Research Paper

PTEN expression is a prognostic marker for patients with non-small cell lung cancer: a systematic review and meta-analysis of the literature

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ABSTRACT

Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is a known tumor suppressor in non-small cell lung cancer (NSCLC). By performing a systematic review and meta-analysis of the literature, we determined the prognostic value of decreased PTEN expression in patients with NSCLC. We comprehensively and systematically searched through multiple online databases up to May 22, 2016 for NSCLC studies reporting on PTEN expression and patient survival outcome. Several criteria, including the Newcastle-Ottawa Quality Assessment Scale (NOS), were used to discriminate between studies. In total, 23 eligible studies with a total of 2,505 NSCLC patients were included in our meta-analysis. Our results demonstrated that decreased expression of PTEN correlated with poor overall survival in NSCLC patients and was indicative of a poor prognosis for disease-free survival and progression-free survival in patients with NSCLC.

INTRODUCTION

Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is a protein that can modulate cell survival and cell cycle progression [1]. In healthy physiological conditions, PTEN can control smooth muscle differentiation [2], mediate angiogenesis [3], maintain Treg cell stability [4], and coordinate retinal neurogenesis [5]. However, PTEN is a tumor suppressor that is commonly down-regulated in many types of cancer [6], including non-small cell lung cancer (NSCLC) [7, 8]. Indeed, inactivation of PTEN can augment invasiveness and anchorage independent growth of NSCLC cells [9]. In addition, in some animal models, PTEN inactivation also accelerates tumorigenesis [10]. On the other hand, exogenously imported PTEN can suppress lung tumorigenesis [11]. Similarly, PTEN upregulation can inhibit NSCLC cell growth and promote partial apoptosis [12].

While many histopathology research studies reported that PTEN downregulation correlates with unfavorable survival prognosis in NSCLC patients [13–16], some studies reached the opposite conclusion [17, 18]. Therefore results in this field seem to be inconsistent. One problem is that most studies assessing the effects of PTEN expression in NSCLC were limited by small sample sizes. Here, we performed a systematic review and statistical meta-analysis of the literature to draw conclusions regarding the prognostic value of PTEN downregulation in NSCLC patients.

RESULTS

Study selection and characteristics

Our initial database search identified 237 potentially relevant records. After the duplicates were removed, 77 records remained, of which 54 were excluded because they failed to meet our inclusion criteria (see Materials and Methods). In the end, a total of 23 studies [13–35] were included in this systematic review (Figure 1). The

main characteristics of these studies are summarized in Table 1 and Table S1. In total, 2,505 NSCLC patients were included in our meta-analysis. According to the different survival outcomes, patients from the 23 eligible studies were divided into 27 datasets: 20 for overall survival (OS), four for disease-free survival (DFS) and three for progression-free survival (PFS) (Table 1 and Figure 1). The Newcastle-Ottawa Quality Assessment Scale (NOS) scores of the included studies ranged from five to eight (with a mean value of 6.22), indicating that these studies were of moderate to high quality (Table S2).

Meta-analysis of OS

The pooled result from 20 datasets revealed significant association between decreased PTEN expression and poor OS in patients with NSCLC (HR = 0.48, 95% CI: 0.43–0.54, $P < 0.001$) (Figure 2). Meanwhile, no obvious heterogeneity was found ($I^2 = 33.1\%$, $P = 0.076$). By successively omitting each study, sensitivity analysis was performed to evaluate the impact of every study on the pooled HR. Results showed that the pooled HRs were no different with the exclusion of any individual study, implying that the result of the meta-analysis of OS is stable (Figure 3).

