Original Article CD133 expression may be useful as a prognostic indicator in glioblastoma multiforme: a meta-analysis

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Abstract: Objective: CD133 has been identified as a putative neoplastic stem cell marker in glioblastoma. However, the clinical and prognostic significance of CD133 in glioblastoma remains controversial. To clarify a precise determinant of the clinical significance of CD133, we performeded a meta-analysis to evaluate the correlation of CD133 expression with prognosis of glioblastoma patients. Methods: A systematic literature search for relevant articles published from 2005 to 2016 was conducted in PubMed, Embase and Cochrane databases. Eligible studies on this subject were included, and then pooled hazard ratios (HRs) with 95% confidence intervals (CIs) were estimated. Publication biaswas assessed by the funnel plots, and heterogeneity and sensitivity were analyzed as well. Results: A total of 715 glioblastoma patients from 10 studies were included. The results of the meta-analyses found that CD133-high expression was an independent prognostic marker correlating with both overall survival (HR=1.81, 95% CI 1.11-2.51) and progression-free survival (HR=1.41, 95% CI 1.03-1.79). In stratified analysis, only high quality studies showed that CD133-high expression was associated with both worse overall survival (HR=2.39, 95% CI 1.77-3.23) and worse progression-free survival (HR=1.97, 95% CI 1.23-2.70) in patients with glioblastoma. Conclusion: Taken together, this study indicats that CD133 is an efficient prognostic factor in eglioblastoma. CD133-high expression is significantly associated with poor prognosis in patients with glioblastoma. CD133 should be recommended as a useful pathological and prognostic biomarker in clinical practice.

Keywords: CD133, cancer stem cell, glioblastoma, meta-analysis

Introduction

Glioblastoma multiforme (GBM), also known as glioblastoma and grade IV astrocytoma, is the most common and most aggressive cancer that begins within the brain [1]. Signs and symptoms are initially non specific. Despite diagnostic options and therapeutic therapies undergoing significant changes, the cancer usually recurs [2]. The most common length of survival following diagnosis is 12 to 15 months with less than 3 to 5% of people surviving greater than five years [2]. Without treatment survival is typically 3 months [3]. About 3 per 100,000 people develop the disease a year [2]. It most often begins around 64 years of age and occurs more commonly in males than females [2].

Cancer stem cells (CSCs) reconstitute tumors with similar histopathological characteristics to

the primary cancer, whereas non-stem cancer cells failed to effect tumor initiation. And, CSCs are believed to play a key role in resistance to chemotherapy and radiotherapy [4, 5]. This new paradigm has promising implications for cancer therapy, as our recently available therapies are more successful at eradicating non-cancer stem cells rather than cancer stem cells [6, 7]. In other words, identification and characterization of CSCs could lead to development of directed and more effective treatments for cancer [8].

Recently, certain cell surface markers have been identified as stem cell markers in cancer; among these, CD133 is considered to be the most robust surface marker for cancer stem cells to date. CD133, also known as prominin-1, is a member of the pentaspan transmembrane glycoprotein family [9]. The CD133+ phenotype was first used to identify and isolate malignant



Figure 1. Flow chart for included articles.

brain tumor stem cells. CD133 is currently identified as a cancer stem cell marker in various solid tumors, such as hepatocellular carcinoma [10], ovarian [11], colon [12] and esophageal carcinoma [13]. These findings suggested that cancer stem cells have the ability to form the bulk of a tumor cell population and confer resistance to conventional therapy.

As regards glioblastoma, the correlation between CD133 expression and the clinicopathological parameters is relatively unclear. In order to address these issues, we performeded a meta-analysis to evaluate the correlation of CD133 expression with prognosis of glioblastoma patients.

Methods

Search strategy

We searched PUBMED, EMBASE and Cochrane Library digital databases for all relevant articles. The search was performed in each database by two independent investigators. The medical subject headings (MeSH) and keywords collected for individually and in combination were as follows: ('glioblastoma multiforme' or 'glioblastoma') AND ('cancer stem cell' or 'neoplastic stem cells) AND ('CD133' or 'prominin-1' or 'AC133'). No language restrictions were imposed. The reference lists of included studies and review articles were also searched to find additional eligible studies. The title and abstract of each study identified in the search was scanned to exclude any clearly irrelevant ones. The remaining articles were browsed to determine whether they contained information on the topic of interest. The reference lists of articles with information on the topic were also reviewed for additional pertinent studies.

