Original Article
Prognostic and diagnostic value of long non-coding RNAs in esophageal squamous cell carcinoma: a systematic review and meta-analysis

Canlin Yang*, Fei Li*, Wenhao Shen*, Zhengdong Wu, Qian Bu, Junxing Huang

*Department of Oncology, Taizhou People's Hospital, Taizhou, Jiangsu, P. R. China. *Equal contributors.*

Abstract: Abnormally-expressed long non-coding RNAs (lncRNAs) may be effective prognostic or diagnostic biomarkers for esophageal squamous cell carcinoma (ESCC). To overcome the shortcomings and inaccuracies of single studies, the clinical value of lncRNAs as predictive ESCC biomarkers was examined through systematic review and meta-analysis. Thirty-five studies, including 26 on clinicopathological features, 27 on prognosis, and 4 on diagnosis were selected from electronic databases. Among ESCC clinicopathological features, HOTAIR and MALAT-1 over-expression correlated with lymph node metastasis (OR = 3.29, 95% CI: 1.18-9.16, P = 0.02; OR = 1.77, 95% CI: 1.04-3.00, P = 0.04, respectively). HOTAIR and AFAP1-AS1 overexpression correlated with tumor-node-metastasis stage (OR = 6.93, 95% CI: 2.79-17.18, P < 0.0001; OR = 2.92, 95% CI: 1.66-5.13, P = 0.0002, respectively). HOTAIR upregulation and MEG3 downregulation correlated with shorter overall survival (HR = 2.01, 95% CI: 1.56-2.58, P < 0.00001; HR = 0.47, 95% CI: 0.25-0.88, P = 0.02, respectively). Evaluation of the clinical performance of all lncRNAs as ESCC predictors yielded a sensitivity of 81%, specificity of 74%, and diagnostic odds ratio of 17.44, with an AUC of 0.87 (95% CI: 0.84-0.90). These results highlight the potential prognostic and diagnostic value of ESCC-related lncRNAs.

Keywords: Esophageal squamous cell carcinoma, long non-coding RNA, prognosis, diagnosis, meta-analysis

Introduction

Esophageal carcinoma (EC) is the ninth most common cancer and the sixth leading cause of cancer deaths, worldwide. Notably, the world witnessed 455,800 new cases and 400,200 death cases in 2012 [1]. Overall 5-year survival of EC ranges from 15% to 25%, remaining low despite continuous progress made in clinical treatment. Its poor prognosis can be attributed to diagnosis at advanced stages and propensity for metastasis. In comparison, early diagnosis often leads to better outcomes [2]. EC can be mainly classified into two categories based on histological type, esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC). In North and Central China, areas with high-prevalence of ESCC, approximately 90% of EC patients develop ESCC [3]. Unfortunately, no efficient diagnostic or prognostic biomarkers of ESCC are currently available. Therefore, identification of novel potential biomarkers for early diagnosis, accurate prognosis, and

therapeutic treatment of ESCC are urgently required.

Long non-coding RNAs (lncRNAs) are a large class of RNA transcripts, longer than > 200 nucleotides, lacking open reading frames [4]. LncRNAs were initially considered 'transcriptional noise', with no biological function. Emerging evidence in recent decades has demonstrated abnormal expression of lncRNAs in a variety of cancers, including ESCC, as well as non-negligible roles for these transcripts in tumorigenesis, invasion, and metastasis [5, 6]. For instance, HOX transcript antisense RNA (HOTAIR) is frequently upregulated in numerous human malignancies, such as breast cancer, gastric cancer, and ESCC [7-10]. In ESCC, HOTAIR upregulation has been associated with poor prognosis [10]. Wang et al. [11] suggested, on the other hand, that serum HOTAIR might serve also as a potential biomarker for ESCC diagnosis, with 56.0% sensitivity (SEN) and 90.0% specificity (SPE). LncRNA metastasis-associat-
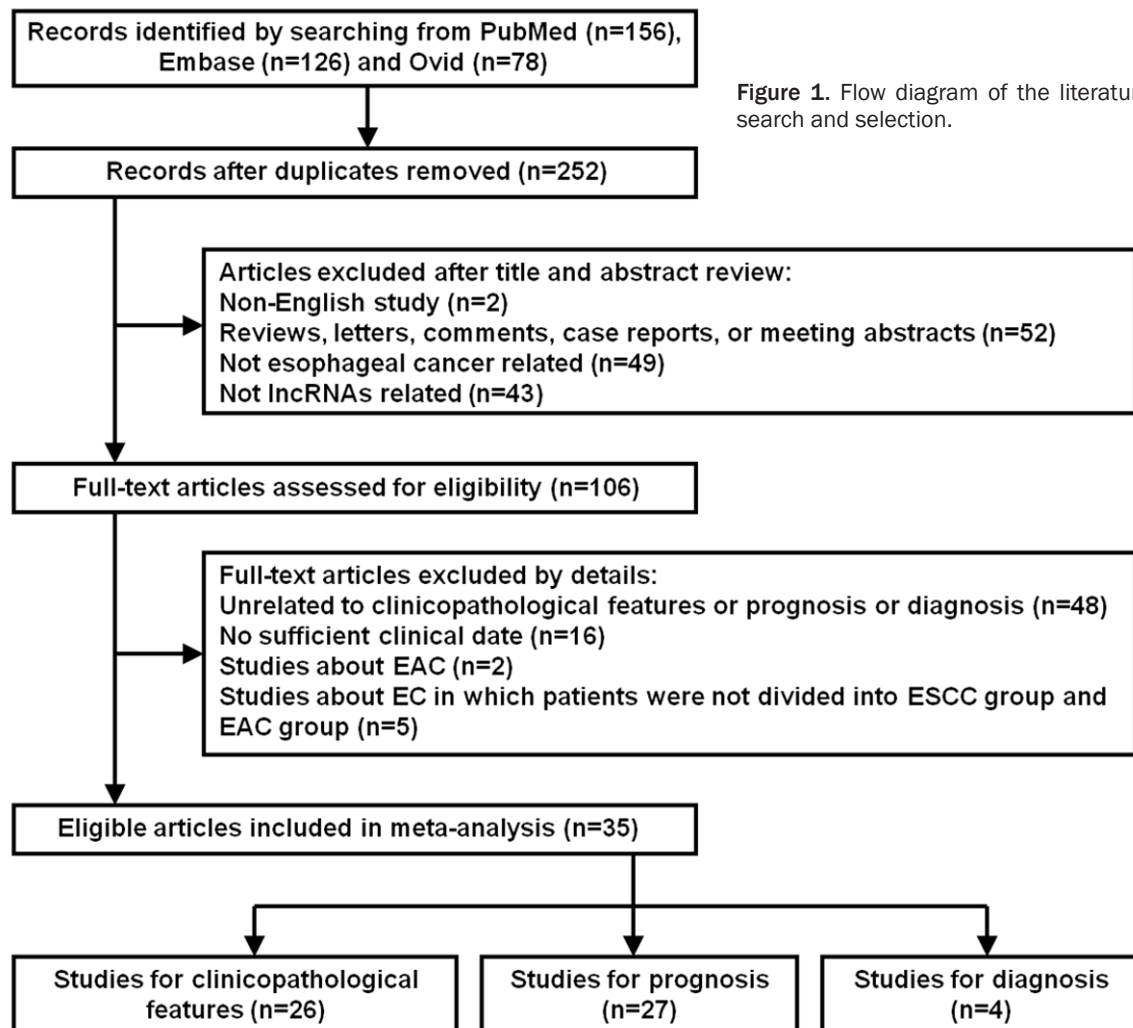
Figure 1. Flow diagram of the literature search and selection.

ed lung adenocarcinoma transcript 1 (MALAT-1), which plays a vital role in lung cancer metastasis, is remarkably upregulated in ESCC patients and correlates with poor survival [12, 13].

Although valuable, conclusions from single studies may be biased or inaccurate due to small sampling sizes and/or inadequate research methods. Because the relevance of lncRNAs could be better explored by expanded patient sampling, this systematic review and meta-analysis was conducted to summarize the results of published studies regarding the clinical value of lncRNAs in ESCC.

Results

Study selection and characteristics

As shown in the flow chart of **Figure 1**, 360 publications were initially identified from PubMed,

Embase, and Ovid databases. Of these, 108 were excluded due to duplicate reporting. After further removal of 146 irrelevant articles upon screening titles and abstracts, 106 full-text articles were assessed for eligibility. At this point, 71 articles that did not meet the selection criteria were further excluded. Finally, a total of 35 studies, involving 3,799 patients, were included for final analysis, of which 26 provided clinicopathological features, 27 provided prognosis data, and 4 provided diagnosis data.

All included articles were published between 2013 and 2017, most carried out in the Chinese population. Expression of lncRNAs was detected by quantitative reverse transcription polymerase chain reaction (qRT-PCR) assay (n = 34) or by *in situ* hybridization assay (ISH) (n = 1). Cut-off values varied from study to study as a result of various cut-off definitions. Specimens
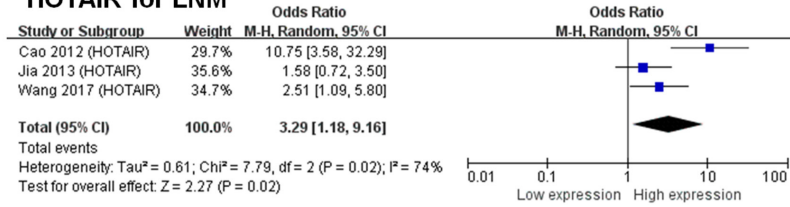
Table 1. Summary of the comparison for *p* values of the association between lncRNAs and clinicopathological features of patients with ESCC

