Prognostic value of abnormally expressed long non-coding RNAs in patients with osteosarcoma: a meta-analysis

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ABSTRACT

Long non-coding RNAs (lncRNAs) contribute to progression of various cancers including osteosarcoma through abnormal regulation of cancer-related cellular processes. Recent researches have shown that various lncRNAs are abnormally expressed in osteosarcoma and associated with the prognosis. With the aim of gaining a better insight into the association between expression level of lncRNAs and prognosis of osteosarcoma, multiple databases including PubMed, Embase, Cochrane Library and Web of Science were carefully searched for available studies up to March 14, 2017. Finally, 19 publications with 1298 patients matched our inclusion criteria and were evaluated in our meta-analysis. The quality of each study was scored using the Newcastle-Ottawa Scale and studies not reaching a minimum threshold were excluded. Results of the meta-analysis demonstrated that abnormal expression level of lncRNAs predicted poor overall survival (pooled HR 3.064, 95% CI: 2.487–3.775) and event-free survival (pooled HR 2.642, 95% CI 1.759–3.970) in osteosarcoma and subgroup analysis showed consistent prognostic value. Furthermore, combining data of Cox multivariable analysis indicated that abnormal expression level of lncRNAs was an independent prognostic marker for overall survival (pooled HR 2.864, 95% CI: 2.246–3.651) in osteosarcoma patients. The clinicopathological parameters analysis further showed that abnormal expression level of lncRNAs was correlated with tumor size, tumor stage, metastasis and differentiation grade of osteosarcoma. Limitations of the meta-analysis included variation of cut-off value definition, Chinese provenance of most studies, publication bias and so on. In conclusion, this meta-analysis suggested that abnormal expression level of lncRNAs has a promising future for predicting the prognosis of patients with osteosarcoma.

INTRODUCTION

Osteosarcoma is an osteoid-producing malignancy of mesenchymal origins. This high-grade tumor is the most common primary bony malignancy and most often originates in the metaphysis of long bones of children and adolescents [1, 2]. The incidence rate of osteosarcoma is about 1.7~4.4/1000,000 per year, and the average male-to-female ratio is 1.22:1 [3]. This devastating tumor is characterized by its high aggressive ability and early systematic metastasis, approximately 20% of osteosarcoma patients present with detectable lung metastatic disease at initial diagnosis, additionally, about 40% of patients free of metastasis will progress to

metastasis in later stages during treatment [4]. With the application of chemotherapy, a combination of neoadjuvant chemotherapy, surgery resection and postoperative chemotherapy has clinically become the standard treatment strategy for osteosarcoma and has significantly improved patients' outcome, the 5-year overall survival of patients with nonmetastatic osteosarcoma of the extremity has approached to 70%~80% over past decades [5, 6]. However, the 5-year overall survival rate of osteosarcoma patients with detectable metastases at diagnosis is as low as 19% [7]. Those patients with recurrent disease also have unsatisfactory 5-year post-relapse-overall-survival (PROS) rate, which is about 30% [8]. The survival of osteosarcoma patients predominantly depends on not only early diagnosis but also timely proper treatment. Therefore, it is highly needed to identify novel diagnostic and prognostic biomarkers for osteosarcoma. Effective prognostic biomarkers predictive of survival or response to therapy will help to predict the outcome more accurately and guide us to select better and more proper treatment options for osteosarcoma patients.

Long non-coding RNAs (lncRNAs) are a class of RNA molecules, which had been dismissed as simply transcriptional 'junk' in the past, with a length of more than 200 nucleotides that with little or no protein coding capacity [9, 10]. LncRNAs, which represent a new frontier in molecular biology, play important roles in regulating gene expression at epigenetic, transcriptional and post-transcriptional levels, and contribute to progression of various cancers through abnormal regulation of cancer-related cellular processes, such as proliferation, invasion, metastasis, apoptosis, etc [11–14]. Recent researches have shown that various lncRNAs are abnormally expressed in tissue and blood sample of osteosarcoma, and the expression level of lncRNAs may be associated with prognosis of osteosarcoma, for example, CCAL, MALAT1, TUG1, UCA1, 91H, etc [15–36]. These discoveries indicated that lncRNAs could be promising biomarkers for predicting the prognosis of osteosarcoma. However, most studies reported so far were limited in discrete outcome and sample size. With the aim of gaining a better insight into the prognostic value of lncRNAs in osteosarcoma. We conducted a quantitative meta-analysis to clarify the prognostic value of abnormally expressed lncRNAs in patients with osteosarcoma.

RESULTS

Included studies and characteristics

According to our search strategy, a total of 263 studies were retrieved from Pubmed, Embase, Cochrane Library and Web of Science and from the reference list of relevant articles. Of these, 129 duplicated articles were excluded; through title and abstract review, studies describing unrelated topics (n = 80) which refers to those studies that were not research articles or those studies unrelated to osteosarcoma, lncRNA , or patient prognosis were removed; after reading the remaining 54 full-text articles, studies lacking relevant data (n = 32), studies presented in language other than English (n = 1), full-text unavailable studies (n = 1), and the studies of which included patients were reported in previous studies (n = 1) were excluded. Eventually, 19 studies (containing 1298 patients) met the inclusion criteria and were evaluated in this analysis [18–36]. The screening process and results was shown in Figure 1.

