

Contents lists available at ScienceDirect

# Journal of Bone Oncology



journal homepage: www.elsevier.com/locate/jbo

**Research** Paper

# Abnormally expressed long non-coding RNAs in prognosis of Osteosarcoma: A systematic review and meta-analysis



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#### ARTICLE INFO

Keywords: LncRNAs Osteosarcoma Prognosis Meta-analysis

#### ABSTRACT

heterogeneity.

*Background:* Numerous studies have reported the relationship between Long non-coding RNAs (LncRNAs) expression and prognosis of osteosarcoma, but less consensus has been reached. Our meta-analysis was conducted to quantitatively assess the relationship between the expression of LncRNAs, prognosis and clinical pathology in osteosarcoma development.

*Methods*: PubMed,Embase,Web of Science,The Cochrane Library,SionMed,CNKI and WanFang databases were carefully searched to identify eligible studies. The pooled hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated to evaluate the prognostic significance of LnCRNAs expression in osteosarcoma. Moreover, meta-regression analysis and subgroup analysis were carried out to explore the potential sources of heterogeneity. *Results*: A total of 20 studies comprising 1749 patients were included in present meta-analysis. The results showed that the over-expression of LnCRNA had a significant correlation with overall survival (OS) (HR = 2.16, 95% CI:1.68–2.79), and was not related to disease free survival (DFS) (HR = 0.71, 95% CI:0.05–9.53). Subgroup analysis further indicated that LnCRNA transcription level was significantly associated with alkaline phosphatase (HR = 2.13, 95% CI:1.58–2.88), tumor size (< 8/ ≥ 8:HR = 1.97, 95% CI: 1.55–2.62), metastasis (yes/no: HR = 2.14,95% CI:1.15–3.97), distant metastasis(presence/absence: HR = 4.02, 95% CI:3.05–5.23) and Enneking stage(IIA / IIB-III:HR = 3.2, 95% CI:2.48–4.14), but not associated with age ( $\leq 25/ > 25$ :HR = 1.01, 95% CI:0.78–1.3), gender(female/male: HR = 1.15, 95% CI: 0.96–1.37), tumor site (femur,tibia/ elsewhere:HR = 1.15, 95% CI:0.04–4.63). *Conclusions:* This study demonstrated that abnormal LncRNA sexpression might be potential prognostic markers to predict worse overall survival in osteosarcoma patients. However, the cut-off values may be the source of

#### 1. Introduction

Osteosarcoma (OS) is a highly malignant tumor of bone in children and adolescents [1], which accounts for about 2.4% of malignant tumors in children, and the incidence of OS is about 1–5 cases per million people per year [2,3]. Most patients are diagnosed as OS under the age of 25 years, and there are more men than women among the OS patients [4,5]. Both the metastasis and mortality rates of OS are high in clinic practice. About 20% OS patients were diagnosed with lung metastases at the time of the first diagnosis, and 80% OS metastases occur in the lung [6]. At present, the treatment of OS is mainly based on the combination of surgical resection and multiple chemotherapeutic drugs [7]. The average treatment rate of OS was 65% [8]. The average five-year survival rate of OS patients without metastasis was about 80% [9,10]. 90% OS patients died of recurrence or metastasis due to the presence of tumor resistance, drug side effects and other causes, and the five-year survival rate of OS patients was only 20–30% [11–13]. So far, the molecular mechanism of OS remains unclear. Therefore, finding

https://doi.org/10.1016/j.jbo.2018.09.005

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Fig. 1. Flow diagram of study selection process.

Table 1	
Comparison of <i>p</i> values of relationships between lncRNAs and clinicopathological features in osteosarcoma.	

Author	Year	LncRNAs	Country	Case number	Cut-off	Expression	Gender	Age	Tumor site	Tumor size	Tumor stage	Metastasis	ALP	Chemotherapy
Jiang	2017	DANCR	China	34	NA	up-regulated	NA	NA	NA	< 0.05	NA	< 0.05	NA	NA
Wen	2017	UCA1	China	151	NA	up-regulated	0.572	0.199	0.804	0.907	0.001	0.007	NA	NA
Cai	2017	HNF1A-AS1	China	72	median	up-regulated	0.215	0.534	0.143	0.311	0.019	0.009	0.128	0.031
O'Leary	2017	PARTICLE	Germany	40	NA	up-regulated	0.030	NA	NA	NA	NA	0.01	NA	NA
Huo	2017	MALAT1	China	46	median	up-regulated	0.759	0.473	NA	0.008	0.058	0.000	NA	NA
Li	2017	XIST	China	145	NA	up-regulated	0.827	0.102	0.886	0.009	0.001	0.009	0.704	NA
Wang	2017	SOX2-OT	China	138	median	up-regulated	0.723	0.115	0.191	0.036	0.008	0.001	NA	NA
Zhou	2016	CCAL	China	46	median	up-regulated	0.555	0.200	0.502	0.134	0.017	0.006	NA	NA
Peng	2016	BANCR	China	84	median	up-regulated	0.509	0.505	0.814	0.008	0.004	0.020	0.366	NA
Ju	2016	BCAR4	China	168	median	up-regulated	0.381	0.494	0.982	0.810	0.002	0.001	0.191	0.841
Chen	2016	BCAR4	China	60	median	up-regulated	0.795	0.436	0.754	0.037	0.041	0.028	NA	NA
Ma	2016	TUG1	China	76	fold-change	up-regulated	0.835	0.701	0.093	0.011	0.002	0.802	0.235	0.020
Gao	2016	MALAT1	China	162	median	up-regulated	0.335	0.202	0.193	0.344	0.000	0.001	NA	NA
Cong	2016	TUSC7	China	82	fold-change	dowm-	0.65	0.473	0.627	NA	0.294	0.087	NA	NA
						regulated								
Uzan	2016	HULC	Brazil	33	ROC	up-regulated	0.999	0.065	0.274	0.67	NA	0.999	NA	NA
Xia	2016	91H	China	67	median	up-regulated	0.497	0.927	0.114	< 0.001	0.015	0.936	NA	0.023
Li	2016	UCA1	China	135	median	up-regulated	0.573	0.339	0.512	0.005	< 0.001	0.002	NA	NA
Tian	2015	MEG3	China	64	median	dowm-	0.614	0.302	0.281	0.076	0.006	0.011	NA	NA
						regulated								
Li	2015	HOTTIP	China	68	median	up-regulated	0.465	0.215	0.161	0.120	0.003	0.016	NA	NA
Sun	2015	HULC	China	78	median	up-regulated	0.492	0.352	0.624	0.496	0.003	0.005	NA	NA

Notes: LncRNA, long non-coding RNA;ALP, alkaline phosphatase;NA, not available.

new molecular markers for early diagnosis and prognosis and therapeutic targets of OS is very important for improving the survival rate of OS patients. Encouragingly, some LncRNAs have recently been reported to play a key role in OS pathogenesis.

LncRNA is a class of non-coding RNAs that are longer than 200 nucleotides in length, with little or no protein coding capacity [14].

#### Table 2

Characteristics of studies included in this meta-analysis.

Studies	LncRNAs	Country	Sample Type	Case number (High/Low)	Method	Cut-off	Tumor stage	outcome	HR availability	NOS
Jiang 2017	DANCR	China	Tissue	34(17/17)	qRT-PCR	NA	NA	OS,DFS	Directly	7
Wen 2017	UCA1	China	Tissue	151 (75/76)	qRT-PCR	NA	IIA-III	OS,DFS	Directly	7
Cai 2017	HNF1A-AS1	China	Tissue	72 (36/36)	qRT-PCR	median	IIA-III	OS	Directly	6
O'Leary 2017	PARTICLE	Germany	Tissue	40 (23/17)	qRT-PCR	NA	NA	OS,MFS	Indirectly	7
Huo 2017	MALAT1	China	serum	46 (18/26)	aRT-PCR	median	I-IV	OS.PFS	Indirectly	6
Li 2017	XIST	China	Tissue	145 (75/70)	qRT-PCR	NA	I-IV	OS	Directly	7
Wang 2017	SOX2-OT	China	Tissue	138 (69/69)	qRT-PCR	median	I-III	OS	Directly	7
Zhou 2016	CCAL	China	Tissue	46 (23/23)	qRT-PCR	median	I-IV	OS	Directly	7
Peng 2016	BANCR	China	Tissue	84 (42/42)	qRT-PCR	median	IIA-III	OS	Directly	7
Ju 2016	BCAR4	China	Tissue	168 (87/81)	qRT-PCR	median	IIA-III	OS	Directly	7
Chen 2016	BCAR4	China	Tissue	60 (30/30)	qRT-PCR	median	I-III	OS,RFS	Directly	7
Ma 2016	TUG1	China	Tissue	76 (41/35)	qRT-PCR	fold-change	I-III	OS,PFS	Directly	8
Gao 2016	MALAT1	China	Tissue	162 (80/82)	qRT-PCR	median	IIA-III	OS	Directly	7
Cong 2016	TUSC7	China	Tissue	82 (13/69)	qRT-PCR	fold-change	early-advanced	OS	Directly	7
Uzan 2016	HULC	Brazil	Tissue	33 (12/21)	qRT-PCR	ROC	NA	OS,EFS	Indirectly	8
Xia 2016	91H	China	serum	67 (34/33)	qRT-PCR	median	I-III	OS	Directly	8
Li 2016	UCA1	China	Tissue	135 (68/67)	qRT-PCR	median	I-III	OS	Directly	7
Tian 2015	MEG3	China	Tissue	64 (32/32)	qRT-PCR	median	I-III	OS	Directly	7
Li 2015	HOTTIP	China	Tissue	68 (34/34)	qRT-PCR	median	IIA-III	OS	Directly	7
Sun 2015	HULC	China	Tissue	78 (39/39)	qRT-PCR	median	IIA-III	OS	Directly	7
					-				-	