Study selection

Eligibility of studies for inclusion was assessed independently by two investigators.

Studies were eligible for inclusion if all the following criteria were fulfilled: (1) The patients were confirmed the diagnosis of glioblastoma; (2) CD133 expression was evaluated by immunohistochemistry, RT-PCR, or Western blot, etc; (3) The data provided must be sufficient to estimate the HR with 95% CI. (4) Articles provided sufficient information on the overall survival (OS), progression-free survival (PFS). Studies that could not meet any one of the above inclusion criteria were excluded. Animal studies, review articles, letters, comments, case reports and unpublished articles were also excluded. When the authors published several studies using the same subjects, only the most recent or the publication including the largest sample size was included.

Data extraction

The full manuscripts of included articles were reviewed by three reviewers independently. Data extracted included f name of the first author, publication year, country of the included subjects, number of cases, cut-off values, adjusted factors, and data for calculating HRs with 95% Cls. For the articles with the same population resources or overlapping data sets,

First author	Country	Cases	Outcomes	Confounded factors	Quality
Dahlrot RH [14]	DMK	239	OS; PFS	N/A	4
He J [18]	CHN	59	PFS	N/A	4
Zeppernick F [21]	GER	95	OS; PFS	Age, WHO grade, and extent of resection.	7
Shibahara I [16]	JPN	112	OS; PFS	10 q loss, 9p homozygous deletion, MGMT, Ki67 labeling index, and total resection.	8
Shin JH [15]	KOR	67	OS; PFS	Age, sex, extent of surgery, IDH1, CD34, et al.	7
Metellus [17]	FRA	48	OS; PFS	Age, extent of surgery, MGMT status.	7
Ardebili SY [19]	SLO	24	OS	N/A	4
Kappadakunnel M [20]	USA	47	OS	N/A	5
Murat A [23]	SWE	80	OS	Age, extent of surgery, chemoradiotherapy.	8
Pallini R [22]	ITA	44	OS; PFS	Ki67 expression, symptom duration, partial surgery, age, MGMT status negative, p53 status positive.	8

 Table 1. Characteristics of included studies

the paper which included the largest population or contained more useful information was included. If some articles revealed the prognosis of esophageal cancer only by Kaplan-Meier curve, the software Engauge Digitizer 4.1 (http://sourceforge.net/projects/digitizer/) was utilized to extract the relevant data.

Study quality

The quality of the randomized controlled trials (RCTs) was assessed by using the Cochrane reviewer's handbook. The methodological quality of RCTs was assessed by means of the Jadad score via three items (method of randomization, blinding and withdrawals/dropouts). The quality of the nonrandomized controlled trials (NRCTs) was assessed using the Newcastle-Ottawa Scale (NOS). Studies were graded on an ordinal star scoring scale with higher scores representing studies of higher quality. The NOS evaluated the quality of studies by examining three aspects of the study design: patient selection, comparability of the study groups, and assessment of outcomes. Two reviewers independently assessed the quality of the studies and disagreement was resolved by consensus.

Statistical analysis

Statistical calculations were all performed using STATA version 13.0 Dichotomous data were presented as HR, with 95% CI. Statistical heterogeneity between studies was assessed with the chi-square statistic and quantified by I^2 , a statistic that represents the percentage of total variation contributed by between-study variation. If the Q test showed a P < 0.05 or the I^2 test exhibited > 50%, indicating significant heterogeneity between studies, the randomeffect model was conducted, or the fixed-effect model was used. Publication bias was examined by using the Begg rank correlation method and the Egger weighted regression method.

Study selection and characteristics

Detailed search steps were described in **Figure 1.** The initial search algorithm retrieved a total of 334 studies according to the inclusion criteria stated above. After titles and abstracts were previewed, only 29 identified studies concerning CD133 and the risk of esophageal cancer were further evaluated. After the removal of all studies that did not meet our criteria, 10 studies [14-23] were finally included in our metaanalysis. The useable data and main characteristics of each article are summarized in **Table 1.** Included articles were published in the period 2008-2014. All the studies were conducted in Asian, European and US population. A total of 715 patients were included.

