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Original Article

Clinical value of glioma-associated oncogene homolog 1 as a prognostic marker in cancer: a meta-analysis

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Abstract: Aberrant activation of the transcription factor glioma-associated oncogene homolog 1 (GLI1), a central effector of the Hedgehog (HH) pathway, is associated with human malignancies. Emerging evidence has shown that overexpressed GLI1 is significantly correlated with clinicopathologic features and poor prognosis in patients with cancer. To evaluate the clinical value of GLI1 as a prognostic marker in human cancers, this meta-analysis collected all relevant articles and explored the association of GLI1 expression levels with prognosis in patients with carcinoma. Literature collection was conducted by searching electronic databases PubMed, Medline, EMBASE, Web of Science, Ovid and Cochrane library (up to July 10, 2015). Pooled hazard ratios (HRs) with corresponding 95% confidence intervals (CIs) were calculated to estimate the strength of the link between GLI1 and clinical prognosis by STATA 12.0 software. 21 eligible studies with a total of 2381 patients were matched to our inclusion criteria. The result showed that overexpression of GLI1 could predict poor overall survival (OS) and disease-free survival (DFS) in patients with cancer, with HR of 2.07 (95% CI 1.83-2.35), HR of 1.81 (95% CI 1.53-2.16), respectively. Residence region (Asia and Europe), cancer type (nervous, digestive and respiratory system carcinoma), measurement methods (IHC and PCR) and methods of analysis (univariate and multivariate analyses), did not alter the predictive value of GLI1 on poor OS among the investigated cancers. This meta-analysis demonstrated that GLI1 may be used as a prognostic marker to predict poor survival of patients with cancer.

Keywords: Meta-analysis, GLI1, prognosis, cancer

Introduction

In 1980, developmental biologists Christiane Nüsslein-Volhard and Eric Wieschaus discovered the hedgehog (HH) signaling pathway in Drosophila (Drosophila melanogaster) [1]. Soon after this, three mammalian orthologs of HH were discovered, namely, Desert hedgehog (DHH), Sonichedgehog (SHH), and Indian hedgehog (IHH). The pathway name is from its polypeptide ligand, an intercellular signalling molecule called HH (desert Hh, sonic Hh, and Indian Hh). Sonic hedgehog (SHH) is the best studied ligand of the vertebrate pathway. When SHH reaches its target cell, it binds to the patched-1 (PTCH1) receptor. PTCH1 has a sterol sensing domain, which has been shown to be essential for suppression of smoothened (SMO) activity. In the absence of ligand, PTCH1 inhibits SMO, a downstream protein in the pathway. The binding of SHH relieves SMO inhibition, leading to activation of the transcription factors Glioma-Associated Oncogene Homolog (GLI), the activators GLI1 and GLI2 and the repressor GLI3 [2]. Sonic hedgehog (Shh) signaling is critically important for embryogenesis and other cellular processes in which GLI transcription factors mediate the terminal effects of the pathway [3]. Mutations or other regulatory errors in the hedgehog pathway are associated with a number of birth defects as well as some cancers [4, 5]. GLI1, in particular, plays a significant role in tumorigenesis, cancer growth and cancer stem cell self-renewal.

Glioma-associated oncogene homolog 1, also known as GLI1, was discovered in 1987 upon investigation into gene amplification in a human glioblastoma cell line [6]. Investigators found a region of chromosome 12 to be amplified; however, this region did not correspond to any known oncogenes. The gene was termed GLI1

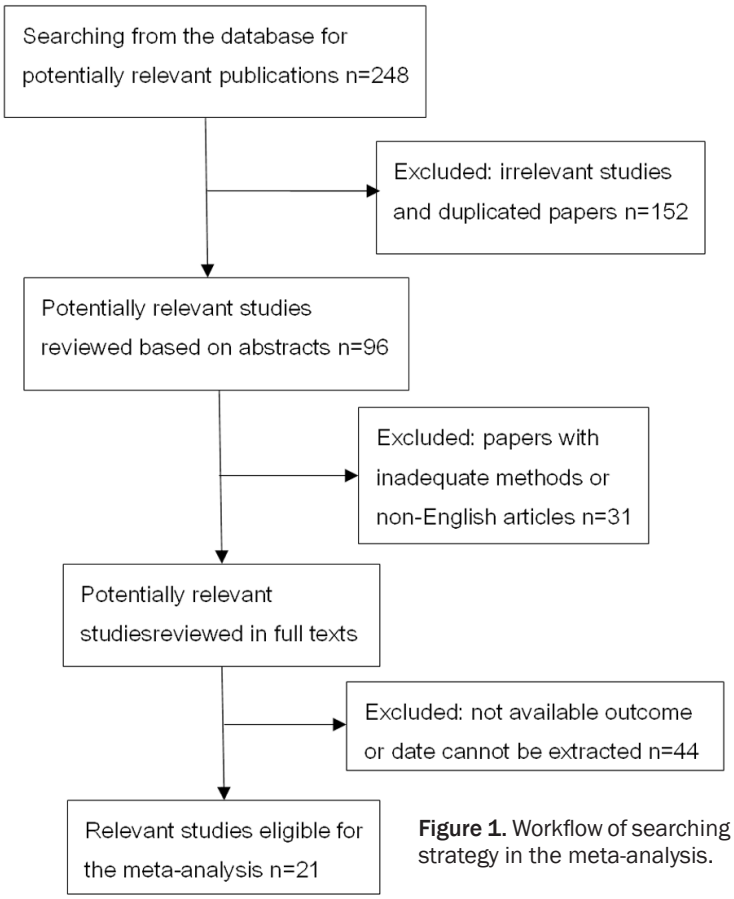

Figure 1. Workflow of searching strategy in the meta-analysis.

