Prognostic value of programmed cell death ligand 1 (PD-L1) for hepatocellular carcinoma: a meta-analysis

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The prognostic role of programmed death ligand-1 (PD-L1) expression in hepatocellular carcinoma (HCC) has been widely studied but the results are controversial. In this comprehensive meta-analysis, we elucidated the clinical value of PD-L1 in HCC. Relevant studies were systematically searched in the Cochrane Library, EMBASE, and PubMed until June 27, 2019. Eligible studies were validated for the prognostic effect of PD-L1 on the overall survival (OS), disease-free survival (DFS), and relapse-free survival (RFS) in HCC using a hazard ratio (HR) and its 95% confidence interval (95% CI). Twenty-three studies with 3529 patients were involved in this meta-analysis. The pooled results revealed that high membrane-bound PD-L1 (mPD-L1) expression was associated with poor OS (HR: 1.42; 95% CI: 1.12–1.80; \(P = 0.004\)) and had no significant correlation with RFS (HR: 1.14; 95% CI: 0.85–1.54; \(P = 0.39\)), and DFS (HR: 1.36; 95% CI: 0.81–2.28; \(P = 0.25\)). The results also indicated that high soluble PD-L1 (sPD-L1) levels were associated with worse OS (HR: 2.93; 95% CI: 2.20–3.91; \(P < 0.00001\)). In addition, high mPD-L1 expression was associated with high alpha-fetoprotein levels (AFP; OR = 1.46; 95% CI: 1.16–1.84; \(P = 0.001\)), hepatitis (OR = 0.72; 95% CI: 0.54–0.98; \(P = 0.03\)), poor tumor differentiation (OR = 0.68; 95% CI: 0.55–0.84; \(P = 0.03\)), and tumor-infiltrating lymphocytes (OR = 3.39; 95% CI: 1.06–10.91; \(P = 0.04\)). The mPD-L1 expression had no significant correlation with age, number of tumors, gender, tumor size, liver cirrhosis, vascular invasion, tumor encapsulation, or TNM stage. The study revealed that high mPD-L1 expression in the tumor tissue and high sPD-L1 levels were associated with shorter OS in HCC. Moreover, overexpression of mPD-L1 was significantly associated with poor tumor differentiation, hepatitis, AFP elevation, and tumor-infiltrating lymphocytes.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide and ranks as the second most prevalent cause of cancer-related mortality [1]. About one-third of the patients with an early stage of HCC receive curative treatment such as surgical resection, liver transplantation, and ablation, while the remaining patients with advanced HCC receive palliative treatment. Viral infection is one of the main causes of liver cirrhosis and HCC [1]. After a chronic viral infection, persistent innate and adaptive immune responses lead to immune tolerance and tumor formation. The programmed cell death-1 (PD-1)/programmed cell death ligand 1 (PD-L1) pathway plays a critical role in the development of chronic infection, in escaping from tumor immune response, and tumor microenvironment formation [2]. PD-1 is a member of the CD28 family and has two ligands PD-1 (PD-L1) and PD-L2 (also known as B7-H1 and B7-DC), which have the characteristics of costimulatory molecules [3]. In cell-mediated immunity, T-cell receptors are activated to recognize the tumor antigen expressed by tumor cell MHC molecules,
and perforin and granzyme are released to attack tumor cells. At the same time, activated T cells release IFN-γ and other cytokines. Under the action of these cytokines, cancer cells up-regulate the expression of PD-L1, making it combine with PD-1 on T cells, weakening the attack of T cells. Tumor cells use a variety of immunosuppressive methods to resist tumor immunity, one of them is the PD-1/PD-L1 axis also known as the ‘immune checkpoint’ [4]. After PD-L1 combines with PD-1, it activates the PD-1/PD-L1 pathway that reduces the anti-tumor activity of T cells and induces T-cell apoptosis. This could be one of many mechanisms responsible for immune tolerance. PD-1 / PD-L1 pathway molecules include membrane-bound PD-1 / PD-L1 (mPD-1/ mPD-L1) and soluble PD-1 / PD-L1 (sPD-1 / sPD-L1). All these molecules play an important immunosuppressive role in tumor T-cell immune response [5–7]. However, their specific roles in tumor microenvironment are different. The role of sPD-L1 in tumor immunity needs further evaluation. It has been found that sPD-L1 could have other sources besides hydrolysis of mPD-L1 [8]. Recently, many studies have found that PD-L1 is related to tumor immunity and overexpression in various solid tumors such as colorectal cancer [9,10], pancreatic cancer [11], lung cancer [12], and melanoma [13]. Similarly, a number of studies have been carried on to examine the association between PD-L1 expression and prognosis of HCC; however, the results have been conflicting [14–17]. In addition, the authors of previous meta-analyses simply taken mPD-L1 and sPD-L1 as one indicator and not studied them separately. The present study aimed to pool the data of various studies to determine the association between PD-L1 expression (sPD-L1 and mPD-L1 separately) and the clinicopathological features and prognosis of HCC.

