Systematic Review and Meta-Analysis

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GLI1 expression in pancreatic ductal adenocarcinoma correlates the clinical significance and prognosis

A meta-analysis

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Abstract

Background: Glioma-associated oncogene homolog 1(GLI1) expression correlates with the clinical significance and prognosis of several cancers. However, the evaluation of the role GLI1 expression plays in pancreatic ductal adenocarcinoma (PDAC) clinicopathological features and outcomes still lacks.

Objective: The present study systemic reviewed the association of GLI1 expression and clinical significance as well as patients survival in PDAC.

Methods: We systematically searched the database of The Cochrane Library, PubMed, Embase, CNKI, Weipu data, and Wanfang data according to the inclusion and exclusion criteria. (The search ended on January 1, 2019; no language restrictions). The Newcastle-Ottawa Scale (NOS) scale was implemented to assess the quality of the literature and the Review Manager 5.3 Software was used to conduct a meta-analysis. Finally, 9 studies, a total of 1058 patients, have been included.

Results: GLI1 is more likely expressed in PDAC tissue rather than para-carcinoma tissue (OR = 2.86, 95%CI = 1.87–4.36, $P < .00001$). GLI1 expression is associated with the TNM stage (OR = 3.11, 95%CI = 2.01–4.79, $P < .00001$), perineural invasion (OR = 2.50, 95%CI = 1.28–4.91, $P = .008$), and lymphatic metastasis (OR = 2.73, 95%CI = 1.71–4.36, $P < .0001$). But the association with differentiation (OR = 1.20, 95%CI = 0.74–1.96, $P = .46$) and tumor size (OR = 2.41, 95%CI = 0.97–6.00, $P = .06$) was not significant. GLI1 expression is related to the worse overall survival in PDACs (HR = 1.68, 95%CI = 1.40–2.02, $P < .00001$).

Conclusion: Positive GLI1 expression promotes the progression and metastasis of PDACs and plays an important role in the clinical significance and the patients survival.

Abbreviations: AJCC = American Joint Committee on Cancer, CSC = cancer stem cells, ENO1 = α-Enolase, GLI1 = glioma-associated oncogene homolog 1, HR = hazard ratio, IHC = immunohistochemistry, NOS = Newcastle-Ottawa Scale, OR = odds ratio, PDAC = pancreatic ductal adenocarcinoma, Shh = sonic hedgehog, Tregs = regulatory T cells.

Keywords: pancreatic ductal adenocarcinoma, GLI1, meta-analysis, prognosis

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a type of cancer with a high mortality rate, occupied the leading cause of cancer-related deaths.[1] This disease is characterized by the difficulty of early diagnosis, a high rate of distant metastasis and poor prognosis.[2] Although the treatment outcome of PDAC has been improved in recent years because of the progression in imaging and endoscopic methods, the overall survival is still relatively low

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with less than 1% 5-year survival. Therefore, identified the practical biomarkers of treating PDAC is of utmost importance now.

The development of PDAC is related to the activation of oncogene and the mutation of the tumor suppressor gene. Some studies have showed that the α-Enolase (ENO1) as a marker which is associated with the migration and metastasis of PDAC. Amedei et al[3] found that there are elevated levels of ENO1-specific Regulatory T cells (Tregs) in PDAC patients which lead to inhibition of antigen specific effector T cells, thus highlighting a possible role in promoting PDAC progression.

Sonic hedgehog (Shh) signaling pathway was regarded to have an essential role in embryonic development, cell proliferation and maintenance of tissue polarity in the past.[4] Currently, it was argued that Shh signaling pathway might be associated with cancer invasion and metastasis by the interaction with cancer stem cells (CSC).[5]

The activation of Shh is related to the multi-steps evolution of several malignancies including small cell lung cancer,[6] gliomas,[7] and prostate cancer.[8] GLI1, the final effective regulator of Shh signaling pathway, is a reliable biomarker that involved in cell survival and proliferation.[9] Recently, studies have proved that the expression level of GLI1 is associated with the clinical characteristic and outcome of a few gastrointestinal malignancies including PDAC,[10] hepatocellular carcinomas[11] and esophageal cancer.[12] However, most of the studies about the relationship between GLI1 expression and PDAC have a limited sample size and the conclusion was inconsistent. Some studies revealed that the positive expression level of GLI1 was positively related to poor outcome and late-stage while other studies yield different conclusions.

