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Original Article

Expression of CXCR4 and non-small cell lung cancer prognosis: a meta-analysis

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Abstract: Purpose: The prognostic value of aberrant C-X-C chemokine receptor type 4 (CXCR4) levels in NSCLC has been described in empirical studies. This meta-analysis evaluates the value of CXCR4 as a prognostic marker for NSCLC and determines the relationship between CXCR4 and clinicopathological features of NSCLC. Methods: A comprehensive search of the English-language literature in PubMed, Embase, Google Scholar and Web of Science was performed. Articles containing sufficient published data to determine an estimate of the hazard ratio (HR) and a 95% confidence interval (95% CI) for over survival (OS) or disease-free survival (DFS) were selected. Of 417 potentially relevant studies, 10 eligible studies (1,334 NSCLC patients) met the inclusion criteria. Results: Overall, high CXCR4 expression was significantly associated with a poor OS rate (HR=1.59, 95% CI=1.36-1.87, *P*<0.001) while the association with DFS was not statistically significant (HR=1.00, 95% CI=0.37-2.69, P=0.993). Stratified analysis by subcellular localization found that CXCR4 overexpression in the non-nucleus predicts poor OS (HR=1.65, 95% CI=1.40-1.95, *P*<0.001) and DFS (HR=3.06, 95% CI=2.15-4.37, *P*<0.001), but elevated CXCR4 expression in the nucleus was positively associated with DFS (HR=0.44, 95% CI=0.26-0.75, *P*=0.002). NSCLC patients with CXCR4 expression were more likely to be diagnosed with adenocarcinoma cancer (OR=1.45, 95% CI=1.07-1.95, *P*=0.016), lymph node involvement (OR=0.69, 95% CI=0.50-0.96, *P*=0.027), and distant metastasis (OR=0.36, 95% CI=0.14-0.93, *P*=0.035). Conclusion: Aberrant overexpression of CXCR4 is associated with worse overall survival, adenocarcinoma histology, distant metastasis, lymph node involvement in NSCLC.

Keywords: NSCLC, CXCR4, prognosis, clinicopathological features, meta-analysis

Introduction

Lung cancer continues to be the leading cause of cancer-related death in both men and women throughout the world and only 15% of lung cancer patients survived 5 years or more [1]. It is known that non-small cell lung carcinoma (NSCLC) accounts for approximately 80% of all lung cancers. Identification of prognostic biomarkers may lead to new therapies. The C-X-C Chemokine receptor type 4 (CXCR4) protein is expressed in NSCLC and may predict prognosis.

CXCR4 receptor is an alpha-chemokine receptor specific for CXCL12 [2]. Many investigators have reported that the CXCR4/CXCL12 axis contributes to cancer progression [3]. During the last five years, several meta-analysis about prognostic value of aberrant CXCR4 levels in the nucleus and/or cytoplasm have been described in breast cancer [4], gastric cancer [5], esophageal cancer [6] and ovarian cancer [7]. To the best of our knowledge, no meta-analysis data on the correlation of CXCR4 expression with the prognosis and survival of patients with NSCLC have been done in the English literature. Therefore, we performed a meta-analysis to evaluate the CXCR4 as a prognostic marker for NSCLC and determine the relationship between CXCR4 and several clinicopathological features of NSCLC.

Methods and materials

Search strategy

Original English articles studying the prognostic value of CXCR4 in NSCLC were identified with a comprehensive literature search in PubMed, Embase, Google Scholar, and Web of Science on September 14, 2014. Studies were identified using the following search terms: "CXCR4" or "C-X-C chemokine receptor type 4" AND "NSCLC" or "non-small lung cancer" or "non-small cell lung cancer" or "non-small cell lung carcinoma" AND "prognosis" or "prognostic" or "outcome" or "survival". All articles were reviewed for inclusion by one reviewer. Another independent review of all articles was conducted by a second reviewers.

Selection criteria

All articles included met the following inclusion criteria: a) articles detecting expression of CXCR4 in tumor tissues; b) articles evaluating the relationship between CXCR4 expression and parameters such as clinicopathological features and prognostic factors of NSCLC; c) articles containing sufficient published data to determine an estimate of the hazard ratio (HR) and a 95% confidence interval (95% CI) for over survival (OS) or disease-free survival (DFS). The exclusion criteria included the following: a) no dichotomous groups of CXCR4 expression; b) Letters to the editor, reviews, comments, duplicated studies and articles published in books.

