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Original Article

Prognostic significance of CCL20 and its receptor CCR6 in cancers: a meta-analysis

Yunhai Li[1], Jing Huang[1], Hongtao Tie[2], Hongyan Huang[1], Hongzhong Li[1], Guosheng Ren[1]

[1]*Chongqing Key Laboratory of Molecular Oncology and Epigenetics, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China;* [2]*Department of cardiothoracic surgery, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China*

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Abstract: Growing evidence from recent publications has shown the association of CC chemokine ligand 20 (CCL20) and CC chemokine receptor 6 (CCR6) with outcomes of cancer, but the results remained controversial. The present meta-analysis was performed to investigate the prognostic value of CCL20 and CCR6 in cancer patients. PubMed and Embase were systematically searched to identify eligible studies. Two investigators independently performed study selection, data extraction and quality assessment. Hazard ratio (HR) and 95% confidence interval (CI) regarding overall survival (OS), disease-free survival (DFS) and relapse-free survival (RFS) were extracted and utilized to calculate pooled effect size. A total of 18 cohort studies with 2429 participants were included. High expression of CCL20 was demonstrated to associate with worse OS (HR = 2.38, 95% CI: 1.94-2.92, $P < 0.001$), DFS (HR = 2.16, 95% CI: 2.01-2.32, $P < 0.001$) and RFS (HR = 2.29, 95% CI: 1.58-3.33, $P < 0.001$) by fixed-effect model. Elevated CCR6 predicted poor OS (HR = 1.50, 95% CI: 1.02-2.20, $P = 0.040$) but not DFS (HR = 1.41, 95% CI: 0.56-3.53, $P = 0.460$) by random-effects model. Moreover, subgroup and sensitivity analyses for OS studies suggested that the results of CCL20 were robust, but caution should be taken for CCR6 analysis because of potential heterogeneity. This meta-analysis suggests that high expression of CCL20 and CCR6 predict poor prognosis in patients with carcinomas. Nevertheless, more well-designed and powerful cohort studies are required to further verify these conclusions.

Keywords: CCL20, CCR6, cancer, prognosis, meta-analysis

Introduction

Cancer as a global health problem contributes to the highest mortality in developed countries and the second highest in developing countries [1]. A total of 1,658,370 new cancer cases and 589,430 cancer deaths are predicted to occur in 2015 in the United States [2]. One of the main reasons for high cancer mortality rates is lack of effective methods of diagnosis and treatment, especially in early stage. Meanwhile, there are few valid biomarkers for prognosis, which could provide critical information for clinical treatment efficacy.

Chemokines are a superfamily of small chemotactic cytokines which are classified into C, CC, CXC and CX3C subfamilies based on the position of conserved cysteine residues and exerts their biological functions by interacting with special transmembrane G-protein-coupled receptor [3-5]. The CC chemokine ligand 20 (CCL20), also named macrophage inflammatory protein-3 alpha (MIP-3α), belongs to the CC subfamily and exhibits chemoattractant properties towards leukocytes [6]. Moreover, increasing evidence indicated that CCL20 plays a crucial role in cancer progression involving tumorigenesis, cell proliferation, angiogenesis, invasion and metastasis [7-9]. CC chemokine receptor 6 (CCR6) is a selective receptor for CCL20 [10]. Previous studies have demonstrated that CCR6 is expressed by dendritic cells, B-lymphocytes cells, memory T-lymphocytes as well as by tumor cells [6]. Currently, high expression of CCR6 has been detected in various cancers such as pancreatic cancer, hepatocellular carcinoma, colorectal malignancy and prostate cancer [11-14]. Dysregulated expression of CCR6 was shown to participate in many physiological progresses of cancer by interacting with CCL20 and closely associate with multiple

clinicopathological features such as tumor size, differentiation and TNM stage which strongly disclosed the potential role of CCL20 and CCR6 in early diagnosis and clinical treatment for cancer [15-17].

More importantly, accumulating data confirmed that elevated CCL20 and CCR6 in various types of cancers predicted distinctively poor outcomes with regard to overall survival (OS), diseases-free survival (DFS) and relapse-free survival (RFS), which suggested that CCL20 and CCR6 might serve as valuable prognostic markers for cancer patients [17-19]. Although both of the biomarkers have been intensively studied, most of the research studied them separately and the results were not fully consistent. Therefore, it is necessary to perform a meta-analysis to summarize those inconsistent literatures.

In the current study, we carried out the first comprehensive meta-analysis to evaluate the role of CCL20 and CCR6 as prognostic biomarkers in cancers. Particularly, we analyzed the prognostic value of CCL20 and CCR6 on OS, DFS and RFS. In addition, subgroup analyses were performed to evaluate whether diseases, specimen types, sample sizes, methods of estimating hazard ratio (HR) as well as quality of included studies influence the prognostic effect of CCL20 and CCR6. Sensitivity analyses were carried out to exam robustness of the results.

