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Prognostic value of long non-coding RNA FOXD2-AS1 expression in patients with solid tumors*

Lu Zhou[a,b,1], Zhi Li[a,b,1], Xinye Shao[a,b], Bowen Yang[a,b], Jing Feng[a,b], Lu Xu[a,b], Yuee Teng[a,b,*]

[a] Department of Medical Oncology, The First Hospital of China Medical University, Shenyang 110001, China
[b] Key Laboratory of Anticancer Drugs and Biotherapy of Liaoning Province, The First Hospital of China Medical University, Shenyang 110001, China

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ABSTRACT

Background: Although increasing evidence has revealed that FOXD2-AS1 overexpression exists in various solid tumors, the value of FOXD2-AS1 as a prognostic marker in such cancers remains uncertain. Accordingly, the present research aimed to assess the association of FOXD2-AS1 with cancer prognosis and predict the biological function of FOXD2-AS1.

Methods: We systematically retrieved PubMed, PMC, Web of Science, EMBASE and Wiley Online Library databases for eligible articles published up to December 2018. Pooled hazard ratios (HRs) and odds ratios (ORs) with 95% confidence intervals (95%CIs) were calculated to evaluate the correlation of FOXD2-AS1 expression with overall survival (OS), disease free survival (DFS) and clinicopathological characteristics. We also used five Gene Expression Omnibus (GEO) datasets from breast cancer patients to explore the relationship between FOXD2-AS1 expression and prognosis. Finally, we validated FOXD2-AS1 expression in various carcinomas and predicted its biological function based on the public databases.

Results: A total of 13 studies with 2502 tumor patients were included. The pooled HRs demonstrated that FOXD2-AS1 overexpression was significantly associated with unfavorable OS (HR = 1.39, 95%CI: 1.23–1.57, $p < 0.001$) and DFS (HR = 2.24, 95%CI: 1.55–3.23, $p < 0.001$) in tumor patients. The pooled ORs indicated that FOXD2-AS1 upregulation was related to large tumor size (OR = 1.53, 95%CI: 1.26–1.85, $p < 0.001$), deep invasion depth (OR = 1.99, 95%CI: 1.53–2.58, $p < 0.001$), distant metastasis (OR = 2.03, 95%CI: 1.69–2.43, $p < 0.001$) and advanced TNM stage (OR = 1.35, 95%CI: 1.06–1.72, $p = 0.0150$), but not to lymph node metastasis nor differentiation. Moreover, a similar pooled result for the OS of breast cancer patients was obtained (HR = 1.55, 95%CI: 1.14–2.11, $p = 0.0052$) by analyzing GEO data. Finally, elevated FOXD2-AS1 expression in various solid tumor tissues was verified based on The Cancer Genome Atlas (TCGA) data. Further functional prediction demonstrated that FOXD2-AS1 may participate in some cancer-related pathways.

Conclusion: Elevated FOXD2-AS1 expression was associated with poor survival in patients with solid tumors and may serve as a potential prognostic biomarker for a variety of cancers.

1. Introduction

Malignant tumors represent a subset of complex diseases that threaten human health and survival around the world [14]. According to the Global Cancer Burden Status Report, there were approximately 18.1 million new cancer cases and 9.6 million cancer deaths worldwide based on 2018 cancer morbidity and mortality estimates [6]. In the past two decades, the molecular basis of various cancers has been deeply explored, contributing to a reduction of approximately 2.4 million carcinoma deaths during this period [26]. At present, the mechanisms of cancer occurrence and development have not been fully elucidated. For this reason, an increasing number of studies aim to identify new cancer-specific biomarkers to explore cancer pathological process and develop better treatment strategies [33].

Long non-coding RNAs (lncRNAs) are a class of RNA transcripts that are more than 200 nt in length and do not contain an open reading frame [12,39]. Recently, accumulating evidence has indicated that lncRNAs play a pivotal role in cancer progression by regulating gene

* This is an original work. Materials containing in this study have never been presented, reported or published.

* Correspondence author at: Department of Medical Oncology, The First Hospital of China Medical University, NO.155, North Nanjing Street, Shenyang, 110001, China.
E-mail address: yeteng@cmu.edu.cn (Y. Teng).

[1] These authors contributed equally to this work.

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expression at the levels of chromatin modification, transcriptional and post-transcriptional regulation [7,8,11,31]. Several studies have found that dysfunction of various lncRNAs is closely related to the proliferation, apoptosis, migration, invasion and drug resistance of tumor cells [1,5,16,17]. Therefore, lncRNAs may represent potential biomarkers with diagnostic, prognostic, and therapeutic value in various human carcinomas.

LncRNA FOXD2-AS1, with a transcript length of 2527 nucleotides, is located on chromosome 1p33. Recently, several studies have found that FOXD2-AS1 is overexpressed in various human cancers [1,8,24,37,41,42], and has been reported to promote tumor cell proliferation, apoptosis, migration and invasion through the Wnt/β-catenin pathway [24], the Notch pathway [38], the PI3K/Akt pathway [28]. Moreover, most studies demonstrated that FOXD2-AS1 overexpression was associated with poor outcomes and certain clinicopathologic features in cancer patients. However, the prognostic value of FOXD2-AS1 in various carcinomas has been limited by small sample sizes and/or methodology. Thus, we conducted this meta-analysis to assess the prognostic value of FOXD2-AS1 in different cancer types by gathering all relevant publications and pooling their results.

2. Materials and methods

2.1. Article retrieval strategy

PubMed, PMC, Web of Science, EMBASE and Wiley Online Library databases were searched for eligible articles published up to December 2018. Retrieval terms included FOXD2-AS1 ('lncRNA FOXD2-AS1' or 'long non-coding RNA FOXD2-AS1' or 'FOXD2 adjacent opposite strand RNA 1') and cancer (tumor or neoplasm or carcinoma) in all possible combinations.

2.2. Study inclusion and exclusion criteria

Inclusive studies should meet the following criteria: (1) All articles were published in English; (2) the patients were grouped based on FOXD2-AS1 expression; (3) the patients' clinicopathological features or survival information such as overall survival (OS) and disease free survival (DFS), were described; (4) the hazard ratios (HRs) for OS or DFS can be extracted from the articles directly or calculated indirectly according to Kaplan-Meier survival curve; (5) The quality of articles were assessed through the Newcastle- Ottawa Scale (NOS) score and the scores should be at least 6.

The exclusion criteria for the meta-analysis were as follows: (1) irrelevant topic articles; (2) non-experimental articles non-experimental articles; (3) the articles lacking of available or sufficient data.

2.3. Data extraction and quality evaluation

The following information was extracted from each eligible inclusive study: first author, publication year, cancer type, sample size, FOXD2-AS1 assay method, cut-off value, clinicalpathological characteristics (differentiation, tumor size, lymph node metastasis, distant metastasis, tumor stage and invasion depth) and survival information (OS or DFS). If univariate and multivariate analysis were performed simultaneously, multivariate analysis was preferred. If only the Kaplan-Meier survival curve was available in the article, the HRs and 95% confidence intervals (95%CIs) were calculated by adopting Engauge Digitizer version 10.1 [32]. Any disagreement was resolved by consensus with a third reviewer.

