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Review

Clinicopathological significance and prognostic role of chemokine receptor CXCR4 expression in pancreatic ductal adenocarcinoma, a meta-analysis and literature review

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ABSTRACT

Background: C-X-C chemokine receptor type 4 (CXCR4) protein level was highly detected in a number of cancer types including pancreatic ductal adenocarcinoma (PDAC). The correlation of CXCR4 expression in PDAC and its clinicopathological characteristics remains inconclusive. This study aims at investigating the relationship of CXCR4 expression and clinicopathological characteristics of PDAC patients using a meta-analysis.

Methods: PubMed, Web of Science, Cochrane Library, EMBASE, and EBSCO databases and Google Scholar from January 2000 to August 2018 were searched. The Review Manager 5.2 was used in the analysis. Odds ratios (ORs) with 95% confidence intervals (CIs) were measured. This study included a total of 11 relevant articles which involved 1439 PDAC patients.

Results: CXCR4 was more frequently overexpressed in PDAC tissues than normal pancreatic samples, OR = 132.07, P = 0.03. The frequency of high CXCR4 expression significantly increased in high grade PDAC than low grade, OR was 1.50, P = 0.03. High CXCR4 expression was more frequently observed in late stage of PDAC than those in early stage, OR was 2.82, P = 0.0009. High CXCR4 expression significantly increased the risk of lymph node and distant metastases in PDAC, OR = 2.69, $p < 0.00001$, and OR = 1.86, p = 0.009 respectively. In addition, high CXCR4 expression was correlated with poor survival in PDAC patients, HR = 1.27, P = 0.05.

Conclusions: CXCR4 overexpression is a valuable risk factor for PDAC. CXCR4 overexpression is a strong prognostic marker correlated with the risk of lymph node involvement and distant metastasis in PDAC patients.

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of most aggressive tumor types and the fourth leading cause of tumor related mortality in developed nations [1]. In the United States, the incidence of PDAC was 12.3/100.000, with 5-year relative poor survival rate of 6.7% in 2011 [2]. Since early symptoms and specific markers for PDAC are usually lacking, most patients are not diagnosed until inoperable advanced stages. Traditionally, PDAC patients' survival rates and prognosis are determined by tumor size, differentiation, lymph node involvement [3], however, those factors are insufficient to predict individual prognosis. Thus, it becomes critical to find reliable molecular markers for early tumor diagnosis and predicting progression of patients with PDAC. Chemokines (CK)s are a family of proinflammatory chemoattractant cytokines that function as the ligands to bind and subsequently activate their receptor, a specific G-protein coupled seven-span transmembrane receptor [4]. They are principle regulators of cell trafficking and adhesion [5–7], survival [8,9], proliferation [10] and gene transcription [11]. C-X-C chemokine receptor type 4 (CXCR4) is one of the receptors to bind to the stromal cell-derived factor-1α (SDF-1 now renamed CXCL12). CXCL12-CXCR4 signaling has been found to be involved in migration of tumor cells to sites of metastasis in various malignancies, such as breast, lung cancers [12–14]. CXCL12-CXCR4 is involved in cell growth, migration, and invasion of PDAC cells. CXCL12/CXCR4 signaling is also critical for regulating pancreatic cancer stem cells and CXCL12-CXCR4 axis has emerged as a novel target for PDAC therapeutics [15–18]. Numerous studies reported higher CXCR4 expression in PDAC than in normal pancreatic tissue [19–21], and correlations between high CXCR4 expression and aggressive tumor behavior, metastasis development, as well as poor prognosis [19,22–26]. However, the clinical relevance of expression of CXCR4 in PDAC remains controversial and the association between CXCR4 expression and

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clinicopathological characteristics is inconclusive due to the relatively small number of tested samples in each study. In the present study, we aimed to perform a meta-analysis from the qualified publications and evaluated CXCR4 expression in PDAC as well as the association of high CXCR4 expression in PDAC with its progression and prognosis.