Subgroup analyses were performed depending on protein and mRNA expression, type of analysis (univariate vs. multivariate), population (Asian vs. non Asian), number of cases (less than 100 vs. more than 100) and year (after 2010 vs. before 2010). Results showed that decreased expression of PTEN protein had an unfavorable prognostic value in NSCLC patients (HR = 0.46, 95% CI: 0.39–0.53, $P < 0.001$). However, although decreased expression of PTEN mRNA also correlated with poor OS in patients with NSCLC, its value did not reach statistical significance (HR = 0.60, 95% CI: 0.34–1.07, $P = 0.084$) (Figure S1, Table 2). The results from analyzing both the univariate and multivariate analysis subgroups indicated that decreased expression of PTEN was associated with poor OS in NSCLC patients (HR = 0.47, 95% CI: 0.37–0.59, $P < 0.001$; HR = 0.47, 95% CI: 0.39–0.56, $P < 0.001$, respectively) (Figure S2, Table 2). In addition, the pooled results of other subgroup analyses showed a similar prognostic value for decreased expression of PTEN (Figures S3–S5, Table 2).

Meta-analysis of DFS/PFS

The pooled result from four datasets of DFS revealed that decreased expression of PTEN was associated with unfavorable DFS in patients with NSCLC (HR = 0.57, 95% CI: 0.44–0.73, $P < 0.001$) (Figure 4). Similarly, the pooled result from three datasets of PFS showed that decreased expression of PTEN also had an unfavorable prognostic value for PFS in NSCLC patients (HR = 0.48, 95% CI: 0.26–0.88, $P = 0.018$) (Figure 4).

Publication bias

Both Begg's and Egger's test were used to evaluate the publication bias of the meta-analysis of OS. The result of Egger's test showed no publication bias ($P = 0.169$) while Begg's test indicated that publication bias might exist ($P = 0.012$). We used the trim-and-fill method to estimate the influence of potential publication bias. As a result, three theoretical studies were added in the meta-analysis of OS (Figure S6). The recalculated pooled result did not change significantly (HR = 0.51, 95% CI: 0.45–0.57, $P < 0.001$), indicating the stability of the result.

DISCUSSION

All 23 studies we analyzed met specific inclusion criteria and had moderate to high quality according to their NOS scores. Overall, 2,505 NSCLC patients were included and the survival data were organized based on overall survival (OS), disease-free survival (DFS) and progression-free survival (PFS). The combined results demonstrated that decreased expression of PTEN correlated with poor OS in NSCLC patients. What's more, we found that decreased expression of PTEN also indicated a poor prognosis for DFS and PFS in patients with NSCLC.

In our meta-analysis, the results from analyzing both the univariate and multivariate subgroups indicated that decreased expression of PTEN was associated with poor OS in patients with NSCLC. However, multivariate analysis ruled out the compounding effects from other clinicopathological factors such as sex, age, tumor size, nodal status and stage, among others [16, 19, 28, 32, 34]. Thus, according to the pooled result from analyzing the multivariate analysis subgroup, expression of PTEN may be considered to be an independent factor of poor prognosis in NSCLC patients. While decreased expression of PTEN protein correlated with poor OS in patients with NSCLC, decreased expression of PTEN mRNA did not. This is not surprising since mRNA levels from an expressed gene do not usually predict the corresponding protein levels [36, 37]. Immunohistochemistry is used as a complementary diagnostic method in 95% of cancer cases [38] because it can benefit surgical and therapeutic decisions at a low cost. Therefore, we recommend clinicians to use PTEN protein expression detected by immunohistochemistry as a prognostic factor to treat NSCLC patients.

Prior to our study, two meta-analyses had been performed to evaluate the association between PTEN expression and the survival of cancer patients. According to the combined results of nine original studies, Chen J. *et al.* concluded that reduced PTEN expression correlated with poor OS in patients with gastric cancer [39]. On the other hand, the meta-analysis of 14 original studies by Cai J. *et al.* found that the expression of PTEN had no

prognostic value for OS in patients with epithelial ovarian cancer [40]. However, in our current meta-analysis, the combined results from 20 original studies showed that decreased expression of PTEN was indeed associated with poor OS in NSCLC patients. These results suggest that the correlation between PTEN expression and OS in cancer patients may differ depending on cancer type.