The present study aims to clarify the relationship of GLI1 expression and patients characteristics and treatment outcome. We systematically reviewed previous studies and evaluated the correlation of GLI1 expression with the clinicopathological features and outcomes.

2. Materials and methods

2.1. Inclusion and exclusion criteria

The full content of each study was evaluated according to the following criteria:

1. Patients had a diagnosis of PDAC;
2. The expression of GLI1 was measured based on PCR or immunohistochemistry (IHC);
3. The clinicopathological features and outcomes were investigated;
4. The correlation of GLI1 expression and the clinicopathological features or prognosis of patients was revealed.

Articles were excluded if:

1. Studies were without any original data;
2. They focused on the cell or animal models;
3. They have not mentioned the required clinicopathological evidence in the literature, or the article was low reliability.

Institutional review board approval and patient informed consent (written/oral) were not required because all analyses were based on previously published studies.

2.2. Literature retrieval and search strategy

The systematic literature was conducted by searching the original article in the Cochrane Library, PubMed, Embase, CNKI, Wanfang Database, and Weipu Information Resources System. The studies were selected using the following keywords

(GLI1 OR Glioma-Associated Oncogene Protein) AND (PDAC OR pancreatic carcinoma OR pancreatic tumor OR pancreatic tumors OR pancreatic neoplasm OR pancreatic neoplasms). The search ended on January 1, 2020, and no language restriction was applied.

2.3. Data extraction and quality assessment

The studies have parallelly filtered, extracted, and collected data by 2 investigators and if arguments occur, the third investigator would take the final decision. The following information was acquired when studies met the inclusion criteria: first authors name, publication year, country, sample size, age, the cut-off value, histology, stage, follow-up period, and detection method. Literature quality was evaluated according to the NOS literature quality evaluation scale. A score of 6 or more is considered to have high quality.

2.4. Statistical analysis

A meta-analysis of the included studies was conducted using Review Manager 5.3 (Cochrane Collaboration, Oxford, UK). The odds ratio (OR), the hazard ratio (HR) and 95% confidence interval were used as the effect indicators to evaluate the correlation between GLI1 expression and main clinical features and prognosis of patients with PDAC. When the heterogeneity is greater than 50%, the random-effect model was adopted. Otherwise, the fixed-effect model was adopted. The funnel plot was applied to evaluate whether there was publication bias.

3. Results

3.1. The selection and characteristics of the included studies

Four hundred eleven studies identified through searching in the Cochrane Library, PubMed, Embase, CNKI, Wanfang Database, and Weipu Information Resources System, 246 studies were excluded due to duplicate or irrelevant studies. After systematically reviewing the Titles and abstracts, 129 studies were excluded for reasons. Then, 27 studies were excluded because of failure to meet the inclusion criteria. Finally, 9 qualified studies,[13–21] contain 1058 patients, were included for meta-analysis (Fig. 1).

The characteristics of the included 9 studies are listed in Table 1. Among these studies, PCR was used in 1 study and IHC was used in 8 studies. 9 studies reported the relationship between GLI1 expression level and the clinical characteristics of patients and 6 studies reported the relationship between GLI1 expression level and patients prognosis. The result of the NOS showed all studies have high quality.

3.2. The correlation of the expression level of GLI1 in PDAC with patients clinical characteristics and prognosis

First of all, 4 studies demonstrated the GLI1 expression level in PDAC compared with the para-carcinoma tissue. The pooled OR with 95% CI, including 203 cancer tissue and 186 para-carcinoma tissue, were shown in Figure 2A. The expression rate of GLI1 in PDAC (53.6%, 109/203) was significantly higher than that in para-carcinoma tissue (29.0%, 54/186). Seven studies, including 193 stage I-II PDAC patients and 239 stage III-IV

