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ORIGINAL RESEARCH

Podoplanin-positive cancer-associated fibroblasts predict poor prognosis in lung cancer patients

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Guoming Hu[1]
Kefang Zhong[1]
Wei Chen[1]
Shimin Wang[2]
Liming Huang[1]

[1]Department of General Surgery (Breast and Thyroid Surgery), [2]Department of Nephrology, Shaoxing People's Hospital (Shaoxing Hospital, Zhejiang University School of Medicine), Zhejiang, China

Background: Cancer-associated fibroblasts (CAFs) are a heterogeneous population, and different subpopulations play differential roles in tumor microenvironment. However, the prognostic role of podoplanin-positive CAFs in human lung cancer still remains controversial.
Methods: Herein, we performed a meta-analysis including 12 published studies with 1,802 patients identified from PubMed and EBSCO to assess the prognostic impact of podoplanin-positive CAFs in lung cancer patients.
Results: We found that podoplanin$^+$ fibroblast infiltration significantly decreased overall survival (OS), disease-free survival (DFS), and progression-free survival in patients. In stratified analyses, podoplanin$^+$ fibroblast infiltration was significantly associated with worse OS and DFS in both squamous cell carcinoma and adenocarcinoma of lung. In addition, high density of podoplanin-positive CAFs significantly correlated with unfavorable clinicopathological features such as lymph node metastasis, and lymphatic, vascular, and pleural invasion of patients.
Conclusion: Podoplanin$^+$ fibroblast infiltration leads to worse clinical outcome in lung cancer patients, implicating that it is a valuable prognostic biomarker and targeting it may have a potential for effective treatment.
Keywords: podoplanin-positive cancer-associated fibroblasts, worse outcome, lung cancer, meta-analysis

Introduction

Lung cancer is the leading cause of cancer-related death worldwide. Accumulating evidence has demonstrated that tumor-infiltrating fibroblasts (also called cancer-associated fibroblasts [CAFs]) were significantly associated with survival of lung cancer patients. However, CAFs are a heterogeneous population, and hence it is important to distinguish among different subpopulations as they may play differential roles in tumor microenvironment (TME).[1] Tumor-infiltrating podoplanin$^+$ fibroblasts, a new subset of CAFs identified recently, have been demonstrated to play specific and significant roles in human lung cancer.

Podoplanin, a well-conserved, mucin-type transmembrane protein, has exerted a variety of functions including regulation of organ development and cell motility.[2] Recent studies have indicated that podoplanin was often upregulated in CAFs in the tumor stroma.[3] Podoplanin$^+$ fibroblasts are often among the early immune cells recruited to tumor sites in response to the stimuli and increase in the TME. In the last decades, multitudinous studies have associated podoplanin-positive CAFs and prognosis in lung cancer patients, but their results were controversial.[4] Thus, it needs in-depth assessment, and furthermore, the potential of these cells as an effective prognostic biomarker and targeted therapy is necessary to be explored.

Correspondence: Guoming Hu; Liming Huang
Department of General Surgery (Breast and Thyroid Surgery), Shaoxing People's Hospital (Shaoxing Hospital, Zhejiang University School of Medicine), 568 Zhongxing North Road, Shaoxing, 312000, China
Tel +86 575 8861 6106
Fax +86 575 8831 1745
Email hgmplj@126.com; shaoxinghlm@126.com

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Herein, we performed this meta-analysis to clarify the association between podoplanin+ fibroblast infiltration and outcomes such as overall survival (OS), disease-free survival (DFS), and progression-free survival (PFS) in lung cancer patients, and thereby provided more evidence on the clinical value of podoplanin-positive CAFs as a prognostic biomarker for lung cancer.

Materials and methods

Search strategy

PubMed and EBSCO were searched for studies to evaluate the density of podoplanin-positive CAFs and survival in lung cancer patients from 1980 to April 15, 2018. The keywords adopted for search were (podoplanin [Title/Abstract] OR fibroblasts [Title/Abstract]) AND (lung [Title/Abstract] OR pulmonary [Title/Abstract]) AND (neoplasms [Title/Abstract] OR tumor [Title/Abstract] OR cancer [Title/Abstract] OR carcinoma [Title/Abstract]).

Inclusion and exclusion criteria

In this meta-analysis, the inclusion criteria were that studies included must have 1) been published as original articles; 2) investigated lung cancer patients; 3) detected podoplanin+ fibroblasts in primary tumor specimens with immunohistochemistry; 4) provided HRs with 95% CI, or Kaplan–Meier curves of podoplanin+ fibroblast density associated with OS, and/or DFS, and/or PFS; and 5) been published in English.

We excluded studies that were not published as research articles or were full texts such as commentary, case report, letters to editors, or conference abstracts. Studies that did not provide sufficient data to estimate HRs, or detected fibroblasts without using the marker "podoplanin", or exhibited metastatic infiltration were also excluded.

End points

In this meta-analysis, OS and DFS were recorded as the primary and PFS as secondary end points. Individual studies defined cut-offs for podoplanin+ fibroblast density and classified patients into high- and low-density groups.

Data extraction

The authors GH and KZ independently reviewed and extracted data such as first author's name, number of patients, median age, time of follow-up, method applied to quantify podoplanin+ fibroblasts, and cut-off value to determine high density of these cells. OS, DFS, PFS, and clinicopathological data including TNM stage, and lymphatic, vascular, and pleural invasion were extracted from the text, tables, or Kaplan–Meier curves.

Quality assessment

The studies included in this meta-analysis were cohort studies. Two independent authors assessed the quality of the included studies with Newcastle–Ottawa Scale (NOS),[5] and achieved consensus for each item with the help of third author. The studies with score 6 or more were recorded as high-quality studies.