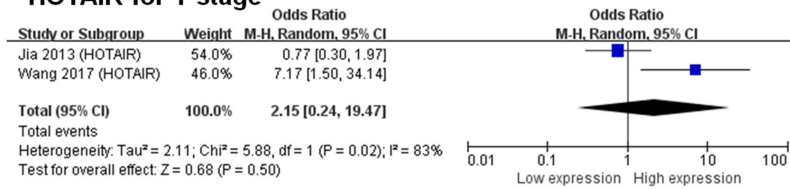| Studies	LncRNAs	Population	Expression	Case number	Cut-off	Age	Gender	Location	Tumor size	Tobacco	Alcohol	Differentiation	LNM	T stage	DM	TNM stage
Bai 2016	H19	Chinese	Up-regulation	64	Fold-change	0.945	0.663	NA	NA	NA	NA	0.409	0,007	0.01	NA	NA
Cao 2012	HOTAIR	Chinese	Up-regulation	78	Fold-change	0.463	0.731	NA	NA	0.815	0.984	NA	0.001	NA	NA	0.001
Cao 2013	PlncRNA-1	Chinese	Up-regulation	73	Mean	0.807	0.067	NA	NA	0.385	0.349	NA	0.04	NA	NA	0.01
Cao 2014	LOC285194	Chinese	Down-regulation	142	Median	0.641	0.478	0.573	0.002	0.603	0.206	0.022	0.013	0.27	0.015	0.018
Cao 2014	SPRY4-IT1	Chinese	Up-regulation	92	Median	0.397	0.288	0.755	NA	0.404	0.09	0.026	0.007	0.01	NA	0.005
Cao 2015	PCAT-1	Chinese	Up-regulation	130	Median	0.213	0.078	NA	NA	0.871	0.859	NA	0.032	0.024	0.975	0.003
Cao 2016	BANCR	Chinese	Up-regulation	142	Median	0.354	0.612	0.196	0.614	0.502	0.733	0.024	0.001	0.355	0.052	0.002
Cao 2016	BC200	Chinese	Up-regulation	70	Median	0.19	0.31	1	NA	NA	NA	0.22	1	0.77	NA	0.45
Chen 2017	RP11-766N7.4	Chinese	Down-regulation	50	Median	0.349	0.23	0.598	NA	NA	NA	NA	0.001	0.034	NA	NA
Fang 2014	FOXCUT	Chinese	Up-regulation	82	Mean	0.022	0.864	0.164	NA	NA	NA	0.001	0.007	0.259	0.001	0.12
Feng 2015	NEAT1	Chinese	Up-regulation	96	Youden index	0.198	0.076	0.257	0.026	NA	NA	0.067	0.035	NA	0.108	0.004
He 2014	3-lncRNA signature	Chinese	High-risk	60	NA	NA	0.34	0.378	NA	0.112	0.356	0.427	0.726	0.227	NA	0.555
He 2016	AFAP1-AS1	Chinese	Up-regulation	70	Fold-change	0.449	0.451	NA	0.04	0.88	0.508	NA	NA	NA	NA	0.01
He 2017	CASC2	Chinese	Down-regulation	133	Mean	0.557	0.198	0.729	0.104	NA	NA	NA	0.016	0.012	NA	NA
Jia 2013	HOTAIR	Chinese	Up-regulation	137	ROC curve	0.324	0.497	NA	NA	NA	NA	NA	0.074	0.775	NA	NA
Li 2014	UCA1	Chinese	Up-regulation	90	Mean	0.574	0.603	0.831	NA	NA	NA	0.001	0.004	NA	NA	0.004
Li 2015	MALAT-1	Chinese	Up-regulation	77	Fold-change	0.76	0.37	NA	NA	NA	NA	0.83	1	0.01	NA	0.28
Tong 2016	AFAP1-AS1	Chinese	Up-regulation	162	Median	0.641	0.463	1	0.738	0.107	1	0.89	0.001	0.185	0.016	0.002
Wang 2013	HOTAIR	Chinese	Up-regulation	93	Staining index	0.272	0.189	NA	NA	NA	NA	0.022	0.005	0.001	0.029	0.001
Wang 2015	ZEB1-AS1	Chinese	Up-regulation	87	Median	0.752	0.307	0.216	0.73	NA	NA	0.002	0.003	0.001	NA	NA
Xu 2016	CASC9	Chinese	Up-regulation	42	Fold-change	0.742	0.282	NA	0.496	NA	NA	0.001	1	1	NA	0.738
Yang 2015	MALAT-1	Chinese	Up-regulation	54	Fold-change	0.984	0.651	NA	0.014	NA	NA	0.991	0.007	NA	NA	NA
Yang 2016	BC032469	Chinese	Up-regulation	45	Fold-change	0.93	NA	0.931	0.0437	0.711	0.615	0.867	0.017	NA	NA	0.026
Yu 2016	TUG1	Chinese	Up-regulation	218	Median	0.078	0.742	0.129	0.129	NA	NA	0.37	0.517	NA	NA	0.127
Zhao 2015	MALAT-1	Chinese	Up-regulation	137	Fold-change	0.571	0.834	0,070	NA	NA	NA	0.168	0.073	0.253	NA	NA
Zhu 2015	CCAT2	Chinese	Up-regulation	229	Median	0.498	0.763	NA	0.506	NA	NA	0.637	0.034	NA	NA	0.032

Abbreviations: LncRNA, long non-coding RNA; NA, not available; ROC, receiver operator characteristic; LNM, lymph node metastasis; DM, distant metastasis; TNM stage, tumor-node-metastasis stage.
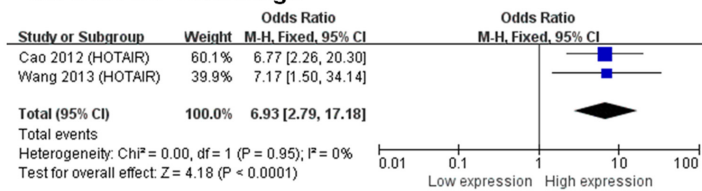
A HOTAIR for LNM

Study or Subgroup	Weight	Odds Ratio M-H, Random, 95% CI
Cao 2012 (HOTAIR)	29.7%	10.75 [3.58, 32.29]
Jia 2013 (HOTAIR)	35.6%	1.58 [0.72, 3.50]
Wang 2017 (HOTAIR)	34.7%	2.51 [1.09, 5.80]
Total (95% CI)	100.0%	3.29 [1.18, 9.16]

Total events
Heterogeneity: Tau² = 0.61; Chi² = 7.79, df = 2 (P = 0.02); I² = 74%
Test for overall effect: Z = 2.27 (P = 0.02)

B HOTAIR for T stage

Study or Subgroup	Weight	Odds Ratio M-H, Random, 95% CI
Jia 2013 (HOTAIR)	54.0%	0.77 [0.30, 1.97]
Wang 2017 (HOTAIR)	46.0%	7.17 [1.50, 34.14]
Total (95% CI)	100.0%	2.15 [0.24, 19.47]

Total events
Heterogeneity: Tau² = 2.11; Chi² = 5.88, df = 1 (P = 0.02); I² = 83%
Test for overall effect: Z = 0.68 (P = 0.50)

C HOTAIR for TNM stage

Study or Subgroup	Weight	Odds Ratio M-H, Fixed, 95% CI
Cao 2012 (HOTAIR)	60.1%	6.77 [2.26, 20.30]
Wang 2013 (HOTAIR)	39.9%	7.17 [1.50, 34.14]
Total (95% CI)	100.0%	6.93 [2.79, 17.18]

Total events
Heterogeneity: Chi² = 0.00, df = 1 (P = 0.95); I² = 0%
Test for overall effect: Z = 4.18 (P < 0.0001)

D MALAT-1 for Differentiation

Study or Subgroup	Weight	Odds Ratio M-H, Fixed, 95% CI
Li 2015 (MALAT-1)	35.3%	0.90 [0.35, 2.28]
Yang 2015 (MALAT-1)	18.4%	1.01 [0.29, 3.52]
Zhao 2015 (MALAT-1)	46.3%	0.36 [0.12, 1.11]
Total (95% CI)	100.0%	0.67 [0.37, 1.22]

Total events
Heterogeneity: Chi² = 1.99, df = 2 (P = 0.37); I² = 0%
Test for overall effect: Z = 1.30 (P = 0.19)

E MALAT-1 for LNM

Study or Subgroup	Weight	Odds Ratio M-H, Fixed, 95% CI
Li 2015 (MALAT-1)	39.8%	1.05 [0.41, 2.73]
Yang 2015 (MALAT-1)	12.8%	4.67 [1.47, 14.79]
Zhao 2015 (MALAT-1)	47.4%	1.58 [0.72, 3.50]
Total (95% CI)	100.0%	1.77 [1.04, 3.00]

Total events
Heterogeneity: Chi² = 3.93, df = 2 (P = 0.14); I² = 49%
Test for overall effect: Z = 2.11 (P = 0.04)

F MALAT-1 for T stage

Study or Subgroup	Weight	Odds Ratio M-H, Random, 95% CI
Li 2015 (MALAT-1)	49.8%	3.54 [1.35, 9.25]
Zhao 2015 (MALAT-1)	50.2%	0.77 [0.30, 1.97]
Total (95% CI)	100.0%	1.64 [0.37, 7.34]

Total events
Heterogeneity: Tau² = 0.93; Chi² = 4.94, df = 1 (P = 0.03); I² = 80%
Test for overall effect: Z = 0.65 (P = 0.52)

G AFAP1-AS1 for Tumor size

Study or Subgroup	Weight	Odds Ratio M-H, Fixed, 95% CI
He 2016 (AFAP1-AS1)	7.0%	3.09 [0.36, 26.92]
Tong 2016 (AFAP1-AS1)	93.0%	1.18 [0.61, 2.28]
Total (95% CI)	100.0%	1.32 [0.71, 2.45]

Total events
Heterogeneity: Chi² = 0.70, df = 1 (P = 0.40); I² = 0%
Test for overall effect: Z = 0.87 (P = 0.38)

H AFAP1-AS1 for Tobacco status

Study or Subgroup	Weight	Odds Ratio M-H, Fixed, 95% CI
He 2016 (AFAP1-AS1)	22.8%	0.92 [0.33, 2.60]
Tong 2016 (AFAP1-AS1)	77.2%	0.56 [0.30, 1.07]
Total (95% CI)	100.0%	0.64 [0.37, 1.11]

Total events
Heterogeneity: Chi² = 0.64, df = 1 (P = 0.43); I² = 0%
Test for overall effect: Z = 1.59 (P = 0.11)

I AFAP1-AS1 for Alcohol status

Study or Subgroup	Weight	Odds Ratio M-H, Fixed, 95% CI
He 2016 (AFAP1-AS1)	19.6%	1.52 [0.44, 5.26]
Tong 2016 (AFAP1-AS1)	80.4%	0.94 [0.46, 1.90]
Total (95% CI)	100.0%	1.05 [0.57, 1.95]

Total events
Heterogeneity: Chi² = 0.44, df = 1 (P = 0.51); I² = 0%
Test for overall effect: Z = 0.16 (P = 0.87)

J AFAP1-AS1 for TNM stage

Study or Subgroup	Weight	Odds Ratio M-H, Fixed, 95% CI
He 2016 (AFAP1-AS1)	28.5%	2.17 [0.68, 6.91]
Tong 2016 (AFAP1-AS1)	71.5%	3.22 [1.69, 6.12]
Total (95% CI)	100.0%	2.92 [1.66, 5.13]

Total events
Heterogeneity: Chi² = 0.34, df = 1 (P = 0.56); I² = 0%
Test for overall effect: Z = 3.73 (P = 0.0002)

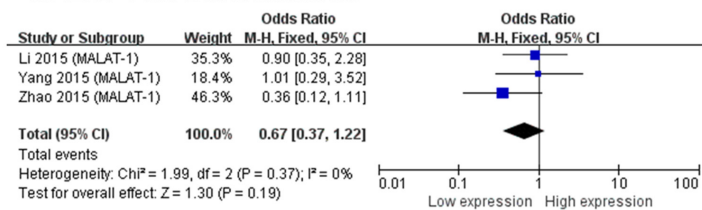**Figure 2.** Forest plots of studies estimating ORs of lncRNAs expression and clinicopathological features of ESCC patients. A. 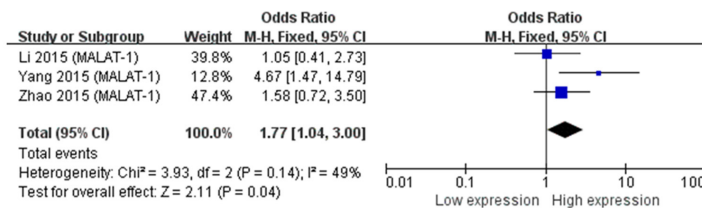HOTAIR for lymph node metastasis; B. HOTAIR for T stage; C. HOTAIR for TNM s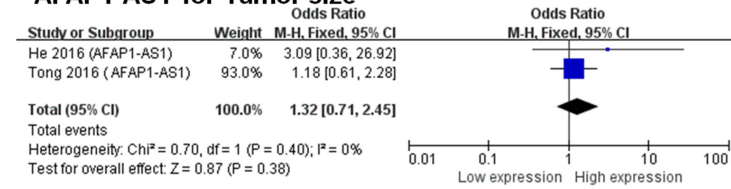tage; D. MALAT-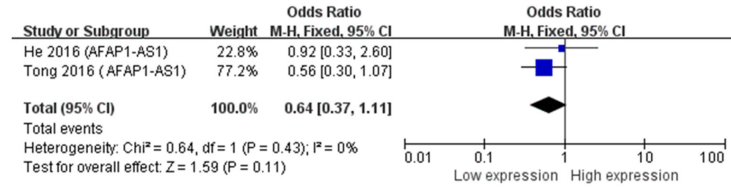1 for differentiation; E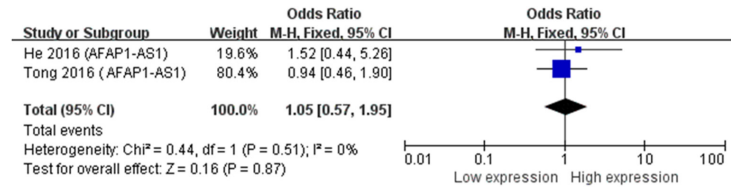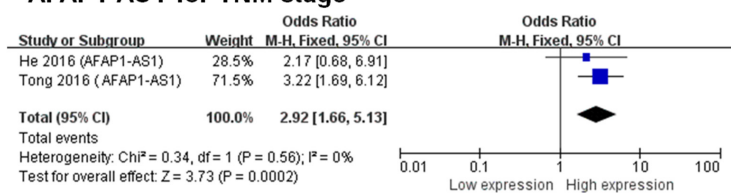. MALAT-1 for lymph node metastasis; F. MALAT-1 for T stage; G. AFAP1-AS1 for tumor size; H. AFAP1-AS1 for tobacco status; I. AFAP1-AS1 for alcohol status; J. AFAP1-AS1 for TNM stage.

