Original Article

Clinicopathological and prognostic significance of the long non-coding RNA HOTAIR high expression in head and neck squamous cell carcinoma: a systematic review and meta-analysis

Gang Yang[1#], Tianyu Huang[1#], Ye Wu[2,3], Jinian Wang[2,3], Zhipeng Pan[4], Yuting Chen[2,3], Faming Pan[2,3*], Yuanyin Wang[1*]

[1]Oral and Maxillofacial Surgery, Stomatologic Hospital & College, Anhui Medical University, Hefei, China; [2]Department of Epidemiology and Biostatistics, School of Public Health, Anhui Medical University, Hefei, China; [3]The Key Laboratory of Major Autoimmune Diseases, Anhui Medical University, Hefei, China; [4]Department of Medical Oncology, the First Affiliated Hospital of Anhui Medical University, Hefei, China

Contributions: (I) Conception and design: G Yang, T Huang; (II) Administrative support: F Pan, Y Wang; (III) Provision of study materials or patients: Y Wu, J Wang, G Yang, F Pan; (IV) Collection and assembly of data: Z Pan, Y Chen, T Huang, Y Wang; (V) Data analysis and interpretation: All authors; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work and should be considered as co-first authors.

[*]These authors contributed equally to this work and should be considered as co-corresponding authors.

Correspondence to: Professor Faming Pan. Department of Epidemiology and Biostatistics, School of Public Health, Anhui Medical University, 81 Meishan Road, Hefei 230032, China; The Key Laboratory of Major Autoimmune Diseases, Anhui Medical University, 81 Meishan Road, Hefei 230032, China. Email: famingpan@ahmu.edu.cn; Professor Yuanyin Wang. Oral and Maxillofacial Surgery, Stomatologic Hospital & College, Anhui Medical University, 81 Meishan Road, Hefei 230032, China. Email: wyy1970548@sohu.com.

Background: Long non-coding RNAs (lncRNAs) have been demonstrated to possess critical biological functions that regulate occurrence and progression of tumors. The HOX transcript antisense intergenic RNA (HOTAIR) is one of the most studied lncRNAs. This study was designed to investigate the association of HOTAIR expressions with clinicopathologic features and prognosis in head and neck squamous cell carcinoma (HNSCC).

Methods: The PubMed, Web of Science, Embase, Cochrane Library and SCOPUS databases were searched. The studies published before September 10, 2021 were screened. Two authors independently screened the literature and extracted data based on inclusion and exclusion criteria. Meta-analysis, bias assessment, sensitivity analysis and subgroup analysis were performed to improve accuracy and reliability.

Results: Seven studies comprising 546 patients were analysed to clarify the relationship between clinicopathologic features and HOTAIR expression, and six studies with 856 patients were applied to evaluate the effects of HOTAIR expressions on the prognosis. After removing those outliers through Galbraith plots and/or sensitivity analysis, the pooled results showed high HOTAIR expressions were associated with high T stage [odds ratio (OR) =2.32, 95% confidence interval (CI): 1.38–3.89, P=0.001], lymphnode metastasis (OR =2.71, 95% CI: 1.57–4.67, P=0.0003), high TNM stage (OR =3.92, 95% CI: 2.28–6.72, P<0.00001), poor histological grade (OR =2.21, 95% CI: 1.02–4.83, P=0.046), poor overall survival (OS) [hazard ratio (HR) =1.74, 95% CI: 1.19–2.56, P=0.005] and poor disease-free survival (DFS) (HR =1.64, 95% CI: 1.09–2.47, P=0.02). Subgroup analyses of T stage, lymphnode metastasis and histological grade identified possible heterogeneity sources, respectively ethnicity, cut-off, and detection methods.

Conclusions: These findings suggest HOTAIR might serve as an excellent prognostic biomarker and predictor of clinicopathologic features in HNSCC.

Keywords: Head and neck squamous cell carcinoma (HNSCC); HOX transcript antisense intergenic RNA (HOTAIR); prognosis; clinicopathologic features; meta-analysis

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Introduction

Head and neck tumors include carcinoma originating in any tissue or organ of the head and neck, with the exception of the brain, eyes, ears, thyroid, and esophagus (1). In addition, more than 90% of head and neck tumors are squamous cell carcinomas (2,3). Head and neck squamous cell carcinoma (HNSCC) is one of the most common malignant tumors in humans and includes a variety of tumors located in the mouth, nose, oropharynx, hypopharynx, and larynx (4,5). It is primarily caused by smoking, alcohol consumption, and human papillomavirus (HPV) infection (6,7). HNSCC has a high morbidity and mortality rate (8,9), and despite great advances in its prevention, diagnosis, and treatment, 5-year overall survival (OS) remains low due to lymphnode metastasis and local recurrence (10,11). In general, tumor clinical stage, lymphnode metastasis, and histological grades are directly related to HNSCC prognosis, but usually do not represent its true evolution. Therefore, to accurately predict HNSCC prognosis, develop therapeutic targets and conduct treatment evaluations, it is important to search for new valuable biomarkers (12).

Long non-coding RNAs (lncRNAs) are a group of RNAs over 200 nucleotides long, and are mostly transcribed by RNA polymerase II (13). LncRNA can play a role in cis and/or trans forms, most of which do not have any protein-coding capacity (14,15). LncRNA was previously defined as "junk" or "noise" (16,17). However, as time passed, lncRNAs have been found to have important biological functions in regulating transcription and gene expression, which can regulate disease occurrence and progression (18). Since they play an important role in all levels of gene expression, lncRNAs have attracted extensive attention in various physiological processes, such as differentiation and metabolism (19-21). Many scholars have studied the process of lncRNA involved in the occurrence and development of HNSCC, the results show that the up-regulation and down-regulation of lncRNA may be involved in the occurrence, development, invasion and metastasis of HNSCC (22,23). LncRNAs affect the clinicopathological features of tumors by participating in these processes and regulating epithelial-mesenchymal transition (EMT) (21,24). And the prognostic value of lncRNA expression in HNSCC remains to be studied.

In HNSCC, some lncRNAs, such as HOTAIR, have been posted to act a key function in tumor development by regulating cell proliferation (25), differentiation (26), and metastasis (27). HOX transcript antisense intergenic RNA (HOTAIR) interacts with the polycomb repressive complex 2 (PRC2) and then participates in the H3 lysine 27 (H3K27) methylation of many genes, which is associated with tumor physiological processes (28). HOTAIR is one of the most widely studied lncRNAs associated with human tumors. Compared with coding genes, lncRNA HOTAIR has clear tissue specificity (29), and exhibits unique characteristics in many types of cancer. HOTAIR is considered a significant biomarker of diagnoses and prognosis evaluations (30). Plus, they can be combined with clinicopathological characteristics to guide which therapeutic strategy to choose (31). Many studies have proven that HOTAIR is related to the occurrence, proliferation, invasion, and metastasis of different types of cancers, including breast cancer (32), lung cancer (33), gastric cancer (34), liver cancer (35), head and neck cancer (27), etc.

Although a number of studies are related to HOTAIR in HNSCC, no conclusive analysis with sufficient evidence has been formulated to summarize these existing studies (36-38). A previous meta-analysis showed that high HOTAIR expressions were associated with poor prognosis in HNSCC [pooled hazard ratio (HR) =1.90, 95% confidence interval (CI): 1.42–2.53, P<0.0001], but this prognostic analysis included only four studies with a total of 271 patients (39). In addition, only 2–3 of these studies evaluated the relationship between HOTAIR and tumor stages, histological grades, and lymph node metastasis. Their results showed that higher levels of HOTAIR expressions were associated with higher tumor stages and lymph node metastasis, but not with histologic grades. However, the number of studies is too small and the heterogeneity was not explained, so the reliability and accuracy of the results are insufficient.

Effect and regulation of HOTAIR on prognosis and clinical malignant phenotype in patients with HNSCC is still lacking convincing evidence-based medicine proof. Therefore, we reviewed the recent data on HOTAIR in relation to HNSCC. To explore the effects of lncRNAs

HOTAIR on clinicopathological features and prognosis of HNSCC, meta-analysis, sensitivity analysis, and subgroup analysis were used in our study to draw more consistent and accurate conclusions. In addition, we added the disease-free survival (DFS) index in the prognostic analysis, and the tumor clinical stage was divided into T stages and TNM stages to analyse them separately. The addition and refinement of these indicators may help us better understand the role of HOTAIR in the occurrence, progression, and prognosis of HNSCC. Therefore, we did not define this study as an update of previous studies, but a new analysis with more research volume and more detailed study methods. We present the following article in accordance with the PRISMA reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-652/rc) (40).