for the glioma tumor in which it was found and was later mapped to a specific region of chromosome 12 at 12q13.3-14.1 [7]. This newly discovered GLI1 gene contained 3,318 base pairs giving rise to a 1,106-residue protein that separates on a polyacrylamide gel to 150-kDa [8]. The known functional domains of full-length GLI1 include the degron degradation signals, SUFU-binding domains, zinc finger domains, the nuclear localization signal, and the transactivation domain [9]. Activated by SMO as mentioned above, GLI1 transports from the cytoplasm into the nucleus, accumulates in the nucleus and binds to the consensus GLI1-binding element within its target genes, leading to activation of a number of genes that regulate important cellular processes, such as, G1 cell cycle progression, cell proliferation and differentiation, anti-apoptosis, tumor progression, metastasis and tumorigenesis [10]. Emerging evidences have shown that overexpressed GLI1 is significantly associated with clinicopathologic features and poor prognosis in patients. Overexpressed GLI1 enhances migration and invasion in ERα negative breast cancer cell lines [11]. GLI1 mediates lung cancer cell proliferation [12], siRNA targeting of GLI1 reduces cell proliferation and tumor size [13]. Elevated GLI1 expression is also found to be significantly associated with invasion and postoperative liver metastasis in colon cancer [14]. Specific knockdown of GLI1 can reduce VEGF production by glioma stem cells reducing their ability to promote angiogenesis in vitro [15], suggesting that GLI1 may serve as an unfavorable prognostic biomarker for patients with cancer.

Recent studies suggested that GLI1 expression was associated with the features of human cancers. However, the sample size was not large enough and the outcomes were relatively discrete. To evaluate the clinical value of GLI1 as a prognostic marker in human cancer more comprehensively, we analyze all previously published data and carried out this meta-analysis.

Material and methods

Search strategy

A systematic literature search of PubMed, Medline, EMBASE, Web of Science, Ovid and Cochrane library. The literature covered was restricted to publications in English. The following key words were used for the search: “GLI1”, “Glioma-Associated Oncogene Homolog 1”, “cancer or carcinoma or tumor or neoplasma or neoplasm or malignancy or sarcoma”, “prognostic or prognosis”, “outcome”, “mortality”, “survival” and “recurrence”. The literature search was stopped at July 10, 2015.

Selection criteria and quality assessment

Inclusion criteria were as follows: (1) information of study population and regions; (2) information of any type of human cancer; (3) description of study design; (4) investigation of the correlation between GLI1 expression level and survival outcome; (5) description of GLI1

Table 1. Main characteristics of included studies

Study	Year	Region	Tumor type	No.	Stage	SO	Cut-off value	Me	Survival analysis	Follow up	Quality score
Chang et al	2015	China	Glioblastoma	135	NA	OS	25% cell staining	1	U+M	24 m	86.4%
Marechal et al (1)#	2014	Belgium	Pancreatic cancer	237	I-IV	OS, DFS	Staining intensity =1	1	U+M	72 m	93.2%
Marechal et al (2)#	2014	France	Pancreatic cancer	234	I-IV	OS, DFS	Staining intensity =1	1	U+M	72 m	93.2%
Marechal et al (3)#	2014	France	Pancreatic cancer	96	I-IV	OS, DFS	Staining intensity =1	1	U+M	72 m	93.2%
Tang et al	2013	China	Hepatocellular carcinoma	108	I-IV	OS, DFS	IRS =6	1	U+M	8-82 m	86.4%
Che et al*	2012	China	Hepatocellular carcinoma	46	NA	OS, DFS	Mean	2	U	1-83 m	72.7%
Jiang et al	2014	China	Pancreatic cancer	90	I-IV	OS	Staining extent scores plus intensity scores =3	2	U	87 m	81.8%
Ciucci et al	2012	Roman	Ovarian Cancer	56	III-IV	OS, DFS	10% cell staining	1	U+M	9-127 m	93.2%
g et al	2014	China	Pancreatic cancer	57	I-III	OS	Staining extent scores plus intensity scores =3	1	U+M	48 m	86.4%
Ishikawa* et al	2014	Japan	Lung adenocarcinoma	102	II-IV	OS	NA	1	U+M	70 m	86.4%
Xu et al	2010	USA	Breast cancer	60	I-IV	OS	Mean of IHC score (stain extent scores mutply intensity scores)	1	U+M	NA	86.4%
Yang et al	2013	Taiwan	Pancreatic cancer	81	I-IV	OS	10% cell staining	1	U+M	NA	81.8%
Li et al	2012	China	Gallbladder carcinoma	93	I-IV	OS	10% cell staining	1	U+M	5-66 m	93.2%
Xu et al	2012	China	Colon cancer	228	I-III	OS, DFS	10% cell staining	1	U+M	5-109 m	93.2%
Haaf et al	2009	Germany	Breast cancer	229	I-IV	OS	IRS =6	2	U	148 m	81.8%
Li et al	2010	China	Hepatocellular carcinoma	32	I-IV	OS	5% cell staining	1	U+M	60 m	86.4%
Chaudary* et al	2011	Canada	Cervical Carcinoma	80	I-IV	DFS	median	1	U+M	10.6 y	93.2%
Zhang et al	2014	China	Hepatocellular carcinoma	58	I-IV	OS	Staining extent scores mutply intensity scores =1	2	U	36 m	81.8%
Hong et al	2014	China	Non-small Cell Lung Cancers	55	I-IV	OS	NA	2	U	120 m	72.7%
Xie et al	2014	China	Gallbladder carcinoma	32	I-IV	OS	IRS =5	2	U	36 m	81.8%
Li et al	2011	China	Glioma	100	II-IV	OS	10% cell staining	1	U + M	1-98 m	86.4%
Saze et al	2012	Japan	Gastric Cancer	41	I-IV	OS	50% cell staining	2	U	NA	72.7%
Buczkowicz et al	2011	Canada	Medulloblastomas	131	NA	OS	Strong staining in 50% of cells	2	U	6.7 y (mean)	81.8%

