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Original Article

Prognostic significance of SOX2 in head and neck cancer: a meta-analysis

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Abstract: Sex determining region Y-box 2 (SOX2) has been identified as a putative cancer stem cells (CSCs) marker in Head and Neck Cancers (HNC). However, the clinicopathological and prognostic significance of SOX2 in HNC patients remains controversial. We reviewed the literature by performing a meta-analysis based on the data from 7 studies (9 cohorts) to evaluate the association between SOX2 and clinicopathological/prognostic parameters in patients with HNC. Pooled hazard ratio (HR) or odds ratio (OR) with its 95% confidence interval (CI) was used as the effect size estimate. Our analysis results suggested that high SOX2 expression predicted unfavorable OS (HR: 1.54, 95% CI: 1.09-2.18) and DFS (HR: 1.54, 95% CI: 1.13-2.10) of patients with HNC. In addition, increased SOX2 was also significantly associated with high tumor grade (OR: 1.86, 95% CI: 1.06-3.28), advanced TNM stage (OR: 4.22, 95% CI: 2.62-6.80), lymph node metastasis (OR: 2.25, 95% CI: 1.50-3.35) and distant metastasis (OR: 1.99, 95% CI: 1.26-3.15). Our study suggested that SOX2 expression can be served as a candidate unfavorable prognostic biomarker for HNC patients, indicating that it might be a potential therapeutic target.

Keywords: SOX2, head and neck cancers, prognosis, meta-analysis

Introduction

Head and neck cancer is one of the most prevalent type of malignancy worldwide, with roughly half million new cases each year, and its incidence is still increasing in several geographic areas and its trend is now affecting younger individuals [1]. The most common histological type of head and neck cancer, including oral cavity, nasopharynx, hypopharynx, larynx and nasal cavity, are squamous cell carcinoma (SCC) [2]. The mortality due to HNC is mainly caused by local recurrence and local metastasis to cervical lymph node, and occasionally by distant organ metastasis [3]. Despite advancements in the field of oncology and great methods of detecting the disease at earlier stages in the last 30 years, we still observe high morbidity and resistance to conventional therapy [4]. It is becoming increasingly evident that an improvement in the survival of HNC requires improved understanding of the high risk of HNC patients who are prone to tumor metastasis and poor prognosis.

Recent studies on the pathobiology of HNC have led to the discovery of a small population of cancer cells that is highly tumorigenic, capable of self-renewal, and behave as tumor progenitor cells. Such behavior is consistent with the features of cancer stem cells (CSCs) [5, 6]. It is believed that existence of CSCs may be the reason for the lack of treatment effectiveness and high relapse and metastasis rate of HNC patients [4]. Targeted elimination of these CSCs has been considered a new conceptual framework for HNC treatment [7, 8].

SOX2, a member of the sex determining region Y-box family, is a key transcription factor involved in maintaining the pluripotency of CSCs in self-renewal and differentiation, and plays a critical role in determining the fate of stem cells [9, 10]. Recent studies indicated that SOX2 was aberrantly expressed in several human tumors including lung cancer, esophageal carcinoma, pancreatic carcinoma, breast cancer, ovarian carcinoma, hepatocellular carcinoma and head and neck cancers [11-15].

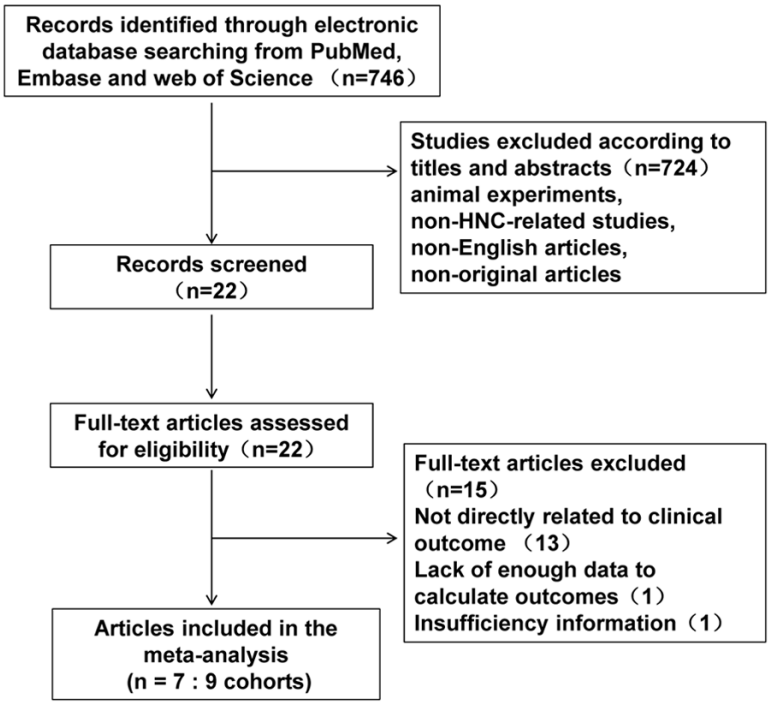


Figure 1. Flow diagram of studies selection procedure.