Among our eligible studies, four [24, 25, 33, 34] reported statistics for DFS and three [22, 26, 30] for PFS. Most of the patients in such studies had undergone surgical resection. Yoo S.B. *et al.* [26] reported a cohort of 288 consecutive NSCLC patients who underwent surgical resection and further received post operative adjuvant chemotherapy and radiation therapy. Among them, some patients received additional EGFR-TKIs, such as gefitinib or erlotinib [26]. In addition, Lim W.T. *et al.* [30] also reported a cohort of NSCLC patients that had only been treated with gefitinib. Overall, our results combining data from both of the aforementioned studies revealed that decreased expression of PTEN was associated with a shorter DFS as well as a shorter PFS. This means that in addition to the prognostic value of decreased PTEN expression on OS, PTEN expression levels can also inform on the effect of treatment for patients with NSCLC.

Tumor suppression by PTEN depends on its negative regulation of the phosphatidylinositol 3-kinase-Akt-mammalian target of rapamycin (PI3K-Akt-mTOR) signaling pathway [41]. Thus, PTEN is regarded as the controller of this pathway [42]. Consequently, when the expression of PTEN is decreased, either inhibiting PI3K or controlling the PI3K-Akt-mTOR pathway in other ways can supplement PTEN's tumor suppression [43–46]. Therefore, we consider that NSCLC patients with decreased expression of PTEN suffer from a subtype of lung cancer and might benefit from individualized treatment plans.

Several limitations should be noticed in our meta-analysis. One of the main limitations is the potential publication bias, stemming from published results being predominantly positive, since all of our included studies were retrospectively designed. Furthermore, patient populations in our study were limited, as patients came only from Asia and North America. Additionally, some of the survival data we used were extracted from survival curves, which may introduce subjective bias. Finally, the studies reporting DFS and PFS were few in number. Therefore, further studies without these biases might strengthen our conclusions. Nonetheless, our