2.4. Statistical methods

To confirm the prognostic value of FOXD2-SA1 expression on patients with cancers, the pooled HRs with 95%CIs for OS or DFS were calculated and assessed. The correlation of FOXD2-AS1 expresssion with clinicopathological features were estimated by the pooled odds ratios (ORs) with 95%CIs. R software version 3.4.3 and Stata software version 12.0 (StataCorp, TX) were used to make the forest graphs in the meta analysis, and the pooled results were considered significant if the 95%CIs did not overlap 1. Pooled HR > 1 implied that high FOXD2-AS1 expression was associated with poor survival. I^2 test and chi-square-based Q test were used to calculate the heterogeneity of publications, and we defined inconsistency index (I^2) > 50% or $p < 0.10$ as significant heterogeneity based on previous research experience [35]. When no significant heterogeneity was found, we selected a fixed-effect model to pool the results. Otherwise, a random effect model was used. Begg's test, Egger's test and funnel plot were adopting to appraise publication bias. Sensitivity analysis was conducted to assess the impact of individual study on the stability of the pooled results.

2.5. GEO data for breast cancer search, download and extraction

We searched the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/) for human breast cancer datasets with more than 100 samples on Affymetrix GPL570 platform up to January 2018. A total of five breast cancer datasets (GSE20685, GSE20711, GSE42568, GSE48390 and GSE65194) containing survival data were obtained. We downloaded the expression profiles and clinical data of the five datasets, and the expression profiles of lncRNAs were available through re-annotation. The FOXD2-AS1 expression data and survival information of patients were extracted from each dataset.

2.6. LncRNA expression and function prediction

We compared the FOXD2-AS1 expression levels in various cancer tissues with normal tissues by utilizing Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn/) which is an online gene analysis tool based on The Cancer Genome Atlas (TCGA) data [30]. Lncactdb 2.0 (http://www.bio-bigdata.net/lncactdb/) is a database that provides comprehensive source of competitive endogenous RNA (ceRNA) regulations for lncRNAs [34]. We identified FOXD2-AS1 relevant ceRNA regulations by Lncactdb 2.0, and used Cytoscape version 3.017 to construct visualized ceRNA network. DAVID is a database of comprehensive bioinformatics databases and analytical tools, which can extract biological features from a large number of gene lists [13]. FOXD2-AS1 function and pathways involved were predicted according to Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses in DAVID.

3. Results

3.1. Description of included studies

The flow chart of our study screening process is presented in Fig. 1. Finally, a total of 13 studies including 2502 patients with 10 types of cancers based on inclusion and exclusion criteria, were qualified for enrollment in this meta-analysis. The included articles were published between 2016 and 2018; sample sizes ranged from 29 to 505. Except for two analyses based on TCGA data, the cancer patients examined were mainly from Asia.

The major characteristics of 13 eligible studies are summarized in Table 1 and Table S1, including digestive system cancers: two gastric cancer (GC) [15,37], one colonic cancer (CRC) [13], one hepatocellular cancer (HCC) [7] and one esophageal squamous cell carcinoma (ESCC) [3]. Other non-digestive system cancers included: two glioma [9,25], two papillary thyroid cancers (PTCs) [18,41], one non-small cell lung cancer (NSCLC) [24], one nasopharyngeal cancer (NPC) [14], one non-muscle-invasive bladder cancer (NMIBC) [28] and one cutaneous melanoma [23]. More than half of the studies chose the median/mean FOXD2-AS1 expression as cut-off value for division of samples into high-expression and low-expression groups. Not all studies contained

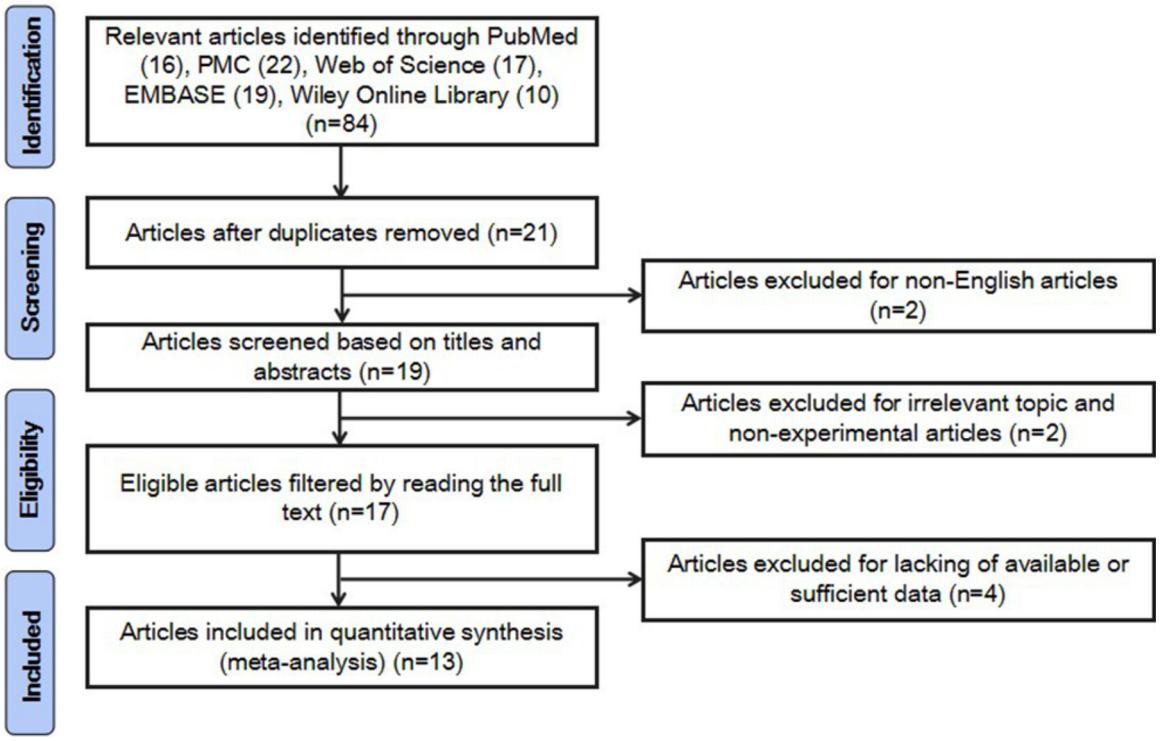

Fig. 1. Flow chart of article retrieval and screening.

the information on survival or clinicopathological characteristics. Ten studies investigated the association of FOXD2-AS1 with OS, and three studies with DFS. The HRs of four studies were extracted directly, while those of seven studies needed to be calculated indirectly according to the aforementioned methods. In the study by Wenqing et al., it was impossible to extract or calculate HRs due to the lack of survival data corresponding to tissue samples [23]. Additionally, the HR calculation with a 95%CI provided by another study [24] was considered inaccurate by more than two researchers and was thus not included in our analysis.

3.2. Association of FOXD2-AS1 expression with survival in various cancers

As shown in Fig. 2, a cumulative meta-analysis to assess the prognostic value of FOXD2-AS1 for OS in patients with solid tumors. There were 10 of 13 included studies with a total of 2227 samples reporting the association between FOXD2-AS1 and OS. FOXD2-AS1 overexpression tended to be significantly associated with poor OS (pooled HR = 1.39, 95%CI: 1.23–1.57, $p < 0.001$) using a fixed effect model due to identified slight heterogeneity ($I^2 = 17\%$, $p = 0.29$) (Fig. 2A). Further, three studies including 337 patients evaluated the correlation between FOXD2-AS1 expression and DFS. The pooled HR was 2.24 (95%CI: 1.55–3.23, $p < 0.001$, fixed-effect), and no heterogeneity existed ($I^2 = 0\%$, $p = 0.4$), implying FOXD2-AS1 may be a negative prognostic factor for DFS (Fig. 2B).