Notes: LncRNA, long non-coding RNA; qRT-PCR, quantities reverse transcription polymerase chain reaction; OS, overall survival; DFS, disease free survival; MFS, metastasis free survival; PFS, progression free survival; RFS, recurrence free survival; EFS, event free survival; HR, hazard ratio; NA, not available.



Fig. 2. Forest plot for the association between LncRNA expression levels with overall survival and disease-free survival in osteosarcoma.

Recent studies showed that LncRNA is widely transcribed in mammalian genome and plays an important role in gene regulation, which is involved in biological processes such as tumor proliferation, invasion, metastasis, apoptosis and drug resistance [15,16]. At present, the most important role of LncRNA may be associated with occurrence of cancers, and some studies have shown significant changes in LncRNA expression levels in colon, liver and lung cancers. Significantly high expressed LncRNA DLEU7-AS1 in patients with colon cancer promotes tumor invasion and metastasis through Wnt/ $\beta$ -catenin signaling pathway [17]. LncRNA HOTTIP can act as oncogenes to enhance the expression of anti-apoptotic factor Bcl-2 and promote chemotherapeutic resistance in small cell lung cancers by sponging miR-216a [18].LncRNA MEG3 inhibits the malignant progression of hepatocellular carcinoma by inhibiting PKM2 activity and  $\beta$ -catenin signaling pathway [19]. Recently, more and more literatures showed that LncRNAs are potential biomarkers for diagnosis and prognosis of OS.

So far, lots of researches have proved that LncRNA such as TUG1 [20], PVT1 [21] and ODRUL [22] are apparently highly expressed in OS patients. Jiang indicated that LncRNA DANCR can upregulate AXL expression by competitively sponging miR-33a-5p, enhance the function of cancer stem cells and promote the invasion and metastasis of OS [23]. Han found that LncRNA ATB is highly expressed in serum of OS patients, and its sensitivity

#### Table 3

General and subgroup analysis of the correlation between LncRNAs expression and overall survival.

Categories	No. of studies	No. of patients	HR(95%CI)for OS	Meta-regression P-value	Heterogeneity	
					I-squared(%)	Chi-squared(P)
OS	20	1749	2.16 (1.68-2.79)		54%	0.0003
Race				0.294		
Asian	18	1676	2.15 (1.63-2.82)		58%	0.001
Not Asian	2	73	2.42 (1.25-4.7)		0%	0.53
No. of patients				0.121		
≥100	6	899	2.41 (1.78-3.27)		4%	0.39
<100	14	777	1.95 (1.4–2.73)		62%	0.001
HR availability				0.957		
Directly	17	1630	2.23 (1.67-2.99)		54%	0.004
Indirectly	3	119	1.66 (1.16-2.37)		13%	0.31
Cut-off values				0.08		
Median	13	1188	2.11 (1.75-2.53)		3%	0.41
Fold-change	2	158	0.97 (0.11-8.24)		90%	0.002
Others	5	403	1.77 (0.71-4.4)		77%	0.002
NOS scores				0.147		
>7	3	176	2.92 (1.81-4.72)		0%	0.98
≤7	17	1573	2.05 (1.54-2.73)		58%	0.001



Fig. 3. Forest plots of studies evaluating hazard ratios of LncRNA UCAI, BCAR4, HULC and MALAT1 and overall survival of osteosarcoma patients.

and specificity are 83.33% and 90%, respectively [24]. Although many studies have assessed the correlation between LncRNA expression and OS prognosis, they are limited due to small sample sizes and different study qualities, and the prognostic value of LncRNA has not been agreed upon. Therefore, we conducted a systematic review and meta-analysis to quantitatively assess the relationship of LncRNAs expression with the prognosis and clinicopathology of OS patients.

#### 2. Material and methods

#### 21. Search strategy

PubMed,EMbase,Web of Science,The Cochrane Library,SionMed,CNKI and WanFang databases were searched to identify eligible studies up to November 11, 2017. The search strategy used both MeSH terms and freetext words to increase the sensitivity of the search. The search terms included: ("Long non-coding RNA", "IncRNA", "LincRNA", "Long ncRNA", "Long intergenic non-coding RNA") AND ("Osteosarcoma", "Osteogenic Sarcoma", "Osteosarcoma Tumor") with the limit to human, detailed search strategy is attached to appendix.

#### 2.2. Inclusion and exclusion criteria

The eligible studies met the following criteria: (1) The clinical study of the expression of LncRNA in osteosarcoma; (2) Patients were confirmed osteosarcoma by pathological or histological examination; (3) qRT-PCR (Quantitative reverse transcription-polymerase chain reaction) was used to detect LncRNA expression in tissues or circulating blood of patients with

#### Table 4

Association between overall survival time and clinicopathological features of patients with osteosarcoma.

clinicopathological Parameters	studies	Univariate analysis				studies	Multivariate analy	sis		
		pooled HR(95%CI)	P-value	I-squared (%)	Chi-squared (P)		Pooled HR (95%CI)	P-value	I-squared (%)	Chi-squared (P)
Age( $\le 25 \text{ vs.} > 25$ )	6	1.01(0.78-1.3)	0.95	0%	0.88					
Gender(Female vs. Male)	11	1.15(0.96-1.37)	0.12	0%	0.97					
Tumor site (femur,tibia vs. elsewhere)	11	1.15(0.94–1.4)	0.17	18%	0.28					
ALP	4	2.13(1.58-2.88)	< 0.00001	22%	0.28					
Chemotherapy(yes vs. no)	3	1.45(0.46-4.63)	0.53	86%	0.0007					
Tumor size( $< 8 \text{ vs.} \ge 8$ )	10	1.97(1.55-2.62)	< 0.00001	0%	0.64	5	1.28 (0.88-1.86)	0.2	64%	0.02
Metastasis(yes vs. no)	4	2.35 (1.69-3.26)	< 0.00001	0%	0.98	7	2.14 (1.15-3.97)	0.02	78%	0.0002
Distant metastasis(presence vs. absence)	7	5.03(3.78–6.69)	< 0.00001	0%	0.85	8	4.02 (3.05–5.23)	< 0.0001	0%	1
Enneking stage(IIA vs. IIB-III)	6	4.01 (3.08-5.23)	< 0.00001	0%	0.64	7	3.2 (2.48-4.14)	< 0.0001	0%	0.63
LncRNA expression	11	2.95 (2.37–3.66)	< 0.00001	0%	0.93	20	2.16 (1.68–2.79)	< 0.00001	54%	0.002

### А

В

### Meta-analysis estimates, given named study is omitted





Meta-analysis estimates, given named study is omitted



Fig. 4. A-B Sensitivity analyses of the studies. (A) Multivariate analysis of overall survival; (B) Univariate analysis of overall survival.



**Fig. 5.** A–D Begg's test for publication bias. (A) Multivariate analysis of overall survival (P = 0.127); (B) Tumor size (P = 1.000); (C) Tumor stage(P = 0.511); (D) Metastasis(P = 0.767).

osteosarcoma; (4) Studies provided sufficient information for extraction or calculation of the individual HR and 95%CI; (5) The association of LncRNAs with survivals was performed. Studies were excluded based on the following criteria: basic research of cell or animal experiment, duplicate articles, conference abstracts, case reports, review articles and letters.

#### 2.3. Data collection and quality assessment

Two reviewers (Delong Chen and Meng Zhang) independently reviewed the eligible publications. The following data was extracted: surname of the first author, year of publication, the type of LncRNAs and expression, country, case number, cut-off value, sample type, outcome, clinicopathological features, outcome of study, HRs with their 95% CIs for OS or DFS and quality score. If the data was unavailable, we contacted study authors to request missing data. The information was extracted and recorded by using a standardized form. Two reviewers (Shan Jiang and Chi Zhou) independently assessed the quality of included studies by using Newcastle-Ottawa Quality Assessment Scale (NOS). If there are differences between the two reviewers, they can be resolved through discussion or finding another reviewer (Peng Chen).

