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Original Article

Association between expression of lysyl oxidase and prognosis in solid tumor patients: a systematic review and meta-analysis

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Abstract: Background: Recently, lysyl oxidase (LOX) has been frequently reported to be overexpressed in various malignancies and to be involved in tumor invasion and migration. However, a prognostic role of LOX in human solid tumor remains unclear. Materials and methods: Eligible studies were gathered by searching on PubMed, Web of Science, Embase and CNKI. Using STATA 12.0 software and Review Manager Version 5.3. Pooled hazard ratios (HRs) and their 95% confidence intervals (CIs) for total and subgroup analyses were calculated to investigate the correlation between expression of LOX and prognosis in solid tumor patients. Results: Thirteen studies with 2235 patients were enrolled in this meta-analysis. The results show that LOX positive expression is associated with poor overall survival (OS) (HR = 1.68, 95% CI: 1.52-1.87) and disease-free survival (DFS) (HR = 2.47, 95% CI: 1.52-4.01). When stratified by tumor type, the influence of LOX overexpression on poor prognosis was found in colorectal cancer, lung cancer, gastric cancer, head and neck cancer and other cancers, but not in ovarian cancer. For subgroup analysis based on study region, TNM stage, sample size and analysis method, the relationship between LOX positive expression and poor OS was significant. Moreover, increased LOX expression was significantly associated with several clinicopathological features, including advanced TNM stage (OR = 2.04, 95% CI: 1.23-3.41), and positive lymph node metastasis (OR = 4.20, 95% CI: 3.23-5.47). Conclusion: LOX overexpression is thus associated with poor prognosis of numerous cancers, and LOX may serve as a biomarker for the progression of solid tumors, which is likely to be a new target for anti-tumor therapy.

Keywords: Lysyl oxidase, prognosis, clinicopathological features, solid tumor, meta-analysis

Introduction

To date, the importance of the microenvironment to pathogenesis is becoming much more acknowledged, and the tumor microenvironment plays a crucial role in tumor initiation and aggression [1]. The interplay between malignant cells and their surrounding microenvironment is critical in all aspects of tumor development, including tumor cell proliferation, epithelial-to-mesenchymal transition (EMT), angiogenesis, migration, invasion, and metastasis [2]. Cancer stages are regulated by mechanisms that depend on the reciprocal interactions among cells at the tumor microenvironment. Benign tumors, which arise from gene mutations in the epithelium, develop to aggressive cancers owing to the alteration of the microenvironment [3]. Extracellular matrix (ECM) is a component of the tumor microenvironment, of which deposition and remodeling can promote tumor progression by destabilization of cell polarity, cell-cell adhesion, and the change of growth factor signaling [4, 5]. ECM remodeling has been regarded as a common characteristic of the processes of tumorigenesis.

The ECM is stabilized by a group of enzymes, namely ECM-modifying proteins. The expression of these ECM-modifying enzymes is closely regulated during normal development, and one of these enzymes-Lysyl oxidase (LOX)-is expressed in various cell types, including fibro-

blasts, adipocytes, osteoblasts, smooth muscle cells, and endothelial cells [6]. LOX is a secreted copper-dependent amine oxidase, which is initially synthesized as a 50-kDa proenzyme (pro-LOX) and then cleaved in the extracellular environment by bone morphogenetic protein 1 to divide into a 30-kDa mature enzyme (LOX) and an 18-kDa propeptide (LOX-PP). Extracellular LOX and LOX-PP can then reenter cells from the extracellular space to exert their biological effects [7]. The most well-known function of LOX is that it can catalyze the oxidative deamination of lysine and hydroxylysine residues to aldehydes, thus initiating covalent crosslinking of collagens and elastin in the ECM [8]. Abnormal expression of LOX is associated with a number of human diseases, especially cancer. In the beginning, LOX was described as a role of tumor suppressor [9, 10], however, more recent studies have shown that LOX production plays a stimulative role in tumor progression and metastasis [11].

In recent years, overexpression of LOX protein by immunolocalization has been reported to be related to prognosis of patients burdened with diverse kinds of solid tumors, including colorectal cancer (CRC) [12, 13], ovarian cancer (OC) [14], larynx cancer (larynx C) [15], gastric cancer (GC) [16, 17], nasopharyngeal carcinoma (NPC) [18], hepatocellular carcinoma (HCC) [19], lung cancer (LC) [20, 21], oral and oropharyngeal squamous cell carcinoma (OSCC) [22], esophageal cancer (EC) [23], head-and-neck squamous cell carcinomas (HNSCC) [24]. However, due to the inconsistency of the results, the prognostic value of LOX in solid tumors is still controversial or inconclusive, and needs to be confirmed by systematic analyses. Thus, this meta-analysis was conducted to assess the correlation of high expression of LOX with survival in human solid tumors, and to illustrate the clinical value of LOX serving as a potential prognostic indicator and therapeutic target for human solid tumors.

Materials and methods

Search strategy

The systematic review and meta-analysis was carried out according to the guideline of the Preferred Reporting Items for Systematic Reviews and Meta-analyses [25, 26]. A systematic electronic search was performed though PubMed, Web of Science, Embase and CNKI database (up to April 2018). The following keywords were used for the search: “lysyl oxidase” or “LOX” (all fields), “cancer” or “tumor” or “malignancy” or “neoplasm” or “carcinoma” (all fields), and “prognosis” or “prognostic” or “outcomes” or “survival” (all fields). No language restrictions were imposed, and citation lists of the included studies were also screened for the comprehensive search.