NA, not available; Me, method (1= HRs obtained directly from publications, 2= HRs extracted from Kaplan-Meier curves); U, univariate; M, multivariate; SO, survival outcome; OS, overall survival; DFS, disease-free survival; M, month; Y, year. #In the study of Marechal 2014, there were three experiments conducted in Belgium, Paris, Marseille, respectively. *In the study of Che 2012, Ishikawa 2014, Chaudary 2011, PCR were used to evaluate the expression of Gli1, the others were immunohistochemistry.

measurement, such as Immunohistochemistry (IHC); (6) description of the relationship betweenGLI1 and overall survival (OS) or disease-free survival (DFS) or other indicators related to survival outcome; (7) description of the cut-off value of GLI1; (8) period of follow-up. The exclusion criteria were as follows: (1) meta-analysis paper; (2) review paper; (3) non-English paper; (4) conference abstract; (5) non-human data; (6) paper lacking all hazard ratio (HR), 95% confidence interval (CI) and *P* value and raw data.

For quality control of a paper, the assessment was performed by two authors, who reached an agreement on all items assessed. The categories of score assessment included the scientific design (five items: study objective definition, study design, outcome definition, statistical consideration, statistical method and test description), laboratory methodology (seven items: blinding in the biological assays performance, tested factor description, tissue sample conservation, description of the relevant test procedure of the biological factor, description of the negative and positive control procedures, test reproducibility control, definition of the level of positivity of the test), generalizability (six items: patient selection criteria, patients' characteristics, initial investigation, treatment description, source of samples, number of unassessable samples with exclusion causes)

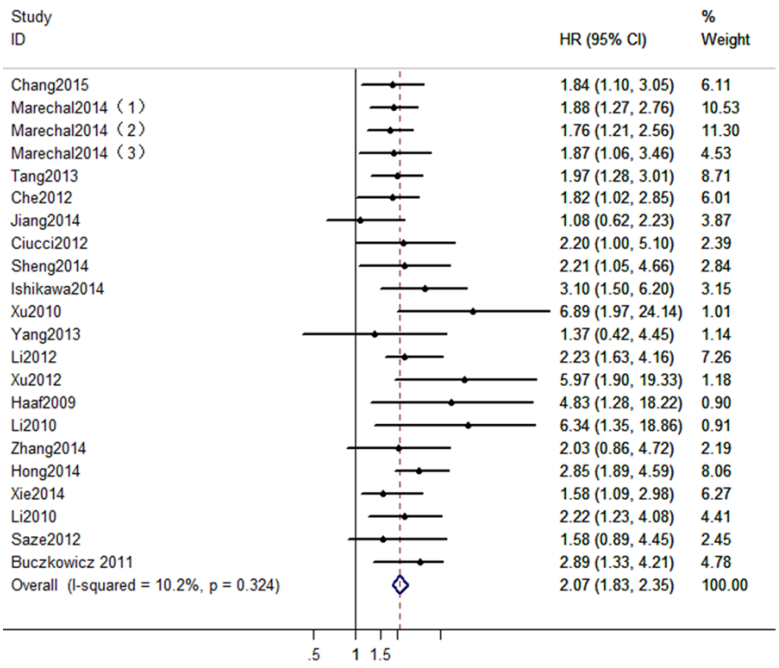

Figure 2. Forest plot for the correlation between GLI1 expression and poor OS in patients with cancers.