Statistical analysis

We combined extracted data into meta-analyses with STATA 12.0 analysis software (Stata Corporation, College Station, TX, USA). Statistical heterogeneity was assessed with the chi-squared based *Q*-test or I^2.[6] Data were pooled based on the random-effect model in the presence of heterogeneity,[7] otherwise, the fixed-effect model was applied.[8] Sensitivity analysis, Begg's funnel plot, and Egger's test[9] were employed to investigate the influence of each study on the pooled results and potential publication bias, respectively. All *P*-values were two-sided and values less than 0.05 were considered to be statistically significant.

Result

Search results and description of studies

A total of 9,860 records were retrieved and the results are exhibited in Figure S1. We ultimately identified 12 studies including 1,802 lung cancer patients for the assessment of podoplanin-positive CAFs,[10–21] and then evaluated all these studies with the NOS. Characteristics of the included studies which satisfied the inclusion criteria and were suitable for data consolidation are shown in Tables 1 and S1.

Meta-analyses

Overall survival

The meta-analysis showed that the elevated density of podoplanin-positive CAFs was significantly associated with decreased OS (HR=1.66, 95% CI 1.20–2.30, P=0.002) in patients with lung cancer (Figure 1).

In stratified analyses by pathologic types of lung cancer, as shown in Figure 2, pooled results indicated that high density of podoplanin-positive CAFs was significantly associated with worse OS in lung adenocarcinoma (AC) (HR=1.81, 95% CI 1.29–2.53, P=0.001); Similar result was observed with regard to podoplanin-positive CAFs and OS in squamous cell carcinoma (SCC) of lung (HR=2.00, 95% CI 1.27–3.15, P=0.003), with little heterogeneity being observed (I^2=31.8%, P=0.231).

Table 1 Main characteristics of the included studies

Study	Year	Tumor type	No of patients	Male/ female	Median age (range) (years)	Cut-offs	Podoplanin+ fibroblast density: high/low	Tumor stage	Median follow-up date (months)	Survival	Quality score (NOS)
Nakasone et al[10]	2018	Lung adenocarcinoma	97	51/46	(40, 85)	≥10% of spindle cells in the stroma	40/57	I–III	NR	OS, DFS	6
Kubouchi et al[11]	2018	Stage IA lung adenocarcinoma	158	76/82	68.8±9.5	≥10% of spindle-shaped cells in the stroma	41/117	IA–IB	82.5 (8, 151)	OS, DFS	7
Yurugi et al[12]	2017	Squamous cell carcinoma of lung	126	115/11	73.9±8.25	≥10% of spindle-shaped cells in the stroma	41/85	I–IIIA	48.0 (1, 137)	OS, DFS	7
Koriyamai et al[13]	2015	Lung adenocarcinoma	87	54/33	64 (41, 78)	≥50% of spindle-shaped cells/0.0625 mm^2	30/57	I–IV	NR	OS, PFS	6
Takahashi et al[14]	2013	Neuroendocrine carcinomas of lung	115	98/17	68 (22, 86)	≥50% of spindle-shaped cells/0.0625 mm^2	47/68	I–IV	52.8	OS, DFS	8
Ono et al[15]	2013	Stage I lung squamous cell carcinoma	142	125/17	66 (58, 80)	≥50% of CAFs in the stroma	44/98	IA–IB	62.4	OS, DFS	7
Neri et al[16]	2012	Stage III lung adenocarcinoma	112	64/48	65.5 (41, 83)	≥10% of stromal fibroblasts/HPF	51/61	III	84	OS	7
Ito et al[17]	2012	Stage I lung adenocarcinoma	304	139/165	<65: 52%; ≥65: 48%	≥10% of spindle cells in the stroma/0.0625 mm^2	105/199	IA–IB	87 (5, 181)	DFS	7
Hoshino et al[18]	2011	Lung adenocarcinoma	112	54/58	NR	≥10% of spindle cells in the stroma/0.0625 mm^2	32/80	NR	≥120	OS, DFS	7
Kitano et al[19]	2010	Lung adenocarcinoma Squamous cell carcinoma of lung	157 109	182/84	65±9.7	≥10% of spindle cells in the stroma/0.0625 mm^2	21/79 31/30	I–IV	NR	OS OS	6
Kawase et al[20]	2008	Lung adenocarcinoma	177	86/91	<70: 70%; ≥70: 30%	≥10% of spindle cells in the stroma	54/123	I–IV	117.6	OS	8
Yoshida et al[21]	2015	Lung adenocarcinoma	106	63/43	(42, 85)	≥10% of spindle cells in the stroma	57/49	I–IV	NR	PFS	6

Notes: Values in parenthesis indicate the shortest and longest time to follow up.
Abbreviations: NOS, Newcastle–Ottawa Scale; NR, not reported; CAF, cancer-associated fibroblasts; HPF, high power field; OS, overall survival; DFS, disease-free survival; PFS, progression-free survival.