Inclusion and exclusion criteria

The eligibility of each study was evaluated independently by two investigators (Zhang CM and Ye J). To be eligible for inclusion in this meta-analysis, a study must meet the following criteria: (1) the cohort design to report the relationship between LOX expression and overall survival (OS) and/or disease-free survival (DFS) in solid tumors; (2) expression of LOX protein was measured in the nucleus and/or cytoplasm of cancer tissue by immunohistochemistry (IHC); (3) the patients was divided into two groups, namely positive LOX groups and negative LOX group, regardless the cutoff value; (4) Hazard ratios (HR) as well as the corresponding 95% confidence intervals (CIs) for survival analysis could be directly obtained or indirectly calculated from existing information [27]; (5) the articles were written as full papers. The following type of studies were excluded: conference abstract, letters, reviews, editorials, basic research, or animal experiments.

Data extraction and quality assessment

Data from eligible studies were extracted independently by two investigators (Zhang CM and Wu JY). Any disagreement between the investigators was resolved by consensus. The following items were extracted from each eligible study: the first author’s name, year of publication, nationality, study region, cancer type, duration period, follow-up time, sample size, cut-off value, number of high LOX expression, blinding status, survival outcomes, HR estimations, and quality scores. Blinding status represented that the evaluation of LOX was blinded to the clinical outcomes. In studies where the HR estimations of univariate and multivariate analyses were both provided, only the latter was applied to the data synthesis because it had taken into account the confounding factors and is more precise.

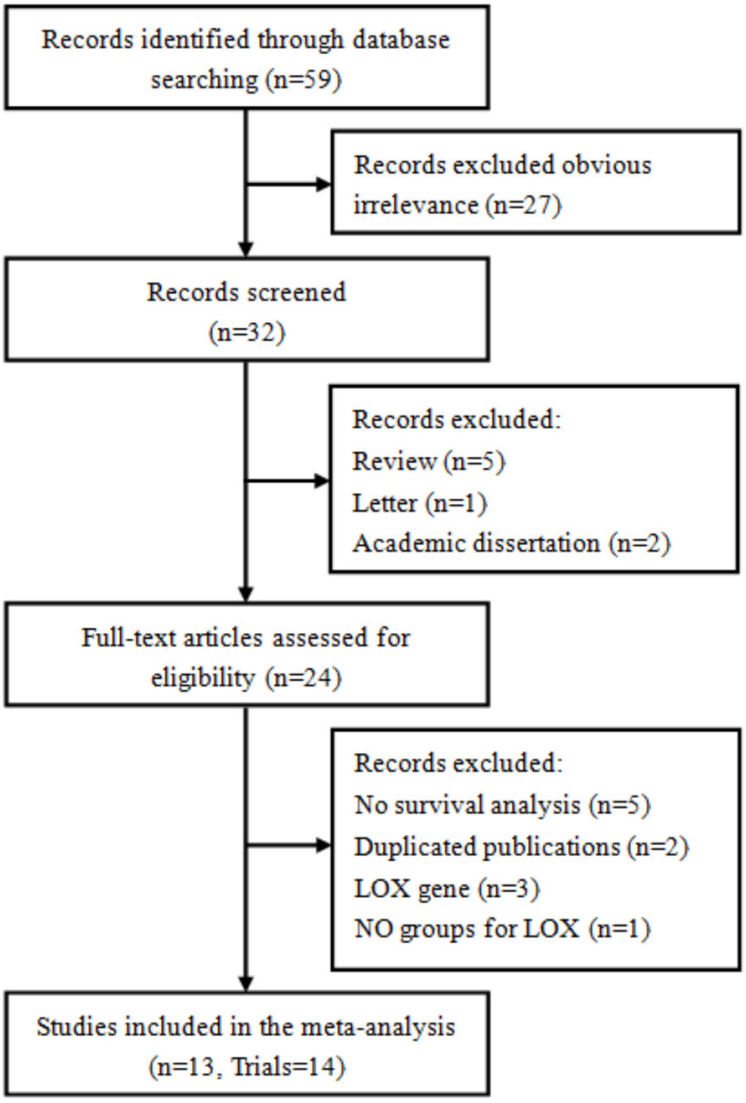

Figure 1. Flow diagram of the study selection process and specific reasons for exclusion in the meta-analysis.

The 9-star Newcastle-Ottawa Scale (NOS) was used to assess the quality of enrolled studies based on the following categories: selection, comparability, and outcome of interest. The total scores of NOS ranged from 0-9, and studies with a score of ≥ 6 were regarded as high quality [28].

Statistical analysis

The combined HR and 95% CI were used to assess the prognostic value of LOX expression in patients with solid tumor based on the data extracted from the included studies. The pooled HR with 95% CI exceeding 1 suggested an increased risk of poor prognosis for patients with LOX overexpression. The results were considered to be statistically significant if $P < 0.05$ through Z-test. Subgroup analyses were conducted according to cancer type (at least two trials must report the same outcome for the same cancer type, otherwise, they were assigned to a subgroup named "Others"), study region, TNM stage, sample size, and analysis method. For the pooled analysis of the correlation between LOX expression and clinicopathological characteristics, the ORs and the corresponding 95% CI were combined to estimate the effect.

Heterogeneity assumption was qualitatively assessed by the Chi-square test based on Q statistic, and was considered statistically significant when $P < 0.05$. Heterogeneity was also quantitatively evaluated through I^2 metric, and I^2 values of 25%, 50% and 75% corresponded to cut-off points for low, moderate, and high degrees of heterogeneity, respectively [29]. When significant heterogeneity was observed among the studies ($I^2 > 50\%$ or $P < 0.05$), a random-effect model was used to calculate the pooled results. Otherwise, a fixed-effect model was applied. Sources of inter-study heterogeneity were also explored using subgroup analysis and sensitivity analysis. Sensitivity analysis validated the stability of the meta-analysis results by omitting each individual study sequentially. A funnel plot with Begg's and Egger's test was applied to assess the potential publication bias [30]. If significant publication bias was found ($P < 0.05$), trim and fill method was applied to validate the robust of the meta-analysis results. Stata 12.0 software and Review Manager Version 5.3 were used for all statistical analyses in this meta-analysis.

