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Review Article

Prognostic and clinicopathological significance of glucose-6-phosphate dehydrogenase in solid tumors: a meta-analysis

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Abstract: The prognostic and clinicopathological role of glucose-6-phosphate dehydrogenase (G6PD) in human solid tumors remains controversial. We conducted this meta-analysis based on a comprehensive literature search to investigate the association between G6PD expression and prognosis in solid tumors. The correlation between G6PD expression and clinicopathological features was also evaluated. A total of 11 studies with 2144 patients were enrolled. The pooled results suggested that elevated G6PD expression contributed to poor overall survival (HR = 1.607; 95% CI, 1.448-1.784) and worse progression in total solid tumors (HR = 1.779; 95% CI, 1.523-2.079). G6PD overexpression was also significantly related to several phenotypes of tumor aggressiveness, including larger tumor size (OR = 1.678; 95% CI, 1.093-2.574), worse histological grade (OR = 2.224; 95% CI, 1.664-2.972), advanced depth of invasion (OR = 1.692; 95% CI, 1.235-2.317), serious clinical stage (OR = 3.192; 95% CI, 2.133-4.776), positive lymph node metastasis (OR = 3.186; 95% CI, 2.232-4.548), and positive distant metastasis (OR = 8.454; 95% CI, 1.930-37.036). Therefore, increased G6PD expression predicts unfavorable prognosis and advanced tumor progression in cancer patients, making it useful as a predictor of prognosis in patients with malignant tumors.

Keywords: G6PD, prognosis, clinicopathological parameter, solid tumor, meta-analysis

Introduction

Reprogramming energy metabolism is one of the key hallmarks of many rapidly growing cancers. Unlike normal cells, tumor cells constantly suffer hypoxic conditions due to the lack of blood supply [1]. Neoplastic cells favor a metabolic shift of aerobic glycolysis through an increased rate of glycolysis and lactic acid fermentation even under normal or high oxygen tension, a phenomenon called Warburg effect [2]. Glycolysis can raise the tolerance of tumor cells to ischemia, resulting in avoidance of apoptosis caused by the inhibition of oxidative phosphorylation (OXPHOS) [3]. Moreover, the metabolic mode of cancer cells changes with the alteration of tumor microenvironment. Furthermore, the metabolic strategy in different cancer types is completely different; for example, some malignant cells breakdown glucose *via* OXPHOS, whereas some perform this process *via* the combination of aerobic glycolysis and OXPHOS [4]. Therefore, tumor cells can continuously adapt to the adverse circumstance and maintain the advantage of selective growth.

Aerobic glycolysis provides cancerous cells with ATP for bioenergetics; however, large amounts of metabolites, such as lipid and nucleotide precursors, are needed to sustainably support the anabolic demands of uncontrolled cell proliferation. To address these biosynthesis requirements, glucose flux through glycolysis can be directly conveyed into the pentose phosphate pathway (PPP) [5]. The PPP, also known as the hexose monophosphate shunt or phosphogluconate pathway, branches from glycolysis at the first committed step. The PPP is composed of the oxidative and non-oxidative branches. The oxidative branch consists of three irreversible reactions that eventually generate

nicotinamide adenine dinucleotide phosphate (NADPH) and ribulose-5-phosphate (Ru5P); whereas the non-oxidative branch contains a series of reversible processes that recruit additional glycolytic intermediates, including fructose-6-phosphate (F6P) and glyceraldehyde-3-phosphate (G3P), to be transformed into pentose phosphates and vice versa [6]. Ru5P can be isomerized to ribose-5-phosphate (R5P) for *de novo* synthesis of DNA and RNA. In rapidly dividing cells, approximately 85% of the pentose phosphates that are synthesized into DNA are derived from the PPP [7]. The NADPH is not only employed for the biosynthesis of fatty acids, but it also functions as an important antioxidant for reactive oxygen species (ROS) detoxification to protect cells from DNA damage [8]. Thus, the PPP is especially vital for cancer cells because it addresses the anabolic demands for cell proliferation and provides anti-oxidative defense for cell survival. The PPP activity is more upregulated in malignant cells compared with normal epithelial cells, and this phenomenon is linked to cancer invasion, metastasis, and resistance to anticancer therapies [9, 10].

Glucose-6-phosphate dehydrogenase (G6PD) is the first and also a rate-limited enzyme in the oxidative branch of the PPP; moreover, it catalyzes the oxidation of glucose-6-phosphate to 6-phosphategluconolactone and generates NADPH. G6PD can exist as a monomer, dimer, tetramer, and even hexamer, but only the dimer and tetramer are bioactive [11]. G6PD is essential to life because it is expressed in nearly all cells, of which its depletion in mammals is lethal for reproduction [12]. In the human body, high levels of G6PD are observed in many normal metabolizing tissues, including the liver, adipose, mammary glands and paranephros [13]. Recently, multiple studies have demonstrated that G6PD is involved in tumor growth, and its overexpression has been found in various cancers, including leukemia, gastric cancer, and renal cell carcinomas [14]. Additionally, G6PD may be one of potential prognostic biomarkers for patients with solid tumors; however, the real prognostic role of G6PD remains controversial. In view of these contradictory results, this meta-analysis is conducted to provide high-level evidence to validate the association between G6PD and prognosis in solid tumors.