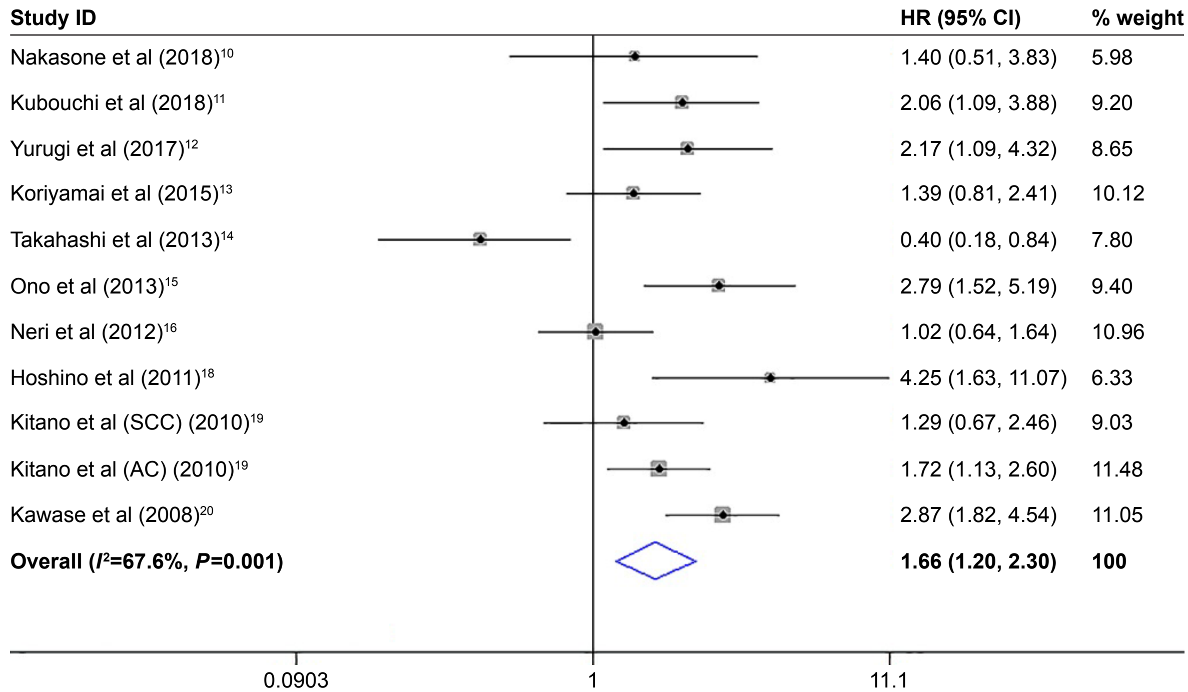

Figure 1 Forest plots describing HR of the association between podoplanin⁺ fibroblast infiltration and OS in lung cancer patients.
Note: Weights are from random-effects analysis.
Abbreviation: OS, overall survival.

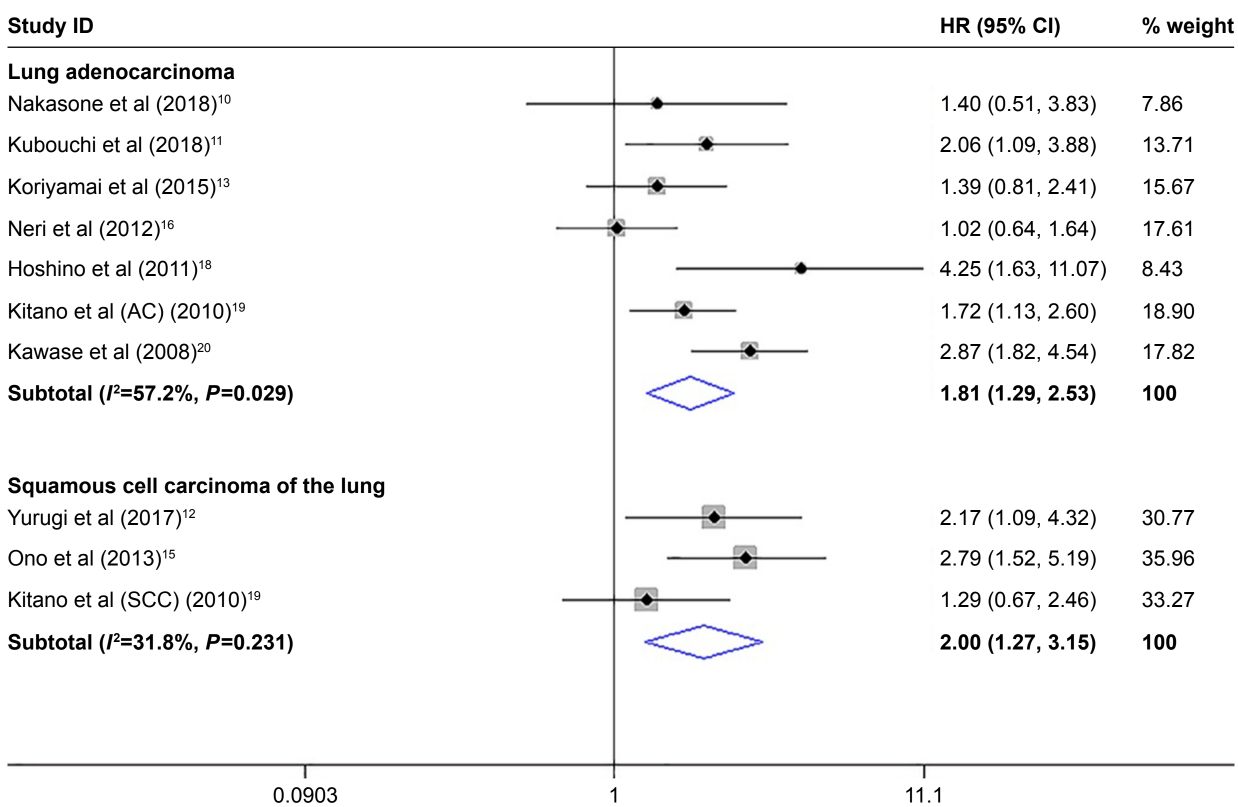

Figure 2 Stratified analyses describing HRs of the association between podoplanin⁺ fibroblast infiltration and OS.
Note: Weights are from random-effects analysis.
Abbreviation: OS, overall survival.