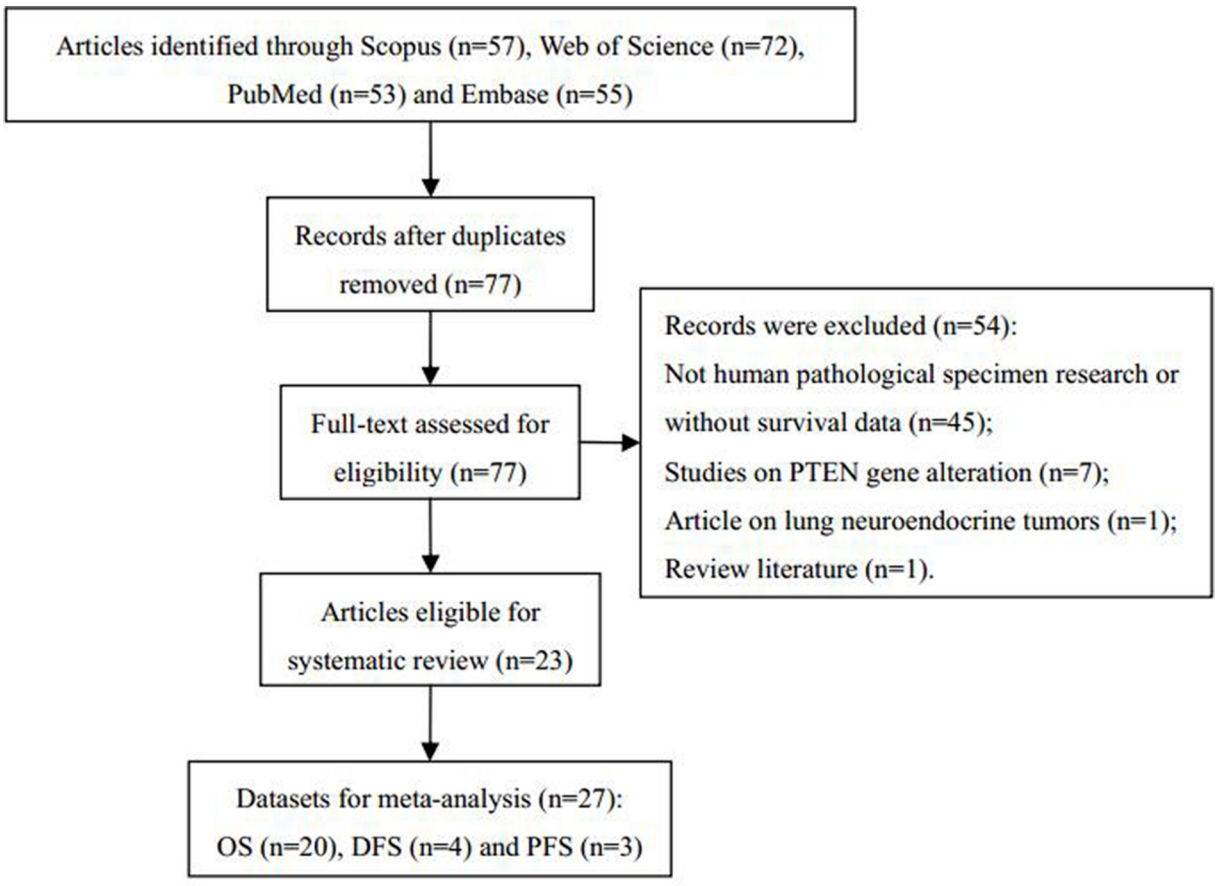

Figure 1: Flow diagram of the selection process in this meta-analysis. OS = overall survival; DFS = disease-free survival; PFS = progression-free survival.

Table 1: Main characteristics of eligible studies

First author	Year	Region	Cases	Expression	Method	Treatment	Outcome	Analysis	HR estimation	Follow-up time
Shen H [13]	2016	China	51	Protein	IHC	Surgery/ EGFR-TKI	OS	Univariate	Survival curves	The max follow-up time was 66.2 months
Wang J [14]	2015	China	92	Protein	IHC	Surgery	OS	Univariate	Survival curves	Median 28 months (range, 3 to 98 month)
Tang YA [15]	2015	Taiwan	133	Protein	IHC	Surgery	OS	Univariate	Survival curves	The max follow-up time was 126.8 months
Li XB [16]	2015	China	68	Protein	IHC	Surgery	OS	Multivariate	Reported data	Median 12.5 months (range, 3.6 to 40.6 months)
Ji Y [19]	2014	China	67	Protein	IHC	Surgery	OS	Multivariate	Reported data	The max follow-up time was 40 months
Shen H [20]	2014	China	46	Protein	IHC	Surgery/ EGFR-TKI	OS	Univariate	Survival curves	The max follow-up time was 66 months
Hu J [21]	2012	China	114	Protein	IHC	Surgery	OS	Univariate	Reported data	Median 40.10 months (range, 2.23 to 67.77 months)
Wang L [22]	2012	China	78	Protein	IHC	Surgery	PFS	Univariate	Survival curves	The max follow-up time was 24 months
An SJ [23]	2012	China	97	Protein	IHC	Surgery	OS	Univariate	Survival curves	Median 53.9 months
Shih MC [24]	2012	Taiwan	119	Protein	IHC	Surgery	OS	Univariate	Survival curves	The max follow-up time was 120 months
Shih MC [24]	2012	Taiwan	119	Protein	IHC	Surgery	DFS	Univariate	Survival curves	The max follow-up time was 120 months
Yanagawa N [25]	2012	Canada	152	Protein	IHC	Surgery	DFS	Multivariate	Reported data	Median 28.56 months (range, 0.84 to 71.64 months)