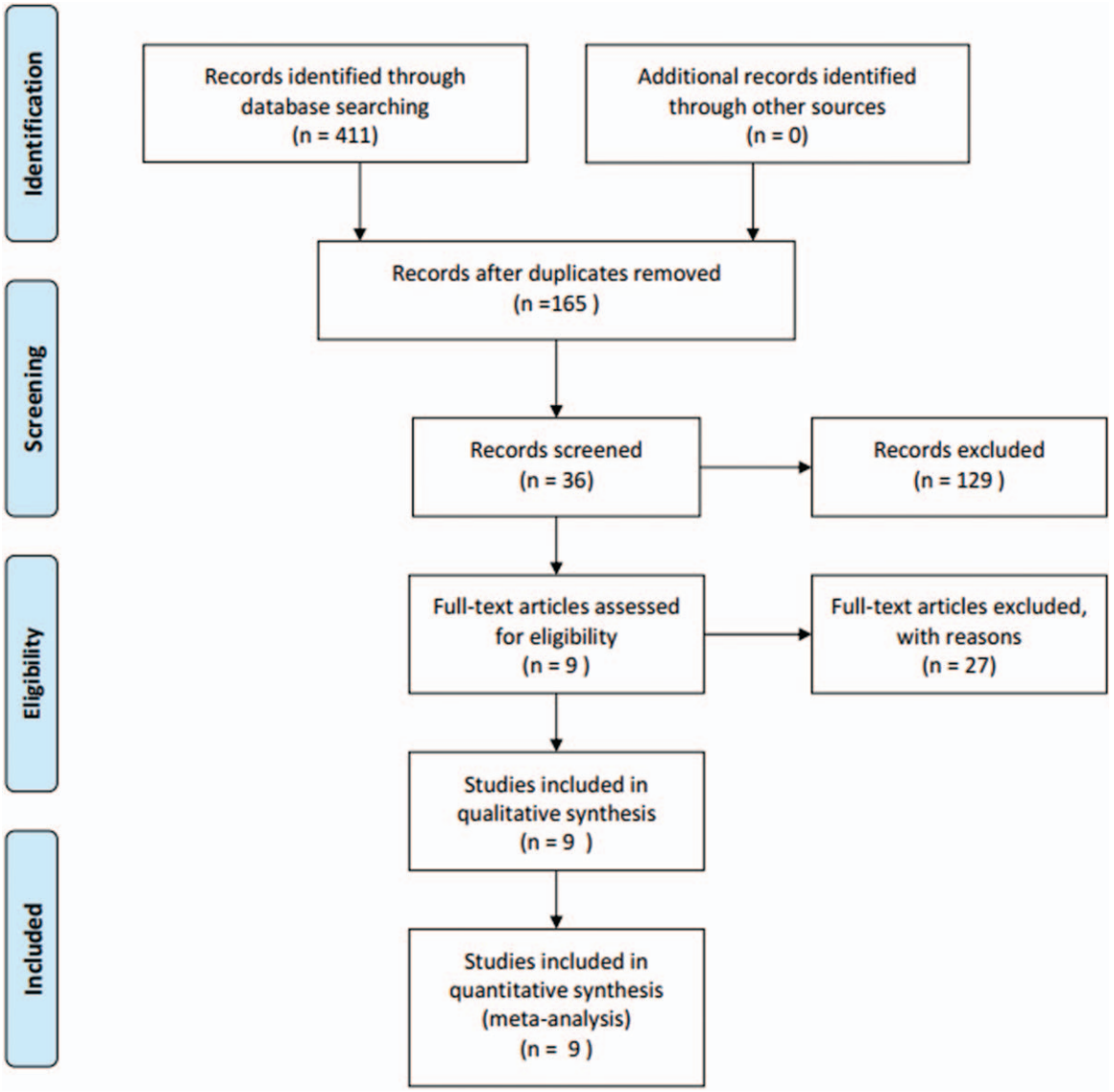

Figure 1. PRISMA-flow diagram for the literature search and exclusion criteria. PRISMA = preferred reporting items for systematic reviews and meta-analysis.

PDAC patients, reported the association of GLI1 expression level and the American Joint Committee on Cancer (AJCC) TNM stage of PDAC patients. The meta-analysis results showed compared with stage I-II PDAC patients, stage III-IV PDAC patients has a higher GLI1 expression level (Fig. 2.B, OR = 3.11, 95%CI = 2.01–4.79, $P < .00001$, $I^2 = 0\%$).

