Clinicopathological and Prognostic Significance of CD44s and CD44v6 Expression in Patients with Glioma: A Systematic Review and Meta-analysis

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Abstract

Background: Cancer stem cell surface marker CD44 has been revealed to promote tumor growth, progression, and metastasis in gliomas. Although the prognostic and clinicopathological value of CD44 standard form (CD44s) and its variant isoform CD44v6 expression in glioma patients has been evaluated in several independent studies, their results remained controversial. Therefore, we performed this meta-analysis to investigate the prognostic and clinicopathological association of CD44s/CD44v6 expression with glioma patients.

Methods: A comprehensive literature search was performed in the electronic databases PubMed, EMBASE, Web of Science, the Cochrane Library, China National Knowledge Infrastructure (CNKI), and the Wangfang Data. The statistical analysis was conducted using Stata 15.0 and Review Manager 5.3.

Results: A total of 43 studies with 2817 glioma patients were included in this meta-analysis. Pooled results indicated that positive expression of CD44s was significantly associated with poorer overall survival (OS, univariate analysis, HR =1.63, 95% CI= [1.16–2.29], P=0.005; multivariate analysis, HR=2.14, 95%CI= [1.21, 3.78], P=0.009), and reduced progression-free survival (PFS) in the univariate analysis (HR=2.09, 95% CI= [1.59, 2.75], P<0.00001), but not with PFS in the multivariate analysis (P>0.05) or tumor recurrence (P>0.05). CD44 expression was significantly upregulated in glioma tissues when compared with non-tumorous brain tissues (CD44s, OR=31.31, 95% CI= [15.22, 64.43], P<0.00001; CD44v6, OR=13.18, 95% CI= [5.51, 31.51], P<0.00001). In particular, CD44 expression was preferentially expressed in high-grade gliomas (grade III-IV vs. grade I-II, CD44s, OR=4.67, 95% CI= [3.18, 6.87], P<0.00001; CD44v6, OR=2.06, 95% CI= [1.21, 3.51], P=0.008). CD44s expression was lower in brain metastases than that in primary gliomas (OR=0.25, 95% CI= [0.10, 0.60], P=0.002), however, higher expression of CD44v6 was detected in brain metastases when compared with primary gliomas (OR=49.44, 95% CI= [13.06, 187.22], P<0.00001).

Conclusions:This meta-analysis revealed the prognostic value of CD44s expression and clinicopathological significance of CD44s/CD44v6 expression in gliomas. Increased CD44s expression can predict worse prognosis of glioma patients. Particularly, CD44s is an independent prognostic factor for poor OS of glioma patients. Both CD44s and CD44v6 were glioma patients predominantly expressed in glioma tissues, especially in high-grade gliomas. Additionally, CD44v6 is a potential diagnostic biomarker for differentiating brain metastases from primary gliomas in individual cases. Therapeutic strategies targeting CD44 in gliomas should be further explored in the future.

Background

Gliomas are the most common malignant primary central nervous system (CNS) tumors, with an average annual age-adjusted incidence rate (AAAIR) of 6.0 cases per 100 000 population from 2010 to 2014 in the United States [1, 2]. According to The 2016 World Health Organization (WHO) Classification of Tumors of the CNS, gliomas can be classified into four grades (WHO grade I-IV) in terms of histopathological features and molecular classification [3]. Despite the dramatic improvements in the diagnosis and treatment of gliomas during the past few decades, the survival rate of malignant glioma patients remained extremely poor due to frequent tumor metastasis, recurrence, and resistance to chemo- and radiation therapy [4-6]. Therefore, it is imperative to explore valid biomarkers to precisely predict the prognosis of glioma patients and effectively provide novel therapeutic targets [7, 8].

Cancer stem cells (CSCs), also known as tumor-initiating cells (TICs), are hypothetically a small subpopulation of cancer cells that possess the capacity for self-renewal and asymmetric cell division as well as drive tumor initiation, progression, metastasis, recurrence and therapeutic resistance [9, 10]. CSCs in the glioma microenvironment, termed glioma stem cells (GSCs), play a critical role in tumorigenesis and recurrence of gliomas [11]. Thus, eradicating CSCs provided a novel target for cancer therapy [12-14]. Some cell surface markers have been reported as CSC markers, such as CD44, CD133, aldehyde dehydrogenase 1 (ALDH1), epithelial cell adhesion molecule (EpCAM) and CD147 [15-20]. High expression of these markers has been generally considered as an indicator of poor prognosis and unfavorable clinicopathological features [21-26]. Among these CSC markers, CD44 is one of the most frequently reported in gliomas.

CD44 is a transmembrane glycoprotein that plays a critical role in mediating cell adhesion and signaling transduction involved in many physiological and pathological processes [27, 28]. CD44 was initially described as a receptor for hyaluronic acid (HA), and the interaction between HA and CD44 receptor promotes cell motility, proliferation, differentiation and survival via PI3K/Akt and MAPK signaling pathways [29-31]. It has been revealed that overexpression of the CD44 standard form (denoted as CD44s) facilitates tumor progression, metastasis, invasion and resistance to apoptosis [28, 32]. In addition, multiple CD44 variant isoforms (CD44v1-CD44v10) generated by alternative mRNA splicing of 10 variable exons in the CD44 gene have also been implicated in tumor invasion, metastasis and drug resistance [33, 34]. Among them, CD44v6 is the most commonly reported splicing variant isoform in gliomas. It was reported that switching between CD44s and CD44 variants may be implicated in mediating epithelial to mesenchymal transition (EMT) [35, 36]. Moreover, CD44s/CD44v6 is recognized as a reliable companion in CSCs maintenance and tumor progression [36].

The prognostic value of CD44 for patients with cancer has been widely validated in various solid tumors, such as hepatocellular carcinoma [37, 38], non-small cell lung cancer [39-41], gastric cancer [42-45], ovarian cancer [46-48], colorectal cancer [49, 50], etc. Some studies have demonstrated that the overexpression of CD44s is a poor prognostic factor for gliomas [51]. Conversely, some other studies concluded that CD44s-positive expression predicted favorable survival outcomes [52, 53]. The prognostic and clinicopathological value of CD44 in glioma patients remained contradictory. To address these discrepancies, we performed this meta-analysis to systematically validate the prognostic and clinicopathological association of CD44 with glioma patients.

Materials And Methods

Search strategies

Meta-analysis was performed in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) Statement [54]. A systematic literature search was conducted in the electronic databases PubMed, EMBASE, Web of Science, the Cochrane Library, China National Knowledge Infrastructure (CNKI), and the Wangfang Data until October 2019, without any limitation of origin and languages. The search strategy included the following terms: “CD44”, “CD44s”, “CD44v6 OR CD44 variant 6”, “glioma OR glial cell tumor OR mixed glioma OR malignant glioma”, “prognosis OR survival OR outcome”. In addition, a manual search of the reference lists of the eligible studies and systematic reviews was performed for potentially relevant studies.

