REVIEW

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Long-Noncoding-RNA *HOTAIR* Upregulation is Associated with Poor Breast Cancer Outcome: A Systematic Review and Meta Analysis

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Abstract

Background: Breast cancer is the most frequent cancer among women worldwide with significant disproportionate mortality rates in developing countries. Although clinical management of breast cancer has been immensely improved, refinement in the prognostic and recurrent markers is still needed. Long non-coding RNAs (lncRNA) *HOTAIR* has recently been associated with poor outcome and is potentially used as a prognostic marker in breast cancer. **Methods:** We comprehensively reviewed studies evaluating lncRNA *HOTAIR* in association with overall and disease-free survivals in breast cancers. Systematic searches were performed in Pubmed, ProQuest, ScienceDirect, Scopus, Google Scholar, Semantic Scholar, Springer, Nature, Sage Journals, and Wiley databases using combination keywords "long non-coding RNA," "*lncRNA*," "*HOX transcript antisense RNA*," "*HOTAIR*," "*breast can-cer*," "*carcinoma mammae*," "*prognosis*," and "survival." Risk of bias score was used to assess quality of studies, I^2 test was conducted to assess heterogeneity. Meta-analysis was performed to compare HOTAIR expression with breast cancer survival rates using STATA v.17 software. **Results:** Of the total 1,504 screened studies, seven studies were included in the meta-analysis involving 533 patients. High expression of HOTAIR was associated with poor survival rates (pooled HR: 1.69; 95%CI: 1.11-2.59; p=0.015), shorter overall survival (OS) (pooled HR: 1.33; 95%CI: 0.78-2.26; p=0.455), poor disease-free survival (DFS) (pooled HR: 2.40; 95%CI: 1.63-3.53; p<0.001), poor distant metastatic-free survival (MFS) (HR: 1.75; 95%CI: 1.13-2.71; p=0.012). In addition, overexpression of *HOTAIR* was associated with positive lymph node infiltration (pooled OR: 2.38; 95%CI: 0.53-10.69; p=0.26) and ductal type cancer (pooled OR: 3.27; 95%CI: 1.15-9.30; p=0.03). **Conclusion:** Upregulation of lncRNA *HOTAIR* is associated with worse DFS aand MFS that can potentially be used as a prognostic marker in breast cancer patients.

Keywords: Long non-coding RNA- lncRNA, HOX transcript antisense RNA- *HOTAIR*, breast cancer

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Introduction

More than two million women are diagnosed with breast cancer and above 600.000 women lose their lives per year in association with the disease [1]. Recent advances in breast cancer treatment using multidisciplinary approach have significantly improved pa-tient prognosis [2, 3]. Several clinical and pathological parameters have been used to deter-mine breast cancer prognosis including stage at diagnosis, histological grades, intrinsic sub-types, and prerequisite metabolic comorbidities [4, 5]. Several algorithms have also been used to determine recurrence, distant metastases, and overall survivals [6, 7]. A recent study have shown potential application of long non-codingRNA panels to determine breast cancer prognosis [8].

Long non-coding RNA (lncRNA) is a class of ncRNAs spanning more than 200 nu-cleotides-length and is transcribed by RNA Polymerase II with lacks open reading frames (ORFs) [9]. Numerous vital biological processes are regulated by lncRNAs including cellu-lar proliferation, apoptosis, transcription of mobile genes, translation, protein modification, the formation of RNA-protein or protein-protein complexes, and post-transcriptional pro-cessing [9]. HOX transcript antisense RNA (*HOTAIR*) is a lncRNA that is highly expressed in breast cancer tissue and plays a role in breast tumorigenesis [10, 11]. It is a 2158-bp lncRNA transcribed from the antisense strand of the *HOXC* gene cluster, located on chromo-some 12q13.13 between the *HOXC11* and *HOXC12* genes [11,

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12]. *HOTAIR* is a crucial regulator of chromatin states and is a mediator of transcriptional silencing [11, 13]. *HOTAIR* will bind to the chromatin modification complex PRC2 (Polycomb repressive complex) at the 5' end (1-300 nt) to recruit and control PCR2 occupancy in genes all over the genome [11, 13]. In addition, *HOTAIR* interacts with E3 ubiquitin ligase, BRCA1, and estradiol to regulate cell proliferation [11, 14].

In breast cancer, lncRNA *HOTAIR* overexpression and the promoter methylation sta-tus are associated with poor cancer prognosis [11, 15, 16]. In an initial study, upregulation of *HOTAIR* in breast cancer patients has been associated with shorter metastatic-free survival and overal survival [11]. Further study shows that prognostic outcomes (overal survival and nodal metastasis) of *HOTAIR* overexpression applies only in estrogen recetor-negative breast cancer patients [17]. *HOTAIR* overexpression has also been associated with lower overal survivial in metastatic breast cancer [18]. However, the prognostic values of HO-TAIR in breast cancer patients remains controversial due to the diferent association with outcome parameters and is often studied in relatively small sample size. We therefore con-ducted a comprehensive meta-analysis to determine whether the mixed evidence can support the association between *HOTAIR* expression with the clinicopathological characteristics and prognosis of breast cancers.

Materials and Methods

Protocol and Eligibility Criteria

The systematic review with meta-analysis in this study was performed following guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-analysis Pro-tocols (PRISMA-P). Informed consent and ethics committee approval were not required as the study did not directly recruit patients and collect personal data. The study was registered in The International Prospective Register of Systematic Reviews (PROSPERO) (https://www.crd.york.ac.uk/prospero/) with registration number CRD42023392412.

The following studies included in this meta-analysis were articles published in Eng-lish without restriction on the year of publication that met the following criteria: (1) Patients diagnosed with breast cancer through histological or pathological examination, and (2) measured expression of lncRNA *HOTAIR* (high versus low), and (3) analyses of *HOTAIR* expression with Survival Outcomes (Overall Survival or Disease-Free Survival or Metastasis Free Survival) that were shown in the table, or Kaplan-Meier curve, or HR values. Research articles in the form of reviews, commentaries, letters, editorials, conference papers, cell line research (in vitro) and animal experiment (in vivo), and duplicated publications or studies without sufficient data were excluded.

Data Sources and Search Strategy

Two authors conducted an independent literature search (January 3-11, 2023) using the following databases: Pubmed, ProQuest, ScienceDirect, Scopus, Google Scholar, Seman-tic Scholar, Springer, Nature, Sage Journals, and Wiley with the following keywords: "long non-coding RNA," "lncRNA," "HOX transcript antisense RNA," "*HOTAIR*," "*breast can-cer*," "*carcinoma mammae*," "*prognosis*," and "*survival*." The literature search terms for each database can be found in Table 1, along with the search method phases for each data-base in Supplementary Data 1.

Study Selection

Two authors reviewed the articles independently, and any disagreements will be re-solved by consensus with a third author. The articles that were collected and duplications were checked. The article titles and abstracts were assessed and Full-Text articles were evaluated to determine if they fulfil the eligibility requirements. Only articles that meet the inclusion criteria will be included in the final systematic review and meta-analysis.

Data Extraction

Two authors extracted data independently and tabulated into the Google Spreadsheet. Any disagreement or discordant was resolved by discussion with a third reviewer. Of the screened studies, identification and year of publication, sample type, lncRNA *HOTAIR* de-tection method, the lncRNA expression level in the low or high category, number of patients, cut-off value, duration of follow-up, HR values (95% CI) for survival outcomes (OS, DFS, MFS) were extracted. In addition, information regarding the clinical characteristics of pa-tients (breast cancer type, histologic tumor grade, tumor size, lymph node involvement, HER2, Estrogen Receptor (ER), and Progesterone Receptor (PR) expression) was also recorded.