Methods

Search strategy

The research was implemented independently by two of the authors in the following online databases: PubMed, Web of Science, Embase, Cochrane Library and SCOPUS. The studies published before September 10, 2021 were screened. MESH terms and free text words were used to search for eligible studies on databases. The search strategy was: (HOTAIR OR "HOX transcript antisense RNA" OR "HOX transcript antisense intergenic RNA" OR "homeobox transcript antisense RNA") AND ("head and neck" OR oropharyngeal OR oropharynx OR oral OR mouth OR gingival OR lip OR palatal OR tongue OR nasopharyngeal OR pharynx OR pharyngeal OR laryngeal OR larynx) AND ("squamous cell carcinoma" OR cancer OR carcinoma OR tumor OR neoplasm).

Inclusion and exclusion criteria

Inclusion criteria were: (I) only English language articles about cohort studies, but there were no restrictions on publication date, region, gender, age or follow-up period; (II) analysis of HOTAIR high expression associations with at least one of the following clinicopathological and prognostic variables in patients diagnosed with HNSCC: OS, DFS, T stage, lymphnode metastasis, TNM stage, histological grade; (III) in the initial literature screen, we found that the data of three pieces about the high expression of HOTAIR and the prognosis of HNSCC were derived from The Cancer Genome Atlas (TCGA) database. After comparison, only the more complete or more recent articles were selected to avoid including duplicated data; (IV) studies including quantitative analysis of tissue sections from surgery through quantitative PCR (qPCR), in situ hybridization (ISH), and fluorescence in situ hybridization (FISH); (V) study requirements for inclusion in survival analysis: all patients did not receive preoperative chemotherapy or radiotherapy, only radical surgery.

Exclusion criteria were: (I) studies with insufficient data; (II) reviews, case reports, small sample studies and conference abstracts; (III) neoplasms were derived from the salivary glands, thyroid and skin; (IV) *in vitro* models or animal experiments.

Data extraction

Data extraction was performed independently by two authors. The following parameters were extracted from each included study: name of the first author, year of publication, patient source, total number and high/low expressions of HOTAIR, tumor sites, detection methods of HOTAIR expressions, cut-off values, tumor clinicopathologic features (T stage, lymphnode metastasis, TNM stage, and histological grades), HRs and 95% CIs for OS and DFS, follow-up periods. If the articles only provide survival curves without directly providing HRs and 95% CIs, we obtained the HRs, 95% CIs and associated statistics from the survival curves or Cox regression analyses using Engauge Digitizer 4.1 software and the methods illustrated by Tierney *et al.* (41). During the data extraction process, any differences were resolved through discussion or based on the opinions of the third author.

Quality evaluation

Quality evaluation of the included studies was conducted independently by two researchers using the Newcastle-Ottawa Scale (NOS). Cohort study quality assessment of NOS contains eight domains, including representativeness of the exposed cohort, selection of the non-exposed cohort, ascertainment of exposure, demonstrations that outcome of interest was not present at the start of study, comparability of cohorts on the basis of the design or analysis, assessment of outcome, follow-ups long enough for outcomes to occur, adequacy of follow-up of cohorts. In each domain, 2–4 prompting items were assessed to conclude an overall evaluation.

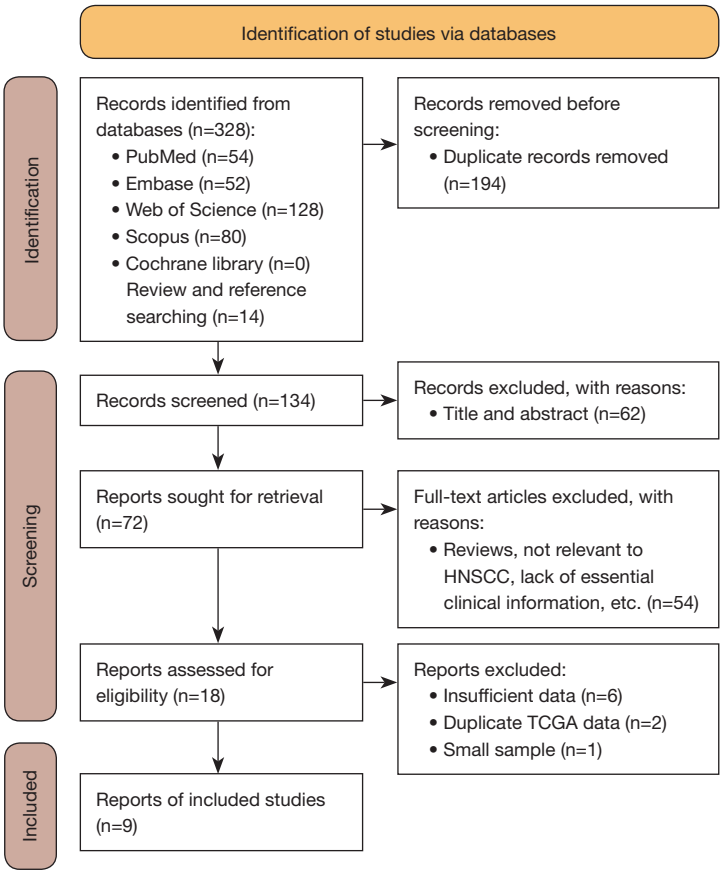

Figure 1 Flow diagram for study selection. HNSCC, head and neck squamous cell carcinoma; TCGA, The Cancer Genome Atlas.

Statistical analysis

Meta analysis was carried out using Review manager v5.3 software (Cochrane Collaboration, Oxford, United Kingdom) and Stata v11.0 software (Stata Corporation, College Station, Texas). Moreover, the relationship between HOTAIR expressions and clinicopathological characteristics was evaluated by referring to odds ratios (ORs) and 95% CIs. The features included tumor T-stage, lymph node metastasis, TNM stage, and histological grades. Meanwhile, HRs and 95% CIs were used to assess any association between HOTAIR expressions and HNSCC prognoses (OS and DFS). For studies that did not report accurate HRs and 95% CIs, Engauge Digitizer V4.1 software was used to extract results from Kaplan-Meier curves.

The presence of heterogeneity among studies was evaluated through Q-testing and P values. If there no evidence of significant heterogeneity (P>0.10 and/or I^2<50%) was observed, a fixed-effects model was adopted. Otherwise, a random-effects model was used to calculate pooled HRs or ORs.

Galbraith diagrams were constructed to identify heterogeneity. In addition, sensitivity analyses were performed to test the reliability of the meta-analysis results. To further identify sources of heterogeneity and confirm the stability of the results, subgroup analyses were performed based on three aspects: patient source (China or India), cut-offs (median, mean or other), and detection methods (PCR or ISH). Finally, funnel plots were constructed, and publication bias was measured by Begg's test. Results with P<0.05 were considered statistically significant.