analyzed in the studies included tissue (n = 32), serum (n = 1), and plasma (n = 2). Notably, 96.3% of eligible studies on prognosis had Newcastle-Ottawa Scale (NOS) scores ≥ 7 (Supplementary Table 1), denoting high quality. In addition, all publications on diagnosis had Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) scores ≥ 4, also indicating high quality (Supplementary Figure 1). However, there were obvious shortcomings in "patient selection" and "index text", suggesting the presence of major bias.

Clinicopathological features

A total of 23 lncRNAs, described in 26 studies, were correlated with clinicopathological features of ESCC. Specifically, expression of AFAP1-AS1 [14, 15], CCAT2 [16], FOXCUT [17], UCA1 [18], ZEB1-AS1 [19], BANCR [20], BC032469 [21], BC200 [22], CASC9 [23], H19 [24], HOTAIR [10, 25, 26], MALAT-1 [13, 27, 28], NEAT1 [29], PCAT-1 [30], PlncRNA-1 [31], SPRY4-IT1 [32], and TUG1 [33] was upregulated, while that of CASC2 [34], LOC285194 [35], and RP11-766N7.4 [36] was downregulated in ESCC. Moreover, a three-lncRNA signature (including the lncRNAs ENST00000435885.1, XLOC_013014, and ENST00000547963.1) [37] was associated with higher risk of ESCC. In contrast, most studies found no correlation between lncRNAs and age, gender, tumor location, and tobacco or alcohol status. Additionally, only a small number of articles reported correlation of ESCC-related lncRNAs and tumor size, differentiation, T-stage, and distant metastasis. In contrast, most studies suggested a prominent correlation of dysregulated lncRNAs with lymph node metastasis (LNM) and tumor-node-metastasis (TNM) stage (**Table 1**).

HOTAIR, MALAT-1, and AFAP1-AS1 were all investigated in at least 2 of the enrolled studies. This meta-analysis was subsequently carried out to assess the strength of the relationship between these lncRNAs and clinicopathological features of ESCC. Extracted study data were divided into different groups based on distinct disease features. Results revealed that HOTAIR upregulation was positively correlated

with LNM (odds ratio [OR] = 3.29, 95% confidence interval [CI]: 1.18-9.16, P = 0.02) and TNM stage (OR = 6.93, 95% CI: 2.79-17.18, P < 0.0001) (**Figure 2A**, **2C**). However, no clear correlation of HOTAIR overexpression with T-stage was found (OR = 2.15, 95% CI: 0.24-19.47, P = 0.50) (**Figure 2B**). There was obvious evidence of interstudy heterogeneity in analyses of LNM (I^2 = 74%, P = 0.02) and T-stage (I^2 = 83%, P = 0.02), but not of TNM stage (I^2 = 0%, P = 0.95). Thus, a fixed effects model was used to analyze the latter. Consequently, evidence linking high HOTAIR expression with LNM should be interpreted with caution.

Marked correlation of MALAT-1 upregulation with LNM (OR = 1.77, 95% CI: 1.04-3.00, P = 0.04) (**Figure 2E**) was detected using a fixed effects model, due to acceptable inter-study heterogeneity (I^2 = 49%, P = 0.14). No significant correlation between MALAT-1 expression and tumor differentiation or T-stage was detected (**Figure 2D**, **2F**).

Next, in the absence of significant interstudy heterogeneity (T stage, I^2 = 0%, P = 0.40; tobacco status, I^2 = 0%, P = 0.43; alcohol status I^2 = 0%, P = 0.51; TNM stage, I^2 = 0%, P = 0.56), a fixed effects model was utilized in analyses related to AFAP1-AS1. Results showed that AFAP1-AS1 upregulation was correlated only with TNM stage (OR = 2.92, 95% CI: 1.66-5.13, P = 0.0002) (**Figure 2G-J**).

Prognosis

A total of 27 studies, encompassing 3,140 ESCC patients, were included in this meta-analysis of the correlation between lncRNA expression and overall survival (OS) (**Table 2**). Of these, 5 studies reported disease-free survival (DFS) data and 2 reported metastasis-free survival/progression-free survival (MFS/PFS) data. High expression of AFAP1-S1 [14], BANCR [20], BC032469 [21], BC200 [22], CCAT2 [16], CFLAR-AS1 [38], FOXCUT [17], HOTAIR [10, 25, 26, 39, 40], Linc00152 [38], MALAT-1 [13, 27], NEAT1 [29], NONHSAT104436 [41], NONHSAT126998 [41], NR_024015 [42], PCAT-1 [30], POU3F3 [38], SPRY4-IT1 [32], TUG1 [33],

Table 2. Summary of lncRNAs used as prognostic biomarkers of ESCC

Studies	LncRNAs	Population	Expression	Case number High	Case number Low	Detected sample	Detection method	Cut-off	Outcomes	MA	HR availability	Follow-up month
Cao 2012	HOTAIR	Chinese	Up-regulation	27	51	FT	qRT-PCR	Fold-change	OS	Yes	Directly	60
Cao 2014	LOC285194	Chinese	Down-regulation	71	71	FT	qRT-PCR	Median	OS/DFS	Yes	Directly	36
Cao 2014	SPRY4-IT1	Chinese	Up-regulation	46	46	FT	qRT-PCR	Median	OS	Yes	Directly	60
Cao 2015	PCAT-1	Chinese	Up-regulation	65	39	FT	qRT-PCR	Median	OS	Yes	Directly	60
Cao 2016	BANCR	Chinese	Up-regulation	71	71	FT	qRT-PCR	Median	OS/DFS	Yes	Indirectly	60
Cao 2016	BC200	Chinese	Up-regulation	35	35	FT	qRT-PCR	Median	OS/DFS	Yes	Directly	48
Chen 2015	HOTAIR	Chinese	Up-regulation	55	64	FT	qRT-PCR	Fold-change	OS	No	Indirectly	72
	LOC645638	Chinese	Down-regulation	52	67	FT	qRT-PCR	Fold-change	OS	No	Indirectly	72
	TMEM106A	Chinese	Down-regulation	59	60	FT	qRT-PCR	Fold-change	OS	No	Indirectly	72
Chen 2017	RP11-766N7.4	Chinese	Down-regulation	29	21	FT	qRT-PCR	Median	OS	No	Indirectly	60
Dong 2016	NR_024015	Chinese	Up-regulation	92	62	FT	qRT-PCR	Fold-change	OS	No	Indirectly	60
Fang 2014	FOXCUT	Chinese	Up-regulation	45	37	FT	qRT-PCR	Mean	OS	No	Indirectly	72
Feng 2015	NEAT1	Chinese	Up-regulation	54	42	FT	qRT-PCR	Youden index	OS	Yes	Directly	78
Guo 2017	MEG3	Chinese	Down-regulation	26	117	FT	qRT-PCR	Fold-change	OS	Yes	Directly	60
Han 2013	HOTAIR	Chinese	Up-regulation	30	70	FT	qRT-PCR	Fold-change	OS	No	Directly	60
He 2014	3-lncRNA signature	Chinese	High-risk	37	23	FT	qRT-PCR	NA	OS	Yes	Directly	72
Huang 2016	ENST00000480669	Chinese	Down-regulation	29	44	FT	qRT-PCR	Fold-change	OS	Yes	Directly	48
	NONHSAT104436	Chinese	Up-regulation	47	26	FT	qRT-PCR	Fold-change	OS	Yes	Directly	48
	NONHSAT112918	Chinese	Up-regulation	45	28	FT	qRT-PCR	Fold-change	OS	Yes	Directly	48
	NONHSAT126998	Chinese	Up-regulation	39	34	FT	qRT-PCR	Fold-change	OS	Yes	Directly	48
Jia 2013	HOTAIR	Chinese	Up-regulation	90	47	FT	qRT-PCR	ROC	OS/MFS	Yes	Directly	80
Li 2014	UCA1	Chinese	Up-regulation	41	49	FT	qRT-PCR	Mean	OS	Yes	Directly	60
Li 2015	MALAT-1	Chinese	Up-regulation	45	32	FT	qRT-PCR	Fold-change	OS/DFS	Yes	Directly	5-92
Lv 2016	MEG3	Chinese	Down-regulation	16	80	FT	qRT-PCR	Fold-change	OS	No	Directly	70-120
Tong 2016	AFAP1-AS1	Chinese	Up-regulation	81	81	FT	qRT-PCR	Median	OS/PFS	Yes	Directly	60
Wang 2013	HOTAIR	Chinese	Up-regulation	49	44	PET	ISH	Staining index	OS	No	Indirectly	70
Wang 2015	ZEB1-AS1	Chinese	Up-regulation	44	43	FT	qRT-PCR	Median	OS/DFS	Yes	Directly	60
Yang 2016	BC032469	Chinese	Up-regulation	35	10	FT	qRT-PCR	Fold-change	OS	No	Indirectly	50
Yu 2016	TUG1	Chinese	Up-regulation	109	109	FT	qRT-PCR	Median	OS	Yes	Directly	60
Zhao 2015	MALAT-1	Chinese	Up-regulation	103	34	FT	qRT-PCR	Fold-change	OS	No	Indirectly	36
Zheng 2016	CFLAR-AS1	Chinese	Up-regulation	114	91	Plasma	qRT-PCR	Fold-change	OS	No	Directly	60
	Linc00152	Chinese	Up-regulation	131	74	Plasma	qRT-PCR	Fold-change	OS	No	Directly	60
	POU3F3	Chinese	Up-regulation	118	87	Plasma	qRT-PCR	Fold-change	OS	No	Directly	60
Zhu 2015	CCAT2	Chinese	Up-regulation	115	114	FT	qRT-PCR	Median	OS	Yes	Directly	60