Materials and methods

Search strategy
We searched the Cochrane Library, EMBASE, and PubMed from its inception until June 27, 2019, using the keywords ('hepatocellular carcinoma' OR 'hepatocarcinoma' OR 'hepatomas' OR 'liver carcinoma' OR 'liver cell carcinoma' OR 'HCC' OR 'liver cancer') AND ('programmed cell death-ligand 1' OR 'PD-L1' OR 'B7-H1').

Selection criteria
The studies satisfying the following criteria were included: (1) all patients were pathologically confirmed to have HCC; (2) tests showed expression of PD-L1 in tumor tissue soluble PD-L1 levels; (3) enough data were available to analyze the relationship between PD-L1 expression, and clinicopathological characteristics and prognosis; and (4) the manuscript should be originally published in English. The exclusion criteria are as follows: (1) case report, review, meta-analysis, and ongoing research; (2) animal experiments on HCC and research involving other tumors; (3) evidence insufficient to show the relationship between PD-L1 expression and survival prognosis; and (4) the samples tested are non-tumor tissues.

Data extraction and quality assessment
Two authors (X.S.L. and J.W.L.) independently extracted the first author's name, publication year, country, clinicopathological characteristics, PD-L1 (test specimen, test method, cut-off value, number of cases with positive expression of PD-L1), follow-up time, OS, DFS, and RFS (HR and 95% CI). According to the Newcastle–Ottawa quality assessment (NOS), two authors independently evaluated the quality of the eligible articles. NOS includes the following three quality parameters: selection, comparability, and the exposure. Any differences between the researchers were resolved through discussion and analysis.

Statistical methods
All data in this meta-analysis were analyzed when using Revman 5.3 software (Revman, Cochrane Collaboration) and Stata 13.0 software (station, College Station), and P value < 0.05 was considered to be statistically significant. Heterogeneity between studies was evaluated by using the Cochrane Q-test and P-value. The data were said to be heterogenous if the chi-square P-value was less than 0.1 or $I^2$ statistics was greater than 50%. If the heterogeneity was significant, random-effect model was adopted; or else, used the fixed-effect model. The potential publication bias was evaluated by a Begg’s funnel chart ($P < 0.05$). A sensitivity analysis was used to assess the source of heterogeneity in the pooled analysis by omitting one study at a time.
Figure 1. Flow diagram showing the study selection process followed in this meta-analysis