### 2.4. Statistical analysis

Review Manager 5.2 (The Cochrane Collaboration, Software Update, Oxford, UK) and stata12.0 (STATA Corporation, College Station, Texas, USA) were used for meta-analysis. The HRs and 95% CI were used to evaluate the association between LncRNAs and prognosis and clinicopathological features. HR > 1 indicated that patients with upregulated LncRNA expression had poor prognosis. On the contrary, HR < 1 meant that patients with decreased LncRNA expression had

better prognosis. We directly extracted if HRs and 95% CIs were reported directly in the articles. Otherwise, HRs and 95% CIs were estimated from existing data or Kaplan–Meier curve using methods previously reported by Tierney et al. Heterogeneity among the eligible studies was assessed with I<sup>2</sup> statistics and chi-square Q test. *P* < 0.05 indicated statistically significant heterogeneity among studies, low heterogeneity: I<sup>2</sup>  $\geq$  25%; moderate heterogeneity: I<sup>2</sup>  $\geq$  50%; high heterogeneity: I<sup>2</sup>  $\geq$  75%. Fixed-effects model was used when I<sup>2</sup> < 50%. Otherwise, the random-effects model was used.

We conducted meta-regression analysis and subgroup analysis according to race, number of patients, HR availability, cut-off values and NOS scores. In order to verify the stability of the pooled results, we carried out sensitivity analysis. The publication bias was identified by Begg test and Egger test. P < 0.05 was considered statistically significant.

#### 3. Results

#### 3.1. Study inclusion and characteristics

As shown in the flow diagram (Fig. 1), 478 articles were initially retrieved from PubMed,Embase, Web of Science, The Cochrane Library, SionMed, CNKI and WanFang databases. 177 duplicated articles were excluded. After the titles and abstracts were scanned, 188 irrelevant articles were removed. According to the inclusion and exclusion criteria, the full texts of remaining 113 articles were read for further evaluation and then 93 articles were excluded. Finally, 20 studies were included in the meta-analysis [25–44].

The correlations between the expression levels of LncRNA and differences in clinicopathological features in osteosarcoma patients were described in Table 1, including gender, age, tumor site, tumor size,



**Fig. 6.** A–D Egger's test for publication bias. (A) Multivariate analysis of overall survival (P = 0.951); (B) Tumor size(P = 0.631); (C) Tumor stage(P = 0.255); (D) Metastasis(P = 0.438).

tumor stage, metastasis, ALP and chemotherapy. The characteristics of 20 included studies were summarized in Table 2. Most of these studies were performed in China (18/20), another two studies were performed respectively in Germany and Brazil. The number of patients in 20 studies ranged from 33 to 168. Specimens were composed of osteosarcoma tissue (n = 18) and serum (n = 2). Among these 20 articles, 20 provide data on correlation between LncRNA expression and overall survival (OS), 2 on correlation between LncRNA expression and disease free survival (DFS) or progression free survival (PFS), 1 on correlation between LncRNA expression and metastasis free survival (MFS), recurrence free survival (RFS) or event free survival (EFS).

#### 3.2. Prognostic value

Twenty studies comprising 1749 patients were included in final analysis to assess the effect of LncRNA expression on OS. As shown in Fig. 2, the heterogeneity test indicated the existence of medium heterogeneity in OS ( $I^2 = 54\%$ , P < 0.00001), the pooled HR was 2.16 (95% CI: 1.68–2.79), which was calculated using random-effects model, the results suggested that a high LncRNA expression was significantly correlated with the poorer OS prognosis. The increased expressions of LncRNA UCA1 [25,38], XIST [26], SOX2-OT [28], MALAT1 [29,36], HNF1A-AS1 [30], PARTICLE [31], CCAL [32], 91H [33], HULC [34,43], BCAR4 [35,39], TUG1 [40], BANCR [41], MEG3 [42] and HOTTIP [44] indicated poorer prognoses of OS, on the contrary, the decreased expressions LncRNA DANCR [27] and TUSC7 [37] indicated better prognoses of OS. A total of 2 studies, including 185 patients, were included in the assessment of the effect of LncRNA expression on DFS (Fig. 2). There was no statistically significant correlation between low expression of LncRNA and prognosis of OS (HR = 0.71, 95%CI: 0.05–9.53,

P = 0.0003,  $I^2 = 92\%$ ).

We conducted meta-regression analysis and subgroup analysis according to race, number of patients, and calculation method of HR value, cut-off values and NOS scores (Table 3). An additional file shows forest plot of HRs for subgroup analysis in more detail (Fig. A.1). In the subgroup analysis, the fold change (HR = 0.97, 95% CI: 0.11–8.24) in cut-off value was not statistically significant, other results showed that expression of LncRNA was correlated with a worse OS prognosis. Meta regression analysis revealed that there were no significant correlations between all relevant stratified factors and the heterogeneity among studies (P value ranged from 0.08 to 0.957).

Of the total 20 LncRNAs, 4 (UCA1, BCAR4, HULC and MALAT1) were investigated in two studies. We found that the higher expressions of LncRNA UCA1 (HR = 2.70, 95% CI: 1.60-4.57), BCAR4 (HR = 2.58, 95% CI: 1.38-4.80), HULC (HR = 2.67, 95% CI: 1.45-4.92) and MALAT1 (HR = 2.41, 95% CI: 1.51-3.83), the poorer prognoses in osteosarcoma (Fig. 3).

#### 3.3. The correlation between LncRNAs and clinicopathological features

We evaluated the correlation between LncRNA expression and clinicopathological features of OS (Table 4). LncRNA transcription level was significantly correlated with alkaline phosphatase (univariate analysis: HR = 2.13, 95% CI: 1.58–2.88, tumor size (univariate analysis: <8 /  $\geq$  8: HR = 1.97, 95% CI: 1.55–2.62), metastasis (univariate analysis: yes/no: HR = 2.35, 95% CI: 1.69–3.26; multivariate analysis: yes/no: HR = 2.14, 95% CI:1.15–3.97), distant metastasis (univariate analysis: presence/absence: HR = 5.03, 95% CI:3.78–6.69; multivariate analysis: presence/absence: HR = 4.02, 95% CI:

3.05–5.23) and Enneking stage (univariate analysis: yes/no: HR = 4.01, 95% CI: 3.08–5.23; multivariate analysis: IIA /IIB-III: HR = 3.2, 95% CI: 2.48–4.14), but not correlated with age (univariate analysis:  $\leq 25$  / > 25: HR = 1.01, 95% CI: 0.78–1.3), gender (univariate analysis: female/male: HR = 1.15, 95% CI: 0.96–1.37),tumor site(univariate analysis: femur,tibia/elsewhere: HR = 1.15, 95% CI: 0.94–1.4),chemotherapy (univariate analysis: yes/no: HR = 1.45, 95% CI: 0.46–4.63) and tumor size (multivariate analysis: < 8 /  $\geq$  8: HR = 1.28, 95% CI: 0.88–1.86). An additional file shows forest plot for the association between overall survival time and clinicopathological features of patients in more detail (Fig. A.2 and A.3). The results showed that the high expression of LncRNA was significantly correlated with the poorer OS prognosis (univariate analysis: HR = 2.95, 95% CI: 2.37–3.66; multivariate analysis: HR = 2.16, 95% CI: 1.68–2.79).

In addition to significant heterogeneity among studies on correlation between high expression of LncRNA and chemotherapy (univariate analysis: yes/no:  $\rm I^2=92\%,\ P=0.0007)$ , tumor size (multivariate analysis: < 8 /  $\geq$  8:  $\rm I^2=64\%,\ P=0.02)$  or metastasis (multivariate analysis: yes/no:  $\rm I^2=78\%,\ P=0.0002)$  no significant heterogeneity was observed among the studies on correlation between high expression of LncRNA and other clinicopathological factors.

#### 3.4. Publication bias and sensitivity analysis

The univariate and multivariate sensitivity analyses of OS showed that the individual study had no obvious influence on the whole study results, and the overall effect had a good stability (Fig. 4.A and B). Both Begg's funnel plot and Egger's test were used to assess publication bias in the meta-analysis. The Begg's funnel plot of the pooled analysis in Fig. 5.A–D was quite symmetric, and no publication bias was detected by Egger's test due to all *P*-values > 0.05 (Fig. 6.A–D).