Table 1 represented the main characteristics of all the included 19 studies ranging from 2015 to 2016. Among these studies, 2 studies had a sample size larger or equal to 100 and 17 studies had a sample size less than 100 patients. 18 studies were from China and 1 study was from Brazil. Quantitative real-time polymerase chain reaction assays (qRT-PCR) were performed to quantify the level of lncRNAs in all of the studies. Specimens were composed of tissue in 18 studies and plasma in 2 studies. Of the total 18 lncRNAs, only lncRNA HULC were investigated by at least two studies, and the remaining 17 lncRNAs were studied in a single report. The cut-off definitions were various in different studies. In 14 studies, the median of lncRNA level were regarded as cut-off value; others based on ROC analysis or fold change. There are 2 articles that reported the negative relationship between expression level of lncRNAs and survival of osteosarcoma patients. 58% (11/19) of the NOS scores for the included studies on prognosis were $\geq$ 7. Cox multivariate analysis was used in 12 studies including 904 patients, and 8 studies used both univariate and multivariate analysis. In 14 studies, none of the patients received chemotherapy or radiotherapy before surgery. 1 study showed patients received multidrug chemotherapy before surgery and the left 4 studies did not mention about it. HR values and 95% CIs could be obtained directly from 10 publications. In the other publications, HR values or 95% CIs should be calculated or retracted from Kaplan–Meier curves. All of 19 studies assessing the prognostic value of 18 abnormally expressed lncRNAs in patients with osteosarcoma. The prognostic value of lncRNAs expression was assessed by analyzing overall survival (OS) in 1298 patients and event free survival (EFS) in 301 patients. The association between lncRNAs expression and clinicpathological characteristics of osteosarcoma was estimated in 79% (15/19) of the studies.

lncRNA expression and prognosis of osteosarcoma

A total of 19 studies reported OS of osteosarcoma based on abnormal expression of lncRNAs in 1298 patients. The pooled hazard ratio (HR) for OS was 3.064 (95% CI: 2.487−3.775, $p < 0.00001$) (Figure 2), which indicated abnormal expression level of lncRNAs

Table 1: Characteristics of studies included in the meta-analysis

Author	Year of publication	Country	lncRNA signatures	Study population (high/low)	Tumor stage	Follow-up (month)	Endpoints	Expression associates with poor prognosis	Assay method	Sample type	Cut-off value	Pre-operation treatment	Survival analysis	NOS score	Method
Xia et al. [36]	2016	China	91H	34/33	I-III [c]	60[#]	OS	High	qRT-PCR	Plasma	Median	None	Univariate Multivariate	8	1
Chen et al. [19]	2016	China	BCAR4	30/30	I-III [b]	40[#]	OS, EFS	High	qRT-PCR	Tissue	Median	None	Multivariate	7	1,2
Zhou et al. [18]	2016	China	CCAL	23/23	I-IV [a]	60	OS	High	qRT-PCR	Tissue	Median	Unclear	Multivariate	6	1
Sun et al. [20]	2016	China	FGFR3-AS1	31/31	I-III [b]	31[#]	OS	High	qRT-PCR	Tissue	Median	None	NA	7	2
Li et al. [26]	2016	China	HIF-2PUT	41/41	IIA-III [a]	80[#]	OS, EFS	High	qRT-PCR	Tissue	Median	None	Multivariate	8	1
Li et al. [22]	2015	China	HOTTIP	34/34	IIA-III [c]	60	OS	High	qRT-PCR	Tissue	Median	None	Univariate Multivariate	6	1
Sun et al. [23]	2015	China	HULC	39/39	IIA-III [c]	60	OS	High	qRT-PCR	Tissue	Median	None	Univariate Multivariate	6	1
Uzan et al.[24]	2015	Brazil	HULC	12/21	NA	100	OS, EFS	High	qRT-PCR	Tissue	ROC	None	Multivariate	7	1,2
Gao et al. [27]	2016	China	MALAT1	80/82	IIA-III [a]	65	OS	High	qRT-PCR	Tissue	Median	None	Univariate Multivariate	7	1
Tian et al. [28]	2015	China	MEG3	32/32	I-III [a]	60	OS	Low	qRT-PCR	Tissue	Median	None	Univariate Multivariate	7	1
Zhao et al. [30]	2016	China	NEAT1	18/19	I-III [c]	60	OS	High	qRT-PCR	Tissue	Median	None	NA	6	2
Zhang et al. [29]	2015	China	ODRUL	38/22	NA	96	OS	High	qRT-PCR	Tissue	Median	Multidrug chemotherapy	NA	6	2
Zhou et al. [31]	2016	China	PVT1	29/24	NA	60	OS	High	qRT-PCR	Tissue	unclear	Unclear	NA	6	3
Ma et al. [34]	2015	China	TUG1	41/35	I-III [b]	44[#]	OS, EFS	High	qRT-PCR	Tissue	Fold change	None	Univariate Multivariate	8	1
Cong et al. [33]	2016	China	TUSC7	82	NA	120	OS	Low	qRT-PCR	Tissue	Fold change	Unclear	NA	6	1
Li et al. [32]	2016	China	UCA1	68/67	I-III [b]	60	OS	High	qRT-PCR	Tissue	Median	None	Univariate Multivariate	8	1,2
Liu et al. [35]	2016	China	ZEB-AS1	25/25	I-III [b]	40	OS, EFS	High	qRT-PCR	Tissue	Median	None	NA	8	2
Zhao et al. [21]	2016	China	HNF1A-AS1	21/22	I-III [a]	60	OS	High	qRT-PCR	Tissue	Median	None	Univariate Multivariate	7	1
Wang et al. [25]	2015	China	HOTAIR	40	IIA-III [c]	50	OS	High	qRT-PCR	Tissue	Fold change	Unclear	NA	6	2

Abbreviations: OS: overall survival; EFS: event free survival; ROC: receiver operating characteristic; NOS: Newcastle-Ottawa Scale; qRT-PCR: quantitative reverse transcription PCR; NA: not available;
Tumor staging: [a]TNM staging, [b]Enneking staging, [c]staging system not stated clearly in paper; Follow-up: [#]median;
Method: 1 denoted as obtaining HRs directly from publications; 2 denoted as HRs calculated from the total number of events and its *p* value; 3 denoted as HRs extracted from Kaplan–Meier curves.