Results

Study characteristics

On initial screening, 689 studies were identified from three databases. After excluding 202 duplicate records, 487 studies were screened for titles and abstracts, and 33 relevant articles were screened for full texts. After a detailed study, 10 studies were excluded due to the following reasons: conference abstracts ($n=6$); liver transplantation ($n=1$); insufficient patients ($n=1$); cholangiocarcinoma ($n=1$); and the peritumoral liver tissue was tested ($n=1$). Finally, 23 articles were included with a NOS score greater than 6 (Figure 1). Eighteen studies determined the mPD-L1 expression in tumor tissues. Among them, 16 studies analyzed the relationship between mPD-L1 expression and OS [14–29], seven studies analyzed the relationship between mPD-L1 expression and DFS [14, 21, 23, 25, 26, 30, 31], and six studies analyzed the relationship between mPD-L1 expression and RFS [15, 19, 20, 22, 27, 28]. Besides, only three studies were conducted in Western countries [20, 24, 31]. The remaining 16 studies were conducted in Asia, of which 12 studies were from China [14, 16–19, 21, 22, 25–29]. In particular, Dai et al. [26] analyzed data from two independent groups, and both of them were included in this meta-analysis. There were five studies analyzing the relationship between the soluble PD-L1 levels and OS [32–36]. Studies didn’t report HR and 95% CIs directly. We used Kaplan–Meier curve to calculate them. Detailed clinicopathological data are shown in Tables 1 and 2.

Survival analysis

The pooled data of 17 studies indicated that overexpression of mPD-L1 was associated with shorter OS (HR: 1.42; 95% CI: 1.12–1.80; $P = 0.004$) with significant heterogeneity ($P < 0.00001$; $I^2 = 77$%; Figure 2A). However, the pooled HR found no significant association between mPD-L1 expression and DFS (HR: 1.36; 95% CI: 0.81–2.28; $P = 0.25$) with heterogeneity ($P < 0.00001$; $I^2 = 82$%; Figure 2B). Six studies with RFS data demonstrated no significant relationship between the expression of mPD-L1 and RFS (HR: 1.14; 95% CI: 0.85–1.54; $P = 0.39$) with significant heterogeneity ($P = 0.004$; $I^2 = 71$%; Figure 2C). The soluble PD-L1 levels was associated with significantly shorter OS (HR: 2.93; 95% CI: 2.20–3.91; $P < 0.000001$) without significant heterogeneity ($P = 0.29$; $I^2 = 19$%; Figure 2D).

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Figure 2. Meta-analysis of PD-L1 and prognosis in HCC

(A) Forest plot between mPD-L1 and overall survival (OS).
(B) Forest plot between mPD-L1 and disease-free survival (DFS).
(C) Forest plot between mPD-L1 and relapse-free survival (RFS).
(D) Forest plot between the soluble PD-L1 levels and overall survival (OS).

| Study or Subgroup | log[Hazard Ratio] | SE | Weight | IV, Random, 95% CI | Year |
|-------------------|------------------|----|--------|-------------------|------|
| Wu 2009           | 0.95             | 0.48| 4.1%   | 2.59 [1.01, 6.22] | 2009 |
| Gao 2009          | -0.49            | 0.22| 8.2%   | 1.62 [1.05, 2.49] | 2009 |
| Umemoto 2014      | 0.68             | 0.41| 4.9%   | 1.93 [0.87, 4.22] | 2014 |
| Kan 2016          | -0.76            | 0.24| 7.0%   | 0.47 [0.29, 0.76] | 2015 |
| Chen 2016         | -0.06            | 0.21| 8.4%   | 0.94 [0.62, 1.42] | 2016 |
| Gabrielson 2016   | -1.47            | 1.29| 0.8%   | 0.23 [0.02, 2.88] | 2016 |
| Xie 2016          | -0.63            | 0.64| 2.7%   | 0.53 [0.15, 1.87] | 2016 |
| Semaan 2017       | 1.04             | 0.48| 4.1%   | 2.83 [1.10, 7.25] | 2017 |
| Jung 2017         | 1.64             | 0.34| 5.9%   | 5.16 [2.65, 10.04]| 2017 |
| Huang 2017        | 0.11             | 0.08| 10.7%  | 1.12 [0.95, 1.31] | 2017 |
| Liu 2019          | 0.19             | 0.05| 11.0%  | 1.17 [1.08, 1.29] | 2019 |
| Zhang 2018        | 1.06             | 0.44| 4.5%   | 2.89 [1.22, 6.94] | 2018 |
| Chang 2019        | -0.27            | 0.41| 4.9%   | 0.76 [0.34, 1.71] | 2018 |
| Dai 2018          | 0.91             | 0.28| 7.0%   | 2.48 [1.44, 4.30] | 2018 |
| Dai* 2018         | 0.86             | 0.29| 6.9%   | 2.36 [1.34, 4.17] | 2018 |
| Hu 2018           | 0.66             | 0.38| 5.3%   | 1.93 [0.92, 4.07] | 2018 |
| Pei 2019          | -0.92            | 0.6  | 3.0%  | 0.40 [0.12, 1.29] | 2019 |