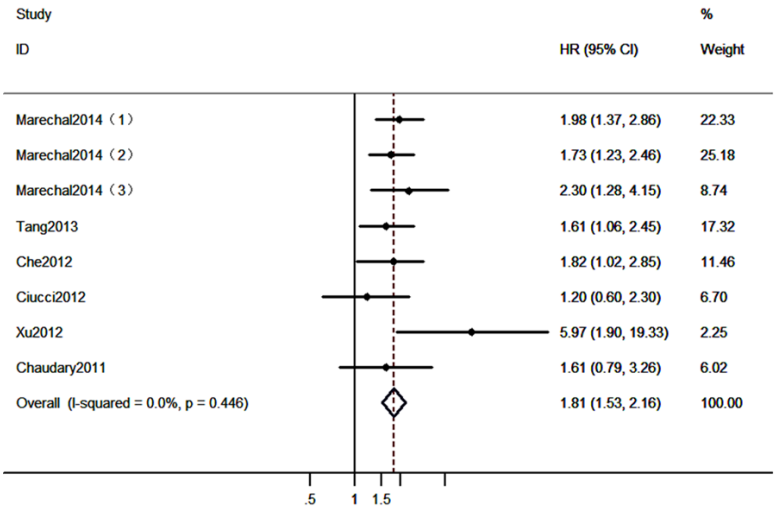

Figure 3. Forest plot for the correlation between GLI1 expression and poor DFS in patients with cancers.

and results analysis (four items: follow-up description, survival analysis according to the biological marker, univariate analysis of the prognostic factors for survival, multivariate analysis of the prognostic factors for survival). Each item was scored as follows: 2 points if it was clearly defined in the article, 1 point if its description was incomplete or unclear and 0 point if it was not defined or was inadequate. The maximum theoretical score was 44 points. The final quality score was presented as percentage, which was calculated using the formula (the sum of the total points divided by 44 and multiplied by 100). An optimal threshold was yet to be defined, which the cut-off point of 85% of the quality scores represented half of the investigated studies. Higher percentages reflected better reporting quality of the paper.

Data extraction

The extracted data included author name, year of publication, country in which study participants were enrolled, the number of patients, study design, the expression level of GLI1, follow-up, cut-off values, HRs of elevated GLI1 for OS, DFS, as well as their 95% CIs and *P* values. There were three methods used to obtain the HRs. In method 1, the HRs were obtained directly from publications. In method 2, the HRs were extracted from Kaplan-Meier curves, the HR estimate was reconstructed by extracting several survival rates at specified times from the survival curves using the Engauge Digitizer software [16-18]. In method 3, the HRs were calculated from the total number of events and its *P* value with the formula: HR = $[P_0/(1-P_0)]/[P_1/(1-P_1)]$, where P_0 represents a 5-year survival rate in the group with low expression of GLI1 and P_1 represents a 5-year survival rate in the group with high expression of GLI1. The formula of 95% CI was exp (lnHR±1.96×SE), where exp = exponential, lnHR = the natural logarithm HR and SE of HR [19]. In this meta-analysis, only method 1, 2 were used to calculate the HRs.

Table 2. A summary of HRs for the overall and subgroup analyses of GLI1 and OS in patients with cancer

Subgroup analyses	Number of studies	Patients number	HR (95% CI)	Heterogeneity I^2	Heterogeneity *P* value
Overall	20	2301	2.07 (1.83-2.35)	10.2%	0.324
Region					
Asia	15	1258	2.06 (1.76-2.41)	12.0%	0.319
Europe	5	1043	2.09 (1.69-2.59)	19.7%	0.279
Cancer type					
Nervous system	3	366	2.23 (1.62-3.08)	0.0%	0.512
Digestive system	12	1433	1.87 (1.67-2.18)	0.0%	0.545
Respiratory system	2	157	2.92 (2.00-4.25)	0.0%	0.844
Measurement methods					
IHC	17	2098	2.00 (1.74-2.30)	8.5%	0.352
PCR	3	203	2.47 (1.83-3.35)	7.0%	0.341
Methods of analysis					
Multivariate	12	1699	2.10 (1.80-2.46)	0.0%	0.468
Univariate	8	602	2.03 (1.63-2.51)	33.7%	0.159

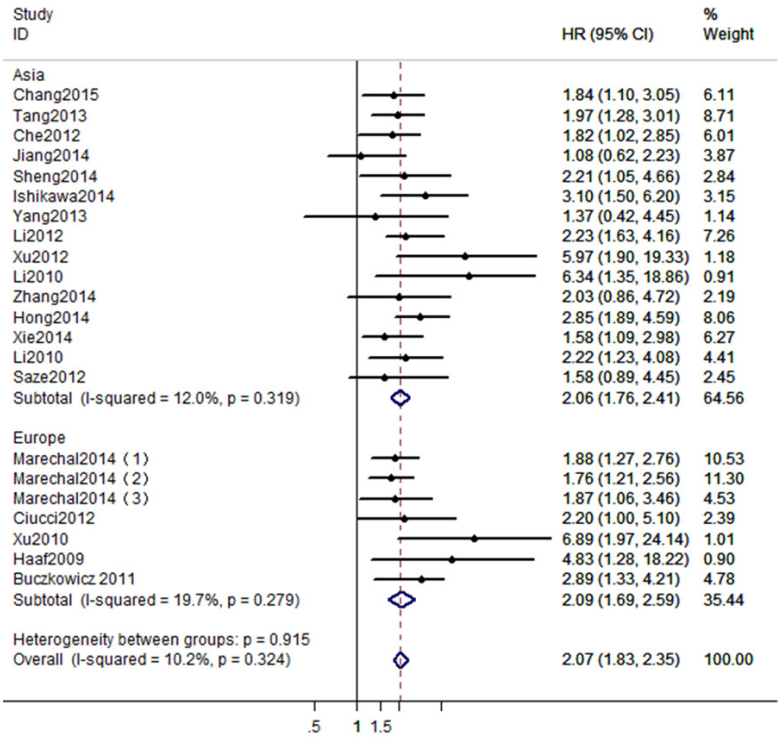

Figure 4. Forest plot of subgroup analysis showed the correlation between GLI1 expression and poor OS in different regions.