was significantly associated with OS in patients with osteosarcoma. The test for heterogeneity gave no significant results ($\chi^2 = 5.90$, $p = 0.997$; $I^2 = 0.0\%$). In order to further explore the association between abnormal expression level of lncRNAs and OS in osteosarcoma patients, subgroup analysis was performed based on the following factors: region (China or Brazil), sample size (more than 100 or fewer than 100), expression level relevant to poor prognosis (high or low), pre-operative treatment (none, yes or unclear) and paper quality (NOS scores ≥ 7 or < 7) (Supplementary Figure 1). The results of subgroup analysis showed the significant association between abnormal expression level of lncRNAs and OS of osteosarcoma patients were not altered with all the factors above. No significant heterogeneity was found across studies in all the subgroup analysis (Table 2). In order to assess publication bias, a Begg's funnel plot was presented for the visual inspection. The funnel plot showed asymmetry and Begg's test ($p < 0.001$) indicated significant publication bias for OS. An Egger's test ($p < 0.001$) was also performed which indicated significance among the included studies (Supplementary Figure 3).

Additionally, in order to ensure the stability of the results, sensitivity analyses were performed including influence analysis, file-drawer analysis and trim and fill analysis. As for the influence analysis, HRs and their 95% CIs did not change significantly after the exclusion of any of the studies (Supplementary Figure 2). Since there was significant publication bias, we performed file-drawer analysis and the calculation showed the fail-safe number (Nfs) should be 776, which was obviously larger than 19, the number of included studies. And the "trim and fill" method was used for further analysis. The result showed that 6 studies evaluating the prognostic value of

Table 2: Results of subgroup analysis of pooled hazard ratios of overall survival of patients with osteosarcoma based on abnormal expression level of lncRNAs

Subgroup analysis	No. of studies	No. of patients	Pooled HR (95% CI)	Heterogeneity	
				I^2	*p*-value
Region					
China	18	1265	3.019 [2.447–3.725]	0.0%	0.999
Brazil	1	33	8.724 [1.497–50.832]	-	-
Sample size					
< 100	17	1001	3.051 [2.440–3.816]	0.0%	0.989
≥ 100	2	297	3.151 [1.766–5.621]	0.0%	0.993
Expression level relevant to poor prognosis					
High	17	1152	3.112 [2.498–3.877]	0.0%	0.992
Low	2	146	2.663 [1.373–5.164]	0.0%	0.693
Pre-operative treatment					
None	14	1017	2.973 [2.358–3.748]	0.0%	0.967
Yes	1	60	3.457 [1.216–9.829]	-	-
Unclear	4	221	3.495 [2.036–6.000]	0.0%	0.978
NOS score					
≥ 7	11	834	3.043 [2.332–3.971]	0.0%	0.948
< 7	8	464	3.098 [2.214–4.334]	0.0%	0.964

abnormally expressed lncRNAs in OS of osteosarcoma patients remained unpublished. The filled meta-analysis for OS (HR 2.772, 95% CI:2.291−3.355, $p < 0.001$) supported our original result (Supplementary Figure 4). All above sensitivity analyses showed our results were stable.

The prognostic value of abnormal expression of lncRNAs in EFS of patients with osteosarcoma was evaluated in 5 studies including 301 patients. We found that abnormal expression level of lncRNAs was significantly associated with EFS (HR 2.642, 95% CI: 1.759–3.970, $p < 0.00001$) (Figure 3). Moderate heterogeneity existed among studies ($\chi^2 = 6.92$, $p = 0.140$; $I^2 = 42.2\%$). Subgroup analysis, sensitivity analysis and appraisal of publication bias were not performed due to the limited number and relative homogeneity of the studies.

Using Cox multivariate analysis in 11 studies including 881 patients, we found that abnormal expression level of lncRNAs was an independent prognostic factor for OS of patients with osteosarcoma (pooled HR 2.864, 95% CI: 2.246–3.651, $p < 0.00001$), and no significant heterogeneity was detected among studies ($\chi^2 = 3.14$, $p = 0.978$; $I^2 = 0.0\%$) (Figure 4). Subgroup analysis was not performed because of the relative homogeneity and similar property of the studies. As for the publication bias, asymmetry was shown on the funnel plot, Begg's test ($p < 0.001$) and Egger's test ($p < 0.001$) showed significant publication bias (Supplementary Figure 3).

Sensitivity analyses were performed likewise, the influence analysis showed no obvious change of the HRs and their 95% CIs after the exclusion of any of the studies (Supplementary Figure 3). The file-drawer analysis showed the fail-safe number (Nfs) should be 285, which was obviously larger than 11, the number of included studies. The "Trim and fill" analysis was performed and the result estimated that none study evaluating the independent prognostic value of abnormal expression of lncRNAs in OS of osteosarcoma remained unpublished. Thus the result of filled meta-analysis maintained unchanged. Based on above results of sensitivity analyses, our results were stable to some extent.

lncRNA expression and clinicopathological characteristics of osteosarcoma

As shown in Table 3, several studies examined the association between lncRNA expression and the clinicopathological characteristics of osteosarcoma. In aspect of tumor staging, TNM staging system and Enneking staging system were used in five and nine studies respectively (Staging system not stated clearly in paper was regard as using Enneking staging). There was a significant association between lncRNA expression and tumor staging of osteosarcoma (Advanced (III/IV) vs Early (I/II) staging: pooled OR 4.592, 95% CI: 2.984−7.068, $p < 0.00001$; pooled OR 4.720, 95% CI: 3.187−6.992, $p < 0.00001$ respectively). Fifteen studies examined the association between lncRNA expression and tumor metastasis, and significant association was

