ORIGINAL RESEARCH

Prognostic significance of epidermal growth factor receptor expression in glioma patients

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Purpose: There is a great controversy regarding the prognostic significance of epidermal growth factor receptor (EGFR) in glioma patients. The current meta-analysis was conducted to evaluate the effect of abnormal EGFR expression on overall survival in glioma patients.

Materials and methods: A comprehensive literature search of PubMed, EMBASE, Google Scholar, Web of Science, and Cochrane Library was conducted. The combined hazard ratio (HR) and its 95% confidence intervals (CIs) were used to evaluate the association between EGFR expression and survival in glioma.

Results: A total of 476 articles were screened, and 17 articles containing 1,458 patients were selected. The quality assessment of the included studies was performed by the Newcastle–Ottawa Scale. Overexpression of EGFR was found to be an indicator of poor prognosis in overall survival in glioma patients (HR =1.72, 95% CI 1.32–2.25, P=0.000, random effect) and glioblastoma multiforme patients (HR =1.57, 95% CI 1.15–2.14, P=0.004, random effect). Subgroup analysis was conducted to explore the source of high heterogeneity.

Conclusion: This meta-analysis indicated that high expression of EGFR may serve as a biomarker for poor prognosis in glioma patients.

Keywords: epidermal growth factor receptor, glioma, meta-analysis, prognosis

Introduction

Epidermal growth factor receptor (EGFR) is a transmembrane tyrosine kinase on chromosome 7p12, containing an extracellular ligand-binding domain, a transmembrane domain, and an intracellular tyrosine kinase domain, belonging to the HER family. The family includes four members (HER1/EGFR, HER2/neu, HER3, and HER4), with downstream signaling pathways regulating a number of processes, including growth, migration, and survival.[1] The pathway is involved in the development and progression of several human malignancies, including non-small-cell lung cancer, gastric cancer, pancreatic cancer, and nasopharyngeal carcinoma.[2–6]

Glioma is the most common primary malignancy of the central nervous system, accounting for over half of all malignant brain tumors in adults.[7] According to the WHO grading (2016), glioma can be divided into four grades. Glioblastoma multiforme (GBM) has the highest grade and the greatest malignancy. The median survival after standard treatment is ~13 months. Median progression-free survival (PFS) is only 7.2 months.[8] The EGFR gene is one of the earliest known carcinogenic genes found in GBM. Its increased transcriptional activity directly causes EGFR expression.[9] Due to its pro-oncogenic effects, it is not surprising that increased EGFR expression is associated with the malignancy of glioma.[10] A study has found that serum levels of EGFR are substantially increased in patients with malignant glioma, suggesting poor survival.[11]

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It has been hypothesized that EGFR may be a potential prognostic biomarker for glioma. However, there remains some controversy as to the prognostic value of EGFR in glioma. Because of the limitations of sample size and research methods, individual studies have not reached consensus on the question. Therefore, this meta-analysis was conducted to evaluate the effect of abnormal EGFR expression on overall survival (OS) in glioma patients.

Materials and methods
Retrieval strategy
Comprehensive literature retrieval was conducted using electronic databases such as PubMed, EMBASE, Google Scholar, Web of Science, and Cochrane Library (up to June 10, 2017). Medical subject headings (MeSH) (Emtree for EMBASE) and free-text words were adopted to balance comprehensiveness and accuracy. The following search terms were used: "Receptor, Epidermal Growth Factor" [Mesh], "Receptor Tyrosine-protein Kinase erbB-1", "Transforming Growth Factor alpha Receptor", "TGF-alpha Receptor", "Epidermal Growth Factor Receptor Kinase", "erbB-1 Proto-Oncogene Protein", "erbB-1 Proto-Oncogene Protein", "Receptor, Transforming-Growth Factor alpha", "Receptor, TGF-alpha", "c-ErbB-1 Protein, Proto-oncogene"; "Glioma" [Mesh], "astrocytoma", "ependymoblastoma", "ependymoma", "glioblastoma", "medulloblastoma", "oligodendroglioma", "optic nerve glioma", "pontine glioma", and "subependymoma", and key words related to survival included prognostic, survival, predictive, outcome, and death. The references of retrieved articles were also screened for eligible studies.

Inclusion and exclusion criteria
Inclusion criteria were as follows: 1) studies on pathological diagnosis of glioma in humans and investigation of EGFR expression in glioma; 2) studies containing hazard ratio (HR) or including survival data such as survival curves to estimate HR and its 95% confidence interval (CI); 3) studies published in English; 4) studies in which detection methods for EGFR were restricted to immunohistochemistry (IHC); and 5) randomized controlled trials, retrospective or prospective studies.

Exclusion criteria were as follows: 1) non-human experiments; 2) letters, case reports, and reviews; 3) duplicated publications; and 4) inability to extract HR or necessary information.

Data extraction and quality assessment
All data were extracted independently by two authors (Junhong Li and Ruofei Liang). Disagreements were resolved

by consensus with a third investigator (Yanhui Liu). The following data were extracted from the eligible studies: last name of the first author, publication year, region, study design, tumor type, assay, sample size, cutoff point, maximum follow-up time, HR, and its 95% CI.

HR and its 95% CI were extracted directly from the publications. If HRs and 95% CI were not available directly, they were calculated from the available survival data or extracted from the Kaplan–Meier curves by using the methods of Parmar et al[12] and Tierney et al.[13] If a study contained both univariate and multivariate analyses, both with HR, the HR provided by multivariate analysis was selected preferentially.