Results

Study selection and features

As shown in *Figure 1*, the literature search yielded a total of 328 studies from the databases (PubMed, Embase, Scopus, Web of Science), and the preliminary list screening (reviews and references). A total of 134 studies remained when

Table 1 Characteristics of included studies

Study	Year	Source	Total	High/low	Sample	Tumor site	Detection method	Follow-up (months)	Cut-off	Outcome	HR	95% CI
Cheng-Zhi Xu (36)	2016	China	73	31/42	Tissue	Head and neck	PCR	250	NA	OS	1.43	0.66–3.10
Dandan Li (42)	2013	China	72	33/39	Tissue	Laryngeal	PCR	60	NA	OS	2.856	1.154–7.071
Detao Tao (43)	2020	China	44	22/22	Tissue	Oral	PCR	–	Median	–	–	–
Fenglian Yang (44)	2021	China	83	46/37	Tissue	Nasopharynx	PCR	–	Mean	–	–	–
Ganesan Arunkumar (37)	2017	India	60	12/43	Tissue	Oral	PCR	–	Mean	–	–	–
Jie Wu (45)	2015	China	50	25/25	Tissue	Oral	PCR	8–60 (median: 37)	Median	OS	1.47	0.43–4.98
Madeleine Sassenberg (46)	2019	TCGA	425	58/367	NA	Head and neck	NA	Median: 23	NA	OS	2.38	1.60–3.55
Yan Nie (38)	2013	China	160	91/69	Tissue	Nasopharynx	ISH	Median: 69	High (SI ≥6); low (SI <6)	DFS; OS	DFS: 1.7; OS: 1.56	DFS: 1.04–2.78; OS: 0.80–3.02
Yansheng Wu (26)	2015	China	76	38/38	Tissue	Oral	PCR	100	Median	DFS; OS	DFS: 1.52; OS: 1.91	DFS: 0.73–3.15; OS: 0.70–5.22

CI, confidence interval; DFS, disease-free survival; HR, hazard ratio; ISH, in situ hybridization; SI, staining index; NA, not applicable; OS, overall survival; TCGA, The Cancer Genome Atlas database.

duplicates were excluded. Then, 62 records were excluded after reading the titles and abstracts, and the remaining 72 were screened for full-text reading. After reading the full text, 18 studies were identified as eligible articles. According to the inclusion and exclusion criteria, 9 pieces of literature were eventually included in this study (26,36-38,42-46).

All of the included studies were published between 2012 and 2021 with sample sizes between 44 and 425 patients and a total of 1,043 patients. Seven studies with a total of 546 patients provided an association between the clinicopathological characteristics of HNSCC patients and HOTAIR expressions (26,36-38,43-45), and 6 studies with a total of 856 patients provided the correlation between HOTAIR expressions and HNSCC prognoses (26,36,38,42,45,46). Among the 9 studies, 7 studies were conducted in China, 1 in India (37), and 1 study involving survival outcomes was derived from TCGA database (46). *Table 1* and Table S1 shows the main characteristics of these included studies.

All the included literature was observational retrospective studies. The bias risks of the included studies were evaluated according to the NOS scale. Among the 9 included pieces of literature, 4 received 8 stars, 3 received 7 stars, 1 received 6 stars (the study involved only clinicopathological features and no follow-ups), and 1 received 5 stars (the data was derived from the TCGA database, and some of the information was not clear), all of which exceeded 5 stars. The specific scores of the included studies are shown in the Table S2. Most of the included studies had NOS scores of 7 to 8, indicating that these studies were of high quality.

Associations between lncRNA HOTAIR and clinicopathological characteristics

T stages

We compared the expressions of HOTAIR at different T stages in patients with HNSCC from 4 studies with 332 patients (36-38,43). Among the patients with T3–T4 stages, 79 had high HOTAIR expressions, and 70 cases exhibited low expressions for HOTAIR. A significant association was found between HOTAIR high expression and advanced T stages in HNSCC (OR =1.93, 95% CI: 1.19–3.13, P=0.008) (*Figure 2A*). Heterogeneity was moderate (I^2=46%, P=0.13). As P=0.13 for the Q test, it was suggested that the heterogeneity among the selected studies was not statistically significant, and fixed effects could be selected for meta-analysis.

According to the Begg's bias test based on the funnel plot (Figure S1A), P=1.000 was obtained, indicating that there

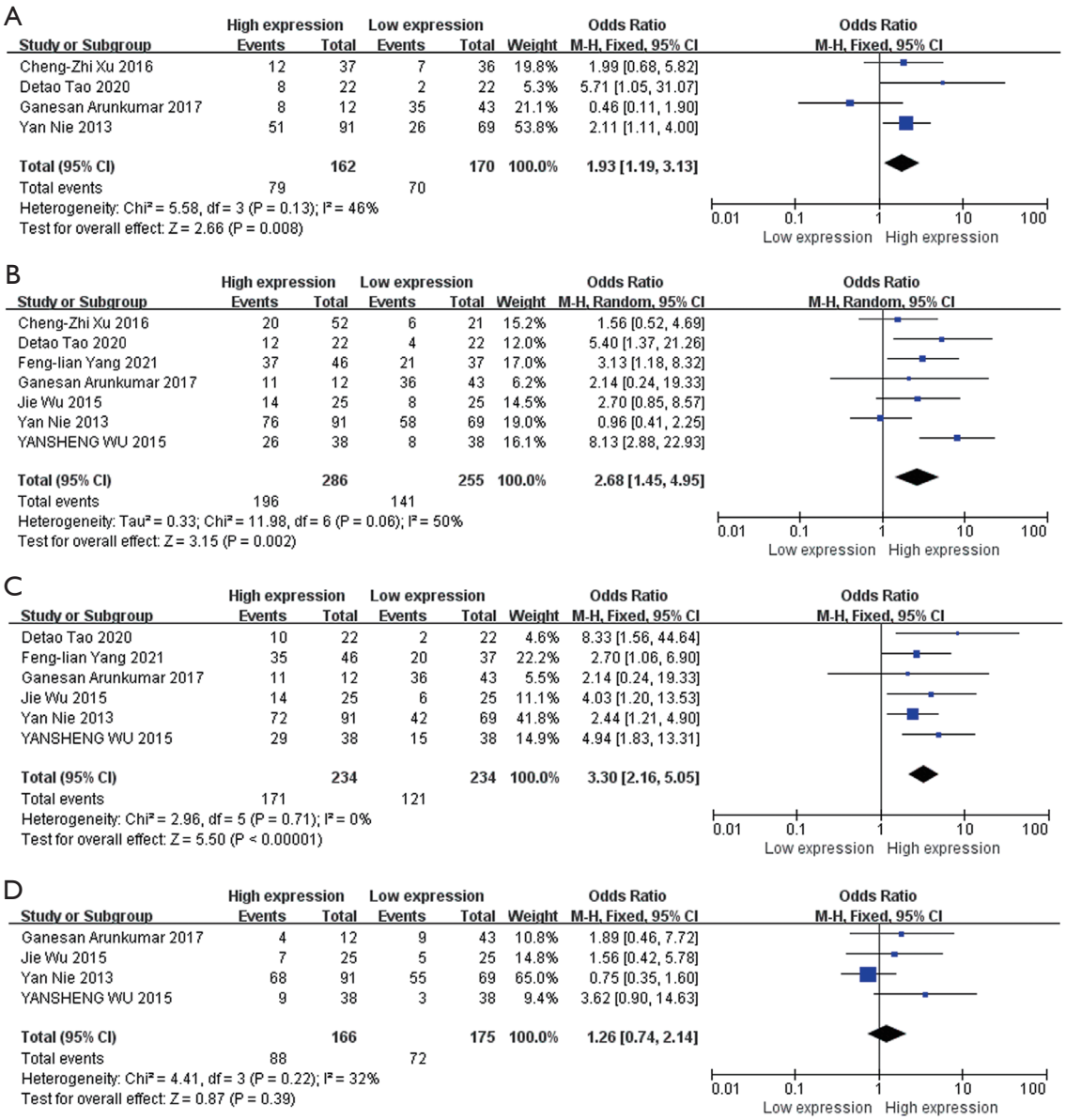

Figure 2 Forest plot of pooled ORs for clinicopathological features and high HOTAIR expression in HNSCC patients. (A) High T stage; (B) lymph node metastasis; (C) high TNM stage; (D) poor histological grades. CI, confidence interval; HNSCC, head and neck squamous cell carcinoma; HOTAIR, HOX transcript antisense intergenic RNA; M-H, Mann-Whitney test; OR, odds ratio.

were no publication biases in the four studies.

Lymphnode metastasis

Next, HOTAIR expression was compared among patients with HNSCC with different Lymphnode metastasis statuses from 7 studies that included in 541 patients (26,36-38,43-45). Among the patients with lymphnode metastasis (N1–N3), 196 patients exhibited high expressions for HOTAIR and 141 patients exhibited low expressions.

The results revealed that high expressions for HOTAIR had a significant association with lymphnode metastasis (OR =2.68, 95% CI: 1.45–4.95, P=0.002) (*Figure 2B*). Significant heterogeneity was found among the studies (I^2=50%, P=0.06); thus, the pooled analysis was estimated using a random-effects model.