Materials and methods

The present meta-analysis was performed according to the guidelines of the Meta-analysis of Observational Studies in Epidemiology group (MOOSE) [20]. Study search, quality assessment, data extraction and statistical analysis were performed by two authors, individually. Any disagreement was resolved by discussing with a third author.

Search strategy

Two reviewers independently searched PubMed and Embase to identify relevant studies published from database inception to April 17, 2015 utilizing the following search terms: (CC chemokine ligand 20 or CCL20 or macrophage inflammatory protein-3-alpha or macrophage inflammatory protein-3α or MIP-3-alpha or MIP-3α or CC chemokine receptor 6 or CCR6) and (cancer or neoplasm or tumor or carcinoma) and (prognosis or survival or outcome). Manual search was performed to further obtain additional publications through the references of relevant literatures.

Inclusion and exclusion criteria

The following criteria must be satisfied in an eligible study: (1) patients were diagnosed with any type of cancer; (2) expression of CCR6 or CCL20 was measured in tumor tissues or blood specimens; (3) the association between CCR6 or CCL20 expression level and survival status was investigated. Articles were excluded according to the following criteria: (1) reviews, meeting articles, letters or laboratory studies; (2) studies dividing participants by non-dichotomous CCR6 and CCL20 levels; (3) lack of key information regarding survival outcomes such as HR, 95% confidence interval (CI) and *P*-value. When several articles about a study or the same patients cohort included in two or more publications were identified, only the latest or complete study was selected.

Quality assessment

Eligible studies were critically assessed according to Newcastle-Ottawa Quality Assessment Scale (NOS) [21] for cohort studies with moderate modification. Each included study was judged on 3 main perspectives: (1) the selection of the study groups, (2) the comparability of the groups and (3) the outcome of the groups. Study with a score of 6 or higher was considered as high quality.

Data extraction and conversion

Data as follows were extracted from all eligible studies: the name of first author, publication year, country of population, sample size, tumor type, specimen type, methods of detecting CCL20 and CCR6 expression, cut-off value and follow-up time and HR of CCL20 and CCR6 for OS, DFS and RFS along with 95% CI. When HR and 95% CI were not directly reported, the number of observed events and the quantity of each group were extracted to compute HR using methods described by Parmar [22]. Alternatively, if only Kaplan-Meier curves were provided, we estimated HR and 95% CI by extracting survival data from the plots [22, 23]. If the article contained insufficient data, we

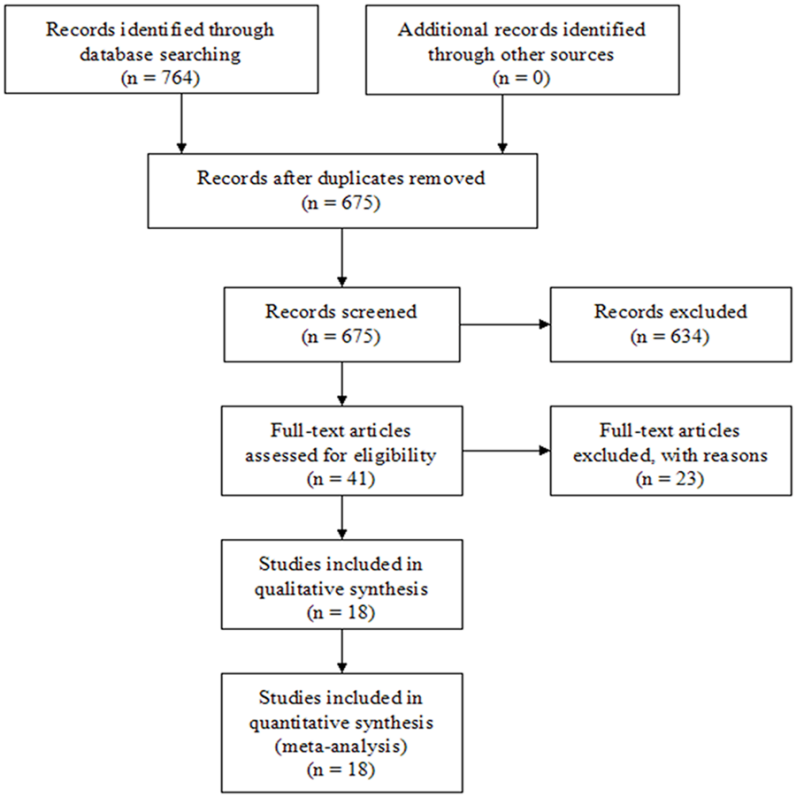

Figure 1. Flow chart of the literature research and selection of included studies.

tried to contact the authors by email to obtain as much useful information as possible.