3.3. Publication bias and sensitivity analysis

Funnel plot, Egger's bias test and Begg's bias test were conducted to evaluate publication bias in the included studies for OS. As presented in Fig. 3A, the shape of the funnel plot was almost symmetrical. In addition, the results of Egger's test ($p = 0.7771$) and Begg's test

Table 1
Main characteristics of the eligible studies in this meta-analysis.

Study	Year	Cancer type	Sample size	FOXD2-AS1 assay	Cut-off value	Outcome	HR estimation method	HR (95%CI)	NOS score
Chengyun L [15]	2016	GC	361	TCGA data	NA	OS	Indirectly	1.44 (0.74-2.81)	6
Jie B [3]	2017	ESCC	147	qRT-PCR	Median	DFS/OS	Directly	1.66 (1.04-2.64)/ 2.68 (1.49–4.82)	8
Gang C [8]	2017	NPC	50	qRT-PCR	Median	OS	Indirectly	1.38 (1.10-1.73)	6
Rong L [24]	2017	NSCLC	45	qRT-PCR	Median	NA	–	NA	7
Fazheng S [25]	2018	Glioma	31	qRT-PCR	Median	OS	Indirectly	1.27 (0.60-2.67)	6
Wenqing R [23]	2018	Cutaneous melanoma	124	qRT-PCR	Median	NA	–	NA	7
Yayuan Z [41]	2018	PTC	84	qRT-PCR	Mean	OS	Indirectly	1.30 (1.08-1.56)	6
Yuhong C [7]	2018	HCC	360	qRT-PCR	Youden index	OS	Directly	1.63 (1.14-2.32)	6
Tongpen X [37]	2018	GC	106	qRT-PCR	Median	DFS	Directly	1.75 (1.04-2.97)	8
Yanyan Z [42]	2018	CRC	481	qRT-PCR	NA	OS	Indirectly	0.88 (0.43-1.82)	6
Feng S [28]	2018	NMIBC	84	qRT-PCR	NA	DFS/OS	Indirectly	2.70 (0.94-7.78)/ 3.36 (1.55-3.23)	8
Huixiao D [9]	2018	Glioma	124	qRT-PCR	NA	OS	Directly	2.45 (1.25-4.78)	7
Wei L [18]	2018	PTC	505	TCGA data	Median	OS	Indirectly	0.54 (0.19-1.53)	6

GC: gastric cancer; ESCC: esophageal squamous cell carcinoma; NPC: especially nasopharyngeal carcinoma; NSCLC: non-small cell lung cancer; PTC: papillary thyroid cancer; HCC: hepatocellular carcinoma; NMIBC: non-muscle-invasive bladder cancer; CRC: colorectal cancer.

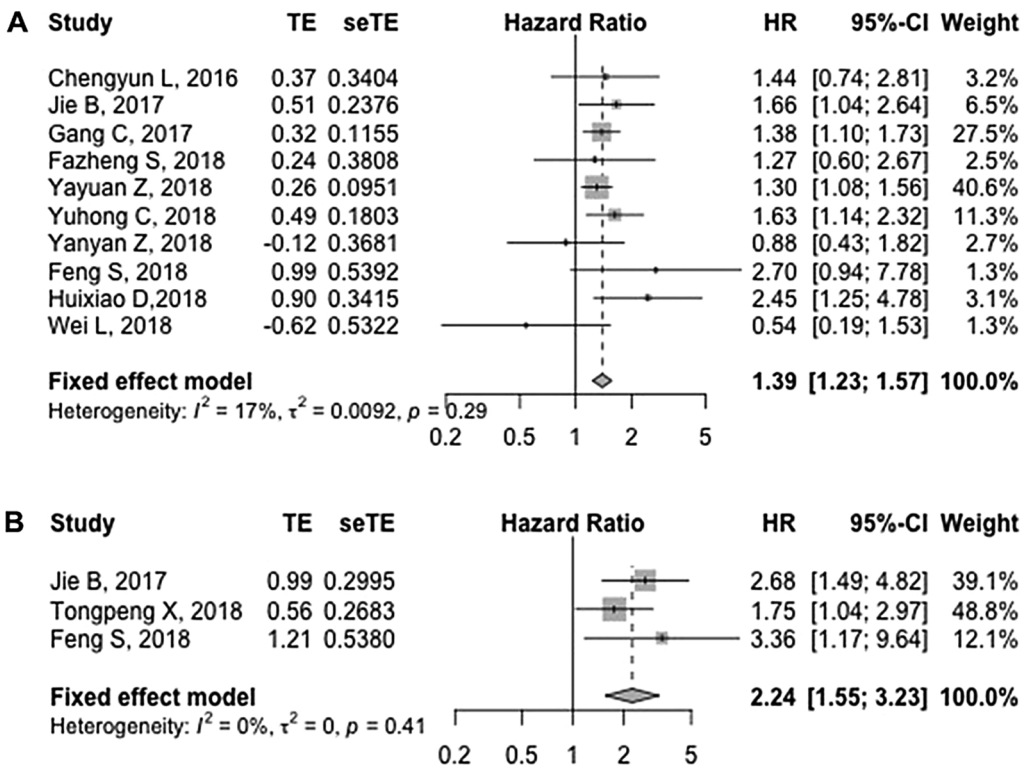

Fig. 2. Forrest plot for the association between FOXD2-AS1 expression and survival in various cancers. (A) overall survival (OS), (B) disease free survival (DFS).

(p = 0.9287) showed no indications of significant publication bias.

A sensitivity analysis was performed to assess the pooled results for OS. The pooled HRs were not significantly affected after removing each study in turn, indicating that the results of this meta-analysis were stable and reliable (Fig. 3B).

3.4. Subgroup analyses

In order to maximize the clinical relevance of FOXD2-AS1 expression with OS, subgroup analyses were conducted according to tumor region, FOXD2-AS1 assay method, sample size, study quality score (NOS) and HR estimation method. Subgroup analysis of tumor region revealed that increased FOXD2-AS1 was associated with poor OS in digestive system tumors (HR = 1.50, 95%CI: 1.18–1.92, p = 0.001,