Selection criteria

The studies included in the present meta-analysis were randomized controlled trials or observational studies (case-control or cohort) that assessed the association between CD44s/CD44v6 expression and the prognostic outcomes or clinicopathological characteristics of patients with glioma. Studies were eligible if they met the following criteria: (a) studies were published as original articles with full text available; (b) diagnosis of glioma was proven by histopathological methods; (c) CD44s/CD44v6 expression was detected by an immunohistochemistry (IHC) or qRT-PCR method based on glioma tissues (instead of serum or other specimens); and (d) the correlation between CD44s/CD44v6 expression and clinicopathological features or prognostic outcomes was analyzed. Studies were excluded from the analyses based on the following criteria: (a) articles were published as reviews, abstracts, case reports, letters or comments; (b) studies were not associated with the topic of the interest; (c) data were obtained from cell lines or animal models; (d) data were analyzed based on public databases, such as TCGA, GEO, Oncomine, etc.; (e) data for estimating the relationship between CD44s/CD44v6 expression and survival outcomes or clinicopathological features were insufficient; and (f) data were from duplicated studies based on the same or similar patient population.

Data extraction

All data from the eligible studies were independently extracted by two investigators (KX and XYZ), and discrepancies in data extraction were resolved by a third investigator (WTG). The following data were collected from each included study in a predefined table: the name of first author, year of publication, country, WHO grades, number of patients, detection method, cut-off value, follow-up periods, clinicopathological parameters and prognostic outcomes (overall survival [OS], progression-free survival [PFS]). Given that some studies displayed the survival data indirectly with a Kaplan-Meier curve, the software Engauge Digitizer version 12 (http://markummitchell.github.io/engauge-digitizer/) was applied to digitize and extract survival data. Since the cut-off value for CD44 expression varied among different studies, we defined the CD44-positive/high group according to the original articles.

Qualitative assessment

The methodological quality of included studies was assessed by two independent investigators (KX and XYZ) using the Newcastle-Ottawa Quality Assessment Scale (NOS) (Supplementary Table S1). According to the guideline, NOS scores of $\geq$6 were determined to be high-quality studies.

Statistical analysis

All analyses were performed by utilizing the software Review Manager 5.3.5 (Cochrane Collaboration, Copenhagen, Denmark) and STATA 15.0 (Stata Corporation, College Station, TX, USA). The odds ratio (OR) with 95% CI were calculated to clarify the correlation between CD44s/CD44v6 expression and clinicopathological characteristics of glioma. $P<0.05$ were considered as statistical significance. To assess the prognostic effect of CD44s expression on glioma patients, the pooled hazard ratio (HR) with 95% CI of OS and PFS were calculated. If a pooled HR >1, it represents a worse prognosis for patients with positive/high CD44s expression, while a pooled HR<1 reflects a favorable prognosis. Heterogeneity among studies was assessed by the chi-square (χ^2) test and I^2 test. When there was no significant heterogeneity ($P>0.05$ or $I^2<50\%$), the fixed-effects model was employed; otherwise, the random-effects model was used. Subgroup analysis was performed to investigate the correlation of CD44s expression with overall survival (univariate analysis) in terms of ethnicity, counting method, cancer type, source of data and NOS score. Sensitivity analyses were performed to examine the robustness of pooled data. Begg’s and Egger’s tests were conducted to assess the potential publication bias.

Results

Description of studies

Detailed steps of literature search and study selection were shown in a flow diagram [54] (Fig. 1). A total of 1153 studies were initially retrieved with search strategies described above. In line with the selection criteria, 762 articles were left after duplicated records removed. After screening the titles and abstracts of identified articles, 555 articles were excluded due to irrelevant topics. The remaining articles were reviewed in full text, 164 articles were excluded, including 64 cell or animal studies, 13 reviews or abstracts, 84 studies without clinicopathological or survival data, and 3 studies based on data from public databases. A total of 43 studies were eventually included in the present meta-analysis. Thirty-one studies including 2205 cases were available for investigating the correlation between CD44s/CD44v6 expression and clinicopathological features of glioma and 15 studies

including 794 cases were available for evaluating the impact of CD44s expression on the survival outcomes of glioma patients. 3 studies were included in both qualitative and quantitative synthesis at the same time. The main characteristics of eligible studies in the quantitative and qualitative synthesis were summarized in Table 1 and Table 2, respectively.

Correlation between CD44s expression and overall survival

Fourteen studies [52, 53, 55-66], including 743 patients, reported overall survival (OS) data using univariate analysis (Table 1). With substantial heterogeneity (P<0.0001, I^2=72.2%), a pooled analysis in a random-effects model showed increased CD44s expression in glioma patients predicted reduced OS (pooled HR =1.63, 95% CI= [1.16, 2.29], P=0.005) (Fig. 2A). The heterogeneity may be generated from the studies by Pinel B *et al* [52] and Wei KC *et al* [53] as CD44s expression was correlated with favorable OS in their studies. When these two studies were omitted, the pooled analysis of the remaining thirteen studies [51] showed no heterogeneity (pooled HR=2.01, 95%CI= [1.66, 2.42], P<0.00001, I^2=0%). Moreover, subgroup analysis of OS on the basis of univariate data was conducted and stratified in terms of ethnicity, counting method, cancer type, source of data and NOS score (Table 3). The results revealed that increased CD44s expression was significantly associated with OS in the following subgroups, including European & American, IHC method, mixed gliomas, survival data from Kaplan-Meier curves and NOS score > 7. No significant association was observed in other subgroups.

In addition, a pooled analysis of four studies [52, 60, 61, 64] including 312 patients investigated the correlation between CD44s expression and OS based on multivariate data (Table 1). Without heterogeneity (P=0.903, I^2=0%), a fixed-effects model showed the similar result (pooled HR=2.14, 95% CI= [1.21, 3.78], P=0.009) (Fig. 2B).

Correlation between CD44s expression and progression-free survival

A total of 235 patients from four studies [60-62, 67] were included in the univariate analysis of the correlation of CD44s expression with progression-free survival (PFS) (Table 1). The pooled result indicated that high expression of CD44s was associated with poorer PFS (pooled HR=2.09, 95% CI= [1.59, 2.75], P<0.00001, I^2=0%) (Fig. 2C). However, according to Iwadate Y *et al*[61], CD44 is not an independent prognostic factor for PFS of patients with GBM in the multivariate Cox regression analysis (HR=2.481, 95% CI= [0.962, 6.402], P=0.0603).

Correlation between CD44s/CD44v6 expression and clinicopathological parameters

Eleven studies [68-78] and three studies [72, 79, 80] compared the differential expression of CD44s and CD44v6 in glioma tissue samples vs. adjacent non-tumorous brain tissue samples (including normal brain tissue and pan-cancer brain tissue), respectively (Table 2). The pooled analysis showed both CD44s and CD44v6 were predominantly expressed in paired glioma tissues than that in adjacent non-tumorous brain tissues (CD44s, OR=31.31, 95% CI= [15.22, 64.43], P<0.00001, I^2=33%; CD44v6, OR=13.18, 95% CI= [5.51, 31.51], P<0.00001, I^2=0%) (Fig. 3A, 3B).

According to the WHOClassifications of Tumors of the CNS, gliomas can be classified into four grades (grade I-V) [3]. Twelve studies [59, 63, 70-73, 75, 76, 78, 81-83] with 737 glioma patients and five studies [69, 84-87] with 406 glioma patients investigated the correlation of CD44s-positive expression and CD44s-high expression (in terms of different cut-off values of CD44s expression) with WHO grades of glioma, respectively (Table 2). The pooled results showed CD44s was significantly overexpressed in grade III-IV group when compared with grade I-II group (positive expression, OR=4.67, 95% CI= [3.18, 6.87], P<0.00001, I^2=23%; high expression, OR=3.51, 95% CI=[2.12, 5.81], P<0.00001, I^2=0%) (Fig. 3C, 3D). Besides, a pooled analysis of five studies [72, 79, 88-90] with 329 glioma patients investigating the association between CD44v6-positive expression and WHO grades of glioma demonstrated that CD44v6 expression was also upregulated in higher tumor grade (grade III-IV vs. grade I-II, OR=1.98, 95% CI= [1.15, 3.40], P=0.01, I^2=0%) (Table 2; Fig. 3E).