Data extraction

Two reviewers independently assessed the articles and resolved discrepancies via discussion and consensus (**Table 1**). Standardized abstraction sheets were used to record data from individual studies. Data retrieved from the literature included first author, year of publication, country of origin, number of analyzed patients, NSCLC subtype, staining methods of CXCR4, cutoff scores, T category (tumor category: T0-2, T3-4), N category (lymph node status), TNM category (I-II, III-IV), distant metastasis, ratio of high/low CXCR4 expression to the study outcomes, and HR estimation. For each study, the HR was estimated using the method reported by Parmar et al. [8]. HR estimates and 95% CIs were either directly obtained from the original article or calculated using parameters such as statistics of observed minus expected events and variance provided in the papers. Otherwise, the number of patients at risk in each group, as well as the number of events and *P* value of the log-rank statistic, was retrieved to allow approximate calculation of the HR estimate and its variance. If the study did not provide the HR but reported the survival curve, survival rates at specified time points were extracted to reconstruct the HR estimate and its variance, with the assumption that the rate of patients censored was constant during the follow-up [9]. Survival rates on the graphical representation of the survival curves were read by Engauge Digitizer version 2.5.

Quality assessment

In this meta-analysis, quality assessment of the cohort studies was performed using the Newcastle Ottawa Scale (NOS), as recommended by the Cochrane Non-randomized Studies Methods Working Group [10]. The NOS contains eight items under three categories: selection (four items, one star each), comparability (one item, up to two stars), and outcome (three items, one star each). Given the variability in the quality of cohort studies found in our initial literature search, we considered studies as of high quality if they scored six stars or more [11].

Statistical analysis

The meta-analysis was performed using STATA version 11 (StataCorp LP, College Station, TX, USA). Combined HR and 95% CI were used to assess the strength of association of CXCR4 expression with OS or DFS; combined OR and 95% CI were used to assess the strength of association of CXCR4 expression with clinicopathological features of NSCLC, $P<0.05$ indicated statistical significance. Heterogeneity was assessed using I^2 and Q statistics. For the I^2 statistic, heterogeneity was interpreted as absent ($I^2<25\%$), moderate ($I^2=25\%$-50%), or extreme ($I^2=50\%$-100%) [12]. For the Q statistic, $P<0.10$ was considered statistically significant for heterogeneity. The pooled HR estimation of each study was calculated using a random-effects model when $P<0.10$ or $I^2\geq50\%$; otherwise ($P>0.10$ and $I^2<50\%$), a fixed-effects model was used [13]. Subgroup analysis was conducted to explore the sources of heterogeneity, and differences between subgroups were assessed using methods described by Deeks et al [14]. To validate the robustness of the meta-analysis findings, sensitivity analysis was

Table 1. Characteristics of eligible studies

First author (Year)	Ref.	source	CXCR4 assay	cases	NSCLC subtype (high/low)	T category (T1/2/3/4)	N category (N+/N-)	Distant metastasis (M1/M0)	TNM (I/II/III/IV)	Gender (high/low)	Cut off (high/low)	OS	DFS	subcellular localization	Prognosis value	NOS score
												HR (95% CI)	HR (96% CI)			
Spano (2004)	29	France	IHC	61	AC32 (8/24)	H: (6/11/0/0)	NR	H: (4/13)	NR	M: 48 (12/36)	Staining 50% (17/44)	0.83 (0.09-8.06)¶	NR	nucleus	positive	7
					N-AC29 (9/20)	L: (22/22/0/0)		L: (15/29)		F: 13 (5/8)						
Song (2008)	28	Korea	IHC	323	AC145 (23/122)	NR	H: (29/19)	H: (24/24)	H: (17/9/21/1)	M: 254 (40/214)	Scores ≥2 (48/275)	1.351 (1.096-1.709)	3.16 (2.10-4.78)¶	cytoplasm nucleus	poor	7
					N-AC178 (25/153)		L: (127/148)	L: (94/181)	L: (128/62/81/4)	F: 69 (8/61)						
Suzuki (2008)	30	Japan	IHC	90	NR	NR	NR	NR	NR	NR	Staining 50% (22/68)	2.22 (1.13-4.37)¶	NR	cytoplasm membrane	poor	6
Iwakiri (2009)	34	Japan	RT-PCR	79	NR	NR	NR	NR	NR	NR	CXCR4>0.11204(40/39)	0.611 (0.226-1.654)	0.651 (0.242-1.748)	nucleus	NS	6
Wagner (2009)	31	USA	IHC	154	AC132 (57/75)	H: (40/14/0/8)	H: (16/46)	H: (4/58)	H: (40/5/13/4)	M: 46 (17/29)	Scores ≥2 (62/92)	NR	0.38 (0.17-0.89)	nucleus	positive	7
					N-AC22 (5/17)	L: (40/33/1/18)	L: (28/64)	L: (2/90)	L: (50/14/26/2)	F: 108 (45/63)						
Wagner* (2009)	31	USA	IHC	154	AC132 (42/90)	H: (24/13/1/9)	H: (13/34)	H: (4/42)	H: (27/4/11/5)	M: 46 (15/31)	Scores ≥2 (47/107)	NR	2.8 (1.4-5.7)	cytoplasm	poor	7
					N-AC22 (5/17)	L: (56/34/0/17)	L: (31/76)	L: (1/106)	L: (63/15/28/1)	F: 108 (32/76)						
Minamiya (2010)	26	Japan	RT-PCR	79	AC79 (37/42)	NR	H: (5/32)	NR	H: (32/1/4/0)	M: 43 (20/23)	CXCR4 mRNA> 2.4 (37/42)	0.73 (0.17-3.10)¶	0.36 (0.13-0.94)	nucleus	positive†	6
					N-AC0		L: (14/28)		L: (25/6/11/0)	F: 36 (17/19)						
Otsuka (2011)	27	Canada	FIHCS	170	AC91 (8/83)	NR	NR	H: (20/9)	NR	M: 86 (19/67)	AQUA score > 3371 (29/141)	2.03 (1.35-3.05)¶	NR	non-nucleus	poor	6
					N-AC79 (21/58)			L: (101/40)		F: 84 (10/74)						
Wang (2011)	35	China	IHC	208	AC90 (56/34)	H: (34/42/33/8)	H: (89/28)	NR	H: (21/49/37/0)	M: 128 (74/54)	Scores ≥2 (117/91)	2.070 (1.365-3.140)	NR	cytoplasm	poor†	9
					N-AC118 (61/57)	L: (49/31/8/3)	L: (53/38)		L: (20/42/29/0)	F: 80 (43/37)						
Franco (2012)	33	Italy	IHC	45	AC16 (14/2)	NR	H: (5/28)	NR	NR	NR	Staining 30% (19/26)	2.40 (0.30-18.95)¶	NR	cytoplasm membrane	poor	6
					N-AC29 (19/10)		L: (1/11)									
AlZobair (2013)	32	China	IHC	125	AC64 (34/30)	NR	NR	H: (26/36)	H: (3/7/26/26)	M: 87 (42/45)	staining 10% (62/63)	2.172 (1.229-3.839)	NR	cytoplasm	poor	7
					N-AC61 (28/33)			L: (5/58)	L: (13/16/29/5)	F: 38 (20/18)						