Abbreviations: LncRNA, long non-coding RNA; NA, not available; FT, frozen tissue; PET, paraffin-embedded tissue; qRT-PCR, quantities reverse transcription polymerase chain reaction; ISH, in situ hybridization; ROC, receiver operator characteristic; OS, overall survival; DFS, disease free survival; MFS, metastasis free survival; PFS, progression free survival; HR, hazard ratio; MA, multivariate analysis.
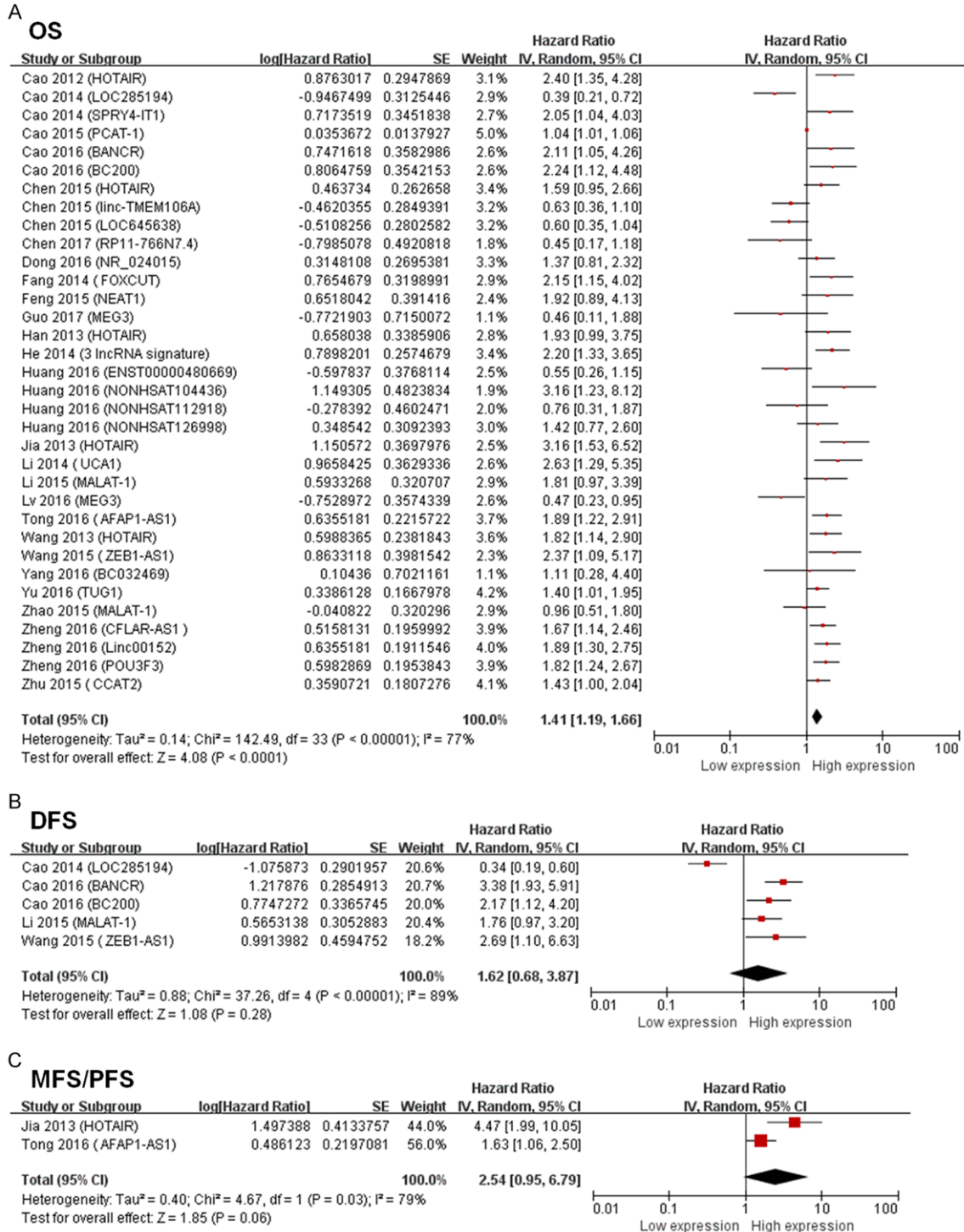
Figure 3. Forest plots of studies evaluating hazard ratios of lncRNAs expression and prognosis of ESCC. A. Overall survival (OS); B. Disease free survival (DFS); C. Metastasis free survival/progression free survival (MFS/PFS).

the three-lncRNA signature [37], UCA1 [18], and ZEB1-AS1 [19], and low expression of EN-ST00000480669 [41], LOC285194 [35], LOC-645638 [39], MEG3 [43, 44], RP11-766N7.4 [36], and linc-TMEM106A [39] was associated with poor prognosis. No statistically significant association was detected between NONHSAT-112918 expression and OS [41]. The pooled
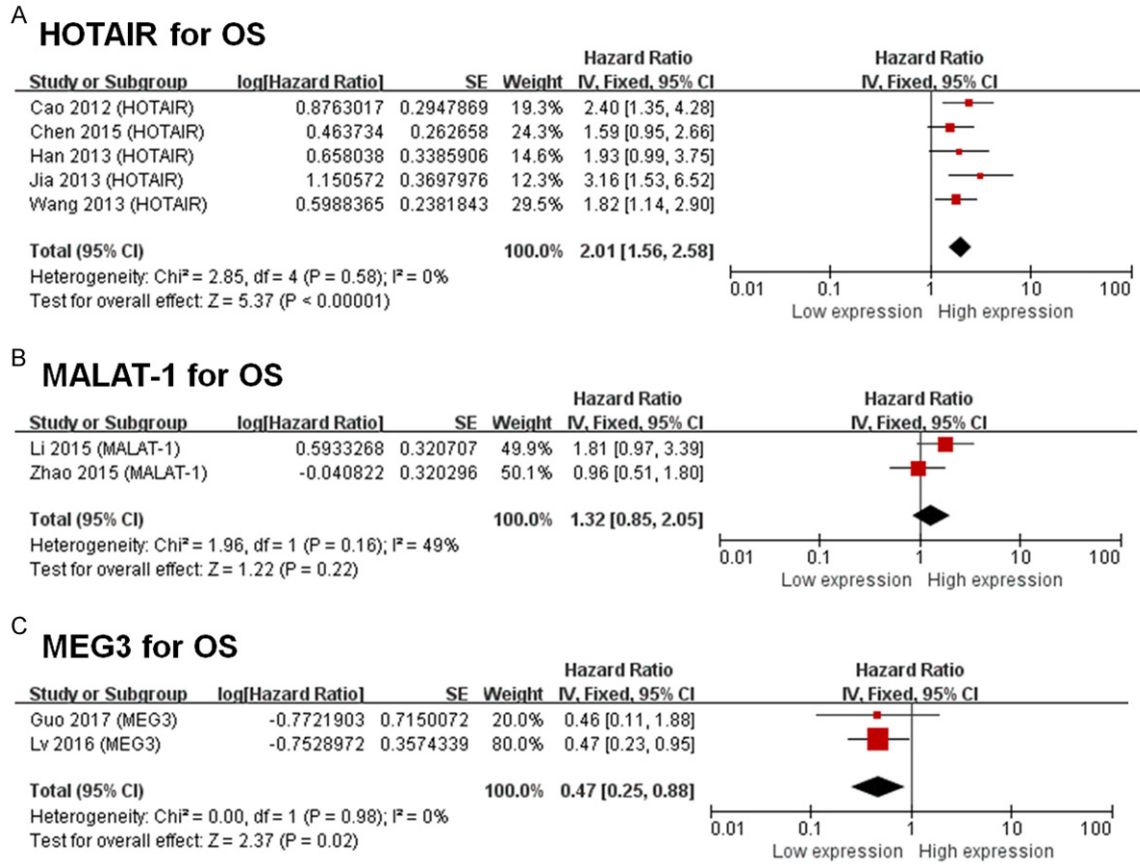
Figure 4. Forest plots of studies evaluating hazard ratios of aberrant expression of lncRNAs and overall survival of ESCC. A. HOTAIR; B. MALAT-1; C. MEG3.

hazard ratio (HR) of the 27 eligible studies assessing the prognostic value of lncRNAs was 1.41 (95% CI: 1.19-1.66, $P < 0.0001$), with apparent evidence of interstudy heterogeneity ($I^2 = 77\%$, $P < 0.00001$) (**Figure 3A**). No significant correlation was found for lncRNAs levels and reported DFS and MFS/PFS data ($P = 0.28$ and $P = 0.06$, respectively). However, respective analyses showed significant interstudy heterogeneity ($I^2 = 89\%$, $P < 0.00001$, and $I^2 = 79\%$, $P < 0.03$, respectively) (**Figure 3B**, **3C**). Thus, again, these conclusions should be interpreted with caution.

Three lncRNAs, HOTAIR, MALAT-1, and MEG3, were investigated by at least 2 studies. Therefore, respective meta-analyses were conducted concerning the relationships between expression of these lncRNAs and ESCC prognosis. Fixed effect models were applied in these three analyses in the absence of distinct interstudy heterogeneity (HOTAIR, $I^2 = 0\%$, $P = 0.58$; MALAT-1, $I^2 = 49\%$, $P = 0.16$; MEG3 $I^2 = 0\%$, $P = $

0.98, respectively). Results revealed that HOTAIR upregulation was correlated with shorter OS (HR = 2.01, 95% CI: 1.56-2.58, $P < 0.00001$) (**Figure 4A**). No significant correlation was observed between MALAT-1 levels and OS (HR = 1.32, 95% CI: 0.85-2.05, $P = 0.22$) (**Figure 4B**). The pooled HR of two studies assessing MEG3 expression indicated that low MEG3 levels were associated with worse OS in ESCC patients (HR = 0.47, 95% CI: 0.25-0.88, $P = 0.02$) (**Figure 4C**).

Diagnosis

Only 4 studies assessed the diagnostic value of lncRNAs on ESCC. These reports evaluated AFAP1-AS1 [14], HOTAIR [11], and POU3F3 [45], as well as the combination of 3 lncRNAs (Linc-00152, CFLAR-AS1, and POU3F3) [38]. Specimens in these 4 studies included tissue (n = 1), serum (n = 1), and plasma (n = 2) (**Table 3**). Pooled sensitivity (SEN) and specificity (SPE) values for diagnosing ESCC were 81% (95%

Table 3. Summary of lncRNAs used as diagnostic biomarkers of ESCC

Studies	LncRNAs	Population	Expression	Detected sample	Detection method	SE (%)	SP (%)	AUC	Case number		QUADAS-2 scores
									Cancer	Control	
Cao 2015	POU3F3	Chinese	Up-regulation	Plasma	qRT-PCR	72.8	89.4	0.842	147	123	5
Tong 2016	AFAP1-AS1	Chinese	Up-regulation	FT	qRT-PCR	79.4	73.3	0.802	162	162	4
Wang 2017	HOTAIR	Chinese	Up-regulation	Serum	qRT-PCR	56	90	0.793	50	20	5
Zheng 2016	Merged 3 lncRNAs	Chinese	Up-regulation	Plasma	qRT-PCR	93.88	64.58	0.765	205	210	5

Abbreviations: LncRNA, long non-coding RNA; FT, frozen tissue; qRT-PCR, quantities reverse transcription polymerase chain reaction; SE, sensitivity; SP, specificity; AUC, area under the curve; QUADAS, quality assessment of diagnostic accuracy studies.