Yoo SB [26]	2011	Korea	288	Protein	IHC	Surgery/ Chemotherapy/ Radiation therapy/ EGFR-TKI	PFS	Multivariate	Reported data	Median 44 months (range, 1 to 84 months)
Cetin Z [27]	2010	Turkey	50	Protein	WB	Surgery	OS	Univariate	Survival curves	The max follow-up time was 34.2 months
Buckingham L [17]	2010	USA	132	Protein	IHC	Surgery	OS	Univariate	Survival curves	The max follow-up time was 60.2 months
Wang C [28]	2009	China	249	Protein	IHC	Surgery	OS	Multivariate	Reported data	The max follow-up time was 83 months
Inamura K [18]	2007	Japan	115	mRNA	PCR	Surgery	OS	Univariate	Survival curves	The max follow-up time was 109.7 months
Zheng H [29]	2007	Japan	143	Protein	IHC	NR	OS	Univariate	Survival curves	Mean 20.6 months (range, 1 to 144 months)
Lim WT [30]	2007	USA	25	Protein	IHC	Gefitinib	PFS	Univariate	Survival curves	The max follow-up time was 100.8 months
Lim WT [30]	2007	USA	25	Protein	IHC	Gefitinib	OS	Univariate	Survival curves	The max follow-up time was 100.8 months
Endoh H [31]	2006	Japan	78	mRNA	PCR	Surgery/ Chemotherapy/ Gefitinib	OS	Univariate	Reported data	Median 4 months (range, 0.8 to 31.3 months)
Tang JM [32]	2006	China	102	Protein	IHC	Surgery	OS	Multivariate	Reported data	Median 25.5 months (range, 3 to 60 months)
Ferraro B [33]	2005	USA	125	mRNA	PCR	Surgery	DFS	Univariate	Survival curves	Median 101 months (range, 39 to 161 months)
Ferraro B [33]	2005	USA	125	mRNA	PCR	Surgery	OS	Univariate	Survival curves	Median 101 months (range, 39 to 161 months)
Bepler G [34]	2004	USA	77	mRNA	PCR	Surgery	OS	Multivariate	Reported data	Median 39.7 months (range, 2.0 to 106.1 months)
Bepler G [34]	2004	USA	77	mRNA	PCR	Surgery	DFS	Univariate	Survival curves	Median 39.7 months (range, 2.0 to 106.1 months)
Goncharuk VN [35]	2004	USA	104	Protein	IHC	Surgery	OS	Univariate	Survival curves	Mean 52 months (range, 5 to 127 months)