Note: ¶Extrapolated from survival curve and HR from univariate; †independent prognostic factors; *Date from the same study of the previous line; Ref., Reference number; IHC, immunohistochemistry; RT-PCR, reverse transcription-polymerase chain reaction; FIHCS, fluorescent immunohistochemical staining; AC, Adenocarcinoma; N-AC, Non-Adenocarcinoma; M, male; F, female; NR, Not reported; OS, over survival; DFS, disease-free survival; NS, No significance.

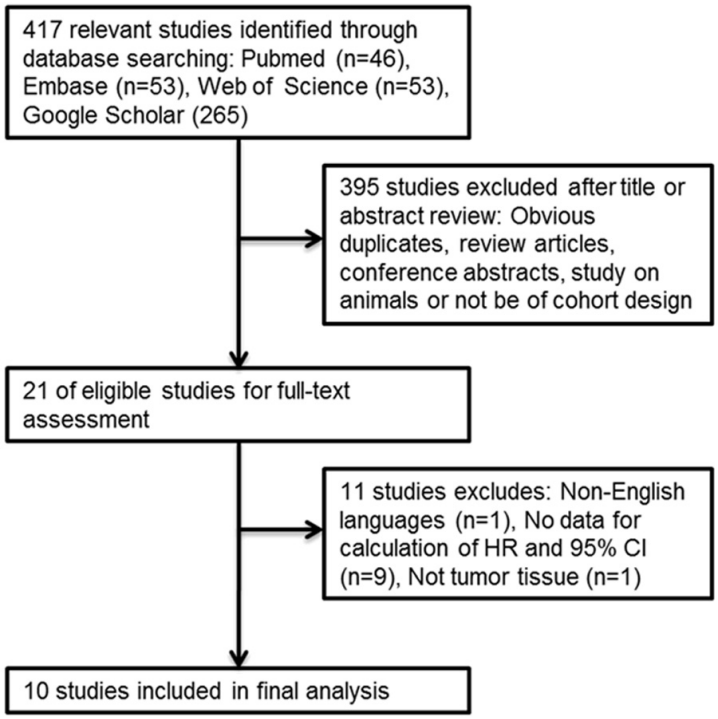

Figure 1. Flow chart of the eligible studies in the meta-analysis.

performed by sequentially omitting each individual study using the "metainf" STATA command. Potential publication bias was evaluated using Begg's asymmetry tests [15]. When the statistical significance of Egger's test results at $P<0.10$, we also conducted a trim and fill analysis [16].

Results

Search results

The study identification and selection processes are presented in **Figure 1**. Initially, 417 relevant studies were identified using the search strategy above. After reading the titles or abstracts, 395 studies were excluded since they were duplicates, review articles, conference abstracts, animal studies or not of cohort design. In the remaining 21 published studies, 11 were excluded because they were published in non-English languages; lack data for calculation of HR and 95% CI; or not detected in tissue samples. Finally, a total of 10 eligible studies were included in this meta-analysis [17-26].