Table 3: Results of meta-analysis of abnormal lncRNA expression and clinicopathological features in osteosarcoma

Clinicopathological features	No. of studies	No. of patients	Pooled OR		Heterogeneity	
			Fixed	Random	I^2	*p*-value
TNM staging (Advanced vs Early)	5	397	4.592 [2.984–7.068]	4.587 [2.978–7.067]	0.0%	0.939
Enneking staging* (Advanced vs Early)	9	637	4.720 [3.187–6.992]	4.680 [3.155–6.941]	0.0%	0.979
Metastasis (Present vs Absent)	15	1013	3.025 [2.274–4.024]	3.014 [2.183–4.162]	13.8%	0.299
Tumor location (Femur/Tibia vs Elsewhere)	15	1013	0.876 [0.670–1.145]	0.885 [0.666–1.176]	6.0%	0.385
Tumor Size (≥ 8 cm vs < 8 cm)	11	805	1.691 [1.276–2.241]	1.806 [1.157–2.820]	54.6%	0.015
Tumor differentiation grade (Poor vs Moderate/Well)	2	181	2.738 [1.453–5.158]	2.737 [1.452–5.157]	0.0%	0.822

*Staging system not stated clearly in paper was regard as using Enneking staging.
Tumor staging: I/II and III/IV were defined as early staging and advanced staging, respectively.

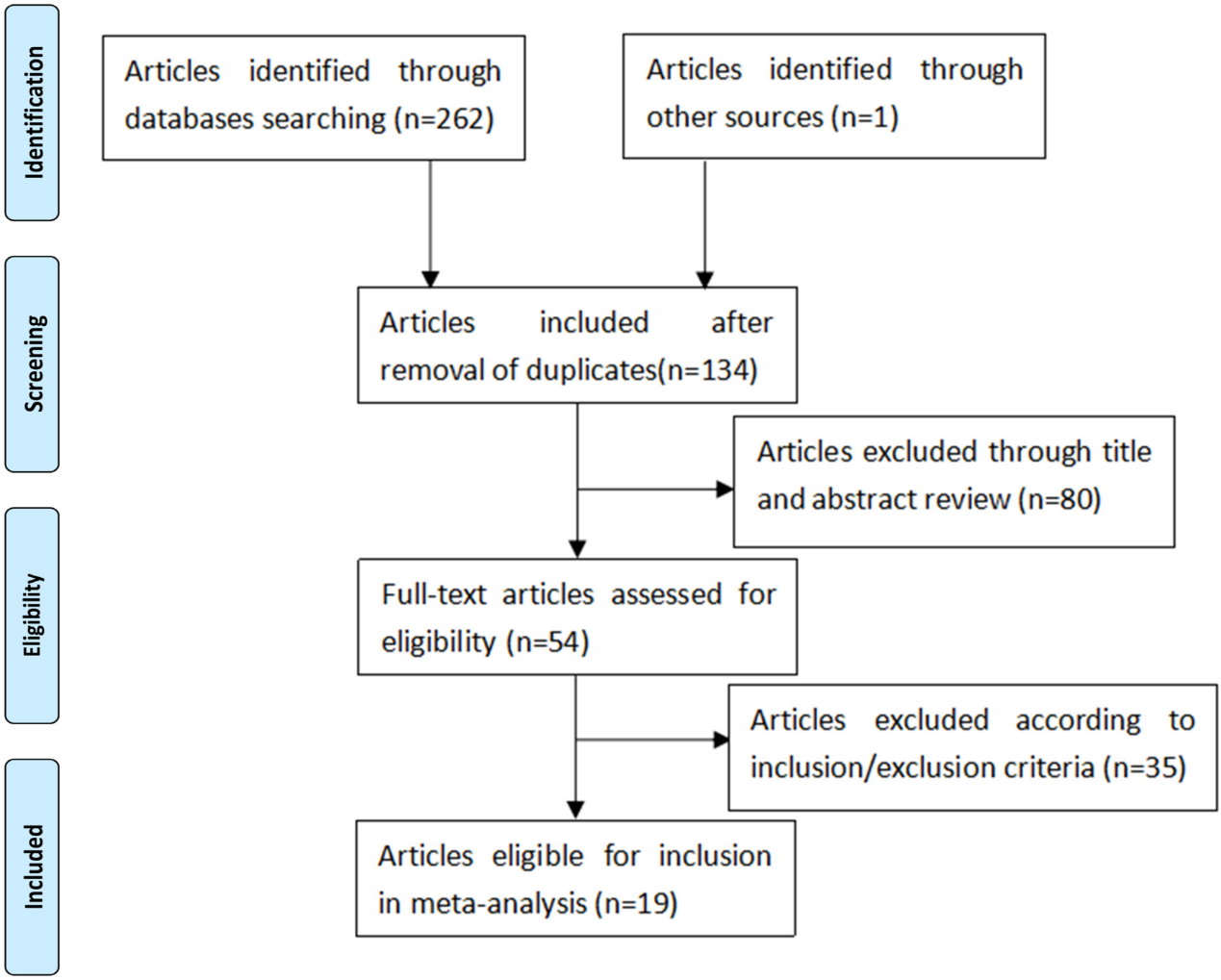

Figure 1: The flow diagram of the studies identified, included and excluded.

observed as pooled OR 3.025 (95% CI: 2.274−4.024, $p < 0.00001$). There were fifteen studies examining the association between lncRNA expression and tumor location, while no significance was observed (pooled OR 0.876, 95% CI: 0.670−1.145, $p = 0.332$). Eleven studies were included to examine tumor size. LncRNA expression was significantly associated with enlarged tumor size (pooled OR 1.806, 95% CI: 1.157−2.820). There were only two studies examining tumor differentiation grade. We observed significant association between lncRNA expression and tumor differentiation grade (pooled OR 2.738, 95% CI: 1.453–5.158, $p < 0.00001$). Corresponding heterogeneity tests were all shown in Table 3. Subgroup analysis, sensitivity analysis and appraisal of publication bias were not performed due to the limited number and relative homogeneity of the studies.