Table 1. Main characteristics of the eligible studies

Study	Region	Cancer type	TNM stage	Location	Duration	Follow up (months)	Number	LOX-high (%)	Blinding status	Survival analysis	Language	Quality
Shi XM 2017 [12]	China	CRC	I-IV	Both	2009-2010	Until Jun 2015	82	62 (75.6%)	Yes	OS (M)	Chinese	8
De Donato M 2017 [14]	Italy	OC	III-IV	Nucleus	NR	Median 42.5 (10-192)	70	47 (67.1%)	Yes	OS (M)	English	7
De Donato M 2017 [14]	Italy	OC	III-IV	Cytoplasm	NR	Median 100 (10-192)	70	32 (45.7%)	Yes	OS (U)	English	7
Lee YS 2017 [15]	Korea	Larynx C	NR	Both	2004-2010	60	100	63 (63.0%)	Yes	OS (M), DFS (M)	English	7
Pen C 2017 [17]	China	GC	I-IV	Both	2002-2011	Median 59.5 (16.8-102.3)	184	92 (50%)	Yes	OS (M), DFS (M)	English	8
Kasashima H 2016 [16]	Japan	GC	NR	Both	NR	NR	544	287 (52.8%)	Yes	OS (M)	English	7
Liu N 2016 [13]	Sweden	CRC	I-III	Nucleus	1987-1990	Median 100 (0-309)	137	118 (86.1%)	Yes	OS (M), DFS (M)	English	8
Hua YJ 2016 [18]	China	NPC	I-IV	Both	2000-2001	Median 71.6 (6-115)	233	144 (61.8%)	Yes	OS (M), DFS (U)	English	6
Zhu JY 2015 [19]	China	HCC	NR	Both	2010-2012	60	146	98 (67.1%)	Yes	OS (M), DFS (U)	English	6
Liu J 2014 [20]	China	LC	I-III	Both	2007-2009	NR	110	79 (71.8%)	Yes	OS (M)	English	8
Wilqus ML 2011 [21]	USA	LC	I-IV	Both	1997-2000	60	166	40 (24.1%)	Yes	OS (M)	English	8
Albinger-Hegyi A 2010 [22]	Switzerland	OSCC	I-IV	Both	1993-2000	Median 81	252	165 (65.5%)	Yes	OS (M)	English	8
Sakai M 2009 [23]	Japan	EC	I-IV	Both	1997-2007	Median 43 (5-125)	122	65 (53.3%)	Yes	OS (U)	English	6
Le QT 2007 [24]	USA	HNSCC	III-IV	Both	NR	Median 58	89	52 (58.4%)	Yes	OS (U)	English	6

M: multivariate analysis; U: univariate analysis; NR: none reported; CRC: colorectal cancer; OC: ovarian cancer; Larynx C: larynx cancer; GC: gastric cancer; NPC: nasopharyngeal; HCC: hepatocellular carcinoma; LC: lung cancer; OSCC: oral and oropharyngeal squamous cell carcinoma; EC: esophageal cancer; HNSCC: head-and-neck squamous cell carcinomas; OS overall survival; DFS: disease-free survival.

Table 2. Meta-analysis of LOX and clinicopathological features in solid tumors patients

Categories	Trials (patients)	OR (95% CI)	I^2 (%)	P_h	Z	P
Gender (male vs. female)	8 (1521)	0.95 (0.75-1.20)	49%	0.06	0.46	0.64
TNM stage (I+II vs. III+IV)	6 (979)	2.04 (1.23-3.41)^R	68%	0.009	2.74	0.006
Depth of invasion						
(T1+T2 vs. T3+T4)	4 (667)	1.19 (0.52-3.22)^R	62%	0.05	0.59	0.56
(T1 vs. T2-T4)	2 (666)	1.96 (0.45-8.51)^R	92%	< 0.001	0.90	0.37
Lymph node metastasis (negative vs. positive)	6 (1111)	4.20 (3.23-5.47)	0%	0.44	10.64	< 0.001
Lymphatic invasion (negative vs. positive)	2 (665)	2.61 (0.88-7.69)^R	76%	0.04	1.74	0.08
Venous invasion (negative vs. positive)	2 (666)	1.53 (0.30-7.71)^R	91%	< 0.001	0.51	0.61

All pooled ORs were calculated from fixed-effect model except for cells marked with (random^R). P_h denotes *P* value for heterogeneity based on Q test; *P* denotes *P* value for statistical significance based on Z test. OR odds ratio; CI confidence interval.