Statistical analysis

HRs was combined to ensure the prognostic value of CCL20 and CCR6. HRs > 1 indicated poor prognosis in cancer patients with high expression of CCL20 and CCR6 and would be considered statistically significant if the 95% CI did not overlap 1. Heterogeneity analysis among eligible studies was carried out utilizing Cochran's Q test and Higgins's I^2 static. Statistical heterogeneity was defined as $P < 0.10$ or $I^2 > 50\%$. If $P > 0.10$ and $I^2 < 50\%$, fixed-effect model was utilized to calculate pooled HR. On the contrary, if statistical heterogeneity was observed, random-effects model was applied to evaluate combined HR. Sensitivity analyses were performed to test the robustness of the results. Subgroup analyses were carried out according to diseases, specimen types, ethnicities, sample sizes, HR estimation methods and NOS scores. Publication bias was assessed by Egger's test and funnel plots among eligible studies [24]. All of the *P* values two-sided; it was considered statistically significance when $P < 0.05$. All analyses were performed by Review Manager software version 5.2 (http://tech.cochrane.org/revman) and Stata software version 12.0 (http://www.stata.com/).

Results

Study selection

A total of 675 articles were identified by initial search algorithm. Among them, 634 articles were excluded by scanning titles and abstracts for the reason that they were reviews, meeting reports, letters or irrelevant to the present study. After reading full texts of the remaining 41 publications, we excluded 23 reports because 21 articles did not report survival data and two articles did not contain key information for evaluating HR. It was worth mentioning that there were two studies [25, 26] included in this meta-analysis, both analyzed the prognostic effect of CCL20 on OS in nasopharyngeal carcinoma and were performed by the same author Chang et al. However, no evidence showed that the same patients were included in both of the studies. Hence, we included the two studies in this meta-analysis. Finally, 18 studies were included [12, 17, 18, 25-39] in the present meta-analysis. The flow chart of the literature research and study selection is shown in **Figure 1**.

Characteristics of included studies

Among the 18 studies published from 2006 to 2015, nine studies investigated CCL20, eight studies investigated CCR6 and there was one article [33] that reported CCR6 and CCL20 together. A total of 1488 participants from China, Japan, France and Israel and 1069 participants from China, Japan and Taiwan were enrolled in CCL20 and CCR6 analyses, respec-

Table 1. Characteristics of the studies included in this meta-analysis for CCL20 and CCR6

Study	Country	Biomarker	Cancer type	Sample size	Specimen type	Assay	Cut-off for high expression	HR estimation	Analysis	Follow-up
Wang 2015	China	CCL20	NSCLC	54	Tissue	IHC	NR	DE	OS	0-50
Cai 2014	China	CCL20	NPC	140	Serum	ELISA	> 110 pg/ml	R	OS	48 (5-61)
Zhang 2014	China	CCL20	NSCLC	203	Serum	ELISA	> 57 pg/ml	DE	RFS	0-24
Cheng 2014	China	CCL20	CRC	213	Tissue	IHC	IRS > 4-6	DE	OS, DFS	61 (3-105)
Itawa 2013	Japan	CCL20	CRC	242	Serum	ELISA	> 28.2 pg/ml	R	OS	47.6 (5.1-142.1)
Ding 2012	China	CCL20	HCC	125	Tissue	IHC	IHC score > 182	R	OS, RFS	47.9 (6-76)
Chang 2011	Taiwan	CCL20	NPC	129	Serum	ELISA	> 6.5 pg/ml	DE	OS	0-60
Chang K 2011	Taiwan	CCL20	OSCC	99	Tissue	IHC	IHC score > 150	R, DE	OS, DFS	0-72
Chang 2008	Taiwan	CCL20	NPC	155	Serum	ELISA	> 65 pg/ml	R	OS	0-80
Wang 2012	China	CCL20,CCR6	Glioma	128	Tissue	IHC	IRS > 5	DE	OS	0-60
Qiu 2015	China	CCR6	HCC	50	Tissue	IHC	Score > 4	DE	OS	0-60
Liu J 2014	China	CCR6	CRC	191	Tissue	IHC	> Median	DE	OS	0-72
Liu 2014	China	CCR6	HCC	212	Tissue	IHC	Score > 4	R	OS	0-72
Minamiya 2011	Japan	CCR6	LA	84	Tissue	RT-PCR	Specific value > 3	R, DE	OS, DFS	0-60
Kirshberg 2011	Israel	CCR6	LA	49	Tissue	IHC	Positive cell > 50%	DE	DFS	0-125
Cassier 2011	France	CCR6	Breast cancer	202	Tissue	IHC	NR	R	OS, RFS	120 (117.6-121.2)
Andre 2006	France	CCR6	Breast cancer	123	Tissue	IHC	Score > 1	R	OS, DFS	156 (3.6-332.4)
Cuhida 2006	Japan	CCR6	HCC	30	Tissue	RT-PCR	> Mean	DE	DFS	0-60

NSCLC, no-small cell lung cancer; NPC, nasopharyngeal carcinoma; CRC, colorectal cancer; HCC, hepatocellular carcinoma; OSCC, oral cavity squamous cell carcinoma; LA, lung adenocarcinoma; IHC, immunohistochemistry; NR, not reported; IRS immunoreactive score, DE data extrapolated; R, reported.