Correlation of CD133 with overall survival

A total of 9 [14-17, 19-23] studies assessed the association between CD133 expression and overall survival in patients with glioblastoma. High level of CD133 expression was significantly associated with poor overall survival (HR=1.41, 95% Cl 1.13-1.68), without any heterogeneity in the data (X^2 =10.19, I^2 =21.5%; P=0.25) (**Figure 2**). In stratified analysis, the



Figure 2. Forest plots of RRs for CD133 and overall survival.

study showed that high level of CD133 expression was associated with poor prognosis, with a risk of 1.34 (95% CI 1.04-1.63) in high quality group and 1.81 (95% CI 1.11-2.51) in low quality group, respectively (**Figure 3**).

Correlation of CD133 with progression-free survival

A total of 7 studies [14-18, 21, 22] assessed the association between CD133 expression and progression-free survival (**Figure 4**). And high level of CD133 expression was significantly associated with poor progression-free survival (HR=1.41, 95% Cl 1.03-1.79), with no heterogeneity in the data (X^2 =6.12, I^2=2.0%; P=0.41). In stratified analysis, only high quality group proposed that high level of CD133 expression was associated with poor progression-free survival (HR=1.97, 95% Cl 1.23-2.70) (**Figure 5**).

Sensitivity analyses

Sensitivity analysis was subsequently performed to detect the influence of individual study on the pooled estimate by omitting one study from the pooled analysis each time. The exclusion of each single study did not significantly change the pooled OR, suggesting that the results of the meta-analysis were robust and credible (Figures S1, S2).

Publication bias

Begg's funnel plot was used to check the existence of publication bias. The plot was symmetric, suggesting that the publication bias was little. There was no evidence of publication bias for asymmetrical shapes existed in neither two groups analyses (Figures S3, S4, S5 and S6).

Discussion

In the last few years, glioblastoma's incidence, diagnostic options and therapeutic therapies have undergone significant changes, but the prognosis remains poor. There is still lacking effective therapy for glioblastoma, due to tumor heterogeneity caused by the existence of cancer stem cells. And many prognostic biomarkers have been identified in glioblastoma which could give assistance to rationalize treatment decisions for individuals. In the year 2014, Molenaar RJ, et al. [24] found that the combination of IDH1 mutations and MGMT methylation

Study		9	6
ID		HR (95% CI) V	Veight
high			
Zeppernick F (2008)		5.62 (2.04, 15.51)).17
Murat A (2008)		2.69 (1.38, 5.26) 2	2.02
Pallini R (2008)		2.22 (1.09, 4.52) 2	2.59
Metellus P (2011)		1.91 (1.19, 3.07) 8	3.61
Shin JH (2013)		2.49 (1.06, 5.85) 1	.33
Shibahara L (2013)	•	1.16 (0.88, 1.54) 6	68.86
Subtotal (I-squared = 36.4%, p = 0.164)		1.34 (1.04, 1.63) 8	34.58
low			
Kappadakunnel M (2010)	-	1.75 (1.06, 2.88) 9	9.19
Ardebili SY (2011)	-	2.18 (1.26, 3.79) 4	1.75
Dahlrot RH (2014)		1.00 (0.21, 4.74) 1	.48
Subtotal (I-squared = 0.0%, p = 0.658)		1.81 (1.11, 2.51) 1	5.42
Heterogeneity between groups: p = 0.222			
Overall (I-squared = 21.5%, p = 0.252)	•	1.41 (1.13, 1.68) 1	00.00
-15.5	0	l 15.5	

Figure 3. Stratified analyses of CD133 expression with overall survival in different quality group.

outperforms either IDH1 mutations or MGMT methylation alone in predicting survival of glioblastoma patients.

Nowadays, CSCs have drawn widespread attention because of their potential roles in tumorigenesis, tumor maintenance, spread and relapse. Such cells are hypothesized to persist in tumors as a distinct population and cause relapse and metastasis by giving rise to new tumors. Several cell surface markers have been identified as stem cell markers in cancer, including CD133, CD90, CD271, CD44, CD24, ABCB5 and ALDH. CD133 is a widely used marker for isolating cancer stem cells in a range of solid tumors. Moreover, CD133 is considered a useful marker to identify the CSCs and its expression has been shown to have prognostic significance in glioblastoma patients.