2. Methods

2.1. Search strategy

We performed a literature search for articles published until August 10, 2018 in PubMed, Web of Science, Cochrane Library, EMBASE, and EBSCO databases and Google Scholar using the search terms: “CXCR4”, “expression”, “pancreatic ductal adenocarcinoma”, “pancreatic cancer”, “PDAC”, and “clinical studies”. There were 149 articles identified from PubMed, 289 articles from Web of Science, 312 articles from EMBASE. One article was identified from Cochrane Library. 25,100 articles were identified from Google Scholar, first 300 of them were screened, since the rest of them were not related to present study. All identified studies were screened by titles and abstracts. Non-relevant and duplicate publications from the different databases were excluded, the remaining papers were assessed in the full text version for in- and exclusion criteria. All clinical studies in English and Chinese except case reports were selected, for instance, cohort studies, case-controls studies, randomized controlled trials (RCTs), and case series. In addition, references of relevant publications were also reviewed to identify potentially eligible studies.

2.2. Selection criteria

The inclusion criteria were: 1) Studies investigating CXCR4 expression and clinicopathological characteristics in PDAC patients; 2) Studies that evaluated the relationship between CXCR4 protein expression and overall survival; 3) Studies that CXCR4 protein expression were examined by immunochemistry; 4) Studies which were published in English and Chinese. The studies were excluded based on the following criteria: 1) Studies in vitro/ex vivo; 2) Reviews, case reports, letters, conference abstracts, editorials, expert opinion; 3) Studies in which same population or overlapping data were used; 4) Studies written in non-English or non-Chinese language.

2.3. Data extraction

We extracted primary study data using a customized data extraction form which included first author's name, time of publication, geographic location, number of cases, clinicopathological stage, grade, size of the tumor, metastasis status, immunohistostaining, CXCR4 antibodies used, and HRs with 95% CIs from multivariate analyses. Data extraction was confirmed independently by the second reviewer (XC).

2.4. Statistical analysis

All analysis was performed with Review Manager 5.2 (Cochrane Collaboration, Oxford, UK). Odds ratios (ORs) with 95% CIs were pooled and compared. For each test, statistics significance was set at a two-sided p values less than 0.05. A chi-square examination was used to determine heterogeneity with significance being set at $P < 0.10$. The I^2 square value is an estimate of the amount of variance due to between-study heterogeneity rather than chance, with a range from 0 to 100% (the Cochran Q statistics). A fixed effect model was used for $I^2 < 50\%$, while a random effect model was used for $I^2 > 50\%$. This work has been reported in line with PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) and AMSTAR (Assessing the methodological quality of systematic reviews) Guidelines.

The analysis was performed to compare the CXCR4 protein expression between PDAC and nonmalignant prostate tissue. In addition, we evaluated the frequency of high CXCR4 protein expression in different grades, different size of the tumor, and the correlation between CXCR4 expression and metastasis status, as well as the relationship between CXCR4 expression and prognosis in patients with PDAC. Publication bias was assessed by using a method reported by Egger et al. [27].