Article Quality Assessment

Two reviewers assessed the quality of the included articles independently and disa-greements were resolved by discussion with a third reviewer. The Newcastle-Ottawa Scale was implemented to evaluate the quality of the included papers. Articles with scores greater than 7 on the Newcastle-Ottawa Scale were considered high quality, while those with scores less than 7 were considered low quality.

Statistical Analysis

All study data will be analyzed using STATA v17.0 software (STATA Corp, College Station, Texas, USA). Using a combination of the Hazard Ratio (HR) and the 95% confi-dence interval (CI), the effect of *HOTAIR* expression on survival outcomes (OS, DFS, MFS) in breast cancer patients was evaluated. Clinicopathological parameters were evaluated us-ing the Odds Ratio (OR) and the 95% confidence interval (CI). When an article only con-tains a Kaplan-Meier curve, the HR and 95% confidence interval (CI) values are calculated using the method described by Tierney et al. All statistical results will be displayed in the form of forest plots. P-values lower than 0.05 are considered statistically significant for all tests performed in this meta-analysis.

Assessment of Heterogeneity

The Cochran's Q test (chi-squared test) and Higgins I2 statistic were used to perform a heterogeneity test by combining HR or OR. Using the Q and I^2 tests, the presence of het-erogeneity among studies was used to assess the risk of bias across studies. A p-value of the Q statistic less than 0.10 or an I^2 greater than 50% indicates apparent heterogeneity in the included articles, so a random-effects model should be used. Conversely, a p-value of the Q statistic greater than 0.10 or I^2 less than 50% indicates no apparent heterogeneity in the in-cluded studies, so a fixed-effects model should be used.

Subgroup Analysis

To comprehensively evaluate the association of *HOTAIR* expression and survival outcome, subgroup analysis was performed using different grouping criteria, such as country, sample size and type, follow-up duration, cut-off value, and source of included data.

Publication bias

Funnel plots were used to assess the publication bias of the included articles and fur-ther analyzed quantitatively using Egger's test.

Sensitivity Analysis

A sensitivity analysis was conducted using STATA v17.0 to determine whether any of the included articles significantly influenced the pool results.

Online Cross Validation

Online cross-validation was performed to validate the prognostic role of lncRNA *HOTAIR* expression in human cancer using Gene Expression Profiling Interactive Analysis (GEPIA) (//gepia.cancer-pku.cn/index.html) based on The Cancer Genome Atlas (TCGA) data (https://cancergenome.nih.gov/).

Results

Study Selection

Systematic search using 9 different databases identified 1504 articles, of which 306 articles were removed due to duplication. A total of 1198 articles were included in the title and ab-stract screening process and 51 articles were retrieved for the full texts. Of these, seven arti-cles presented sufficient data to be included in the metaanalysis [18-24]. The flowchart for the selection process was shown in Figure 1.

Quality and Risk of Bias

The quality and risk of bias were assessed using the Newcastle-Ottawa Scales as summarized in Table 2. All of the 7 articles (100%) had scores above or equal to 7 indicat-ing relatively low risk of bias.

Study Characteristics

Characteristics of the seven studies that included a total of 533 patients were tabulat-ed in Table 3. Three studies were conducted in China [23, 24] and a study