Table 3. Summary of the meta-analysis results

Categories	Trials	HR (95% CI)	I^2 (%)	P_h	Z	P
OS (All)	14 (2235)	1.68 (1.52-1.87)	0%	0.53	9.68	< 0.001
Study region						
Eastern countries	8 (1521)	1.68 (1.49-1.90)	0%	0.44	8.31	< 0.001
Western countries	6 (714)	1.69 (1.37-2.07)	1%	0.41	4.97	< 0.001
Cancer type						
CRC	2 (219)	1.95 (1.00-3.78)^R	66%	0.09	1.96	0.05
OC	2 (70)	1.42 (0.97-2.08)	0%	0.54	1.78	0.07
LC	2 (276)	2.09 (1.41-3.09)	0%	0.63	3.67	< 0.001
GC	2 (728)	1.66 (1.41-1.94)	0%	0.89	6.24	< 0.001
HNC	4 (674)	1.83 (1.41-2.38)	47.0%	0.13	4.52	< 0.001
Others	2 (268)	1.57 (1.78-2.11)	0%	0.67	3.04	0.002
TNM stage						
I-IV	6 (1039)	1.66 (1.47-1.89)	12.6%	0.33	7.88	< 0.001
I-III	2 (247)	2.24 (1.43-3.52)	0%	0.33	3.53	< 0.001
III-IV	3 (159)	1.49 (1.12-1.98)	0%	0.77	2.76	0.006
NR	3 (790)	1.84 (1.34-2.54)	5%	0.35	3.77	< 0.001
Sample size						
≥ 150	5 (1379)	1.73 (1.50-1.98)	15.6%	0.32	7.64	< 0.001
< 150	9 (856)	1.63 (1.39-1.92)	0%	0.54	5.96	< 0.001
Analysis method						
Multivariate	11 (2024)	1.74 (1.55-1.96)	0.3%	0.44	9.21	< 0.001
Univariate	3 (281)	1.48 (1.17-1.87)	0%	0.82	3.23	0.001
DFS (All)	5 (800)	2.47 (1.52-4.01)	76.6%	< 0.01	3.65	< 0.001

CRC: colorectal cancer; OC: ovarian cancer; LC: lung cancer; GC: gastric cancer; HNC: head and neck cancer, including larynx cancer, nasopharyngeal, oral and oropharyngeal squamous cell carcinoma, and head-and-neck squamous cell carcinomas; Others: including hepatocellular carcinoma and esophageal cancer. R: random-effect model.

Results

Description of included studies

The process of literature search is shown in **Figure 1**. Initially, 59 papers were generated in the primary electronic search in the major databases. According to the inclusion criteria, 13 full-text articles with 14 trials published from 2007 to 2017 were finally retrieved for our meta-analysis. In total, 2235 patients from various regions (China [12, 17-20], Japan [16, 23], Korean [15], western countries [13, 14, 21, 22, 24]) with 10 distinct cancers (CRC [12, 13], OC [14], Larynx C [15], GC [16], NPC [18], HCC [19], LC [20, 21], OSCC [22], EC [23], HNSCC [24])

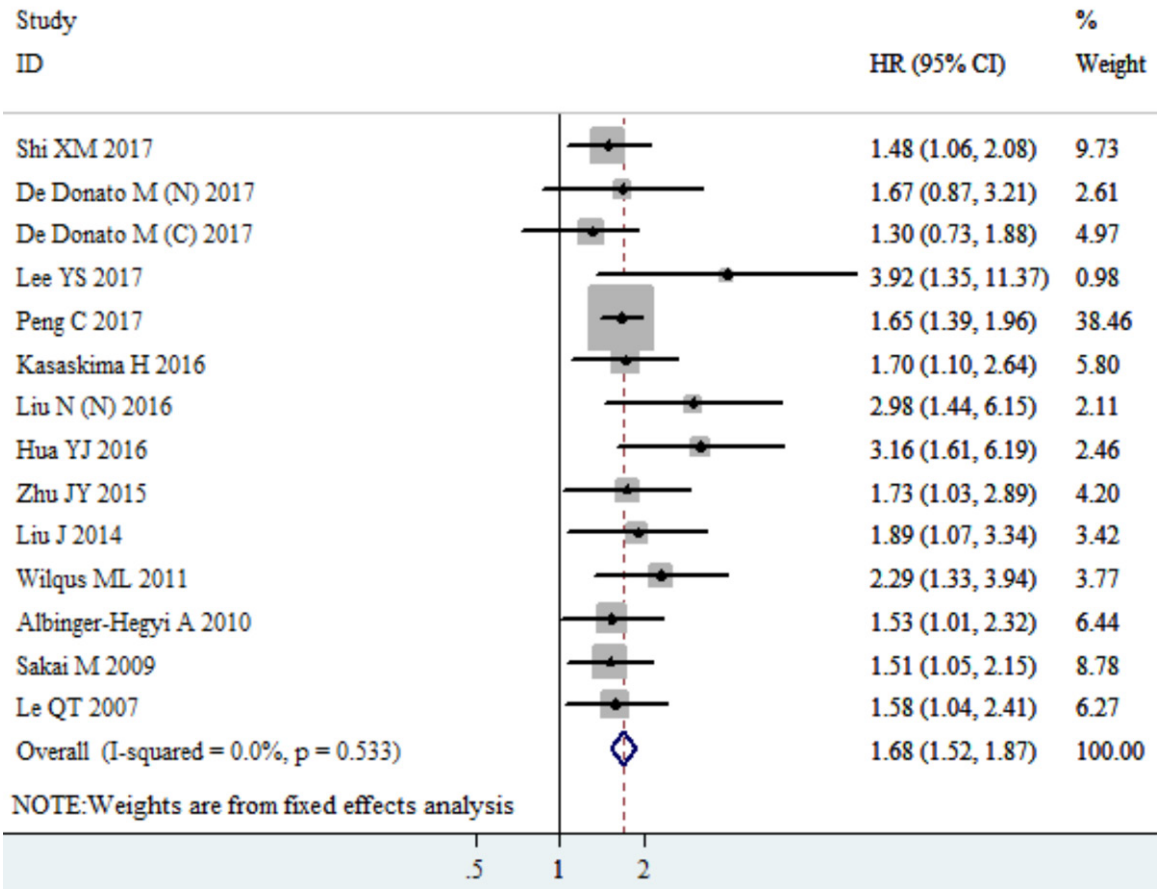

Figure 2. Forest plots of the overall outcomes for overall survival (OS). Hazard ratios (HRs) for each trial are represented by the squares, and the horizontal lines crossing the square stand for the 95% confidence intervals (CIs). The diamonds represent the estimated pooled effect of the overall outcome for OS in all solid tumors. All *P* values are two-sided.

were included in these studies. Thirteen articles with 14 trails reported the outcome of OS, and 5 articles reported DFS. HRs and the corresponding 95% CIs were obtained by multivariate analysis in 11 trials and through univariate or Kaplan-Meier curves in 3 trials, while those of DFS were achieved by multivariate analysis in 3 trials and through univariate or Kaplan-Meier curves in 2 trials. According to quality standards, all cohort studies were of high quality with scores of 6 or more. The main characteristics of the included studies are listed in **Table 1**.