DISCUSSION

Osteosarcoma, which is often fatal in both children and adolescents and accounts for 5 % of child malignancies and 9 % of cancer-related deaths in children, is the most common primary malignancy of bone [2, 3]. Although advances in tumor treatment strategy have significantly raised the survival rate of osteosarcoma patients, the survival of osteosarcoma patients with metastatic disease or recurrences and those in advanced stage is still quite poor [3, 6–8]. Thus, novel promising biomarkers of osteosarcoma that can help in diagnosis and prognosis evaluation are still urgently needed. They will help to detect osteosarcoma patients at early stage and contribute to recognize those patients whose tumor will resist chemotherapy or whose tumor will metastasize or recur. These potential biomarkers predictive of survival or response to therapy will be helpful to guiding the individualized treatment of osteosarcoma patients and finally improve their outcome. In recent years, studies have demonstrated that lncRNAs are involved in various biological processes of osteosarcoma, including progression and metastasis, and aberrant expression of multiple lncRNAs was found to have the potential value for predicting outcome of osteosarcoma patients [37, 38].

In this meta-analysis, we examined the prognostic value of abnormally expressed lncRNAs in osteosarcoma

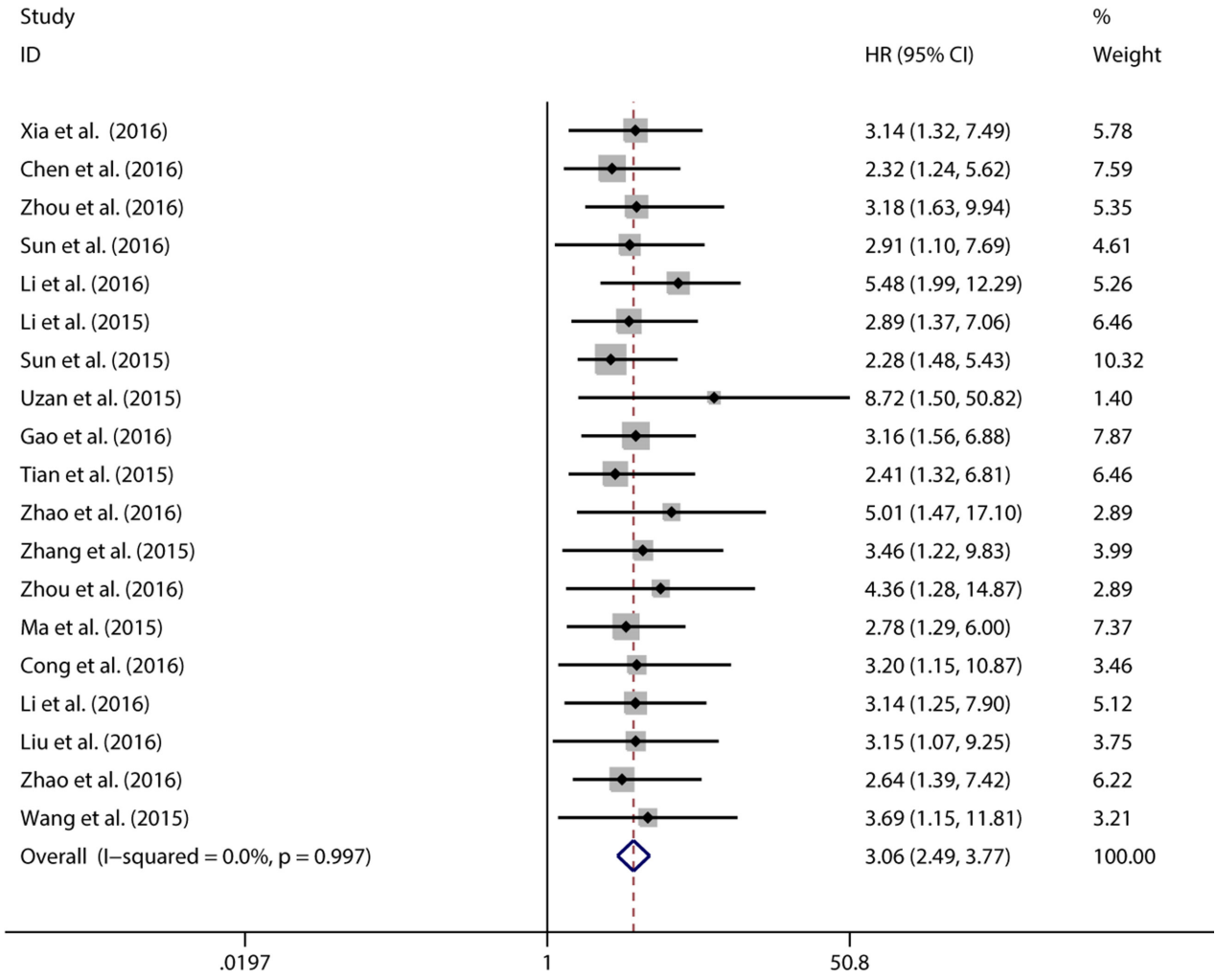

Figure 2: Meta-analysis of the pooled HRs of overall survival (OS) with abnormally expressed lncRNAs.