Frequent recurrence of high-grade gliomas is an inevitable problem in clinical practice despite a combined treatment with surgery, chemo- and radiation therapy [91]. Three studies [58, 59, 76], including 137 primary glioma samples and 66 recurrent glioma samples, investigated the relationship between CD44s expression and recurrence of glioma (Table 2). The pooled result showed there was no significant difference in CD44s expression between recurrent and primary gliomas (OR=1.70, 95% CI= [0.87, 3.33], P=0.12, I^2=0%) (Fig. 3F).

Brain metastases (BM) is a major neurological complication of advanced cancer that frequently originate from lung, breast, and skin by means of hematogenous dissemination [92, 93]. Six studies [68, 73, 87, 94-96] with 254 glioma patients and five studies [80, 87, 96-98] including 339 glioma patients investigated the differential expression of CD44s and CD44v6 in brain metastases vs. primary glioma tissues, respectively (Table 2). The pooled analysis revealed that CD44s-positive expression in brain metastases was lower than that in primary gliomas (OR=0.25, 95% CI= [0.10, 0.60], P=0.002, I^2=13%) (Fig. 3G). However, with substantial heterogeneity (P=0.03, I^2=62%), a random-effects model showed that a remarkably increased expression level of CD44v6 was noted in brain metastases when compared with primary gliomas (pooled OR=49.92, 95% CI= [8.93, 279.06], P<0.00001) (Fig. 3H).

Sensitivity analysis and publication bias.

A sensitivity analysis was performed to verify the stability of each pooled study (Fig. 4). As shown in Table 4, no individual study could statistically significantly alter the combined results of survival outcomes and clinicopathological parameters, except the study by Hou CX *et al*[60] which can affect the pooled HR of CD44s expression on OS in multivariate analysis (Fig. 4B), the study by Du Q *et al* [79] included in the pooled analysis of the

correlation between CD44v6 expression and WHO grades of glioma (Fig. 4H), and the study by Wei HQ *et al* [87] which can affect the pooled OR of CD44s expression between brain metastases and primary gliomas (Fig. 4J).

Begg's test (Fig 5A-5C, S1) and Egger's test (Fig 5D-5F, S2) were conducted to evaluate the potential publication bias of survival outcomes and clinicopathological parameters. As shown in Table 4, p values assessed by Begg's test and Egger's test were all greater than 0.05 except the evidence of significant publication bias (P<0.05) in three pooled studies (Fig 5). Therefore, the trim and fill method was utilized to evaluate the potential impacts of publication bias. For the pooled analysis of the association of CD44s expression with PFS of gliomas in the univariate analysis (Begg's test, P=0.089; Egger's test, P=0.011), a filled funnel plot was generated by trim and fill analysis including two imputed studies, and the meta-analysis incorporating these two imputed studies demonstrated the similar result (adjusted HR=1.956, 95% CI= [1.518–2.521]; P<0.001) (Fig. 6A). Similarly, trim and fill analysis including two imputed studies generated a symmetrical funnel plot for the pooled analysis of CD44v6 expression between brain metastases and primary gliomas, and the meta-analysis incorporating these two imputed studies demonstrated the semblable result (adjusted HR=23.222, 95% CI= [5.415–99.595]; P<0.001) (Fig. 6B). Intriguingly, despite a significant publication bias in the analysis of the association between CD44s expression and tumor recurrence (Begg's test, P=1.000; Egger's test, P=0.017), the trim and fill analysis revealed that no trimming was performed and thus pooled data remained unchanged. In conclusion, the results of the three pooled studies were robust in spite of significant publication bias.

Discussion

CD44, a commonly reported CSC marker, plays a critical role in mediating malignant transformation via inducing tumor cell adhesion, invasion, and metastasis [28, 99]. Upregulated expression of CD44 has been reported to be closely associated with tumor initiation, progression, metastasis, and treatment resistance in various cancers [28, 32, 35, 99]. Previous studies indicated the potential prognostic value and clinicopathological significance of CD44 expression in glioma patients. However, the eligible studies included in this meta-analysis were diversified and the results were contradictory [52, 53, 55-66]. The present meta-analysis systematically analyzed the prognostic impact of CD44s expression on glioma patients. Pooled results demonstrated that positive expression of CD44s was significantly associated with shorter OS and reduced PFS of glioma patients in the univariate analysis. Particularly, CD44s is an independent factor for OS of glioma patients. Overexpression of CD44s facilitates tumor cell migration through cell-extracellular matrix interactions. Furthermore, CD44s functions as a common upstream regulator in a signaling network consisting of AKT, ERK and Hippo-YAP pathways regulating the expression of downstream genes to mediate tumor cell growth and proliferation [100, 101]. These mechanisms may account for tumor invasion and early progression in gliomas.

In addition, the correlation between CD44s/CD44v6 expression and clinicopathological features of glioma patients was also analyzed in this meta-analysis. CD44s and CD44v6 expression were barely detected in adjacent non-tumorous brain tissues, including normal brain tissues and pan-cancer brain tissues. Both CD44s and CD44v6 expression were significantly overexpressed in paired glioma tissues when compared with adjacent non-tumorous brain tissues. In contrast to a relatively low rate of CD44v6 expression in primary gliomas, CD44v6 seems to be preferentially expressed in recurrent gliomas. According to a study by Du Q *et al* [79], CD44v6 expression was upregulated in recurrent gliomas comparing with primary gliomas (OR=7.70, 95% CI= [2.28–26.03]; P=0.001). However, there was no significant difference in CD44s expression between primary gliomas and recurrent gliomas in this meta-analysis. Moreover, both CD44s and CD44v6 expression strongly correlated with higher tumor grade. It suggested that both CD44s and CD44v6 were predominantly expressed in glioma tissues, particularly in high-grade gliomas. Intriguingly, CD44s expression was lower in brain metastases from other sides, such as lung carcinomas, breast carcinomas, and melanomas, than that in primary gliomas. Conversely, CD44v6 expression was remarkably higher in brain metastases when compared with primary gliomas. It indicated that CD44v6 is a potential diagnostic marker for differentiating brain metastases from primary gliomas in individual cases. These results demonstrated that CD44s participates in tumor initiation, proliferation, and progression. In the meantime, CD44v6 plays a vital role in promoting tumor invasion, metastasis, and recurrence.

Gliomas are a clinically and molecularly heterogeneous group of primary brain tumors [3, 102]. Diffuse gliomas can be stratified into prognostically distinct subgroups in terms of some genetic alterations in primary gliomas, such as mutations in isocitrate dehydrogenase (IDH) 1 and 2, methylation of the O^6-methylguanine DNA methyltransferase (MGMT) and codeletion of chromosome arms 1p and 19q [3]. Subgroup analysis of the prognostic and clinicopathological value of CD44 expression in diffuse gliomas in terms of IDH mutation status, MGMT methylation status or 1p/19q codeletion status were not performed in this meta-analysis because no eligible studies reported relevant data. In addition, GBMs can be classified into four distinct molecular subtypes, proneural, neural, classical and mesenchymal GBM based on dominant gene expression patterns [103]. Further studies to determine the prognostic and clinicopathological correlation between CD44 expression and four subtypes of GBMs are warranted. Moreover, the correlation of CD44 expression with some clinicopathological parameters, such as age, gender, KPS score, and adjuvant therapy, was not evaluated due to a lack of sufficient eligible data for pooled analysis.