Table 1

Process of literature screening and results.

Study	Year	Country	Sample size	Median age	Detection method	Cut-off value	TNM Stage	Follow-up period
Zhou et al	2018	China	69	58.3	IHC	Score ≥ 3	I-IV	NR
Yang et al	2013	China	81	58	IHC	Score ≥ 3	I-IV	NR
Wen et al	2015	China	36	NR	IHC	IRS≥ 5	I-IV	NR
Sheng et al	2013	China	57	NR	IHC	Score ≥ 2	I-IV	1200 days
Marechal et al 1	2015	Belgium	237	63	IHC	Score ≥ 2	I-III	72 months
Marechal et al 2	2015	France	234	64	IHC	Score ≥ 2	I-III	72 months
Marechal et al 3	2015	France	96	63	IHC	Score ≥ 2	I-III	48 months
Jiang et al	2014	China	90	62	IHC	Score ≥ 3	I-IV	100 months
Guo et al	2007	China	25	60	PCR	NR	I-IV	NR
Chen et al	2015	China	48	58.6	IHC	IRS > 6	I-IV	25 months
Abula et al	2015	China	85	58	IHC	Score ≥ 2	I-IV	NR

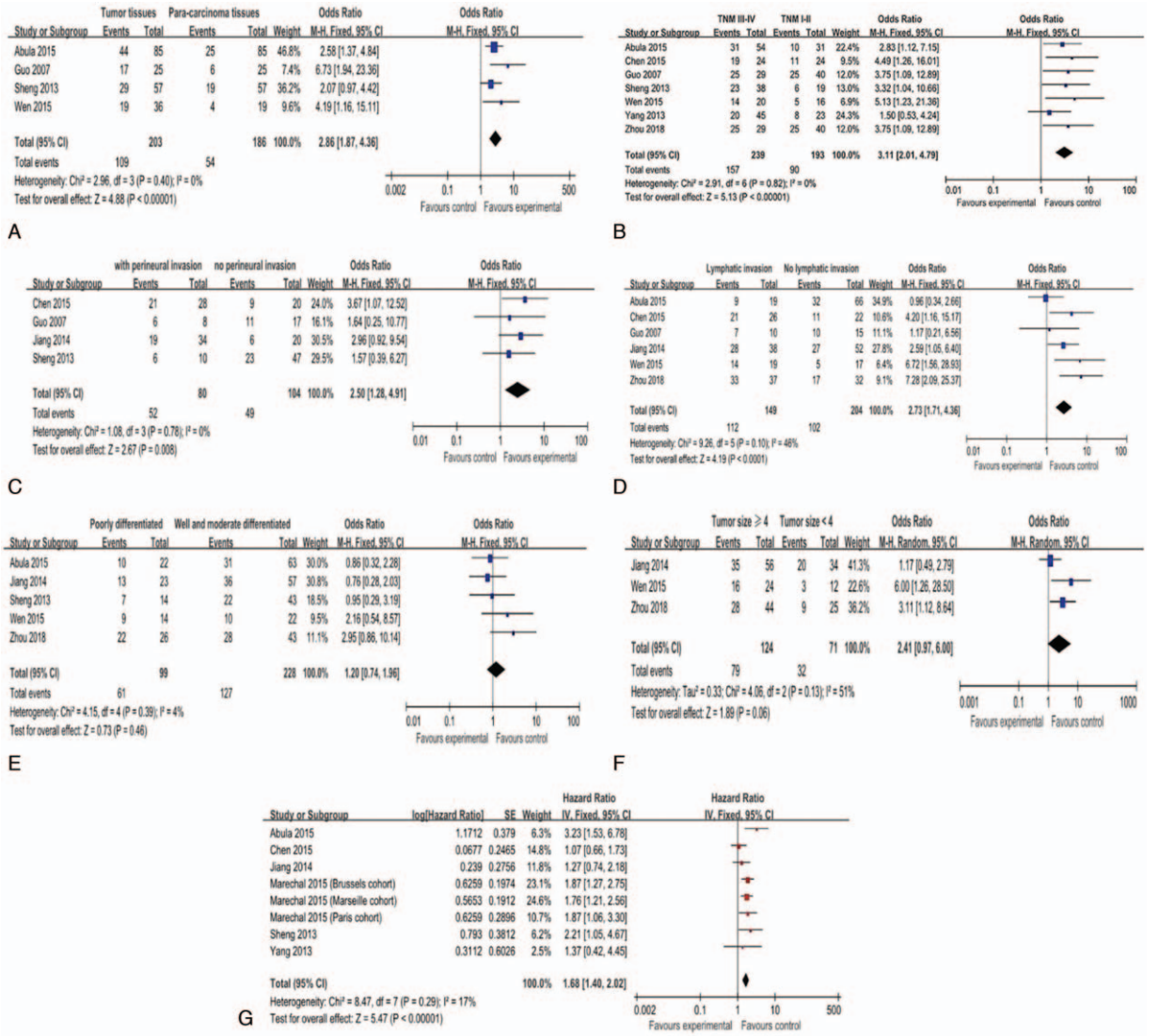

Figure 2. The forset plots of results. A.The forset plot of GLI1 expression with tumor tissues and para-carcinoma tissues. B. The forset plot of GLI1 expression and the TNM stage of pancreatic cancer patients. C.The forset plot of GLI1 expression and the perineural invasion. D. The forset plot of GLI1 expression and the lymphatic invasion of pancreatic cancer. E. The forset plot of GLI1 expression and the differentiated of pancreatic cancer patients. F. The forset plot of GLI1 expression and the tumor size of pancreatic cancer patients. G. The forset plot of GLI1 expression and the prognosis of pancreatic cancer patients.

Then, we investigated the relationship between GLI1 expression level and the perineural invasion situation of PDAC patients. Eighty PDAC patients with perineural invasion and 104 PDAC patients without perineural invasion were included. The expression level of GLI1 in PDAC with perineural invasion was higher than that in PDAC without perineural invasion (Fig. 2.C, OR = 2.50, 95%CI = 1.28–4.91, $P = .008$, $I^2 = 0\%$).