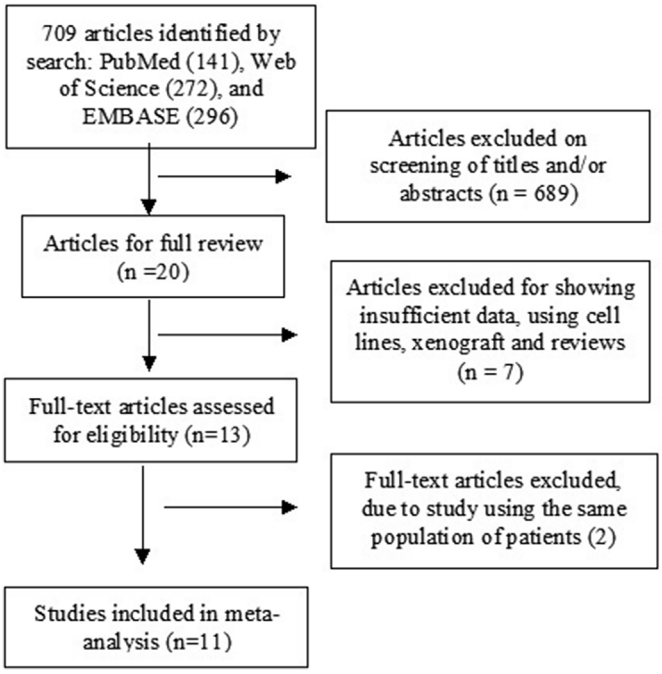

Fig. 1. Flow chart of study selection.

3. Results

3.1. Identification of eligible studies

A total of 11 studies that meet the inclusion criteria were identified in the literature search and 1439 patients were included in the data analysis (Fig. 1 and Table 1).

3.2. Quality of included studies

Newcastle Ottawa Quality Assessment Scale (NOQAS) was used for assessing Methodological quality of included articles. This scale was used to allocate a maximum of nine points for the quality of selection, comparability, exposure, and outcomes for study participants. The NOS scores ranged from 0 to 9, and a score ≥ 7 indicates a good quality. Of the studies, seven scored eight points, two scored seven points and two scored six points. Hence, the studies were of a relatively high quality (data not shown).

3.3. Study characteristics

Eleven studies published from 2000 to 2015 were eligible for meta-analysis. A total of 1439 PDAC patients from China, Germany, Japan and Belgium were included. Their basic characteristics were summarized in Table 1. The “Events” in all figures means the number of PDAC patients with high level of CXCR4 expression.

The correlation between the high level of CXCR4 expression and the risk of PDAC incidence.

The high level of CXCR4 expression was significantly correlated with the increased risk of PDAC incidence. The pooled OR from three studies including 327 PDAC patients and 279 normal pancreas controls was shown in Fig. 2A (OR = 132.07, 95% CI = 1.78–9806.85,

Table 1
Main Characteristics of included studies and CXCR4 overexpression with clinicopathologic data.

Author	Year	Country	Sample Size	Grade (L/H)	Size T1-2/T3-4	LN status (−/+)	TNM Stage (I + II/III)	Follow-up (median)	CXCR4 Ab
Wu [20]	2015	China	131	40/84; 28/47	26/59; 42/72	23/63; 45/68	59/119; 9/12		Abcam
Wang [57]	2013	China	48	25/30; 16/18		10/14; 31/34	11/16; 30/32		San Diego, CA. USA
Kure [58]	2012	Japan	105					17.2mo (0.4–153.1)	Abcam, ab2074
Park [23]	2012	Korea	88						
Liao [59]	2012	China	97	38/83; 6/14	49/53; 42/44	25/53; 33/44	1/6; 43/91		clone 12G5, dilution R&D Systems
Bachet [60]	2012	France	471					54mo	R&D Systems Inc.
Zhong [21]	2012	China	30	12/17; 12/13		6/12; 18/18	10/12; 17/18		
Gebauer [19]	2011	Germany	249	116/139; 99/110	70/82; 145/167	78/94; 135/154			clone 12G5; R&D Systems
Marechal [22]	2009	Belgium	78	32/56; 7/15	6/10; 33/61	7/23; 32/48			R&D Systems Inc.
Wehler [24]	2006	Germany	90	19/61; 11/39		7/30; 23/71	5/27; 25/76		CIO115, Capralogics, USA
Koshiba [49]	2000	Japan	52			11/18; 26/34	8/13; 29/39		

L: Low, H: High, LN:Lymph Node, mo: month.

P = 0.03, I^2 = 91%), suggesting that the association is statistically significant. These findings that CXCR4 was highly expressed in PDAC tumor tissues than that in normal tissues indicated that CXCR4 could be a risk factor for PDAC.