Alternative splicing of 10 variable exons in *CD44* gene produced ten CD44 variant isoforms (CD44v1-CD44v10) [33, 34]. Except for CD44v6, the prognostic and clinicopathological significance of other CD44 variant isoforms in glioma patients remains unclear because limited studies on these CD44 variant isoforms have been reported. So far, only one study by Li R *et al*[104]investigated the association of the mRNA level of CD44v9 detected by the qRT-PCR method with the prognosis and clinicopathological features of gliomas. It revealed that elevated expression of CD44v9 was correlated with poorer median OS (P<0.05), reduced median recurrence-free survival (RFS, P<0.05), higher WHO grade (grade III-IV vs. grade I-II, OR=2.77, 95%CI=

[1.17, 6.57], P=0.02), larger tumor size (T>5 cm vs. T≤5 cm, OR=2.77, 95%CI= [1.17, 6.57], P=0.02) and cystic changes (yes vs. no, OR=7.39, 95%CI= [2.81, 19.40], P<0.0001). Hence, the diagnostic and prognostic value of CD44 variant isoforms in glioma patients remains to be further evaluated.

Of note, several studies reported the positive correlation between CD44 and some other biomarkers in gliomas, such as CD29/Integrin β-1 (encoded by *ITGB1*) [70], osteopontin (OPN, encoded by *SPP1*) [79] and metalloproteinase-9 (encoded by *MMP9*) [97]. Thus, we performed gene expression correlation analysis using online database Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn/index.html), and the results showed mRNA expression level of *CD44* gene is positively correlated with the expression of *ITGB1*(r=0.6, P<0.001), *SPP1*(r=0.5, P<0.001) and *MMP9* (r=0.23, P<0.001) in gliomas. Co-expression of CD44 and relevant biomarkers provides a novel approach to more precisely predicting the prognosis of gliomas. This warrants further studies on investigating the diagnostic and prognostic value of co-expression of CD44 and relevant biomarkers.

There were some potential limitations to this meta-analysis. First, survival data extracted from Kaplan-Meier curves are less dependable than data directly obtained or calculated from original data available in the articles. Second, the criterion of positive or high expression of CD44 was verified among different studies, which may lead to the heterogeneity of studies. Finally, limited studies were included in the evaluation on the correlation of CD44s expression with OS (multivariate analysis) and PFS (univariate analysis), which may lead to unreliable results. Therefore, more studies remain to be added in these pooled analyses to draw robust and reliable conclusions.Despite the limitations mentioned above, the present meta-analysis still revealed the prognostic value of CD44s expression and clinicopathological significance of CD44s/CD44v6 expression in glioma patients.

Conclusions

This meta-analysis revealed the prognostic value of CD44s expression and clinicopathological significance of CD44s/CD44v6 expression in gliomas. Increased CD44s expression can predict worse prognosis of glioma patients. Particularly, CD44s is an independent prognostic factor for poor OS of glioma patients. Both CD44s and CD44v6 were predominantly expressed in gliomas tissues, especially in high-grade gliomas. CD44s participates in tumor initiation, proliferation, and progression while CD44v6 plays a critical role in promoting tumor invasion, metastasis, and recurrence of gliomas. Further studies to evaluate CD44 as a therapeutic target for gliomas are warranted.

Abbreviations

AAAIR: average annual age-adjusted incidence rate; ALDH1: aldehyde dehydrogenase 1; CD44s: CD44 standard form; CD44v: CD44 variant isoform; CI: confidence interval; CNKI: China National Knowledge Infrastructure; CNS: central nervous system; CSCs: cancer stem cells; EpCAM: epithelial cell adhesion molecule; GSCs: glioma stem cells; HA: hyaluronic acid; HR: hazard ratio; IDH: isocitrate dehydrogenase; IHC: immunohistochemistry; M: multivariate analysis; MGMT: O6-methylguanine DNA methyltransferase; NOS: Newcastle-Ottawa Quality Assessment Scale; OR: odds ratio; OS: overall survival; PFS: progression-free survival; PRISMA: Preferred Reporting Items for Systematic Review and Meta-Analysis; U: univariate analysis; WHO: World Health Organization; CD44s: CD44 standard form; CNS: central nervous system; AAAIR: average annual age-adjusted incidence rate; WHO: World Health Organization; CSCs: cancer stem cells; GSCs: glioma stem cells; ALDH1: aldehyde dehydrogenase 1; EpCAM: epithelial cell adhesion molecule; HA: hyaluronic acid; CD44v: CD44 variant isoform; PRISMA: Preferred Reporting Items for Systematic Review and Meta-Analysis; CNKI: China National Knowledge Infrastructure; IHC: immunohistochemistry; OS: overall survival; PFS: progression-free survival; OR: odds ratio; HR: hazard ratio; NOS: Newcastle-Ottawa Quality Assessment Scale; IDH: isocitrate dehydrogenase; MGMT: O^6-methylguanine DNA methyltransferase; CI: confidence interval; M: multivariate analysis; U: univariate analysis

Declarations

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Author's Contributions

KX and XYZ contributed conception and design of the study, analysis and interpretation of data; KX, XYZ, CSX, LYZ and KG drafted the manuscript; LYZ and ML provided technical and material support; ZXL contributed to study supervision. All authors contributed to manuscript revision, read and approved the submitted version.

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Availability of data and materials

The data supporting this study are from previously reported articles, which have been cited as references. The processed data are available in this article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1: Characteristics of the studies included in the quantitative analysis