Moreover, 6 studies, including 149 PDAC patients with lymphatic metastasis and 204 PDAC patients without lymphatic metastasis, reported the association of GLI1 expression level and the lymphatic metastasis of PDAC patients. The expression level of GLI1 in patients with lymphatic metastasis was higher than that without lymphatic metastasis (Fig. 2.D, OR = 2.73, 95% CI = 1.71–4.36, $P < .00001$, $I^2 = 46\%$).

In addition, we found the high expression level of GLI1 has no significant association with the tumor differentiation (Fig. 2.E, OR = 1.20, 95%CI = 0.74–1.96, $P = .46$) and size (Fig. 2.F, OR = 2.41, 95%CI = 0.97–6.00, $P = .06$).

Subsequently, we identified the correlation of GLI1 expression and the prognosis of PDAC patients. The results showed high expression level of GLI1 was significantly associated with a worse prognosis (Fig. 2.G, HR = 1.68, 95%CI = 1.40–2.02, $P < .00001$, $I^2 = 17\%$).

At the same time, we also performed a subgroup analysis that included only western study cohort data (Belgium Brussels, France Paris, and France Marseille). The result showed the same results, the high expression level of GLI1 associated a bad prognosis (see Figure, Supplemental Content, http://links.lww.com/MD/E465, HR = 1.82, 95%CI = 1.43–2.32, $P < .00001$, $I^2 = 0\%$).

3.3. The sensitivity analysis and assessment of the publication bias

Sensitively analysis suggested that no single study could affect the pooled ORs and HRs in the meta-analysis. Funnel plots