Table 2. Study quality assessment based on Newcastle-Ottawa Scale (NOS)

Study	1	2	3	4	5A	5B	6	7	8	Scores
Wang 2015	*	*	*				*	*	*	6
Cai 2014	*	*	*	*	*	*	*	*	*	9
Zhang 2014	*	*	*	*	*	*	*		*	8
Cheng 2014	*	*	*		*	*	*	*		7
Itawa 2013	*	*	*		*	*	*	*	*	8
Ding 2012	*	*	*	*	*	*	*	*	*	9
Chang 2011	*	*	*	*			*	*	*	7
Chang K 2011	*	*	*	*	*	*	*	*		8
Chang 2008	*	*	*	*	*	*	*	*		8
Wang 2012	*	*	*		*	*	*	*	*	8
Qiu 2015	*	*	*		*	*	*	*	*	8
Liu J 2014	*	*	*		*	*	*	*		7
Liu 2014	*	*	*		*	*	*	*	*	8
Minamiya 2011	*	*	*	*	*	*	*	*	*	9
Kirshberg 2011	*	*	*	*			*	*		6
Cassier 2011	*	*	*	*	*	*	*	*	*	9
Andre 2006	*	*	*				*	*	*	6
Cuhida 2006	*	*	*	*	*	*	*	*	*	9

tively. The median sample size for CCL20 was 134.5 (range from54 to242) and that for CCR6 was 123 (range from 30 to 212). The included studies investigated a wide range of cancers including nasopharyngeal carcinoma, lung cancer, hepatocellular carcinoma, oral cavity squamous cell carcinoma, breast cancer, colorectal cancer and glioma. The majority of the studies detected CCR6 and CCL20 in cancerous tissue, yet five studies examined CCL20 in blood serum. Enzyme-linked immunosorbent assay (ELISA), immunohistochemistry (IHC) and reverse transcription-polymerase chain reaction (RT-PCR) were major methods to detect the expression of CCL20 and CCR6. It was notable that there was no identical standard about the cut-off value of CCL20 or CCR6 expression. For CCL20, the most common outcomes were OS which included nine studies, among them two studies reported OS and DFS together and one reported OS and RFS together. Besides, one study only reported RFS for CCL20. As for CCR6, there were four studies investigating OS, three reporting OS combined with DFS and one combined with RFS. In addition, two studies regarded DFS as primary outcome for CCR6. The main characteristics of eligible studies are summarized in **Table 1**.

Quality assessment

Specifically, we assessed representativeness of the exposed cohort, selection of the non-exposed cohort, ascertainment of exposure, outcome of interest, comparability of cohorts, assessment of outcome and adequacy of follow up for each study. The scores of the 18 studies ranged from 6 to 9. The results of quality assessment are shown in **Table 2**.

Data synthesis: CCL20

For nine studies involving 1285 cancer patients analyzing the relationship between CCL20 expression and OS, statistical heterogeneity was not observed (I^2 = 0%, P = 0.82). Therefore, fixed-effect model was applied to calculate the pooled HR with the corresponding 95% CI. The result showed that high expression of CCL20 significantly correlated with worse OS in various types of cancers (HR = 2.38, 95% CI: 1.94-2.92, P < 0.001) (**Figure 2A**).

Subsequently, subgroup analyses were carried out according to the diseases, specimen types, sample sizes, HR estimation methods and NOS scores. None of the subgroups had significantly different combined HR compared to overall analysis. All results of subgroup analyses are summarized in **Table 3**. Moreover, sensitivity analysis was performed by omitting one study at a time and the pooled HRs were ranged from 2.29 (95% CI: 1.86-2.82) to 2.51 (95% CI: 1.99-3.16) which further indicated that the result of OS analysis was robust.

Although there were only two studies including 312 patients reported the effect of CCL20 on DFS and two containing 328 patients on RFS, we analyzed the combined HR and the results showed that pooled HR for DFS was 2.16 (95% CI: 2.01-2.32, P < 0.001) and for RFS was 2.29 (95% CI: 1.58-3.33, P < 0.001), providing hints that elevated CCL20 was associated with reduced DFS and RFS in cancer patients (**Figure 2B** and **2C**).

Data synthesis: CCR6

Seven studies with 990 participants were subjected to evaluate the association between CCR6 and OS. The random-effects model was used to calculate the pooled HR due to evident heterogeneity detected among the seven stud-