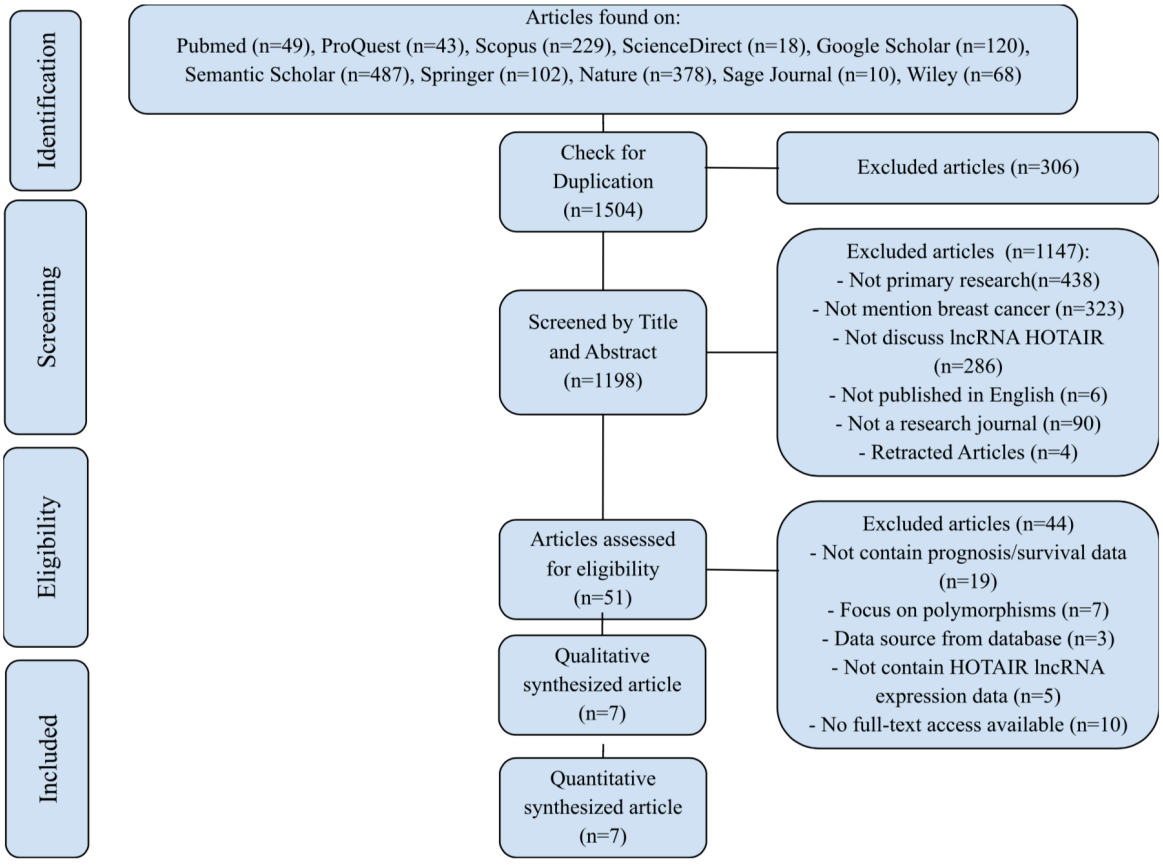

Figure 1. Screening and Selection of Studies Included in Systematic Review

Table 1. Keyword Search in Each Database

PubMed (n=49)
("long noncoding rna"[Title/Abstract] OR "lncRNA"[Title/Abstract]) AND ("hox transcript antisense rna"[Title/Abstract] OR "HOTAIR"[Title/Abstract]) AND ("breast cancer"[Title/Abstract] OR "carcinoma mammae"[Title/Abstract]) AND ("prognosis"[MeSH Terms] OR "prognosis"[All Fields] OR "prognoses"[All Fields] OR ("mortality"[MeSH Subheading] OR "mortality"[All Fields] OR "survival"[All Fields] OR "survival"[MeSH Terms] OR "survivability"[All Fields] OR "survivable"[All Fields] OR "survivals"[All Fields] OR "survive"[All Fields] OR "survived"[All Fields] OR "survives"[All Fields] OR "surviving"[All Fields]))
ProQuest (n=43)
TITLE,ABSTRACT,IF((Long noncoding RNA) OR (lncRNA)) AND TITLE,ABSTRACT,IF((HOX transcript antisense RNA) OR (HOTAIR)) AND TITLE,ABSTRACT,IF((Breast Cancer) OR (Carcinoma Mammae)) AND ((Prognos*) OR (Surviv*))
Scopus (n=229)
((TITLE-ABS-KEY (long AND noncoding AND rna)) OR (TITLE-ABS-KEY (lncrna))) AND ((TITLE-ABS-KEY (hox AND transcript AND antisense AND rna)) OR (TITLE-ABS-KEY (hotair))) AND ((TITLE-ABS-KEY (breast AND cancer)) OR (TITLE-ABS-KEY (carcinoma AND mammae))) AND ((ALL (surviv*)) OR (ALL (prognos*)))
ScienceDirect (n=18)
((Prognosis) OR (Survival)) Title, abstract, keywords: ((Long noncoding RNA) OR (lncRNA)) AND ((HOX transcript antisense RNA) OR (HOTAIR)) AND ((Breast Cancer) OR (Carcinoma mammae))
Google Scholar (n=120)
“breast cancer”, "hotair", "HOX transcript antisense RNA", "lncRNA", "survival", "prognosis", intitle:"breast cancer"
Semantic Scholar (n=487)
"breast cancer", "HOTAIR", "HOX transcript antisense RNA", "lncRNA", "survival", "prognosis"
Springer (n=103)
((Long noncoding RNA) OR (lncRNA)) AND ((HOX transcript antisense RNA) OR (HOTAIR)) AND ((Prognos*) OR (Surviv*)) in All Field with ((Breast Cancer) OR (Carcinoma Mammae)) in Title Field
Nature (n=378)
((Long noncoding RNA) OR (lncRNA)) AND ((HOX transcript antisense RNA) OR (HOTAIR)) AND ((Breast Cancer) OR (Carcinoma Mammae)) AND ((Prognos*) OR (Surviv*))
Sage Journals (n=10)
((Long noncoding RNA) OR (lncRNA)) AND ((HOX transcript antisense RNA) OR (HOTAIR)) AND ((Prognos*) OR (Surviv*)) in All Content Field with ((Breast Cancer) OR (Carcinoma Mammae)) in Title Field
Wiley (n=68)
[[All: breast cancer] OR [All: carcinoma mammae]] AND [[All: long noncoding rna] OR [All: lncrna]] AND [[All: hox transcript antisense rna] OR [All: hotair]] AND [[Publication Title: breast cancer] OR [Publication Title: carcinoma mammae]] AND [[All: prognos*] OR [All: surviv*]]

was performed consecutively in Italy [20], Denmark [22], Iran [19], and Egypt [18]. Six studies measured *HOTAIR* expression in the primary tumor tissues [19-24], and only a study measured the ex-pression in plasma [18]. Measurement for *HOTAIR* epression were various including qRT-PCR [19, 18, 21, 23, 24]. RNA-ISH [21], and microarray [22]. Number of included samples (sample sizes per study) were also various ranging from 15-165 patients. The cut-off value of *HOTAIR* expression varied using ROC Curve [19], Median [18], Youden Index [21], and Density Plot [22], and three studies did not mention the cut-off values. Four studies reported follow-up with a duration of 5 years [20, 21, 23, 24] and the rest three studies did specifical-ly report the median duration of

Table 2. Quality Assessment of Eligible Studies (Newcastle-Ottawa Scale)

Article	Selection			Comparability		Outcome			
	Adequacy of case definition	Number of case	Representativeness of the cases	Ascertainment of exposure	Ascertainment of detection method	Ascertainment of cut-off	Assessment of outcome	Adequate follow-up	Total
Arshi S (2019)	1	1	1	1	1	1	1	0	7
Collina F (2019)	1	1	1	1	1	0	1	1	7
El-Helkan B (2022)	1	1	1	1	1	1	1	0	7
Liang H (2019)	1	1	1	1	1	1	1	1	7
Sørensen KP (2013)	1	1	1	1	1	1	1	0	7
Tang S (2019)	1	1	1	1	1	0	1	1	7
Wang Y (2020)	1	1	1	1	1	0	1	1	7

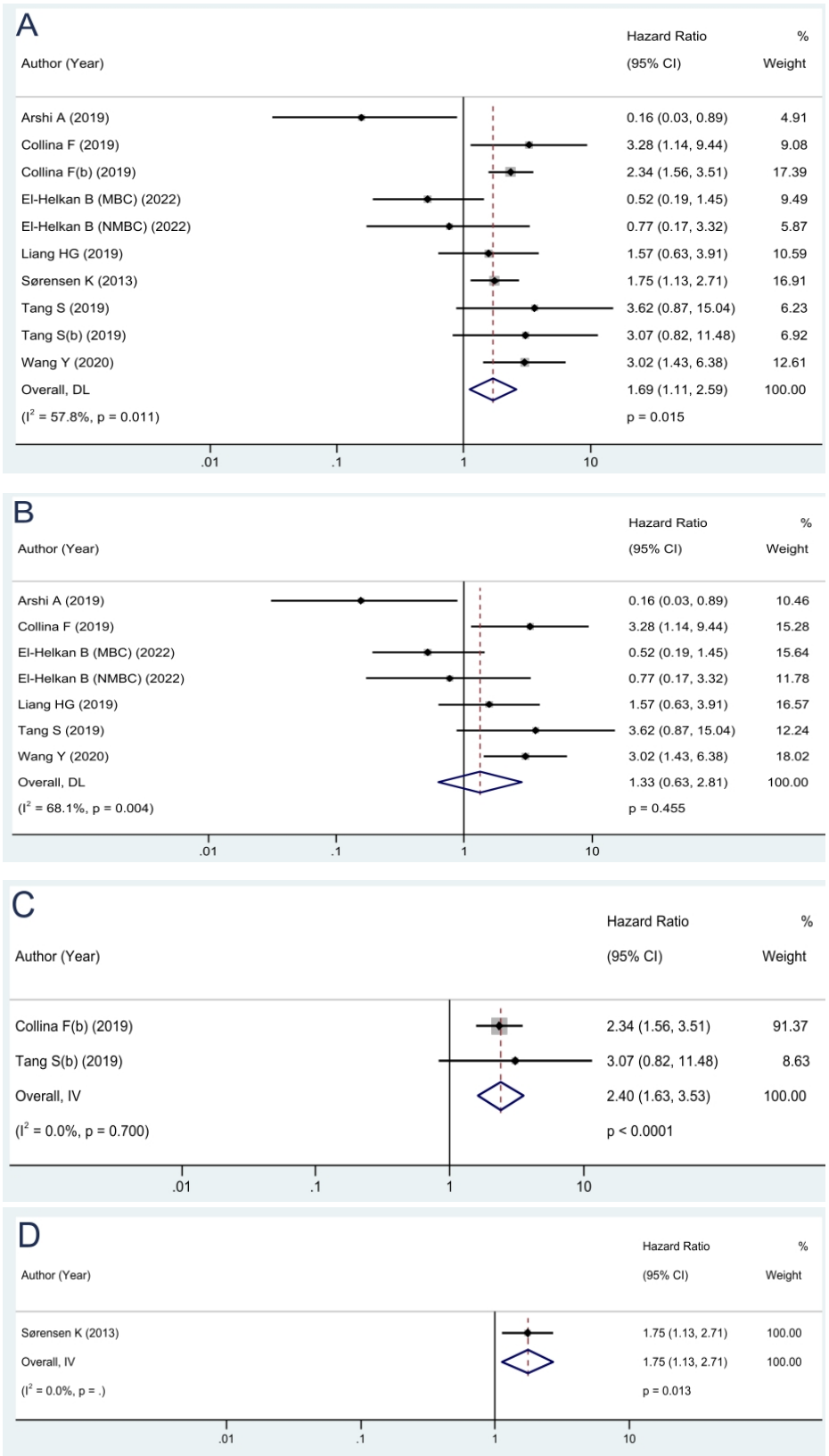

Figure 2. Comparison of *HOTAIR* Expression and the association with Survival Outcomes. Comparison of HOTAIR expression in breast cancer patients with (A) 5-years survival rates; (B) Overall survival; (C) Disease-free survival (DFS); (D) Metastasis-free survival (MFS). Hazard ratio (HR).