Study characteristics

Main characteristics of the 10 studies are listed in **Table 1**. They were published during the last decade. A total of 1,334 patients with NSCLC from China, Italy, Japan, France, Canada, Korea and USA were enrolled. These studies were then divided into nuclear subgroup, in which CXCR4 expression was only in nucleus [17, 20, 22, 25], and non-nuclear subgroup (studies detected in cytoplasm and/or membrane [21-24, 26], cytoplasm and nucleus [19] and non-nucleus [18]) in our present meta-analysis. In addition, the expression of CXCR4 was identified by immunohistochemistry (IHC) in 7 studies [19-24, 26], by quantitative fluorescent IHC in 1 study [18] and by reverse transcription-polymerase chain reaction (RT-PCR) analysis in 2 studies [17, 25]. TNM stage was reported in six studies, among which 64.8% were stage I or II, while the other 35.2% were stage III or IV. The definition of overexpressed CXCR4 staining varied among the studies. Among all of the included studies, HRs and 95% CIs for OS were obtained directly from 4 original articles. Same number of articles provided HRs and 95% CIs for DFS. For the remaining studies, HRs and 95% CIs were calculated or extrapolated from Kaplan-Meier curves. The mean value of the article quality was 6.7 out of 9 stars (**Table 1**).

Correlation of CXCR4 expression with clinicopathological parameters

We only chose the studies [19-24, 26] which CXCR4 expression examined by IHC to evaluate the correlation of CXCR4 expression with gender, Adenocarcinoma (AC), T category, N category, TNM stage and distant metastasis (**Figure 2**). The pooled OR indicated that CXCR4 expression did not have significant correlation with gender (OR=1.03, 95% CI=0.76-1.39, $P = 0.857$ and $I^2=0$ fixed-effect), T category (OR=0.66, 95% CI=0.22-2.03, $P=0.472$ and $I^2=81$ random-effect) or TNM stage (OR=0.64, 95% CI=0.39-1.04, $P=0.073$ and $I^2=60.7$ random-effect). However, NSCLC with CXCR4 expression was associated with AC (OR=1.45, 95% CI=1.07-1.95, $P=0.016$ and $I^2=0$ fixed-effect), N category (OR=0.69, 95% CI=0.50-0.96, $P=0.027$ and $I^2=39.5$ fixed-effect) and distant metastasis (OR=0.36, 95% CI=0.14-0.93,