Correlation between LOX and clinicopathological features

The relationships between LOX intensity and clinicopathological features are presented in **Table 2**. There were significant relationships between the high expression of LOX and some phenotypes of tumor progression, including advanced TNM stage (OR = 2.04, 95% CI: 1.32-3.41, *P* = 0.006, random effects), and positive lymph node metastasis (OR = 4.20, 95% CI: 3.23-5.47, *P* < 0.001, fixed effects). This result indicated that LOX overexpression in a tumor tissue may promote tumor aggressiveness. However, there was no significant relationship between LOX expression and gender (OR = 0.95, 95% CI: 0.75-1.20, *P* = 0.64, fixed effects), depth of invasion (T1+T2 vs. T3+T4, OR = 1.19, 95% CI: 0.52-3.22, *P* = 0.56, random effects; T1 vs. T2-T4, OR = 1.96, 95% CI: 0.45-8.51, *P* = 0.37, random effects), lymphatic invasion (OR = 2.61, 95% CI: 0.88-7.69, *P* = 0.08, random effects), and venous invasion (OR = 1.53, 95% CI: 0.30-7.71, *P* = 0.61, random effects).

Impact of LOX on prognosis

The main results of the analysis on the association between LOX expression and prognosis are

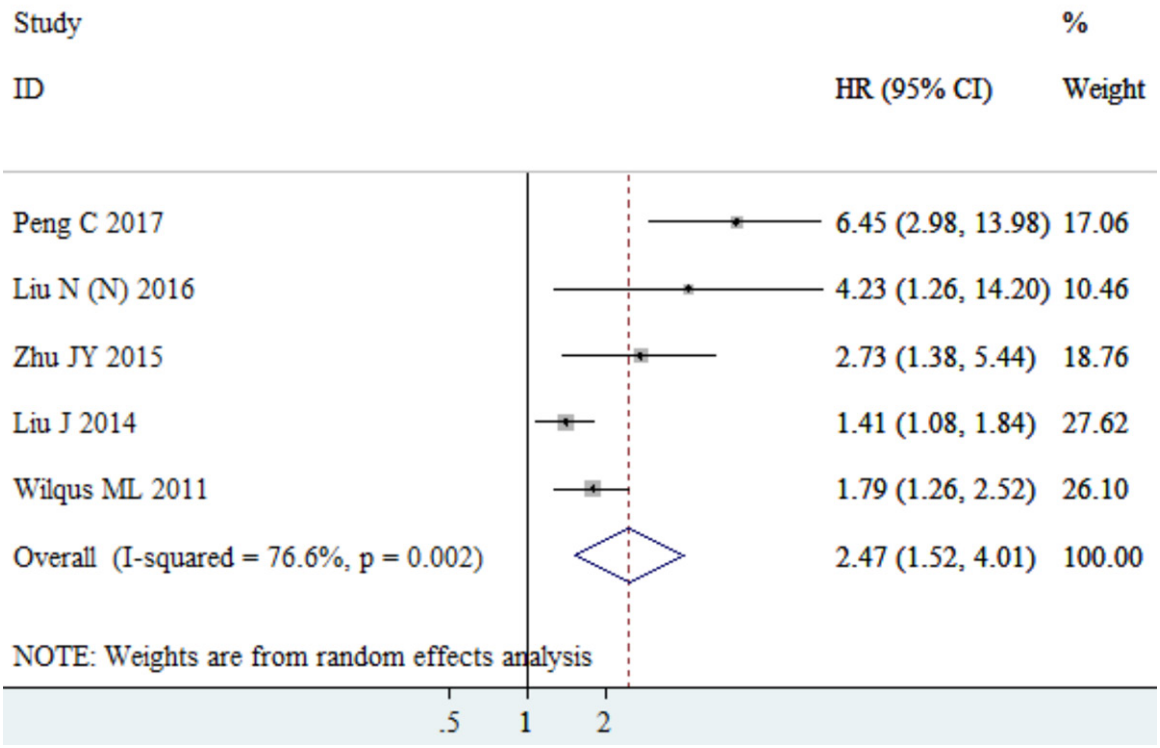

Figure 3. Forest plots of the overall outcomes for disease-free survival (DFS). Hazard ratios (HRs) for each trial are represented by the squares, and the horizontal lines crossing the square stand for the 95% confidence intervals (CIs). The diamonds represent the estimated pooled effect of the overall outcome for DFS in all solid tumors. All *P* values are two-sided.