CI: 77%-84%) and 74% (95% CI: 70%-78%), respectively, with significant evidence of heterogeneity (I^2 = 93.9%, P < 0.001 and I^2 = 90.1%, P < 0.001, respectively) (**Figure 5A**, **5B**). Therefore, a random effects model was used to estimate the overall performance of lncRNAs in diagnosing ESCC. Results indicated a pooled positive likelihood ratio (PLR) of 3.66 (95% CI: 2.44-5.50), a pooled negative likelihood ratio (NLR) of 0.26 (95% CI: 0.15-0.46), and a pooled diagnostic odds ratio (DOR) of 17.44 (95% CI: 10.43-29.1) (**Figure 5C-E**). Furthermore, a summary receiver operator characteristic (SROC) curve was constructed. The corresponding area under the curve (AUC) was 0.87 (95% CI: 0.84-0.90) (**Figure 6**). These results indicate that some lncRNAs are highly valuable in diagnosing ESCC. Marked heterogeneity could be observed in this analysis. However, subgroup analysis or meta-regression was not carried out, given the small study number and small sampling size. As a result, the diagnostic effects of lncRNAs on ESCC should be further validated in future studies with larger sample sizes.

Publication bias

No publication bias among the enrolled studies was detected, according to Begg's tests (P > 0.05; **Figure 7A-C**) [46]. No conclusive graph could be generated for data derived from groups containing up to 3 related studies due to small study number. Thus, publication bias was not assessed in such groups. Moreover, publication bias among eligible studies on diagnosis was evaluated using Deeks' funnel plot asymmetry test [47]. Here, P = 0.64 also indicates no presence of publication bias in the meta-analysis (**Figure 7D**).

Discussion

ESCC is one of the leading causes of cancer related deaths, worldwide [3]. Therefore, novel

effective biomarkers for ESCC diagnosis and prediction of lymph node status, metastasis, and survival are urgently needed. Emerging studies in recent years have pointed out that dysregulated lncRNAs act as oncogenes or tumor suppressors in ESCC [48], representing potential diagnostic and prognostic biomarkers, as well as promising therapeutic targets in ESCC [10, 11]. However, limitations imposed by small sample sizes and noisiness of microarray data have yielded inconsistent conclusions. This systematic review and meta-analysis aimed at assessing the clinical value of lncRNAs in ESCC was, therefore, conducted. To the best of our knowledge, this is the first article comprehensively describing the correlation of lncRNAs expression with prognosis and diagnosis of patients with ESCC.

This meta-analysis began by evaluating the correlation between lncRNAs expression and important clinicopathological features of ESCC. Results revealed that aberrant expression of lncRNAs is associated with both high TNM stage and LNM. HOTAIR was the most extensively studied lncRNA among enrolled studies. Pooled data results suggest that high HOTAIR expression is positively correlated with LNM and high TNM stage in ESCC patients. Not surprisingly, a recent meta-analysis about HOTAIR in ESCC indicated that HOTAIR upregulation displays a remarkable correlation with positive LNM (risk ratios [RR] = 1.96, 95% CI: 1.07-3.60, P = 0.03, random effects model) [49], agreeing partly with present results. Although these data seem to indicate that HOTAIR is an outstanding biomarker for predicting LNM and TNM stage in ESCC, obvious heterogeneity observed in their analyses and present analyses (I^2 = 75% and I^2 = 74%, respectively), suggesting the need for further validation of the predictive value HOTAIR in ESCC, especially for LNM. In addition to HOTAIR, two other lncRNAs, MALAT-1 and AFAP1-AS1, were investigated in at least
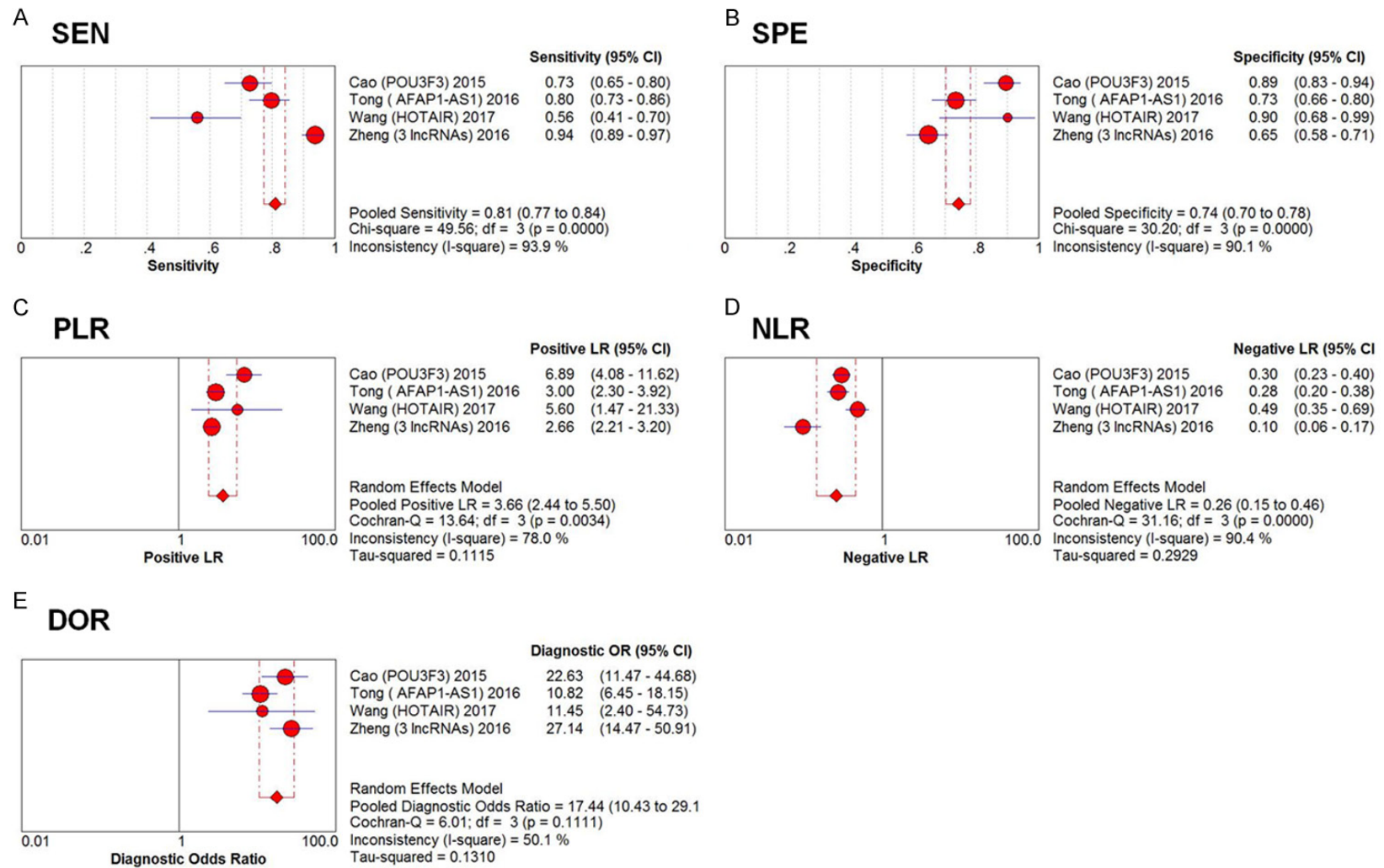
Figure 5. Forest plots of studies evaluating pooled diagnostic indexes of lncRNAs for diagnosis of ESCC. A. Sensitivity (SEN); B. Specificity (SPE); C. Positive likelihood ratio (PLR); D. Negative likelihood ratio (NLR); E. Diagnostic odds ratio (DOR).
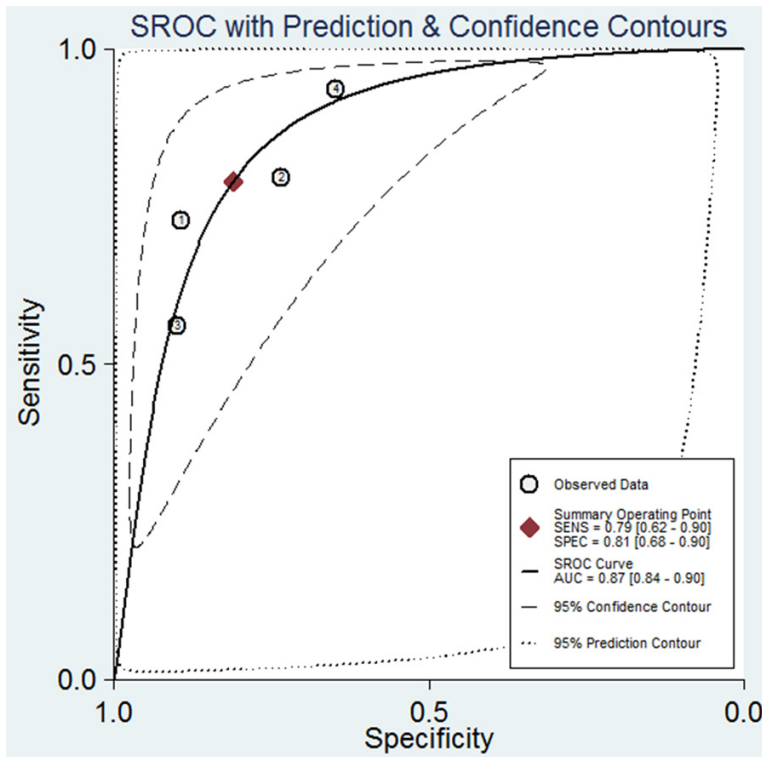
Figure 6. Summary receiver operating characteristic curves (SROC) for lncRNAs expression outline in diagnosis of ESCC. (1) POU3F3; (2) AFAP1-AS1; (3) HOTAIR; (4) Merged 3 lncRNAs (Linc00152, CFLAR-AS1 and POU3F3).

2.587, 95% CI: 2.054-3.259, $P < 0.001$). A similar conclusion was also reached by Miao et al. [51] in their meta-analysis involving 63 studies on various solid carcinomas (HR = 2.21, 95% CI: 1.77-2.74, $P < 0.00001$). While the present study observed no statistically significant correlation between MALAT-1 expression and ESCC outcomes, strong correlation between low MEG3 expression and poor ESCC prognosis was evident in this analysis. Therefore, based on present and past analyses, the conclusion that HOTAIR and MEG3 may serve as promising biomarkers for predicting survival of ESCC patients can be drawn.

2 studies and also analyzed in the present work. Results indicated that high MALAT-1 expression is correlated with LNM, while increased cellular expression of AFAP1-AS1 is markedly correlated with TNM stage. Considering the small sample sizes of corresponding studies, however, the clinical values of MALAT-1 and AFAP1-AS1 in ESCC should be further validated.