Through the Begg's bias test, P=0.764, indicating that

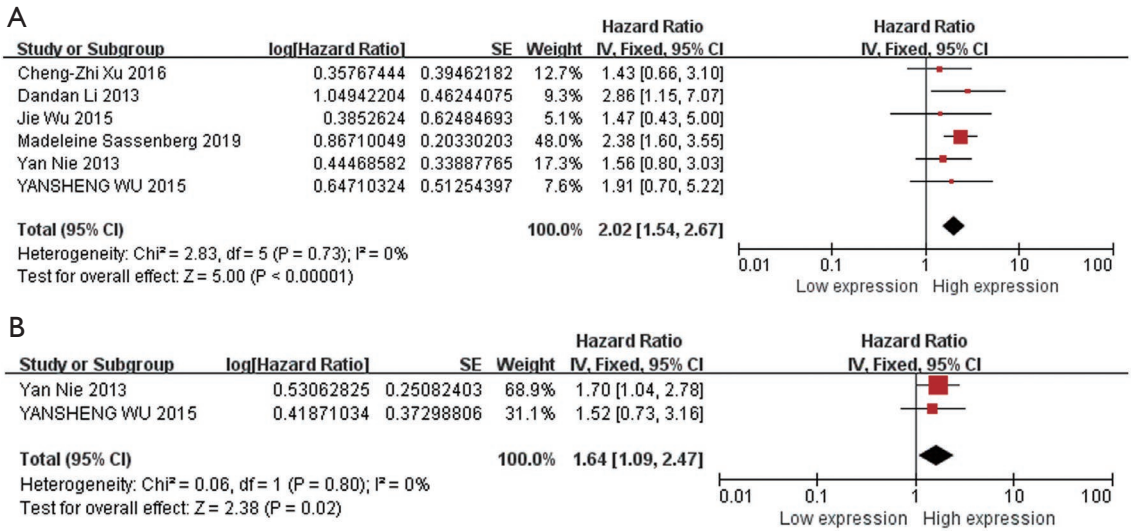

Figure 3 Forest plot of pooled HRs for prognosis and high HOTAIR expression in patients with HNSCC. (A) OS; (B) DFS. DFS, disease-free survival; HR, hazard ratio; HNSCC, head and neck squamous cell carcinoma; HOTAIR, HOX transcript antisense intergenic RNA; OS, overall survival; SE, standard error.

there were no publication biases in the 6 pieces of literature (Figure S1B).

TNM stages

In total, 468 patients across 6 studies were assessed to determine the relationships between TNM stages and HOTAIR expressions (26,37,38,43-45). Among the patients with high TNM stages (III–IV), 171 exhibited high HOTAIR expressions, and 121 exhibited low HOTAIR expressions.

The results revealed that HOTAIR high expression was significantly related to advanced TNM stages (OR =3.30, 95% CI: 2.16–5.05, P<0.00001) (*Figure 2C*). No heterogeneity was discovered between studies (I^2=0%, P=0.71); therefore, the fixed-effect model was applied for pooled analyses.

As shown in the Funnel plot (Figure S1C), the Begg's test was used to conduct publication bias. The Begg's test analysis indicated there was no publication bias in these 6 studies due to the value of P=0.260.

Histological grades

The relationship between tumor histological grades and HOTAIR expressions was compared across 341 patients from 4 studies (26,37,38,45). Among the patients with poor histological grades, 88 had high HOTAIR expressions, and 72 had low HOTAIR expressions. However, the difference was not statistically significant (OR =1.26, 95% CI: 0.74–2.14, P=0.39) (*Figure 2D*). No apparent heterogeneity was found in the studies (I^2=32%, P=0.22); therefore, the fixed-effect model was applied for pooled analysis.

As shown in the Figure funnel plot (Figure S1D), the Begg's test indicated there were no publication biases in these 4 studies due to the value of P=0.308.

Association between lncRNA HOTAIR and prognosis

OS

Among the included studies, 6 reported the OS of 856 patients with HNSCC, and this data was pooled for further analysis (26,36,38,42,45,46). The pooled results for the association between high HOTAIR expressions and OS revealed that higher expressions of HOTAIR correlates with worse survival in HNSCC patients (HR =2.02, 95% CI: 1.54–2.67, P<0.00001) (*Figure 3A*). No heterogeneity between studies was detected (I^2=0%, P=0.73).

Through the Begg's bias test, P=0.707, indicating that there were no publication biases in the 6 pieces of literature (Figure S2A).

DFS

Then we analyzed the pooled HRs of two studies with 236 patients to assess the correlation between high HOTAIR expression and DFS in patients with HNSCC (26,38). Because no heterogeneity (I^2=0%, P=0.80), the fixed-effects model was chosen. Compared with the low HOTAIR

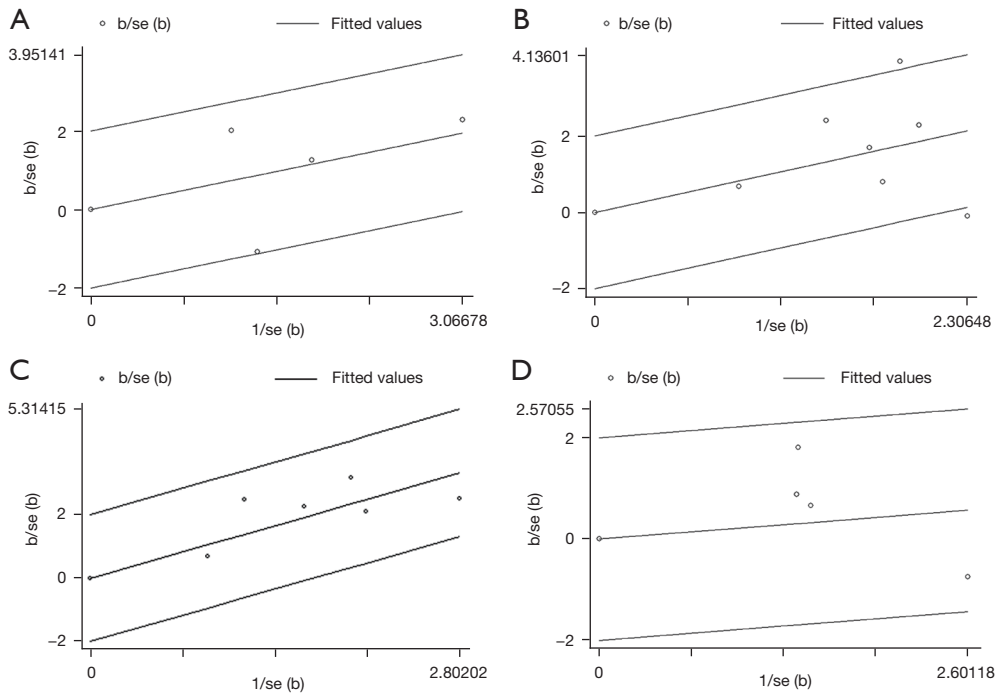

Figure 4 Galbraith plot. (A) High T stage; (B) lymph node metastasis; (C) high TNM stage; (D) poor histological grades. se, standard error.

expression group, the high HOTAIR expression group had a statistically significant reduced DFS (HR =1.64, 95% CI: 1.09–2.47, P=0.02) (*Figure 3B*).

According to the Begg's bias test, P=1.000 was obtained, indicating that there were no publication biases in these 2 studies (Figure S2B).

Heterogeneity identification and sensitivity analysis of individual studies

A Galbraith plot of the association between high HOTAIR expression and clinicopathological characteristics, including high T stages, lymphnode metastasis, high TNM stages, and poor histological grades in HNSCC, was constructed to examine the contribution of individual studies to measure heterogeneity and identify outliers (*Figure 4*). An approximate 95% CI is the area between the two intermittent parallel lines ±2 units above or below the regression line that begins from the origin. Studies outside the CI were identified as outliers.

To make sure the conclusions are credible, we conducted sensitivity analysis, deleting one study at a time for analysis to determine whether any of the studies affected the final pooled results (*Figure 5*).

In combination with the aforementioned Galbraith plots and sensitivity analysis, we found the following outliers. Meanwhile, we removed these outliers and conducted a secondary analysis to obtain the new forest plots.