#### 4. Discussion

Osteosarcoma has become a cancer with the highest mortality rate in children and adolescents, which has characteristics such as local corrosion and systemic metastasis. Although the five-year survival rate of OS patients without metastasis is 80%, the measures to treat the metastatic OS are very limited. LncRNA is widely involved in biological processes such as tumor proliferation, invasion, metastasis, apoptosis and drug resistance. Investigating the expression and clinical significance of LncRNA in OS patients can provide a basis for prevention, diagnosis and treatment of OS in clinic. Therefore, we conducted a meta-analysis to assess the relationship of LncRNAs expression with the prognosis and clinicopathology of OS patients.

Recent studies have shown that LncRNA is closely correlated with the OS prognosis. LncRNA FOXC2-AS1 can increase the expression of FOXC2 transcription factors and promote the drug resistance of Adriamycin and multidrug resistance of OS cells [45]. High LncRNA HOST2 expression is a biomarker of poor prognosis in OS patients, it affects the proliferation, migration, invasion and apoptosis of OS cells [46]. In this meta-analysis, a total of 20 studies comprising 1749 patients were included into the final analysis. Our results showed that high LncRNA expression was significantly correlated with a poorer prognosis in OS patients, which suggested that LncRNA plays an important role in the prognosis of OS. The same results were also found in the meta-analysis of other cancers such as colon cancer [47], cervical cancer [48] and prostate cancer [49]. However, there was moderate heterogeneity in multivariate analysis of overall survival (I<sup>2</sup> = 54%, P < 0.00001), so we performed meta-regression analysis and subgroup analysis according to race, number of patients, HR availability, cut-off values and NOS scores. The results of Meta regression analysis showed that there were no significant correlations between these factors and the heterogeneity in this study, but there was still a significant heterogeneity in the subgroup analysis. In subgroup analysis, the median cutoff value (HR = 2.11, 95% CI: 1.75-2.53; heterogeneity: P = 0.41,

 $I^2 = 3\%$ ) was significantly correlated with worse OS, but had no significant correlation with fold-change (HR = 0.97, 95% CI: 0.11–8.24; heterogeneity: P = 0.002,  $I^2 = 90\%$ ). Significant heterogeneity existed among studies of cut-off value, fold-change and OS, and the reason maybe that fewer cases were included in studies.

In the result of our study, high expressions of LncRNA UCA1, BCAR4, HULC and MALAT1 were reported in two literatures, which predicted a worse overall survival rate in OS patients. Li found that HIF-1 $\alpha$  enhances the expression of LncRNA UCA1 and promotes the proliferation of OS cells by inhibiting the PTEN/Akt signaling pathway [50]. The study by Kong et al. showed that LncRNA HULC exerts a sponge effect on miR-122. Over-expression of miR-122 promotes PI3K/AKT, JAK/STAT and Notch signaling pathways, down-regulates HNF4G expression and enhances tumor invasion and metastasis [51]. LncRNA MALAT1 plays an important role in the OS progression through modulating the enhancers of zeste homolog 2 (EZH2). MALAT1 provides a new target for the treatment of OS by inhibiting E-cadherin expression and enhancing  $\beta$ -catenin expression [52]. Therefore, LncRNA UCA1, BCAR4, HULC, and MALAT1 are independent risk factors for poor prognosis in OS patients.

We also evaluated the relationship between LncRNA expression and the clinicopathological features of OS. An univariate analysis showed that LncRNA transcription levels were not associated with age ( $\leq 25/$ > 25), gender (male/female), tumor site (femur, tibia/elsewhere) and chemotherapy (yes/no), but were significantly correlated with ALP, tumor size ( $< 8/ \geq 8$ ), tumor metastasis (yes/no), tumor distant metastasis (presence/absence) and Ennking stage (IIA /IIB-III). However, a multivariate analysis showed that there was no significant association between LncRNA transcription level and tumor size ( $< 8/ \geq 8$ ), which may be one of the sources of heterogeneity.

In addition, there are still some deficiencies in this meta-analysis: (1) In order to reduce the bias caused by different methods of detecting LncRNA expression, we only included the studies using qRT-PCR method to detect LncRNA expression, which may bias the results due to different primers. (2) Different methods of HR extraction may also lead to bias. There were certain differences in HR and 95% CI between indirect and direct extractions. (3) The majority of populations were Asians included in this study, which may lead to biased results due to geographical differences. (4) Other factors such as different follow-up time and cut-off values will also result in bias.

In conclusion, this study shows that there is a significant correlation between the expression of LncRNA and the overall survival rate of OS patients, and it affects the prognosis of OS patients, suggesting that LncRNA may play an important role in the occurrence and development of OS. To further confirm our conclusion, a prospective high-quality study with a large sample size is needed to verify the role of LncRNA expression in the OS prognosis.

#### Acknowledgments

We owe our thanks to Yaolong Chen of Lanzhou University for his work on revising this manuscript. This work was financially supported by the National Natural Science Foundation of China (NSFC) (No. 81603641) and Guangdong Provincial Science and Technology Project (No. 2017A020213030).

### Author contributions

Peng Chen designed the study. Delong Chen and Peng Chen wrote the manuscript. Delong Chen, Meng Zhang, Shan Jiang and Chi Zhou collected and extracted data. Haibin Wang and Bing Fang revised the manuscript. All authors read and approved the final manuscript.

#### **Conflict of interest**

The authors have no conflict of interest to declare.

### Appendices

				Hazard Ratio	Hazard Ratio
Study or Subgroup	log[Hazard Ratio]	SE	Weight	IV, Random, 95% CI	IV, Random, 95% Cl
3.2.1 Asian					
2015 Li(HOTTIP)	1.060218	0.418869	5.0%	2.89 [1.27, 6.56]	
2015 Sun(HULC)	0.822859	0.331293	6.2%	2.28 [1.19, 4.36]	
2015 Tian(MEG3)	0.881699	0.418798	5.0%	2.41 [1.06, 5.49]	
2016 Chen(BCAR4)	0.839842	0.386315	5.4%	2.32 [1.09, 4.94]	
2016 Cong(TUSC7)	-1.16155	0.572258	3.4%	0.31 [0.10, 0.96]	-
2016 Gao(MALAT1)	1.149622	0.37932	5.5%	3.16 [1.50, 6.64]	
2016 Ju(BCAR4)	1.169381	0.556342	3.5%	3.22 [1.08, 9.58]	
2016 Li(UCA1)	1.144541	0.4705	4.4%	3.14 [1.25, 7.90]	
2016 Ma(TUG1)	1.022451	0.392122	5.3%	2.78 [1.29, 6.00]	
2016 Peng(BANCR)	1.076367	0.4899	4.2%	2.93 [1.12, 7.66]	
2016 Xia(91H)	1.144223	0.442841	4.7%	3.14 [1.32, 7.48]	
2016 Zhou(CCAL)	1.158452	0.460337	4.5%	3.18 [1.29, 7.85]	
2017 Cai (HNF1A-AS1)	0.968504	0.329814	6.2%	2.63 [1.38, 5.03]	
2017 Huo (MALAT1)	0.368109	0.158227	9.0%	1.44 [1.06, 1.97]	-
2017 Jiang (DANCR)	-1.73161	0.6553	2.8%	0.18 [0.05, 0.64]	
2017 Li (XIST)	1.75128464	0.688729	2.6%	5.76 [1.49, 22.22]	
2017 Wang (SOX2-OT)	0.506215	0.237152	7.7%	1.66 [1.04, 2.64]	
2017 Wen ( UCA1)	0.92267	0.326347	6.3%	2.52 [1.33, 4.77]	
Subtotal (95% CI)			91.5%	2.15 [1.63, 2.82]	•
Heterogeneity: Tau <sup>2</sup> = 0.18;	Chi <sup>2</sup> = 40.33, df = 17	(P = 0.001);	I <sup>2</sup> = 58%		
fest for overall effect: Z = 5.4	47 (P < 0.00001)				
.2.2 Not Asian					
016 Uzan(HULC)	1.064711	0.441976	4.7%	2.90 [1.22, 6.90]	
2017 O'Leary (PARTICLE)	0.628609	0.5271	3.8%	1.88 [0.67, 5.27]	
Subtotal (95% CI)			8.5%	2.42 [1.25, 4.70]	•
leterogeneity: Tau <sup>2</sup> = 0.00;	Chi <sup>2</sup> = 0.40, df = 1 (P	= 0.53); I <sup>2</sup> =	0%		
fest for overall effect: Z = 2.6	61 (P = 0.009)				
otal (95% CI)			100.0%	2.16 [1.68, 2.79]	•
Heterogeneity: Tau <sup>2</sup> = 0.16;	Chi <sup>2</sup> = 41.05, df = 19	(P = 0.002);	I <sup>2</sup> = 54%	the second se	
est for overall effect: Z = 5.9	99 (P < 0.00001)	S ()		L 5-1	J. UT U.T T TU T
and fam and anna an aliffanan a		0 0 7 0	17 0.00	Fai	rours (experimental) Favours (control)