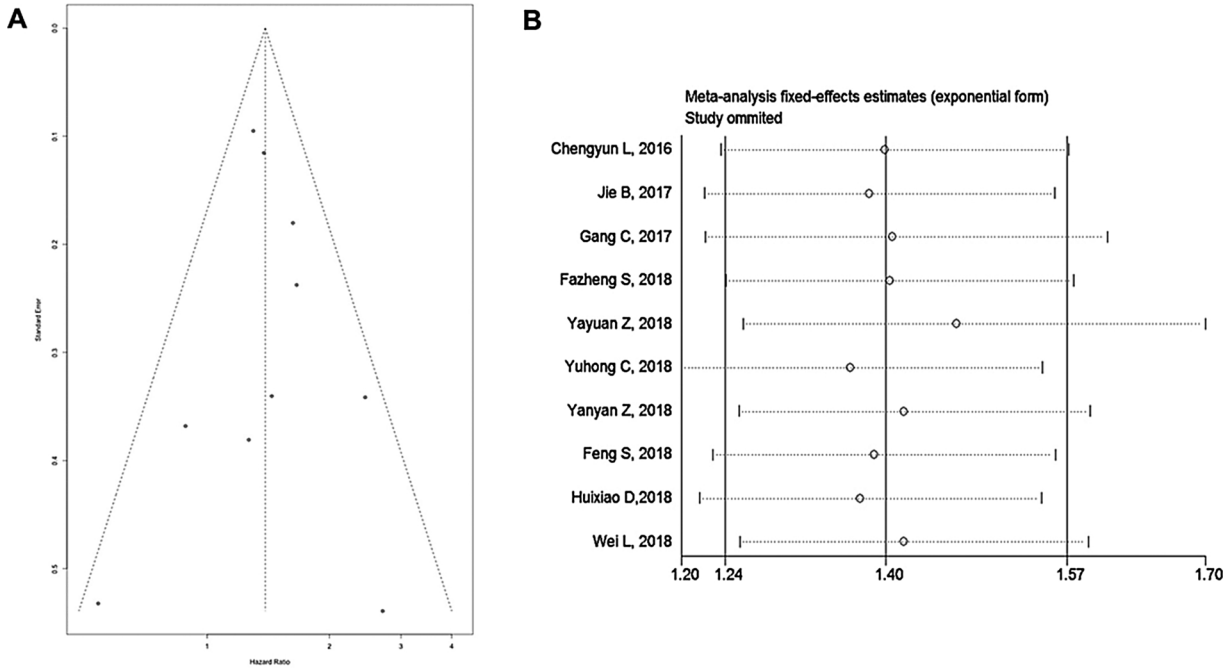

Fig. 3. (A) Funnel plot of the publication bias for the correlation between FOXD2-AS1 expression and OS in various cancers. (B) Sensitivity analysis of the impact of individual study on the pooled HR for OS.

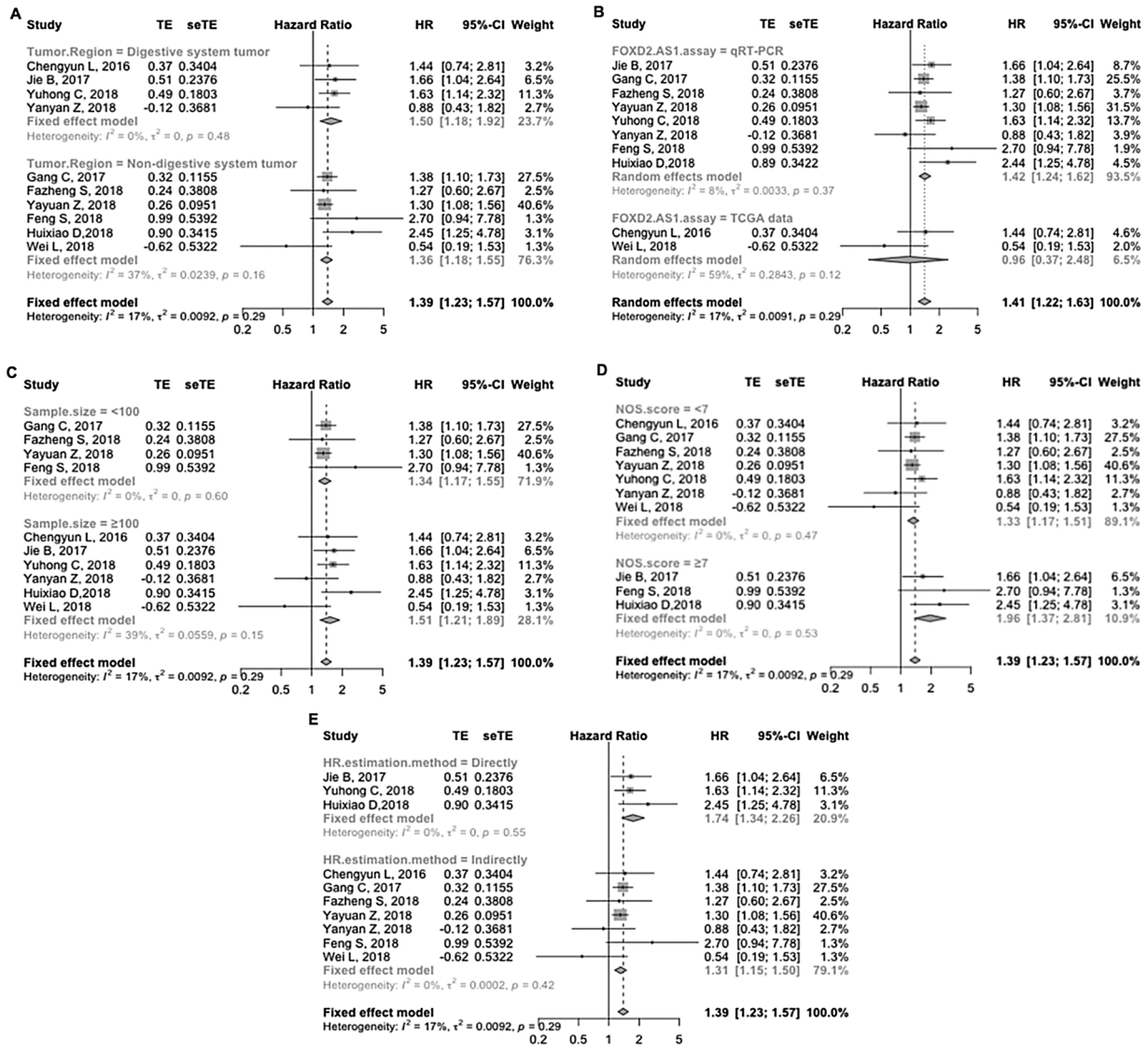

Fig. 4. Subgroup analyses of the relationship between FOXD2-AS1 expression and OS by (A) tumor region, (B) FOXD2-AS1 assay method, (C) sample size, (D) NOS score and (E) HR estimation method.

fixed-effect) and non-digestive system tumors (HR = 1.36, 95%CI: 1.18–1.55, p < 0.001, fixed-effect) (Fig. 4A). In subgroup analysis of FOXD2-AS1 assay, the relation between FOXD2-AS1 and OS was observed only in the qRT-PCR detection group (HR = 1.42, 95%CI: 1.24–1.62, p < 0.001, random-effect). However, no similar result was obtained in the TCGA data group (HR = 0.96, 95%CI: 0.37–2.48, p = 0.9294, random-effect) (Fig. 4B). Subsequently, subgroup analysis based on sample size indicated that FOXD2-AS1 served as a poor prognostic biomarker in the sample size < 100 group (HR = 1.34, 95%CI: 1.17–1.55, p < 0.001, fixed-effect) and ≥100 group (HR = 1.51, 95%CI: 1.21–1.89, p < 0.001, fixed-effect) (Fig. 4C). In addition, neither NOS score nor HR estimation method altered the predictive value of FOXD2-AS1 expression for OS of cancer patients (Fig. 4D and E). A subgroup analysis of DFS was not performed because of the limited number of studies.