Statistical analysis

All analyses were performed using Stata 12.0 software. The HRs with the corresponding 95% CIs were used to estimate the strength of the link between GLI1 and clinical prognosis. The HRs with their 95% CIs and *P* values were collected from the original articles. However, if not available, we calculated the HRs and their 95% CIs using previously reported methods, as indicated above. A random-effect model was applied if heterogeneity was observed, whereas a fixed-effect model was used in the absence of between-study heterogeneity. The factors contributing to heterogeneity were analyzed by subgroup analysis, meta-regression or sensitivity analysis by a sequential omission of each individual study. The test for heterogeneity of combined HRs was carried out using a χ^2-based Cochran *Q* test and Higgins I^2 statistic. A *P* value of <0.05 or an I^2 value of >50% was considered statistically significant. Publication bias was evaluated using a funnel plot with Begg's bias indicator test [20].

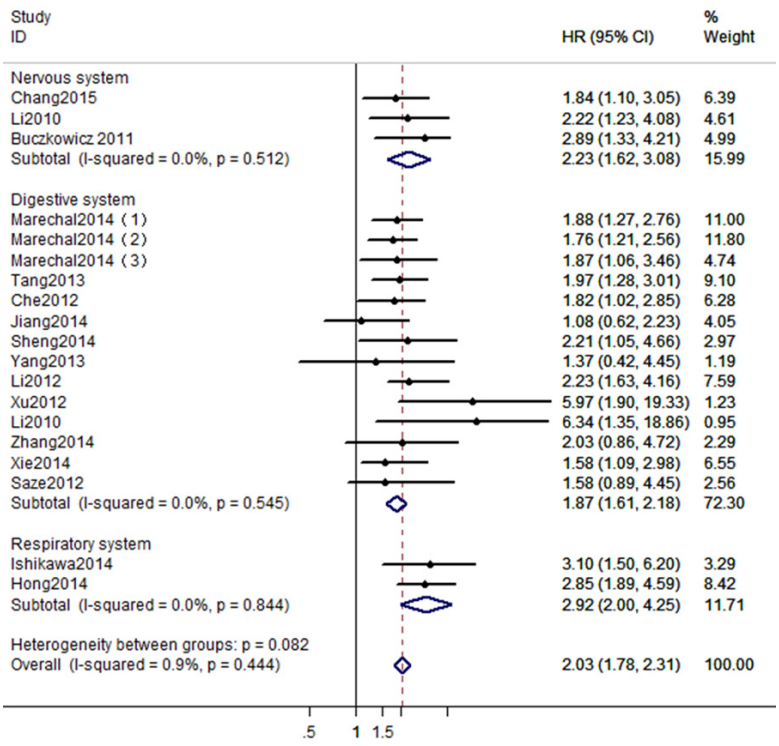

Figure 5. Forest plot of subgroup analysis showed the correlation between GLI1 expression and poor OS in different cancers.

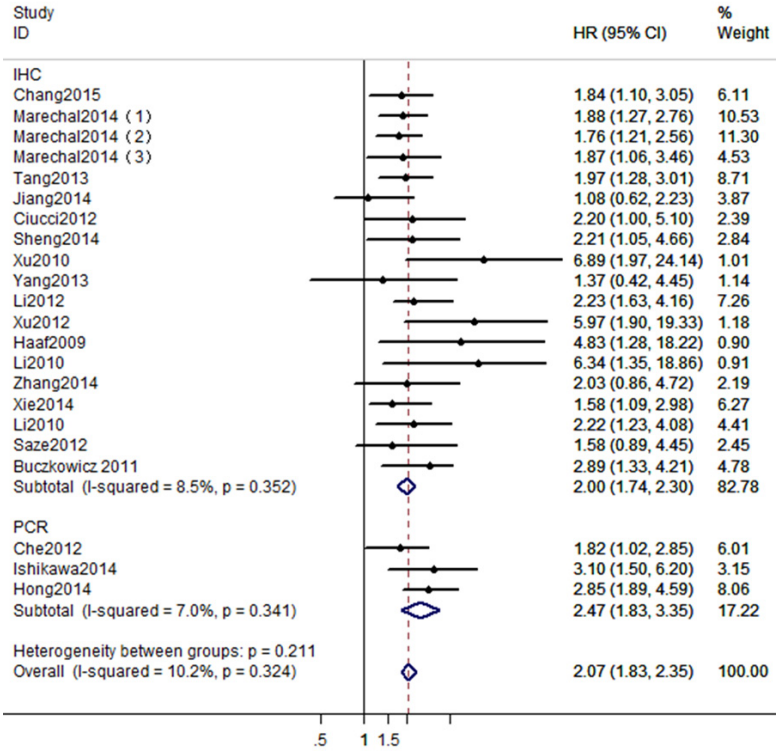

Figure 6. Forest plot of subgroup analysis showed the correlation between GLI1 expression and poor OS in studies with different measurement methods.