Total (95% CI) 100.0% 1.42 [1.12, 1.80]  
Heterogeneity: Tau² = 0.13; Chi² = 70.69, df = 16 (P < 0.00001), I² = 77%  
Test for overall effect: Z = 2.90 (P = 0.004)

| Study or Subgroup | log[Hazard Ratio] | SE | Weight | IV, Random, 95% CI | Year |
|-------------------|------------------|----|--------|-------------------|------|
| Gao 2009          | 0.58             | 0.21| 14.4%  | 1.79 [1.18, 2.70] | 2009 |
| Xie 2016          | -0.42            | 0.4 | 11.6%  | 0.66 [0.30, 1.44] | 2016 |
| Sideras 2017      | -1.2             | 0.44| 11.0%  | 0.30 [0.13, 0.71] | 2017 |
| Jung 2017         | 1.32             | 0.48| 10.4%  | 3.74 [1.46, 9.58] | 2017 |
| Chang 2017        | 0.74             | 0.37| 12.1%  | 2.10 [1.01, 4.33] | 2017 |
| Chang 2018        | -0.43            | 0.26| 13.7%  | 0.65 [0.38, 1.06] | 2018 |
| Dai* 2018         | 0.86             | 0.29| 13.2%  | 2.36 [1.34, 4.17] | 2018 |
| Dai 2018          | 0.91             | 0.28| 13.4%  | 2.48 [1.44, 4.30] | 2018 |

Total (95% CI) 100.0% 1.36 [0.81, 2.28]  
Heterogeneity: Tau² = 0.44; Chi² = 38.59, df = 7 (P < 0.00001), I² = 82%  
Test for overall effect: Z = 1.16 (P = 0.25)

| Study or Subgroup | log[Hazard Ratio] | SE | Weight | IV, Random, 95% CI | Year |
|-------------------|------------------|----|--------|-------------------|------|
| Umemoto 2014      | 0.44             | 0.32| 12.5%  | 1.55 [0.83, 2.91] | 2014 |
| Gabrielson 2016   | -1.14            | 0.51| 6.8%   | 0.32 [0.12, 0.87] | 2016 |
| Chen 2016         | -0.22            | 0.19| 20.0%  | 0.80 [0.56, 1.14] | 2016 |
| Huang 2017        | 0.09             | 0.07| 26.1%  | 1.09 [0.95, 1.26] | 2017 |
| Hu 2018           | 0.77             | 0.29| 12.9%  | 2.16 [1.22, 3.81] | 2018 |
| Liu 2018          | 0.33             | 0.17| 20.6%  | 1.39 [1.00, 1.94] | 2018 |

Total (95% CI) 100.0% 1.14 [0.85, 1.54]  
Heterogeneity: Tau² = 0.08; Chi² = 17.37, df = 5 (P = 0.004), I² = 71%  
Test for overall effect: Z = 0.86 (P = 0.39)

| Study or Subgroup | log[Hazard Ratio] | SE | Weight | IV, Fixed, 95% CI | Year |
|-------------------|------------------|----|--------|-------------------|------|
| Chang, B 2019     | 0.57             | 0.26| 27.4%  | 1.77 [1.02, 3.08] | 2019 |
| Finkelman 2016    | 1.21             | 0.37| 15.7%  | 3.35 [1.62, 6.93] | 2016 |
| Han 2019          | 1.22             | 0.48| 9.3%   | 3.39 [1.32, 8.68] | 2016 |
| Iwn 2018          | 1.43             | 0.3 | 23.8%  | 4.18 [2.32, 7.52] | 2018 |
| Zeng 2011         | 1.16             | 0.3 | 23.8%  | 3.19 [1.77, 5.74] | 2011 |