The quality of the eligible articles was evaluated on the Newcastle–Ottawa Scale (NOS), including assessments of patient selection, study comparability, follow-up time, and relevant outcome. NOS scores ranged from 0 to 9, with high-quality study defined as a score of >6.
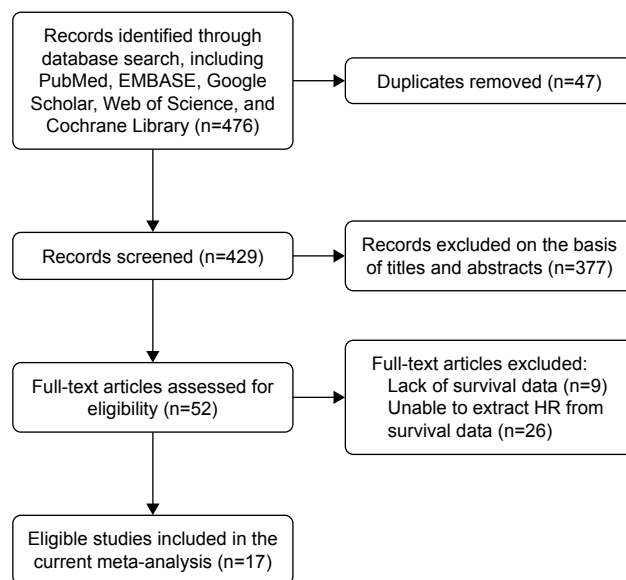
Statistical analysis
HR and 95% CIs were used to evaluate the association between EGFR expression and OS in glioma. An HR of >1 indicated worse survival with elevated EGFR expression, and a 95% CI not overlapping 1 was taken to be statistically significant. Statistical heterogeneity of the included studies was assessed by I^2 statistics and chi-square test, and I^2 value >50% or $P_{\text{heterogeneity}}$ <0.05 indicated substantial heterogeneity. The random-effects model was used to estimate the pooled HR if heterogeneity appeared. Otherwise, the fixed-effect model was applied.[14] All P-values were two-sided, and P<0.05 was considered to be statistically significant. Sensitivity analysis was also performed to evaluate the stability and reliability of the combinative results. The funnel plot with Begg's test and Egger's test was applied to evaluate publication bias. STATA 12.0 (StataCorp LP, College Station, TX, USA) was used to perform statistical analysis.

Results
Characteristics of the included studies
A total of 476 articles were screened from the databases, and 17 eligible articles including 1,458 patients were selected (Figure 1 and Table 1).[15–31] The publication dates ranged from 1998 to 2015, sample size from 18 to 199, and maximum follow-up time from 25 to 180 months. On NOS scoring, five articles were rated 6 points, one article was rated 7 points, and only one article was rated 8 points. Among all the included studies, only two were prospective studies, and the rest were retrospective studies. In most studies, specimens were fixed with formalin and embedded with paraffin. The treatment

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┌─────────────────────────────┐
│ Records identified through  │      ┌──────────────────────────┐
│ database search, including  │─────▶│ Duplicates removed (n=47)│
│ PubMed, EMBASE, Google      │      └──────────────────────────┘
│ Scholar, Web of Science, and│
│ Cochrane Library (n=476)    │
└─────────────────────────────┘
              │
              ▼
┌─────────────────────────────┐      ┌──────────────────────────┐
│ Records screened (n=429)    │─────▶│ Records excluded on the  │
│                             │      │ basis of titles and      │
│                             │      │ abstracts (n=377)        │
└─────────────────────────────┘      └──────────────────────────┘
              │
              ▼
┌─────────────────────────────┐      ┌──────────────────────────┐
│ Full-text articles assessed │─────▶│ Full-text articles       │
│ for eligibility (n=52)      │      │ excluded:                │
│                             │      │ Lack of survival data    │
│                             │      │ (n=9)                    │
│                             │      │ Unable to extract HR from│
│                             │      │ survival data (n=26)     │
└─────────────────────────────┘      └──────────────────────────┘
              │
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┌─────────────────────────────┐
│ Eligible studies included   │
│ in the current meta-analysis│
│ (n=17)                      │
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Figure 1 Flow diagram of the study selection process.
Abbreviation: HR, hazard ratio.

of tumor tissues was not clearly defined in two studies. IHC was performed in all studies. Of the 17 articles, seven reported the available HR and 10 provided the Kaplan–Meier curves. Considering glioma subgroups, 10 studies focused on GBM, two studies focused on ependymoma, two focused on low-grade glioma (LGG), one focused on astrocytoma, one included anaplastic astrocytoma (AA) and GBM, and one included glioma of various subgroups and grades. No study reported disease-free survival or PFS.

Prognostic significance of EGFR expression in glioma patients

A total of 1,458 glioma patients were included in the survival analysis. The pooled HR was 1.72 (95% CI 1.32–2.25, $P=0.000$, random effect; Figure 2). High expression of EGFR had a significant relationship with poor prognosis for glioma patients.

Due to the emergence of high heterogeneity in the study ($I^2=79.8\%$, $P_h=0.000$), a subgroup analysis was conducted to further explore the source of heterogeneity. The subgroups included tumor types, publication years, region, study design, sample size, cutoff value, maximum follow-up time, source of HR, and quality score (Table 2). High EGFR expression was significantly associated with reduced OS in GBM (HR 1.57, 95% CI 1.15–2.14, $P=0.004$) and mixed gliomas (HR 1.95, 95% CI 1.37–2.77, $P=0.000$), but not in LGG (HR 1.67, 95% CI 0.037–7.62, $P=0.508$). There was substantial heterogeneity in GBM ($I^2=83.8\%$, $P_h=0.000$), while heterogeneity was not found in LGG ($I^2=0\%$, $P_h=0.731$) and mixed gliomas ($I^2=0\%$, $P_h=0.522$). In subgroups of publication

years, study design, sample size, source of HR, and quality score, the following predicted poor survival: publication year <2010 (HR 1.95, 95% CI 1.37–2.77, $P=0.000$); publication year ≥2010 (HR 1.52, 95% CI 1.10–2.10, $P=0.011$); retrospective study (HR 1.75, 95% CI 1.39–2.19, $P=0.000$); sample size <100 (HR 1.99, 95% CI 1.54–2.57, $P=0.000$); sample size ≥100 (HR 1.43, 95% CI 1.04–2.97, $P=0.027$); HRs extracted from the Kaplan–Meier curves (HR 2.10, 95% CI 1.71–2.58, $P=0.000$); quality score <7 (HR 1.37, 95% CI 1.00–1.87, $P=0.048$); and quality score ≥7 (HR 1.72, 95% CI 1.32–2.25, $P=0.000$).