3.5. Association of FOXD2-AS1 expression with clinicopathological features

FOXD2-AS1 expression for different clinicopathological features is presented in Table 2. Five studies including 536 samples were used to analyze the relationship between FOXD2-AS1 expression and tumor size. The pooled OR demonstrated that the elevated FOXD2-AS1 was highly correlated with large tumor size (OR = 1.53, 95%CI: 1.26–1.85, p < 0.001, fixed-effect), and no significant heterogeneity was observed (I^2 = 38.7%, p = 0.164) (Fig. 5A). Similarly, pooled OR demonstrated that FOXD2-AS1 expression was associated with invasion depth

Table 2
FOXD2-AS1 expression for various clinicopathological features of cancers.

Clinicopathological characteristics	No.of studies	Sample size	Subgroup	FOXD2-AS1 expression	
				High	Low
Tumor size	5	536	Large	142	90
			Small	130	174
Distant metastasis	3	314	Positive	37	5
			Negative	120	152
Lymph node metastasis	4	372	Positive	103	79
			Negative	88	102
TNM stage	3	288	III + IV	94	64
			I + II	56	74
Differentiation	5	486	Poor	102	81
			Well	150	153
Invasion depth	3	309	Deep	106	60
			Shallow	46	97

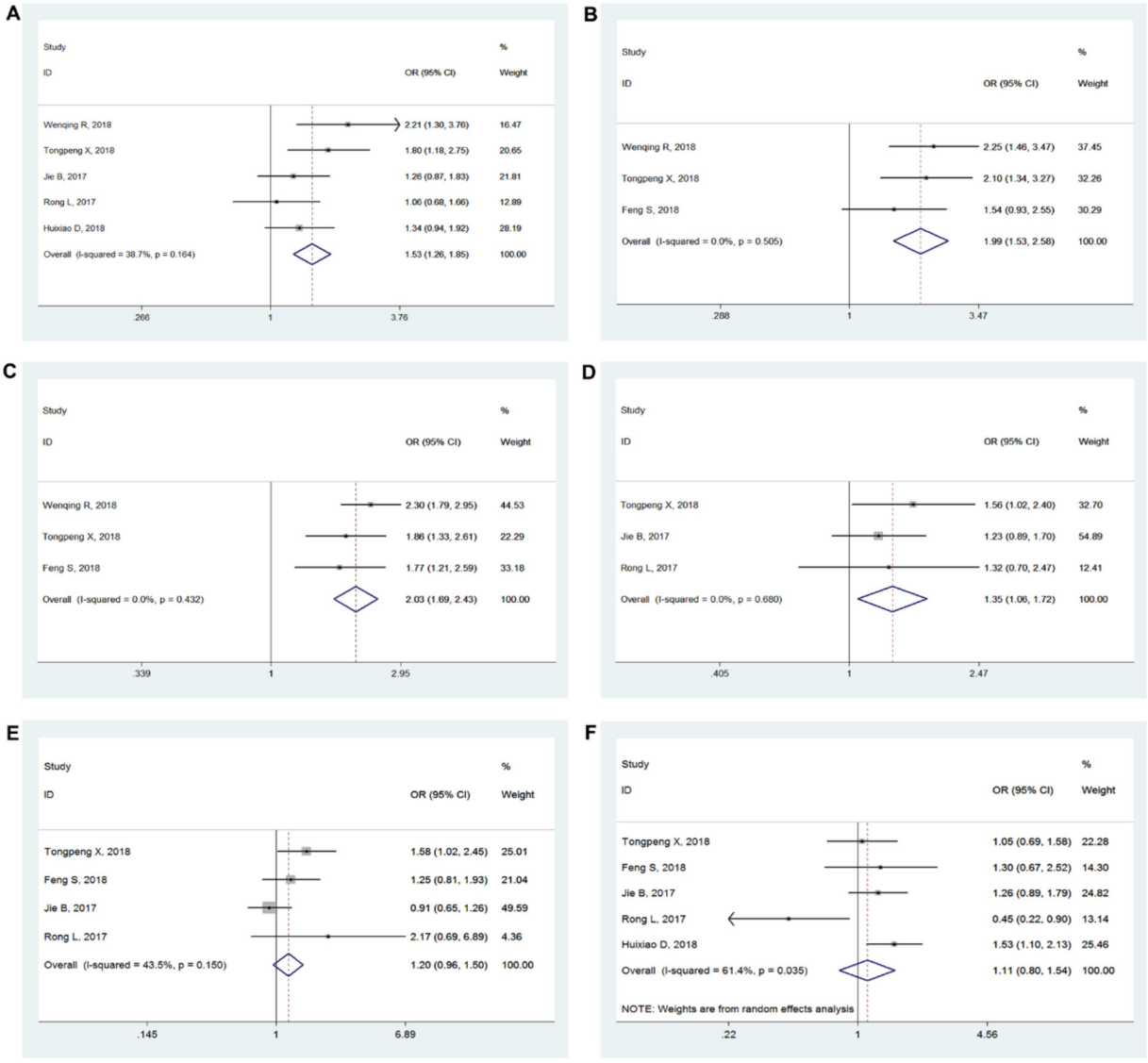

Fig. 5. Forest plots for assessing the association of FOXD2-AS1 expression with clinicopathological features. (A) tumor size, (B) invasion depth, (C) distant metastasis, (D) TNM stage, (E) lymph node metastasis, (F) differentiation.

Table 3
Basic characteristics of the five GEO datasets of breast cancer.

GEO dataset	Region	Sample size	FOXD2-AS1 cut-off value	Outcome	HR	HR (95%CI)
GSE20685	China (TaiWan)	327	4.0025	OS	1.854	1.1837-2.9038
GSE20711	Canada	88	3.2376	OS	0.933	0.4244-2.0511
GSE42568	Ireland	104	3.4315	OS	2.217	1.0999-4.4687
GSE48390	China (TaiWan)	81	4.5456	OS	0.765	0.2328-2.5140
GSE65194	France	130	4.0161	OS	1.196	0.4704-3.0412

(OR = 1.99, 95%CI: 1.53–2.58, p < 0.001) (Fig. 5B). A fixed effect model was adopted because of no significant heterogeneity existed (I^2 = 0%, p = 0.505). Furthermore, high FOXD2-AS1 expression was significantly related to distant metastasis (OR = 2.03, 95%CI:1.69–2.43, p < 0.001, fixed-effect) (Fig. 5C) and advanced TNM stage (OR = 1.35, 95%CI = 1.06–1.72, p = 0.0150, fixed-effect) (Fig. 5D). As shown in Fig. 5E and F, there was no significant correlation of FOXD2-AS1 expression with lymph node metastasis (OR = 1.20, 95%CI: 0.96–1.50, p = 0.1093, fixed-effect) and differentiation (OR = 1.11, 95%CI: 0.80–1.54, p = 0.5324, random-effect).