However, SOX2 expression pattern and the correlation with clinicopathological features and clinical outcome were highly variable among cancers. Some studies revealed that expression of SOX2 conferred a better prognosis [16-18], but others found an association with worse clinical outcome as well as adverse clinical parameters, including recurrence, lymph node and distant metastasis [2, 19-21]. Based on these controversies, a meta-analysis was conducted in order to gain deeper insight into the clinicopathological and prognostic significance of SOX2 in HNC.

Materials and methods

Search strategy

PubMed, Embase and Web of Science were used to search for the original articles analyzing the prognostic value of SOX2 in human cancer, by means of keywords variably combined: ("SOX2" OR "SOX-2" OR "Sex determining region Y-box 2" OR "SRY-Related HMG-Box Gene 2") AND ("cancer" OR "carcinoma" OR "neoplasm" OR "tumor" OR "malignancy") AND ("prognosis" OR "prognostic" OR "outcome" OR "survival"). Last search was updated on 1 July 2014, and no lower date limit was used. Reports in English were eligible for inclusion. The reference list was also checked for relevant articles. Investigators were contacted and asked to supply additional data when essential data were unavailable from original literatures.

Eligibility criteria

All candidate articles were reviewed by two independent reviewers (ZYD and JYS), and discrepancies were resolved by discussion. Inclusion criteria were as follows: (i) The diagnosis of Head and Neck Cancers was made based on pathological examination; (ii) disease-free survival (DFS), overall survival (OS) and other clinicopathological indicators were the main outcomes of interest; (iii) SOX2 expression status was detected by immunohistochemistry (IHC); (iv) the values of hazard ratios (HRs) and 95% CI between SOX2 expression and survival status could be obtained from the literature directly or recalculated based on the survival curve in the articles; and (v) for duplicate articles, only the most complete and/or recently published one was included. Exclusion criteria were as follows: (i) abstracts, letters, editorials, expert opinions, reviews and case reports; (ii) studies with insufficient data for estimating HR and 95% CI; (iii) literature which failed to present the cut-off value defining "elevated SOX2"; (iv) literature written in language other than English; (v) non-human research.

Data extraction

Data extraction was performed independently by two authors (GCL and BQH) from eligible studies. Controversial problems were resolved by discussion and consensus. Two investigators reviewed all of researches that met inclusion and exclusion criteria. In order to ensure the quality of the meta-analysis, we followed the guidelines provided by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [22]. Information retrieved from the researches included

Table 1. Characteristics of included studies

First author	Year	Country	Malignant disease	Histological type	Stage (I II/III IV)	Sample size (male)	Follow-up median (months)	Outcome indexes	Design of Data Collection	Detection method	Staining pattern	Cut-off value
Luo et al.	2013	China	Nasopharyngeal carcinoma	SCC	33/89	122 (92)	60.1 (8-92)	OS	Retrospective	IHC	nucleus	Score ≥ 6 (0-9)
Wang et al.	2012	China	Nasopharyngeal carcinoma	SCC	40/68	108 (76)	86.4	OS	Retrospective	IHC	nucleus	Score ≥ 9 (0-12)
Ge et al.	2010	China	Hypopharyngeal carcinoma	SCC	7/78	85 (84)	52 (7-69.5)	OS/DFS	Retrospective	IHC	nucleus	Score ≥ 4 (0-7)
Dai et al.	2014	China	Salivary gland adenoid cystic carcinoma	ACC	NA	131 (75)	38.6 (3-62)	OS/DFS	Retrospective	IHC	nucleus	Score ≥ 6 (0-9)
Du et al.	2013	China	Oral tongue carcinoma	SCC	NA	82 (55)	67 (4-81)	OS/DFS	Retrospective	IHC	nucleus	Score ≥ 2 (0-7)
Tang et al.	2011	China	Laryngeal carcinoma	SCC	65/96	161 (152)	46.3 (8-60)	OS	Retrospective	IHC	nucleus	Score ≥ 6 (0-7)
Márquez et al.	2013	Spain	Hypopharyngeal carcinoma	SCC	7/95	102 (99)	14 (0-95)	OS/DFS	Retrospective	IHC	nucleus	> 5%
	2013	Spain	Laryngeal carcinoma	SCC	13/54	67 (67)	37 (1-97)	OS/DFS	Retrospective	IHC	nucleus	> 5%
	2013	Spain	Sinonasal carcinoma	SCC	4/47	51 (37)	18 (1-211)	OS/DFS	Retrospective	IHC	nucleus	> 5%

Abbreviations: OS, overall survival; DFS, disease-free survival; IHC, Immunohistochemistry; SCC, squamous cell carcinoma; ACC, adenoid cystic carcinomas.

author, publication year, country of population, sample size, histological type, tumor stage, outcome indexes, detection method , SOX2 location, cut-off value, follow-up time, HR and their 95% CI.

Quality assessment

Study quality was assessed independently by two investigators (ZYD and DHW), by means of reading and evaluating according to Newcastle-Ottawa quality assessment scale (NOS) [23]. NOS scores of ≥ 6 were assigned as high-quality studies. Any disagreement was addressed by joint discussion.