Test for overall effect: Z = 5.99 (P < 0.00001) Test for subarous differences: Chi<sup>2</sup> = 0.11. df = 1 (P = 0.74). I<sup>2</sup> = 0%

#### B

Α

- 127 8 1270				Hazard Ratio	Hazard Rat	io
Study or Subgroup	log[Hazard Ratio]	SE	Weight	IV, Random, 95% Cl	IV, Random, 9	5% CI
3.1.1 < 100						2
2015 Li(HOTTIP)	1.060218	0.418869	5.0%	2.89 [1.27, 6.56]		
2015 Sun(HULC)	0.822859	0.331293	6.2%	2.28 [1.19, 4.36]		
2015 Tian(MEG3)	0.881699	0.418798	5.0%	2.41 [1.06, 5.49]		2-00
2016 Chen(BCAR4)	0.839842	0.386315	5.4%	2.32 [1.09, 4.94]	1000	(514)
2016 Cong(TUSC7)	-1.16155	0.572258	3.4%	0.31 [0.10, 0.96]		
2016 Ma(TUG1)	1.022451	0.392122	5.3%	2.78 [1.29, 6.00]		
2016 Peng(BANCR)	1.076367	0.4899	4.2%	2.93 [1.12, 7.66]	1	
2016 Uzan(HULC)	1.064711	0.441976	4.7%	2.90 [1.22, 6.90]		Sere Of
2016 Xia(91H)	1.144223	0.442841	4.7%	3.14 [1.32, 7.48]		10 10 10 10 10 10 10 10 10 10 10 10 10 1
2016 Zhou(CCAL)	1.158452	0.460337	4.5%	3.18 [1.29, 7.85]		
2017 Cai ( HNF1A-AS1)	0.968504	0.329814	6.2%	2.63 [1.38, 5.03]		
2017 Huo ( MALAT1)	0.368109	0.158227	9.0%	1.44 [1.06, 1.97]		
2017 Jiang (DANCR)	-1.73161	0.6553	2.8%	0.18 [0.05, 0.64]		
2017 O'Leary (PARTICLE)	0.628609	0.5271	3.8%	1.88 [0.67, 5.27]		
Subtotal (95% CI)			70.0%	1.95 [1.40, 2.73]	•	·
Heterogeneity: Tau² = 0.23; Test for overall effect: Z = 3.9	Chi <sup>2</sup> = 34.10, df = 13 34 (P < 0.0001)	(P = 0.001);	I <sup>2</sup> = 62%			
3 1 2 >100						
2016 Coo/MALAT1)	1 1 406 22	0 27022	E EQ.	24614 60 6 641		
	1.149022	0.57932	0.0%	3.10[1.00, 0.04]		
2010 30(0CAR4)	1.109301	0.330342	3.3 %	3.22 [1.00, 3.30]		
2010 EI(OCAT)	1.144041	0.4700	4.4 70	5.14 [1.25, 7.80]		
	0.5000404	0.000729	2.0%	1 66 14 04 0 641		
2017 Wang ( 30A2-01 )	0.000210	0.237132	6.20	1.00 [1.04, 2.04]		
Subtotal (95% CI)	0.92207	0.320347	30.0%	2.02 [1.33, 4.77]		
	01-12-540 df-5 (D	0.000.17	10/	2.41[1.70, 3.27]		
Heterogeneity: Tau-= 0.01, 1	CHF = 5.19, UI = 5 (P	= 0.39), IF =	4 %			
lest for overall effect: $Z = 5.6$	o7 (P < 0.00001)					
Fotal (95% CI)			100.0%	2.16 [1.68, 2.79]	•	6
Heterogeneity: Tau <sup>2</sup> = 0.16;	Chi <sup>2</sup> = 41.05, df = 19	(P = 0.002);	I <sup>2</sup> = 54%			10 10
Test for overall effect: Z = 5.9	39 (P < 0.00001)			-	0.01 0.1 1 avoure [experimental] Fee	IU IU
Test for subaroup difference	es: Chi <sup>2</sup> = 0.84. df = 1	(P = 0.36).	I <sup>2</sup> = 0%	г	avours (experimental) Fav	ours (control)

Fig. A.1. Forest plot of HRs for subgroup analysis of the correlation between LncRNAs expression and overall survival. (A) Race; (B) Number of patients; (C) HR availability; (D) Cut-off values; (E) NOS scores.

D

Study or Subgroup

С					U.S. A. D. A.
Study or Subaroup	Iod[Hazard Ratio]	SE	Weight	W Random 95% CL	Mazard Katto
3.4.1 Directly	log nazara ratio	51	Treight	IV, I WINNOW, SS // CI	
2015 Li(HOTTIP)	1.060218	0 418869	5.0%	2 89 [1 27 6 56]	<b>-</b> _
2015 Sun(HULC)	0.822859	0.331293	6.2%	2 28 [1 19 4 36]	_ <b></b>
2015 Tian(MEG3)	0.881699	0 418798	5.0%	2 41 [1 06 5 49]	<b>.</b>
2016 Chen(BCAR4)	0.839842	0.386315	5.4%	2.32 [1.09, 4.94]	
2016 Cong(TUSC7)	-1.16155	0.572258	3.4%	0.31 [0.10, 0.96]	
2016 Gao(MALAT1)	1.149622	0.37932	5.5%	3.16 [1.50, 6.64]	
2016 Ju(BCAR4)	1,169381	0.556342	3.5%	3.22 [1.08, 9.58]	
2016 LI(UCA1)	1,144541	0.4705	4.4%	3.14 [1.25, 7.90]	<del></del>
2016 Ma(TUG1)	1.022451	0.392122	5.3%	2.78 [1.29, 6.00]	<del></del>
2016 Peng(BANCR)	1.076367	0.4899	4.2%	2.93 [1.12, 7.66]	
2016 Xia(91H)	1.144223	0.442841	4.7%	3.14 [1.32, 7.48]	
2016 Zhou(CCAL)	1.158452	0.460337	4.5%	3.18 [1.29, 7.85]	
2017 Cai (HNF1A-AS1)	0.968504	0.329814	6.2%	2.63 [1.38, 5.03]	
2017 Jiang (DANCR)	-1.73161	0.6553	2.8%	0.18 [0.05, 0.64]	
2017 Li (XIST)	1.75128464	0.688729	2.6%	5.76 [1.49, 22.22]	
2017 Wang (SOX2-OT)	0.506215	0.237152	7.7%	1.66 [1.04, 2.64]	
2017 Wen (UCA1)	0.92267	0.326347	6.3%	2.52 [1.33, 4.77]	
Subtotal (95% CI)			82.5%	2.23 [1.67, 2.99]	•
Heterogeneity: Tau <sup>2</sup> = 0.19; (	Chi <sup>2</sup> = 34.62, df = 16	(P = 0.004);	I <sup>2</sup> = 54%		
Test for overall effect: Z = 5.4	1 (P < 0.00001)				
3.4.2 Indirectly					
2016 Uzan(HULC)	1.064711	0.441976	4.7%	2.90 [1.22, 6.90]	
2017 Huo (MALAT1)	0.368109	0.158227	9.0%	1.44 [1.06, 1.97]	-
2017 O'Leary (PARTICLE)	0.628609	0.5271	3.8%	1.88 [0.67, 5.27]	
Subtotal (95% CI)			17.5%	1.66 [1.16, 2.37]	◆
Heterogeneity: Tau <sup>2</sup> = 0.02; (	Chi <sup>2</sup> = 2.31, df = 2 (P	= 0.31); I <sup>2</sup> =	13%		
Test for overall effect: Z = 2.7	6 (P = 0.006)				
Total (95% CI)			100.0%	2.16 [1.68, 2.79]	•
Heterogeneity: Tau <sup>2</sup> = 0.16; 0	Chi <sup>2</sup> = 41.05, df = 19	(P = 0.002);	I <sup>2</sup> = 54%		
Test for sucrell effect: 7 - 5.0	0 /D = 0.00004	//			U.U1 U.1 1 10 100

Test for overall effect: Z = 5.99 (P < 0.00001) Test for subaroup differences: Chi<sup>2</sup> = 1.60, df = 1 (P = 0.21), l<sup>2</sup> = 37.5%