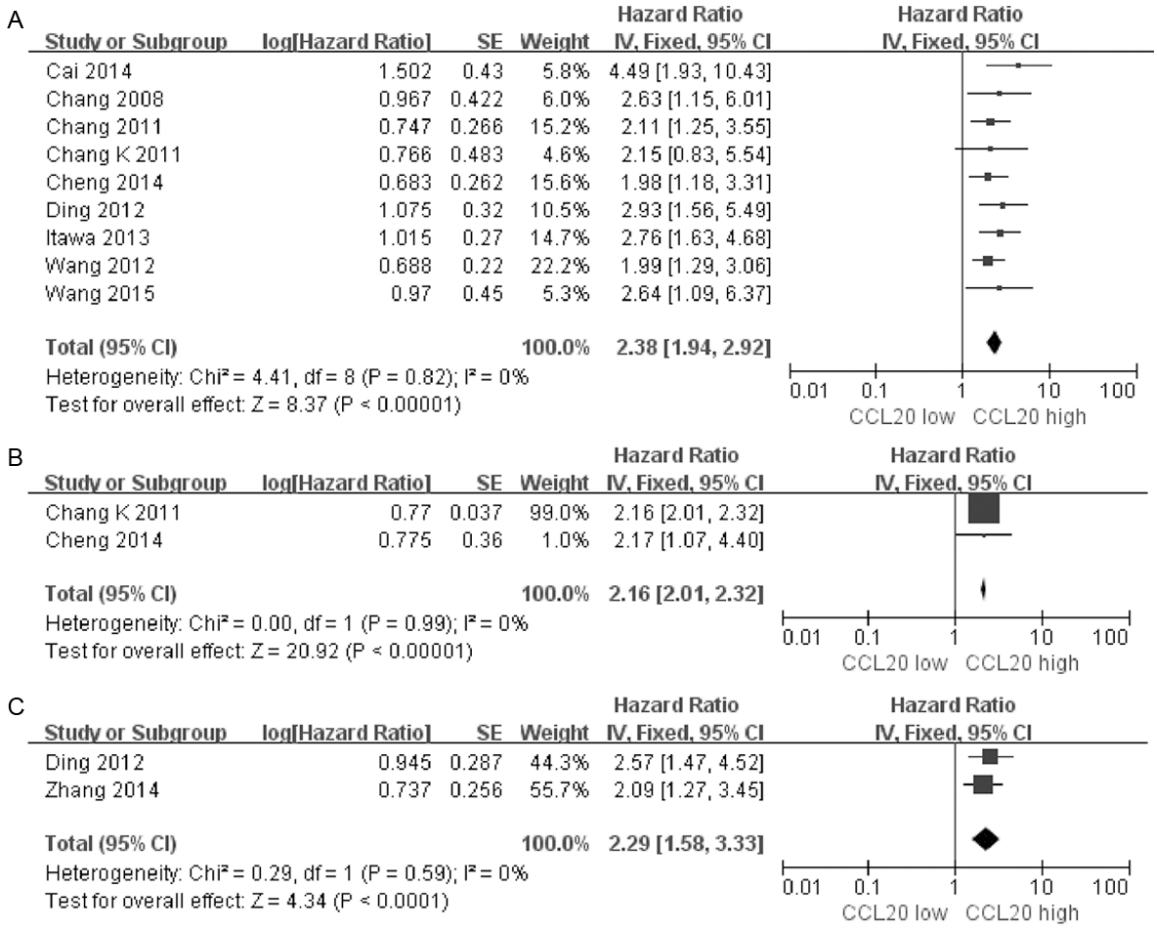

Figure 2. Forest plots of the analyses about CCL20. Survival data were reported as OS (A), DFS (B) and RFS (C).

ies (I^2 = 77%, P < 0.001). The result showed that high expression of CCR6 moderately predicted poor OS (HR = 1.50, 95% CI = 1.02-2.20, P = 0.040) (**Figure 3A**).

In order to analyze the source of heterogeneity and robustness of the result, subgroup analyses were performed based on ethnicity, types of cancer, sample sizes, HR estimation methods and NOS scores (**Table 3**). To a large extent heterogeneity was reduced within two non-Asian ethnicity studies (I^2 = 0%, P = 0.48), three non-digestive system tumor studies (I^2 = 54%, P = 0.12) and four digestive system tumors studies (I^2 = 62%, P = 0.05), which indicated that ethnicity and diseases were the factors responsible for high heterogeneity. Besides, sensitivity analysis was also performed to explore the heterogeneity. Yet obvious heterogeneity still existed by omitting one study at a time with the combined HRs ranging from 1.32 (95% CI: 0.91-1.93) to 1.71 (95% CI: 1.21-2.40) which demonstrated that the statistical heterogeneity was not generated by one study, and that no individual study was dominant in the pooled HR.

Four studies exploring the relationship between CCR6 and DFS were utilized to pool HR by random-effects model due to prominent heterogeneity (I^2 = 79%, P = 0.003). However, the combined HR was calculated to be 1.41 (95% CI: 0.56-3.53, P = 0.46), showing no significant connection between high expression of CCR6 and DFS (**Figure 3B**). Regretfully, there was only one study [30] reporting the impact of CCR6 on RFS which suggested that CCR6 was not associated with RFS in multivariate analysis (HR = 1.93, 95% CI: 0.99-3.77, P = 0.55).

Publication bias

Both funnel plot and Egger's test were used to evaluate the publication bias of the OS studies.