follow-up. Clinical outcomes were reported using OS [18, 20, 21, 23, 19], DFS [23], and MFS [22]. HR and 95% CI values were calculated and pre-sented in 3 studies [22, 18, 19] and the other 4 studies, HR and 95% CI were extracted from Kaplan-Meier curves [20, 21, 24] .

Clinicopathological data of breast cancer patients

Clinicopathological data of breast cancer patients involved in this systematic review were summarized from seven available studies as shown in the Table 4. Two studies differ-entiated histological types of breast

Table 4. Clinicopathological Variables of Breast Cancer Patients Analyzed for *HOTAIR* Expression

Author	Year	Breast Cancer Type				Histologic Tumor Grade				Tumor Size				LNM				HER2				ER				PR			
		Ductal		Others		3		1 and 2		>2 cm		<2 cm		(+)		(-)		(+)		(-)		(+)		(-)		(+)		(-)	
		H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L
Arshi S27	2019					2	2	5	6	0	4	7	4	0	4	7													
Collina F28	2019	21	95	3	44	41	6	104	12	20	5	69	42	26	44	20	70												
El-Helkan B29	2022																												
Liang HG30	2019																												
Sørensen KP31	2013																					53	67	26	18				
Tang S32	2019	8	3	4	5					11	8	1	0	12	3	0	5	5	5	6	4	8	5	3	4	3	3	8	6
Wang Y23	2020													11	4	8	12	9	7	10	9	11	9	8	7	12	10	7	6

Abbreviations: LNM; Lymphatic Node Metastasis;

Table 3. Characteristics of Study Included in Systematic Review and Meta-Analysis

Author	Year	Country	Research Sample	Detection Method of lncRNA HOTAIR	Total Patients	Expression of lncRNA HOTAIR High	Expression of lncRNA HOTAIR Low	Cut-off Value	Duration follow-up	HR and 95% CI	P Value
Arshi A27	2019	Iran	Tumour Tissue	qRT-PCR	15	7	8	ROC Curve	N/A	OS: 0.156 (0.031-0.887)	0.048
Collina F28	2019	Italia	Tumour Tissue	RNA ISH	163	47	116	N/A	Five years	OS: 3.28 (1.14-9.44)	0.99
										DFS: 2.34 (1.56-3.51)	0.872
El-Helkan B29	2022	Egypt	Blood Plasma	qRT-PCR	MBC: 28	13	15	Median	N/A	OS: 0.52 (0.19-1.45)	0.2
					NMBC: 23	9	14			OS: 0.77 (0.17-3.32)	0.7
Liang HG30	2019	China	Tumour Tissue	qRT-PCR	84	40	44	Youden Index	Five years	OS: 1.57 (0.63-3.91)	0.0007
Sørensen K31	2013	Denmark	Tumour Tissue	Microarray	164	79	85	Density plot	N/A	MFS: 1.747 (1.125-2.712)	0.012
Tang S32	2019	China	Tumour Tissue	qRT-PCR	20	11	9	N/A	Five years	OS: 3.62 (0.87-15.04)	0.0463
										DFS: 3.07 (0,82-11.48)	0.0481
Wang Y33	2020	China	Tumour Tissue	qRT-PCR	35	19	16	N/A	Five years	OS: 3.02 (1.43-6.38)	<0.05

Abbreviations: OS, Overall Survival; DFS, Disease-Free Survival; MFS, Metastasis-Free Survival; HR, Hazard Ratio; Qrt-PCR, Quantitative Reverse Transcription Polymerase Chain Reaction; N/A: Not Available; CI, Confidence Interval

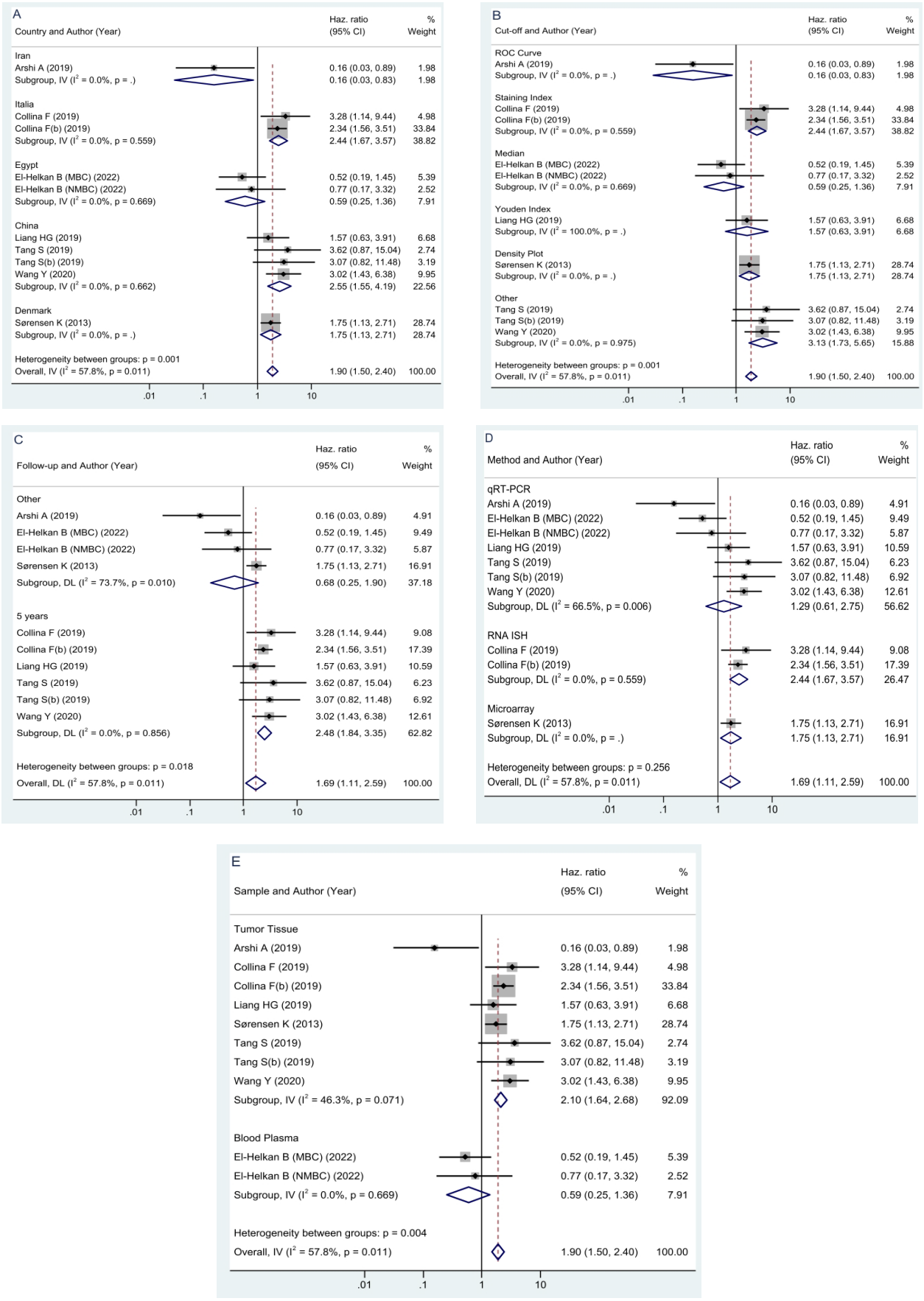

Figure 3. Subgroup Analyses of *HOTAIR* Expression and Survival According to the (A) country, (B) cut-off point of HOTAIR expression, C) 5-year survival, (D) methods of HOTAIR expression analyses, (E) primary samples.