Apparent heterogeneity existed in the subgroup of publication year ≥2010 ($I^2=80.2\%$, $P_h=0.000$), prospective study ($I^2=81.1\%$, $P_h=0.000$), sample size ≥100 ($I^2=85.5\%$, $P_h=0.000$), HRs that were reported ($I^2=69.5\%$, $P_h=0.003$), and quality score ≥7 ($I^2=84.8\%$, $P_h=0.000$). Low heterogeneity existed in the subgroup of publication year <2010 ($I^2=45.6\%$, $P_h=0.065$) and retrospective study ($I^2=45.8\%$, $P_h=0.024$). Sample size <100 ($I^2=0\%$, $P_h=0.501$) and quality score <7 ($I^2=0\%$, $P_h=0.618$) did not show heterogeneity.

The subgroup region included three variables: Europe, America, and Asia, where Europe (HR 1.86, 95% CI 1.32–2.61, $P=0.000$) and Asia (HR 1.83, 95% CI 1.04–3.23, $P=0.037$) were significantly related to worse prognosis, but not related to America (HR 1.32, 95% CI 0.98–1.79, $P=0.070$). The heterogeneity of America ($I^2=0\%$, $P_h=0.546$), Europe ($I^2=53.3\%$, $P_h=0.036$), and Asia ($I^2=85.7\%$, $P_h=0.000$) gradually increased. In the two subgroups of cutoff value and maximum follow-up time, each subitem gave significance for predicting prognosis. The heterogeneity of cutoff value <20% ($I^2=28.2\%$, $P_h=0.223$) and cutoff value ≥20% ($I^2=43.9\%$, $P_h=0.129$) was relatively low, while there was substantial heterogeneity in the subgroup of cutoff value determined by other modes and of unavailable cutoff value ($I^2=83.3\%$, $P_h=0.000$). There was substantial heterogeneity in the subgroup of maximum follow-up time <100 months ($I^2=81.8\%$, $P_h=0.000$), while there was no substantial heterogeneity in the subgroup of maximum follow-up time ≥ 100 months ($I^2=0\%$, $P_h=0.976$) and unavailable maximum follow-up time ($I^2=0\%$, $P_h=0.944$).
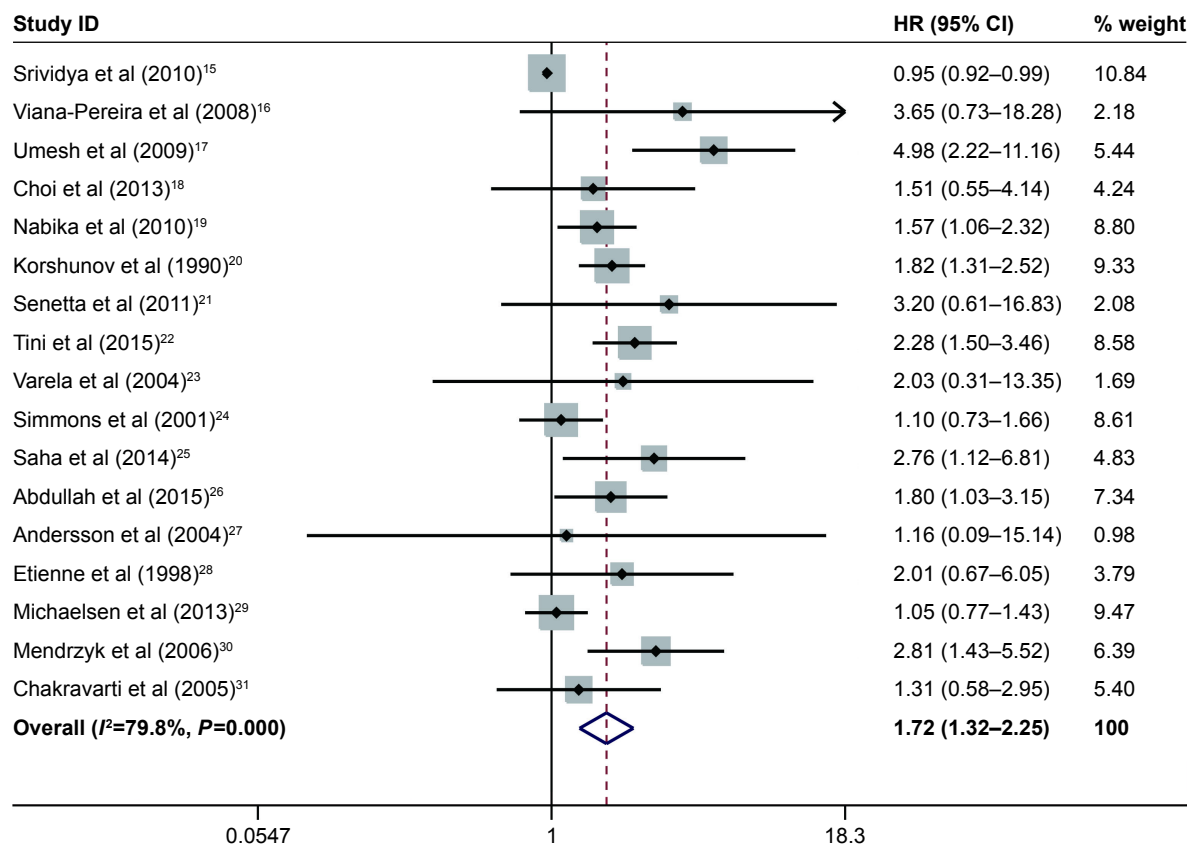
Prognostic significance of EGFR expression in GBM patients

GBM is one of the most common gliomas with the highest degree of malignancy. It accounts for a large proportion of the included studies, and therefore, a meta-analysis was conducted to determine the prognostic significance of EGFR expression in GBM patients. Ten studies including 1,074 patients were considered in the survival analysis. The pooled HR was