Table 3. Subgroup analyses of CCL20 and CCR6 on OS

Group by	Subgroup	Studies (N)	Heterogeneity		Meta-analysis model	Pooled HR	95% CI	*P*-value
			I^2	*P*-value				
CCL20/Specimen type	Serum	4	0%	0.520	Fixed	2.66	1.94-3.64	< 0.001
	Tissue	5	0%	0.860	Fixed	2.20	1.69-2.87	< 0.001
CCL20/Disease	Colorectal cancer	2	0%	0.380	Fixed	2.33	1.61-3.36	< 0.001
	NPC	3	10%	0.330	Fixed	2.61	1.76-3.85	< 0.001
CCL20/Sample size	Number > 150	3	0%	0.650	Fixed	2.37	1.70-3.32	< 0.001
	Number < 150	6	0%	0.610	Fixed	2.39	1.85-3.08	< 0.001
CCL20/HR estimation	Reported	4	0%	0.780	Fixed	3.01	2.15-4.20	< 0.001
	DE	5	0%	0.990	Fixed	2.08	1.61-2.68	< 0.001
CCL20/NOS score	NOS score ≥ 8	6	0%	0.640	Fixed	2.54	1.97-3.28	< 0.001
	NOS score ≤ 7	3	0%	0.860	Fixed	2.12	1.51-2.97	< 0.001
CCR6/Ethnicity	Asian	5	78%	0.001	Random	1.73	1.09-2.76	0.020
	Non-Asian	2	0%	0.480	Fixed	1.00	0.69-1.44	1.000
CCR6/Disease	Digestive system	4	62%	0.050	Random	2.10	1.49-2.97	<0.001
	Non-digestive system	3	54%	0.120	Random	0.82	0.47-1.44	0.490
CCR6/Sample size	Number >150	3	76%	0.020	Random	1.82	1.10-3.01	0.020
	Number <150	4	82%	0.001	Random	1.22	0.61-2.43	0.580
CCR6/HR estimation	Reported	3	86%	0.001	Random	1.51	0.68-3.34	0.310
	DE	4	75%	0.008	Random	1.48	0.89-2.46	0.130
CCR6/NOS score	NOS score ≥ 8	5	78%	0.001	Random	1.62	0.92-2.86	0.100
	NOS score ≤ 7	2	76%	0.040	Random	1.23	0.74-2.05	0.430

No significant publication bias was observed for the nine studies of CCL20 ($P = 0.159$) or the seven studies of CCR6 ($P = 0.198$) by Egger's test and funnel plots (**Figure 4**).

Discussion

In the present meta-analysis, we investigated the evidence that demonstrated the prognostic value of CCL20 and CCR6 to obtain a further understanding of the two biomarkers. The results demonstrated that high expression of CCL20 was significantly related to poor OS, DFS and RFS. Moreover, subgroup and sensitivity analyses further clarified the reliability of the results. As for CCR6, we proved that elevated CCR6 predicted worse OS but not DFS. Nevertheless, conclusion should be interpreted cautiously and needed to be refined for several reasons. Above all, the number of eligible cohort studies was insufficient to draw a completely convincing conclusion to date. Besides, all the included studies reported multiple cancers that might be a source of potential heterogeneity. Therefore, caution should be taken when apply the conclusion to a specific cancer.

Interestingly, among all the included studies in this meta-analysis, there was one article [33] that reported the prognostic value of co-expression of CCL20 and CCR6 which demonstrated that CCL20-high/CCR6-high expression was correlated with worse OS and was identified as an independent prognostic factor by Cox proportional hazard model for patients with gliomas. Considering the close relationship between CCL20 and CCR6, we speculate that combining both of the biomarkers might strengthen the prognostic effect of survival status for cancer patients. Hence, many more studies are needed to be performed to investigate the prognostic value of CCL20 combined with its special receptor not only in gliomas but also in some other carcinomas.

Recently, there were reviews paying attention to the crucial roles of both of the biomarkers in cancer progression and prognosis. A review by Huang et al. found that CCL20 and CCR6 were associated with hepatocellular carcinoma involving low differentiation and poor prognosis [40]. Ghadjar et al. reviewed studies focusing on colorectal cancer and showed that interaction of CCL20 and CCR6 participated in colorec-