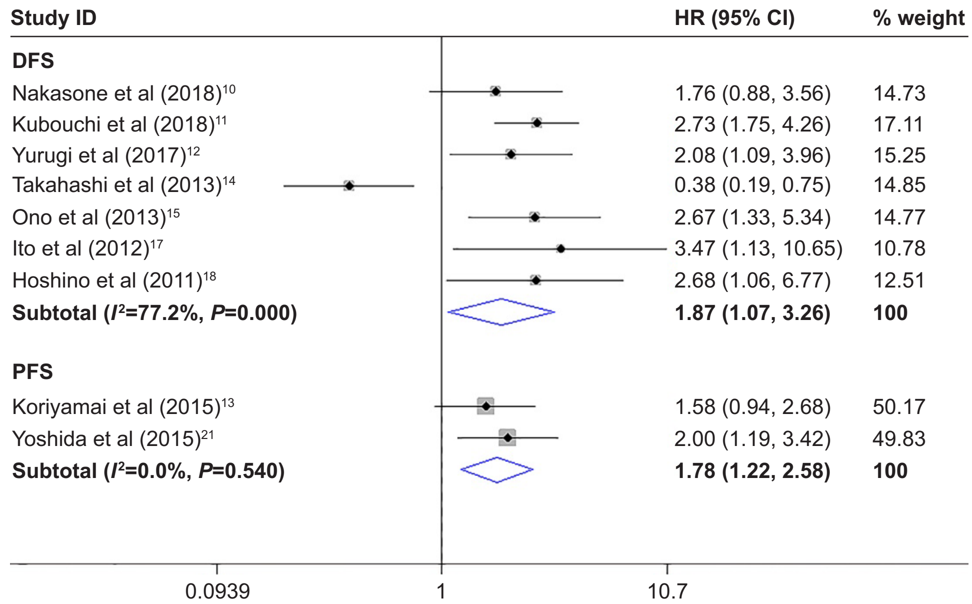

Figure 3 Forest plots describing HR of the association between podoplanin+ fibroblast infiltration and DFS and PFS in lung cancer patients.
Note: Weights are from random-effects analysis.
Abbreviations: DFS, disease-free survival; PFS, progression-free survival.

Disease-free survival and progression-free survival

Meta-analysis showed that podoplanin+ fibroblast infiltration was significantly associated with decreased DFS (HR=1.87, 95% CI 1.07–3.26, *P*=0.027) and PFS (HR=1.78, 95% CI 1.22–2.58, *P*=0.002) in lung cancer patients (Figure 3).

As for DFS, in stratified analyses by pathologic types, we found that increased density of podoplanin+ fibroblasts within tumor was significantly associated with worse DFS in lung AC (HR=2.52, 95% CI 1.81–3.51, *P*=0.000), with no heterogeneity existing among included studies (I^2=0.0%, *P*=0.689). Similar result was observed between podoplanin+ fibroblast infiltration and DFS in SCC of the lung (HR=2.33, 95% CI 1.45–3.74, *P*=0.000) (Figure S2).

We further investigated whether podoplanin-positive CAFs correlated with clinicopathological features such as lymph node metastasis and lymphatic invasion of lung cancer. We found that increased density of these cells was significantly associated with lymph node metastasis (OR=1.99, 95% CI 1.35–2.94, *P*=0.001); lymphatic (OR=2.10, 95% CI 1.06–4.13, *P*=0.032), vascular (OR=3.83, 95% CI 1.03–14.21, *P*=0.044), and pleural invasion (OR=2.19, 95% CI 1.03–4.64, *P*=0.041) (Figure 4); and also with tumor size (OR=0.46, 95% CI 0.32–0.66, *P*=0.000) and smoking (OR=2.44, 95% CI 1.39–4.27, *P*=0.002) status, but not with age (dichotomized according to an age of 70 years) (OR=0.81, 95% CI 0.46–1.42, *P*=0.463) or tumor differentiation (OR=0.24, 95% CI 0.01–4.15, *P*=0.324) of patients (Figure S3).

Sensitivity analysis

Sensitivity analysis indicated that each included study had no influence on the overall HR for OS or DFS (Figure S4).

Publication bias

There was no publication bias existing between podoplanin-positive CAFs and OS (*P*=0.876) or DFS (*P*=0.491) in patients by Funnel plot and Egger's test.

Discussion

Fibroblasts play a crucial role in maintaining the structural integrity of connective tissues by continuously secreting precursors of the extracellular matrix (ECM). In the past decades, although many studies have correlated podoplanin-positive CAFs with prognosis of lung cancer patients, their results were not consistent but rather controversial. In the present meta-analysis, we found that podoplanin+ fibroblast infiltration had a negative prognostic effect associated with survival in lung cancer, especially in AC and SCC of lung. In addition, increased density of podoplanin-positive CAFs was significantly associated with lymph node metastasis; lymphatic, vascular, and pleural invasion; tumor size, and smoking status. We believe that our study is the first to provide meaningful statistical evidence exhibiting the important prognostic value of podoplanin-positive CAFs as a cancer promoter in lung cancer patients.