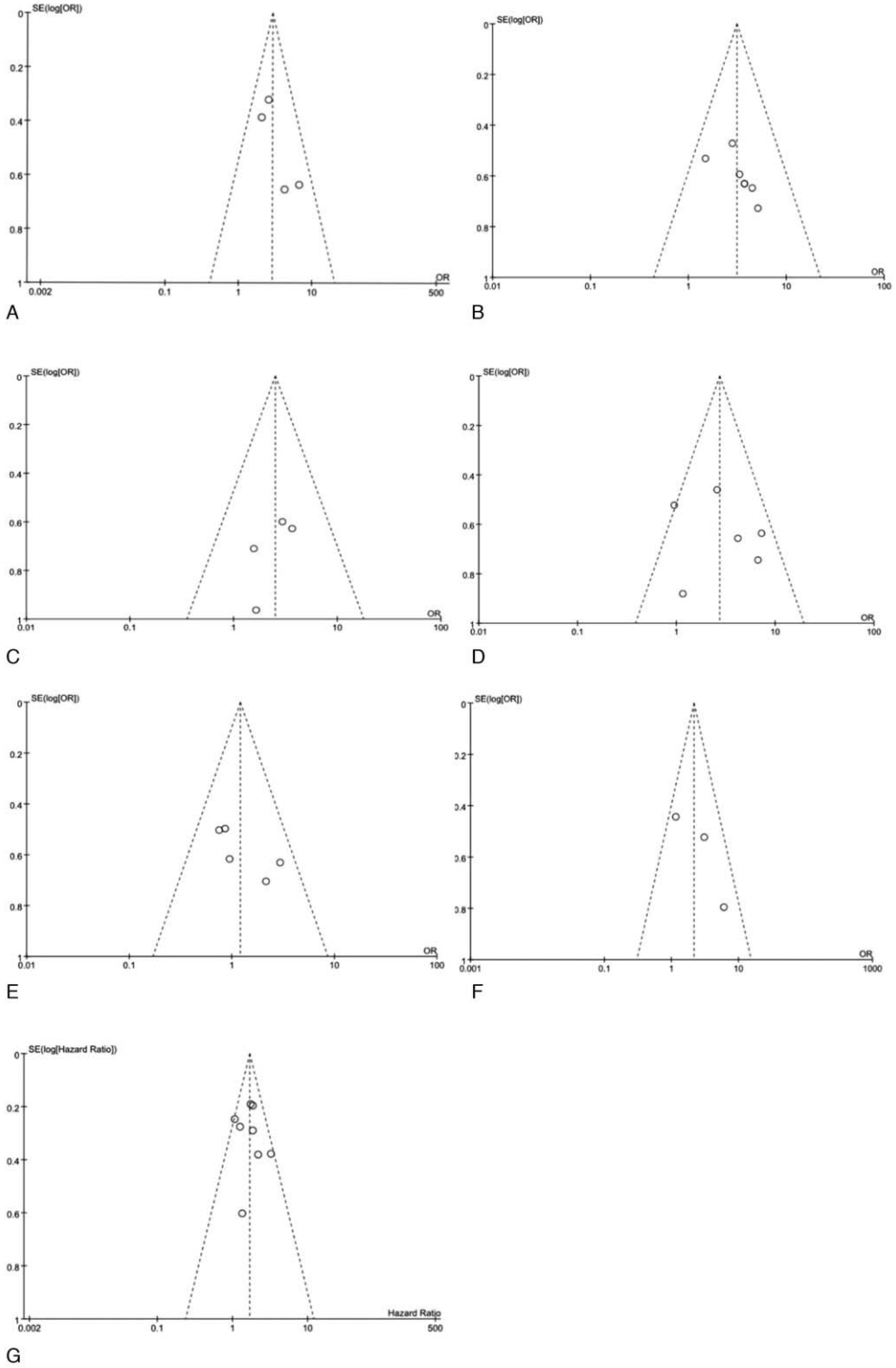

Figure 3. The funnel plots of results. A.The funnel plot of GLI1 expression with tumor tissues and para-carcinoma tissues. B. The funnel plot of GLI1 expression and the TNM stage of pancreatic cancer patients. C.The funnel plot of GLI1 expression and the perineural invasion. D. The funnel plot of GLI1 expression and the lymphatic invasion of pancreatic cancer. E. The funnel plot of GLI1 expression and the differentiation of pancreatic cancer patients. F. The funnel plot of GLI1 expression and the tumor size of pancreatic cancer patients. G. The funnel plot of GLI1 expression and the prognosis of pancreatic cancer patients.

(Fig. 3) demonstrated no evidence of obvious asymmetry and did not illustrate strong statistical evidence of publication bias. The evidence rating for this article is provided in the supplementary material (see Table, Supplemental Content, http://links.lww.com/MD/E466, which illustrates the evidence rating results).