3.6. Meta-analysis for FOXD2-AS1 expression in breast cancer

Breast cancer is one of the most common malignancies and is the second leading cause of cancer death among females worldwide [26]. Recently, accumulating studies have reported that the abnormal expression of some lncRNAs plays an important role in the occurrence and progression of breast cancer [16,19,21]. However, no studies on FOXD2-AS1 expression in breast cancer are reported at present. Therefore, we further explored whether abnormal FOXD2-AS1 expression has a similar effect in breast cancer by utilizing the breast cancer data from GEO database. In total, five GEO datasets (GSE20685, GSE20711, GSE42568, GSE48390, GSE65194) with 730 breast cancer

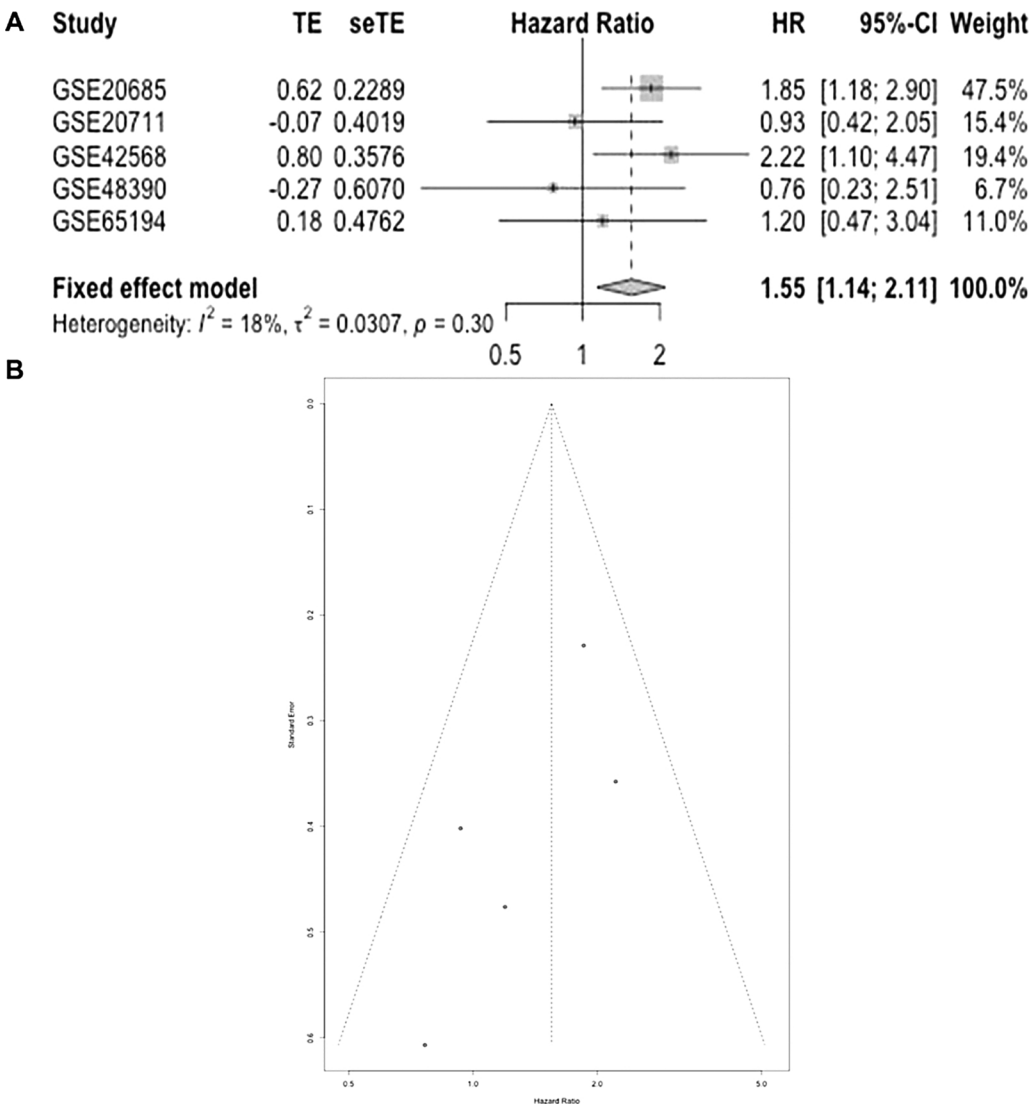

Fig. 6. (A) Forest plot of the correlation between FOXD2-AS1 expression and OS in breast cancer. (B) Funnel plot of the publication bias for OS in breast cancer.

patients were enrolled in our analysis. The basic characteristics of the five GEO datasets were generalize in Table 3. The pooled HR demonstrated that FOXD2-AS1 overexpression was significantly associated with poor OS of breast cancer patients (HR = 1.55, 95%CI: 1.14–2.11, p = 0.0052) when applying a fixed effect model due to slight heterogeneity (I^2 = 18%, p = 0.30) (Fig. 6A). As the funnel plot shows in Fig. 6B, no significant publication bias was noted.

3.7. TCGA data verification of FOXD2-AS1 expression

To further verify the increased expression of FOXD2-AS1 in a variety of malignant solid tumors, we used GEPIA online data for further population analysis. As shown in Fig. 7, the expression of FOXD2-AS1 was increased significantly in nine different types of tumor tissues compared with paired normal tissues. In addition to the cancers mentioned previously, data on cholangio carcinoma, lymphoid neoplasm diffuse large B-cell lymphoma, pancreatic adenocarcinoma, rectum adenocarcinoma and thymoma were included in this analysis.

3.8. Prediction of FOXD2-AS1 function

In order to further understand the molecular mechanism of FOXD2-AS1 overexpression affecting the prognosis of various cancers, we predicted its possible biologic function and involved signaling pathways of FOXD2-AS1 using Lncactdb 2.0 and DAVID analytic tools. First, the ceRNA regulations for FOXD2-AS1 were identified through Lncactdb 2.0 online prediction, and then a FOXD2-AS1-miRNA-mRNA network was constructed by using cytoscape software (Fig. 8). Based on the ceRNA regulation mechanism, we obtained a list of mRNAs that indirectly reflected the function of FOXD2-AS1. Then the list of mRNAs was entered into DAVID and the top 20 of KEGG pathways and the top 10 of GO terms were obtained (Fig. 9). As presented in Fig. 9A, KEGG pathway enrichment analysis indicated that FOXD2-AS1 may be involved in the signal pathways acknowledged to be closely related to cancer, such as the PI3K-Akt signaling pathway, viral carcinogenesis, FoxO signaling pathway, cell cycle, TNF signaling pathway, p53 signaling pathway and signaling pathways regulating the pluripotency of stem cells.

4. Discussion

Increasing evidence has showed that lncRNAs could acted as oncogenes and/or tumor suppressor genes in various tumor tissues, cells and microenvironments, and they are expected to become a novel class of biomarkers for cancer prognosis [43,44]. In recent years, FOXD2-AS1 has been reported by most research to be up-regulated in different types of solid tumors and negatively affect the survival outcomes [8,9,25,28]. However, one published study based on thyroid cancer data from TCGA

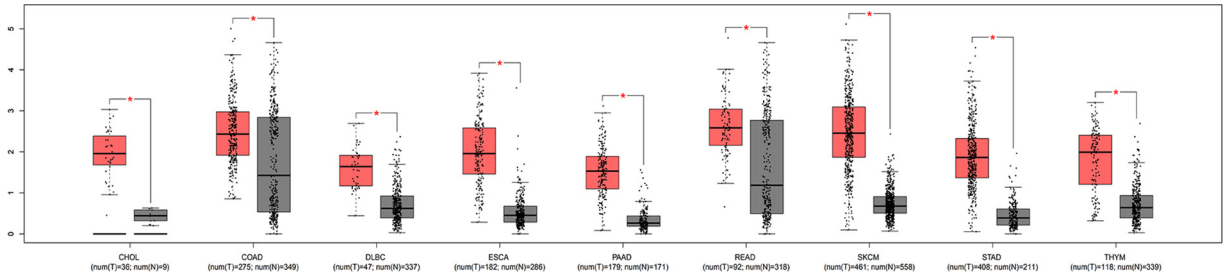

Fig. 7. FOXD2-AS1 expression in different types of tumor tissues compared with paired normal tissues. CHOL: cholangio carcinoma; COAD: colon adenocarcinoma; DLBC: lymphoid neoplasm diffuse large B-cell lymphoma; ESCA: esophageal carcinoma; PAAD: pancreatic adenocarcinoma; READ: rectum adenocarcinoma; SKCM: skin cutaneous melanoma; STAD: stomach adenocarcinoma; THYM: thymoma.