We thought that the following reasons could possibly be responsible for the close association between increased

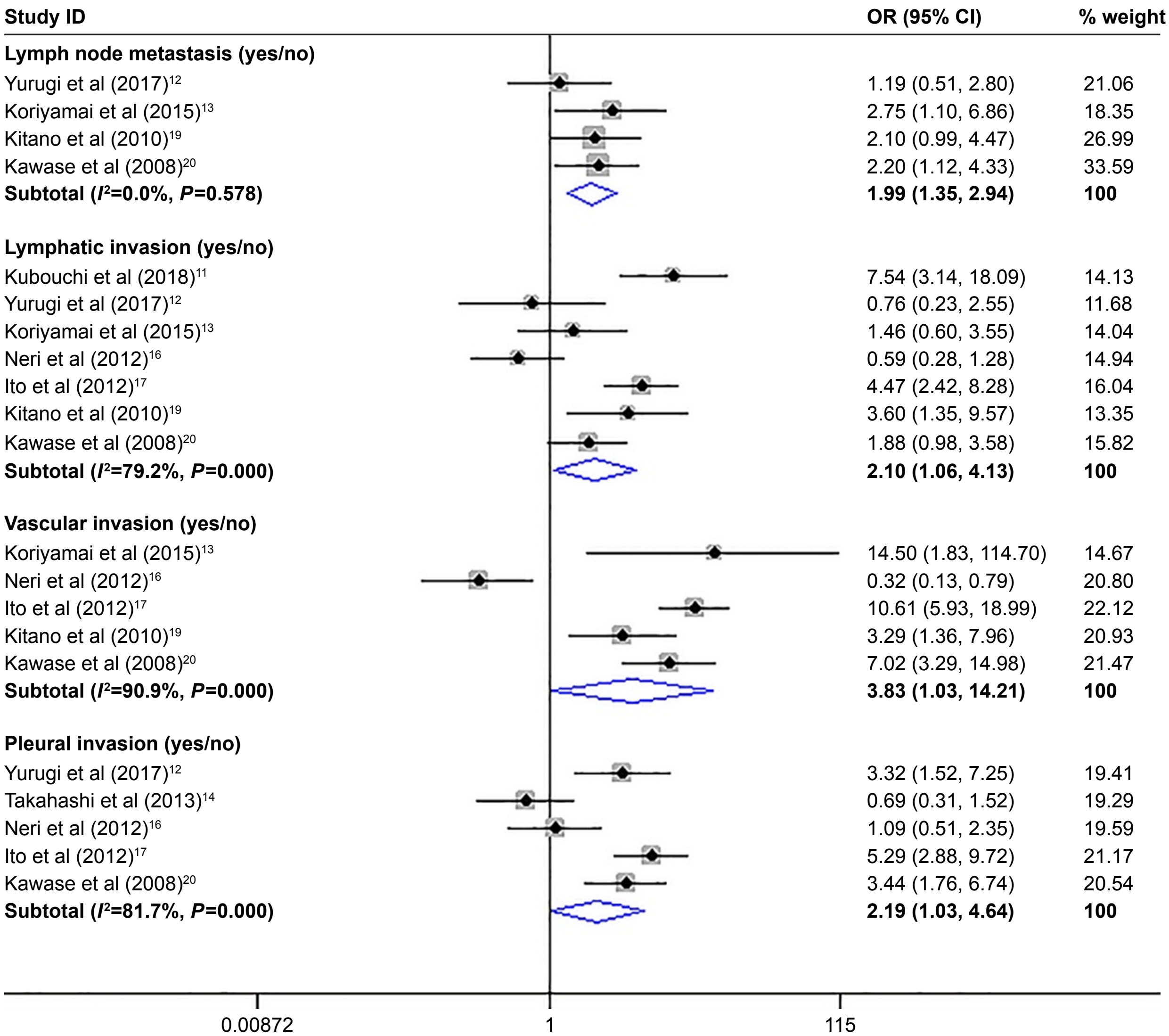

Figure 4 Forest plots indicating ORs of the association between podoplanin+ fibroblast infiltration and clinicopathological features such as lymph node metastasis of lung cancer.

Note: Weights are from random-effects analysis.

podoplanin-positive CAFs and decreased survival of patients identified in this study: Activated fibroblasts are able to promote tumor cell invasion, proliferation, and survival through releasing growth factors, cytokines,[22] and ECM-degrading proteases such as matrix metalloproteinases.[23] More importantly, podoplanin expressed in fibroblasts can enhance the ability of these cells to promote motility and survival of neighboring tumor cells through increased RhoA activity, especially in AC cells.[24] Podoplanin-positive CAFs can synthesize and release angiogenic factors including IL-8 and TNF-α as well as VEGF which promote neoangiogenesis, thereby facilitating tumor growth.[25] In addition, they can also produce varied amounts of immunosuppressive cytokines such as TGF-β1, IL-6, and IL-10 to inhibit antitumor immunity mediated by effector T cells,[25] recruit tumor-associated macrophages via CCL2 secretion, and decrease the activation of effector T cells through their acquisition of adhesion molecules such as intercellular adhesion molecule–1 (ICAM-1),[26] and thereby establishing immunosuppressive microenvironment. Thus, it is reasonable to conclude that the podoplanin-positive CAFs are able to promote tumor progression, thus decreasing survival. However, one included study reported that the presence of podoplanin-positive CAFs within tumor predicted favorable prognosis in high-grade neuroendocrine carcinomas,[14] suggesting that these cells might possess antitumor property. However, further investigation is needed to validate such result.

Previous studies have demonstrated that many cancer types are rich in CAFs, such as pancreatic cancer, and can facilitate a desmoplastic TME, hindering antitumor agents from infiltrating into tumor and thereby dampening treatment efficacy to a greater extent.[27] Researchers have developed several therapeutic strategies to target fibroblasts such as nano-delivery of fraxinellone and nanoparticle-mediated trapping of Wnt family member 5A to remodel the TME in preclinical studies, yielding somewhat promising results.[28,29] We think our finding may provide a new strategy for effective lung cancer treatment.

There were some limitations in this study. First, morphometric analyses for podoplanin-positive CAFs used in individual included studies were not consistent. Second, there was only one study reporting the relevant data for OS in neuroendocrine carcinomas of the lung; therefore, we could not get a combined result for it. Finally, studies with negative results may not be published, which can cause potential publication bias.