Table 1 The main characteristics of the included 17 studies in the meta-analysis

Authors	Publication year	Region	Study design	Tumor type	Range	Detected sample	Assay	Number of patients (male)	Median/mean* (range) or Mean ± SD	Cutoff point (%)	Maximum follow-up time (m)	Survival analysis	Hazard ratios	Quality score	Author's attitude
Srividya et al[15]	2010	India	Prospective	GBM	NA	Tumor tissue/FFPE	IHC	140	47 (18–65)	NA	34	OS	Report	8	Negative
Viana-Pereira et al[16]	2008	Portugal	Retrospective	GBM	NA	Tumor tissue/FFPE	IHC	27	NA	10	93	OS	Report	7	Negative
Umesh et al[17]	2009	India	Retrospective	GBM	2002	Tumor tissue/FFPE	IHC	54 (41)	46.15* (16–80)	20	53	OS	K–M curve	7	Positive
Choi et al[18]	2013	Korea	Retrospective	GBM	1997–2006	Tumor tissue/FFPE	IHC	30 (18)	55.5 (24–77)	10	NA	OS	K–M curve	7	Positive
Nabika et al[19]	2010	Japan	Retrospective	AA and GBM	1995–2005	Tumor tissue/FFPE	IHC	59 (35)	61.2±13.3	30	NA	OS	Report	7	Positive
Korshunov et al[20]	1999	Russia	Retrospective	GBM	1997–1998	Tumor tissue/FFPE	IHC	168 (95)	50 (18–73)	NA	60	OS	K–M curve	7	Positive
Senetta et al[21]	2011	Italy	Retrospective	Ependymoma	2001–2006	Tumor tissue/FFPE	IHC	22 (6)	38.7* (18–63)	20	109	OS	K–M curve	7	Positive
Tini et al[22]	2015	Italy	Retrospective	GBM	2008–2013	Tumor tissue/FFPE	IHC	144	63 (35–84)	50	100	OS	K–M curve	7	Positive
Varela et al[23]	2004	Argentina	Retrospective	LGG	1995–2000	Tumor tissue/FFPE	IHC	25 (16)	37 (18–72)	10	140	OS	K–M curve	6	Positive
Simmons et al[24]	2001	USA	Retrospective	GBM	1987–1995	Tumor tissue/FFPE	IHC	110 (68)	55	NA	75	OS	Report	6	Negative
Saha et al[25]	2014	India	Prospective	Astrocytoma	2009–2012	Tumor tissue/FFPE	IHC	57 (45)	NA	15.40	25	OS	K–M curve	7	Positive
Abdullah et al[26]	2015	USA	Retrospective	GBM	2003–2013	Tumor tissue	IHC	47	NA	NA	72	OS	K–M curve	6	Positive
Andersson et al[27]	2004	Sweden	Retrospective	LGG	NA	Tumor tissue/FFPE	IHC	18	NA	20	180	OS	K–M curve	6	Positive
Etienne et al[28]	1998	France	Retrospective	Glioma	1985–1995	Tumor tissue/FFPE	IHC	40	NA	4.60	120	OS	K–M curve	6	Positive
Michaelsen et al[29]	2013	Denmark	Retrospective	GBM	2005–2010	Tumor tissue/FFPE	IHC	199	NA	10	60	OS	Report	7	Negative
Mendrzyk et al[30]	2006	Russia	Retrospective	Ependymoma	1987–2003	Tumor tissue	IHC	163	NA	NA	120	OS	Report	7	Positive
Chakravarti et al[31]	2005	USA	Retrospective	GBM	NA	Tumor tissue/FFPE	IHC	155	NA	MOD 26.3	60	OS	Report	7	Negative

Abbreviations: AA, anaplastic astrocytoma; FFPE, formalin fixed and paraffin embedded; GBM, glioblastoma multiforme; IHC, immunohistochemistry; K–M curve, Kaplan–Meier curve; LGG, low-grade glioma; m, months; MOD, mean optical density; NA, not available; OS, overall survival.

Study ID	HR (95% CI)	% weight
Srividya et al (2010)[15]	0.95 (0.92–0.99)	10.84
Viana-Pereira et al (2008)[16]	3.65 (0.73–18.28)	2.18
Umesh et al (2009)[17]	4.98 (2.22–11.16)	5.44
Choi et al (2013)[18]	1.51 (0.55–4.14)	4.24
Nabika et al (2010)[19]	1.57 (1.06–2.32)	8.80
Korshunov et al (1990)[20]	1.82 (1.31–2.52)	9.33
Senetta et al (2011)[21]	3.20 (0.61–16.83)	2.08
Tini et al (2015)[22]	2.28 (1.50–3.46)	8.58
Varela et al (2004)[23]	2.03 (0.31–13.35)	1.69
Simmons et al (2001)[24]	1.10 (0.73–1.66)	8.61
Saha et al (2014)[25]	2.76 (1.12–6.81)	4.83
Abdullah et al (2015)[26]	1.80 (1.03–3.15)	7.34
Andersson et al (2004)[27]	1.16 (0.09–15.14)	0.98
Etienne et al (1998)[28]	2.01 (0.67–6.05)	3.79
Michaelsen et al (2013)[29]	1.05 (0.77–1.43)	9.47
Mendrzyk et al (2006)[30]	2.81 (1.43–5.52)	6.39
Chakravarti et al (2005)[31]	1.31 (0.58–2.95)	5.40
Overall (I^2=79.8%, P=0.000)	1.72 (1.32–2.25)	100

0.0547   1   18.3

Figure 2 Meta-analysis of pooled HRs of OS in gliomas.
Note: Weights are from random-effects analysis.
Abbreviations: CI, confidence interval; HR, hazard ratio; OS, overall survival.

1.57 (95% CI 1.15–2.14, P=0.004, random effect; Figure 3). This indicated that high expression of EGFR was significantly associated with poor prognosis in GBM patients. Similarly, subgroup analysis was performed because of high heterogeneity (I^2=83.8%, P_h=0.000). The grouping and results of subgroup analysis are displayed in Table 3.

Sensitivity analysis and publication bias

All the studies were sequentially removed to determine whether a single study had significant influence on pooled HR and to verify the stability and reliability of HR estimates. It was found that the pooled HRs were not significantly influenced by any individual study (Figures 4 and 5).

Publication bias was assessed using the funnel plot (Figure 6), Begg's and Egger's tests. The funnel plot was asymmetrical. Begg's test (z =0.54, $P > |z|$ =0.592) revealed no publication bias among the 17 eligible studies, while Egger's test (t=5.41, $P > |t|$ =0.000) showed clear bias.