Study	Year	Country	Cancer types (WHO Grade)	Cases/ Controls	Median age [Range] (years)	Follow-up time	Counting Method	Cut-off value (positive/high)	Survival outcomes	Source of data	NOS score
Bhat KPL, *et al* [55]	2013	USA	GBM(IV)	37/29	56.9 [18.9-84.2]	6.1-673 weeks	qRT-PCR	median	OS(U)	curve	7
Bien MS, *et al* [56]	2018	Germany	GBM(IV)	24/24	67	NR	qRT-PCR	median	OS(U)	direct	7
Guadagno E, *et al* [57]	2016	Italy	GBM(IV)	14/9	[41-75]	3-55 months	IHC	A1>1	OS(U)	available data	9
Hagel C, *et al* [59]	1999	Germany	OD (II-III) GBM(IV)	21/38	Male: 41.2 [19-65], Female: 49.3 (20-70)	NR	IHC	B1>1	OS(U)	curve	7
Hagel C, *et al* [58]	2004	Germany	GBM(IV)	22/20	51.2 [18-70]	NR	IHC	B1>1	OS(U)	curve	7
Hou CX, et al [60]	2019	Japan	Glioma (II-III)	56/56	NR	NR	qRT-PCR	median	OS(U), OS(M), PFS(U)	direct+curve	9
Iwadate Y, *et al* [61]	2017	Japan	GBM(IV)	34/36	NR	NR	IHC	B1>2	OS(U), OS(M), PFS(U), PFS(M)	curve+direct	9
Jin J, *et al*[67]	2013	China	A(I-III), GBM(IV)	21/20	[6-79]	NR	IHC	A2>2.4	PFS(U)	curve	7
Nishikawa M, *et al*[62]	2018	Japan	GBM(IV)	6/6	66 [44-79]	4-46 months	qRT-PCR	median	OS(U), PFS(U)	survival data	7
Pinel B, *et al*[52]	2017	France	GBM(IV)	56/18	60 [30-83]	1-114 months	IHC	B1>1	OS(U), OS(M), PFS(U)	curve+direct	7
Ranuncolo SM, *et al*[63]	2002	Argentina	LGA(II), AA(III), GBM(IV)	57/24	NR	NR	IHC	A3>1	OS(U), OS(M)	curve+direct	8
Sooman L, *et al* [66]	2015	Sweden	AA(III), AOD(III), GBM(IV)	6/18	NR	NR	IHC	A4>1	OS(U)	curve	7
Tsidulko AY, *et al* [64]	2017	Russia	AA(III), AOA(III), GBM(IV)	27/29	48	NR	qRT-PCR	median	OS(U), OS(M)	curve+direct	9
Wang W, *et al* [65]	2017	USA	GBM(IV)	20/23	NR	NR	IHC	B1>1	OS(U)	curve	7
Wei KC, *et al* [53]	2010	China	GBM(IV)	21/22	NR	NR	qRT-PCR	median	OS(U)	direct	7

GBM: glioblastoma multiforme, **OD**: oligodendroglioma, **A**: astrocytoma, **LGA**: low-grade astrocytoma, **EPN**: ependymoma, **AA**: anaplastic astrocytoma, **AOD**: anaplastic oligodendroglioma, **AOA**: anaplastic oligoastrocytoma. **IHC**: immunohistochemistry, **qRT-PCR**: quantitative real-time reverse transcription polymerase chain reaction. **NR**: not reported. **OS**: overall survival, **PFS**: progression-free survival, **U**: univariate analysis. **M**: multivariate analysis. **NOS**: Newcastle-Ottawa Scale.

A: positive cell percentage, **A1**: scored 0 (0 %), 1 (1-10 %), 2 (11–50 %), 3 (>50 %); **A2**: scored 0-100; **A3**: scored 0 (<10%), 1 (10-50%), 2 (51-70%), 3 (>70%); **A4**: scored 0 (<2%) 1 (2-10%), 2 (11-25%), 3 (26-50%), 4 (51-75%), 5 (>75%). **B**: staining intensity, **B1**: scored 0 (absence of staining), 1 (weak staining), 2 (moderate staining), 3 (strong staining).

Table 2: Characteristics of eligible studies in the qualitive synthesis

Study	Year	Country	WHO Grade	Indicators reported	Cases	Method	Cut-off value (positive/high)
Bar JK, *et al* [88]	2014	Poland	I-IV	□	92	IHC	A1*B1>0
Bouvier LC, *et al* [81]	2000	France	II-III	□	28	IHC	A2>0
Cao WD, *et al* [68]	1999	China	IV	□□	50	IHC	A3>0
Celiku O, *et al* [69]	2017	USA	I-IV	□□	52	IHC	A4*B1>median
Chen S, *et al* [97]	2006	China	III-IV	□	80	IHC	A5>0
Chen ZG, *et al*[70]	2011	China	I-IV	□□	70	IHC	A6*B1>0
Dong C [71]	2012	China	I-IV	□□	91	IHC	A7+B1>2
Du Q, *et al* [79]	2013	China	I-IV	□□	120	IHC	A2+B1>3
Frank S, *et al* [94]	1996	Germany	NR	□	51	IHC	B1>0
Hagel C, *et al* [59]	1999	Germany	II-IV	□□	114	IHC	B1>0
Hagel C, *et al* [58]	2004	Germany	IV	□	58	IHC	B1>0
Hu WP, *et al* [72]	2004	China	I-IV	□□□□	82	IHC	A8*B1>1
Huang HB, *et al* [82]	2006	China	I-IV	□	58	IHC	A3>0
Jiang H, *et al* [89]	2005	China	I-IV	□	61	IHC	A9*B1>1
Li H, *et al* [95]	1993	Switzerland	IV	□	32	IHC	NR
Lin ZX, *et al* [73]	2000	China	I-IV	□□□	66	IHC	A8*B1>1
Liu YJ, *et al* [74]	2005	China	I-IV	□	50	IHC	NR
Niu GM, *et al* [75]	2004	China	I-IV	□□	50	IHC	A10>0
Oz B, *et al* [83]	2000	Turkey	I-IV	□	52	IHC	B2>0
Popova SN, *et al* [84]	2014	Sweden	I-IV	□	180	IHC	A11>2
Ranuncolo SM, *et al*[63]	2000	Argentina	I-IV	□	84	IHC	A12>0
Shen H, *et al* [98]	2000	China	III-IV	□	115	IHC	A13>0
Tews DS, *et al* [85]	1998	Germany	IV	□	45	IHC	A14>2
Valkonen M, *et al* [86]	2018	Finland	II-IV	□	120	IHC	B1>1
Wei HQ, *et al* [87]	1999	China	I-IV	□□□	45	IHC	A14>0
Xu J, *et al* [76]	2005	China	I-IV	□□□	48	IHC	A15>0
Yan SJ, *et al* [96]	2001	China	NR	□□	49	IHC	B1>0
Yang SW, *et al* [77]	2011	China	III-IV	□	70	IHC	A16>0
Yoshida T, *et al*[78]	2001	Japan	I-IV	□□	63	IHC	B1>0
Zhao J [90]	2001	China	II-III	□	59	IHC	A17>0
Zhao ZX [80]	2001	China	NR	□□	70	IHC	A16>0

Indicators reported: CD44s expression (□) and CD44v6 expression (□) in glioma tissues vs. non-tumorous brain tissues; CD44s-positive expression (□), CD44s-high expression (□) and CD44v6 expression (□) in WHO Grade III-IV vs. WHO Grade I-II; CD44s expression (□) in recurrent gliomas vs. primary gliomas; CD44s expression (□) and CD44v6 expression (□) in brain metastases vs. primary gliomas.

A: percentage of positive cells. **A1**: scored 0 (<10%), 1 (10-40%), 2 (41-60%), 3 (>60%); **A2**: scored 0 (0%), 1 (<25%), 2 (25–50%), 3 (>50%); **A3**: scored 0 (≤5%), 1 (>5%); **A4**: scored 0-100; **A5**: scored 0 (<5%), 1 (5–50%), 2 (>50%); **A6**: scored 0 (<5%), 1 (5-25%), 2 (26–50%), 3 (51-75%), 4 (>75%); **A7**: scored 0 (<10%), 1 (10-24%), 2 (25–49%), 3 (50-74%), 4 (≥75%); **A8**: scored 0 (<5%), 1 (5–20%), 2 (21-60%), 3 (>60%); **A9**: scored 0 (<10%), 1 (10-50%), 2 (51-80%), 3 (>80%); **A10**: scored 0 (0%), 1 (>0%); **A11**: scored 0 (0%), 1 (<10%), 2 (10–50%), 3 (>50%); **A12**: scored 0 (<10%), 1 (10-50%), 2 (51-70%), 3 (>70%); **A13**: scored 0 (<25%), 1 (≥25%); **A14**: scored 0 (0%), 1 (1-25%), 2 (26–50%), 3 (51-75%), 4 (>75%); **A15**: scored 0 (<10%), 1 (10-49%), 2 (≥50%); **A16**: scored 0 (<10%), 1 (10-24%), 2 (25–49%), 3 (≤50%); **A17**: scored 0 (≤10%), 1 (11-50%), 2 (>50%).