(I) After removing the study of Ganesan Arunkumar *et al.* (37), heterogeneity was not detected (I^2=0%, P=0.53), and a significant association was found between HOTAIR high expressions and high T stages in HNSCC (OR =2.32, 95% CI: 1.38–3.89, P=0.001) (*Figure 6A*).

(II) The study from Yan Nie *et al.* (38) and Yansheng Wu *et al.* (26) were deleted to determine the relationship between lymphnode metastasis and HOTAIR expressions. Due to heterogeneity no longer being detected (I^2=0%, P=0.72) a fixed-effect model was used to replace the previous random-effects model. The more accurate and reliable correlation results between HOTAIR and lymphnode metastasis can be observed (OR =2.71, 95% CI: 1.57–4.67, P=0.0003) (*Figure 6B*).

(III) After deleting the study of Nie *et al.* (38) relating the association between TNM stages and HOTAIR expressions, the pooled data revealed that the new results (OR =3.92, 95% CI: 2.28–6.72, P<0.00001) and heterogeneity were not detected (I^2=0%, P=0.76)

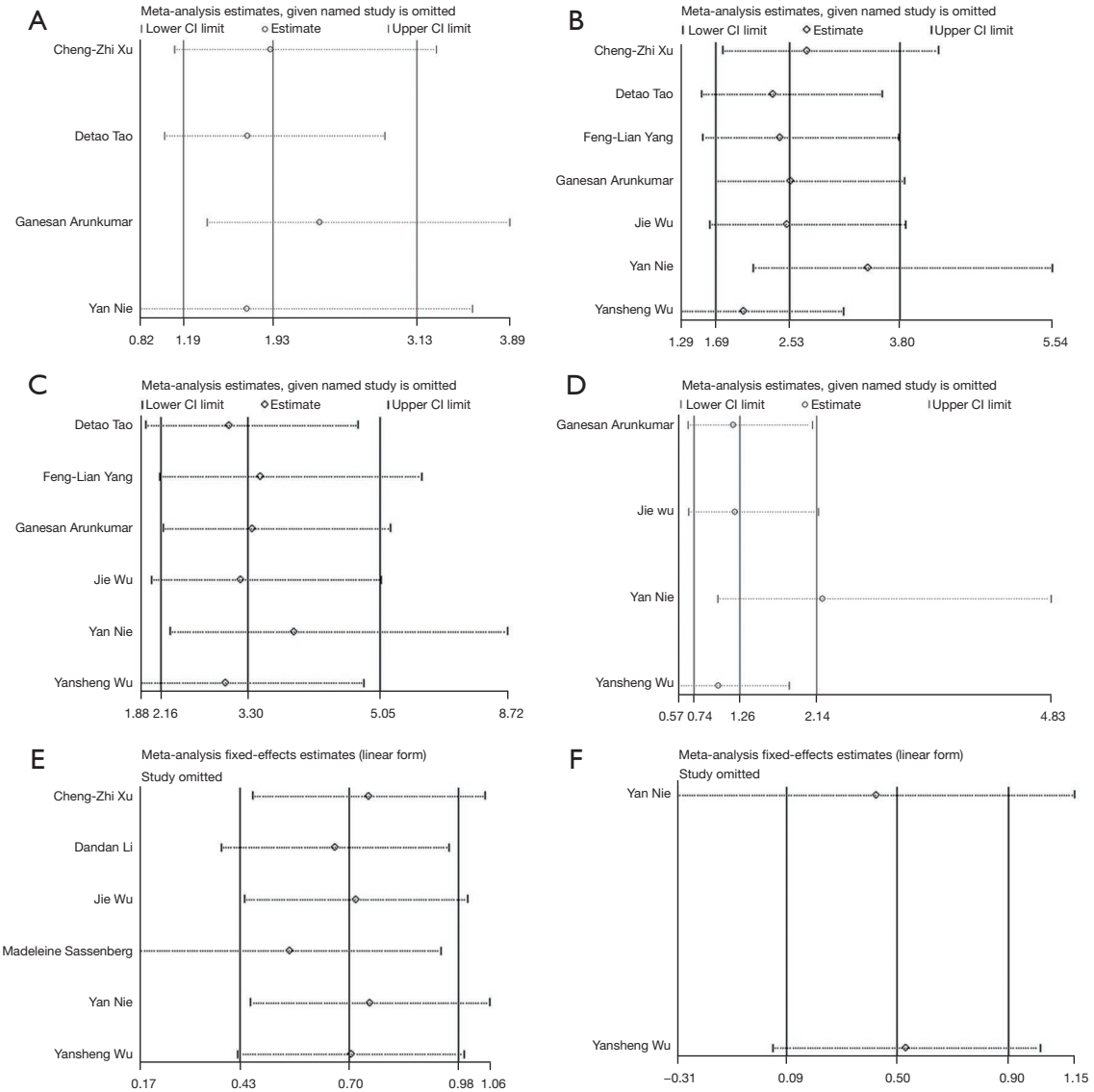

Figure 5 Sensitivity analyses of the studies. (A) High T stage; (B) lymph node metastasis; (C) high TNM stage; (D) poor histological grades; (E) OS; (F) DFS. CI, confidence interval; OS, overall survival; DFS, disease-free survival.

(*Figure 6C*).

(IV) After removing the study of Nie *et al.* (38), heterogeneity was not detected (I^2=0%, P=0.67), a statistically significant association was found between HOTAIR high expressions and poor histological grades in HNSCC (OR =2.21, 95% CI: 1.02–4.83, P=0.046). (Note: The forest plot provided in this paper was generated by Revman software, and the p-value retains two decimal places, but in STATA software, we obtained P=0.05) (*Figure 6D*).

(V) The study from Madeleine Sassenberg *et al.* (46) was removed, then we pooled the results again to reveal the correlation between high HOTAIR expressions and OS in HNSCC patients (HR =1.74, 95% CI: 1.19–2.56, P=0.005), (I^2=0%, P=0.81) (*Figure 6E*).

Subgroup analysis

T stages

The four pieces were divided into two groups according to the source of the study population, and meta-analysis was performed respectively. The results are shown in the *Figure* 7.