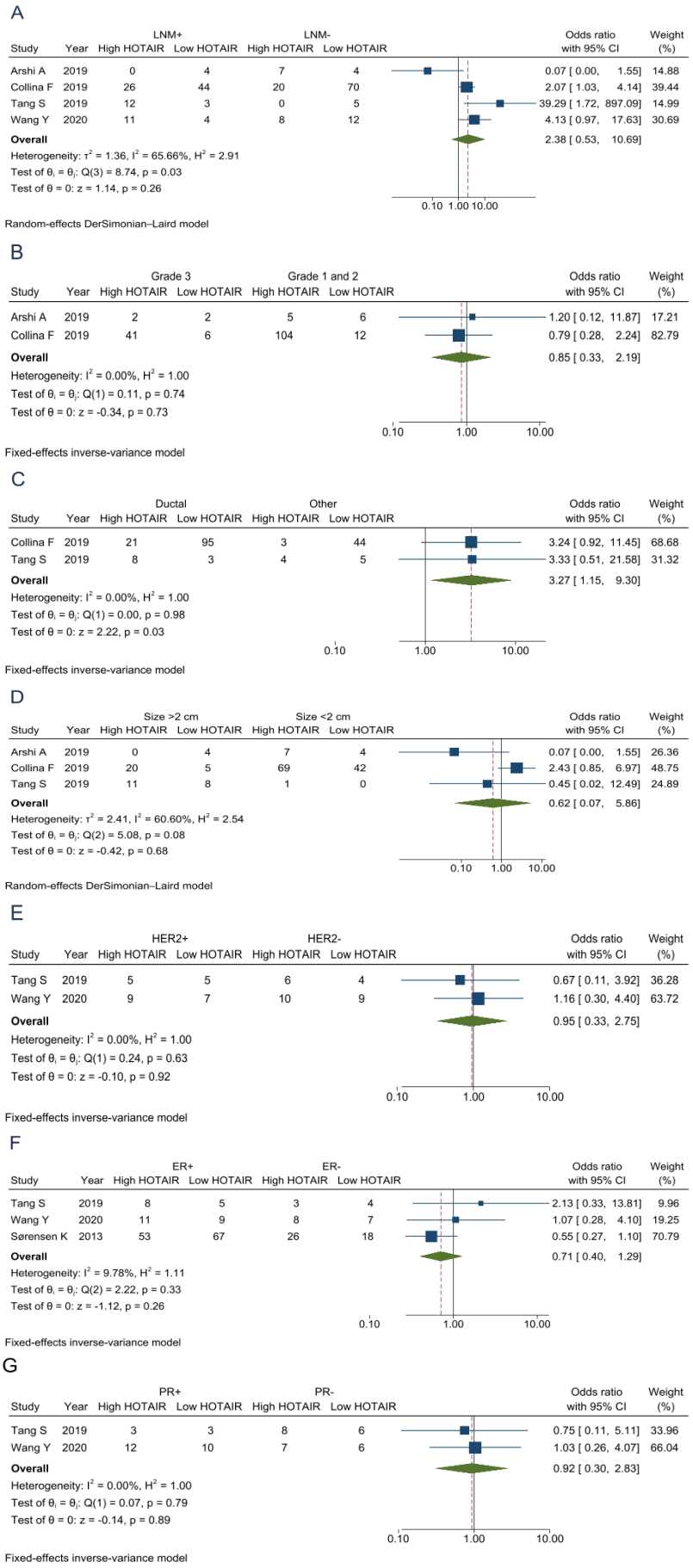

Figure 4. Comparison of *HOTAIR* Expression with Overall Survival According to (A) axillary lymph node status, (B) histological grades, C) histological type, (D) tumor size, (E) *Her2*-expression, (F) *ER* expression, and (G) *PR* expression.