Statistical analysis

Hazard ratio (HR) and 95% confidence intervals (95% CI) were obtained directly from each literature or from estimation according to the methods by Parmer [24] and Tierney [25]. For the analysis of the relationship between SOX2 and clinicopathological parameters, odds ratios (OR) and 95% CI were combined as the effective value. Statistical heterogeneity between cohorts was evaluated by x^2 test and inconsistency index (I^2) and was considered significant when x^2 *P*-value < 0.1 or I^2 > 50%. In the absence of statistically significant heterogeneity, the Mantel-Haenszel method in the fixed-effect model was used for the Meta analysis. Otherwise, the DerSimonian-Laird method in the random-effect model was selected. Publication bias was evaluated graphically by Begg's funnel plot analysis and then statistically using Egger's test with significant publication bias defined as $P < 0.05$. All analyses were performed with Review Manager Version 5 (RevMan, Cochrane Collaboration, Oxford, England) and Stata version 12.0 (StataCorp LP, College Station, TX).

Results

Selection and characteristics of studies

A total of 746 articles were identified initially using the search strategy above. Studies excluded with animal experiments, non-NHC-related studies, non-English articles, non-original articles, only 22 publications met the inclusion criteria for the present analysis (**Figure 1**). Of the 22 candidate studies, 13 studies were not directly related to specific outcomes, 1 did not provide enough data for estimating the HR and 95% CI and 1 failed to present complete information about the follow-up time and cut-off value. Thus 7 studies (9 cohorts) [2, 19, 20, 26-29] published between 2010 and 2014 were included in our meta-analysis investigating OS/DFS or pathological features. The total number of patients included was 909, with sample sizes ranging from 51 to 161 patients. The median follow-up period ranged from 18 to 86.4 months. As the studies by Márquez [2] included three cohorts focused on three types of HNC and reported their clinical outcome separately, we marked them as Márquez (HPC), Márquez (LC) and Márquez (SNC) respectively in the following analysis. The characteristics of the included studies were summarized in **Table 1**. Six studies were from China, one study (three cohorts) from Spain. HR and 95% CI were produced directly by the multivariate analysis in five of the enrolled cohorts (Table S1). For the remaining studies, HRs and 95% CIs were calculated from Kaplan-Meier curves. NOS score was above 6 in all cohorts (Table S2).

SOX2 expression and OS in HNC patients

There were 9 cohorts presenting the data of SOX2 and OS in HNC patients. The pooled estimates demonstrated a significant relationship between elevated SOX2 and shorter OS (HR = 1.59, 95% CI = 1.09-2.18, $P = 0.01$), with significant heterogeneity between studies (I^2 = 44%, $P = 0.07$) (**Figure 2A**).

To explore the heterogeneity, further subgroup analysis by different histological type suggested that both subgroups did not alter the prognostic role of SOX2 in OS (SCC: HR = 1.33, 95% CI = 1.03-1.74, $P = 0.03$, I^2 = 40%, $P = 0.11$ and ACC: HR = 2.64, 95% CI = 1.20-5.78, $P = 0.02$). When different cancer types were considered, SOX2 was only a negative prognostic marker in patients diagnosed with laryngeal carcinoma (LC) (HR = 1.79, 95% CI = 1.05-3.14, $P = 0.03$, $I^2 = 0$, $P = 0.67$). When grouped according to the regional distribution, Asian (China) cohorts with increased SOX2 expression suggested the significant results (HR = 1.64, 95% CI = 1.08-2.50, $P = 0.02$, I^2 = 47%, $P = 0.006$). We then focused on the median follow-up time in each cohort, those with a median follow-up time over 36 month studies suggested the significant relationship (HR = 1.54, 95% CI = 1.16-2.05, P = 0.003, I^2 = 37%, $P = 0.15$). In subtotal analyses of the sample size, the pooled outcome of those with a research object over 100 patients