Discussion

EGFR plays an important role in tumor growth, participating in cell motility, adhesion, invasion, and angiogenesis.[32]

In recent years, EGFR has been a focus of numerous studies on tumor prognosis. Similar meta-analyses involving gastric cancer, non-small-cell lung cancer, nasopharyngeal carcinoma, pancreatic cancer, and esophageal adenocarcinoma have shown poor prognosis associated with EGFR expression.[32–36] Comparatively speaking, biomarker studies in glioma have been relatively recent. There have been a large number of inconsistent and even contradictory results regarding the prognostic significance of EGFR in glioma. Among the included studies, 12 suggested that high expression of EGFR indicates poor prognosis, while five others were negative or uncertain on the question. In addition to the 17 studies included in the current meta-analysis, the studies that were excluded according to the inclusion criteria also showed a substantial degree of polarization. High expression of EGFR was not found to predict the poor prognosis of glioma in the studies of Reis-Filho et al,[37] Bouvier-Labit et al,[38] Smith et al,[39] and Dorward et al,[40] while other studies suggested that elevated EGFR predicted reduced OS.[41–46]

Similarly, as the direct source of EGER expression, EFEG amplification was also used as a prognostic marker of glioma. The literature also returned two levels of differentiation.

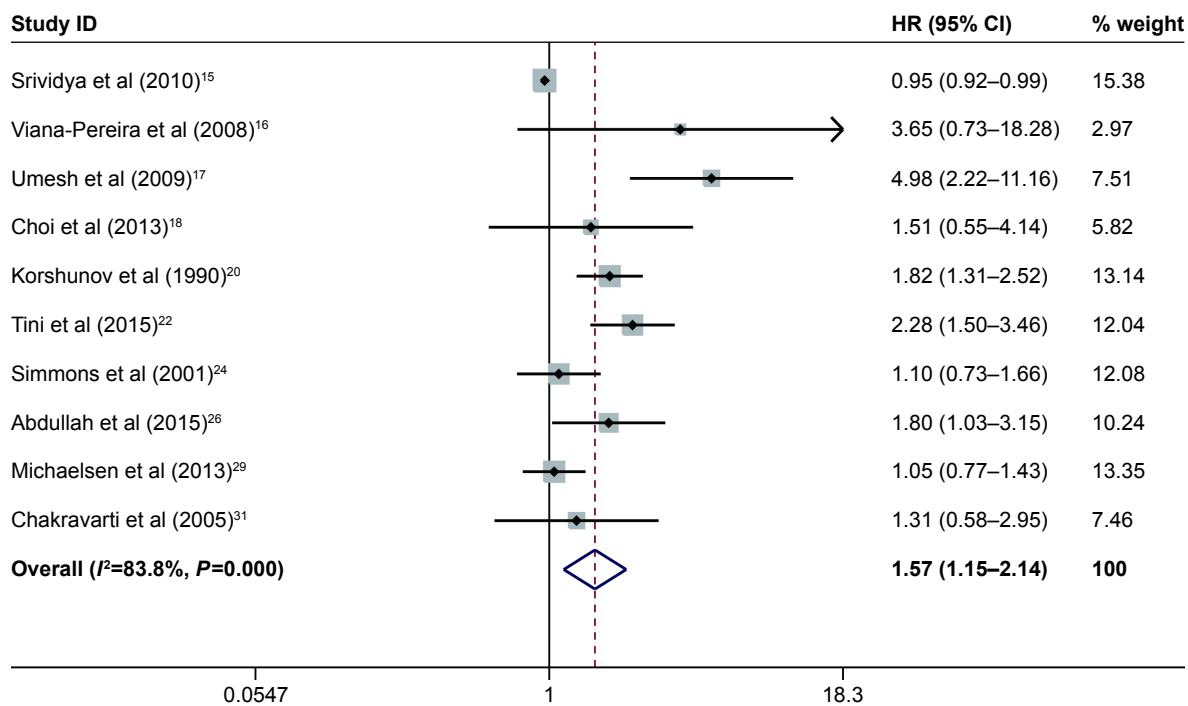**Table 2** Meta-analysis results of subgroups for the OS of gliomas

Subgroup analysis	Number of studies	HR (95% CI)	P-value	Heterogeneity	
				I^2 (%)	P_h
OS	17	1.72 (1.32–2.25)	0.000	79.8	0.000
Tumor types					
GBM	10	1.57 (1.15–2.14)	0.004	83.8	0.000
LGG	2	1.67 (0.37–7.62)	0.508	0	0.731
Mixed gliomas	5	1.95 (1.45–2.63)	0.000	0	0.522
Publication year					
<2010	9	1.95 (1.37–2.77)	0.000	45.6	0.065
≥2010	8	1.52 (1.10–2.10)	0.011	80.2	0.000
Region					
Europe	8	1.86 (1.32–2.61)	0.000	53.3	0.036
America	4	1.32 (0.98–1.79)	0.070	0	0.546
Asia	5	1.83 (1.04–3.23)	0.037	85.7	0.000
Study design					
Prospective	2	1.47 (0.53–4.08)	0.460	81.1	0.021
Retrospective	15	1.75 (1.39–2.19)	0.000	46.8	0.024
Sample size					
<100	10	1.99 (1.54–2.57)	0.000	0	0.501
≥100	7	1.43 (1.04–1.97)	0.027	85.5	0.000
Cutoff value					
<20%	6	1.57 (1.02–2.43)	0.041	28.2	0.223
≥20%	5	2.30 (1.49–3.55)	0.000	43.9	0.129
Others and NA	6	1.46 (1.01–2.10)	0.044	83.3	0.000
Maximum follow-up time					
<100 m	9	1.55 (1.13–2.12)	0.006	81.8	0.000
≥100 m	6	2.36 (1.71–3.26)	0.000	0	0.976
NA	2	1.56 (1.08–2.25)	0.016	0	0.944
Source of HR					
Report	7	1.28 (0.98–1.66)	0.072	69.5	0.003
Kaplan–Meier curves	10	2.10 (1.71–2.58)	0.000	0	0.658
Quality score					
<7	5	1.37 (1.00–1.87)	0.048	0	0.618
≥7	12	1.82 (1.31–2.51)	0.000	84.8	0.000

Abbreviations: CI, confidence interval; GBM, glioblastoma multiforme; HR, hazard ratio; LGG, low-grade glioma; m, months; NA, not available; OS, overall survival.