3.4. CXCR4 expression in the progression of PDAC

The strong CXCR4 expression was more frequently observed in high grade PDAC than those in low grade. The pooled OR from seven studies including 726 PDAC patients was 1.50, 95% CI = 1.04–2.16, P = 0.03, I^2 = 27% (Fig. 2B). The result suggested that the high level of CXCR4 expression was significantly correlated with local progression and proliferation of the primary PDAC. Fig. 2C showed the frequency of high CXCR4 expression in stage III/IV and stage I/II of PDAC. The pooled OR from six studies was 2.82 with 95% CI = 2.82–5.18, P = 0.0009, I^2 = 0%. The data indicated that the high level of CXCR4 expression was significantly correlated with the late stage (stage III/IV) of PDAC patients. Fig. 3A showed that the rate of high CXCR4 expression were increased in the PDAC with positive lymph node involvement compared to those with negative lymph node involvement. The pooled

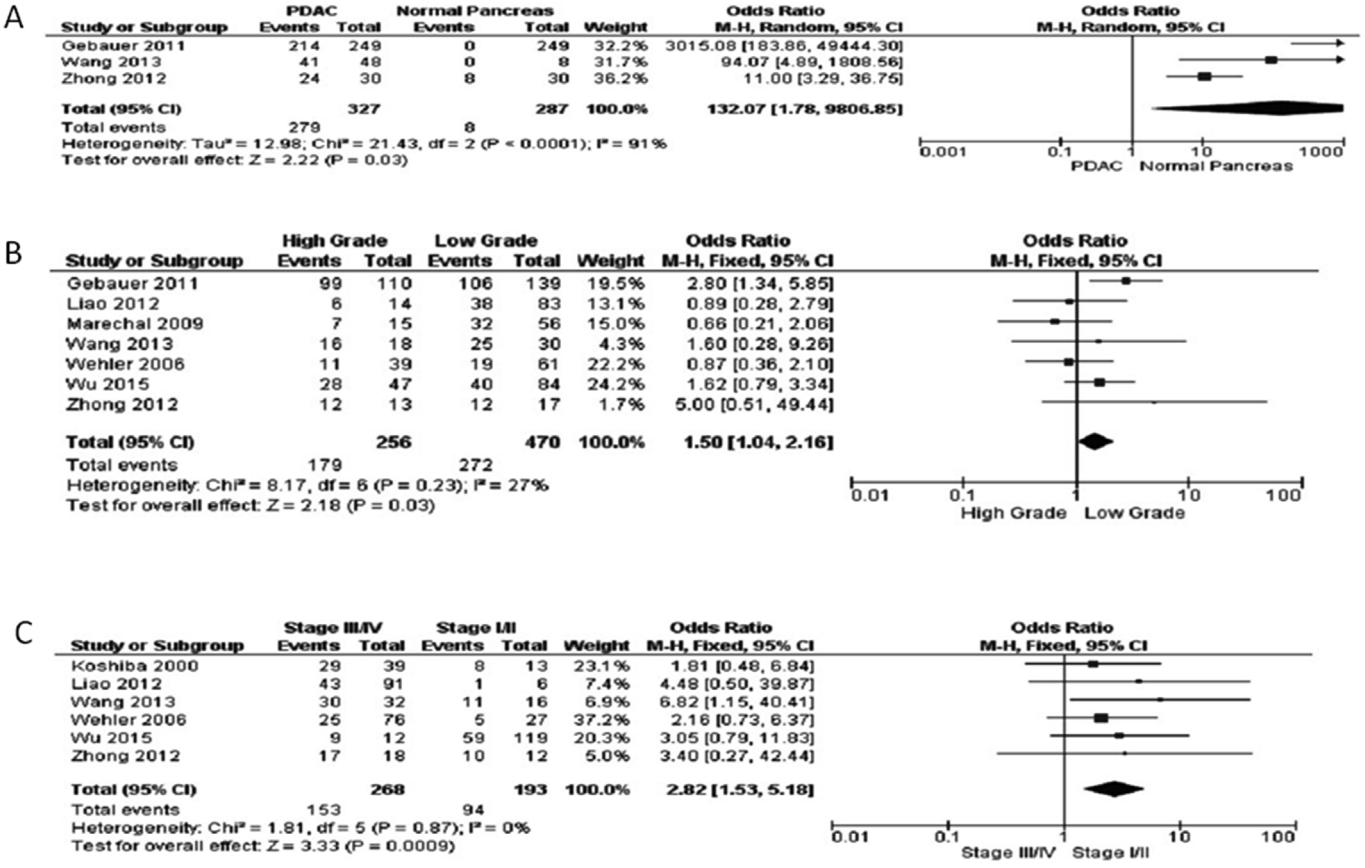

Fig. 2. A. Funnel plot of CXCR4 expression in PDAC and normal pancreas tissues. B. Funnel plot of CXCR4 expression in high and low grade of PDAC. C. Funnel plot of CXCR4 expression in different stages of PDAC.