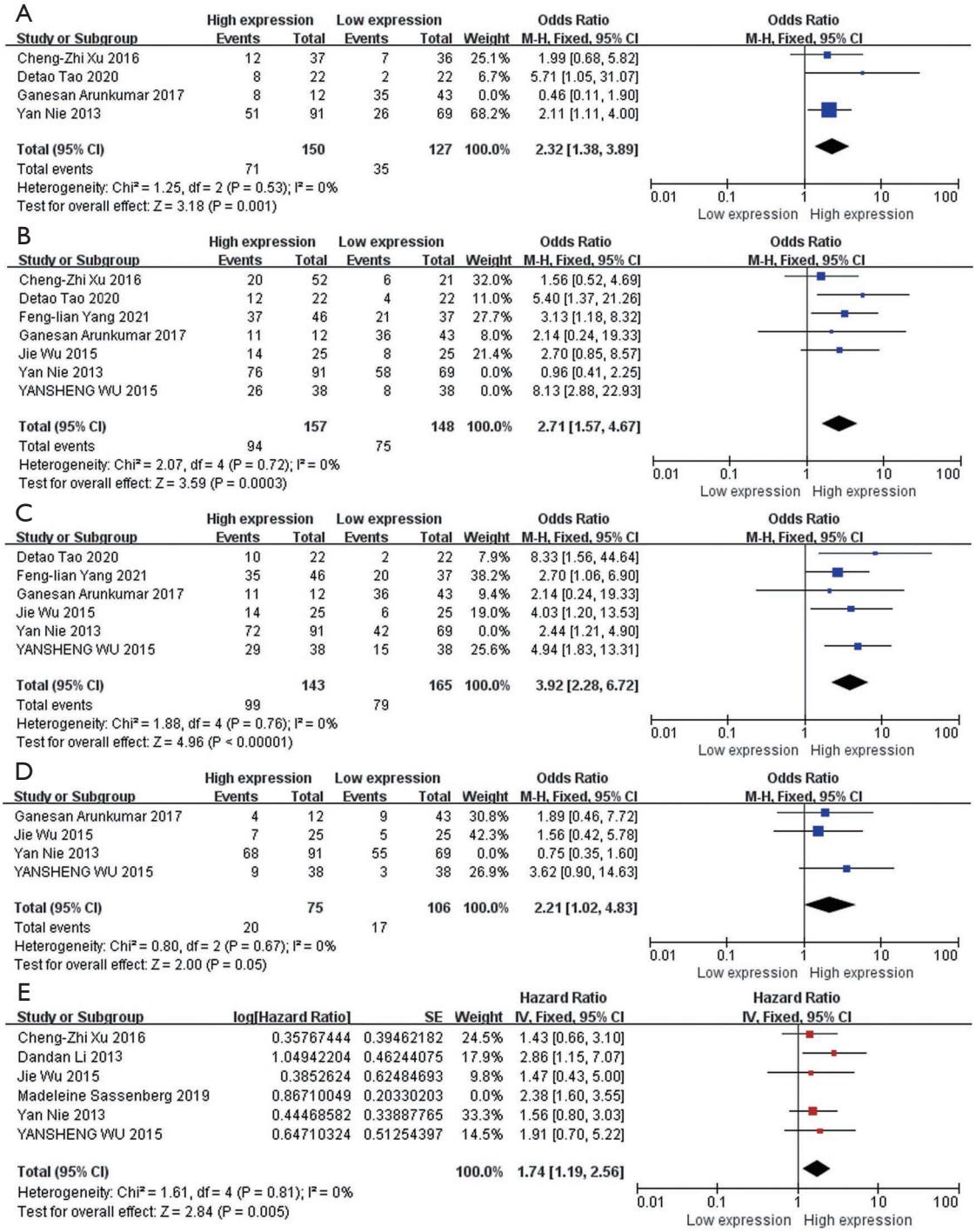

Figure 6 Forest plot with outliers removed. (A) High T stage; (B) lymph node metastasis; (C) high TNM stage; (D) poor histological grades; (E) OS. CI, confidence interval; M-H, Mann-Whitney test; OS, overall survival.

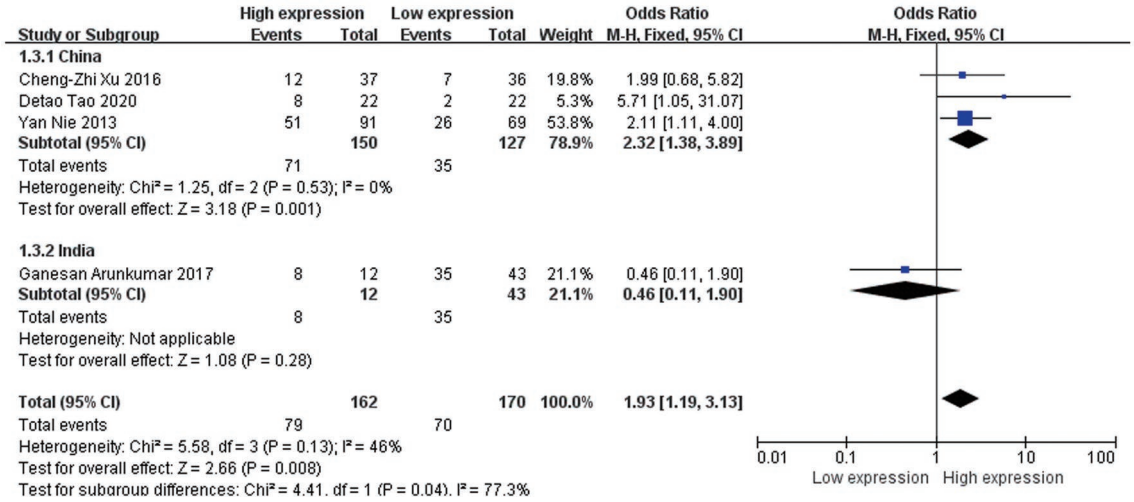

Figure 7 Subgroup analysis on the basis of racial differences in HNSCC patients with high T stage. CI, confidence interval; M-H, Mann-Whitney test; HNSCC, head and neck squamous cell carcinoma.

Based on the aforementioned subgroup analysis, the overall intergroup heterogeneity was strong, reaching moderate heterogeneity (I^2=46%, P=0.13), but in the China group, I^2=0%, P=0.53, and there was a significant difference between subgroups (I^2=77.3%, P=0.04), suggesting that ethnicity had a significant impact on the meta-analysis results.

The effect size OR of the China group was 2.32 and significant (OR =2.32, 95% CI: 1.38–3.89, P=0.001), implying that there was a significant relationship between high expressions of HOTAIR and high T stages in the China group, and the risk of high T stage was 2.32 times higher in the high expression group than in the low expression group. Second, although there is only one literature on an Indian group, its OR =0.46 (95% CI: 0.11–1.90, P=0.28). This suggests that racial differences may influence HOTAIR expressions in patients with high T stages. Clearly, more studies are needed to further verify this view.

Lymphnode metastasis

The seven studies were divided into three groups based on cut-off differences for HOTAIR gene expressions, and the results were analysed respectively. We divided them into a median group, mean group, and other (*Figure 8*).

The results from the subgroup analysis showed that the intergroup heterogeneity was significant (I^2=50%, P=0.06), and there was a significant difference between subgroups (I^2=78.9%, P=0.009), suggesting that the differences in cut-offs had a powerful impact on the meta-analysis results.

The pooled ORs for the cut-offs: median group (OR =5.07, 95% CI: 2.59–9.93, P<0.00001), and the mean group (OR =2.94, 95% CI: 1.20–7.18, P=0.02) support the conclusion that HOTAIR high expressions were significantly related to positive lymph node metastasis. No heterogeneity was found in the two subgroups. However, in the other group, the analysis assessing the association between HOTAIR high expressions and positive lymphnode metastasis showed no statistically significant differences (OR =1.15, 95% CI: 0.59–2.26, P=0.68), although the heterogeneity test showed a reduced heterogeneity (I^2=0%, P=0.49).

Histological grades

According to different detection methods, the four pieces were divided into a PCR group and ISH group, and meta-analyses were performed respectively (*Figure 9*).

Based on the results from the subgroup analysis, intergroup heterogeneity was present (I^2=32%, P=0.22), but after grouping, heterogeneity was not detected (I^2=0%, P=0.67) in the PCR group, and a statistically significant association was found between HOTAIR high expressions and poor histological grade in HNSCC (OR =2.21, 95% CI: 1.02–4.83, P=0.05). The risk of poor histological grades was 2.21 times higher in the high expression group than in the low expression group.

The OR of the ISH group was 0.75 (OR =0.75, 95% CI: 0.35–1.60, P=0.46). Although there is only one study in the ISH group, which means nothing for the analysis, the difference between the two groups was significant (I^2=77.3%, P=0.04).

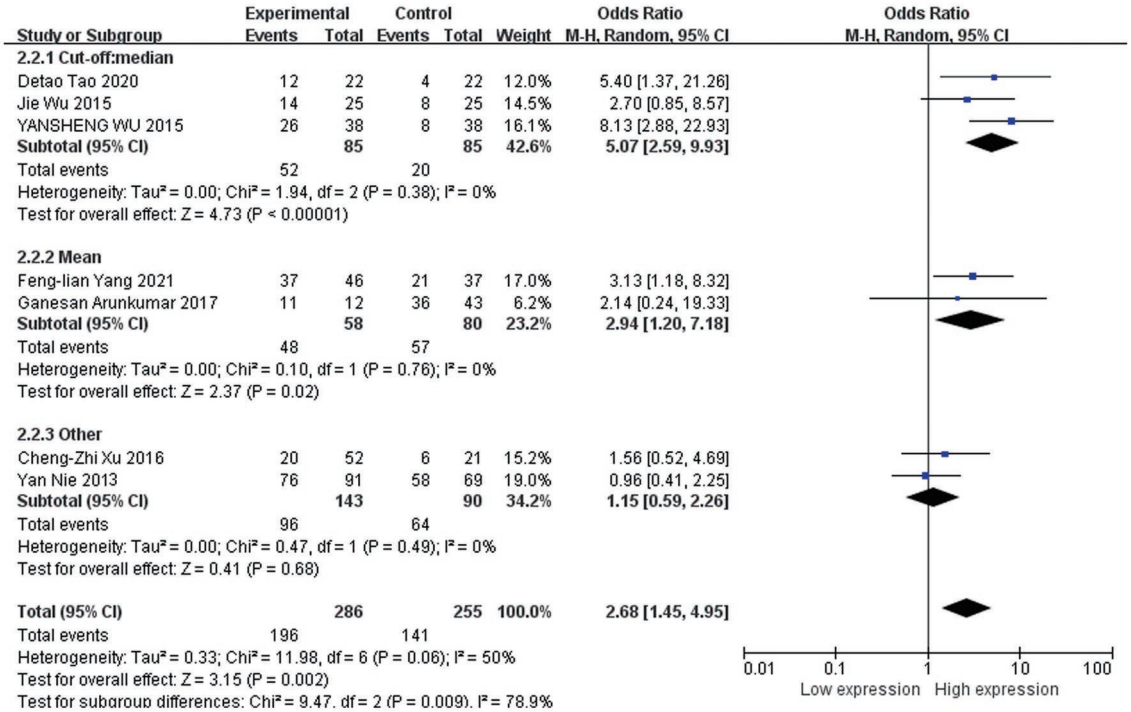

Figure 8 Subgroup analysis on the basis of cut-off differences in HNSCC patients with positive lymphnode metastasis. CI, confidence interval; M-H, Mann-Whitney test; HNSCC, head and neck squamous cell carcinoma.