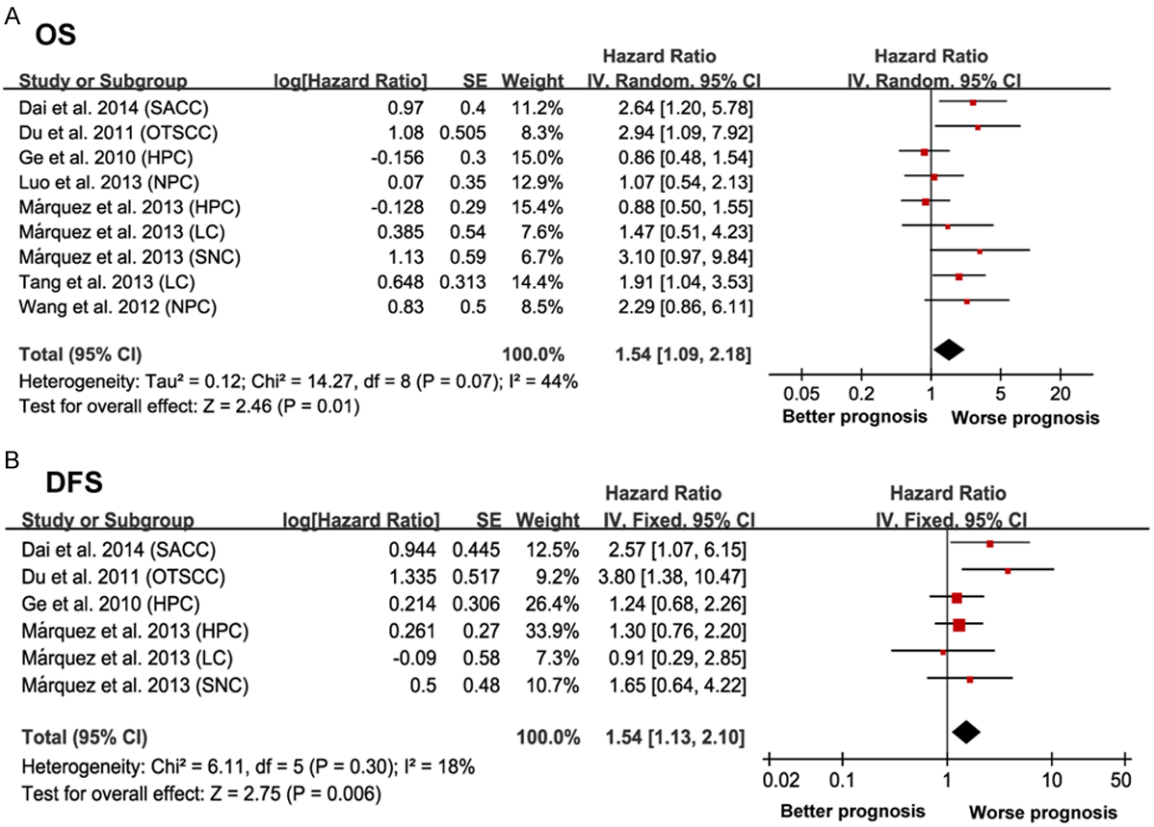


Figure 2. Forrest plots evaluating association between increased SOX2 expression and clinical outcomes in HNC. A. Forrest plot to assess the overall effect of SOX2 on OS in HNC patients. B. Forrest plot to assess the overall effect of SOX2 on DFS in HNC patients. Results are presented as individual and pooled hazard ratio (HR), and 95% confidence interval (CI).

subgroup showed increased SOX2 expression was significantly associated with an unfavorable OS (HR = 1.45, 95% CI = 1.07-1.97, P = 0.02, I^2 = 47%, P = 0.11) (**Table 2**).

SOX2 expression and DFS in HNC patients

A total of six cohorts focused on SOX2 expression and DFS in HNC patients. The pooled estimates demonstrated a significant relationship between elevated SOX2 and poor DFS (HR = 1.54, 95% CI =1.13-2.10, P = 0.006) without significant heterogeneity (I^2 = 18%, P = 0.30) (**Figure 2B**).

SOX2 expression and HNC clinicopathological features

To gain further insight into the value of SOX2 as a biomarker, we investigated the association of positive SOX2 expression with various clinicopathological indicators (Figure 3 and Table S3). A fixed-effect model revealed associations between SOX2 expression and advanced TNM stage (III-IV) (OR = 4.22, 95% CI = 2.62-6.80, P < 0.00001, **Figure 3B**), lymph node metastasis (OR = 2.25, 95% CI = 1.50-3.35, P < 0.0001, **Figure 3D**) and distant metastasis (OR = 2.09, 95% CI = 1.31-3.34, P = 0.002, **Figure 3F**), However, no significant association was observed between SOX2 expression and tumor local recurrence (OR = 0.62, 95% CI = 0.29-1.36, P = 0.24, **Figure 3E**). A random-effect model revealed an association between SOX2 expression and high tumor grade (T3-T4) (OR=1.86, 95% CI = 1.06-2.18, P = 0.03, **Figure 3A**) but there was no significant correlation between SOX2 expression and histological grade (poor) (OR = 0.97, 95% CI = 0.51-1.85, P = 0.92, **Figure 3C**). These findings indicate that SXO2 expression implies a poor prognosis in HNC patients with advanced TNM stage or high tumor grade, as well as serving as an indicator of lymph node and distal organ metastasis.

Sensitivity analysis

Sensitivity analysis was performed through the sequential omission of individual studies. The

Table 2. Subgroup analysis of the studies reporting the prognostic value of SOX2 expression

Outcome	Subgroups	Cohorts	HR (95% CI)	P	Model	Heterogeneity	
						I^2	P
OS	All	9	1.54 (1.09-2.18)	0.01	Random	44%	0.07
	Cancer type						
	LC	2	1.79 (1.05-3.14)	0.03	Fixed	0	0.67
	HPC	2	0.87 (0.58-1.31)	0.5	Fixed	0	0.95
	NPC	2	1.38 (0.79-2.24)	0.26	Fixed	36%	0.21
	Histological type						
	SCC	8	1.33 (1.03-1.74)	0.03	Fixed	40%	0.11
	ACC	1	2.64 (1.20-5.78)	0.02	Fixed	N/A	N/A
	Region						
	Asia	6	1.64 (1.08-2.50)	0.02	Random	47%	0.09
	Europe	3	1.18 (0.75-1.87)	0.48	Fixed	48%	0.15
	Median follow-up time						
	≥ 36 month	7	1.54 (1.16-2.05)	0.003	Fixed	37%	0.15
	< 36 month	2	1.49 (0.44-5.01)	0.52	Random	73%	0.06
	Sample size (n)						
	≥ 100	5	1.45 (1.07-1.97)	0.02	Fixed	47%	0.11
	< 100	4	1.67 (0.84-3.31)	0.14	Random	55%	0.08

Abbreviations: HR, hazard radio; CI, confidence internal; OS, overall survival; SCC, squamous cell carcinoma; ACC, adenoid cystic carcinomas; LC, laryngeal carcinoma; HPC, hypopharyngeal carcinoma; NPC, nasopharyngeal carcinoma.

corresponding pooled estimates of the relation of SOX2 expression to clinicopathological and prognostic outcomes were not altered significantly for any study factor after sequentially excluding each study, demonstrating that our data are stable and reliable.