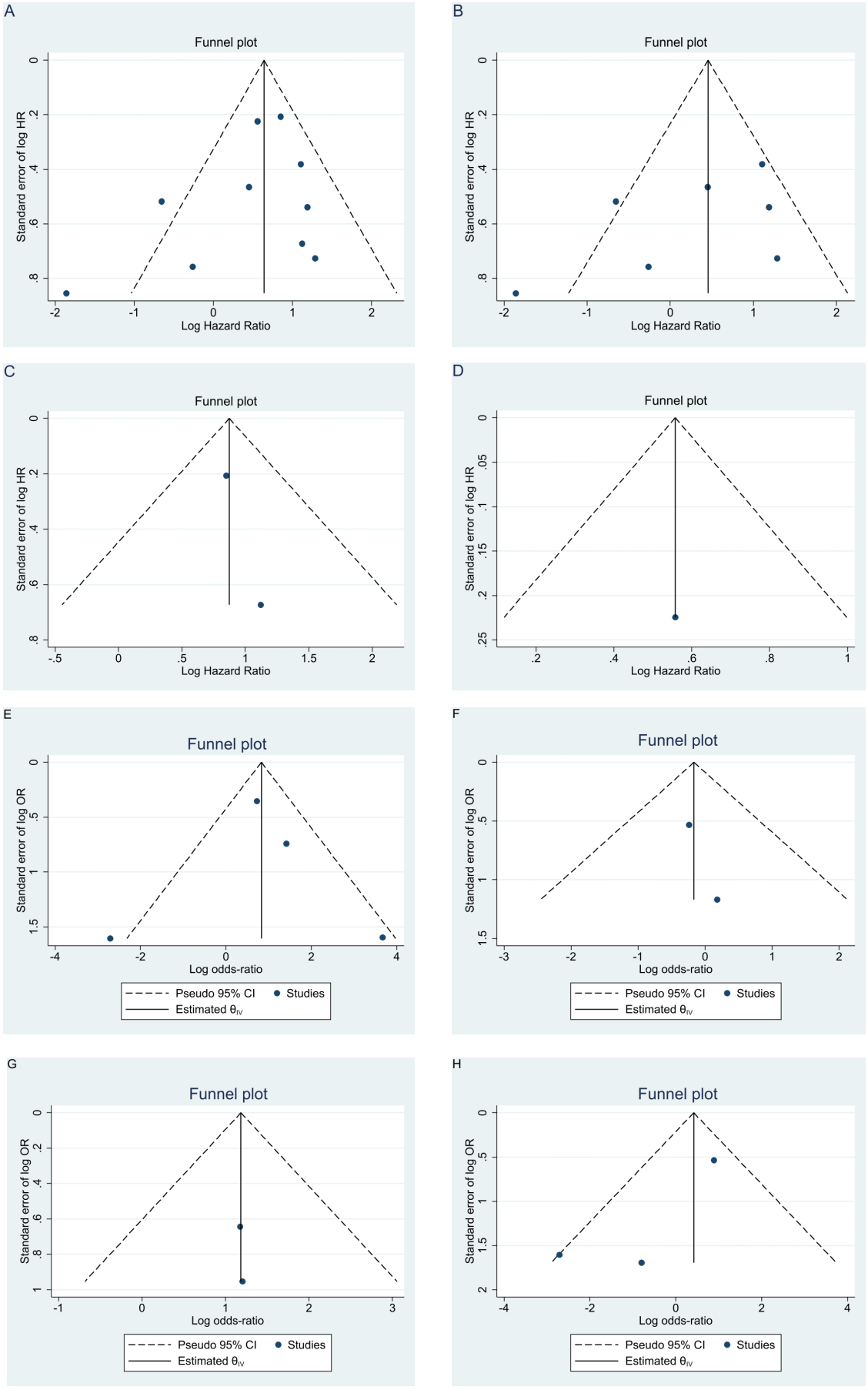

Figure 5. Funnel Plots for association between *HOTAIR* Expression and (A) Survival out-comes; (B) Overall survival; (C) Disease-free survival; (D) MFS; (E) Axillary lymph node status; (F) Histopathological grades; (G) Histological types; (H) Tumor Size; (I) *HER2* expression; (J) *ER* expression; (K) *PR* expression.

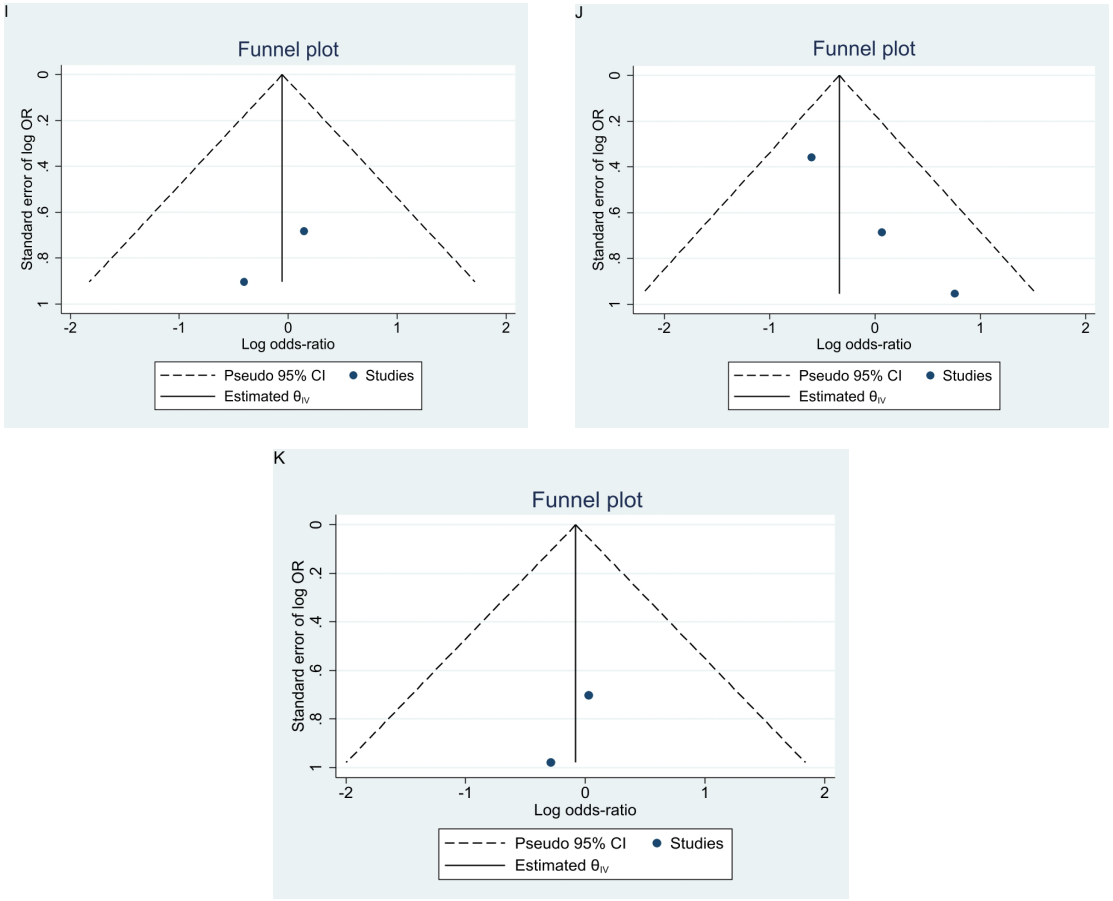

Figure 5. Funnel Plots for association between *HOTAIR* Expression (I) *HER2* expression; (J) *ER* expression; (K) *PR* expression.

cancer [20, 23]. Histological grades were presented in two studies [19, 20]. Information of Her2 status [23, 24] and expression progesterone recep-tors [23, 24] were shown by two studies. Expression of estrogen receptor and tumor size were reported in 3 studies (as shown in the Table 4).

Association of HOTAIR lncRNA expression and breast cancer survival

In 10 studies with relevant overall survival data, patients with higher *HOTAIR* ex-pression were significantly associated with worse overall survivals (HR: 1.69; 95% CI: 1.11-2.59, P = 0.015, Figure 2A). However, omitting 3 studies without complete survival rates, *HOTAIR* overexpression was also associated with worse survival although was not statisti-cally significant (HR: 1.33; 95% CI: 0.63-2.81, P = 0,455, Figure 2B). In two studies as-sessing disease-free survival (DFS), higher *HOTAIR* expression was significantly associated with shorter DFS (HR 2.40; 95% CI: 1.63-3.53, P<0.0001, (Figure 2C). In a study assessing metastasis-free Survival (MFS), higher *HOTAIR* expression was associated with worse MFS (HR 1.75; 95% CI: 1.13-2.71, P=0.013, Figure 2D).

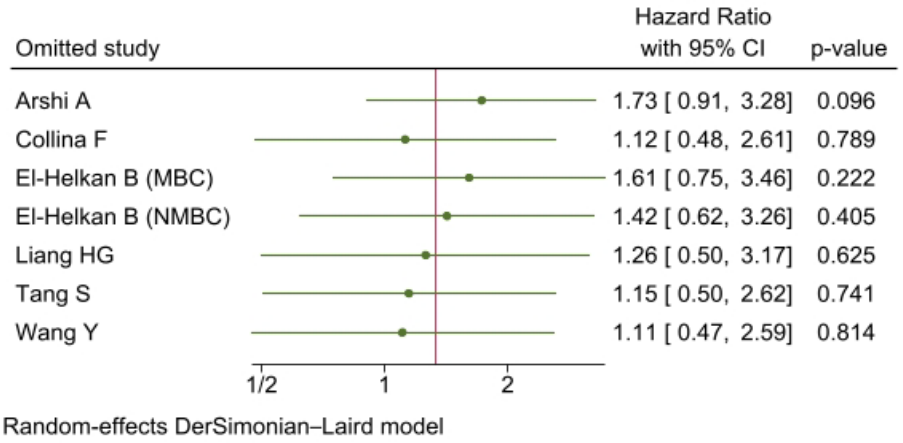

Figure 6. Sensitivity Tests of Individual Study Effects on Pool HR for the Association of *HOTAIR* Upregulation and Overall Survival

