In our study, *SOX2* was amplified in 4.4% ESCC, less than 10–18% in other ESCC reports (13,14,19). These variations in frequency are likely due to (I) the molecular method applied for detection of *SOX2* copy number status, (II) the threshold set to distinguish gene amplification, (III) the differences between ESCC cohorts, and (IV) tumor heterogeneity (21). A key finding of this study was the observation that green signals (*Chromosome 3* gain) were found in 12.9% ESCC, among which 6.2% tumors with *SOX2* copies were ≥4 in more than 30% cells. If 6.2% patients were also regarded as *SOX2* amplification, the percentage of 10.6% (6.2%+4.4%) was consistent with other reports in ESCC. However, it is possible that there are multiple targets for *chromosome 3* gain, and co-amplification of adjacent oncogenes can have a synergistic effect. Other genes reported to be putative targets in *chromosome 3* include *PIK3CA* (22,23), *SKIL* (24), *TERC* (25), *DCUN1D1* (26), *TP63* (27), and *EVI1* (28). For example, *PIK3CA*, which is located at the 2.6 Mb centromeric side of *SOX2* and *DCUN1D1*, which is located at the 1.2 Mb telomeric side of *SOX2*, are reported to be molecular biomarkers in many tumors (14,29). Therefore, the division of *SOX2* amplification and *chromosome 3* gain should be necessary. In clinicopathological analysis, *SOX2* amplification was higher in later pTNM stage, however, *chromosome 3* gain was higher in earlier pTNM stage (P=0.039).

In our univariate and multivariate analyses, *SOX2* amplification was an independently poorer prognostic factor (DFS, P<0.001, HR 2.638, 95% CI, 1.581–4.403;

Table 3 Univariate and multivariate analysis for disease-free survival (DFS) and overall survival (OS)

	DFS		OS	
	P value	HR (95% CI)	P value	HR (95% CI)
Univariant factor analysis				
Sex	0.601	1.090 (0.789–1.506)	0.419	1.148 (0.821–1.607)
Age	0.918	1.014 (0.781–1.316)	0.917	0.986 (0.757–1.285)
Smoking	0.431	1.112 (0.854–1.448)	0.206	1.188 (0.909–1.552)
Tumor size	0.433	1.110 (0.856–1.439)	0.300	1.150 (0.883–1.497)
Tumor site	0.918	0.986 (0.758–1.283)	0.734	1.048 (0.801–1.370)
Differentiation	0.064	1.281 (0.986–1.663)	0.102	1.249 (0.957–1.630)
Vessel invasion	0.002	1.580 (1.186–2.104)	0.003	1.559 (1.163–2.090)
Nerve invasion	0.030	1.344 (1.029–1.755)	0.009	1.437 (1.096–1.884)
pTNM stage	<0.001	2.603 (2.005–3.381)	<0.001	2.634 (2.019–3.436)
Adjuvant therapy	0.113	1.282 (0.943–1.742)	0.149	1.258 (0.921–1.720)
Gene copy number variation	<0.001		<0.001	
chromosome 3 gain	0.003	0.486 (0.300–0.789)	0.003	0.474 (0.289–0.779)
SOX2 amplification	<0.001	2.638 (1.581–4.403)	<0.001	2.608 (1.562–4.355)
SOX2 expression	0.171		0.239	
Low	0.060	0.741 (0.543–1.013)	0.091	0.760 (0.553–1.045)
High	0.503	0.897 (0.653–1.232)	0.592	0.915 (0.663–1.265)
Multivariant factor analysis				
Vessel invasion	0.441	1.127 (0.832–1.526)	0.538	1.102 (0.809–1.502)
Nerve invasion	0.355	1.138 (0.865–1.496)	0.129	1.240 (0.940–1.636)
pTNM stage	<0.001	2.410 (1.830–3.173)	<0.001	2.416 (1.826–3.198)
Gene copy number variation	<0.001		<0.001	
chromosome 3 gain	0.003	0.485 (0.299–0.785)	0.003	0.473 (0.288–0.777)
SOX2 amplification	0.010	1.973 (1.174–3.315)	0.018	1.874 (1.116–3.148)

OS, P<0.001, HR 2.608, 95% CI, 1.562–4.355), and *chromosome 3* gain was an independently better prognostic factor (DFS, P=0.003, HR 0.486, 95% CI, 0.300–0.789; OS, P=0.003, HR 0.474, 95% CI, 0.289–0.779). This is the first study simultaneously explored the prognostic significance of *SOX2* amplification and *chromosome 3* gain in a relatively large-scale ESCC cohort study using the FISH method. SOX2 amplification was detected more frequently in SCC than in adenocarcinomas (19). Therefore, *SOX2* amplification was believed to be an important mechanism of tumor progression in a considerable subset of SCC (30). In LSCC, *SOX2* amplification is associated with indicators of favorable prognosis (10). In HNSCC, *SOX2* copy number gain is associated with improved patients' prognosis (9). In SSCC, patients with *SOX2* amplification experienced a significantly higher incidence of recurrence (12). In vulvar SCC, *SOX2* amplification was not associated with OS (31). None of the above studies analyzed the prognostic difference between *chromosome 3* gain and *SOX2* amplification. Unlike other SCCs, gene focus within 3q26-3q33 containing candidate oncogenes, including *TP63* (6.5–20%) (13,32) and *PIK3CA* (7–40.7%) (13,33), were amplified to higher levels in ESCC. We speculated why *chromosome 3* gain is associated with a better prognosis might due to *chromosome 3* gain

includes multiple oncogenes. The prognostic significance of these gene amplifications is not well known in ESCC, and needs to be explored in the future.

SOX2 expression was also evaluated in our ESCC patients with a negative rate of 46.4%, low expression rate of 28.9% and high expression rate of 24.7%. Consistent with our results, reports from the literature demonstrated that SOX2 expression rate was 22.8–93% (11,16,17). SOX2 expression occurred more frequently than *SOX2* amplification. SOX2 expression was significantly associated with *SOX2* amplification or *chromosome 3* gain (P=0.007). Among patients with *SOX2* amplification or *chromosome 3* gain, high SOX2 expression was higher than low SOX2 expression and negative. However, SOX2 expression was not associated with DFS and OS (P=0.159 and 0.230), which was inconsistent with *SOX2* amplification or chromosome 3 gain. This is because *SOX2* amplification is only one of the mechanisms capable of up-regulating gene expression. Namely, SOX2 expression can be up-regulated not only by amplification, but also at the transcription and translation levels. To date, SOX2 expression has been documented in other studies about ESCC. In Wang' s study, high SOX2 expression was significantly associated with higher histological grade and poorer clinical survival (16), however, Maehara *et al.* demonstrated that the negative expression of SOX2 appeared to be an independently poor prognostic factor (OR 7.05, 95% CI, 1.27–39.0) (17). In terms of the above results, more detailed analyses are needed to elucidate the prognostic significance of SOX2 expression.

To our knowledge, there is limited study correlating *SOX2* amplification and *chromosome 3* gain with its clinical significance in patients with ESCC. In the present study, *SOX2* amplification is an independently poorer prognostic factor, but *chromosome 3* gain is an independently favorable prognostic factor. SOX2 expression was significantly associated with copy number variation (P=0.007), but not associated with DFS and OS. In summary, these results suggested that *SOX2* amplification and *chromosome 3* gain might play important roles in tumor development and could serve as an independent predictor of poorer and better prognosis for ESCC.

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Footnote

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