B: intensity of staining. **B1**: scored 0 (absence of staining), 1 (weak staining), 2 (moderate staining), 3 (strong staining). **B2**: scored 0 (absence of staining), 1 (weak staining), 2 (moderate to strong staining).

IHC: immunohistochemistry. **NR**: not reported.

Table 3: Subgroup analysis of OS based on univariate data

Categories	Studies (n)	Heterogeneity		Effect models	HR (95% CI)	p values
		I^2 (%)	p values			
Ethnicity						
Asian	4	85	0.0001	Random	1.81 (0.65-5.09)	0.26
European & American	10	65	0.002	Random	1.58 (1.12-2.23)	0.008
Counting method						
IHC	8	68	0.003	Random	1.55 (1.03-2.32)	0.04
qRT-PCR	6	80	<0.0001	Random	1.72 (0.90-3.27)	0.10
Cancer type						
GBM only	9	79	<0.00001	Random	1.39 (0.85-2.27)	0.19
Mixed Gliomas	5	0	0.64	Fixed	2.13 (1.59-2.85)	<0.00001
Source of data						
Curve	9	69	0.001	Random	1.71 (1.18-2.49)	0.005
Direct	5	80	0.0005	Random	1.50 (0.69-3.27)	0.31
NOS score						
7	9	80	<0.00001	Random	1.48 (0.87-2.50)	0.15
8-9	5	0	0.45	Fixed	1.94 (1.48-2.54)	<0.00001

Table 4: Sensitivity analysis and publication bias				
	Sensitivity analysis		Publication bias (p value)	
	HR/OR fluctuation	95% CI fluctuation	Begg's test	Egger's test
CD44s expression and survival outcomes				
OS (univariate analysis)	1.543-1.816	1.089-2.433	0.913	0.777
OS (multivariate analysis)	1.821-2.485	0.853-4.999	1.000	0.962
PFS (univariate analysis)	2.017-2.399	1.494-3.616	0.089	0.011
CD44s expression and clinicopathological parameters				
tumor vs. non-tumorous tissues	26.548-39.954	12.572-92.369	0.276	0.076
tumor grade (positive vs. negative expression)	4.813-5.303	2.806-7.931	0.451	0.431
tumor grade (high vs. low expression)	3.003-4.010	1.769-7.970	0.086	0.160
recurrent gliomas vs. primary gliomas	0.949-1.892	0.280-3.938	1.000	0.017
brain metastases vs. primary gliomas	0.185-0.495	0.066-1.428	0.452	0.686
CD44v6 expression and clinicopathological parameters				
tumor vs. non-tumorous tissues	12.113-14.235	1.559-121.002	1.000	0.821
tumor grade (positive vs. negative expression)	1.663-2.186	0.892-3.872	0.221	0.168
brain metastases vs. primary gliomas	35.907-103.179	5.323-548.058	0.027	0.014

Figures

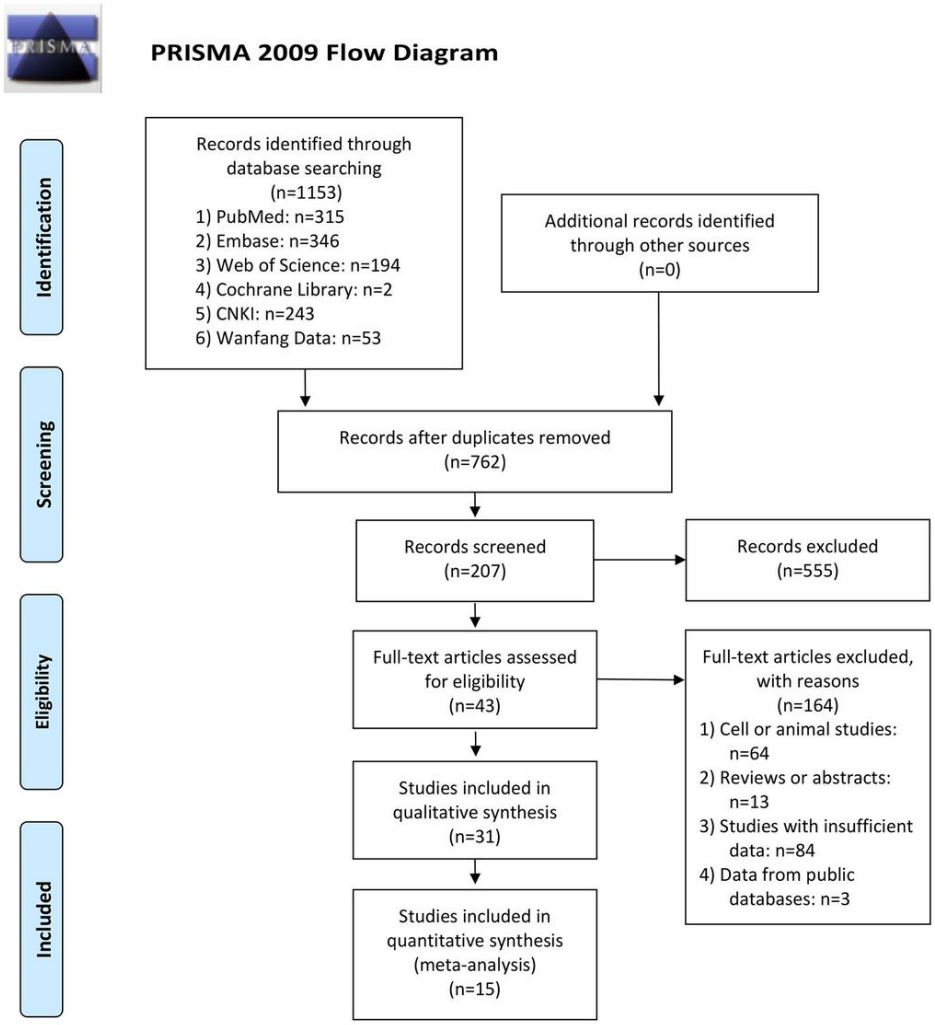

Figure 1

Flow diagram of the literature search and study selection in the meta-analysis.