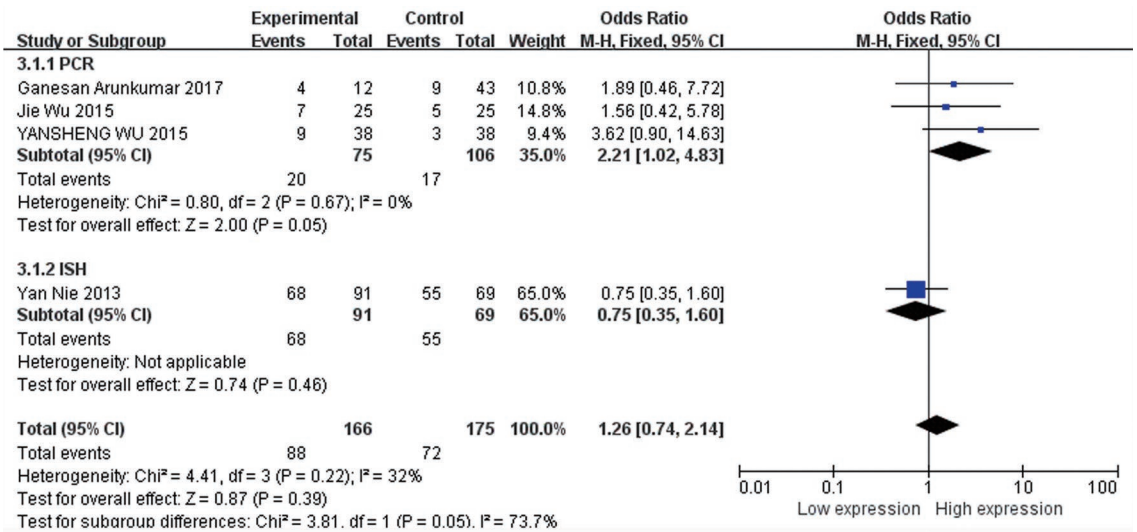

Figure 9 Subgroup analysis on the basis of detection methods in HNSCC patients with poor histological grade. CI, confidence interval; ISH, in situ hybridization; M-H, Mann-Whitney test; HNSCC, head and neck squamous cell carcinoma.

Discussion

Identifying potential biomarkers may be crucial in improving prognosis and predicting patient survival outcomes (47,48). Dysregulation of lncRNAs expressions has been found in a variety of cancers, among which HOTAIR is one of the most studied lncRNAs (49,50). The role of lncRNAs in HNSCC has been extensively studied, of course, in addition to HOTAIR, many other lncRNAs play an important role in HNSCC and can be used as biomarkers for predicting tumor stage and prognosis. Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) may promote the invasion and metastasis of oral squamous cell carcinoma through EMT process, and its overexpression is related to poor prognosis and significantly shortened OS (51,52). Urothelial carcinoma antigen 1 (UCA1) inhibits the growth and metastasis of oral squamous cell carcinoma cells by activating Wnt/β-catenin, and its overexpression may be related to lymph node metastasis (53). The low expression of maternally expressed gene 3 (MEG3) is associated with lymph node metastasis and TNM stage in patients with HNSCC, and may induce poor prognosis, on the contrary, its overexpression leads to the inhibition of tumor cell proliferation and invasion (54-56).

HOTAIR, as a valuable biomarker reflecting clinical parameters and predicting prognosis, is of great clinical significance in HNSCC. Some studies have shown that HOTAIR expression is positively correlated with tumor volume in oral squamous cell carcinoma (57). Wang *et al.* found that in advanced T stage or clinical stage, the expression of HOTAIR was significantly higher than that in early stage (58). In previous studies, HOTAIR expression was increased in patients with lymph node metastasis (58,59). Metastasis is one of the main causes of death in patients with HNSCC. Therefore, HOTAIR may be used to predict the survival rate of patients. Clinically, these biomarkers can be used to monitor disease exacerbation, and help to choose treatment methods. In view of this, we conducted the systematic review and meta-analysis. Our objective was to investigate whether HNSCC patients with high expressions of HOTAIR, compared to those with low expressions, had a worse prognosis (OS, DFS) and/or clinicopathological characteristics (tumour T stage, lymphnode metastasis, TNM stage, histological grades).

We performed a meta-analysis of 7 studies involving 546 patients to clarify the relationship between clinicopathologic features and HOTAIR expression in HNSCC patients, and 6 studies comprising 856 HNSCC patients to evaluate the effects of HOTAIR expressions on the prognosis. We found that an accordant effect of high HOTAIR expressions on poor OS and worse clinicopathologic features.

The results indicated that high expressions of HOTAIR was a risk factor for OS in HNSCC (HR =2.02, 95% CI: 1.54–2.67, P<0.00001). In addition, we also found that HOTAIR was significantly correlated with DFS (HR =1.64, 95% CI: 1.09–2.47, P=0.02). These results suggest that lncRNA HOTAIR can be used as a biomarker for head and neck tumor prognosis. However, larger studies are still needed. Only two studies were pooled when assessing the DFS results, resulting in poor reliability. When evaluating OS outcomes, more studies were expected to be included, but because some studies did not provide accurate HR values or Kaplan-Meier curves, and two data from TCGA were excluded to avoid duplication (60,61). In the end, we only included 7 studies, but the study population was indeed much larger than the previous meta-analysis.

Meanwhile, we evaluated the relationships between HOTAIR expressions and HNSCC clinicopathological characteristics. We discovered that high HOTAIR expressions were more likely to lead to high T stages (OR =1.93, 95% CI: 1.19–3.13, P=0.008), lymphnode metastasis (OR =2.68, 95% CI: 1.45–4.95, P=0.002), high TNM stages (OR =3.30, 95% CI: 2.16–5.05, P<0.00001). However, there were no correlations in poor histological grades (OR =1.26, 95% CI: 0.74–2.14, P=0.39).

After deleting a study by heterogeneity identification and sensitivity analysis, fortunately, a statistically significant correlation was found between HOTAIR high expressions and poor histological grades (OR =2.21, 95% CI: 1.02–4.83, P=0.05). However, the deleted literature has the largest weight, and the sample size of the remaining three studies is relatively small, so the number of included studies needs to be increased to generate more reliable results.

The heterogeneity tests and sensitivity analysis of other indicators are as aforementioned. Sensitivity analyses can assess the stability of the results. After the outliers were removed, studies that previously existed heterogeneity are no longer heterogeneous, but in these analyses, after excluding the studies that had a large impact on heterogeneity or sensitivity, there were no significant changes in the pooled results of the remaining studies, only a slight variation in the value of the results, but no qualitative changes, indicating that despite heterogeneity existing, the meaning of the results is relatively stable. The applicability of our results to other ethnic groups may be

limited because the majority of the remaining studies were conducted in China.

Then in subgroup analysis, it is worth noting that the elevated HOTAIR levels are highly associated with high T stages in China group, but not in Indian group, suggesting that genetic, ethnic, and environmental factors may influence the progression of squamous cell carcinoma. Previous studies have shown differences in disease risks and prognoses among ethnic groups with head and neck cancer (62,63). But unfortunately, most of our studies were conducted in China, only one from India, which could influence the validity and evidential capability of the results. Hence further large-scale studies should be conducted among different ethnic groups.