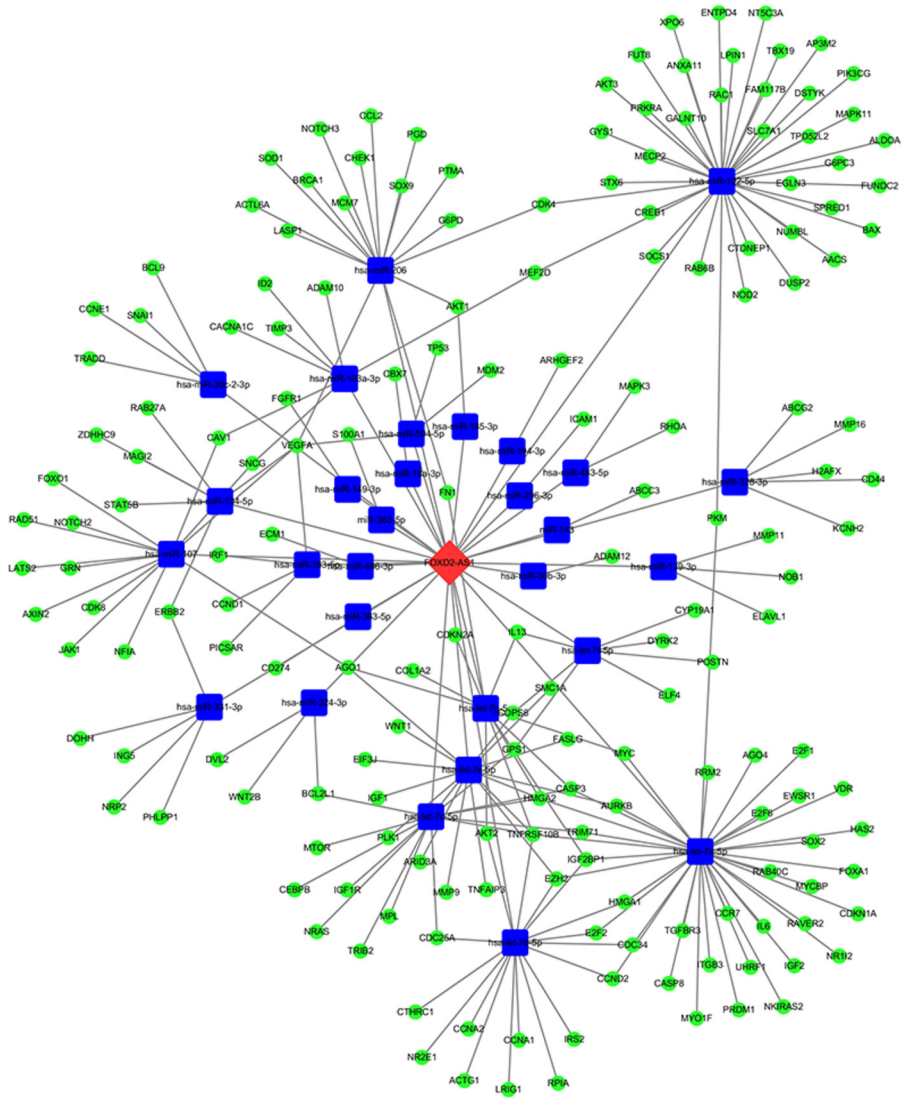

Fig. 8. Construction of FOXD2-AS1-mediated ceRNA network. FOXD2-AS1-mediated ceRNA network is comprised of 29 miRNAs and 179 mRNAs. Blue square represents miRNA, green round stands for mRNA, red rhombus is FOXD2-AS1.

found that patients with lower FOXD2-AS1 expression had shorter survival time [18]. In addition, several published studies were not good enough in quality. For instance, the sample size of certain studies was too small [8,28,41], and some studies only provide survival curves, but lack exact HR and p values. Besides, most studies only performed univariate analysis but lacked multivariate analysis [28,37,42]. To this end, we conducted a meta-analysis to assess the correlation of FOXD2-AS1 expression with survival from various cancer patients. The value of meta-analysis is reflected in that pooling the results of multiple studies can compensate for the lack of precision in most independent studies [45]. Our pooled results revealed that FOXD2-AS1 overexpression was related to poor prognosis of cancers.

To date, the correlations between FOXD2-AS1 and related clinicopathological features in different types of cancer remain to be clarified. For example, FOXD2-AS1 overexpression in GC, NMIBC and cutaneous melanoma was associated with T stage [23,28,37]. And, high