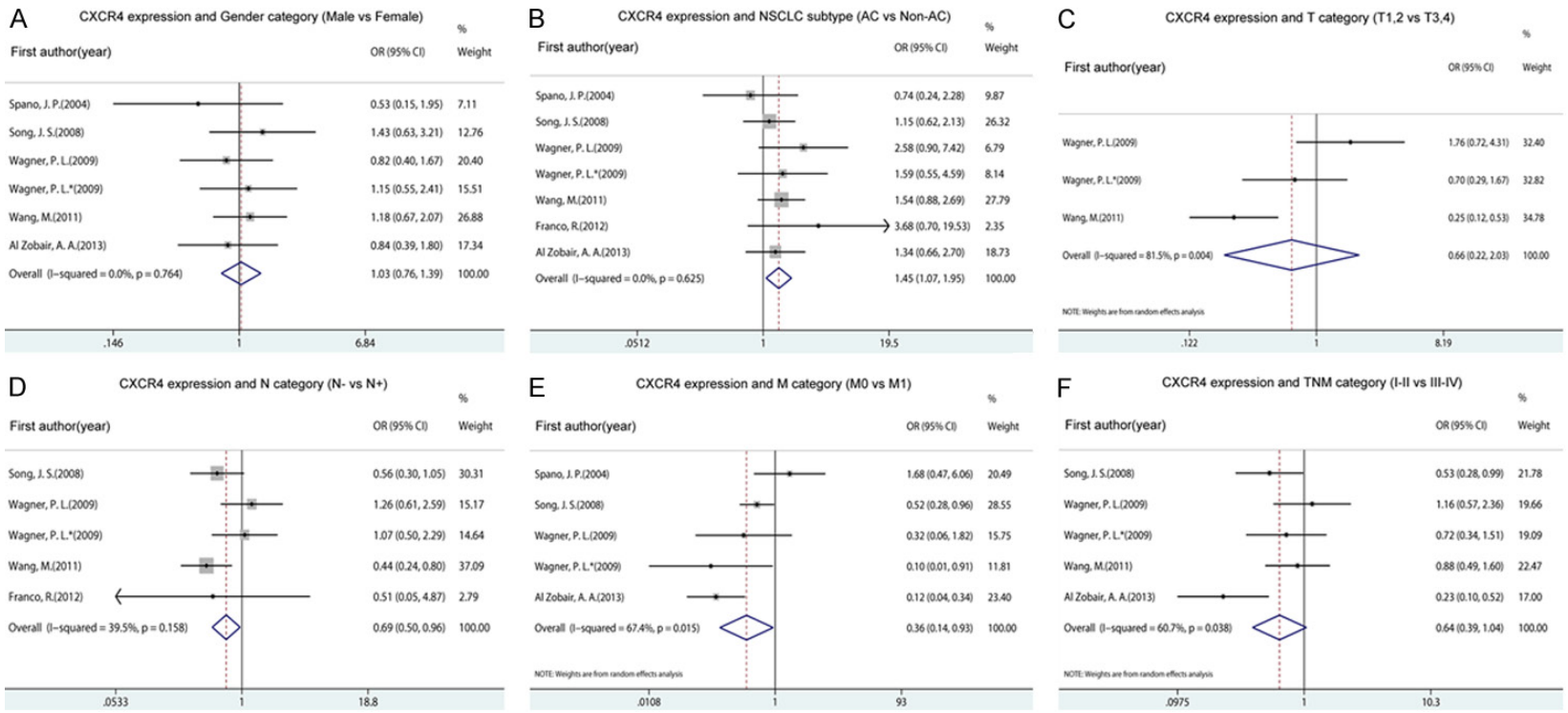

Figure 2. Forest plots of CXCR4 expression and the clinicopathological features of the patients with NSCLC. A. Gender. B. NSCLC subtype. C. Tumor depth. D. Status of lymph node. E. Distant metastasis. F. TNM staging.

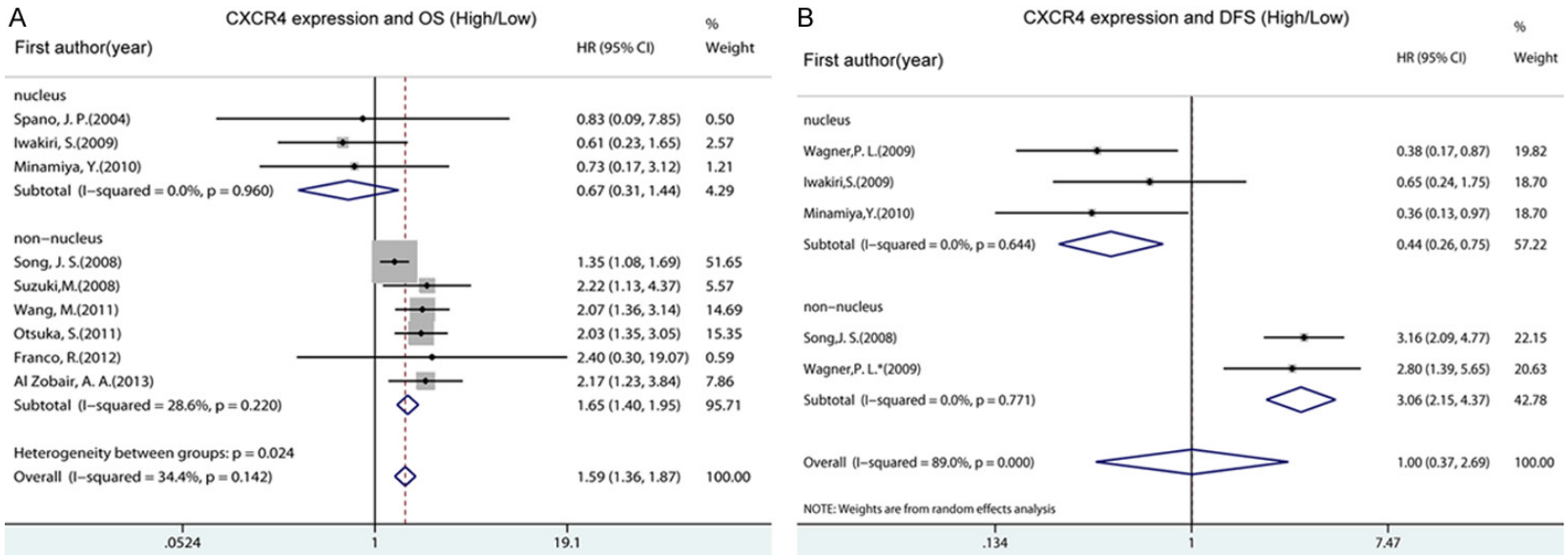

Figure 3. Forest plots of HRs for Overall Survival (A) and Disease-free Survival (B) among the included studies.