Some studies showed that EGFR amplification was related to worse survival,[47–50] while other studies returned the opposite result.[51–53] Hobbs et al[54] showed that low-to-moderate EGFR amplification was an independent adverse prognostic variable, while high-level EGFR amplification did not play a role in a similar model. In the study of EGFR gene polymorphisms, Costa et al[55] found that EGFR variants "-191C/A" and "intron 1 (CA)n repeat" were prognostic markers in GBM patients. Li et al[56] conducted a similar gene polymorphism study. In the molecular studies of the Tunisian population, both EGFR amplification and EGFR overexpression predicted significantly poor OS.[57] The study on pediatric glioma indicated that no association was apparent between EGFR expression level and either PFS or OS.[58,59] All the abovementioned studies have provided rigorous experimental design and accurate and reliable data analysis but contradictory results. A previous meta-analysis of EGFR

amplification done by Chen et al[60] concluded that there is not enough evidence to suggest that EGFR amplification has prognostic value in GBM patients.

In addition to being a potential prognostic factor, EGFR is also a potential target for the treatment. Some reports claim that 97% of primary GBM show EGFR amplification.[61] Because of the important role of EGFR signaling in the pathogenesis of malignant tumor, a growing number of studies have been devoted to developing therapeutic strategies targeting EGFR aberrant activity. As a transmembrane tyrosine kinase, small molecule tyrosine kinase inhibitors (TKIs) may theoretically inhibit EGFR expression and function in malignant tumor. The mechanism would involve displacement of ATP from the catalytic pocket of the enzyme to inhibit kinase activity. Monoclonal antibodies may play a role by combining with the extracellular domain of EGFR.[9] Both the methods have been applied in clinical

Study ID		HR (95% CI)	% weight
Srividya et al (2010)[15]		0.95 (0.92–0.99)	15.38
Viana-Pereira et al (2008)[16]		3.65 (0.73–18.28)	2.97
Umesh et al (2009)[17]		4.98 (2.22–11.16)	7.51
Choi et al (2013)[18]		1.51 (0.55–4.14)	5.82
Korshunov et al (1990)[20]		1.82 (1.31–2.52)	13.14
Tini et al (2015)[22]		2.28 (1.50–3.46)	12.04
Simmons et al (2001)[24]		1.10 (0.73–1.66)	12.08
Abdullah et al (2015)[26]		1.80 (1.03–3.15)	10.24
Michaelsen et al (2013)[29]		1.05 (0.77–1.43)	13.35
Chakravarti et al (2005)[31]		1.31 (0.58–2.95)	7.46
Overall (I^2=83.8%, *P*=0.000)		**1.57 (1.15–2.14)**	**100**

0.0547   1   18.3

Figure 3 Meta-analysis of pooled HRs of OS in GBM.
Note: Weights are from random-effects analysis.
Abbreviations: CI, confidence interval; GBM, glioblastoma multiforme; HR, hazard ratio; OS, overall survival.

Table 3 Meta-analysis results of subgroups for the OS of GBMs

Subgroup analysis	Number of studies	HR (95% CI)	*P*-value	Heterogeneity	
				I^2 (%)	P_h
OS	10	1.57 (1.15–2.14)	0.004	83.8	0.000
Publication years					
<2010	5	1.88 (1.15–3.09)	0.012	68.0	0.014
≥2010	5	1.36 (0.95–1.97)	0.096	82.2	0.000
Region					
Europe	4	1.69 (1.09–2.63)	0.018	73.7	0.010
America	3	1.31 (0.96–1.78)	0.086	0	0.381
Asia	3	1.85 (0.63–5.41)	0.262	88.1	0.000
Study design					
Prospective	1	0.95 (0.92–0.99)	0.027	/	/
Retrospective	9	1.70 (1.26–2.30)	0.001	64.5	0.004
Sample size					
<100	4	2.50 (1.38–4.50)	0.002	42.7	0.156
≥100	6	1.32 (0.97–1.80)	0.078	84.4	0.000
Cutoff value					
<20%	3	1.25 (0.77–2.04)	0.370	22.4	0.276
≥20%	2	3.11 (1.15–2.14)	0.003	64.9	0.092
Others and NA	5	1.31 (0.93–1.86)	0.126	80.4	0.000
Maximum follow-up time					
<100 m	8	1.47 (1.07–2.02)	0.017	82.0	0.000
≥100 m	1	2.28 (1.50–3.46)	0.000	/	/
NA	1	1.51 (0.55–4.14)	0.424	/	/
Source of HR					
Report	5	0.96 (0.92–1.00)	0.077	0.4	0.404
Kaplan–Meier curves	5	2.13 (1.59–2.85)	0.000	33.0	0.202
Quality score					
<7	2	1.36 (0.84–2.18)	0.211	48.1	0.165
≥7	8	1.65 (1.13–2.41)	0.009	86.2	0.000

Abbreviations: CI, confidence interval; GBM, glioblastoma multiforme; HR, hazard ratio; m, months; NA, not available; OS, overall survival.