IHC: Immunohistochemistry; WB: Western blot; PCR: Polymerase chain reaction; NR: No report; EGFR-TKI: Epidermal growth factor receptor-tyrosine kinase inhibitor; OS: Overall survival; DFS: disease-free survival; PFS: progression-free survival; HR: Hazard ratio.

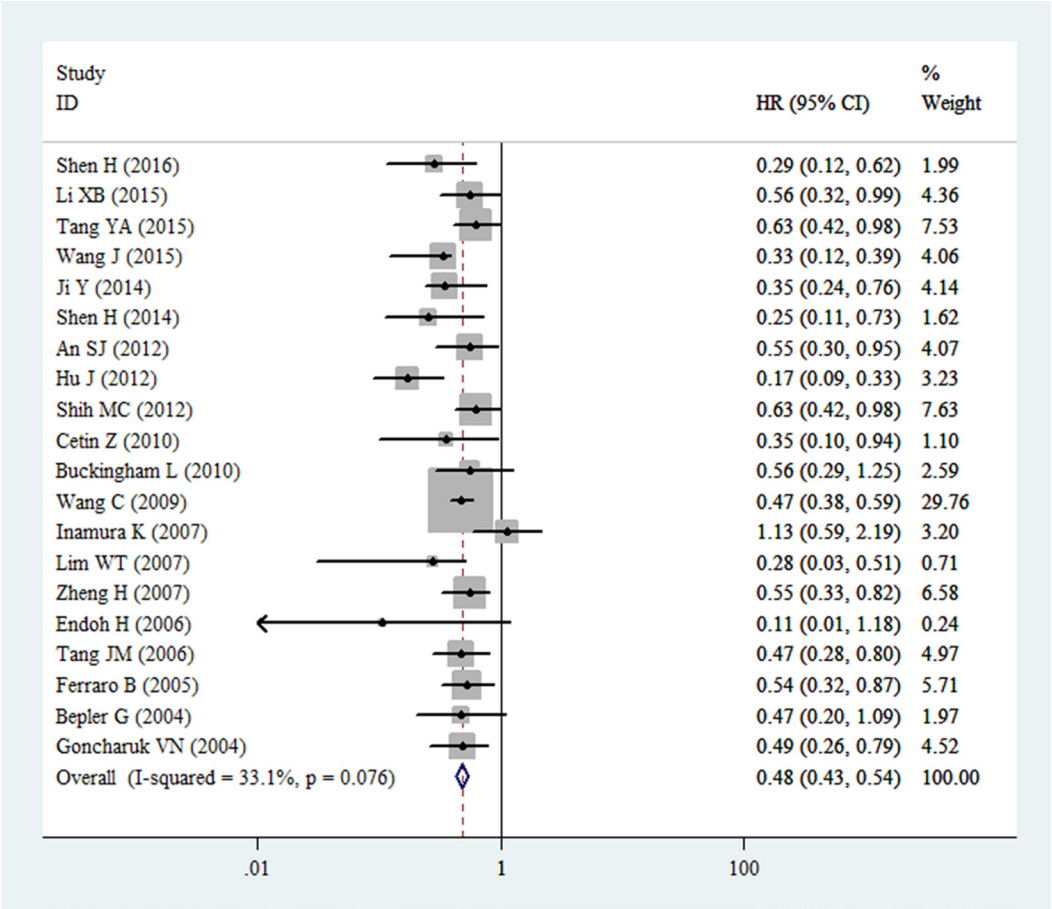

Figure 2: Forest plot for the relationships between decreased expression of PTEN and OS in patients with NSCLC. HR = hazard ratio; CI = confidence interval.

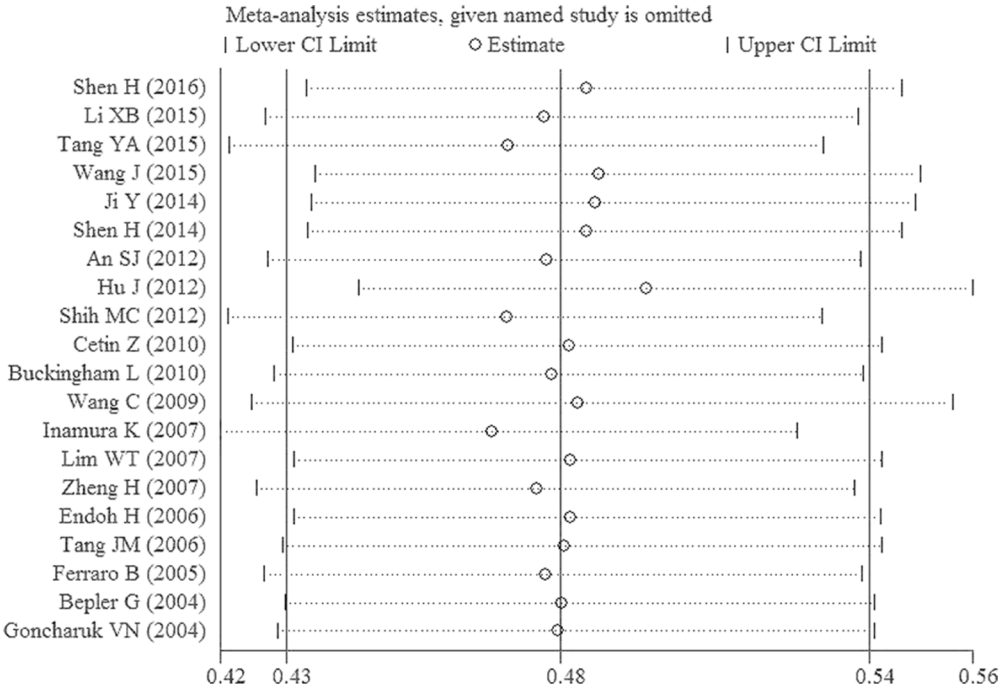

Figure 3: Sensitivity analysis on the relationship between decreased expression of PTEN and OS in patients with NSCLC. CI = confidence interval.