Table 2. Meta-analysis of CXCR4 overexpression and prognosis in NSCLC

Categories	Datasets (No. of patients	HR (95% CI)	I^2 (%)	P_h	*P*	95% PI	P_{sub}
Overall survival	9 (1180)	1.59 (1.36, 1.87)	34.4	0.142	<0.001	na	
Subcellular localization							0.024
nucleus	3 (219)	0.67 (0.31, 1.44)	0	0.960	0.302	na	
non-nucleus	6 (961)	1.65 (1.40, 1.95)	28.6	0.220	<0.001	na	
Ethnicity							0.225
Non-Asian	3 (276)	1.99 (1.34, 2.95)	0	0.773	0.001	na	
Asian	6 (904)	1.52 (1.28, 1.81)	50.5	0.072	<0.001	(0.70-2.64)	
Assay							0.037
IHC	6 (852)	1.58 (1.33, 1.89)	18.9	0.290	<0.001	na	
RT-PCR	2 (158)	0.65 (0.28, 1.47)	0	0.843	0.298	na	
Sample size							0.047
<80	4 (264)	0.78 (0.38,1.60)	0	0.712	0.497	na	
>80	5 (916)	1.65 (1.40,1.94)	41.9	0.142	<0.001	na	
Analysis							0.183
univariate	5 (445)	1.94 (1.39, 2.70)	0	0.639	<0.001	na	
multivariate	4 (735)	1.50 (1.25, 1.80)	62	0.048	<0.001	(0.31, 3.02)	
Disease-free survival	5 (789)	1.00 (0.37, 2.69)^R	89	<0.001	0.993	(-1.81, 4.87)	
Subcellular localization							<0.001
nucleus	3 (312)	0.44 (0.26, 0.75)	0	0.644	0.002	na	
non-nucleus	2 (477)	3.06 (2.15, 4.37)	0	0.771	<0.001	na	
Ethnicity							0.148
Non-Asian	2 (308)	1.04 (0.15, 7.39)	92.3	<0.001	0.965	(-25.35, 32.89)	
Asian	3 (481)	0.95 (0.22, 4.17)	90.6	<0.001	0.951	(-4.65, 9.04)	
Assay							<0.001
IHC	3 (631)	1.56 (0.48, 5.07)	90.3	<0.001	0.460	(-3.81-9.36)	
RT-PCR	2 (158)	0.48 (0.24, 0.97)	0	0.406	0.042	na	
Sample size							<0.001
<80	2 (158)	0.48 (0.24, 0.97)	0	0.406	0.042	na	
>80	3 (631)	1.56 (0.48, 5.07)	90.3	<0.001	0.460	(-3.81-9.36)	
Analysis							<0.001
univariate	1 (323)	3.16 (2.10-4.78)	na	na	<0.001	na	
multivariate	4 (466)	0.73 (0.25, 2.09)	83.2	<0.001	0.554	(-2.29, 4.63)	

Note: All pooled HRs were derived from fixed-effects model except for cells marked with (random R). P_h, value for heterogeneity based on Q test; *P*, value for statistical significance based on Z test; 95% PI, the distribution of true effect sizes; P_{sub}, subgroup difference; Assay, the detected methods; IHC, Immunohistochemistry; RT-PCR, Reverse Transcription-polymerase Chain Reaction; CI, Confidence Interval; na, not applicable.

P=0.035 and I^2=67.4 random-effect) (Table S1).

It is worth mentioning that in the staining pattern subgroup analysis, non-nuclear CXCR4 expression had a significant association with TNM stage (OR=0.55, 95% CI=0.32-0.94, *P*=0.029 and I^2=58.5 random-effect), AC (OR=1.44, 95% CI=1.03-2.00, *P*=0.031 and I^2=0 fixed-effect), N category (OR=0.59, 95% CI=0.41-0.86, *P*=0.005 and I^2=9.2 fixed-effect) and distant metastasis (OR=0.22, 95% CI=0.07-0.75, *P*=0.015 and I^2=71.1 random-effect). However, CXCR4 expression in nucleus had no significant association with tumor characteristics (data not shown).

Impact of CXCR4 expression on 5-year OS and DFS rates

Nine studies (1,180 patients) were pooled into the meta-analysis of OS. As shown in **Figure**

3A, overall, high CXCR4 expression was statistically associated with a poor OS rate (HR=1.59, 95% CI=1.36-1.87, P<0.001 and I^2=34.4 fixed-effect). Elevated CXCR4 expression in non-nucleus was significantly associated with poor OS (HR=1.65, 95% CI=1.40-1.95, P<0.001 and I^2=28.6 fixed-effect). There was not significant association between OS and overexpression of CXCR4 in nucleus. Differences between the two subgroups (nucleus and non-nucleus) were statistically significant (P_{sub}=0.024).

Four studies (635 patients) were used in the meta-analysis of DFS. As shown in **Figure 3B**, overall, no significant association between overexpression of CXCR4 and poor DFS was found in these studies (HR=1, 95% CI=0.37-2.69, P=0.993 and I^2=89 random-effect). However, high CXCR4 expression in non-nucleus predicts poor DFS (HR=3.06, 95% CI=2.15-4.37, P<0.001 and I^2=0 fixed-effect). Interestingly, high CXCR4 expression in nucleus was significantly associated with positive DFS (HR=0.44, 95% CI=0.26-0.75, P=0.002 and I^2=0 fixed-effect). Differences between the two subgroups were statistically significant (P_{sub}<0.001).

Additional results from the subgroup analyses can be found in **Table 2**. The results suggested that differences between the two subgroups of different categories (assay-detected methods or patients in study) were all statistically significant no matter for OS or for DFS. However, differences between the analysis subgroups (univariate vs multivariate) were only statistically significant (P_{sub}<0.001) in DFS.