## Meta-analysis estimates, given named study is omitted
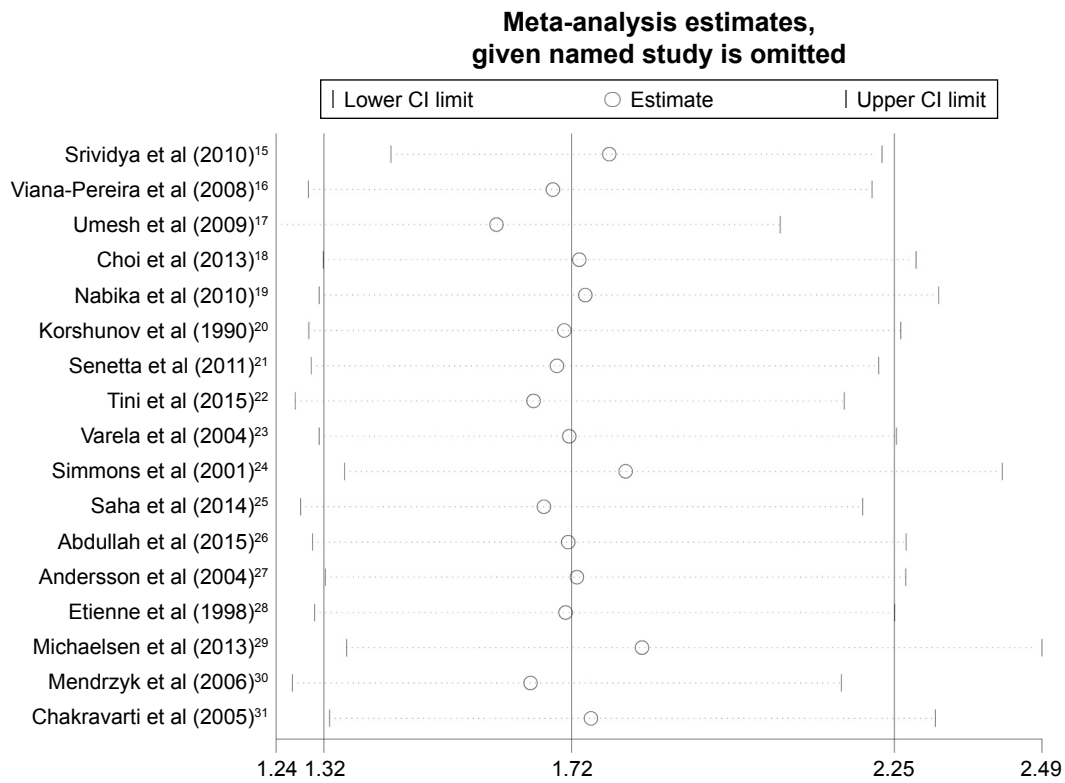
Figure 4 Sensitivity analysis of the relationship between EGFR expression and overall survival in glioma patients.
Abbreviations: CI, confidence interval; EGFR, epidermal growth factor receptor.

Meta-analysis estimates, given named study is omitted
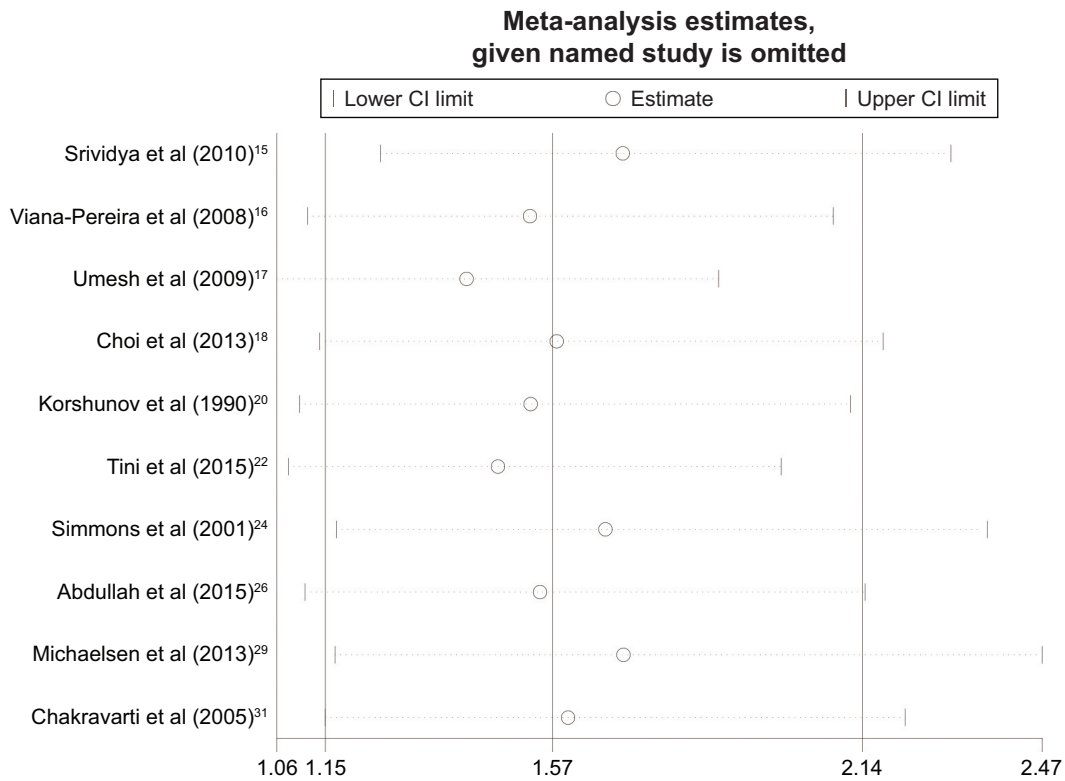
Figure 5 Sensitivity analysis of the relationship between EGFR expression and overall survival in GBM patients.
Abbreviations: CI, confidence interval; EGFR, epidermal growth factor receptor; GBM, glioblastoma multiforme.
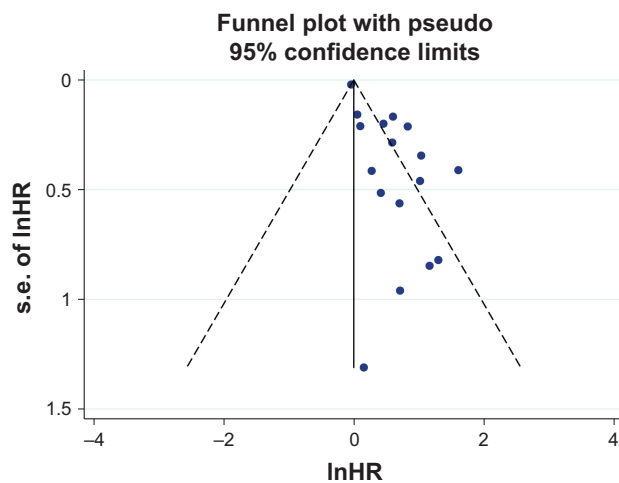
Figure 6 The funnel plot for all the included studies for overall survival.
Abbreviations: s.e., standard error; lnHR, natural logarithm hazard ratio.

practice with remarkable results in the treatment of non-small-cell lung cancer and colon cancer. However, the use of TKIs and monoclonal antibodies in glioma is challenged. Preusser et al[62] concluded in their clinical trial that only a small number of malignant glioma patients benefited from EGFR inhibitor monotherapy. Bevacizumab has a tendency to increase the risk of adverse effects in people older than 65 years, and there is not enough evidence to recommend the use of bevacizumab in pediatric glioma patients.[8] Combined with other treatments, such as temozolomide and radiation therapy, stereotactic surgery may be helpful in controlling glioma. Newer and safer targeted therapies have yet to be developed to improve treatment efficacy.