Results

Data selection and characteristics of eligible studies

Based on the study design, our search with key terms disclosed 248 articles by July 10, 2015. The titles and abstracts were reviewed, and 152 irrelevant studies and duplicates were excluded. 75 studies were eliminated from the remaining 96 because different statistics methods had been used or the articles were not in English. After data extraction, 21 studies with a total of 2381 patients [21-41], were matched to our inclusion criteria and were eligible for the meta-analysis (**Figure 1**).

The clinical characteristics of the 21 included studies are summarized in **Table 1**. There were 19 studies for OS and 6 for DFS in the meta-analysis. Participants in 15 studies were Asian and others were European. Various cancers were recorded in our study, including hepatocellular carcinoma, pancreatic cancer, etc. All specimens examined were tissues. The cut-off values included in the studies were inconsistent due to different detection methods. HRs with the corresponding 95% CIs were extracted from univariate analysis and the graphical survival plots in 8 studies, and multivariate analysis in 13 studies.

Association of GLI1 expression with prognosis in human cancer

First, we investigated whether GLI1 was predictive for the survival (OS, DFS) of patients with cancer. The elevated

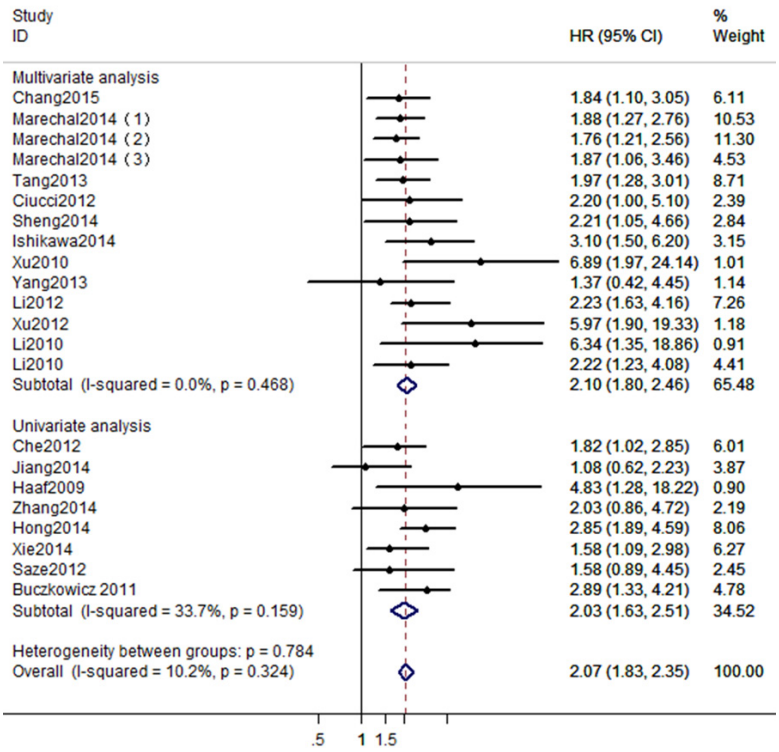

Figure 7. Forest plot of subgroup analysis showed the correlation between GLI1 expression and poor OS in studies with different methods of analysis.

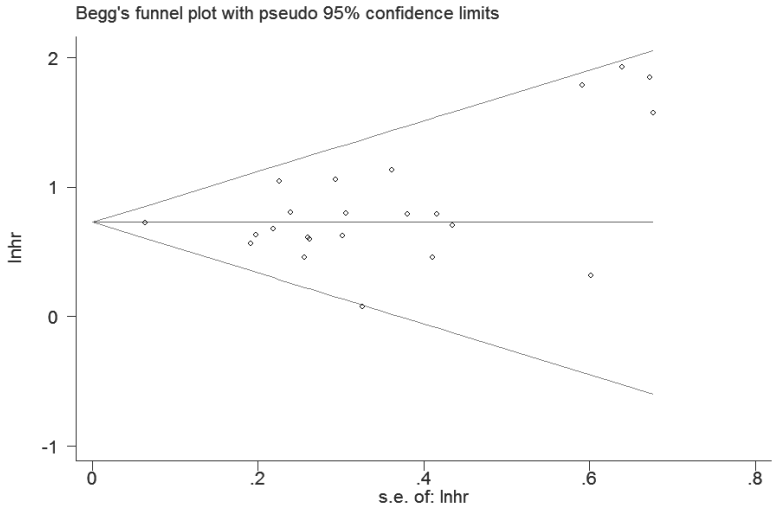

Figure 8. Funnel plot for the publication bias test of the included studies for GLI1 expression and overall survival.

expression of GLI1 was found to be significantly associated with poor OS (HR 2.07, 95% CI 1.83-2.35) and poor DFS (HR 1.81, 95% CI 1.53-2.16). There was no evidence of statistically significant heterogeneity in OS (P =0.324) (**Figure 2**) and DFS (P =0.446) (**Figure 3**).