Total (95% CI) 100.0% 2.93 [2.26, 3.91]  
Heterogeneity: Chi² = 4.96, df = 4 (P = 0.29), I² = 19%  
Test for overall effect: Z = 7.35 (P < 0.00001)
Table 1 Characteristics of eligible studies involving the mPD-L1 expression in tumor tissue

| Study | Country | Sample size | Stage | Method | Case number (High/Low) | Cut-off | Outcome | Follow-up time |
|-------|---------|-------------|-------|--------|-----------------------|---------|---------|---------------|
| Gao et al. [14] | China | 240 | I–III | IHC, IPP software | 60/180 | >75% | OS; DFS | Median time 60.8 month |
| Wu et al. [18] | China | 71 | I–IV | IHC, IPP software | 35/36 | | OS | 80 months |
| Umemoto et al. [15] | Japan | 80 | I–IV | IHC, intensity of the staining scoring | 37/43 | Score 3 | RFS; OS | Median time 2427 days |
| Kan et al. [16] | China | 128 | I–IV | IHC, positive cell rate | 105/23 | >25% | OS | Median time 10 months |
| Chen et al. [19] | China | 217 | I–III | IHC, positive cell rate | 52/165 | >5% | RFS, OS | >5 years |
| Gabrielson et al. [20] | America | 58 | I–IV | IHC, intensity and distribution | 19/39 | N | RFS, OS | 84 months |
| Xie et al. [21] | China | 90 | I–III | IHC, positive cell rate | 15/75 | 75% | OS | 150 months |
| Chang, H. et al. [30] | Korea | 146 | T1–T4 | IHC, slide scanner for positive cell rate | 80/60 | | Median | DFS | 153 months |
| Dai et al. [26] | China | 90 | N | IHC, Staining intensity | 37/53 | Score 2–3 | OS; DFS | 72 months |
| Dai et al. [26] | China | 90 | N | IHC, Staining intensity | 44/46 | Score 2–3 | OS; DFS | 72 months |
| Jung et al. [23] | Korea | 85 | I–IV | IHC, staining percentage and intensity scoring | 23/62 | 3–5 | OS; DFS | 125 months |
| Semaan et al. [24] | Germany | 176 | T1–T4 | IHC, Tissue studio v.2.1 semiquantitative software | 88/88 | | Median | OS | 60 months |
| Sideras et al. [31] | Netherlands | 146 | N | IHC, staining intensity scoring | 121/25 | | Cut-off with the lowest 2 log likelihood | DFS | > 100 months |
| Chang, B. et al. [25] | China | 145 | N | IHC, positive cell rate | 40/105 | >5% | OS; DFS | 120 months |
| Hu et al. [27] | China | 136 | I–III | IHC, positive cell rate of membranous staining | 26/110 | >1% | RFS, OS | 60 months |
| Liu et al. [28] | China | 453 | I–IV | IHC, positive cell rate | 87/366 | ≥5% | RFS, OS | 132 months |
| Pei et al. [17] | China | 143 | N | IHC, positive cell rate of membranous staining | 19/124 | >1% | OS | 87 months |
| Huang et al. [22] | China | 411 | I–III | IHC, positive cell rate | 78/333 | ≥5% | RFS, OS | 1000 months |
| Zhang et al. [29] | China | 46 | I–IV | IHC, positive cell rate of membranous staining | 21/25 | 2–3 | OS | 5 years |

Dai * included data from Dai et al. (25) control group. Dai data from Dai et al. (25) experimental cohort.