Publication bias

A Begg's funnel plot was presented for the visual assessment of overt publication bias for the included cohorts in SOX2 (**Figure 4**). The funnel plot did not showed obvious asymmetry for OS (Pr > |Z| = 0.175, **Figure 4A**) and DFS (Pr > |Z| = 0.452, **Figure 4B**). The *P* value of Egger's test also indicated that there was not any publication bias in OS (*P* = 0.101) and DFS (*P* = 0.267) among these included studies. In addition, publication bias was also not observed among studies with regard to clinicopathological indicators (Table S3).

Discussion

CSCs are defined as a small subpopulation of cancer cells that constitute a pool of self-sustaining cells with the exclusive ability to cause the heterogeneous lineages of cancer cells that comprise the tumor [30]. Correlation between the presence of CSCs in HNC and prognosis has been corroborated by numerous studies since 2007, when Prince described CSCs in HNC [6, 31]. Recently, there are growing evidences suggest that stable expression of CSC markers in HNC could promote tumor cell growth, anti-apoptosis and metastasis, therefore play an important role in carcinogenesis and contributed to tumor aggressiveness and poor outcome [32].

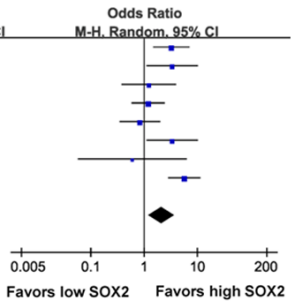
SOX2 has been proven to be a key regulator for maintaining the pluripotency and self-renewal of CSCs in HNC. The role of the SOX2 in the carcinogenesis is attributed to their properties involved in the regulation of cell differentiation, proliferation, and survival in multiple essential processes [33]. Although overexpressed SOX2 has been wildly demonstrated in HNC, the role of SOX2 as a prognostic marker is still a matter of debate. In order to detect the precise relationship between SOX2 expression and the prognostic significance of HNC, we extracted the eligible data into groups, including DFS, OS and clinicopathological indicators.

In this meta-analysis, we first assessed the association of high SOX2 expression with OS and DFS in HNC patients. Our analysis suggest-

A **Tumor grade (T3-T4)**

Study or Subgroup	SOX2 high expression Events	Total	SOX2 low expression Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Dai et al. 2014 (SACC)	42	82	12	49	15.1%	3.24 [1.48, 7.08]
Du et al. 2011 (OTSCC)	20	51	5	31	11.7%	3.35 [1.11, 10.18]
Ge et al. 2010 (HPC)	51	67	13	18	11.1%	1.23 [0.38, 3.97]
Luo et al. 2013 (NPC)	37	68	27	54	15.9%	1.19 [0.58, 2.44]
Márquez et al. 2013 (HPC)	27	39	46	63	14.0%	0.83 [0.35, 2.00]
Márquez et al. 2013 (LC)	20	51	5	31	11.7%	3.35 [1.11, 10.18]
Márquez et al.2013 (SNC)	6	7	40	44	4.5%	0.60 [0.06, 6.31]
Tang et al. 2013 (LC)	49	66	32	95	16.1%	5.67 [2.83, 11.39]
Total (95% CI)		431		385	100.0%	**2.10 [1.20, 3.68]**
Total events	252		180			

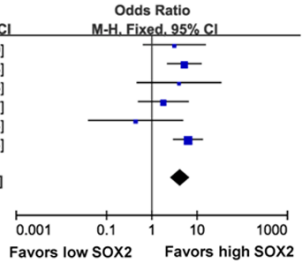
Heterogeneity: Tau² = 0.39; Chi² = 18.79, df = 7 (P = 0.009); I² = 63%
Test for overall effect: Z = 2.58 (P = 0.010)



B **TNM stage (III-IV)**

Study or Subgroup	SOX2 high expression Events	Total	SOX2 low expression Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Ge et al. 2010 (HPC)	63	67	15	18	7.9%	3.15 [0.64, 15.59]
Luo et al. 2013 (NPC)	59	68	30	54	24.7%	5.24 [2.17, 12.68]
Márquez et al. 2013 (HPC)	38	39	57	63	6.2%	4.00 [0.46, 34.56]
Márquez et al. 2013 (LC)	24	28	30	39	20.0%	1.80 [0.49, 6.57]
Márquez et al. 2013 (SNC)	6	7	41	44	9.0%	0.44 [0.04, 4.94]
Tang et al. 2013 (LC)	55	66	42	95	32.1%	6.31 [2.94, 13.54]
Total (95% CI)		275		313	100.0%	**4.22 [2.62, 6.80]**
Total events	245		215			