Because of the limited articles about DFS, stratifying analyses were only conducted on the correlation between GLI1 and OS. Subgroup analyses were performed based on residence region (Asia and Europe), cancer type (nervous system carcinoma, digestive system carcinoma and respiratory system carcinoma), measurement methods (IHC and PCR) and methods of analysis (univariate analysis and multivariate analyses). Main results of subgroup analyses for OS were listed in **Table 2**. We detected a significant correlation between overexpressed GLI1 and poor OS in patients with cancer in Asia (HR =2.06; 95% CI 1.76 to 2.41) and Europe (HR =2.09; 95% CI 1.69 to 2.59). There was no evidence of statistically significant heterogeneity across the studies within the subgroups of Asia (P =0.319) and Europe (P =0.279) (**Figure 4**).

The elevated expression of GLI1 was found to be significantly associated with poor OS in patients with nervous system malignancies (HR =2.23; 95% CI 1.62 to 3.08), digestive system malignancies (HR =1.87; 95% CI 1.61 to 2.18) and respiratory system carcinoma (HR =2.92; 95% CI 2.00 to 4.25). There was no evidence of statistically significant heterogeneity within the subgroups of patients with nervous system malignancies (P =0.512), digestive system malignancy (P =0.545) and respiratory system carcinoma (P =0.844) (**Figure 5**).

Next, we examined the measurement methods in the studies and found that the measurement methods did not change the result of the esti-

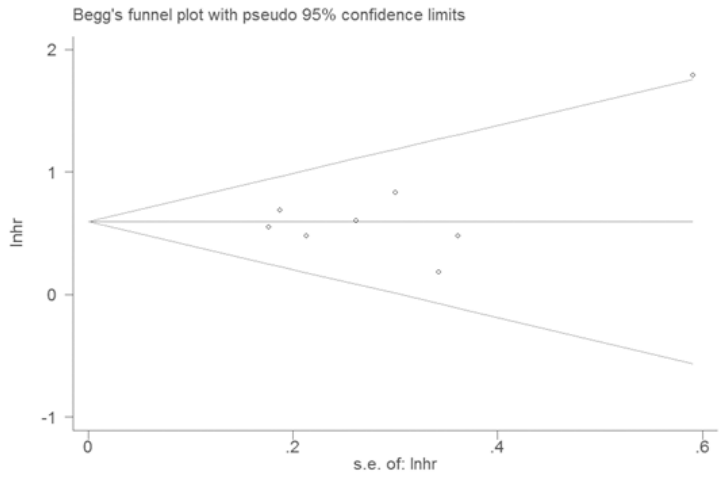

Figure 9. Funnel plot for the publication bias test of the included studies for GLI1 expression and disease-free survival.

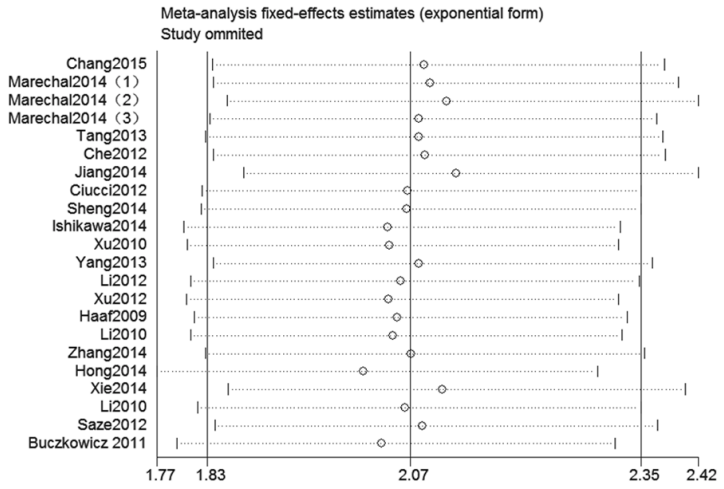

Figure 10. Sensitivity analysis of the pooled HR of GLI1 expression for overall survival for the included studies.

mated HR (HR =2.00; 95% CI 1.74 to 2.30 and HR =2.47; 95% CI 1.83 to 3.35, respectively) and that there was no evidence of statistically significant heterogeneity across the studies within the subgroups with IHC or PCR (P =0.352 and P =0.341, respectively) (**Figure 6**).

Using different methods of analysis, we obtained similar results for the association of GLI1 expression with OS with multivariate analysis (HR =2.10; 95% CI 1.80 to 2.46) and univariate analysis (HR =2.03; 95% CI 1.63 to 2.51). No evidence of statistically significant heterogeneity was found across the studies (P =0.468 by multivariate analyses and P =0.159 by univariate analysis) (**Figure 7**).

Publication bias

The potential publication bias was assessed using Begg's funnel plot. The funnel plot showed that there was no significant asymmetry. *P* value were assessed by Begg's test and the results showed no significant publication bias concerning the prognostic value of GLI1 in OS (**Figure 8**) and DFS (P =0.088 and P =0.711, respectively) (**Figure 9**).