Publication bias analysis and Sensitivity analysis

Begg's tests indicated that there was no evidence of significant publication bias for the studies included in our meta-analysis (P=0.221-1.000, respectively). However, Egger's test demonstrated a publication bias among the studies regarding HR of DFS with a P value of 0.058 (P<0.10). But we did not discover any unpublished studies after performing the "trim and fill" analysis. However, based on stratification by subcellular localization, no publication bias of DFS was found (Table S2). It might due to limited number of studies on the relationship between non-nuclear CXCR4 expression and NSCLC. Moreover, in order to gauge results stability, a sensitivity analysis, in which one study was deleted at a time, was performed. Both of the corresponding pooled ORs and HRs were essentially unchanged, suggesting the robustness of our results.

Discussion

Several meta-analysis found that high level of CXCR4 appears to be associated with increased malignancy across cancers, as witnessed by the correlation with adverse characteristics such as poor patient survival [19, 21, 27, 28]. An increasing number of studies suggest a possible role for the CXCL12/CXCR4 axis in the metastatic evolution of NSCLC, and its potential use as prognostic markers and drug targets [19, 29-32]. Despite many studies showing that the presence of CXCR4 in the cytoplasm and/or nucleus is associated with a poor prognosis in some types of cancers such as breast, esophagus, stomach and colon, the predictive value of CXCR4 in NSCLC is controversial. In our meta-analysis, we attempt to evaluate the value of CXCR4 as a prognostic marker for NSCLC and determine the relationship between CXCR4 and clinicopathological features such as gender, NSCLC histologic subtype, distant metastasis and status of lymph node.

In recent years, Otsuka et al. initially suggested that a gender-dependent difference in clinical outcome based on CXCR4 overexpression in stage IV NSCLC. Interestingly, this poor outcome is disproportionately represented in the female population [18]. Subsequently, the sex differences in CXCR4 activity were proposed, along with evidence potentially linking estrogen receptor(ER) expression and activity to CXCR4 function [33]. Moreover, ERs and Progesterone receptors (PRs) are present in stage IV NSCLC tissue samples, and are associated with both CXCR4 expression and overall survival [34]. But our meta-analysis did not show clear relationship between CXCR4 expression and gender. Certainly, these different results may be owing to few advanced stage NSCLC patients in the eligible studies.

Higher expression of CXCR4 was observed in adenocarcinoma subtype compared to non-adenocarcinoma samples [35] and was an independent predictor of a better prognosis in patients with lung adenocarcinoma [17]. Amazingly, cytomembranous expression of

CXCR4 in adenocarcinoma of the lung is an independent risk factor associated with worse DFS, whereas nuclear staining confers a survival benefit. These findings are consistent with a model in which CXCR4 promotes tumor cell proliferation and metastasis when present in the cytoplasm or cell membrane, whereas localization of this molecule in the nucleus prevents it from exerting these effects [22]. Our results also suggested that CXCR4 expression was related to distant metastasis, status of lymph node and Adenocarcinoma in non-nuclear subgroup but not in nuclear subgroup.

Strong CXCR4-positive nuclear staining was associated with a significantly better outcome in NSCLC [20, 22], while cytomembranous expression of CXCR4 in adenocarcinoma of the lung is an independent risk factor associated with worse disease-free survival [22]. Our present study has shown that CXCR4 is very promising for prognosis prediction. For OS, the pooled HR of higher CXCR4 expression was 1.59 (95% CI=1.36-1.87, P<0.001), which could predict poorer survival in NSCLC. When grouped according to the subcellular localization of CXCR4 in studies, we found that patients with higher CXCR4 expression of non-nuclear subgroup showed a significantly poorer survival than those with lower expression. High nuclear expression of CXCR4 was associated with better survival in NSCLC, but no significant difference was observed for overall survival (P=0.302). Similarly, high CXCR4 expression of non-nuclear subgroup showed a significantly worse disease-free survival, while CXCR4-positive nuclear staining was remarkably associated with a significantly better outcome in NSCLC. Zeelenberg et al. [36] once reported the retention of CXCR4 in intracellular compartments (endoplasmic reticulum) of T-cell hybridoma reduced metastasis and increased the survival of mice. So the nuclear location of CXCR4 may inhibit the signal provided by CXCL12 and result in decreased cell proliferation and metastasis. But the mechanism remains controversial and needs further exploration.

However, several points should be concerned about the clinical application of our findings. First of all, an explicit definition should be made about the cut-off value of CXCR4 level for increased survival risk. Secondly, the studies in our analysis used tumor tissue, and tissue may not be easy to obtain and therefore might not allow one to monitor a patient's progress. Circulating markers are more acceptable than tissue markers because they can be assayed before surgery and be monitored throughout the life. Of potential interest is the observation high levels of CXCR4 could also be detected in patient serum [24, 27]. More studies should be conducted in future to evaluate the prognostic value of CXCR4 level in serum. The lastly, the studies included in the meta-analysis used various IHC protocols (choice of CXCR4 antibody, dilutions of antibodies and other relevant information), and experimental IHC protocols could have confounded the results. For routine clinical application in the future, the above-mentioned problems should be addressed.