Table 2: Meta-analysis results for the association between decreased expression of PTEN and OS in patients with NSCLC

Categories	Subgroups	Number of datasets	HR (95% CI)	*P*-Value	Heterogeneity	
					I^2	*P*-Value
All [F]		20	0.483 (0.429–0.543)	< 0.001	33.1%	0.076
Expression [R]	Protein	16	0.456 (0.389–0.535)	< 0.001	24.9%	0.173
	mRNA	4	0.604 (0.340–1.070)	0.084	50.4%	0.110
Analysis [R]	Univariate	15	0.466 (0.368–0.589)	< 0.001	47.8%	0.020
	Multivariate	5	0.469 (0.393–0.558)	< 0.001	0.0%	0.852
Population [R]	Asian	15	0.461 (0.376–0.566)	< 0.001	49.0%	0.017
	Non Asian	5	0.502 (0.372–0.677)	< 0.001	0.0%	0.929
Cases [R]	Less than 100	10	0.396 (0.311–0.503)	< 0.001	0.0%	0.721
	More than 100	10	0.523 (0.421–0.649)	< 0.001	51.9%	0.028
Year [R]	After 2010	9	0.412 (0.307–0.554)	< 0.001	56.0%	0.020
	Before 2010	11	0.506 (0.436–0.589)	< 0.001	0.0%	0.514

[F] For fixed-effects model; [R] for random-effects model; HR: Hazard ratio; CI: Confidence intervals.