shown in **Table 3**. Summary of the reported HRs for OS from the 14 individual trials indicated that LOX positive expression was significantly associated with poor OS (HR = 1.68, 95% CI: 1.52-1.87, *P* < 0.001, fixed effects, **Figure 2**) with a low heterogeneity (I^2 = 0.0%, *P* = 0.53). When the eligible studies were grouped by study region, significant correlation not only existed in the Eastern countries (HR = 1.68, 95% CI: 1.49-1.90, *P* < 0.001, fixed effects), but also in the Western countries (HR = 1.69, 95% CI: 1.37-2.07, *P* < 0.001, fixed effects). With regard to subgroup analysis based on cancer type, the predictive role of LOX positive expression on unfavorable OS in patients with CRC (HR = 1.95, 95% CI: 1.00-3.78, *P* = 0.05, random effects), LC (HR = 2.09, 95% CI: 1.41-3.09, *P* < 0.001, fixed effects), GC (HR = 1.66, 95% CI: 1.41-1.94, *P* < 0.001, fixed effects), head and neck cancer (HNC, including larynx C, NPC, OSCC, and HNSCC) (HR = 1.83, 95% CI: 1.41-2.38, *P* < 0.001, fixed effects) and others (including HCC and EC) (HR = 1.57, 95% CI: 1.78-2.11, *P* = 0.002, fixed effects), but not in patients with OC (HR = 1.42, 95% CI: 0.97-2.08, *P* = 0.07, fixed effects). With regard to TNM stage, LOX positive expression predicted shorter OS for patients with stage I-IV (HR = 1.66, 95% CI: 1.47-1.89, *P* < 0.001, fixed effects), I-III (HR = 2.24, 95% CI: 1.43-3.52, *P* < 0.001, fixed effects), III-IV (HR = 1.49, 95% CI: 1.12-1.98, *P* = 0.006, fixed effects), none reported (HR = 1.84, 95% CI: 1.34-2.54, *P* < 0.001, fixed effects). Moreover, the significant correlation of LOX overexpression and worse OS did not change regardless of the subgroup analyses of sample size and analysis method (**Table 3**).

Additionally, five studies reported the survival endpoint of DFS, of which the pooled result indicated that positive expression of LOX was significantly corrected with poor DFS (HR = 2.47, 95% CI: 1.52-4.01, *P* < 0.001, random effects **Figure 3**), and with extreme heterogeneity (I^2 = 76.6%, *P* < 0.001).

Sensitivity analysis and publication bias

Sensitivity analysis indicated that no single point estimate of the omitted individual dataset

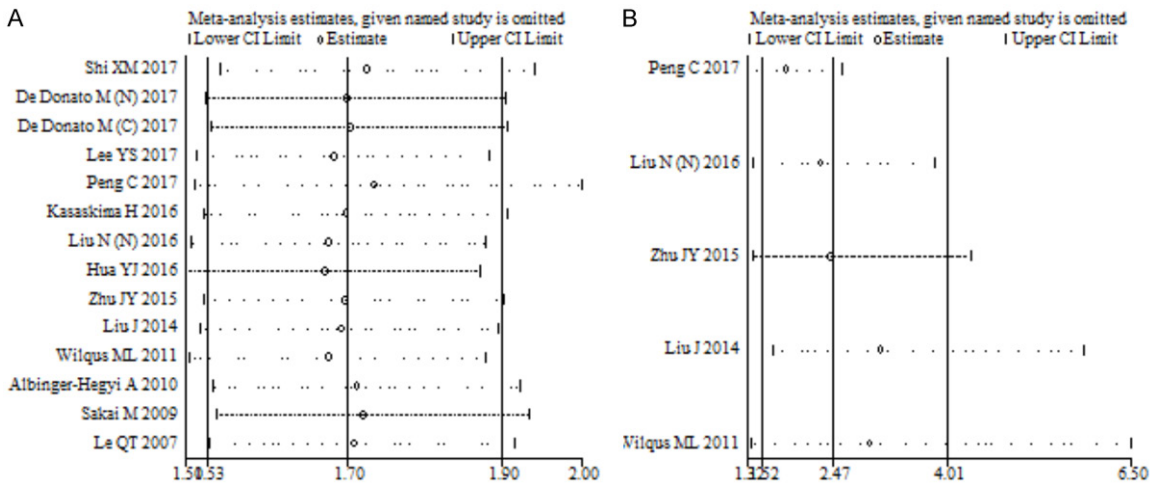

Figure 4. Effects of individual studies on pooled hazard ratios (HRs) for LOX expression and survival in solid tumors. A. Result of sensitivity analysis for pooled OS estimation. B. Result of sensitivity analysis for pooled DFS estimation.

lay outside the 95% CI of the combined analysis based on the overall HR estimate of OS (**Figure 4A**) and DFS (**Figure 4B**), and the results were stable and reliable.

Although there was no publication bias by Egger's test (P = 0.054) concerning the pooled result of OS, a significant publication bias was found by Begg's test (P = 0.005). Furthermore, the funnel plot showed a certain degree of apparent asymmetry (**Figure 5A**). The trim-and-fill analysis showed that one non-published studies were needed to balance the funnel plot for OS (**Figure 5B**), and the adjusted HR and 95% CI was slight changed but remains significant (HR = 1.69; 95% CI = 1.52-1.88; P < 0.001; fixed effects), suggesting that potential publication bias had minimal impact on the overall outcome. With regard to DFS, a significant publication bias was found by Egger's test (P = 0.042) but not by Begg's test (P = 0.086), which was also confirmed by the funnel plot shape (**Figure 5C**). After the trim-and-fill analysis was performed, two non-published studies were needed to add into the funnel plot (**Figure 5D**), and the adjusted HR and 95% CI remained significant (HR = 1.78; 95% CI = 1.07-2.95; P = 0.03; random effects), indicating the robustness of the overall results.

Discussion

We conducted this first meta-analysis to examine the association between LOX expression and prognosis as well as clinicopathological features in solid tumors. The combined result from 13 eligible studies with 2235 patients demonstrates that high LOX expression levels are associated with poor OS in patients with solid tumor, regardless of the study region, TNM stage, sample size, or analysis method. For subgroup analysis based on tumor type, the relationship between LOX positive expression and poor OS remained significant in patients with CRC, LC, GC, HNC, and Others, but not in OC patients, mainly due to the lack of sample size and the existence of heterogeneity, so more studies were needed to consolidate or overthrow the conclusion. Moreover, although the result concerning OS in the CRC subgroup was critical with a 95% CI of 1.00 to 3.78, many meta-analyses would consider this result statistically significant [31, 32]. Thus, the pooled HR estimates indicated that high LOX expression corresponded to poor OS in CRC patients. In addition, the pooled outcome based on 5 trials with 800 cases revealed that elevated LOX expression was significantly associated with worse DFS. Considering that the number of included studies was limited, we did not perform a subgroup analysis for the survival endpoint. Though with extreme heterogeneity, the prognostic significance of LOX on predicting DFS was not weakened by sensitivity analysis. Therefore, LOX may serve as an independent, negative biomarker for prognosis in solid tumors.