The subgroup analysis results of lymphnode metastasis groups confirmed that the differences of gene expression cut-offs had a powerful impact on the results. The conclusion that HOTAIR high expressions were significantly related to positive lymph node metastasis was obtained in the median group and mean group. In the two studies with the staining index (SI) as the cut-off point and the unexplained situations (36,38), pooled results showed no statistically relevant differences. Different cut-off points were associated with high/low gene expression sample sizes, which could be inferred to influence the determinations of the correlations between gene expressions and a clinical feature to some extent. This suggests that in future studies, we should pay attention to the heterogeneity caused by differences in cut-off definitions in single studies, which may affect results.

Among the four studies involving histological grading, three used PCR as the detection method for lncRNA, and only one used ISH. The results of these four studies showed that there were no correlations between high HOTAIR expressions and poor histological grades, but after subgrouping, the pooled results revealed a positive correlation. It may provide thoughts for future research and analysis that different detection methods of gene expression may affect identifying the relationship between HOTAIR expressions and histological grades. ISH can be used to detect the expressions and changes of oncogenes, tumor suppressor genes, and various functional genes at the transcriptional level. Some scholars have studied its sensitivity and specificity (64). As for why the different results were produced in the study from Nie *et al.* (38), more research data needs to be supplemented to demonstrate whether it is caused by differences in detection methods.

There is much evidence that HOTAIR plays a key role in cancer progression and metastasis. Li *et al.* showed that HOTAIR promoted the malignant growth of human hepatocellular carcinoma stem cells by inhibiting SETD2 expressions and its phosphorylation (35). Song *et al.* demonstrated that HOTAIR promoted the development of gastric cancer by regulating and inhibiting the transcription of E-cadherin (28). These studies all showed that high expressions of HOTAIR may activate the characteristics of cancer stem cell (65). Although the mechanisms of tumorigenesis and progression in HNSCC have not been fully elucidated, we verified the negative correlation between high HOTAIR expressions and tumor prognosis by pooled analysis of clinical data.

Conclusions

In conclusion, our meta-analysis confirmed the prognostic role of HOTAIR in HNSCC. High HOTAIR expressions predicted low clinical survival rates and were associated with worse clinicopathologic features. These results revealed that, except for clinical stages and histologic grades of HNSCC patients, HOTAIR may be an excellent biomarker to guide prognosis and therapeutic strategy.

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Footnote

Reporting Checklist: The authors have completed the PRISMA reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-652/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-652/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are

appropriately investigated and resolved.

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Table S1 Clinicopathological features of included studies

Study	High T stage				Positive Lymphnode metastasis				Poor histological grade				High TNM stage			
	High expression		Low expression		High expression		Low expression		High expression		Low expression		High expression		Low expression	
	Events	Total	Events	Total	Events	Total	Events	Total	Events	Total	Events	Total	Events	Total	Events	Total
Cheng-Zhi Xu	12	37	7	36	20	52	6	21	–	–	–	–	–	–	–	–
Detao Tao	8	22	2	22	12	22	4	22	–	–	–	–	10	22	2	22
Feng-lian Yang	–	–	–	–	37	46	21	37	–	–	–	–	35	46	20	37
Ganesan Arunkumar	8	12	35	43	11	12	36	43	4	12	9	43	11	12	36	43
Jie Wu	–	–	–	–	14	25	8	25	7	25	5	25	14	25	6	25
Yan Nie	51	91	26	69	76	91	58	69	68	91	55	69	72	91	42	69
Yansheng Wu	–	–	–	–	26	38	8	38	9	38	3	38	29	38	15	38

Table S2 Newcastle-Ottawa quality assessment scale for cohort studies

Study	Selection	Comparability	Outcome	Total
Cheng-Zhi Xu	★★★★	★★	★★	8
Dandan Li	★★★★	★	★★★	8
Detao Tao	★★★★	★★	★	7
Feng-lian Yang	★★★	★★	★★	7
Ganesan Arunkumar	★★★★	★	★	6
Jie Wu	★★★★	★	★★★	8
Madeleine Sassenberg	★★	★	★★	5
Yan Nie	★★★★	★	★★★	8
Yansheng Wu	★★★★	★	★★	7

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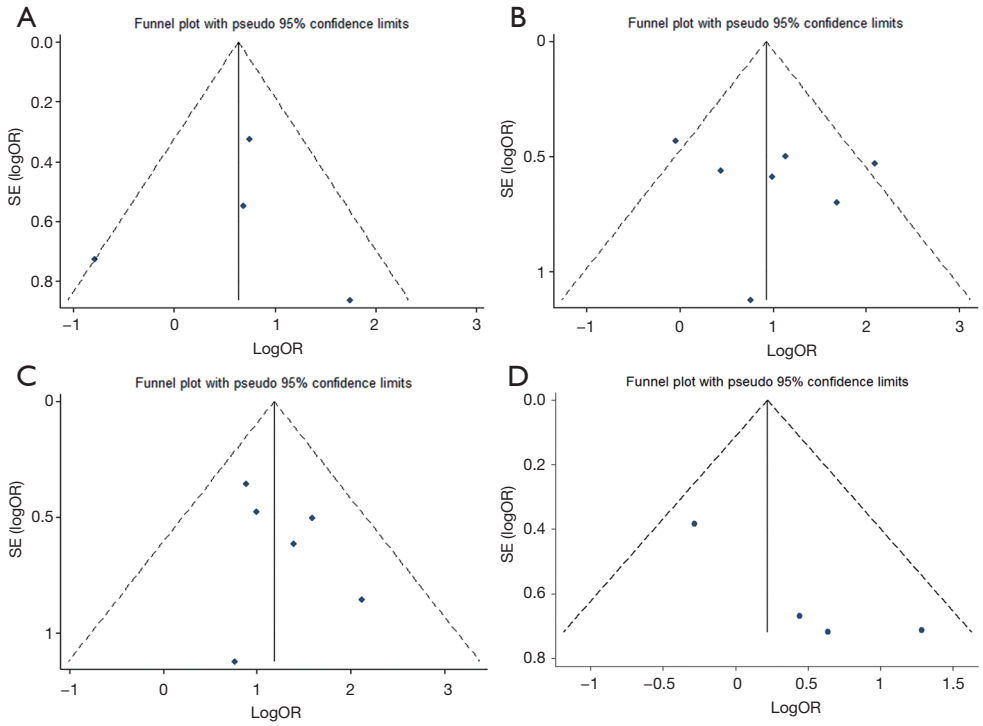

Figure S1 Funnel plot for publication bias tests in high T stage (A), lymph node metastasis (B), high TNM stage (C), and poor histological grades (D). SE, standard error; OR, odds ratio.

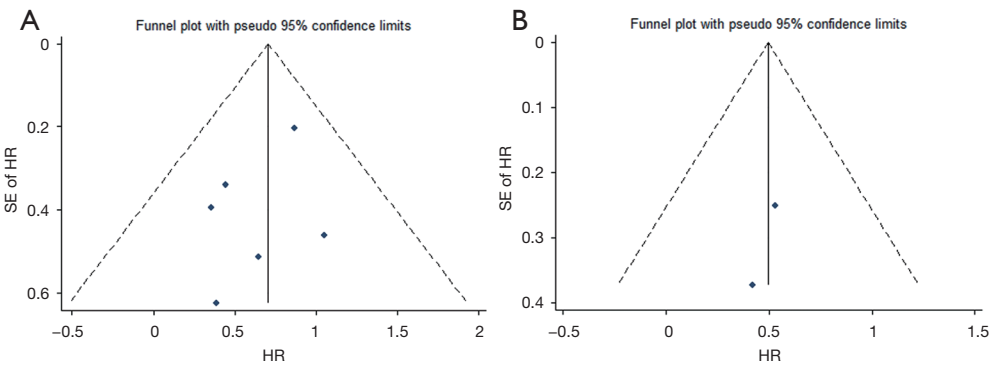

Figure S2 Funnel plot for publication bias tests in OS (A) and DFS (B). DFS, disease-free survival; HR, hazard ratio; OS, overall survival; SE, standard error.

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