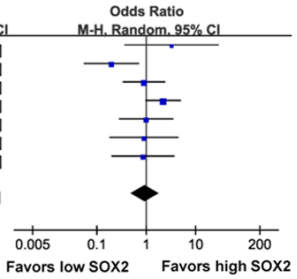
Heterogeneity: Chi² = 6.45, df = 5 (P = 0.26); I² = 23%
Test for overall effect: Z = 5.92 (P < 0.00001)



C **Histological grade (poor)**

Study or Subgroup	SOX2 high expression Events	Total	SOX2 low expression Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Du et al. 2011 (OTSCC)	5	51	1	31	6.9%	3.26 [0.36, 29.30]
Ge et al. 2010 (HPC)	6	67	6	18	14.4%	0.20 [0.05, 0.71]
Luo et al. 2013 (NPC)	57	68	46	54	18.9%	0.90 [0.33, 2.43]
Márquez et al. 2013 (HPC)	19	39	19	63	21.8%	2.20 [0.96, 5.03]
Márquez et al. 2013 (LC)	5	28	7	39	14.7%	0.99 [0.28, 3.53]
Márquez et al. 2013 (SNC)	3	7	20	44	10.9%	0.90 [0.18, 4.50]
Tang et al. 2013 (LC)	3	66	5	95	12.4%	0.86 [0.20, 3.72]
Total (95% CI)		326		344	100.0%	**0.97 [0.51, 1.85]**
Total events	98		104			

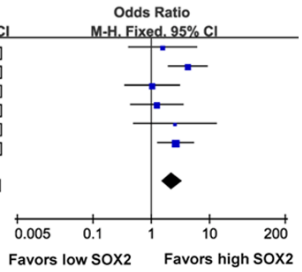
Heterogeneity: Tau² = 0.32; Chi² = 10.80, df = 6 (P = 0.09); I² = 44%
Test for overall effect: Z = 0.10 (P = 0.92)



D **Lymph node metastasis**

Study or Subgroup	SOX2 high expression Events	Total	SOX2 low expression Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Ge et al. 2010 (HPC)	16	67	3	18	11.0%	1.57 [0.40, 6.12]
Luo et al. 2013 (NPC)	39	68	13	54	18.9%	4.24 [1.93, 9.32]
Márquez et al. 2013 (HPC)	33	39	53	63	19.1%	1.04 [0.34, 3.12]
Márquez et al. 2013 (LC)	20	28	26	39	19.0%	1.25 [0.43, 3.59]
Márquez et al. 2013 (SNC)	3	7	10	44	4.8%	2.55 [0.49, 13.34]
Tang et al. 2013 (LC)	24	66	17	95	27.2%	2.62 [1.27, 5.42]
Total (95% CI)		275		313	100.0%	**2.25 [1.50, 3.35]**
Total events	135		122			

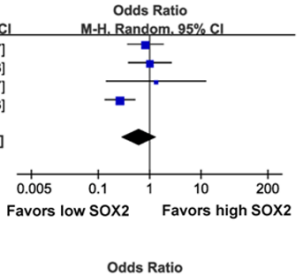
Heterogeneity: Chi² = 6.04, df = 5 (P = 0.30); I² = 17%
Test for overall effect: Z = 3.95 (P < 0.0001)



E **Local recurrence**

Study or Subgroup	SOX2 high expression Events	Total	SOX2 low expression Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Márquez et al. 2013 (HPC)	23	39	40	63	30.3%	0.83 [0.36, 1.87]
Márquez et al. 2013 (LC)	13	28	18	39	26.7%	1.01 [0.38, 2.68]
Márquez et al. 2013 (SNC)	6	7	36	44	9.5%	1.33 [0.14, 12.67]
Tang et al. 2013 (LC)	16	66	52	95	33.5%	0.26 [0.13, 0.53]
Total (95% CI)		140		241	100.0%	**0.62 [0.29, 1.36]**
Total events	58		146			

Heterogeneity: Tau² = 0.35; Chi² = 7.33, df = 3 (P = 0.06); I² = 59%
Test for overall effect: Z = 1.19 (P = 0.24)



F **Distant metastasis**

Study or Subgroup	SOX2 high expression Events	Total	SOX2 low expression Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Dai et al. 2014 (SACC)	35	82	8	49	23.1%	3.82 [1.59, 9.15]
Luo et al. 2013 (NPC)	12	68	3	54	11.1%	3.64 [0.97, 13.65]
Márquez et al. 2013 (HPC)	17	39	24	63	41.7%	1.26 [0.56, 2.83]
Márquez et al. 2013 (LC)	8	28	10	39	24.1%	1.16 [0.39, 3.45]
Total (95% CI)		217		205	100.0%	**2.09 [1.31, 3.34]**
Total events	72		45			

Heterogeneity: Chi² = 5.13, df = 3 (P = 0.16); I² = 42%
Test for overall effect: Z = 3.08 (P = 0.002)

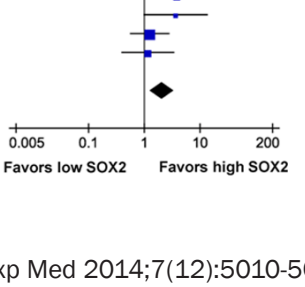


Figure 3. Forest plots showing results of studies on the association between elevated SOX2 and clinicopathological parameters in HNC patients. Forrest plots display the correlation between SOX2 expression and high tumor grade (T3-T4) (A), advanced TNM stage (III-IV) (B), histological grade (poor) (C), lymph node metastasis (D), local recurrence (E) and distant metastasis (F) in HNC. Results are presented as individual and pooled odds ratio (OR), and 95% confidence interval (CI).