and the relation between lncRNAs and clinicopathological characteristics. We examined 19 independent studies comprising data from a total of 1298 patients. We found that abnormal expression level of lncRNAs was associated with OS in patients with osteosarcoma (pooled HR 3.064, 95% CI: 2.487−3.775, $p < 0.00001$). In detail, of all studies included, subgroup analysis indicated that the significant association did not alter with factors of the region, sample size, lncRNA expression level relevant to poor prognosis (high or low), pre-operative treatment or paper quality. And poorer EFS was found related to abnormal expression level of lncRNAs (HR 2.642, 95% CI: 1.759−3.970, $p < 0.00001$). Moreover, by combining HRs from studies using Cox multivariate analysis we found abnormal expression level of lncRNAs was an independent prognostic factor for OS of patients with osteosarcoma (pooled HR 2.864, 95% CI: 2.246−3.651, $p < 0.00001$).

In the aspect of clinicopathological characteristics of osteosarcoma, our results revealed that abnormal expression level of lncRNAs was significantly associated with advanced tumor staging, enlarged tumor size, tumor metastasis and poor tumor differentiation grade, but not related with tumor location.

In our study, a few limitations should be underlined. First, the cut-off value dividing lncRNA expression into high and low groups were different among studies, although most of them were set to median. Second, the staging system for osteosarcoma was regarded as Enneking staging system, when it was not stated clearly in publication, because Enneking staging system are widely used as clinical staging system for osteosarcoma in China now. Third, we only searched English papers. And most studies were from China, the results might mainly represent Chinese patients. Chinese provenance of most studies essentially affected generalizability. Fourth, differences of paper quality among the studies might cause bias in the meta-analysis although subgroup analysis or sensitive analysis did not show obvious change. Fifth, HRs of six studies could not be directly obtained from the publications. Calculating them ourselves or to reconstruct the survival curves to extract the HR estimates might not be precise enough. Sixth, nearly all of the studies included in this analysis reported positive results, so our results might overestimate the prognostic value of abnormally expressed lncRNAs in osteosarcoma to some degree. Therefore, large-scale, multicenter, and high-quality studies are highly needed to confirm our findings. In our opinion, with the updating of gene chip and microarray platform technology, such as TCGA and GEO databases, and an explosion of lncRNAs research in osteosarcoma, a significant extension of our finding and re-analysis, which will include more patients from different regions of the world, would be accomplished in near future.

In summary, this meta-analysis evaluated the prognostic value of abnormally expressed lncRNAs in patients with osteosarcoma. We demonstrated abnormal expression level of lncRNAs was closely associated with poor outcomes of osteosarcoma and it was an independent

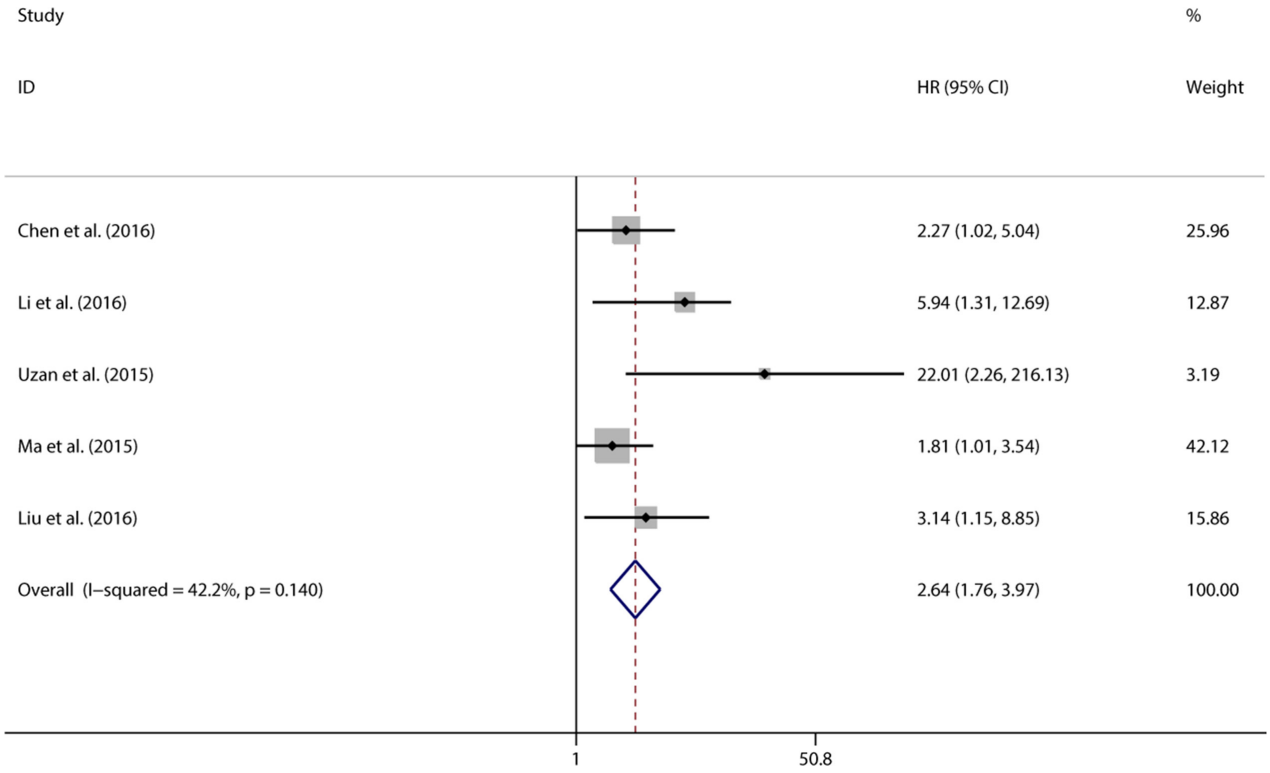

Figure 3: Meta-analysis of the pooled HRs of event-free-survival (EFS) for osteosarcoma patients with abnormally expressed lncRNAs.

prognostic factor for overall survival. Abnormal expression level of lncRNAs also reflected some malignant clinicopathological characteristics of osteosarcoma. Despite the limitations mentioned above, although there is a long way to clinical application, we believe that abnormally expressed lncRNAs will be potential prognostic biomarkers for osteosarcoma patients. With future development of lncRNAs research in osteosarcoma, it will play a role in guiding the individualized treatment of osteosarcoma patients in near future.