4. Discussion

The activation of Shh signal transduction pathway is associated with embryonic development, supporting stem cells, and cell growth while the abnormal activation and mutations of Shh pathway components could lead the cancer development. GLI1 is one of the vital downstream transcription factors in Shh signal transduction pathways. The cellular expression level of GLI1 could directly influence the target-gene transcription and mediate the proliferation, differentiation, and metastasis of cancer cells.[22] Shh signal transduction pathway plays its role through Hh-GLI1 pathway in PDAC and the aberrant expression of GLI1 results in the acceleration of the progression of PDAC. Li et al found that the up-regulated of GLI1 promoted the epithelial-mesenchymal transition process and the progression of PDAC cell, suggesting a higher likelihood of malignant level.[23]

The role GLI1 played in the advance and outcome of PDAC patients is still controversial and different studies yield different outcomes. For GLI1 expression, 3 studies[13,16,18] concludes that GLI1 significantly expressed higher in PDAC tissues than para-carcinoma tissues while Sheng et al[20] demonstrates obscure results in GLI1 expression state in PDAC tissues. For the relationship between GLI1 expression level and TNM stage, there were generally agreed that GLI1 was higher expressed in TNM III-IV PDAC patients compared with TNM I-II PDAC patients while Yang et al[21] suggested there was no correlation between these 2. One of 4 studies showed that GLI1 expression was higher in PDAC with perineural invasion,[17] and others showed the opposite view.[13,15,20] Although we found PDAC with perineural invasion has a higher expression level of GLI1, the data were not sufficient, and thus more correlational researches need to be performed. For perineural invasion, the present result concluded that GLI1 was more likely has a positive expression level in advanced PDAC. Currently, there was a heated discussion about the association of GLI1 and the lymphatic metastasis state. Abula and Guo et al[13,18] suggested the correlation of GLI1 and lymphatic metastasis state did not exist. However, our results revealed that GLI1 was higher expressed in PDAC patients with lymphatic metastasis, suggesting that GLI1 played a vital role in the lymphatic metastasis of PDAC.

The present research reveals the relationships of GLI1 expression and the development, progression, and patients outcome of PDAC by integrating the relative data of 9 qualified research. Generally, GLI1 plays an essential role in the proliferation, differentiation, and metastasis of cancer cells of PDAC. The expression level of GLI1 is significantly up-regulated in PDAC tissues and is directly related to the TNM stage, lymphatic and distant metastasis. The positive expression rates of GLI1 in stage III-IV PDAC is significantly higher than stage I-II PDAC, suggesting GLI1 participants the PDAC development and metastasis.

Our study also has some deficiencies. Firstly, the amounts of the included studies were limited, making the results of the present study less convincing. Secondly, the detection methods, the cut-off value, and sample size of the included researches exist differences and inevitable heterogeneity. Thirdly, the studies we included only a small percentage (3 of 11) came from the west, more clinical data is warranted form abroad. Therefore, the role GLI1 played in the PDAC still needs further investigation and larger clinical studies. However, because of the slow detection speed of PCR and IHC, it is difficult for us to obtain larger clinical study data more efficiently, so it is necessary to develop a more rapid test. These are issues that need to be addressed further.

Above all, the present study is the first study that reveals the association between the expression level of GLI1 and the clinical characteristic and prognosis of PDAC. GLI1 is positively expressed in PDAC. Moreover, its expression level is associated with the differentiated degree, lymphatic metastasis, TNM stage, and poor prognosis. GLI1, as an important downstream transcription factor of the Shh pathway, could be used as a target for anticancer treatment. The present study reveals GLI1 has the potential to be an important target and factor of patients pathogenesis and survival outcome, building the reliable foundation of future personal treating.

Author contributions

Data curation: Xiaolin Hu.
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Investigation: Ye Chen.
Methodology: Ruidan Li, Ye Chen, Xiaolin Hu.
Resources: Ruidan Li.
Supervision: Ruidan Li.
Validation: Ruidan Li.
Visualization: Zheran Liu.
Writing - original draft: Zheran Liu.
Writing - review & editing: Ruidan Li.

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