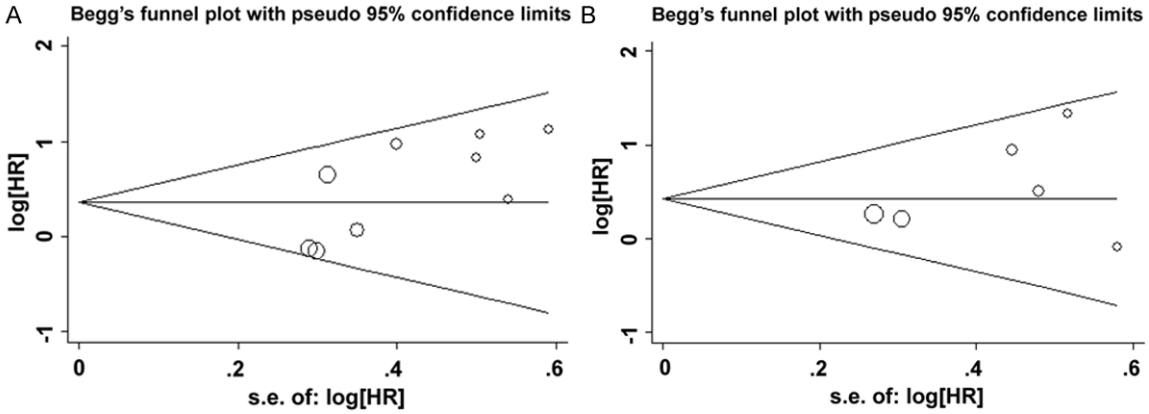


Figure 4. Begg's funnel plots for the evaluation of potential publication bias in the impact of SOX2 expression on the clinical outcome of HNC. A. Begg's funnel plots of publication bias test for the overall merged analysis of OS. Each point represents a separate study; B. Begg's funnel plots of the publication bias test for the overall merged analysis of DFS.

ed that elevated SOX2 was associated with poor OS and DFS in the indicated studies. Though with significant heterogeneity, most of the prognostic value was not undermined between SOX2 expression and OS by subgroup analysis based on different histological type, different tumor sites, region of the studied population, median follow-up time and sample size. Taken all these in to consideration, SOX2 was a promising prognostic marker helpful for the clinical decision-making process regarding HNC treatment and outcomes.

Regardless of progress in the HNC treatment recently, the survival rate of five years after diagnosing advanced HNC remains insufficient, approximately 50% [34]. One reason for high mortality associated with the advanced stage HNC is locoregional lymph node metastases due to the presence of a rich lymphatic network and the overall high number of lymph nodes in the neck region [35, 36]. More important, the increasingly mortality of HNC should be also ascribable to local recurrence and distant metastasis, which emerged as the predominant cause of death in the face of the achievement of excellent local control for HNC [37]. In the present study, we also carried out pooled analyses of the association between SOX2 expression and clinicopathological features. The results indicated that high expression of SOX2 was closely correlated with high tumor grade, advanced TNM stage, lymph node and distant metastasis.

There has been growing evidences suggest that CSCs potential for epithelium- mesenchymal transition (EMT) during metastasis formation. CSCs might strongly resemble cells that have undergone an EMT, attributing these cells a role in local invasion [38, 39]. As a key regulator of CSCs, SOX2 is proposed to play a role in the EMT in HNC, and confer invasive and metastases capacity on tumor cells. Luo [26] indicated overexpression of SOX2 in Nasopharyngeal Carcinoma (NPC) was significantly associated with high expression of N-cadherin, but adversely with low E-cadherin expression. Particularly, the distributions of SOX2 staining were more frequently located in the invasive front of tumors, and these cells often exhibited a fibroblast-like, spindle-shaped phenotype which was correlated strongly with EMT in tumor tissues. In the present analysis, high expression of SOX2 proteins in HNC was correlated significantly with a majority of tumor aggressive behaviors, such as local invasion, lymph node metastasis and distant metastasis. However, there was no significant association between SOX2 expression and tumor local

recurrence, which could be ascribe to lack of efficient data focused on this correlation in the candidate studies and further investigation should be conducted to verify their relationship.

Several sources of heterogeneity should be considered in the present study. Pooled HRs from different articles with various cut-off values may partly account for the inter-study heterogeneity. Meanwhile, all of the studies included in our meta-analyses were retrospective and their experimental design may, to some extent, contribute to the heterogeneity. Besides, the heterogeneity could also be attributed to the differences in the histological types, tumor types and their treatments, the sample sizes, the regional distribution, the durations of follow-up and the inconsistency of clinicopathological parameters. We had conducted subgroup analysis and sensitive analysis to evaluate potential sources of bias and the observed inter-study heterogeneity. Furthermore, a meta-regression was also performed to find out the heterogeneity. Unfortunately, there were no variables analyzed in the meta-regression contributed to the heterogeneity.