3.3.1 Median						
2015 Li(HOTTIP)	1.060218	0.418869	5.0%	2.89 [1.27, 6.56]		
2015 Sun(HULC)	0.822859	0.331293	6.2%	2.28 [1.19, 4.36]		
2015 Tian(MEG3)	0.881699	0.418798	5.0%	2.41 [1.06, 5.49]		
2016 Chen(BCAR4)	0.839842	0.386315	5.4%	2.32 [1.09, 4.94]		
2016 Gao(MALAT1)	1.149622	0.37932	5.5%	3.16 [1.50, 6.64]		
2016 Ju(BCAR4)	1.169381	0.556342	3.5%	3.22 [1.08, 9.58]		_
2016 Li(UCA1)	1.144541	0.4705	4.4%	3.14 [1.25, 7.90]		-
2016 Peng(BANCR)	1.076367	0.4899	4.2%	2.93 [1.12, 7.66]		-
2016 Xia(91H)	1.144223	0.442841	4.7%	3.14 [1.32, 7.48]		-
2016 Zhou(CCAL)	1.158452	0.460337	4.5%	3.18 [1.29, 7.85]		-
2017 Cai ( HNF1A-AS1)	0.968504	0.329814	6.2%	2.63 [1.38, 5.03]		
2017 Huo (MALAT1)	0.368109	0.158227	9.0%	1.44 [1.06, 1.97]	-	
2017 Wang (SOX2-OT)	0.506215	0.237152	7.7%	1.66 [1.04, 2.64]	-	
Subtotal (95% CI)			71.1%	2.11 [1.75, 2.53]	•	
Heterogeneity: Tau <sup>2</sup> = 0.00; Chi <sup>2</sup>	= 12.42, df = 12 (	P = 0.41); I <sup>2</sup>	= 3%			
Test for overall effect: Z = 7.97 (F	<sup>o</sup> < 0.00001)					
3.3.2 Others						
2016 Uzan(HULC)	1.064711	0.441976	4.7%	2.90 [1.22, 6.90]		
2017 Jiang (DANCR)	-1.73161	0.6553	2.8%	0.18 [0.05, 0.64]		
2017 Li (XIST)	1.75128464	0.688729	2.6%	5.76 [1.49, 22.22]		
2017 O'Leary ( PARTICLE)	0.628609	0.5271	3.8%	1.88 [0.67, 5.27]		
2017 Wen (UCA1)	0.92267	0.326347	6.3%	2.52 [1.33, 4.77]		
Subtotal (95% CI)			20.2%	1.77 [0.71, 4.40]		
Heterogeneity: Tau <sup>2</sup> = 0.81; Chi <sup>2</sup>	= 17.26, df = 4 (F	? = 0.002); I <sup>z</sup>	= 77%			
Test for overall effect: Z = 1.22 (F	P = 0.22)					
3.3.3 fold-change				19.000 control 000		
2016 Cong(TUSC7)	-1.16155	0.572258	3.4%	0.31 [0.10, 0.96]		
2016 Ma(TUG1)	1.022451	0.392122	5.3%	2.78 [1.29, 6.00]		
Subtotal (95% CI)			9 704	0 0 7 10 44 0 241		
	an an 12 an an	and the first	0.1 70	0.97 [0.11, 0.24]		
Heterogeneity: Tau <sup>2</sup> = 2.14; Chi <sup>2</sup>	= 9.91, df = 1 (P =	= 0.002); I <sup>2</sup> =	90%	0.37 [0.11, 0.24]		

 $\label{eq:constraint} \begin{array}{l} \mbox{Total (95\% CI)} & \mbox{100.0\%} \\ \mbox{Heterogeneity: Tau^2 = 0.16; Chi^2 = 41.05, df = 19 (P = 0.002); l^2 = 54\% \\ \mbox{Test for overall effect: $Z = 5.99 (P < 0.00001)$ \\ \mbox{Test for subarous differences: Chi^2 = 0.63, df = 2 (P = 0.73), l^2 = 0\% \end{array}$ 



0.01

0.1

10

1

100

2.16 [1.68, 2.79]

Study or Subgroup	Iod[Hazard Ratio]	SE	Woight	Hazard Ratio	Hazard Ratio
3.5.1 >7	ioginazara radoj	52	Treight	IV, Rundoni, Soli Ci	
2016 Ma(TUG1)	1.022451	0.392122	5.3%	2.78 [1.29, 6.00]	
2016 Uzan(HULC)	1.064711	0.441976	4.7%	2.90 [1.22, 6.90]	
2016 Xia(91H)	1.144223	0.442841	4.7%	3.14 [1.32, 7.48]	
Subtotal (95% CI)			14.7%	2.92 [1.81, 4.72]	•
Heterogeneity: Tau <sup>2</sup> = 0.00; (	Chi <sup>2</sup> = 0.04, df = 2 (P	= 0.98);   <sup>2</sup> =	0%		
Test for overall effect: Z = 4.3	89 (P < 0.0001)	10.01			
3.5.2 ≤7					
2015 LI(HOTTIP)	1.060218	0.418869	5.0%	2.89 [1.27, 6.56]	
2015 Sun(HULC)	0.822859	0.331293	6.2%	2.28 [1.19, 4.36]	
2015 Tian(MEG3)	0.881699	0.418798	5.0%	2.41 [1.06, 5.49]	
2016 Chen(BCAR4)	0.839842	0.386315	5.4%	2.32 [1.09, 4.94]	
2016 Cong(TUSC7)	-1.16155	0.572258	3.4%	0.31 [0.10, 0.96]	
2016 Gao(MALAT1)	1.149622	0.37932	5.5%	3.16 [1.50, 6.64]	
2016 Ju(BCAR4)	1.169381	0.556342	3.5%	3.22 [1.08, 9.58]	
2016 Li(UCA1)	1.144541	0.4705	4.4%	3.14 [1.25, 7.90]	
2016 Peng(BANCR)	1.076367	0.4899	4.2%	2.93 [1.12, 7.66]	
2016 Zhou(CCAL)	1.158452	0.460337	4.5%	3.18 [1.29, 7.85]	
2017 Cai (HNF1A-AS1)	0.968504	0.329814	6.2%	2.63 [1.38, 5.03]	
2017 Huo ( MALAT1)	0.368109	0.158227	9.0%	1.44 [1.06, 1.97]	
017 Jiang (DANCR)	-1.73161	0.6553	2.8%	0.18 [0.05, 0.64]	
017 Li (XIST)	1.75128464	0.688729	2.6%	5.76 [1.49, 22.22]	
2017 O'Leary (PARTICLE)	0.628609	0.5271	3.8%	1.88 [0.67, 5.27]	
2017 Wang (SOX2-OT)	0.506215	0.237152	7.7%	1.66 [1.04, 2.64]	
2017 Wen ( UCA1)	0.92267	0.326347	6.3%	2.52 [1.33, 4.77]	
Subtotal (95% CI)			85.3%	2.05 [1.54, 2.73]	•
Heterogeneity: Tau <sup>2</sup> = 0.19; (	Chi <sup>2</sup> = 38.42, df = 16	(P = 0.001);	I <sup>2</sup> = 58%		
Fest for overall effect: Z = 4.9	)4 (P < 0.00001)				
otal (95% CI)			100.0%	2.16 [1.68, 2.79]	•
Heterogeneity: Tau <sup>2</sup> = 0.16; (	Chi <sup>2</sup> = 41.05, df = 19	(P = 0.002);	I <sup>2</sup> = 54%	0.01	0.1 1 10 1
estitur overall effect. Z = 5.9	19 (F < 0.00001)	-		, Favou	rs [experimental] Favours [control]

Test for overall effect: Z = 5.99 (P < 0.00001) Test for subaroup differences: Chi<sup>2</sup> = 1.54. df = 1 (P = 0.22). i<sup>2</sup> = 34.9%

Fig. A.1. (continued)

А				Hazard Ratio		Hazar	d Ratio		
Study or Subgroup	log[Hazard Ratio]	SE	Weight	IV, Fixed, 95% C	1	IV, Fixed	d, 95% C	Î.	
2015 LI(HOTTIP)	0.193921	0.344507	14.2%	1.21 [0.62, 2.38	1]	80			
2015 Sun(HULC)	-0.11205	0.225364	33.3%	0.89 [0.57, 1.39	ŋ	1	-		
2015 Tian(MEG3)	-0.02532	0.401462	10.5%	0.97 [0.44, 2.14	]	10	-		
2016 Gao(MALAT1)	-0.19723	0.407282	10.2%	0.82 [0.37, 1.82	!]		1		
2016 Ma(TUG1)	0.34359	0.350028	13.8%	1.41 [0.71, 2.80	I)		•		
2017 Cai (HNF1A-AS1)	-0.04082	0.305874	18.1%	0.96 [0.53, 1.75	1]		-		
Total (95% CI)			100.0%	1.01 [0.78, 1.30	1		•		
Heterogeneity: Chi <sup>2</sup> = 1.78	8, df = 5 (P = 0.88); I <sup>2</sup>	= 0%			-		-	10	400
Test for overall effect: Z =	0.06 (P = 0.95)				0.01 Favours	[experimental]	Favour	rs (conti	rol]