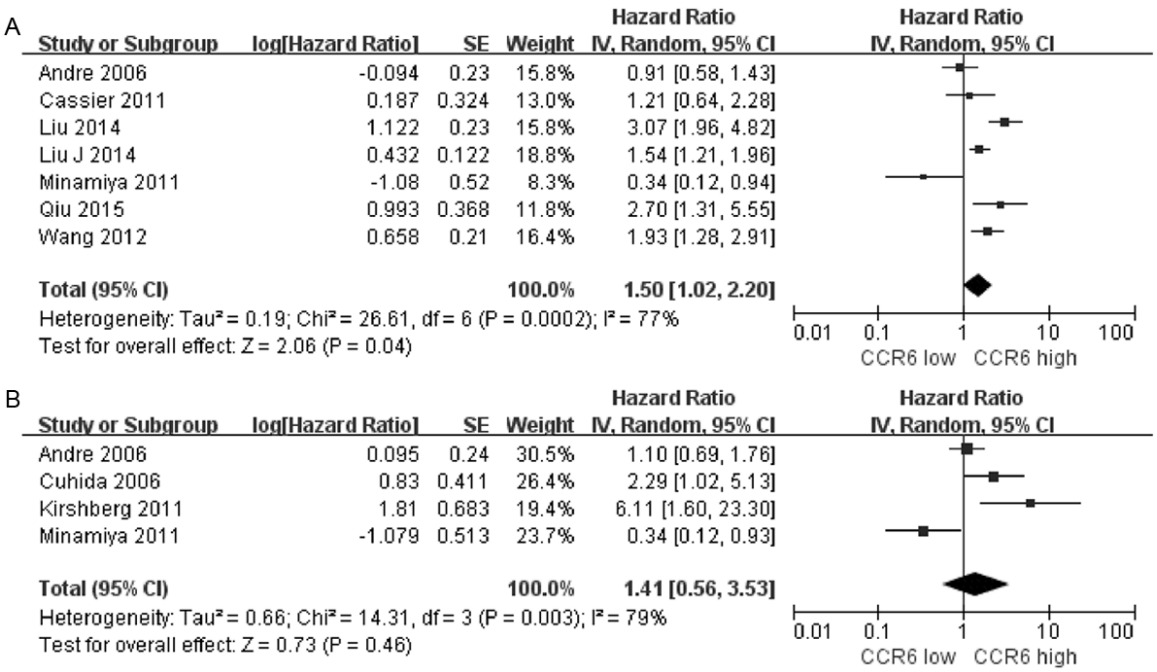

Figure 3. Forest plots of the analyses about CCR6. Survival data were reported as OS (A) and DFS (B).

tal liver metastasis which accounted for the high mortality in patients [19]. However, both of them just simply summarized poor effect of CCL20 and CCR6 rather than systematically analyzed relevant literatures or provided exact pooled HR which could effectively show the prognostic value. Likewise, there were two studies that investigated the relationship between CCL20 or CCR6 and prognostic status providing no HRs or key information for calculating it, one performed by Hou et al. [41] demonstrated that expression of CCL20 in hepatocellular carcinoma closely corrected with OS ($P < 0.001$) and RFS ($P < 0.001$), the other carried out by Zhang et al. [42] showed that patients with higher CCL20 will suffer a shorter period of time ($P = 0.0198$) in non small cell lung cancer. Compared to those publications, the present meta-analysis systematically summarized 18 high-quality cohort studies and offered convincing evidence by pooling HRs.

It is well known that CCL20 and its receptor CCR6 are responsible for the recruitment of immature dendritic cells to inflammatory environment [6]. Currently, emerging publications have demonstrated that the ligand-receptor pair CCL20/CCR6 was utilized by cancer cells for multiple physiological functions involving tumorigenesis, angiogenesis, invasion and metastasis [40, 43-45]. Moreover, reports revealed that Akt, ERK-1/2, SAPK/JNK and MAPKs signal pathways could be activated by CCL20 and resulted in a significant increase of cell proliferation and migration [46]. Zeng et al. demonstrated that CCL20/CCR6 promoted cell invasion and migration via activating NF-κB and stimulating the expression and secretion of MMP-3 [47]. CCR6 was illustrated to play a crucial role in liver metastasis of colon, thyroid and ovarian tumors with a potential mechanism that CCR+ caner cells could be attracted and selected by liver that constitutively expressed CCL20 [48]. Additionally, high expression of CCL20 and CCR6 has been shown in multiple tumors such as breast cancer, lung cancer, colorectal malignancy, hepatocellular carcinoma [49-52]. Previous studies suggested that CCL20 and CCR6 were demonstrated to be remarkably overexpressed compared with the normal cancer tissues, and that elevated CCL20 and CCR6 were not only significantly associated with multiple physiological functions but also closely related to clinicopathological characteristics [53, 54].

More importantly, a series of cohort studies disclosed that high expression of CCL20 or CCR6 was strongly correlated with prognosis in various types of cancers. Cheng et al. conclud-