Table 2 Characteristics of eligible studies involving the soluble PD-L1 levels

| Study | Country | Sample size | Stage | Method | Case number (High/Low) | Cut-off | Outcome | Follow-up time |
|-------|---------|-------------|-------|--------|-----------------------|---------|---------|---------------|
| Zeng et al. [32] | China | 109 | I–III | Flow cytometric analysis | N | | OS | 36 months |
| Finkelmeier et al. [33] | Germany | 215 | I–IV | ELISA | 63/152 | 0.8 ng/ml | OS | 1464 days |
| Chang, B. et al. [35] | China | 120 | I–II | Antibody array assay | 35/85 | 11.2 μg/ml | OS; DFS | 1600 days |
| Han et al. [36] | China | 81 | I–IV | ELISA | 37/43 | 5.471 ng/ml | OS; DFS | 120 months |
| Kim et al. [34] | Korea | 53 | I–IV | ELISA | 43/10 | 1.315 pg/ml | OS | 72 months |

Association between mPD-L1 expression in the tumor tissue and clinicopathological features of HCC

Table 3 demonstrates the relationship between mPD-L1 expression and clinicopathological features. We found that high expression of mPD-L1 correlated with AFP (OR = 1.46; 95% CI: 1.16–1.84; P = 0.001), history of hepatitis (OR: 0.72; 95% CI: 0.54–0.98; P = 0.03), tumor differentiation (OR: 0.68; 95% CI: 0.55–0.84; P = 0.03), and tumor-infiltrating lymphocytes (OR: 3.39; 95% CI: 1.06–10.91; P = 0.04; Figure 3A–D). However, the high expres-
Figure 3. The association between mPD-L1 and clinicopathological features in HCC

(A) Forest plot of HR for alpha-feto protein (AFP) levels. (B) Forest plot of HR for hepatitis history. (C) Forest plot of HR for tumor differentiation. (D) Forest plot of HR for CD8 + TILs.
Table 3 Association between high mPD-L1 and clinicopathological features

| Characteristics       | Studies | Cases | Odds ratio (95% CI) | P     |  | Heterogeneity |
|-----------------------|---------|-------|---------------------|-------|----|----------------|
| Age (>50 vs ≤50)     | 10      | 1734  | 1.17 [0.94, 1.45]   | 0.17  | 0% | 0.54           |
| Gender (male/female) | 13      | 2345  | 1.22 [0.93, 1.62]   | 0.16  | 12%| 0.32           |
| AFP (high/low)       | 10      | 1680  | 1.46 [1.16, 1.84]   | 0.001 | 0% | 0.46           |
| Tumor size (<5 vs ≥5)| 10      | 2015  | 0.82 [0.67, 1.02]   | 0.07  | 40%| 0.09           |
| Liver cirrhosis (yes/no) | 8  | 1105  | 0.99 [0.58, 1.69]   | 0.96  | 61%| 0.01           |
| Hepatitis (yes/no)   | 12      | 2291  | 0.72 [0.54, 0.98]   | 0.03  | 0% | 0.62           |
| Tumor number (multiple/single) | 8 | 1634  | 0.92 [0.69, 1.22]   | 0.54  | 40%| 0.11           |
| Vascular invasion (yes/no) | 7 | 1558  | 0.99 [0.55, 1.78]   | 0.97  | 73%| 0.001          |
| TNM stage (advanced/early) | 9 | 1860  | 1.23 [0.97, 1.56]   | 0.05  | 48%| 0.09           |
| Differentiation (well/poor) | 11 | 2217  | 0.68 [0.55, 0.84]   | 0.0003| 3% | 0.41           |
| CD8+ TILs (high/low) | 3       | 772   | 3.39 [1.06, 10.91]  | 0.04  | 88%| 0.0003         |
| Tumor encapsulation (no/yes) | 3 | 787    | 0.97 [0.66, 1.42]   | 0.86  | 79%| 0.009          |

The association between high mPD-L1 and OS was demonstrated not significantly correlated with age, sex, tumor size, liver cirrhosis, vascular invasion, number of tumors, tumor encapsulation, or TNM stage.