Regarding the biological properties of CSCs, several studies indicated that evaluation of CD133 expression in glioblastoma tissue could

be useful in the future as a novel prognostic factor. Shin JH, et al. [15] indicated that CD133 expression was associated with poor overall survival (OS) and progression-free survival. Shibahara I, et al. [16] revealed that CD133 expression was significantly higher in distant than in local recurrence. Of the factors to predict the timing of recurrence, high CD133 expression was associated with shorter time to distant recurrence in both univariate and multivariate analyses. Zeppernick F, et al. [21] suggested that CD133-positive cells was an independent risk factor for tumor regrowth and time to malignant progression in WHO grade 2 and 3 tumors. In contrast, Dahlrot RH, et al. [14] found that the expression of CD133 did not correlate with WHO grade, and there was no association with overall survival (OS). Such conflicting findings made it difficult to access CD133 as a useful marker to adequately predict the pathological features and prognostic outcomes in clinical practice. So based on the previous literatures, this meta-analysis demonstrated that high level of CD133 expression was asso-



Figure 4. Forest plots of RRs for CD133 and progression-free survival.

ciated with poor overall survival and progression-free survival in patients with glioblastoma. In addition, stratified analysis proposed that only studies with high quality showed that high CD133 expression was associated with both poorer overall survival and poorer progressionfree survival. Thus, these results suggested that level of CD133 expression is correlated with a number of parameters that are traditionally associated with poor prognosis.

Several restrictions of our study also need to be considered. First, the numbers of the studies included in the current meta-analysis are relatively small, and may not consider all confounding factors though they reported adjusted estimates. Second, all the studies are based on Asian and Western population, none from African countries. Further studies are needed to investigate the role of CSCs in on African countries. As we know, there are significant differences such as etiology, biology features, clinical types, and prognosis in the risk of glioblastoma in different ethnic groups within a given geographical area. Third, in the metaanalyses of clinical parameters, variability in definitions, outcomes, measurements and experimental procedures may contribute to between-study heterogeneity. Finally, no attempt was made to identify unpublished work and grey literature, for example university theses or conference proceedings. As a result, publication bias may have influenced the results. And only English literatures were included in this study, it was possible that our findings were biased for many non-English literatures were not included.

In conclusion, despite the abovementioned limitations, this meta-analysis showed that high level of CD133 expression was significantly correlated with poor prognosis of glioblastoma. Thus, CD133 may have a predictive role and be helpful tool in the management of patients with glioblastoma. Further studies on CD133 and its potential as a marker for glioblastoma prognosis in clinical practice are required.

Disclosure of conflict of interest

None.

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Study			%
ID		HR (95% CI)	Weight
high			
Zeppernick F (2008)	-	• 4.67 (1.94, 11.23)	0.68
Pallini R (2008)	-	- 2.10 (1.05, 4.21)	5.89
Metellus P (2011)		1.80 (1.40, 3.50)	13.34
Shin JH (2013)	-	1.71 (0.75, 3.88)	6.01
Shibahara L (2013)	+ *	2.90 (1.10, 7.80)	1.31
Subtotal (I-squared = 0.0%, p = 0.768)	\diamond	1.97 (1.23, 2.70)	27.24
low			
He J (2011)		2.84 (1.72, 7.60)	1.70
Dahlrot RH (2014)	-	1.16 (0.79, 1.70)	71.06
Subtotal (I-squared = 18.4%, p = 0.268)		1.20 (0.75, 1.65)	72.76
Heterogeneity between groups: p = 0.080			
Overall (I-squared = 2.0%, p = 0.409)		1.41 (1.03, 1.79)	100.00
-11.2	0	11.2	
	1.54		

Figure 5. Stratified analyses of CD133 expression with progression-free survival in different quality group.

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CD133 expression and glioblastoma multifo



Figure S1. Sensitivity analysis of CD133 and overall survival.



Figure S2. Sensitivity analysis of CD133 and progression-free survival.

CD133 expression and glioblastoma multifo



Figure S3. Begg's funnel plot for CD133 and overall survival.



Figure S4. Egger's publication bias plot for CD133 and overall survival.



Figure S5. Begg's funnel blot for CD133 and progression-free survival.



Figure S6. Egger's publication bias plot for CD133 and progression-free survival.