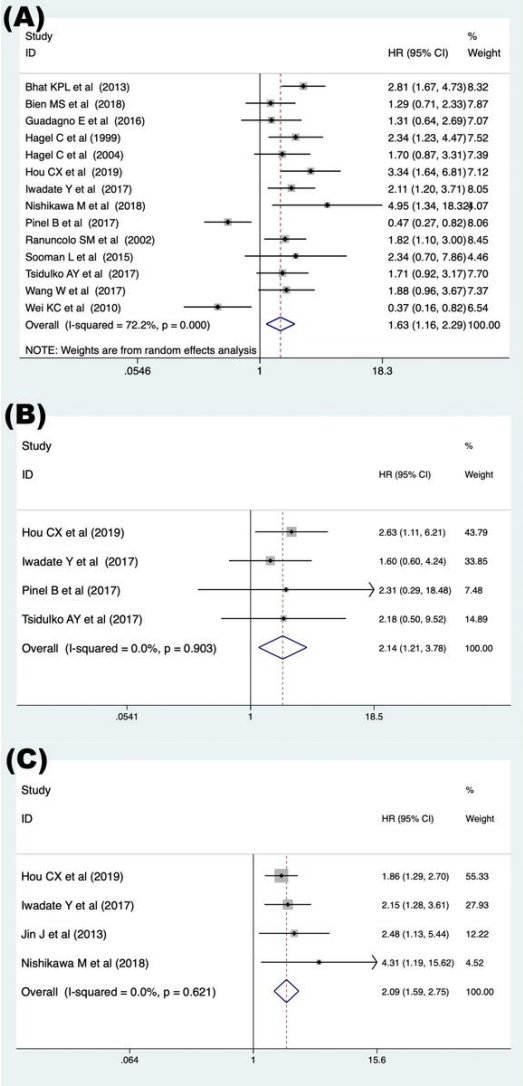

Figure 2

Forest plots of the correlation of CD44s expression with survival outcomes. (A): The correlation of CD44s expression with OS in the univariate analysis; (B) The correlation of CD44s expression with OS in the multivariate analysis; (C): The correlation of CD44s expression with PFS in the univariate analysis.

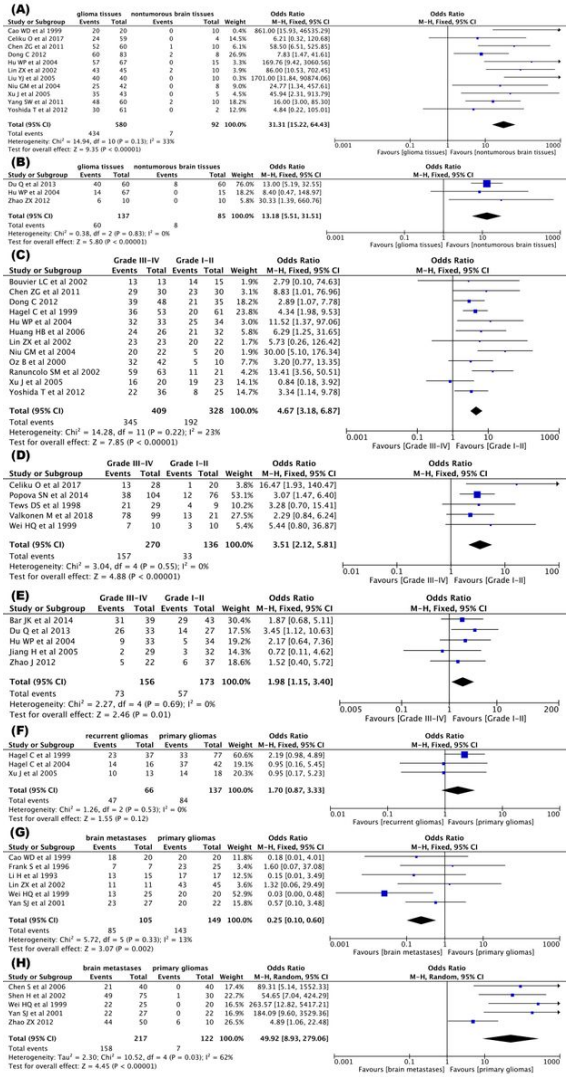

Figure 3

Forest plots of the correlation between CD44s/CD44v6 expression and clinicopathological parameters. (A-B): CD44s (A) and CD44v6 (B) expression in glioma tissues and nontumorous brain tissues; (C-E): CD44s-positive (C), CD44s-high (D) and CD44v6-postive (E) expression in grade III-IV group and grade I-II group; (F): CD44s expression in recurrent gliomas and primary gliomas; (G-H): CD44s (G) and CD44v6 (H) expression in brain metastases and primary gliomas.

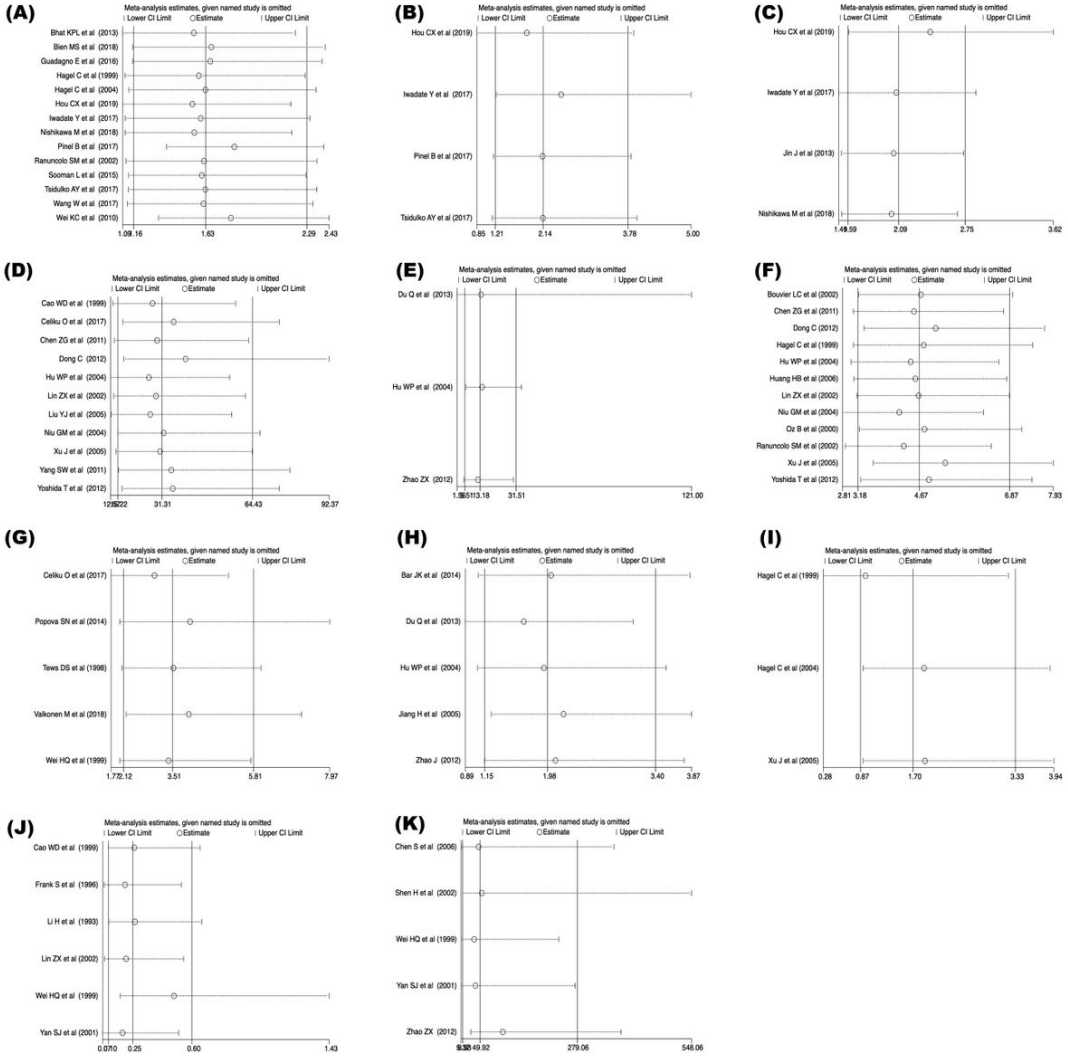

Figure 4

Sensitivity analysis. (A-B) The correlation of CD44s expression with OS in the univariate analysis (A) and multivariate analysis (B); (C) The correlation of CD44s expression with PFS in the univariate analysis; (D-E) CD44s (D) and CD44v6 (E) expression in glioma tissues and nontumorous brain tissues; (F-H): CD44s-positive (F), CD44s-high (G) and CD44v6-postive (H) expression in grade III-IV group and grade I-II group; (I): CD44s expression in recurrent gliomas and primary gliomas; (J-K): CD44s (J) and CD44v6 (K) expression in brain metastases and primary gliomas.