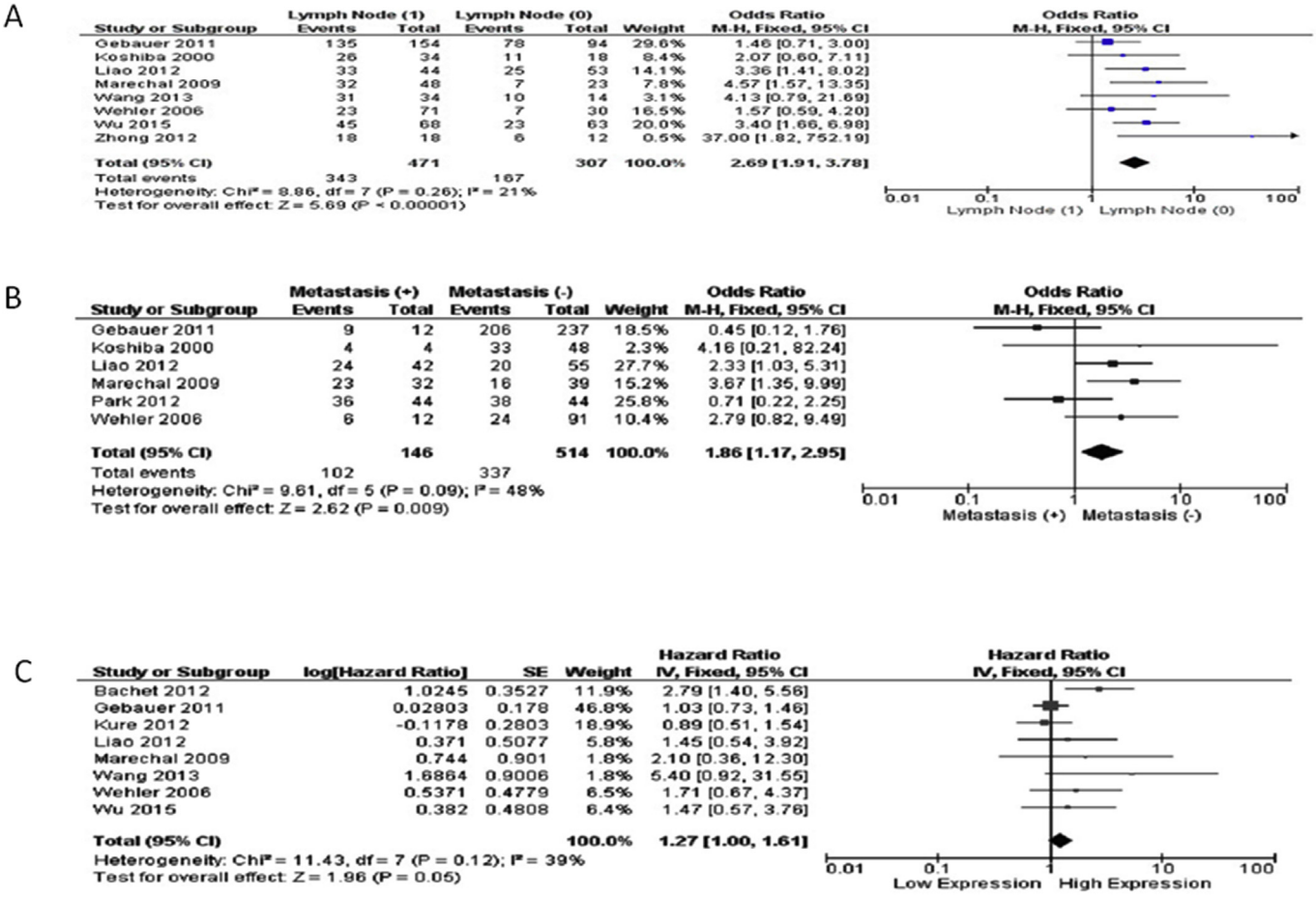

Fig. 3. A. Funnel plot of CXCR4 expression in PDAC with lymph node positive and negative involvement. B. Funnel plot of CXCR4 expression in PDAC with positive and negative distant metastasis. C. Funnel plot for the association of CXCR4 expression with overall survival of PDAC patients.

OR from eight studies was OR = 2.69 95%CI = 1.91–3.78, p < 0.00001 I^2 = 21%, indicating high level of CXCR4 expression was significantly associated with lymph node metastasis. High level of CXCR4 expression was significantly more frequently observed in PDAC with distant metastasis than those just having limited local lesion. The pooled OR from six studies was 1.86, 95% CI = 1.17–2.95, P = 0.009, I^2 = 48% (Fig. 3B). These data suggest that CXCR4 expression increased the risk of lymph node and distant metastases in PDAC patients.

3.5. Association of high CXCR4 expression with poor survival outcome

Eight included studies evaluated the relationship between the level of CXCR4 expression and overall survival (OS) in patients with PDAC. The pooled results (Fig. 3C) showed that the poor prognosis in patients of PDAC was with high CXCR4 expression (HR = 1.27, 95% CI = 1.00–1.61, P = 0.05, I^2 = 39%), indicating that high CXCR4 expressions is a poor prognostic factor for patients with PDAC.

3.6. Sensitivity analyses and publication bias

We removed one study at a time to examine the result stability, which was called a sensitivity analysis. The pooled ORs stayed unchanged, suggesting the stability of our analyses. The symmetric funnel plots implied no publication biases in the meta-analysis (Fig. 4).

4. Discussion