Publication bias and sensitivity analyses

No evidence for apparent publication bias for the analysis between mPD-L1 and OS was found (Begg's test: $P = 0.536$; Figure 4A). Likewise, no apparent publication bias was found for RFS and DFS analysis (Figure 4B,C). Meanwhile, for the analysis of clinicopathological features (Figure 4D–F), we found no obvious publication bias. Additionally, there was no apparent publication bias for analysis involving sPD-L1 (Figure 4G). Sensitivity analysis showed that none of the studies remarkably affected the combined HR for OS, DFS, RFS, AFP, tumor differentiation, and hepatitis (Figure 4A–F). Because the number of studies of tumor-infiltrating lymphocytes analyses was small, we didn’t test its publication bias and sensitivity analyses.

Discussion

As an immune checkpoint, the PD-1/PD-L1 pathway plays an important role in regulating the expression of PD-L1 on immune cells, preventing T-cell mediated immune response, and regulating T-cell activation, proliferation, and cytokine release. Immune checkpoint inhibitors have been effective in the treatment of Hodgkin lymphoma and advanced melanoma [37]. PD-L1 is also an important target for immunotherapy. Nivolumab, a PD-1 blocking antibody, has been approved as a second-line treatment for HCC in patients who fail to respond to sorafenib treatment [38].

In the previous meta-analysis, Li et al. [39] pointed out the association between PD-L1 overexpression and worse OS and DFS. However, they failed to include sufficient studies. Additionally, it mistakenly regarded sPD-L1 and mPD-L1 as the same indicators. Two studies in this meta-analysis, Zeng et al. [32] and Finkelmeier et al. [33], adopted the ELISA method to test the level of sPD-L1, which were different from other studies, and those studies measured the expression of mPD-L1 in the tumor tissue, so the value of its pooled analysis was limited. In another meta-analysis by Liu et al. [40], the authors demonstrated that mPD-L1 expression had no correlation with OS and RFS, which may have resulted from the error in HR extraction from the Kaplan–Meier curves or the combining of RFS and DFS as the same outcome. Besides, it did not further explore the role of sPD-L1 in the prognosis of HCC patients.

In our meta-analysis, 23 articles were included, involving 3529 patients. We divided them into two subgroups. We grouped the studies where the mPD-L1 expression was determined in tumor cells using the IHC method together. In this group, we found significant correlation between high expression of mPD-L1 and worse OS. However, no significant correlation of mPD–L1 expression with DFS and RFS was found. High expression of mPD-L1 correlated with high AFP levels, history of hepatitis, and poor tumor differentiation. The high mPD-L1 expression positively correlated with CD8 + TILs, which confirmed the critical role that PD-L1 played in activating and proliferating T cells. In the other group, the soluble PD-L1 levels had significant correlation with poor OS. We used the HR reported in the article as much as possible for the pooled analysis. In studies were HR was not mentioned, it was extracted...
Figure 4. Begg’s funnel plot for publication bias test

(A) Begg’s funnel plot for publication bias in studies reporting OS and mPD-L1; $P = 0.536$. (B) Begg’s funnel plot for publication bias in studies reporting DFS and mPD-L1; $P = 0.711$. (C) Begg’s funnel plot for publication bias in studies reporting RFS and mPD-L1; $P = 0.707$. (D) Begg’s funnel plot for publication bias in studies reporting AFP levels and mPD-L1; $P = 0.283$. (E) Begg’s funnel plot for publication bias in studies reporting hepatitis history and mPD-L1; $P = 0.537$. (F) Begg’s funnel plot for publication bias in studies reporting tumor differentiation and mPD-L1; $P = 0.755$. (G) Begg’s funnel plot for publication bias in studies reporting OS and sPD-L1; $P = 0.564$. 
There was heterogeneity in our analysis due to several reasons. Taking the research by Gao et al. as an example [14], mouse anti-PD-L1 antibody was used for the IHC analysis; however, they did not distinguish the PD-L1 staining of HCC tumor cells in cell membrane and cytoplasm, and differentiated the 'high' and 'low' PD-L1 expression according to the density of staining. Other studies, such as that of Hu et al. [27] adopted a cutoff value of $\geq 1\%$ tumor positive cells. The difference in the definition of mPD-L1 overexpression may be one of the sources of heterogeneity. At present, tumor positive cells $\geq 1\%$ is the most widely accepted cutoff value for high expression [41], which was applied in the clinical trials of Nivolumab [38]. Most of the studies in the current meta-analysis used this cutoff value. Another reason for heterogeneity could be that the detection methods and antibodies were different in different studies. During

from the Kaplan–Meier curves by two investigators independently. Any disputes were further verified and discussed by the two investigators.
the analysis between sPD-L1 and OS, we found that there were differences in the measurement methods of sPD-L1 in the included studies, which might have resulted in different cut-off values, leading to certain heterogeneity in our study.