In conclusion, increased density of podoplanin-positive CAFs leads to an unfavorable clinical outcome in lung cancer patients, implicating that it is a valuable prognostic biomarker and targeting it may have a potential for effective treatment.

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Author contributions

GH conceived the study, participated in its design, extracted data, performed the statistical analysis, and drafted the manuscript. KZ participated in data extraction. WC and SW participated in the statistical analysis. LH participated in the design of the study. All authors contributed to the paper revision, agreed to be accountable for all aspects of the work and approved the final version. Guoming Hu and Kefang Zhong are co-first authors.

Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

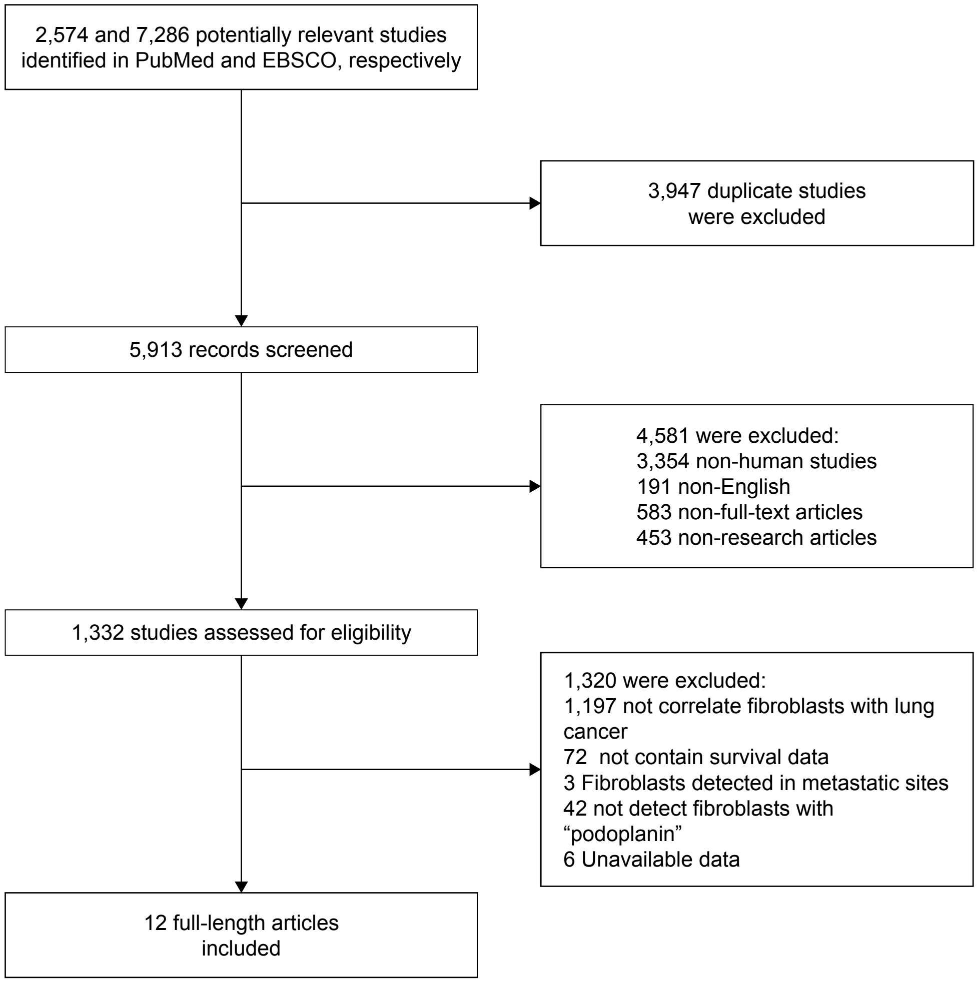

Figure S1 Flowchart diagram of study selection.

Table S1 Characteristics of the included studies for OR analysis of clinicopathological features

Study	Year	Tumor type	No of patients	Age (<70/≥70 years)	Podoplanin+ fibroblast density: high/low	Lymph node metastasis (yes/no)	Lymphatic invasion (yes/no)	Vascular invasion (yes/no)	Pleural invasion (yes/no)	Tumor size (≤3/>3 cm)	Tumor differentiation (well-moderate/poor)	Smoking (yes/no)
Kubouchi et al[2]	2018	Stage IA lung adenocarcinoma	158	H: (17/24); L: (55/62)	41/117	NR	H: (18/23); L: (11/106)	NR	NR	NR	H: (29/39); L: (109/8)	H: (30/11); L: (42/75)
Yurugi et al[3]	2017	Squamous cell carcinoma of lung	126	NR	41/85	H: (11/30); L: (20/65)	H: (4/15); L: (22/63)	NR	H: (22/19); L: (22/63)	H: (14/27); L: (42/43)	NR	H: (39/2); L: (82/3)
Koriyamai et al[4]	2015	Lung adenocarcinoma	87	NR	30/57	H: (19/11); L: (22/35)	H: (16/14); L: (25/32)	H: (29/1); L: (38/19)	NR	NR	NR	NR
Takahashi et al[5]	2013	Neuroendocrine carcinomas oflung	115	H: (20/27); L: (41/27)	47/68	NR	NR	NR	H: (14/33); L: (26/42)	NR	NR	NR
Neri et al[7]	2012	Stage III lung adenocarcinoma	112	NR	51/61	NR	H: (28/23); L: (41/20)	H: (33/18); L: (52/9)	H: (32/19); L: (37/24)	NR	H: (34/14); L: (43/18)	H: (32/19); L: (35/26)
Nakasone et al[1]	2018	Lung adenocarcinoma	97	NR	40/57	NR	NR	NR	NR	NR	NR	H: (29/11); L: (25/32)
Ito et al[8]	2012	Stage I lung adenocarcinoma	304	NR	105/199	NR	H: (35/70); L: (20/179)	H: (61/44); L: (23/176)	H: (39/66); L: (20/179)	H: (68/37); L: (161/38)	NR	NR
Kitano et al[10]	2010	Lung cancer	266	NR	92/174	H: (41/51); L: (64/110)	H: (38/36); L: (78/75)	H: (36/36); L: (46/104)	NR	NR	NR	NR
Kawase et al[11]	2008	Lung adenocarcinoma	177	H: (40/14); L: (84/39)	54/123	H: (23/31); L: (31/92)	H: (29/25); L: (47/76)	H: (43/11); L: (44/79)	H: (29/25); L: (31/92)	H: (23/31); L: (77/46)	NR	H: (35/19); L: (50/73)