CXCR4, an alpha-chemokine receptor that exclusively interacts with the endogenous ligand CXCL12 [28], has been observed in at least 23 epithelial, mesenchymal, and hematopoietic cancers, suggesting its importance of this ligand/receptor axis in tumor initial, aggressiveness and metastasis [29–34]. CXCL 12 acts as the unique ligand to bind and subsequently activates CXCR4, a seven-transmembrane domain G protein-coupled receptor. CXCL 12/CXCR4 induces several different downstream pathway in tumorigenesis and progression, including Ras, PI3 kinase extracellular signal-regulated kinase, phospholipase C/mitogen-activated protein kinase (MAPK), p38 MAPK, and stress-activated protein kinase/c-Jun N-terminal kinase, eventually facilitating tumor cell growth, migration, neo-angiogenesis and dissemination [35–40]. CXCR4 expression has been demonstrated in pancreatic cancer cell lines and in human PDAC tissues. CXCL12/CXCR4 axis may promote the progression of PDAC via induction of activation of signaling pathways, such as AKT [41], MAPK [42], NF-κB [41,43], HIF-1α [43]. CXCL12/CXCR4 axis may also facilitate the progression of PDAC via induction of angiogenesis and lymphangiogenesis [44]. Recent studies of combination therapies with CXCR4 antagonists have revealed improved outcomes, and inhibiting CXCL12/CXCR4 axis may prove crucial to effectively treat PDAC at both the primary tumor and metastases [45]. In the present study, we pooled three studies with a total of 327 patients, and the rate of high CXCR4 expression was evaluated in PDAC tissue versus normal pancreatic tissues. The data indicated that the frequency of high CXCR4 expression significantly increased in PDAC compared to normal pancreatic tissues. These findings suggest CXCR4 could be a molecular marker for diagnosis of PDAC.