In one of the studies by Chang et al. [25] with 1404 HCC patients involved, 1259 subjects underwent surgical resection alone while the remaining 145 patients were treated with surgery followed by post-operative CIK cell immunotherapy (surgery-CIK group). The results suggested that in surgery-CIK group high expression of mPD-L1 and tumor infiltrating lymphocytes (TILs) significantly correlated with OS and DFS, while in surgery-only group, PD-L1 expression did not significantly correlate with OS. In another very similar study, a cohort study of 448 HCC patients (217 cases of hepatectomy alone and 231 cases of CIK cell transfusion after hepatectomy) revealed that the OS of PD-L1 positive patients in the combined treatment group was better, but no significant difference existed in the surgery-only group [19]. Based on the two studies, no association was observed between PD-L1 expression and OS in hepatectomy alone group. In tumor microenvironment, both tumor cells and immune cells could produce sPD-L1, so the sPD-L1 levels had only moderate correlation with the mPD-L1 expression in the tumor tissues [36]. Further studies are required to explore the role of sPD-L1 in the tumor immunity. We hypothesize that there may be an important role of PD-L1 expression in identifying patients likely to benefit from the immunotherapy for HCC.

In the present study, we found significant association between the soluble PD-L1 levels and OS. In clinical practice, only a small number of HCC patients undergo surgical resection, and liver biopsy is not routinely performed. Hence, obtaining tumor tissues for determining mPD-L1 expression by immunohistochemistry in clinical practice is difficult. On the contrary, determination of soluble PD-L1 is easier and showed a high prognostic value. Therefore, we recommend that future studies should focus on the prognostic role of soluble PD-L1 in HCC.

Although we systematically evaluated the relationship between PD-L1 expression and clinical features and prognosis, there still exist some deficiencies. First, studies from Asia accounted for majority of the included studies, thus probably limiting the results’ applicability to the rest of the world. Second, there was a lack of detailed analysis on the subsequent treatment after surgery. Most of the studies simply described the treatment after surgery and did not consider the differences between the treatments while making the final analysis. Finally, a few studies lacking original data made us unable to analyze the relationship between mPD-L1 expression and the clinical features in detail, such as the TNM stage, tumor size, cirrhosis, vascular invasion etc.

Conclusions
The present meta-analysis supported that mPD-L1 overexpression in tumor tissues was highly associated with poor OS, although the short comings found in studies included were true. Moreover, high expression of mPD-L1 was associated with high AFP levels, hepatitis history, poor tumor differentiation, and TILs. The soluble PD-L1 levels were also significantly associated with poor OS. PD-L1 may be useful as a prognostic biomarker for HCC in future.

Competing Interests
The authors declare that there are no competing interests associated with the manuscript.

Funding
The authors declare that there are no sources of funding to be acknowledged.

Author Contribution
J.T. designed this study. L.-X.S. and L.-J.W. conducted data extraction. At last, L.-X.S. analyzed and wrote the manuscript of the article through the software. All the authors contributed to this article.

Acknowledgements
This study was not supported by grants from any funding sources.

Abbreviations
95% CI, 95% confidence interval; AFP, alpha-fetoprotein; DFS, disease-free survival; HCC, hepatocellular carcinoma; HR, hazard ratio; mPD-L1, membrane-bound PD-L1; OS, overall survival; PD-L1, programmed death ligand-1; RFS, relapse-free survival; sPD-L1, soluble PD-L1.
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