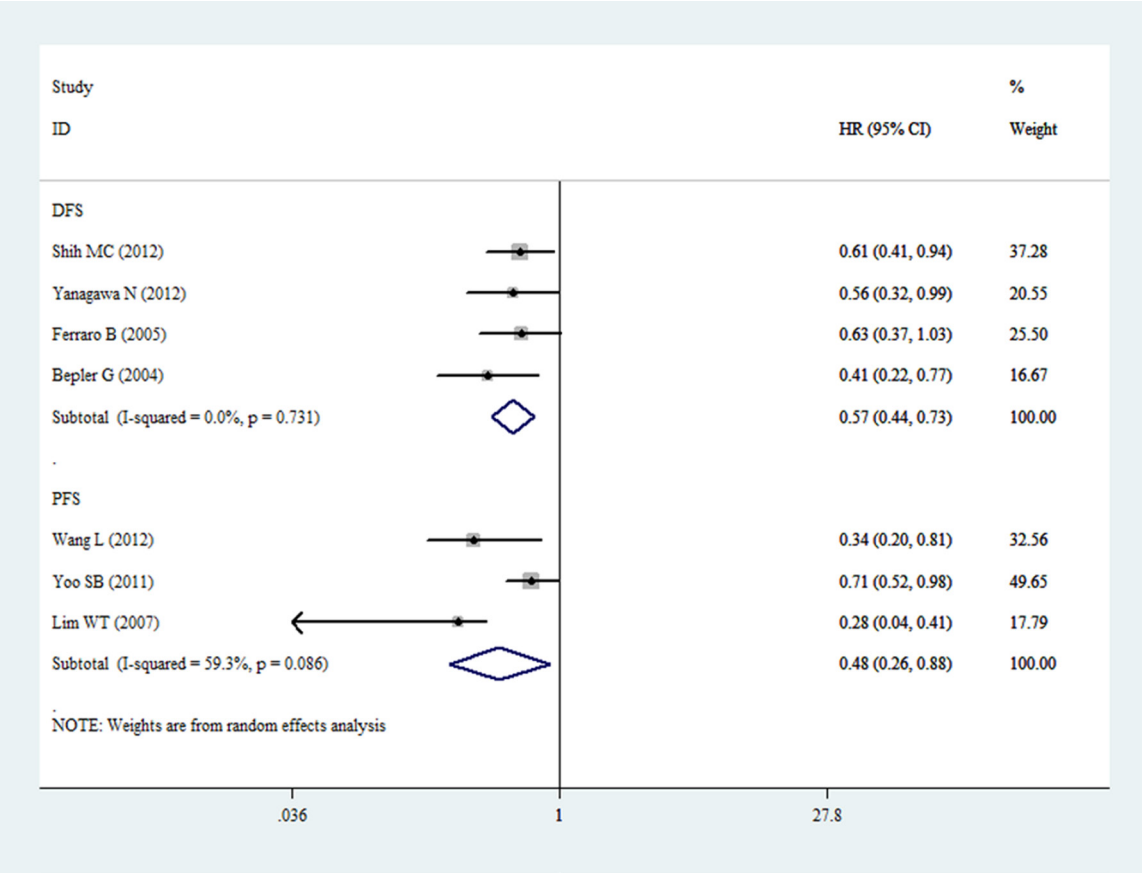

Figure 4: Forest plot for the relationships between decreased expression of PTEN and DFS/PFS in patients with NSCLC. DFS = disease-free survival; PFS = progression-free survival; HR = hazard ratio.

meta-analyses showed that decreased expression of PTEN predicted a shorter OS, DFS and PFS in the populations of patients with NSCLC analyzed.

MATERIALS AND METHODS

Search strategy

We systematically searched in the online Scopus, Web of Science, PubMed and Embase databases (updated until May 22, 2016) with the restrictions of English language and original article. Two investigators (Jian Xiao and Bi-Xiu He) independently screened all titles and abstracts to identify eligible studies. The search terms used included "PTEN", "NSCLC" and derivative terms (File S1). Manual searches of the included studies and published reviews were also conducted.

Study selection

In this meta-analysis, studies were selected according to the following criteria: (1) original studies measured the PTEN expression in patients with NSCLC; (2) reported the correlation between PTEN expression and patient survival; (3) reported the hazard ratios (HRs), and their corresponding 95% confidence intervals (CIs) could be obtained. Of note, we would include the most complete report if the same authors reported repeated results. However, unpublished studies, meeting abstracts, case reports, comments, letters, meta-analyses and literature reviews were excluded.

Data extraction

Two raters independently extracted the primary information using a standardized form and disagreements were discussed until a consensus was reached. Except for the HRs and their corresponding 95% CIs, the following information categories were also extracted: first author, year of publication, region of population, number of cases, PTEN expression, test method, survival outcome, analysis method, HR estimation, follow-up time and cut-off value. When both multivariate and univariate analyses of the survival results were reported, we extracted the HRs and their corresponding 95% CIs from multivariate analyses. However, when HR and its corresponding 95% CI were not reported as calculated data, they were estimated using the previously reported methods [47, 48], according to other relevant information (e.g., survival curves).

Quality assessment

The Newcastle-Ottawa Quality Assessment Scale (NOS) was used to assess the quality of the included studies and was conducted by two independent investigators. Disagreements were resolved by discussion. In brief, NOS is comprised of three parameters of quality: selection, comparability, and outcome assessment. Furthermore, each study received a total score between 0 and 9, with a NOS score of 7 or above considered as high quality and a NOS score of 3 or below considered as low quality [48–50]. Details of the quality assessment of included studies are provided in File S2 and Table S2.

Statistical analysis

We used Stata 12.0 (StataCorp LP) and R software (https://www.r-project.org/) to perform all statistical analyses. HRs and their corresponding 95% CIs were calculated for all of the survival outcomes. When the pooled HR was lower than 1, we considered that the decreased expression of PTEN was associated with unfavorable survival in patients with NSCLC. Heterogeneity analysis was conducted using Cochran's Q test and Higgins' I-squared statistic and Heterogeneity was defined either as $I^2 > 50\%$ or $P < 0.05$. A random-effects model was used when heterogeneity was present; otherwise, the fixed-effects model was used. The stability of the pooled HR results was assessed by the sensitivity analysis. Publication bias was evaluated using Begg's and Egger's tests. If publication bias existed, we applied the trim-and-fill method. For all of our results, $P < 0.05$ (two-tailed) was defined to be statistically significant.

CONFLICTS OF INTEREST

The authors declared no conflicts of interest.

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