Regarding the prognostic value of aberrantly expressed lncRNAs on ESCC, results revealed that specific lncRNAs are associated with shortened survival. Specifically, overexpression of 21 lncRNAs, along with underexpression of another 4 lncRNAs, was found to be associated with poor prognosis. Of those, only 3 dysregulated lncRNAs, HOTAIR, MALAT-1, and MEG3, were assessed by more than 2 studies. Present meta-analysis revealed that high HOTAIR expression is a strong predictor of poor OS in ESCC patients. Consistent with this conclusion, Wang et al. [50] linked HOTAIR upregulation with poor OS in patients with digestive system malignancies, including ESCC (pooled HR =

In recent years, development of endoscopic procedures and surgical techniques has improved prognosis for ESCC patients. However, the o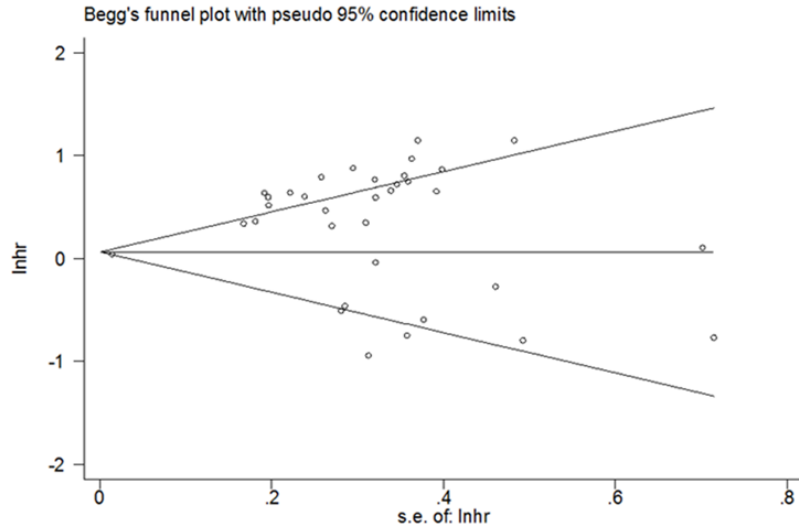verall 5-year survival for advanced ESCC remains very low [3]. The lack of effective diagnostic biomarkers is clearly associated with delayed ESCC detection. Moreover, diagnosis of ESCC still relies on the pathological method, which is linked to potential tissue damage. Three out of the 4 eligible studies on ESCC diagnosis were carried out on serum or plasma specimens, indicating that lncRNAs may function as novel noninvasive blood markers for ESCC detection. Moreover, pooled data illustrated high overall sensitivity (81%) and specificity (74%) of lncRNAs in distinguishing patients with ESCC from healthy controls, with a SROC's AUC of 0.87. Therefore, although available data is scarce and additional validation studies are clearly required, lncRNAs can be considered highly efficient diagnostic biomarkers for ESCC.

HOTAIR, first discovered by Rinn and his collaborators in 2007, is a lncRNA located in chromosome 12q13.13 between the HOXC11 and HOXC12 genes [52]. As the most widely studied lncRNA in ESCC, its overexpression has been detected in both cancer tissues and blood sam-
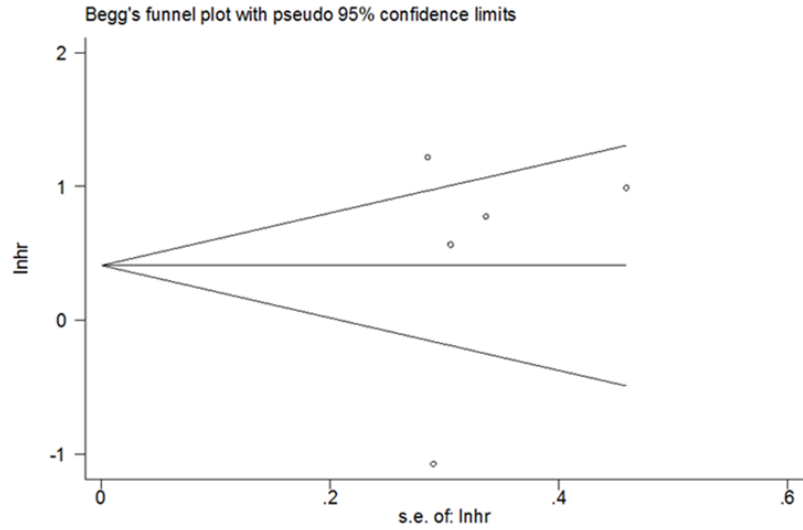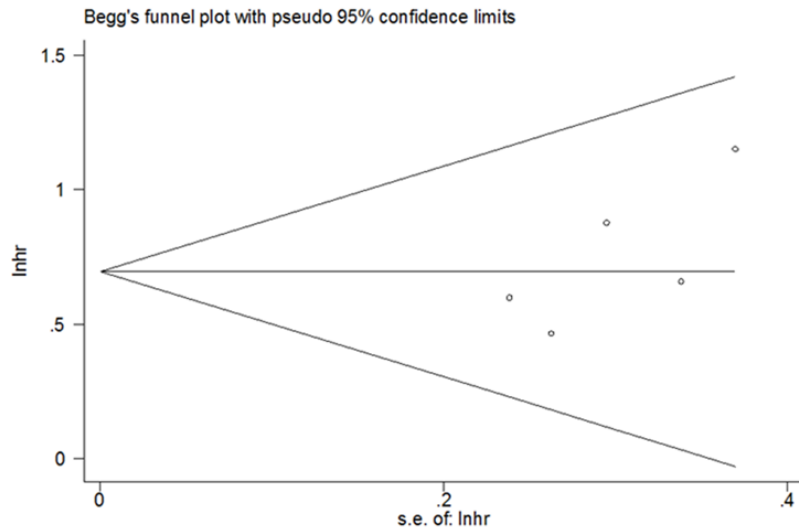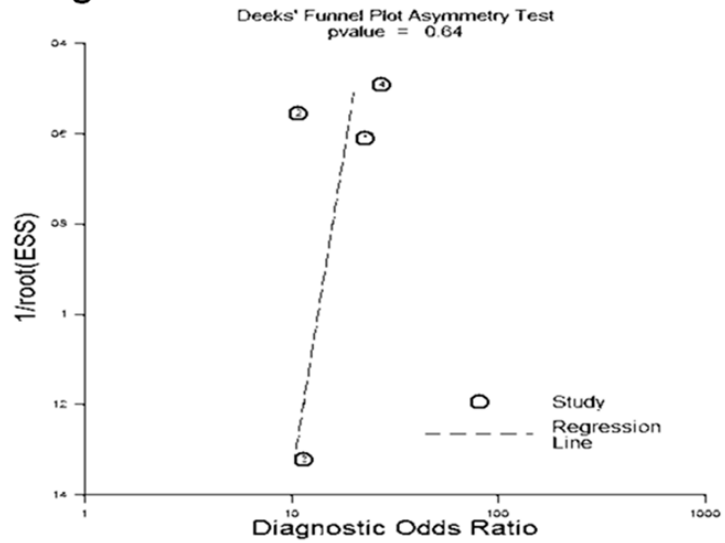
A
OS
Begg's funnel plot with pseudo 95% confidence limits

B
DFS
Begg's funnel plot with pseudo 95% confidence limits

C
HOTAIR for OS
Begg's funnel plot with pseudo 95% confidence limits

D
Diagnosis
Deeks' Funnel Plot Asymmetry Test
pvalue = 0.64

Figure 7. Begg's and Deeks' funnel plots for publication bias. A. Overall survival (OS), *P* = 0.260; B. Disease free survival (DFS), *P* = 0.806; C. HOTAIR for OS, *P* = 0.221; D. Diagnosis, *P* = 0.64.

ples from ESCC patients [10, 11, 25, 26, 40]. Accordingly, this study suggests high clinical value for HOTAIR in ESCC diagnosis and LNM, TNM stage, and OS prediction. HOTAIR functions as a scaffold for binding polycomb repressive complex 2 (PRC2) and lysine-specific demethylase 1 (LSD1) complexes, which could lead to H3K27 methylation and H3K4 demethylation, thus resulting in transcriptional repression of differentiation genes [53]. Cao et al. [26] demonstrated that suppressing HOTAIR expression in ESCC cells impaired cell invasiveness and migration, while increasing apoptosis. Moreover, another study suggested that upregulation of HOTAIR promoted ESCC cell proliferation and tumor metastasis in a mouse xenograft model [40]. These findings support a crucial role for HOTAIR in tumorigenesis and development of ESCC.

The present study had some limitations. First, no publications with negative results were included in the analysis, which may have led to hidden publication bias. Second, patients enrolled in this meta-analysis were predominately Asian, which may restrict generalization of present conclusions to other ethnicities. Third, qRT-PCR was used in all but one study to detect lncRNAs and selection of qRT-PCR primers and cut-off values to distinguish low and high lncRNAs levels differed between studies, even for the same lncRNA. Fourth, different selection of survival endpoints and insufficient follow-ups may have resulted in interstudy heterogeneity. Fifth, 8 articles enrolled in this study did not directly provide HRs and 95% CIs. As a result, HRs were calculated by extracting data from Kaplan-Meier curves, which may have given rise to variance errors. Sixth, diagnostic analysis results presented evident heterogeneity. No subgroup or meta-regression analysis could be conducted due to small study numbers and sample sizes.

In conclusion, this is the first meta-analysis to assess the clinical value of lncRNAs in ESCC. Despite the abovementioned limitations, present results suggest that specific lncRNAs, especially HOTAIR, are useful prognostic and diagnostic biomarkers for ESCC. Further comprehensive and large-scale studies are necessary to validate present findings.

Materials and methods

Systematic review

This meta-analysis was carried out in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [54].

Literature retrieval strategies

PubMed, Embase, and Ovid databases were comprehensively searched through August 22, 2017, to identify all primary studies evaluating the clinical value of candidate lncRNAs as ESCC biomarkers. The following search terms were used: ("esophageal carcinoma" or "esophageal neoplasm" or "esophageal tumor" or "esophageal squamous cell carcinoma" or "ESCC") and ("Long non-coding RNA" or "Long intergenic non-coding RNA" or "lncRNA" or "LincRNA"). References in relevant articles (including review articles) were also reviewed.

Inclusion and exclusion criteria

Inclusion criteria used to determine study eligibility were as follows: (1) Studies in which all patients were diagnosed with ESCC; (2) Studies in which patients were divided into ESCC group and EAC group if ESCC and EAC patients both existed; (3) Studies that detected lncRNAs expression in ESCC tissues or blood samples; (4) Studies detecting the association of lncRNAs with ESCC clinicopathological features, prognosis, or diagnosis; and (5) Studies providing sufficient information for extraction or calculation of individual OR, HR, and 95% CI. Articles meeting the following exclusion criteria were removed: (1) Duplicate publications; (2) Non-English studies; (3) Reviews, letters, comments, case reports, or meeting abstracts; (4) Sample size of less than 40 cases; and (5) Studies without complete data.

Data extraction and quality assessment

Eligible articles were reviewed by two investigators (Canlin Yang and Fei Li), independently, and any disagreements were resolved by discussion with a third investigator (Wenhao Shen). Information extracted included name of

author, publication date, population, type of lncRNAs, status of lncRNA expression, detection methods, follow-up period, clinicopathological parameters, HRs with 95% CIs for survival analysis, and diagnostic results. Validation group results were extracted for studies containing both training and validation tests. HRs and matched 95% CIs were calculated from Kaplan-Meier curves based on the methods illustrated by Tierney et al. [55].

The methodological quality of studies assessing prognosis was evaluated using the Newcastle-Ottawa Scale (NOS) [56]. The NOS score had a maximum of nine points and studies with scores ≥ 7 points were considered high quality. The quality of studies assessing diagnosis was evaluated in accordance with the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) in Review Manager 5.3 (The Nordic Cochrane Center, Rigshospitalet, Denmark) [57]. The QUADAS-2 tool consisted of four key domains: patient selection, index test, reference standard, and flow and timing, which were applied to aid judgment on risk of bias and concerns about applicability; it had a maximum score of 7 points, and studies with a score ≥ 4 were rated as high quality.