Results from our study must be interpreted within the limitations of included studies. Firstly, although we strived to extract valid data from survival curves, in which HRs were not directly measured, these indirect data were less reliable than direct data from the original articles because these calculated HRs were the result of univariate analyses and might contain some deviations. Secondly, SOX2 expression in the indicated studies was measured mainly using IHC and these results were strongly dependent upon methodological factors, such as primary antibody and secondary antibody concentration. Meanwhile, there was also a large difference in the definition of cut-off values among the studies, and this can be a source of potential bias. Thirdly, high quality researches with complete reports, including clinicopathological and survival data, were limited, which may compromise our conclusions.

In conclusion, SOX2 expression exhibited the significant association with survival outcome and clinicopathological parameters in HNC. It can be used to figure out the high risk patients who may benefit less from the antitumor therapies and to adjust the management strategy accordingly. Whereas, given the limitation of the current analysis, the large prospective clinical studies based on homogeneous series of patients were needed to further confirm the prognostic value of SOX2.

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Disclosure of conflict of interest

None.

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Table S1. Multivatiate analysis for disease-free and overall survival in our included studies

First author & publishing year	Multivariable analysis	Case number		OS		DFS	
		high expression n (%)	low expression n (%)	HR (95% CI)	P	HR (95% CI)	P
Luo *et al.* 2013 (NPC)	Yes	43 (35.2%)	79 (64.8%)	1.072 (0.556-2.064)	0.836	NA	NA
Ge *et al.* 2010 (HPC)	Yes	67 (78.8%)	18 (21.2%)	0.855 (0.477-1.532)	0.599	1.239 (0.681-2.254)	0.485
Dai *et al.* 2014 (SACC)	Yes	82 (62.6%)	49 (37.4%)	2.65 (1.21-5.20)	0.035	2.57 (1.44-5.07)	0.042
Du *et al.* 2011 (OTSCC)	Yes	51 (62.2%)	31 (37.8%)	2.94 (1.097-7.889)	0.032	3.8 (1.378-11.850)	0.011
Tang *et al.* 2013 (LC)	Yes	66 (41.0%)	95 (59.0%)	1.911 (1.037-3.522)	0.038	NA	NA
Wang *et al.* 2012 (NPC)	NA	29 (26.9%)	79 (73.1%)	NA	NA	NA	NA
Márquez *et al.* 2013 (HPC)	NA	39 (38.2%)	63 (61.8%)	NA	NA	NA	NA
Márquez *et al.* 2013 (LC)	NA	28 (41.8%)	39 (58.2%)	NA	NA	NA	NA
Márquez *et al.* 2013 (SNC)	NA	7 (13.7%)	44 (86.3%)	NA	NA	NA	NA

Table S2. Assessment of Newcastle-Ottawa Scale methodological quality of cohort studies

Study	Selection				Comparability	Outcome			Score
	Representativeness of the exposed cohort	Selection of non-exposed cohort	Ascertainment of exposure	Outcome not present at start		Assessment of outcome	Follow-up length	Follow-up adequacy	
Dai et al. (SACC)	★	★	★	★	★★	★	★	-	8
Du et al. (OTSCC)	★	★	★	★	★	★	★	-	7
Ge et al. (HPC)	★	★	★	★	★	★	★	-	7
Luo et al. (NPC)	★	★	★	★	★★	★	★	★	9
Márquez et al. (HPC)	★	★	★	★	★★	-	★	★	8
Márquez et al. (LC)	★	★	★	★	★★	-	★	★	8
Márquez et al. (SNC)	★	★	★	★	★★	-	★	★	8
Tang et al. (LC)	★	★	★	★	★★	★	★	-	8
Wang et al. (NPC)	★	★	★	★	★	-	★	-	6

Newcastle-Ottawa Quality Assessment Scale: study can have 1 star (★) for meeting each criterion, except that comparability (design or analysis) can have a maximum of 2 stars. For comparability in this study: 1 star if controlled for Age, gender, grade, etc.; 2 stars if also controlled for other important variables such as recurrence or metastasis.

Table S3. Main meta-analysis of association of SOX2 expression with clinicopathological indicators

Clinicopathological Indicators	Cohorts	OR (95% CI)	*P*	Model	Heterogeneity		Publication bias	
					I^2	P_{het}	Begg's *P*	Egger's *P*
Tumor grade (T3-T4)	8	2.10 (1.20-3.68)	0.01	Random	63%	0.009	0.174	0.177
TNM stage (III-IV)	6	4.22 (2.62-6.80)	< 0.00001	Fixed	23%	0.26	0.707	0.341
Histological grade (poor)	7	0.97 (0.51-1.85)	0.92	Random	44%	0.09	0.784	0.581
Lymph node metastasis	6	2.25 (1.50-3.35)	< 0.0001	Fixed	17%	0.30	0.707	0.108
Distant metastasis	4	2.09 (1.31-3.34)	0.002	Fixed	36%	0.21	0.734	0.348
Local recurrence	4	0.62 (0.29-1.36)	0.24	Fixed	42%	0.16	1.000	0.541