MATERIALS AND METHODS

Literature retrieval strategy

The present review was performed in accordance with the PRISMA statement Supplementary Table 1 (2009) and the standard guidelines for meta-analyses and systematic reviews of tumor marker prognostic studies [39–41]. The research databases PubMed, Embase, Cochrane Library and Web of Science were independently searched by two researchers (Shidai Mu and Yu Huang) to obtain all relevant articles about the prognostic value of abnormally expressed lncRNAs in patients with osteosarcoma. The literature search ended in March 14, 2017. The search strategy used both MeSH terms and free-text words to increase the sensitivity of the search. The search strategy was: ("long non-coding RNA or lncRNA or RNA long non-coding or lincRNA or long intergenic non-coding RNA") AND ("osteosarcoma or osteogenic sarcoma"). All included studies were retrieved in English database. We also retrieved articles from other sources, for example, retrieving from the reference lists of relevant articles. Conflicts were solved through group discussion.

Inclusion and exclusion criteria

Studies included in this analysis had to meet the following inclusion criteria: 1) Patients were pathologically diagnosed with osteosarcoma. 2) The expression of lncRNAs was determined in tissues or plasma samples from patients using quantitative reverse transcription polymerase chain reaction (qRT-PCR). 3) Patients were divided into high and low lncRNA expression groups, the prognostic value of one lncRNA was investigated and the relationship between lncRNA expression and survival was examined. 4) Sufficient data or the survival curve was provided to estimate hazard ratios (HRs) for survival rates and their 95% confidence intervals (95% CIs).

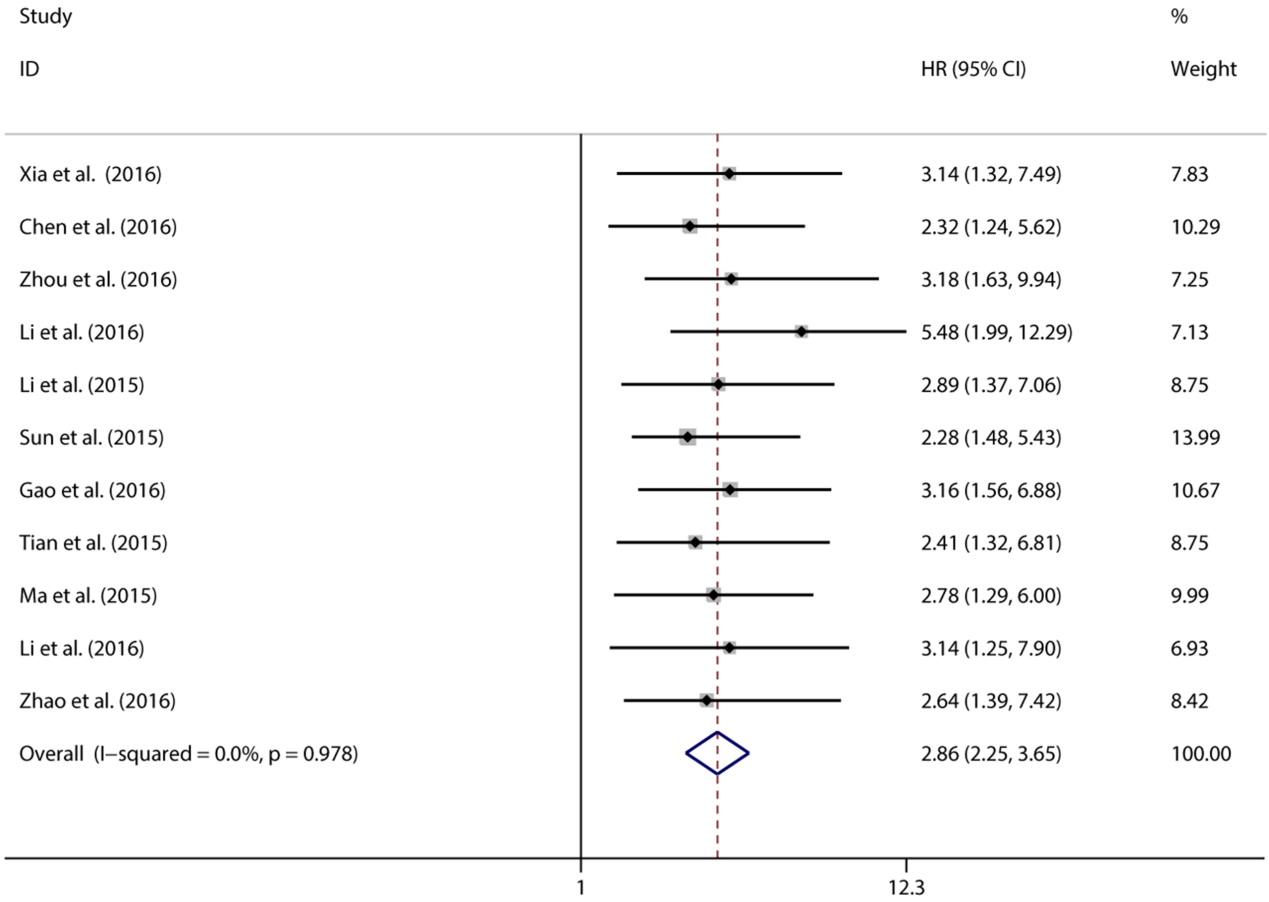

Figure 4: Meta-analysis of the independent role of abnormal expression level of lncRNAs in overall survival (OS) of osteosarcoma.