There is a considerable degree of heterogeneity among various studies. Heterogeneity emerged from differences in experimental items, overall experimental design, and analyzed indicators. There was substantial heterogeneity (I^2=79.8%, P_h=0.000) in pooled HR in the current meta-analysis. Similar results in pooled HR were observed in the GBM group (I^2=83.8%, P_h=0.000). Although the final results suggested poor prognostic role of EGFR, high heterogeneity substantially reduces the reliability of the results. The operations from setting include criteria, subgroup analysis and sensitivity analysis were used to ensure the reliability of the final results, in other words, to provide a strong reference. All the included studies used HR with 95% CI as the index of survival analysis. With I^2 and P_h as indicators of heterogeneity, subgroup analysis suggested high heterogeneity in tumor types, publication year, region, sample size, maximum follow-up time, source of HR, and quality score, as follows: GBM (I^2=83.8%,

P_h=0.000), publication year ≥2010 (I^2=80.2%, P_h=0.000), Asia (I^2=85.7%, P_h=0.000), sample size ≥100 (I^2=85.5%, P_h=0.000), and other methods to divide cutoff value and cutoff value not available (I^2=83.3%, P_h=0.000), maximum follow-up time <100 months (I^2=81.8%, P_h=0.000), and quality score ≥7 (I^2=84.8%, P_h=0.000). There was low heterogeneity in Europe (I^2=53.3%, P_h=0.036) when HR was reported (I^2=69.5%, P_h=0.003). The abovementioned items were the actual source of heterogeneity; other items showed low or no heterogeneity. Among 22 items in the nine subgroups, only three had HR with no statistical significance, including LGG (HR 1.67, 95% CI 0.37–7.62, P=0.508), America (HR 1.32, 95% CI 0.98–1.79, P=0.070), and HR that were reported (HR 1.28, 95% CI 0.98–1.66, P=0.072). This suggested a stable and reliable final result.

In the GBM subgroup analysis, there was substantial heterogeneity in items such as publication year ≥2010 (I^2=82.2%, P_h=0.000), Europe (I^2=73.7%, P_h=0.010), Asia (I^2=88.1%, P_h=0.000), sample size ≥100 (I^2=84.4%, P_h=0.000), other methods to divide cutoff value (I^2=80.4%, P_h=0.000), unavailable cutoff value (I^2=80.4%, P_h=0.000), maximum follow-up time <100 months (I^2=82.0%, P_h=0.000), and quality score ≥7 (I^2=86.2%, P_h=0.000). The condition was basically consistent with HR subgroup analysis of the whole set of glioma. Among 19 items in the eight subgroups, pooled HR of eight items showed no statistical significance, and the reliability and stability of the pooled results were lower than that of the entire group of glioma. The greater heterogeneity of the GBM group (83.8% vs 79.8%) is one of the reasons for these results.

In sensitivity analysis, individual studies were sequentially removed, and the bottom limits of 95% CI of pooled HR of other studies were all larger than 1, suggesting that individual studies had limited impact on the entire study. The pooled HR had statistical significance, and the entire study was stable.

Asymmetry of the funnel plot and Egger's test demonstrated the existence of publication bias (the most common source of bias) affecting the authenticity and reliability of the data to some extent. First, positive results are easier to publish. Of the 17 studies, five were negative, and the inclusion of negative results would theoretically reduce publication bias. Second, repeatedly published theses will also lead to bias, if a single-center or multiple-center results are both published and included. Repeated calculation would lead to larger weight for some studies, affecting the reliability of

the clinical research. The research institutions and localities were carefully checked, and overlapping of institutions or experimental data was not found. Next, the small sample size studies return low test efficiencies. In other words, positive results may be caused by opportunities, which may not provide a reliable basis for clinical practice. In the subgroup analysis, HR was merged with a sample size of 100 as the boundary. Our results suggested larger HR and lower P in the subgroup with a sample size of <100, without heterogeneity, while greater heterogeneity was observed in the subgroup with large sample size (≥100). This confirmed that bias was caused by small sample size from the side. There may be many other unknown confounding factors that play a role in publication bias.

As a meta-analysis of prognostic analysis, there are inevitable limitations in several aspects which should be further discussed. First, the areas and population involved in this study are irregular (Africa, the Middle East, South America, and other regions). In addition, China, with a large population, was not considered. Second, there are differences in diagnostic standard and inspection methods in studies on glioma at different times. The accuracy of IHC results is improved by the optimization of the equipment and test methods with the passage of time. The diverse definitions of cutoff values among the studies may also lead to bias. Among experimental designs, there were no randomized controlled trials, which might have improved the accuracy of the analysis. However, there were two prospective studies, which are superior to retrospective studies. Finally, patients in several studies underwent standard therapy with temozolomide plus radiotherapy after surgery[15,22,29] and some patients did not undergo uniform treatments, while the remaining studies failed to report postoperative treatments. This too may result in heterogeneity of survival data.

Conclusion

Our meta-analysis suggested that EGFR may serve as a useful biomarker for poor prognosis in glioma patients. In the future, targeted therapy aiming at EGFR signaling pathways may help slow the disease progression of glioma and prolong OS. To verify this result, studies with large sample sizes, covering extensive areas, with more reasonable experimental designs, more standardized diagnostic criteria, and standardized postoperative treatment are required.

Acknowledgments

Disclosure

The authors report no conflicts of interest in this work.

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