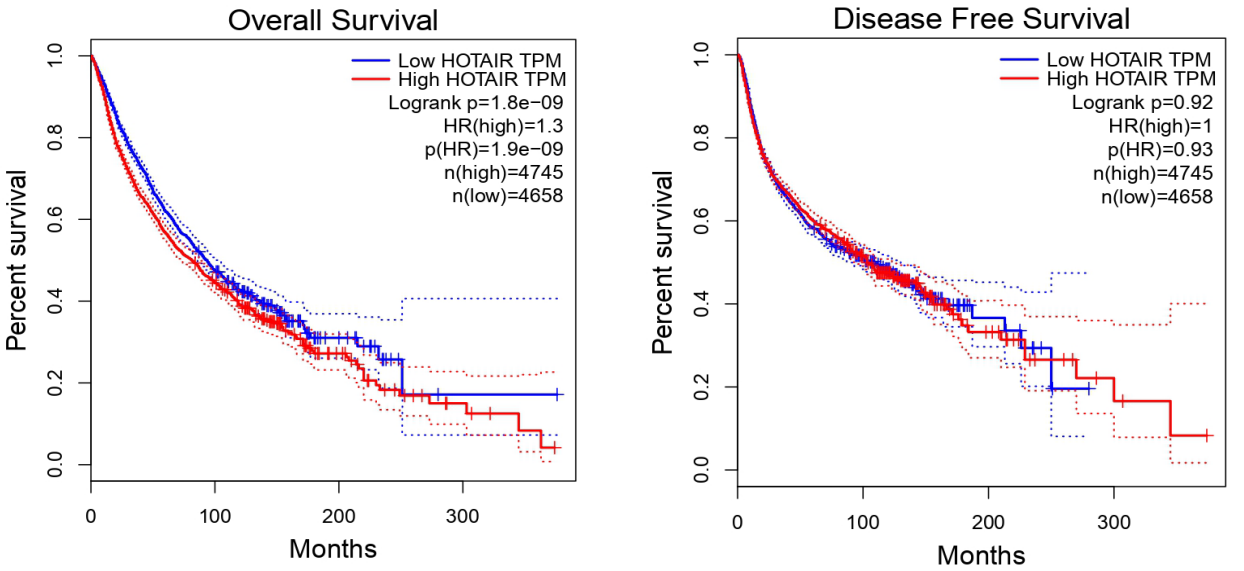

Figure 7. Confirmation Analyses using TCGA Database using Kaplan-Meier Plots of Breast Cancer Overall Survival (A) and disease-free survival (B) according to the *HOTAIR* Expression

Table 5. Association of *HOTAIR* Expression and Clinicopathological Variables of Breast Cancer Patients

Variable	Number of Studies (n)	Patients (n)	*HOTAIR* Expression Group		OR (95% CI)	P Value	Heterogeneity		Model
			High	Low			I2 (%)	P	
Lymph Node Metastasis (Positive versus Negative)	4	228	49 versus 35	55 versus 91	2.38 (0.53-10.69)	0.26	65.66	0.03	Random
HistologyTumor Grade (3 versus 1 and 2)	2	178	43 versus 109	8 versus 18	0.85 (0.33-2.19)	0.73	0	0.74	Fixed
Type (Ductal versus Other)	2	183	29 versus 7	98 versus 49	3.27 (1.15-9.30)	0.03*	0	0.98	Fixed
Tumor Size (>2 cm versus <2 cm)	3	171	31 versus 77	17 versus 46	0.62 (0.07-5.86)	0.68	60.6	0.08	Random
HER2 (Positive versus Negative)	2	55	14 versus 16	12 versus 13	0.95 (0.33-2.75)	0.92	0	0.63	Fixed
ER (Positive versus Negative)	3	219	72 versus 37	81 versus 29	0.71 (0.40-1.29)	0.26	9.78	0.33	Fixed
PR (Positive versus Negative)	2	55	15 versus 15	13 versus 12	0.92 (0.30-2.83)	0.89	0	0.79	Fixed

Notes: *, Statistically significant

We then performed subgroup analysis according to the country of origin, cut-off val-ue, study design, length of follow-up, methods used to measure expression, and type of pri-mary samples used in *HOTAIR* expression analysis. In the country-based subgroup, high *HOTAIR* expression in Italy was correlated with poor survival outcomes (HR: 2.44; 95% CI: 1.67-3.57, P<0.001, Figure 3A). Over-expression of *HOTAIR* was also associated with poor-er survival in China (HR: 2.55, 95% CI: 1.55-4.19, P= 0.001, Figure 3A). However, higher *HOTAIR* expression was not associated with better survivals in Egypt (HR: 0.59; 95% CI: 0.25-1.36, P = 0.217, Figure 3A).

In studies using different cut-off values to define overexpression, higher expression of *HOTAIR* was associated with poor survivals (HR: 2.62; 95% CI: 1.91-3.61, P<0.001, Figure 3B). However, in studies using median as a cutoff, higher *HOTAIR* than median was as-sociated with better survival outcomes (HR: 0.59; 95% CI: 0.25-1.36, P=0.217, Figure 3B). In studies with information of 5 years-survival, high expression of *HOTAIR* was associated with shorter survivals (pooled HR = 2.48; 95% CI: 1.81-3.35, P = 0.001, Figure 3D). In stud-ies without information of follow-up duration, high expression of *HOTAIR* was not signifi-cantly associated with poor survival (Random effect pooled HR: 0.68; 95%

Table 6. Assessment of Publication Bias

Variable	Egger Test	
	(P>|t|)	(P>|z|)
Survival Outcomes	0.2052	0.1679
OS	0.1952	0.1349
DFS	N/A	0.6998
MFS	N/A	N/A
Lymph Node Metastasis	0.8654	0.8477
Histology Tumor Grade	N/A	0.7437
Breast Cancer Type	N/A	0.9807
Tumor Size	0.2876	0.0393
HER2	N/A	0.6262
ER	0.3771	0.1372
PR	N/A	0.7932

Abbreviation; N/A, Not Available

CI: 0.25-1.90, P=0.466, Figure 3D).

In studies using qRT-PCR to measure *HOTAIR* expression, higher *HOTAIR* expres-sion was not significantly associated with poorer survival (Random-effects model, pooled HR = 1.29; 95% CI: 0.61-2.75, P = 0.505, Figure 3E). In studies using RNA ISH methods, higher *HOTAIR* expression was associated with worse survival outcomes (pooled HR: 2.44; 95% CI: 1.67-3.57, P<0.001). In studies measured expression in primary tumor tissues, high-er *HOTAIR* expression was associated with worse survival (pooled HR = 2.10; 95% CI: 1.64-2.68, P<0.001). Higher *HOTAIR* expression in plasma was not significantly associated with worse survival (pooled HR = 0.59; 95% CI: 0.25-1.36, P=0.217, Figure 3).

Association of lncRNA HOTAIR expression with clinicopathological characteristics of breast cancer patients

Association of any clinicopathological variables with *HOTAIR* expression was ana-lyzed by 5 studies (summarized in the Table 5). Elevated HOTAIR expression was not asso-ciated with positive axillary lymph node infiltration (pooled OR: 2.38; 95%CI: 0.53-10.69, P =0.26), high histological grade (Grade 3) (pooled OR: 0.85; 95%CI: 0.33-2.19, P=0.73), larger tumor size than 2 cm (pooled OR: 0.62; 95% CI: 0.07-5.86, P= 0.68), Her2 positive (pooled OR: 0.95; 95% CI: 0.33-2.75, P= 0.92), estrogen receptor positive (pooled OR: 0.71; 95%CI: 0.40-1.29, P= 0.26), and progesterone receptor positive (pooled OR: 0.92; 95%CI: 0.30-2.83, P=0.89). High *HOTAIR* expression was significantly associated with ductal histo-logical type (pooled OR: 3.27; 95%CI: 1.15-9.30, P=0.03, Figure 4).