Sensitivity analysis

Moreover, the sensitivity analysis showed that the pooled HR of OS (**Figure 10**) and DFS were reliable (**Figure 11**). The exclusion of any individual study did not change the pooled HR significantly.

Discussion

This study disclosed the prognostic value of GLI1, a central effector of the Hedgehog pathway involved in cancer metastasis and progression. This meta-analysis of published clinical studies, using a detailed search strategy and predetermined selection criteria, provided convincing evidence that aberrant activation of GLI1 is predictive of poor patient survival in various types of cancer.

The prognostic role of GLI1 in cancer was evaluated in this meta-analysis of 21 studies including 2381 participants. Elevated GLI1 expression was indicative of poor prognosis in patients with cancer. The pooled HR for OS was 2.07 (95% CI: 1.83-2.35; P<0.001), and the pooled HR for DFS was 1.81 (95% CI: 1.53-2.16; P<0.001). Subgroup analyses, including residence region (Asia and Europe), cancer type (digestive system carcinoma, respiratory system carcinoma and nervous system carcinoma), measurement methods (IHC and PCR) and methods of analysis (univariate analysis and multivariate analyses), showed that these factors did not alter the predictive value of GLI1 on poor OS among the investigated cancers. Furthermore, Begg's test showed no significant publication bias concerning the

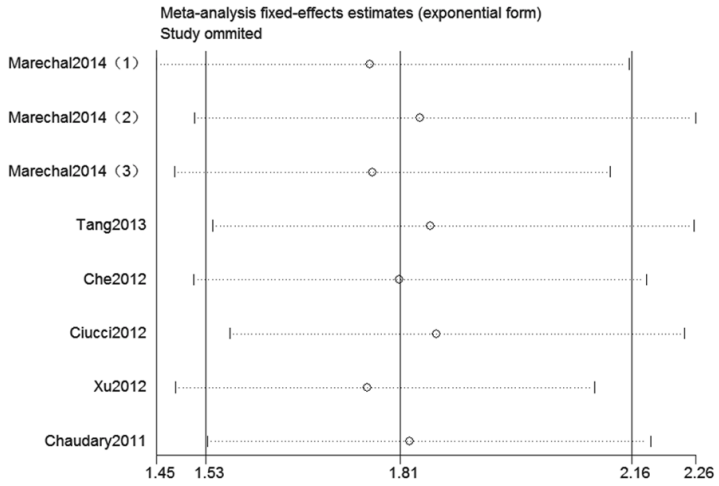

Figure 11. Sensitivity analysis of the pooled HR of GLI1 expression for disease-free survival for the included studies.

prognostic value of GLI1 in OS and DFS, sensitivity analysis showed that the pooled HR of OS and DFS were reliable. Therefore, this meta-analysis supports the outcomes of many studies which found that GLI1 is amolecular predictor for poor OS and DFS in patients with cancers.

The function and role of GLI1 in cancer has been extensively investigated. GLI1 regulates processes involved in all six of the traditional hallmarks of cancer. GLI1 protects against apoptosis by inducing anti-apoptotic proteins, such as B-cell lymphoma 2 (BCL2) [42]. GLI1 promotes cell invasion and metastasis through induction of epithelial-mesenchymal transition (EMT) markers such as SNAIL1 [43], C-terminal binding protein 2 (ctBP2) [44], transforming growth factor β (TGFβ), rat sarcoma viraloncogene homolog (RAS) and wingless-type MMTV integration site family (WNT) [45]. Replicative immortality can be achieved through GLI1-mediated regulation of human telomerase reverse transcriptase (hTERT) protein expression [46]. GLI1 can promote proliferation by inducing expression of Ki67, proliferating cell nuclear antigen (PCNA) and mitotic spindle assembly checkpoint protein L1 (MAD2L1) [47]. Finally, GLI1 stimulates new blood vessel formation by enhancing expression of the potent pro-angiogenic protein cysteine-rich protein 61 (CYR61) [48]. Based on these studies and owing to its functions, GLI1 can be an unfavorable factor for survival in patients with cancer.

A few limitations in our analysis due to the discrete data across these clinical studies have to be addressed. First, the criteria for calculating the cut-off value were not the same in different studies. Second, the inclusion of a relatively small number of studies in different regions might have decreased the applicability of our results across different ethnicities. Third, only summarized data rather than individual patient data were used. Furthermore, some of the HRs were calculated by reconstructing survival curves rather than directly obtained from the primary studies. Therefore, it is possible that our results might overestimate the predictive role of GLI1 in patients with cancer.

In summary, this meta-analysis shows that elevated GLI1 expression is common to various types of cancer and that it is significantly associated with poor OS and DFS. Furthermore, the functional role of GLI1 in the regulation of cell proliferation, apoptosis, and metastasis suggests that GLI1 may play a key role in the development and the progression of cancer. Thus, GLI1 can be used as a prognostic marker to predict poor survival outcome in patients with cancer. More clinical studies investigating different malignancies should be undertaken to have a better understanding of GLI1 as a prognostic marker in cancer.

Disclosure of conflict of interest

None.

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