Statistical analysis

All statistical analyses were performed using Review Manager 5.3 (The Nordic Cochrane Center, Rigshospitalet, Denmark), STATA 12.0 (Stata Corporation, College Station, TX, USA), and Meta-Disc 1.4 (XI Cochrane Colloquium, Barcelona, Spain). ORs and 95% CIs were employed to assess the correlation of lncRNAs expression with clinicopathological features. HRs and 95% CIs were applied to evaluate the relationship between lncRNAs expression and survival. HRs and 95% CIs were extracted directly from original articles, or calculated from Kaplan-Meier survival curves using Engauge Digitizer 4.1 software, in accordance with the method proposed by Tierney et al. [55]. However, this last method might have generated errors due to variation. An HR > 1 implies worse survival for patients in the high lncRNAs expression group, while an HR < 1 indicates poor survival for patients in the low lncRNAs expression group. $P < 0.05$ is considered as statistically significant in estimating OR and HR when the 95% CI did not cover the value "1". Furthermore, SEN, SPE, PLR, NLR, DOR, AUC and their 95% CIs, together with SROC curves were used

to evaluate the value of lncRNAs in diagnosing ESCC. Heterogeneity of analyses was tested using Cochran's Q test and Higgins' (I-squared) statistic [58]. $I^2 > 50\%$ or $P < 0.1$ suggests significant heterogeneity among the studies considered. A fixed-effects model was used in the presence of acceptable heterogeneity of related studies. Otherwise, a random-effects model was adopted. Publication bias was evaluated using Begg's tests and Deeks' tests with funnel plots and $P < 0.05$ is deemed as statistically significant.

Disclosure of conflict of interest

None.

Abbreviations

AUC, area under the curve; CI, confidence interval; DOR, diagnostic odds ratio; DFS, disease-free survival; EAC, esophageal adenocarcinoma; EC, esophageal carcinoma; ESCC, esophageal squamous cell carcinoma; HOTAIR, HOX transcript antisense RNA; HR, hazard ratio; ISH, in situ hybridization; LncRNA, long non-coding RNA; LNM, lymph node metastasis; LSD1, lysine-specific demethylase 1; MA, multivariate analysis; MALAT-1, metastasis-associated lung adenocarcinoma transcript 1; MFS, metastasis-free survival; NA, not available; NLR, negative likelihood ratio; NOS, Newcastle-Ottawa Scale; OR, odds ratio; OS, overall survival; PET, paraffin-embedded tissue; PFS, progression-free survival; PLR, positive likelihood ratio; PRC2, polycomb repressive complex 2; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; qRT-PCR, quantitative reverse transcription polymerase chain reaction; QUADAS, quality assessment of diagnostic accuracy studies; ROC, receiver operator characteristic; RR, risk ratio; SEN, sensitivity; SPE, specificity; SROC curve, summary receiver operating characteristic curve; TNM stage, tumor-node-metastasis stage.

Address correspondence to: Dr. Junxing Huang, Department of Oncology, Taizhou People's Hospital, No. 366 Taihu Road, Taizhou 225300, Jiangsu, P. R. China. Tel: +86-523-86361458; Fax: +86-523-86-225199; E-mail: hjxtz@sina.cn

References

[1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.

[2] Pennathur A, Gibson MK, Jobe BA and Luketich JD. Oesophageal carcinoma. Lancet 2013; 381: 400-412.

[3] Lagergren J, Smyth E, Cunningham D and Lagergren P. Oesophageal cancer. Lancet 2017; 390: 2383-2396.

[4] Guttman M and Rinn JL. Modular regulatory principles of large non-coding RNAs. Nature 2012; 482: 339-346.

[5] Abraham JM and Meltzer SJ. Long noncoding RNAs in the pathogenesis of Barrett's esophagus and esophageal carcinoma. Gastroenterology 2017; 153: 27-34.

[6] Schmitt AM and Chang HY. Long noncoding RNAs in cancer pathways. Cancer Cell 2016; 29: 452-463.

[7] Yu X and Li Z. Long non-coding RNA HOTAIR: a novel oncogene (review). Mol Med Rep 2015; 12: 5611-5618.

[8] Gokmen-Polar Y, Vladislav IT, Neelamraju Y, Janga SC and Badve S. Prognostic impact of HOTAIR expression is restricted to ER-negative breast cancers. Sci Rep 2015; 5: 8765.

[9] Lee NK, Lee JH, Park CH, Yu D, Lee YC, Cheong JH, Noh SH and Lee SK. Long non-coding RNA HOTAIR promotes carcinogenesis and invasion of gastric adenocarcinoma. Biochem Biophys Res Commun 2014; 451: 171-178.

[10] Lv XB, Lian GY, Wang HR, Song E, Yao H and Wang MH. Long noncoding RNA HOTAIR is a prognostic marker for esophageal squamous cell carcinoma progression and survival. PLoS One 2013; 8: e63516.

[11] Wang W, He X, Zheng Z, Ma X, Hu X, Wu D and Wang M. Serum HOTAIR as a novel diagnostic biomarker for esophageal squamous cell carcinoma. Mol Cancer 2017; 16: 75.

[12] Gutschner T, Hammerle M, Eissmann M, Hsu J, Kim Y, Hung G, Revenko A, Arun G, Stentrup M, Gross M, Zornig M, MacLeod AR, Spector DL and Diederichs S. The noncoding RNA MALAT1 is a critical regulator of the metastasis phenotype of lung cancer cells. Cancer Res 2013; 73: 1180-1189.

[13] Cao X, Zhao R, Chen Q, Zhao Y, Zhang B, Zhang Y, Yu J, Han G, Cao W, Li J and Chen X. MALAT1 might be a predictive marker of poor prognosis in patients who underwent radical resection of middle thoracic esophageal squamous cell carcinoma. Cancer Biomark 2015; 15: 717-723.

[14] Zhou XL, Wang WW, Zhu WG, Yu CH, Tao GZ, Wu QQ, Song YQ, Pan P and Tong YS. High expression of long non-coding RNA AFAP1-AS1 predicts chemoradioresistance and poor prognosis in patients with esophageal squamous cell carcinoma treated with definitive chemoradiotherapy. Mol Carcinog 2016; 55: 2095-2105.

[15] Luo HL, Huang MD, Guo JN, Fan RH, Xia XT, He JD and Chen XF. AFAP1-AS1 is upregulated and promotes esophageal squamous cell carcinoma cell proliferation and inhibits cell apoptosis. Cancer Med 2016; 5: 2879-2885.

[16] Zhang X, Xu Y, He C, Guo X, Zhang J, He C, Zhang L, Kong M, Chen B and Zhu C. Elevated expression of CCAT2 is associated with poor prognosis in esophageal squamous cell carcinoma. J Surg Oncol 2015; 111: 834-839.

[17] Pan F, Yao J, Chen Y, Zhou C, Geng P, Mao H and Fang X. A novel long non-coding RNA FOXCUT and mRNA FOXC1 pair promote progression and predict poor prognosis in esophageal squamous cell carcinoma. Int J Clin Exp Pathol 2014; 7: 2838-2849.

[18] Li JY, Ma X and Zhang CB. Overexpression of long non-coding RNA UCA1 predicts a poor prognosis in patients with esophageal squamous cell carcinoma. Int J Clin Exp Pathol 2014; 7: 7938-7944.

[19] Wang YL, Bai Y, Yao WJ, Guo L and Wang ZM. Expression of long non-coding RNA ZEB1-AS1 in esophageal squamous cell carcinoma and its correlation with tumor progression and patient survival. Int J Clin Exp Pathol 2015; 8: 11871-11876.

[20] Liu Z, Yang T, Xu Z and Cao X. Upregulation of the long non-coding RNA BANCR correlates with tumor progression and poor prognosis in esophageal squamous cell carcinoma. Biomed Pharmacother 2016; 82: 406-412.

[21] Lu C, Yang L, Chen H and Shan Z. Upregulated long non-coding RNA BC032469 enhances carcinogenesis and metastasis of esophageal squamous cell carcinoma through regulating hTERT expression. Tumour Biol 2016; [Epub ahead of print].

[22] Zhao RH, Zhu CH, Li XK, Cao W, Zong H, Cao XG and Hu HY. BC200 LncRNA a potential predictive marker of poor prognosis in esophageal squamous cell carcinoma patients. Onco Targets Ther 2016; 9: 2221-2226.

[23] Pan Z, Mao W, Bao Y, Zhang M, Su X and Xu X. The long noncoding RNA CASC9 regulates migration and invasion in esophageal cancer. Cancer Med 2016; 5: 2442-2447.

[24] Tan D, Wu Y, Hu L, He P, Xiong G, Bai Y and Yang K. Long noncoding RNA H19 is up-regulated in esophageal squamous cell carcinoma and promotes cell proliferation and metastasis. Dis Esophagus 2017; 30: 1-9.

[25] Ge XS, Ma HJ, Zheng XH, Ruan HL, Liao XY, Xue WQ, Chen YB, Zhang Y and Jia WH. HOTAIR, a prognostic factor in esophageal squamous cell carcinoma, inhibits WIF-1 expression and activates Wnt pathway. Cancer Sci 2013; 104: 1675-1682.

[26] Chen FJ, Sun M, Li SQ, Wu QQ, Ji L, Liu ZL, Zhou GZ, Cao G, Jin L, Xie HW, Wang CM, Lv J, De W, Wu M and Cao XF. Upregulation of the long non-coding RNA HOTAIR promotes esophageal squamous cell carcinoma metastasis and poor prognosis. Mol Carcinog 2013; 52: 908-915.

[27] Yao W, Bai Y, Li Y, Guo L, Zeng P, Wang Y, Qi B, Liu S, Qin X, Li Y and Zhao B. Upregulation of MALAT-1 and its association with survival rate and the effect on cell cycle and migration in patients with esophageal squamous cell carcinoma. Tumour Biol 2016; 37: 4305-4312.

[28] Hu L, Wu Y, Tan D, Meng H, Wang K, Bai Y and Yang K. Up-regulation of long noncoding RNA MALAT1 contributes to proliferation and metastasis in esophageal squamous cell carcinoma. J Exp Clin Cancer Res 2015; 34: 7.

[29] Chen X, Kong J, Ma Z, Gao S and Feng X. Up regulation of the long non-coding RNA NEAT1 promotes esophageal squamous cell carcinoma cell progression and correlates with poor prognosis. Am J Cancer Res 2015; 5: 2808-2815.

[30] Shi WH, Wu QQ, Li SQ, Yang TX, Liu ZH, Tong YS, Tuo L, Wang S and Cao XF. Upregulation of the long noncoding RNA PCAT-1 correlates with advanced clinical stage and poor prognosis in esophageal squamous carcinoma. Tumour Biol 2015; 36: 2501-2507.

[31] Wang CM, Wu QQ, Li SQ, Chen FJ, Tuo L, Xie HW, Tong YS, Ji L, Zhou GZ, Cao G, Wu M, Lv J, Shi WH and Cao XF. Upregulation of the long non-coding RNA PlncRNA-1 promotes esophageal squamous carcinoma cell proliferation and correlates with advanced clinical stage. Dig Dis Sci 2014; 59: 591-597.

[32] Xie HW, Wu QQ, Zhu B, Chen FJ, Ji L, Li SQ, Wang CM, Tong YS, Tuo L, Wu M, Liu ZH, Lv J, Shi WH and Cao XF. Long noncoding RNA SPRY4-IT1 is upregulated in esophageal squamous cell carcinoma and associated with poor prognosis. Tumour Biol 2014; 35: 7743-7754.

[33] Jiang L, Wang W, Li G, Sun C, Ren Z, Sheng H, Gao H, Wang C and Yu H. High TUG1 expression is associated with chemotherapy resistance and poor prognosis in esophageal squamous cell carcinoma. Cancer Chemother Pharmacol 2016; 78: 333-339.