### В

Study of Subaroun	leafUererd Detic)	er.	Mainht	Hazard Ratio		Н	azard Rati	0	
Study of Subgroup	logi Hazard Katioj	35	weight	IV, Fixed, 95% C		IV,	fixed, 95%		
2015 Li(HOTTIP)	-0.06614	0.348521	6.8%	0.94 [0.47, 1.85	]		Sec. 2		
2015 Sun(HULC)	0.167208	0.181046	25.2%	1.18 [0.83, 1.69	]		-		
2015 Tian(MEG3)	0.274597	0.463084	3.8%	1.32 [0.53, 3.26	]			2	
2016 Gao(MALAT1)	0.348542	0.32745	7.7%	1.42 [0.75, 2.69	]		-		
2016 Ju(BCAR4)	0.207014	0.265482	11.7%	1.23 [0.73, 2.07	]		-		
2016 Li(UCA1)	0.113329	0.392851	5.3%	1.12 [0.52, 2.42	]		-		
2016 Ma(TUG1)	-0.30111	0.379611	5.7%	0.74 [0.35, 1.56	]		-		
2016 Peng(BANCR)	0.135405	0.447077	4.1%	1.15 [0.48, 2.75	]		-		
2016 Xia(91H)	-0.09431	0.404272	5.0%	0.91 [0.41, 2.01	]		-		
2017 Cai (HNF1A-AS1)	0.01094	0.332197	7.5%	1.01 [0.53, 1.94	]		-		
2017 Wang (SOX2-OT)	0.297137	0.21954	17.1%	1.35 [0.88, 2.07	1				
Total (95% CI)			100.0%	1.15 [0.96, 1.37	1		٠		
Heterogeneity: Chi <sup>2</sup> = 3.28	3, df = 10 (P = 0.97); l	<sup>2</sup> =0%				-	-	1	
Test for overall effect: Z =	1.55 (P = 0.12)				Favours	0.1 (experime	ntal] Favo	ours (cont	rol]

### С

				Hazard Ratio	Hazai	d Ratio
Study or Subgroup	log[Hazard Ratio]	SE	Weight	IV, Fixed, 95% Cl	IV, Fixe	d, 95% Cl
2015 Li(HOTTIP)	-0.14618	0.361118	7.9%	0.86 [0.43, 1.75]		•
2015 Sun(HULC)	-0.05975	0.220095	21.3%	0.94 [0.61, 1.45]	-	<b>-</b>
2015 Tian(MEG3)	0.125751	0.435894	5.4%	1.13 [0.48, 2.66]		
2016 Gao(MALAT1)	-0.29571	0.361971	7.9%	0.74 [0.37, 1.51]	i	
2016 Ju(BCAR4)	0.845868	0.329222	9.5%	2.33 [1.22, 4.44]		
2016 Li(UCA1)	0.521766	0.388268	6.9%	1.69 [0.79, 3.61]	ĺ l	
2016 Ma(TUG1)	-0.11653	0.406715	6.2%	0.89 [0.40, 1.98]	1	•
2016 Peng(BANCR)	0.539413	0.398638	6.5%	1.71 [0.79, 3.75]		
2016 Xia(91H)	-0.21072	0.462184	4.8%	0.81 [0.33, 2.00]	i —	
2017 Cai (HNF1A-AS1)	-0.1948	0.375357	7.3%	0.82 [0.39, 1.72]		•
2017 Wang (SOX2-OT)	0.378436	0.253636	16.1%	1.46 [0.89, 2.40]	l	<b>+•</b>
Total (95% CI)			100.0%	1.15 [0.94, 1.40]		•
Heterogeneity: Chi <sup>2</sup> = 12.1	2, df = 10 (P = 0.28);	I <sup>2</sup> = 18%				
Test for overall effect: Z =	1.37 (P = 0.17)				Favours (experimental)	Favours (control)

#### D

Study or Subgroup	log[Hazard Ratio]	SE	Weight	Hazard Ratio IV, Fixed, 95% C	Haz I IV, Fix	ard Ratio (ed, 95% Cl	
2016 Ju(BCAR4)	1.018847	0.272688	31.4%	2.77 [1.62, 4.73]	1		
2016 Ma(TUG1)	0.746688	0.356666	18.3%	2.11 [1.05, 4.24]	Ì	-	
2016 Peng(BANCR)	0.340037	0.272688	31.4%	1.40 [0.82, 2.40]	1	+	
2017 Cai (HNF1A-AS1)	1.024248	0.351493	18.9%	2.79 [1.40, 5.55]	]		
Total (95% CI)			100.0%	2.13 [1.58, 2.88]	1	٠	
Heterogeneity: Chi <sup>2</sup> = 3.8-	4, df = 3 (P = 0.28); I <sup>2</sup>	= 22%					400
Test for overall effect: Z = 4.95 (P < 0.00001)					Favours (experimenta	al] Favours (co	ntrol]

Fig. A.2. Forest plot for the association between overall survival time and clinicopathological features of patients with osteosarcoma in univariate analysis. (A) Age  $(\le 25 \text{ vs. 25});$  (B) Gender (Female vs. Male); (C) Tumor site (femur,tibia vs elsewhere); (D) ALP; (E) Chemotherapy (yes vs. no); (F) Tumor size (< 8 vs.  $\ge 8$ ); (G) Metastasis (yes vs. no); (H) Distant metastasis (presence vs. absence); (I) Enneking stage (IIA vs. IIB-III).

				Hazard Ratio	Hazard Ratio		
Study or Subgroup	log[Hazard Ratio]	SE	Weight	IV, Random, 95% Cl	IV, Rand	om, 95% Cl	
2016 Ma(TUG1)	0.875469	0.362673	33.7%	2.40 [1.18, 4.89]		-	
2016 Xia(91H)	-0.8675	0.410571	32.6%	0.42 [0.19, 0.94]			
2017 Cai (HNF1A-AS1)	1.073978	0.363235	33.7%	2.93 [1.44, 5.96]		-	
Total (95% CI)			100.0%	1.45 [0.46, 4.63]	-		
Heterogeneity: Tau <sup>2</sup> = 0.9	0; Chi <sup>2</sup> = 14.62, df = 2	2 (P = 0.000	7); I <sup>2</sup> = 88	i%			400
Test for overall effect: Z =	0.63 (P = 0.53)			-	U.U1 U.1 Favouro Ioveorimontal	1 10 Equation	100

## F

Study or Subgroup	log[Hazard Batio]	SE	Weight	Hazard Ratio		Hazard Ratio			
2015 Li(HOTTIP)	0.88665593	0 47207394	6.7%	2 43 10 96 6 12	1	14,11		-	
2015 Sun(HULC)	0.3155404	0.31261407	15.3%	1 37 [0 74 2 53	1				
2015 Tian(MEG3)	0.75940298	0.53030167	5.3%	2.14 [0.76, 6.04	1			- 22	
2016 Gao(MALAT1)	0.81580719	0.55893451	4.8%	2.26 [0.76, 6.76	i			2.3	
2016 Ju(BCAR4)	1.21491274	0.41134276	8.8%	3.37 [1.50, 7.55	i			121	
2016 LI(UCA1)	1.13172464	0.51505123	5.6%	3.10 [1.13, 8.51	ì		-		
2016 Ma(TUG1)	1.02245093	0.53338157	5.2%	2.78 [0.98, 7.91	ì		-	- 12	
2016 Peng(BANCR)	0.84929586	0.37626876	10.5%	2.34 [1.12, 4.89	ì			2	
2017 Cai (HNF1A-AS1)	-0.1381133	0.59581963	4.2%	0.87 [0.27, 2.80	1	20	•		
2017 Wang (SOX2-OT)	0.55100741	0.21101246	33.5%	1.73 [1.15, 2.62	]		-		
Total (95% CI)			100.0%	1.97 [1.55, 2.51	1		٠		
Heterogeneity: Chi <sup>2</sup> = 6.97	7, df = 9 (P = 0.64); I <sup>2</sup> :	= 0%				1		10	400
Test for overall effect: Z =	5.56 (P < 0.00001)				Favours	experimen	tal] Favou	rs (conti	rol]

## G

				Hazard Ratio	Hazar	d Ratio	
Study or Subgroup	log Hazard Ratio	SE	Weight	IV, Fixed, 95% CI	IV, Fixed	95% CI	
2016 Gao(MALAT1)	0.816249	0.307527	29.6%	2.26 [1.24, 4.13]	ĺ.	-	
2016 Ma(TUG1)	0.824175	0.349735	22.9%	2.28 [1.15, 4.53]	Ú	-	
2016 Xia(91H)	1.011601	0.393973	18.0%	2.75 [1.27, 5.95]	Č.		
2017 Cai (HNF1A-AS1)	0.816249	0.307527	29.6%	2.26 [1.24, 4.13]	ĺ,	-	
Total (95% CI)			100.0%	2.35 [1.69, 3.26]	2 Гала ал	•	
Heterogeneity: Chi <sup>2</sup> = 0.2	D, df = 3 (P = 0.98); I <sup>2</sup>	= 0%					0 40
Test for overall effect: Z = 5.10 (P < 0.00001)				F	Favours [experimental]	Favours (d	control]