Although we have conducted a broad electronic search, there was still significant publication

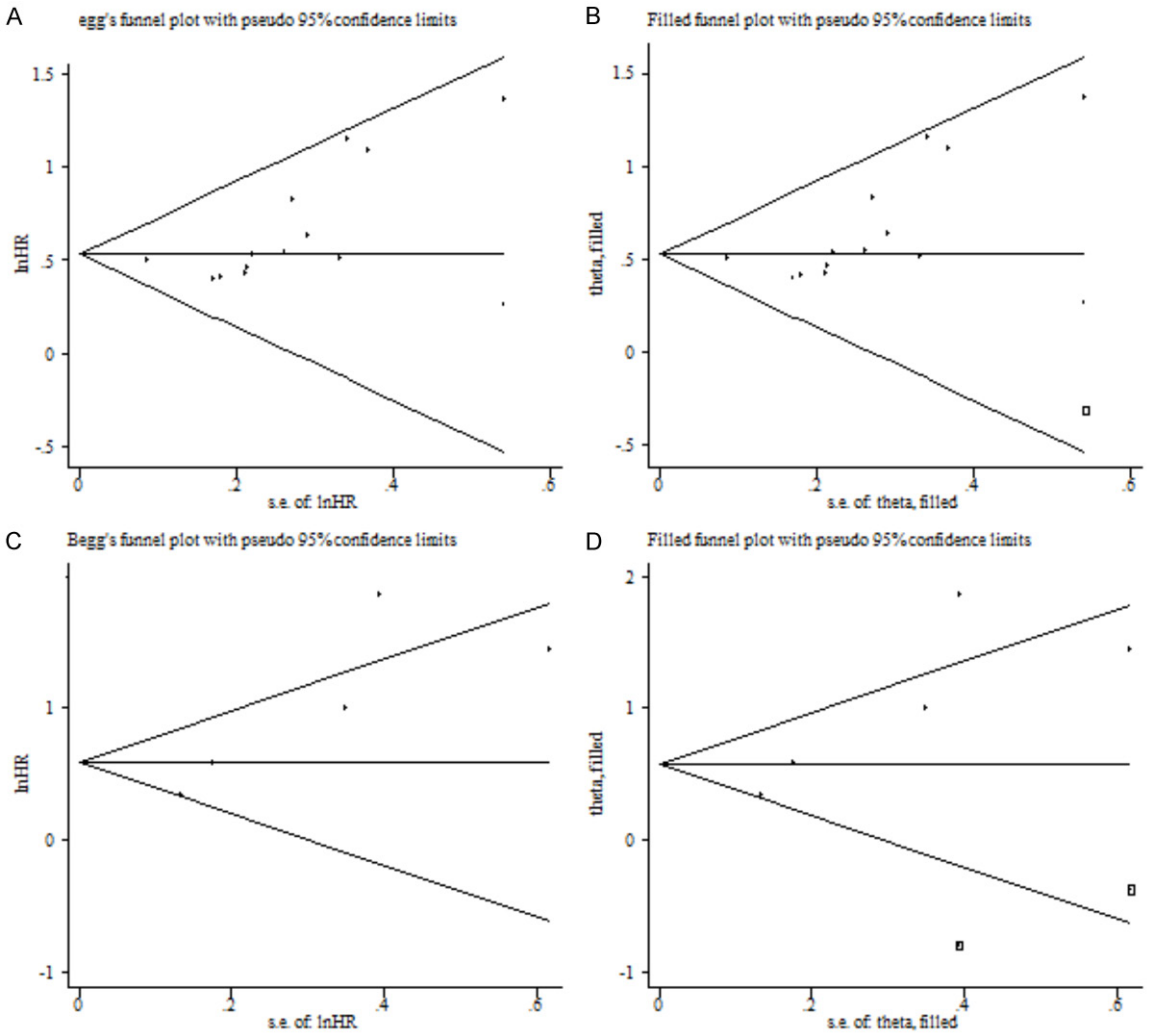

Figure 5. Begg's funnel plots for assessment of potential publication bias in studies of LOX in patients with solid tumor. Each study represented by one circle. The horizontal line represented the pooled effect estimate. A. Funnel plot of publication bias for studies reporting overall survival. B. Funnel plot adjusted with trim and fill methods for studies reporting overall survival. C. Funnel plot of publication bias for studies reporting disease-free survival. D. Funnel plot adjusted with trim and fill methods for studies reporting disease-free survival.

bias among studies concerning the outcomes of OS and PFS, which may have inflated the overall results. So we applied a trim-and-fill analysis to recalculate the combined outcomes, and the adjusted HRs as well as 95% CIs reinforced the prognostic role of LOX in solid tumors and remained statistically, indicating our results were robust and reliable. We also analyzed the correlation between LOX and the clinicopathological features to further investigate the prognostic impact of LOX on solid tumors. The synthesized data showed that overexpression of LOX was positively associated with advanced TNM stage and positive lymph node metastasis. All these factors have been reported to be meaningful variables related to cancer progression and result in short-term survival. Herein, elevated LOX intensity is closely correlated with more aggressive tumor behavior, and cancer patients with advanced clinical stage or positive lymph node metastasis would benefit most from LOX evaluation to make clinical decisions.