[34] He J, Tian H, Liu K and Ying J. Long non-coding RNA CASC2 serves as an onco-suppressor in human esophageal squamous cell carcinoma through inhibition of NF-κB pathway. Int J Clin Exp Med 2017; 10: 2800-2808.

[35] Tong YS, Zhou XL, Wang XW, Wu QQ, Yang TX, Lv J, Yang JS, Zhu B and Cao XF. Association of decreased expression of long non-coding RNA LOC285194 with chemoradiotherapy resistance and poor prognosis in esophageal squamous cell carcinoma. J Transl Med 2014; 12: 233.

[36] Yao GL, Pan CF, Xu H, Wei K, Liu B, Zhai R and Chen YJ. Long noncoding RNA RP11-766N7.4 functions as a tumor suppressor by regulating epithelial-mesenchymal transition in esophageal squamous cell carcinoma. Biomed Pharmacother 2017; 88: 778-785.

[37] Li J, Chen Z, Tian L, Zhou C, He MY, Gao Y, Wang S, Zhou F, Shi S, Feng X, Sun N, Liu Z, Skogerboe G, Dong J, Yao R, Zhao Y, Sun J, Zhang B, Yu Y, Shi X, Luo M, Shao K, Li N, Qiu B, Tan F, Chen R and He J. LncRNA profile study reveals a three-lncRNA signature associated with the survival of patients with oesophageal squamous cell carcinoma. Gut 2014; 63: 1700-1710.

[38] Hu HB, Jie HY and Zheng XX. Three circulating LncRNA predict early progress of esophageal squamous cell carcinoma. Cell Physiol Biochem 2016; 40: 117-125.

[39] Wei G, Luo H, Sun Y, Li J, Tian L, Liu W, Liu L, Luo J, He J and Chen R. Transcriptome profiling of esophageal squamous cell carcinoma reveals a long noncoding RNA acting as a tumor suppressor. Oncotarget 2015; 6: 17065-17080.

[40] Li X, Wu Z, Mei Q, Li X, Guo M, Fu X and Han W. Long non-coding RNA HOTAIR, a driver of malignancy, predicts negative prognosis and exhibits oncogenic activity in oesophageal squamous cell carcinoma. Br J Cancer 2013; 109: 2266-2278.

[41] Yao J, Huang JX, Lin M, Wu ZD, Yu H, Wang PC, Ye J, Chen P, Wu J and Zhao GJ. Microarray expression profile analysis of aberrant long noncoding RNAs in esophageal squamous cell carcinoma. Int J Oncol 2016; 48: 2543-2557.

[42] Han L, Liu S, Liang J, Guo Y, Shen S, Guo X, Dong Z and Guo W. A genetic polymorphism at miR-526b binding-site in the lincRNA-NR_024015 exon confers risk of esophageal squamous cell carcinoma in a population of North China. Mol Carcinog 2017; 56: 960-971.

[43] Dong Z, Zhang A, Liu S, Lu F, Guo Y, Zhang G, Xu F, Shi Y, Shen S, Liang J and Guo W. Aberrant methylation-mediated silencing of lncRNA MEG3 functions as a ceRNA in esophageal cancer. Mol Cancer Res 2017; 15: 800-810.
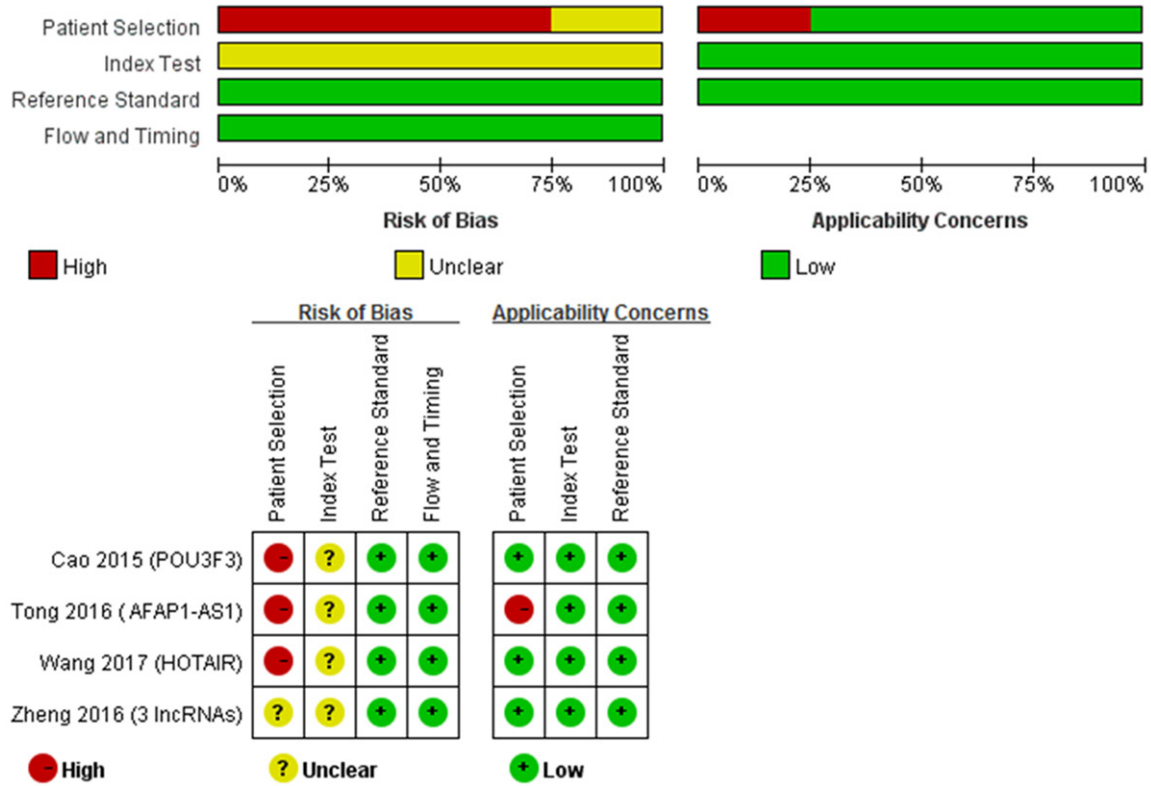
[44] Lv D, Sun R, Yu Q and Zhang X. The long non-coding RNA maternally expressed gene 3 activates p53 and is downregulated in esophageal squamous cell cancer. Tumour Biol 2016; [Epub ahead of print].

[45] Tong YS, Wang XW, Zhou XL, Liu ZH, Yang TX, Shi WH, Xie HW, Lv J, Wu QQ and Cao XF. Identification of the long non-coding RNA POU3F3 in plasma as a novel biomarker for diagnosis of esophageal squamous cell carcinoma. Mol Cancer 2015; 14: 3.

[46] Egger M, Davey Smith G, Schneider M and Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997; 315: 629-634.

[47] Deeks JJ, Macaskill P and Irwig L. The performance of tests of publication bias and other sample size effects in systematic reviews of diagnostic test accuracy was assessed. J Clin Epidemiol 2005; 58: 882-893.

[48] Shen WJ, Zhang F, Zhao X and Xu J. LncRNAs and esophageal squamous cell carcinoma-implications for pathogenesis and drug development. J Cancer 2016; 7: 1258-1264.

[49] Song W and Zou SB. Prognostic role of lncRNA HOTAIR in esophageal squamous cell carcinoma. Clin Chim Acta 2016; 463: 169-173.

[50] Wang S and Wang Z. Prognostic value of long noncoding RNA HOTAIR in digestive system malignancies. J Gastroenterol Hepatol 2015; 30: 1123-1133.

[51] Miao Z, Ding J, Chen B, Yang Y and Chen Y. HOTAIR overexpression correlated with worse survival in patients with solid tumors. Minerva Med 2016; 107: 392-400.

[52] Rinn JL, Kertesz M, Wang JK, Squazzo SL, Xu X, Brugmann SA, Goodnough LH, Helms JA, Farnham PJ, Segal E and Chang HY. Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. Cell 2007; 129: 1311-1323.

[53] Cai B, Song XQ, Cai JP and Zhang S. HOTAIR: a cancer-related long non-coding RNA. Neoplasma 2014; 61: 379-391.

[54] Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, Ioannidis JP, Clarke M, Devereaux PJ, Kleijnen J and Moher D. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. J Clin Epidemiol 2009; 62: e1-34.

[55] Tierney JF, Stewart LA, Ghersi D, Burdett S and Sydes MR. Practical methods for incorporating summary time-to-event data into meta-analysis. Trials 2007; 8: 16.

[56] Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. Eur J Epidemiol 2010; 25: 603-605.

[57] Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, Leeflang MM, Sterne JA and Bossuyt PM. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med 2011; 155: 529-536.

[58] Higgins JP, Thompson SG, Deeks JJ and Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003; 327: 557-560.

Supplementary Table 1. Quality assessment of eligible studies (Newcastle-Ottawa Scale)

Studies	LncRNAs	Representativeness of the exposed cohort	Selection of the non exposed cohort	Ascertainment of exposure	Demonstration that outcome of interest was not present at start of study	Comparability of cohorts on the basis of the design or analysis	Assessment of outcome	Was follow-up long enough for outcomes to occur	Adequacy of follow-up of cohorts	Scores
Cao 2012	HOTAIR	1	1	1	1	1	1	1	1	8
Cao 2014	LOC285194	1	1	1	1	2	1	1	1	9
Cao 2014	SPRY4-IT1	1	1	1	1	2	1	1	1	9
Cao 2015	PCAT-1	1	1	1	1	2	1	1	1	9
Cao 2016	BANCR	1	1	1	1	2	1	1	1	9
Cao 2016	BC200	1	1	1	1	1	1	1	0	7
Chen 2015	HOTAIR/LOC645638/TMEM106A	1	1	1	1	1	1	1	1	8
Chen 2017	RP11-766N7.4	0	1	1	1	1	1	1	1	7
Dong 2016	NR_024015	1	1	1	1	1	1	1	1	8
Fang 2014	FOXCUT	1	1	1	1	1	1	1	1	8
Feng 2015	NEAT1	1	1	1	1	1	1	1	1	8
Guo 2017	MEG3	1	1	1	1	2	1	1	1	9
Han 2013	HOTAIR	1	1	1	1	1	1	1	1	8
He 2014	3 lncRNA signature	1	1	1	1	2	1	1	1	9
Huang 2016	ENST00000480669/NONHSAT104436/ NONHSAT112918/NONHSAT126998	1	1	1	1	1	1	0	0	6
Jia 2013	HOTAIR	1	1	1	1	1	1	1	1	8
Li 2014	UCA1	1	1	1	1	1	1	1	1	8
Li 2015	MALAT-1	1	1	1	1	1	1	1	1	8
Lv 2016	MEG3	1	1	1	1	2	1	1	0	8
Tong 2016	AFAP1-AS1	1	1	1	1	2	1	1	1	9
Wang 2013	HOTAIR	1	1	1	1	1	1	1	1	8
Wang 2015	ZEB1-AS1	1	1	1	1	1	1	1	1	8
Yang 2016	BC032469	0	1	1	1	2	1	0	1	7
Yu 2016	TUG1	1	1	1	1	1	1	1	1	8
Zhao 2015	MALAT-1	1	1	1	1	1	1	0	1	7
Zheng 2016	CFLAR-AS1/Linc00152/POU3F3	1	1	1	1	1	1	1	1	8
Zhu 2015	CCAT2	1	1	1	1	1	1	1	0	7

Supplementary Figure 1. Quality assessment of included studies for prognosis by QUADAS-2. It summarizes "risk of bias" and "applicability concerns" through judging each domain for each eligible study.