Publication bias and sensitivity analysis

To assess publication bias, direct visual inspection and formal tests of funnel plot were performed. No significant publication bias for all studies comparing *HOTAIR* expres-sion with certain variables including survival outcome (P>|t|=0.298; P>|z|=0. 1679), overall survival (P>|t|=0.221; P>|z|=0.1349), DFS (P>|z|=0.6998), lymph node involvement (P>|t|=0.8654; P>|z|=0.8477), histology tumor grade (P>|z|=0. 7437), breast cancer type (P>|z|=0.9807), tumor size (P>|t|=0.2876P>|z|), HER2 (P>|z|=0.6262), and ER (P>|t|=0.3771; P>|z|=0.1372), PR (P>|z|=0.7932), as shown in the Figure 5 and Table 6.

In sensitivity analysis, excluding one study did not significantly remove the observed heterogeneity. As shown in Figure 6, omission of study 1[19] or 7 [24] caused a relatively larger influence (when compared with other studies) on the estimation of the overall effect size. Omitting study 1 caused the overall Hazard Ratio to increase by roughly 0.40, whereas omitting study 7 caused the overall Hazard Ratio to decrease by roughly 0.22.

Cross-validation using TCGA database

To further explore the association of *HOTAIR* expression in breast cancer, cross-validation using The Cancer Genomic Atlas (TCGA) data was performed. Patients with high expression of lncRNA *HOTAIR* were significantly associated with shorter Overall Sur-vival (OS) (HR=1.3, P<0.05). However, *HOTAIR* overexpression was not significantly asso-ciated with breast cancer patient's DFS (HR=1, P=0.93) as shown in the Figure 7.

Discussion

HOTAIR is a lncRNAs in which the overexpression in primary breast cancer is in-volved in the initiation, growth, angiogenesis, progression, drug resistance, recurrence, and poor prognosis by regulating multiple downstream targets in several signaling pathways [25]. In vitro models, *HOTAIR* plays an important role in breast cancer carcinogenesis through modification of chromatin remodeling [26, 11], regulation of estradiol and androgen recep-tors [22], and synchronization of EMT signaling pathway [27].

Our study performed comprehensive review and meta-analysis on *HOTAIR* expres-sion and breast cancer prognosis. Upregulation of *HOTAIR* are significantly associated with shorter overal survival, lower survival rate, and more pathologically aggressive. In the risk of bias, most of included studies have adjusted confounding variables although substatian degress ofheterogenetiy was observed in some outcome variables. The observed associations suggest that *HOTAIR* plays a significant role in the progression of breast cancer. A plausible biological mechanism is that *HOTAIR* binds to the chromatin modification complex PRC2 (Polycomb repressive complex) at the 5' end (1-300 nt) to further recruit and influence PRC2 occupancy to the other gene targets [13]. *HOTAIR* also binds to lysine-specific histone de-methylase 1A (LSD1) at the 3' end (1500-2146 nt) as a chromatin modifier to initiate gene silencing [28]. The interaction of *HOTAIR* with chromatin-modifying enzymes can further promote epigenetic activation or silencing of gene expression. *HOTAIR* also functions as a molecular scaffold through H3K27 trimethylation (PRC2 activity) and H3K demethylation (LSD1 activity) to silence of target genes and represses their expression [11, 13]. *HOTAIR* is also associated with abnormal DNA methylation profiles in cancer [13, 29]. In breast cancer, combining *HOTAIR* overexpression and methylation status is a significant predictor of poor prognosis [30]. In ER-positive cells, *HOTAIR* interacts with several ERE elements to regu-late transcription factors [31]. In addition, *HOTAIR* regulates epithelial to mesenchymal transition (EMT) thus contributing to the cell invasion and metastasis. Exposure of trans-forming growth factor beta 1 (TGF-β1) could trigger *HOTAIR* expression and EMT for-mation. *HOTAIR* inhibition is also able to reverse TGF-β1 in the induction of EMT and breast cancer cell ability in the colony-forming capacity [32].

HOTAIR upregulation is suggested from direct transcriptional activation by classical oncogenes including myocardin-related transcription factor-A, a Rho signaling responsive co-activator of serum response factor (SRF) regulated by the Rho GTPase-actin signaling pathway [27]. In addition, SRF induces *HOTAIR* gene promoter activity on CArG box (CC(A/T)6GG sequences)-dependent manner in breast cancer cells [27, 33]. FOXA1 and FOXM1, two members of the forkhead box (FOX)

transcription factor family, can also acti-vate *HOTAIR* expression in breast cancer [28].

HOTAIR has also been associated with regulation of anti-apoptotic protein Bcl-w. *HOTAIR* can upregulate Bcl-w expression by sequestering miR-206 at the post-transcriptional level in breast cancer cells [34]. The *HOTAIR* gene promoter can also bind to IRF1 (Interferon Regulatory Factor-1), which can induce its inhibition in breast cancer cells [35].

We also performed independent analysis using TCGA data and showed that *HOTAIR* overexpression with poor overall breast cancer survival. Further study is needed to confirm the association of *HOTAIR* overexpression and poor breast cancer outcomes and whether it can be used as independent prognostic markers as well as targeted therapy. The strength of this study lies in the involvement of studies measuring *HOTAIR* expression in the primary tumor tissues and plasma as well as inclusion of studies with association of different clinical outcome parameters. However, there are some limitations in the current analyses including HRs are calculated based on the KM curve and the estimation might be less accurate. There is a lack of diversity in the regions or country-origins conducting the studies. Most studies are from China, so it is unclear whether our results also apply in other countries. Different studies have inconsistent definitions of cut-off values for *HOTAIR* expression. The data ob-tained were from online databases, so to further determine the clinical application value of *HOTAIR*, these findings need to be further confirmed in a larger sample size. In addition, most study designs are retrospective cohorts that might have selection patient bias. To our notice, this is the first systematic review to address potential *HOTAIR* expression for clini-cal application as a prognostic marker in breast cancer patients.

In conclusion, breast cancer patients with higher *HOTAIR* expression are generally associated with worse overall survivals (study number 10, HR: 1.69; 95% CI: 1.11-2.59, P = 0.015), shorter disease-free survival (DFS) (HR 2.40; 95% CI: 1.63-3.53, P<0.0001), and shorter Metastasis-free Survival (MFS) (HR 1.75; 95% CI: 1.13-2.71, P=0.013).

Author Contribution Statement

SS and KGP performed systematic literature search and, title and abstract tabulation, full text screening and data extraction. KGP and SLA wrote manuscript and all authors provided feedback and approved the final version of manuscript.

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Ethics approval and consent

The study did not directly recruit study participants. The study protocol for project 'Breast cancer in Indonesia' has been approved by local university ethical council (KE/FK/15434/EC/2022).

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Abbreviation

CI: confidence interval
DFS : disease-free survival
DNA: Deoxyribose nucleid acid
EMT: epithelial-to-mesenchymal transition
lcRNA: long-noncoding ribonucleic acid
MFS: metastasis-free survival
OS: overall survival
PROSPERO: The International Prospective Register of Systematic Reviews
RNA: ribonucleic acid
TCGA: The Cancer Genome Atlas

Conflict of Interest

There is no conflict of interest in this research

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