There are some limitations to the present meta-analysis. First, the number of samples and studies are relatively small. What's more, some of the eligible studies had insufficient information to estimate the HRs of OS or DFS and the data of clinical pathological features are also very limited. Second, marked heterogeneity existed in DFS studies, possibly due to the various cellular locations. Moreover, the applied method for detecting CXCR4 expression, the cutoff values as well as the duration of follow-up were so different that may another source of variance. Although differences between the analysis subgroups were statistically significant (P_{sub}<0.001) in DFS, but the number of studies in univariate subgroup was limited. The data suggested that analysis method of HR might not be the induced factor. We could not perform subgroup analysis to explore this influence because few studies offered concrete data. Finally, the concentrations of different antibodies could influence the result.

In conclusion, our meta-analysis identified that aberrant overexpression of CXCR4 is associated with survival, distant metastasis, status of lymph node and histologic subtype in NSCLC. Furthermore, elevated CXCR4 expression in cytoplasm and whole tumor cells is markedly related to poor outcome. However, when elevated CXCR4 expression in nucleus, it may be a favorable prognostic factor in NSCLC. Further large-scale clinical researches should be performed to investigate the precise prognostic significance of CXCR4 in NSCLC, especially in different histological types and nucleus.

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Disclosure of conflict of interest

None.

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Table S1. Meta-analysis of CXCR4 overexpression and clinicopathological features in NSCLC patients

Categories	Studies (No. of patients	OR (95% CI)	I^2 (%)	P_h	Z	*P*	95% PI
Gender (male/female)	5 (871)	1.03 (0.76, 1.39)	0	0.764	0.18	0.857	na
NSCLC subtype (AC/Non-AC)	6 (916)	1.45 (1.07, 1.95)	0	0.625	2.41	0.016	na
T category (T1+2/T3+4)	2 (362)	0.66 (0.22, 2.03)^R	81	0.004	0.72	0.472	(-3.20, 5.44)
N category (N-/N+)	4 (730)	0.69 (0.50, 0.96)	39.5	0.158	2.21	0.027	na
Distant metastasis (M0/M1)	4 (663)	0.36 (0.14, 0.93)^R	67.4	0.015	2.11	0.035	(-1.91, 2.98)
TNM stage (I+II/III+IV)	4 (810)	0.64 (0.39, 1.04)^R	60.7	0.038	1.8	0.073	(-0.59, 2.02)

Note: All pooled ORs were derived from fixed-effects model except for cells marked with R; R denotes random-effects model; P_h denotes *P* value for heterogeneity based on Q test; *P* denotes *P* value for statistical significance based on Z test; 95% PI denotes the distribution of true effect sizes; OR, Odd Ratio; CI, Confidence Interval.

Table S2. Results of Egger's test and Begg's test and trim and fill analysis

Comparison	Egger's test			Begg's test		Trim and Fill			
	t	P_e	95% CI	Z	P_b	SFP	n	t&fHR/OR (t&f 95% CI)	ΔHR/OR
Overall survival	-0.04	0.966	(-1.81, 1.74)	0.73	0.466	neither	0	1.59 (1.36, 1.87)	0
Disease-free survival	-3	0.058	(-13.75, 0.41)	1.22	0.221	neither	0	1.00 (0.37, 2.69)	0
Gender (male/female)	-1.4	0.233	(-5.09, 1.67)	0.38	0.707	neither	0	1.03 (0.76, 1.39)	0
NSCLC subtype (AC/Non-AC)	0.91	0.406	(-1.66, 3.47)	0	1	left	1	1.38 (1.02, 1.86)	-0.07
T category (T1+2/T3+4)	3.13	0.197	(-62.10, 102.65)	1.04	0.296	left	2	0.26 (0.18, 0.37)	-0.40
N category (N-/N+)	-0.28	0.796	(-6.98, 5.84)	0.73	0.462	neither	0	0.69 (0.50, 0.96)	0
Distant metastasis (M0/M1)	0.49	0.66	(-5.55, 7.55)	0.24	0.806	neither	0	0.36 (0.14, 0.93)	0
TNM stage (I+II/III+IV)	-1.09	0.356	(-24.55, 12.03)	0.73	0.462	neither	0	0.64 (0.39, 1.04)	0

Note: SFP denotes the side of the funnel plot where samples are imputed: L= left, R= right, N= neither; n denotes the number of samples imputed by trim and fill analysis; t&f HR/OR (t&f 95% CI) denotes trim and fill adjusted hazard ratio/odd ratio (trim and fill adjusted 95% confidence interval); ΔHR/OR denotes the difference between hazard ratio/odd ratio without trim and fill adjustment and hazard ratio/odd ratio with trim and fill adjustment; P_e for Egger's test; P_b for Begg's test.