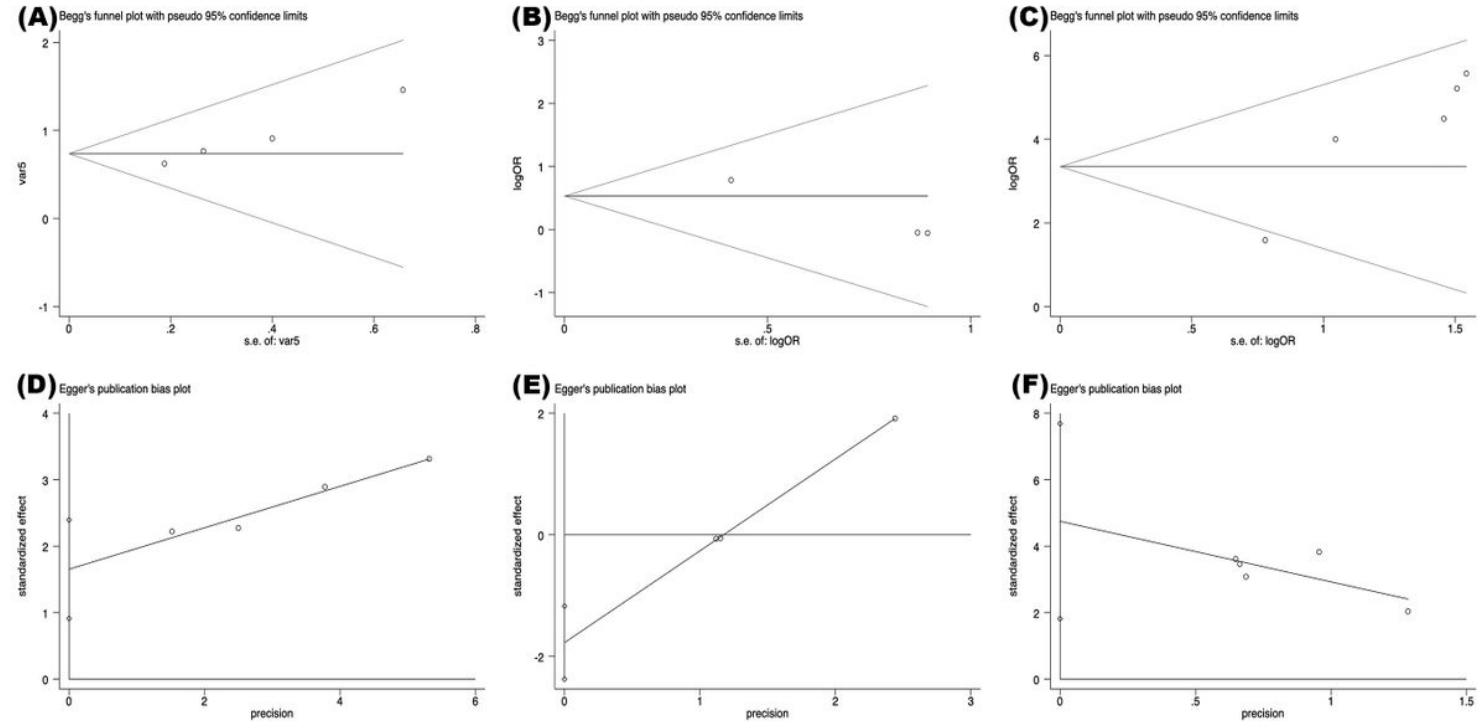

Figure 5

Publication bias. (A, D): The correlation of CD44s expression with PFS in the univariate analysis (A, Begg's test; D, Egger's test); (B, E): CD44s expression in recurrent gliomas and primary gliomas (B, Begg's test; E, Egger's test); (C, F): CD44v6 expression in brain metastases and primary gliomas (C, Begg's test; F, Egger's test).

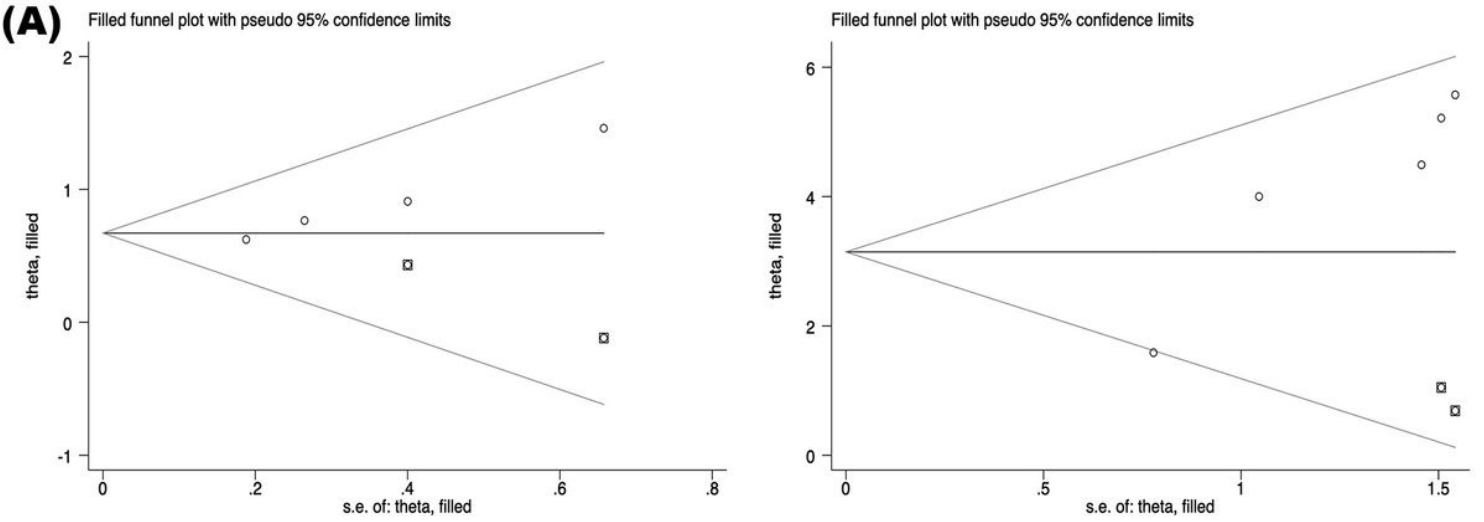

Figure 6

Trim and fill analysis. (A): The correlation of CD44s expression with PFS in the univariate analysis; (B): CD44v6 expression in brain metastases and primary gliomas.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TablesS1.docx
- renamed90716.png
- renamed6a4ab.png