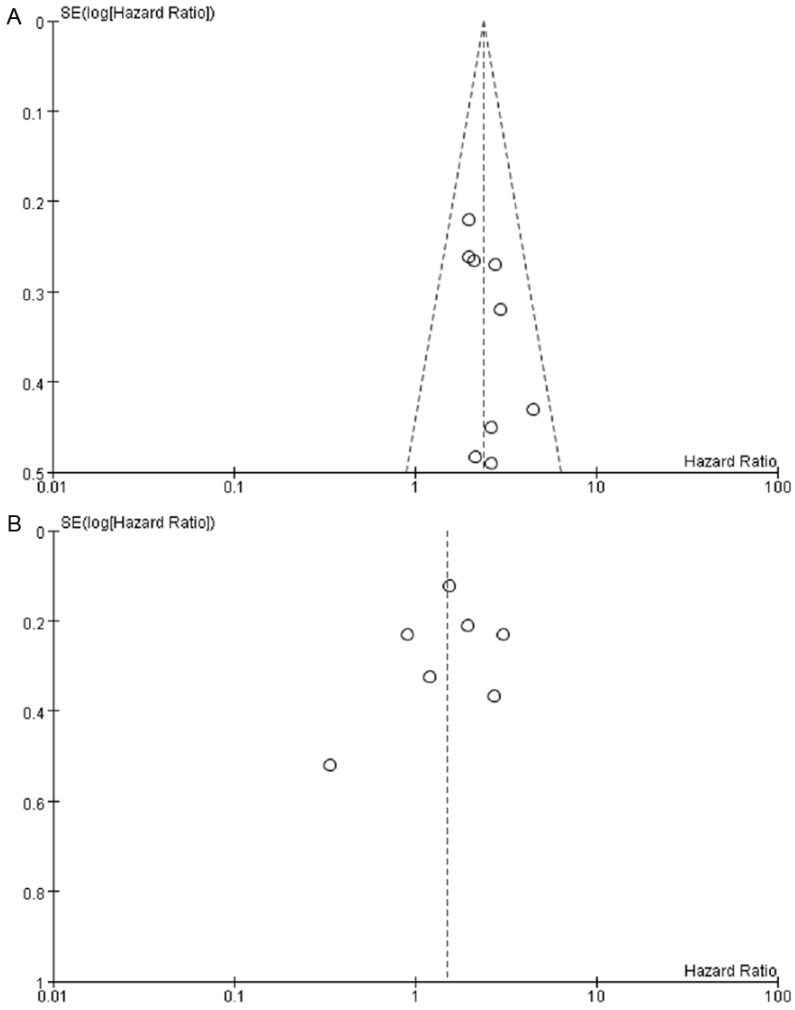

Figure 4. Funnel plots for publication bias of the included OS studies about CCL20 and CCR6. OS studies of CCL20 (A) and OS studies of CCR6 (B).

ed that patients with high CCL20 level had poorer OS and DFS compared to those with lower level of CCL20 in colorectal cancer [34]. At the same time, another group demonstrated that high expression CCL20 in hepatocellular carcinoma was an independent risk factor for prognosis by multivariate analyses [37]. Moreover, up-regulated CCR6 could effectively predict prognosis in patients with colorectal cancer, hepatocellular carcinoma and glioma [12, 27, 33]. However, stronger expression of CCR6 was also proved to be an independent predictor of better prognosis in lung adenocarcinoma [28]. Though previous studies were shedding light on the correlation between prognosis and the expression of CCL20 and CCR6, there was no quantitative analysis that was carried out to help determine their prognostic value. To the best of our knowledge, this is the first meta-analysis performed to gather those scattered and discrepant results and pool the significant prognostic effects of the two biomarkers.

However, our meta-analysis does have several limitations. Firstly, the total number of patients included in the studies was relatively small with only 1488 and 1069 for CCL20 and CCR6, respectively, and the sample sizes of several studies were rather small. For instance, the studies of Kirshberg et al. [29], Cuhida et al. [31] and Qiu et al. [17] included no more than 50 patients. Therefore, to this issue many more high quality studies with larger sample sizes are needed to further strengthen our conclusion. Secondly, potential heterogeneity was observed among CCR6 studies, which could not be effectively reduced via subgroup and sensitivity analyses. Hence, we used random-effects model to combine HRs to minimize the influence of heterogeneity on pooled effect size. Thirdly, survival data of several studies, such as Cheng et al. [34], Qiu et al. [17] and Liu et al. [27], could only be estimated by survival curves according to Parmar [22]. Although the estimated data approximates true values, it might weaken the reliability of the results. In addition, we failed to obtain the HRs of two studies [41, 42] that might enhance the convincingness of our meta-analysis despite our utmost efforts to contact the authors.

In summary, despite the limitations above, our study strongly suggests that high expression of CCL20 and CCR6 are associated with poor prognosis in various carcinomas. Considering the widespread involvement of CCL20 and CCR6 in tumor progression and the close rela-

tionship between outcomes of cancers and high expression of CCL20 and CCR6, both of the biomarkers may serve as crucial indicators that can effectively predict prognosis for cancer patients as well as potential candidates that can help to guide clinical treatment. In addition, more well-designed clinical trials with relatively larger sample sizes are needed to be performed to further verify the prognostic value of CCL20 and CCR6 in the near future.

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Disclosure of conflict of interest

None.

Address correspondence to: Hongzhong Li and Guosheng Ren, Chongqing Key Laboratory of Molecular Oncology and Epigenetics, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China. E-mail: tibetlhz@126.com (HZL); rengs726@126.com (GSR)

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