Above all, LOX positive expression was associated with poor prognosis and tumor aggression. Here are some possible reasons that can explain the results.

First, the relationship between tissue fibrosis and malignancy has drawn much attention. The aberrant ECM stiffness in tissue fibrosis, main-

ly induced by an increased collagen deposition and deregulation of covalent cross-linking modifications has been clearly proven to involve in cancer progression [33]. Cancer cells in a stiff microenvironment are prone to be more proliferative and migratory into surrounding matrix [34]. Overexpression of active LOX could strengthen ECM stiffness by increasing cross-linking of collagens and elastin [35]. Also, LOX-mediated ECM cross-linking functions in coordination with matrix metalloproteinase activity, thereby leading to ECM remodeling, and subsequently, promoting tumor metastasis [36]. Moreover, secreted LOX has been shown to engage in the recruitment of inflammatory cells to distant sites, resulting in the formation of the premetastatic niche and malignancy metastasis [37].

Second, the hypoxic microenvironment present in solid tumors larger than 1 cm^3 due to insufficient blood supply could be considered as an inducer of tumor metastasis and is strongly relevant to poor prognosis [38]. Hypoxia is largely mediated by the targets of hypoxia-inducible factors (HIFs). Activation of HIFs facilitates its binding to the hypoxia-response element to induce cell proliferation and secretion of angiogenesis factors, such as vascular endothelial growth factor (VEGF) [4]. The combination of HIF, LOX, and VEGF enhances cancer cells to grow faster, resulting in a metastatic spread of malignancies [39]. Furthermore, LOX, among the hypoxia gene signature, has been shown to be regulated by HIF-1α, and contributes to hypoxia-induced metastasis in several cancers [40]. Silencing of LOX could impair the hypoxia-induced cancer cell invasion, and the overexpression of mature LOX can also rescue the decreased invasive ability, suggesting secreted LOX plays key roles in the process of tumor progression and has an impact on the prognosis [41].

Third, increasing evidence shows the importance of the LOX in transforming epithelial neoplasms towards their more aggressive phenotypes [42]. On one hand, LOX is a potent chemokine inducing directional migration in various cell types. LOX expression is found to be raised in advanced tumors with distant metastasis compared with primary tumors in breast cancer [43]. On the other hand, LOX is actively involved in the process of EMT, which is believed to be a critical step in cancer cell dissemination and metastasis [44]. EMT is a process by which epithelial cells lose their cell-cell adhesion and polarity, as well as gain the migratory and invasive properties to become mesenchymal stem cells. EMT is characterized by decreased intensity of epithelial markers, e.g. E-cadherin, and elevated expression of mesenchymal markers, e.g. vimention [45]. Both *in vitro* and *in vivo* experiments have demonstrated that, LOX downregulation significantly upregulates the E-cadherin level and downregulates the vimention level, which shows that LOX contributes to the induction of EMT during cancer cell invasion and metastasis [41].

Fourth, as LOX has been reported to facilitate migration, invasion, and metastasis of malignant cells through its capacity to regulate collagen cross-linking and ECM stiffening, it can be a potential molecular target for anti-cancer treatment [46]. Elevated LOX activity has been shown to reduce drug diffusion under hypoxic conditions and consequently decrease the efficacy of cytotoxic treatment in 3D tumor models [47]. Therefore, targeting the activities of LOX could significantly enhance the therapeutic efficacy in the treatment of various malignant diseases. In a cervical cancer study, LOX protein expression and catalytic activity were upregulated following exposure to hypoxia in malignant cells [48]. The LOX inhibitor, β-amunopropionitrile (βAPN) could block the EMT phenomenon of cervical cancer cells, thus abolishing metastasis of several cancers [49]. Another study showed that miR30a downregulates LOX expression and inhibits anaplastic thyroid cancer progression [50]. Moreover, LOX expression was remarkably increased not only at the mRNA and protein level, but also at enzymatic activity level in the hypoxic A549 cells, compared with normoxic A549 cells. Inhibition of LOX resulted in the reduction of the ability to repair double-stranded breaks, promotion of apoptosis, relief of G2/M cycle arrest, and eventually reduction of hypoxia-induced radioresistance in the hypoxic A549 cells [51], which suggests that LOX might be novel potential therapeutic target in the management of malignance.

There are several limitations in this study that should be acknowledged. To begin with, LOX expression was determined by IHC in all enrolled searches of this meta-analysis, however, the cut-off value to determine the positive or negative expression of LOX were inconsis-

tent in different studies, potentially resulting in heterogeneity. Therefore, a more unified standard should be defined in the future. Second, several individual HRs were indirectly achieved from survival curves or univariate analysis, which may be less reliable than the actual HRs directly obtained from published data. Third, although we did not impose limitations in language, only studies in English and Chinese were included in the meta-analysis. Fourth, several characteristics of the included studies were extremely different, including analysis patterns, follow-up times, and LOX detection, which may also led to heterogeneity that affect our results. Finally, anticancer therapy also has certain effect on the survival time of cancer patients. However, whether LOX is an independent prognostic factor from clinical treatment is still unclear because the included literatures failed to control the latter.

Conclusion

In conclusion, the results of our meta-analysis suggest that high LOX expression is associated with poor prognosis and some unfavorable clinicopathological features in patients with solid tumors. LOX may be a valuable prognostic biomarker and a useful treatment target in malignancies. The development of therapeutic regimen against LOX might be a reasonable approach to control cancer progression and prolong patient's life. However, due to the limitation of this work, the results should be cautiously interpreted. Further researches with large sample and prospective design are needed to validate the clinical value of LOX.

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Disclosure of conflict of interest

None.

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