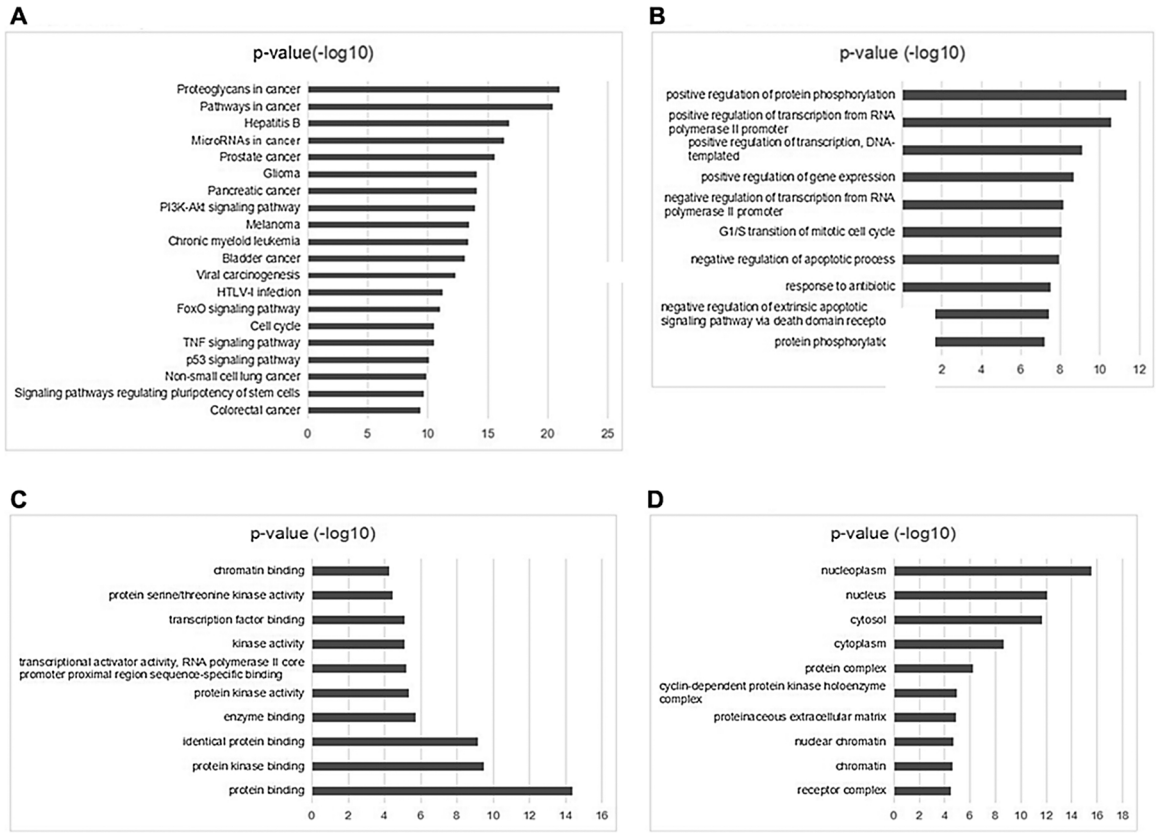

Fig. 9. KEGG pathway and GO term enrichment for FOXD2-AS1. (A) KEGG Pathway; (B) Biological Process; (C) Molexular Function; (D) Cellular Components.

expression of FOXD2-AS1 in cutaneous melanoma and GC was correlated with lymph node metastasis (LNM) [37] and distant metastasis (DM) [23], respectively. Moreover, upregulated FOXD2-AS1 was observed to be related to differentiation in NSCLC and glioma [9,24]. Thus, we analyzed the correlation between FOXD2-AS1 expression and clinicopathological parameters of cancers. Here, our results indicated that the patients with high FOXD2-AS1 expression had a higher risk of large tumor size, deep invasion depth, DM and advanced TNM stage, whereas no association was found with LNM and poor differentiation. Significant heterogeneity (I^2 = 61.4%, p = 0.035) was observed in the differentiation subgroup that may attribute to the various definitions of differentiation in the studies of various cancers. Except for the subgroup based on TCGA data, most pooled results of subgroup analyses of tumor region, FOXD2-AS1 assay method, sample size, NOS score and HR evaluation method, were stable and reliable. In addition, due to a lack of breast cancer related studies, a meta-analysis based on five GEO datasets of breast cancer were conducted to evaluate whether FOXD2-AS1 expression had similar predictive value in breast cancer. The pooled HR for OS showed that high FOXD2-AS1 expression was also associated with poor OS in breast cancer patients.

Recently, increasing evidence has indicated that lncRNAs could be detected in human body fluids such as plasma and serum by qRT-PCR [2,36]. Therefore, circulating lncRNAs, which resist degradation by nucleases and exist stably in the circulatory system, have gained attention as novel tumor biomarkers [27,29]. For example, lncRNA H19 from plasma may be a potential biomarker for breast cancer [40]. In most published studies, FOXD2-AS1 expression in tumor tissues was detected by qRT-PCR, and could predict the prognosis of various cancers. Thus, we inferred that FOXD2-AS1 may also serve as a potential circulating biomarker for the diagnosis and prognosis of some cancers, which requires a large number of experiments to be verified.

LncRNAs can indirectly regulate the expression and function of target genes via ceRNA. Thus, we constructed a ceRNA network to assess the potential function and molecular mechanism of FOXD2-AS1 in cancers. Among the predicted KEGG pathways, the 'FoxO signaling pathway' is involved in the regulation of multiple cancers [10], the 'PI3K-Akt signaling pathway' is considered one of the most frequently activated pathways in human cancer [20], the 'TNF signaling pathway' affects the proliferation and apoptosis of human leukemia cells [22], and the 'p53 signaling pathway' is involved in inducing the apoptosis of breast cancer cells in response to anticancer drugs [4]. The results of our functional analysis indicated that target genes indirectly affected by FOXD2-AS1 may be involved in some of these signaling pathways, and promoted the proliferation, invasion and metastasis of tumor cells. In most published studies, FOXD2-AS1 was localized to the cytoplasm and acted as a ceRNA adsorbing anti-cancer miRNAs. For example, Yayuan et al. reported that FOXD2-AS1 up-regulated KLK7 expression by adsorbing miR-485-5p, leading to the proliferation and migration of papillary thyroid cancer cells [41]. Qing et al. found that FOXD2-AS1 could induce gemcitabine resistance in bladder cancer cells through the FOXD2-AS1/miR-143/ABCC3 axis [1]. In addition, FOXD2-AS1 was reported to regulate the expression of both CDC42 and CCND2 by interacting with miR-185-5p, which promoted the proliferation and metastasis of colorectal cancer and glioma cells respectively [25,42]. However, for the lncRNAs mainly located in the nucleus, they interact directly with RNA or protein to affect the expression and function of the downstream target genes. Tongpeng et al. found that FOXD2-AS1 bound EZH2 and LSD1 proteins to form a complex which was recruited to the EphB3 promoter region. Afterwards, decreased transcription of EphB3 was shown to promote the growth of gastric cancer cells [37]. Moreover, another study on bladder cancer elucidated a new regulatory mode of lncRNAs. Feng et al. demonstrated that the transcriptional activity of TRIB3 was repressed via FOXD2-AS1 binding with TRIB3 promoter to form RNA-DNA complexes, which led to Akt activation. Subsequently, E2F1 expression was up-regulated by activated Akt, and binded to the promoter region of FOXD2-AS1 to promote its

transcription by forming a FOXD2-AS1/Akt/E2F1 positive feedback loop [28]. Together, further investigating the functions and signaling pathways of FOXD2-AS1 could provide new therapeutic strategies for cancer patients.

Certain unavoidable limitations in this meta-analysis should be noted. Firstly, most of the included studies were from Asia, thus our pooled results may best represent predictive value in Asian populations. However, we compensated for this racial limitation by supplementing analysis with TCGA and GEO data. Secondly, because of the number of studies and cancer types included in our analysis were limited, more studies with larger sample size and cancer types are required. Although most of the results did not show significant publication bias, the detection power of publication bias within a small number of studies is limited. The HRs with 95%CIs in some publications were also calculated from survival curves indirectly, which may affect the accuracy of the results and increase the heterogeneity. Additionally, since some articles with negative results may not have been published yet, the value of FOXD2-AS1 expression in predicting prognosis of cancers may be overestimated. Finally, we predicted the biological function and signaling pathways involving FOXD2-AS1 only using ceRNA mechanism, however FOXD2-AS1 may also have other broader functions through other regulatory mechanisms, such as cis-regulation or trans-regulation.

In summary, in spite of the aforementioned limitations, this meta-analysis confirmed that FOXD2-AS1 overexpression was significantly associated with an unfavorable OS and DFS in the patients with various cancers. Furthermore, FOXD2-AS1 was significantly related with severe clinicopathological features of cancers, such as a large tumor size, deep invasion depth, distant metastasis and advanced TNM stage. The results of functional prediction demonstrated that FOXD2-AS1 may be involved in many cancer-related signaling pathways which could affect the proliferation, invasion and metastasis of tumor cells. Therefore, FOXD2-AS1 may serve as a potential prognostic biomarker for a variety of cancers, but further large-sample studies are needed.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prp.2019.152449.

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