Studies were excluded from the analysis if they met any of the following exclusion criteria: 1) They were retracted articles, letters, case reports, reviews, meta-analysis, commentary, conference reports or expert opinions. 2) Articles presented in languages other than English. 3) The article was not found in full text. 4) The article was a repeated study or included patients was reported in a previous study. 5) Did not provide sufficient descriptions of the required data or the data could not be received from the original article or from the authors. 6) Sample population consisted of less than 30 cases. 7)Used nonhuman samples. All the eligible studies were carefully identified by the same two researchers (Fashuai Wu and Deyao Shi), and discrepancies were resolved by discussing with a third researcher (Feng Gao).

Quality of all included studies was assessed independently by four researchers (Fashuai Wu, Deyao Shi, Feng Gao and Xiangcheng Qing) according to the Newcastle-Ottawa Scale (NOS), which contains 9 items, and disagreements were resolved through discussion. Studies with NOS scores more than 7 were considered to be of high quality. And studies not reaching a minimum threshold (NOS scores <=5) would be excluded from the analysis to guarantee the quality of included papers.

Data extraction

Four researchers (Fashuai Wu, Deyao Shi, Shidai Mu and Yu Huang) extracted data independently, and conflicts were resolved through discussion. For each eligible article, extracted information included: author, journal name, year of publication, lncRNA signature, expression associates with poor prognosis, NOS score, method of obtaining HRs and characteristics of the study population (including country of the population enrolled, number of patients (high/low), tumor size, tumor location, tumor differentiation grade, tumor stage, lymph node metastasis, distant metastasis, follow up (month), endpoint, assay method, sample type, cut off value, pre-operation treatment and survival analysis). The primary outcome was overall survival (OS), which was measured from the initiation of therapy until death from any cause. The secondary endpoint was event-free survival (EFS). Data regarding disease-free survival (DFS), progress-free survival (PFS), recurrence-free survival (RFS) were obtained from articles, and were redefined as EFS, which was measured from the date of initiation of therapy to the date of recurrence or metastasis [42, 43].

The effect of lncRNA expression on survival was assessed using HR. We extracted HRs following a methodology suggested previously [44]. HRs and their 95% CIs were extracted directly from the publication, if provided by the authors. Otherwise, we calculated the HRs and their 95% CIs from the published data including *p* values of the log-rank tests and number of events. Besides, we used the Engauge Digitizer version 9.8 to read survival rates in Kaplan–Meier Curves, then we inputted the extracted survival rates into the spreadsheet set up by Tierney JF et al. to obtain HRs and their 95% CIs, assuming that patients were censored at a constant rate during follow-up [45]. If possible, we asked for original data directly from the authors of the relevant studies.

Statistical methods

1) Pooled HRs and their associated 95% CIs were merged using a fixed-effect model (Mantel-Haenszel). While the random-effect model was applied if significant heterogeneity was present. An HR > 1 indicates that the patients have a poor prognosis. 2) The clinicopathological characteristics were assessed by the pooled odds ratios (ORs). 3) The test for heterogeneity of combined HRs and ORs was performed using a χ^2 based Cochran *Q* test and Higgins I^2 statistic. A *p* value of less than 0.05 or an I^2 value of larger than 50% was considered statistically significant. 4) The presence of publication bias was evaluated by using both Begg's test and Egger's test. A *p* value of less than 0.05 was considered statistically significant [46]. 5) Sensitivity analyses including influence analysis, file-drawer analysis (fail-safe number) and trim and fill analysis, were used to examine the stability of results. By excluding any of the whole studies one at a time, influence analysis was used to trace the potential heterogeneity sources. To evaluate the influence of publication bias, file-drawer analysis (fail-safe number) and trim and fill analysis were carried out. Fail-safe number (Nfs) was firstly prompted by Rosenthal in 1979 [47]. Nfs refers to the number of unpublished studies needed to reverse the result of a meta-analysis and Nfs was calculated by the specific formula according to Rosenthal's article. When Nfs is much larger than actual included studies, we consider the result is stable. The trim and fill method is a nonparametric data augmentation technique firstly proposed by Duval and Tweedie [48]. This rank-based data augmentation technique can be used to estimate the number of missing studies that might exist in a meta-analysis and the effect that these studies might have had on its outcome. The method formalizes the use of funnel plots and yields an effect adjusted for funnel plot asymmetry. Once the number of 'missing' studies is estimated, a recalculation was performed to assess the weighted mean effect size and its variance when the 'missing' studies are included [49, 50]. 6) Statistical analysis and graphical representation were performed using Stata software statistical software version 14.0 (Stata Corporation, College Station, TX, USA).

Author contributions

F.W. and D.S. contributed equally to this study and share the first authorship. S. M. and Y.H independently

searched the databases. F.W., D.S. and F.G. identified the eligible studies. F.W., D.S., F.G. and X.Q assessed the quality of included studies. F.W., D.S., S.M. and Y.H. extracted the data. F.W., and D.S. analysed the data and made the figures and tables. F.W., D.S. and Z.S. wrote the manuscript, and all authors commented on it at every stage. Z.S. oversaw the study.

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CONFLICTS OF INTEREST

The authors declare no Conflicts financial interests.

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