				Hazard Ratio		Hazard	l Ratio	
Study or Subgroup	log[Hazard Ratio]	SE	Weight	IV, Fixed, 95% C		IV, Fixed	, 95% CI	
2015 LI(HOTTIP)	1.553079	0.409518	12.6%	4.73 [2.12, 10.55]	1			
2015 Sun(HULC)	1.548201	0.299277	23.5%	4.70 [2.62, 8.46]	l.		-	
2015 Tian(MEG3)	1.312648	0.346056	17.6%	3.72 [1.89, 7.32]	l.			
2016 Ju(BCAR4)	1.474763	0.588272	6.1%	4.37 [1.38, 13.84]	Í.			
2016 Li(UCA1)	1.477733	0.497924	8.5%	4.38 [1.65, 11.63]	1			
2016 Peng(BANCR)	1.687509	0.512835	8.0%	5.41 [1.98, 14.77]	Í.			
2017 Wang ( SOX2-OT )	2.001615	0.298283	23.7%	7.40 [4.12, 13.28]	l			
Total (95% CI)			100.0%	5.03 [3.78, 6.69]	<u>.</u>		•	
Heterogeneity: Chi <sup>2</sup> = 2.67	, df = 6 (P = 0.85); I <sup>2</sup> :	= 0%			-			400
Test for overall effect: Z = 11.13 (P < 0.00001)					0.01	U.T.	Г – 10 Ганана (алан)	100

Study or Subaroup	log[Hazard Ratio]	log[Hazard Ratio] SE		Hazard Ratio	Hazard Ratio			
2015 Li(HOTTIP)	1 330782	0 297828	20.6%	3 78 [2 11 6 78]		- IVITIACU		
2015 Sun(HULC)	1.155623	0.307347	19.3%	3.18 [1.74, 5.80]				
2016 Gao(MALAT1)	1.169071	0.305345	19.6%	3.22 [1.77, 5.86]				
2016 Ju(BCAR4)	2.020222	0.465299	8.4%	7.54 [3.03, 18.77]				
2016 Peng(BANCR)	1.687509	0.61412	4.8%	5.41 [1.62, 18.01]				
2017 Wang (SOX2-OT)	1.507405	0.258538	27.3%	4.51 [2.72, 7.49]				
Total (95% CI)			100.0%	4.01 [3.08, 5.23]			٠	
Heterogeneity: Chi <sup>2</sup> = 3.42	2, df = 5 (P = 0.64); I <sup>2</sup> :	= 0%		85 in 888			10	4.00
Test for overall effect: Z =	10.28 (P < 0.00001)			F	avours lex	0.1 1 (perimental)	Favours (contr	roll

Fig. A.2. (continued)

1				Hazard Ratio	Haza	rd Ratio	
Study or Subgroup	log[Hazard Ratio]	SE	Weight	IV, Random, 95% CI	IV, Random, 95% Cl		
2016 Li(UCA1)	0.14236724	0.08372175	33.2%	1.15 [0.98, 1.36]			
2016 Peng(BANCR)	0.7006192	0.40283719	13.8%	2.01 [0.91, 4.44]			
2017 Jiang (DANCR)	-0.97816614	0.51028169	10.1%	0.38 [0.14, 1.02]		+	
2017 Wang (SOX2-OT)	0.19803085	0.22117197	24.1%	1.22 [0.79, 1.88]		-	
2017 Wen ( UCA1)	0.80244992	0.30291178	18.8%	2.23 [1.23, 4.04]		-	
Total (95% CI)			100.0%	1.28 [0.88, 1.86]		٠	
Heterogeneity: Tau <sup>2</sup> = 0.10	0; Chi <sup>2</sup> = 11.25, df = 4	(P = 0.02); I <sup>2</sup> =	= 64%			1 10	400
Test for overall effect: Z =	1.27 (P = 0.20)			F	Favours (experimental	Favours [cont	rol]

#### B

				Hazard Ratio	Hazai	d Ratio
Study or Subgroup	log[Hazard Ratio]	SE	Weight	IV, Random, 95% C	I IV, Rande	om, 95% Cl
2016 Gao(MALAT1)	1.42359	0.394616	14.5%	4.15 [1.92, 9.00	]	
2016 Ma(TUG1)	0.828552	0.350858	15.2%	2.29 [1.15, 4.55	]	
2016 Xia(91H)	1.018847	0.393255	14.5%	2.77 [1.28, 5.99	]	
2016 Zhou(CCAL)	1.34443	0.391914	14.5%	3.84 [1.78, 8.27	]	
2017 Cai (HNF1A-AS1)	0.971915	0.322481	15.6%	2.64 [1.40, 4.97	]	
2017 Jiang (DANCR)	-1.83258	0.563705	11.7%	0.16 [0.05, 0.48	] —	
2017 Wen ( UCA1)	1.058137	0.417504	14.1%	2.88 [1.27, 6.53	]	
Total (95% CI)			100.0%	2.14 [1.15, 3.97	1	•
Heterogeneity: Tau <sup>2</sup> = 0.5	4; Chi <sup>2</sup> = 26.86, df = 8	6 (P = 0.000	2); I <sup>2</sup> = 78	1%		
Test for overall effect: Z = 2.40 (P = 0.02)					Favours [experimental]	Favours [control]

#### С Hazard Ratio Hazard Ratio Study or Subgroup log[Hazard Ratio] IV, Fixed, 95% CI IV, Fixed, 95% CI SE Weight 2015 LI(HOTTIP) 1.475907 0.402922 12.7% 4.38 [1.99, 9.64] 2015 Sun(HULC) 1.338679 0.299752 22.9% 3.81 [2.12, 6.86] 2015 Tian(MEG3) 1.211346 0.348589 17.0% 3.36 [1.70, 6.65] 2016 Ju(BCAR4) 1.355835 0.580544 6.1% 3.88 [1.24, 12.11] 2016 Li(UCA1) 1.607235 0.520095 7.6% 4.99 [1.80, 13.83] 2016 Peng(BANCR) 1.584735 0.564548 6.5% 4.88 [1.61, 14.75] 1.474305 0.555905 6.7% 4.37 [1.47, 12.99] 2017 Li (XIST) 2017 Wang (SOX2-OT) 1.383791 0.31707 20.5% 3.99 [2.14, 7.43] Total (95% CI) 100.0% 4.02 [3.03, 5.32] Heterogeneity: Chi<sup>2</sup> = 0.66, df = 7 (P = 1.00); I<sup>2</sup> = 0% 0.01 0.1 10 100 Test for overall effect: Z = 9.68 (P < 0.00001) Favours [experimental] Favours [control]

#### D

				Hazard Ratio		Haza	rd Ratio	
Study or Subgroup	log[Hazard Ratio]	SE	Weight	IV, Fixed, 95% Cl	5	IV, Fixe	d, 95% Cl	
2015 Li(HOTTIP)	1.185096	0.268883	23.8%	3.27 [1.93, 5.54]				
2015 Sun(HULC)	1.070213	0.308535	18.0%	2.92 [1.59, 5.34]				
2016 Gao(MALAT1)	0.976068	0.273827	22.9%	2.65 [1.55, 4.54]				
2016 Ju(BCAR4)	1.829376	0.468573	7.8%	6.23 [2.49, 15.61]				
2016 Peng(BANCR)	1.685473	0.512213	6.5%	5.40 [1.98, 14.72]				
2017 Wang (SOX2-OT)	0.813593	0.410065	10.2%	2.26 [1.01, 5.04]				
2017 Wen ( UCA1)	1.211048	0.401185	10.7%	3.36 [1.53, 7.37]				
Total (95% CI)			100.0%	3.20 [2.48, 4.14]			•	
Heterogeneity: Chi <sup>2</sup> = 4.37, df = 6 (P = 0.63); l <sup>2</sup> = 0%							1 10	100
Test for overall effect: Z = 8.88 (P < 0.00001)					Favours	0.1 (experimental	Favours [cor	ntrol]

Fig. A.3. Forest plot for the association between overall survival time and clinicopathological features of patients with osteosarcoma in multivariate analysis. (A) Tumor size ( $< 8 \text{ vs.} \ge 8$ ); (B) Metastasis (yes vs. no); (C) Distant metastasis (presence vs. absence); (D) Enneking stage (IIA vs. IIB–III).

### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jbo.2018.09.005.

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