Abbreviations: NR, not reported; H, high; L, low.

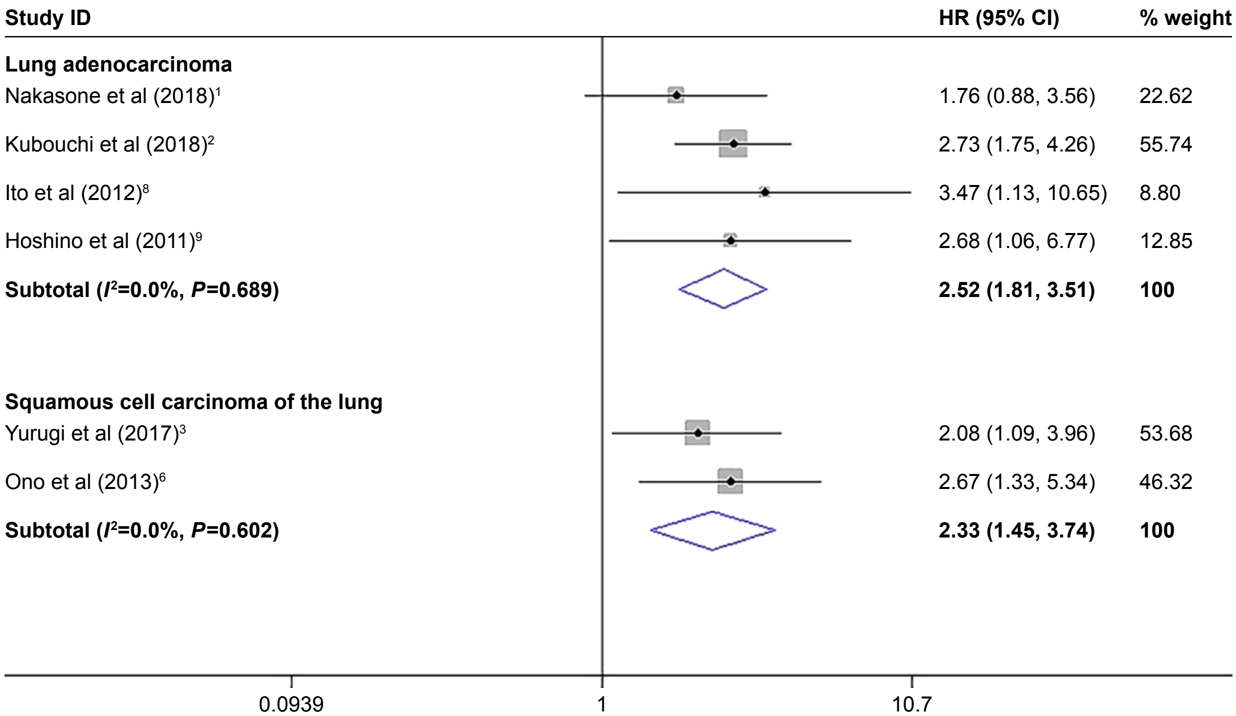

Figure S2 Stratified analyses describing HRs of the association between podoplanin⁺ fibroblast infiltration and DFS.
Abbreviation: DFS, disease-free survival.