CXCR4 induces downstream signaling by several pathways in carcinogenesis and progression. CXCR4/CXCL12 activates various intracellular signaling transduction pathways and downstream effectors that promote cell survival, proliferation, chemotaxis, migration, and

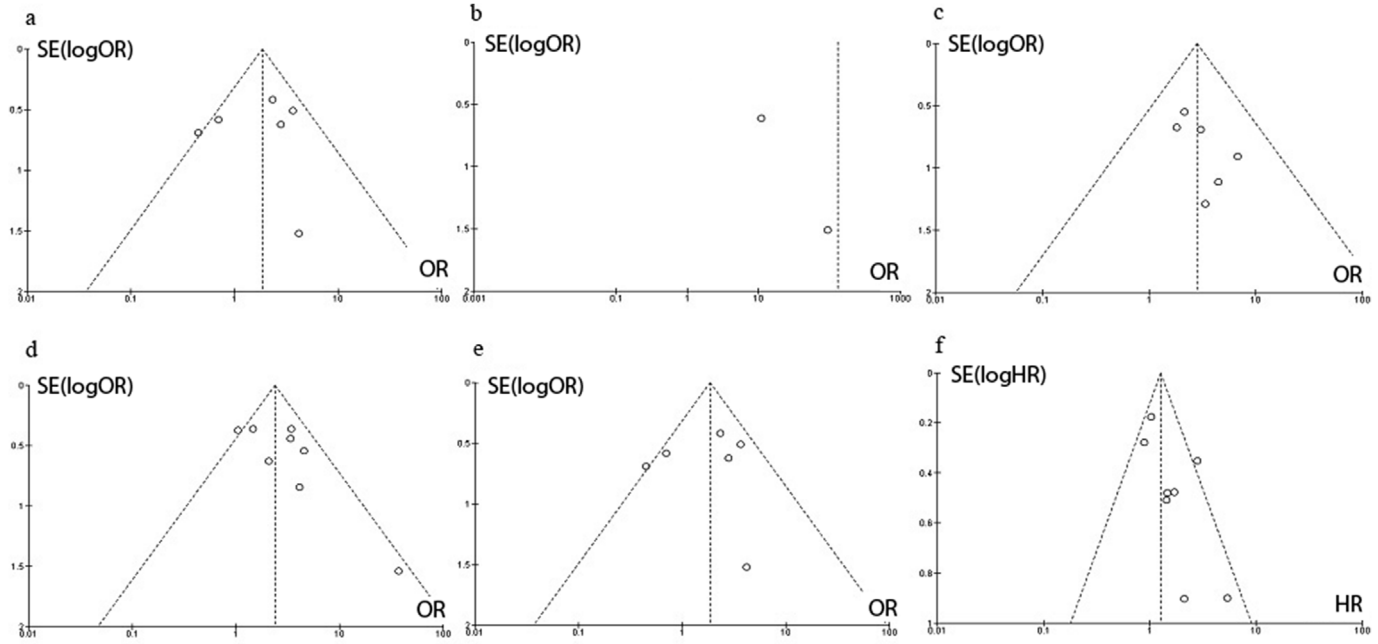

Fig. 4. Funnel plot for publication bias. a: CXCR4 expression in PDAC and normal pancreas tissues; b: Funnel plot of CXCR4 expression in high and low grade of PDAC; c: CXCR4 expression in different stages of PDAC d: CXCR4 expression in different lymph node metastasis status; e: CXCR4 expression in different distant metastasis status; f: the association of CXCR4 expression with overall survival in PDAC patients. Y-axis represents the Standard Error, X-axis represents Order Ratio or Hazard Ratio, area of the circle represents the weight of individual study.

adhesion [46]. Several studies evaluated the correlation of elevated CXCR4 expression and PDAC differentiation, the results showed inconsistent due to small power. We pooled seven studies and included 726 patients, the data showed that high CXCR4 expression was more frequently observed in high grade PDAC than in low grade one. Moreover, pooled data from six studies revealed that high CXCR4 expression was more frequently observed in late stage of PDAC than early stage.

Metastasis is a multistage process that involves tumor cells to escape from the primary tumor, survive in circulation, seed at distant sites, and grow [47]. Findings from in vitro and *in vivo* models underline the critical role of CXCR4/CXCL12 receptor ligand system for pancreatic cells. Various human tissues and cells express SDF/CXCL12, such as lungs, liver, lymph node, epithelial cells, bone marrow and stromal cells [32,48]. CXCR4 contributes to the cells migration, matrix degradation and tissue invasion through CXCR4/CXCL12 interaction [25,26,49,50], thus it correlates with more aggressive behavior of many cancers [14,51,52] and predicts worse outcome. However, studies about the association of CXCR4 expression and lymph node involvement in PDAC showed inconsistent results due to limited number of patients involved. Our data from eight studies, including 809 patients suggested that high CXCR4 expression was significantly correlated with the risk of lymph node involvement. Moreover, our finding, from six studies with 760 patients indicated that high CXCR4 expression was significantly associated with distant metastasis of PDAC. The use of CXCR4 monoclonal antibody was able to reduce PDAC invasion and metastasis in mouse models [53,54]. Interestingly, Hermann et al. discovered that two distinct subsets of CD133 positive cancer stem cells based on their ability to form metastasis, only the one with additional CXCR4 expression was capable of driving metastasis [55]. CXCL12, which is expressed at a high level in lymph node and liver, can initiate and facilitate the homing of pancreatic cancer in these tissues [56]. Taken together, the evidence indicates CXCR4 plays a pivotal role in the process of PDAC metastasis.

Furthermore, we investigated the relationship between CXCR4 expression and overall survival, suggesting high CXCR4 expression was strongly associated with poor prognosis in patients with PDAC. The finding indicates CXCR4 expression is a poor prognosis factor which is consistent with previous studies.

The finding should be interpreted in view of certain limitations. First, although included studies were not selected randomly and most of them were retrospective, most were of sufficient quality (Newcastle-Ottawa Scale > 7). The possibility of selection and publication bias cannot be excluded, however, the funnel charts were largely symmetric (Fig. 4), suggesting no publication biases existed in the meta-analysis. Survival data were extracted from Kaplan-Meier curves, a bias might be introduced. Some analysis was only in a few cases such as the comparison between PDAC patients and normal pancreas controls, hence, cautions should be taken about the conclusion.

In summary, CXCR4 overexpression is a valuable risk factor for PDAC. CXCR4 overexpression is a strong prognostic marker associated with the risk of lymph node involvement and distant metastasis in patients with PDAC indicating a worse prognosis in patients with PDAC.

Ethical approval

There is no any ethical approval needed in this study.

Author contribution

YD and YWD participated in the design of the study, identify related studies, reviewed and extracted data from eligible studies. YWD drafted the manuscript.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Trial registry number

D: CRD42019119694.

Title: Prognostic role of chemokine receptor CXCR4 expression in pancreatic ductal adenocarcinoma, a meta-analysis and literature review.

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Credit author statement

Dr. Ding and Dr. Du contributed substantially to the study and design, acquisition of data, and analysis and interpretation of data. Dr. Ding contributed substantially to the acquisition, analysis, interpretation of data and performed the statistical analysis. The corresponding author had full access to all data and the final responsibility for the decision to submit the article for publication. All authors read and approved the final manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijsu.2019.03.009.

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