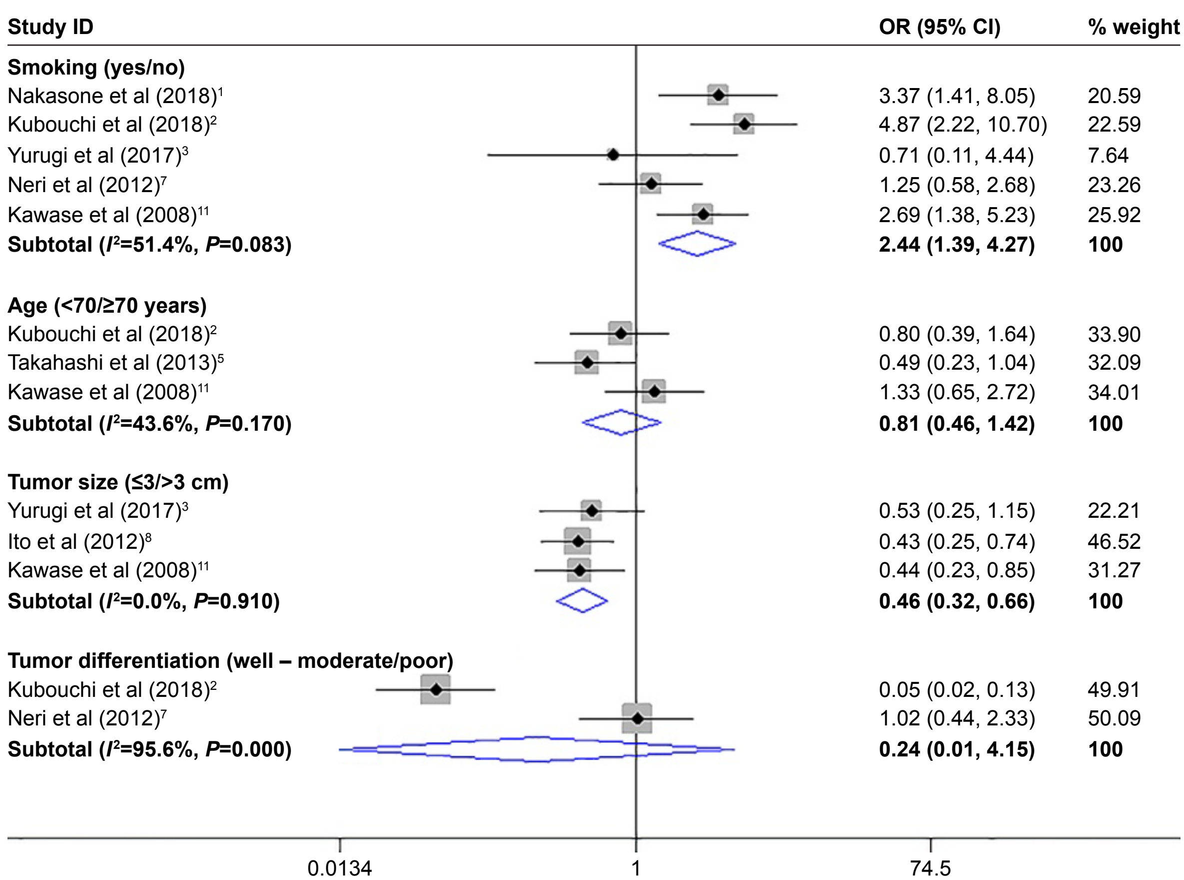

Figure S3 Forest plots indicating ORs of the association between podoplanin⁺ fibroblast infiltration and other clinicopathological features such as tumor size.
Note: Weights are from random-effects analysis.

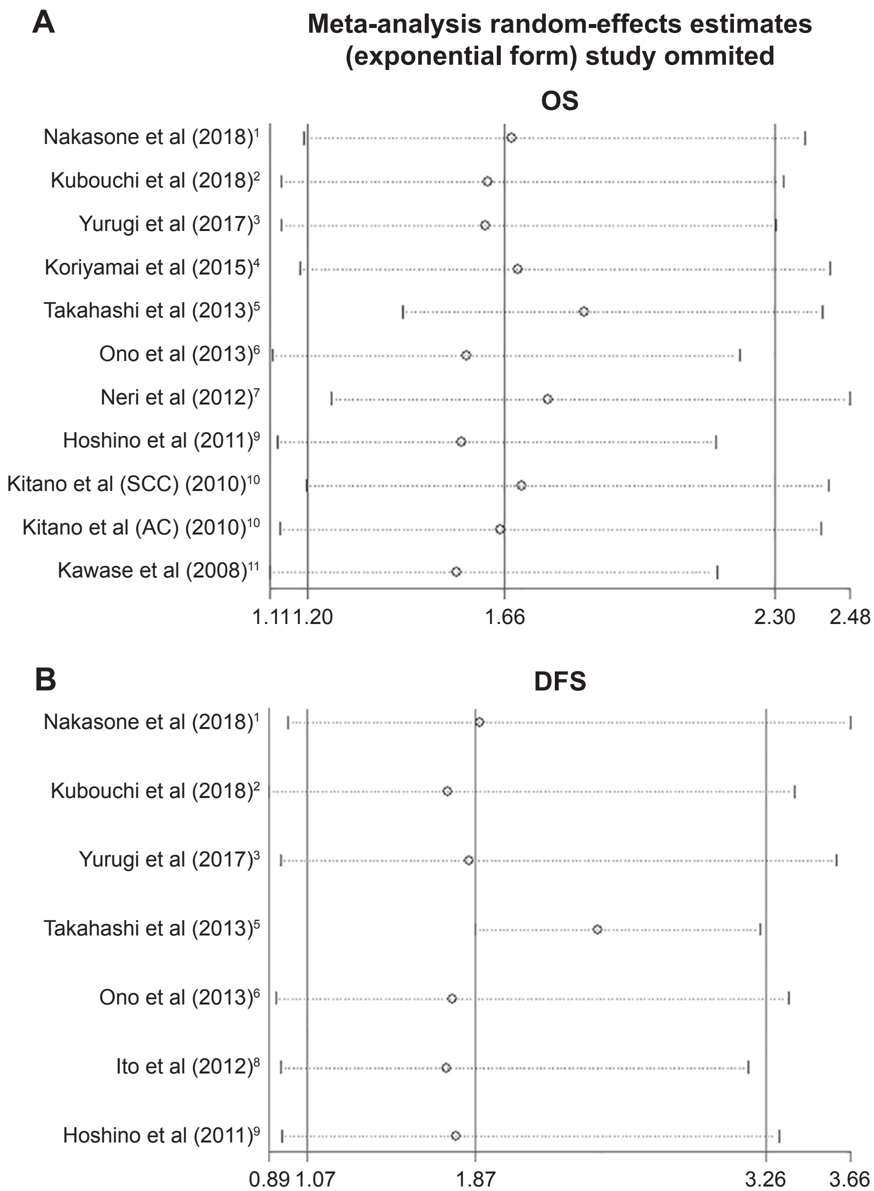

Figure S4 Plots describing the influence of individual studies on the overall HRs for OS (**A**) and DFS (**B**) in lung cancer patients.
Abbreviations: DFS, disease-free survival; OS, overall survival.

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