Methods

Search strategy

This meta-analysis was performed according to the Preferred Reported Items for Systematic Reviews and Meta-analysis (PRISMA) [15]. A comprehensive literature search was conducted through the electronic databases of PubMed, Embase, Web of Science, Cochrane Library, and China National Knowledge Infrastructure by the end of February 15, 2019. The search terms were as follows: ("G6PD", "G6PDH", or "glucose-6-phosphate dehydrogenase"), and ("tumor", "cancer", "carcinoma", or "malignance"), and ("survival", "prognosis", or "mortality"). The reference lists of relevant articles were also manually checked to identify more potential studies.

Inclusion and exclusion criteria

Studies were considered eligible for this meta-analysis if they met the following criteria: 1) cohort studies that investigated the association between G6PD expression and prognosis in solid tumors; 2) studies that detected G6PD protein in tumor tissue by immunohistochemistry (IHC) before treatment; 3) studies that reported the survival outcomes of overall survival (OS) and progression-free survival (PFS); 4) studies with sufficient data to extract or calculate the hazard ratio (HR) and 95% confidence interval (CI); 5) studies with sample size no less than 50; and 6) studies written in full text without language restriction. Publications were excluded based on the following criteria: duplicate publications, reviews, conference abstract, case reports, animal experiments, basic studies and inefficient data.

Data extraction and quality assessment

Two authors (Wen JY and Chen L) independently extracted information from all eligible publications, and any discrepancy was resolved by discussion. Data collected from individual studies included the following: first author's name, year of publication, original country of study population, recruitment time, follow-up period, cancer type, number of patients, cut-off value, number of high G6PD expression, analysis method, HR estimation, quality scores, and clinicopathological parameters. HRs and the corresponding 95% CIs of multivariate analyses were

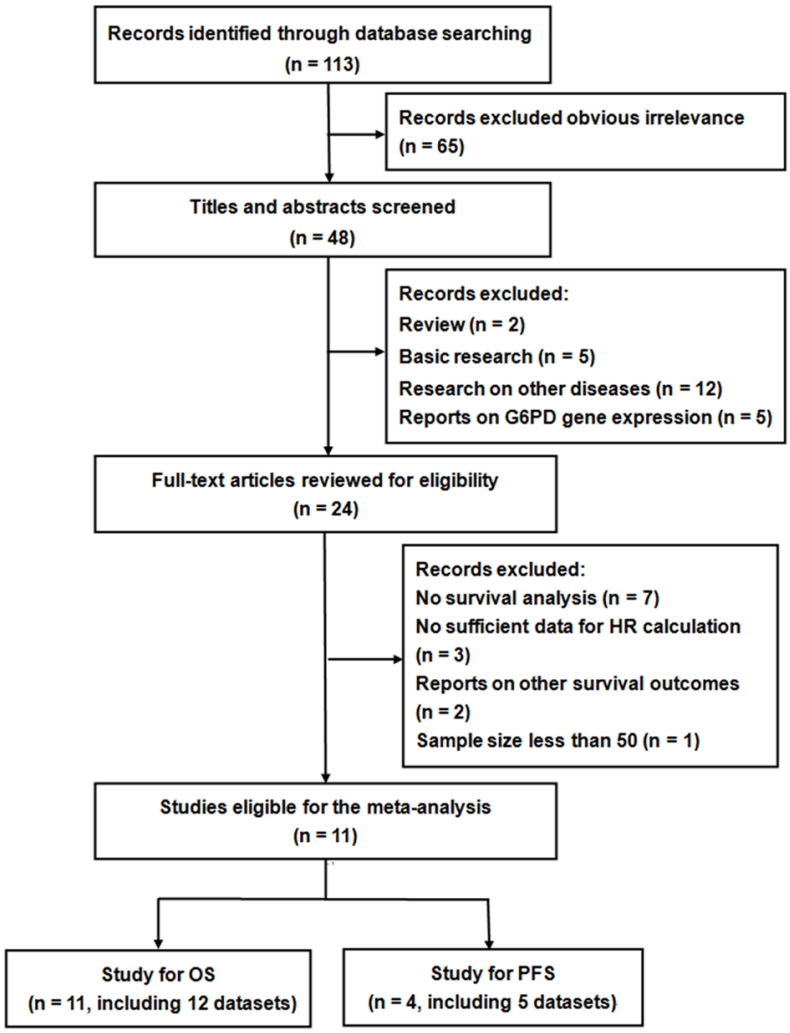

Figure 1. Flow diagram of the study selection process. Abbreviation: G6PD, Glucose-6-phosphate dehydrogenase; HR, hazard ratio; OS, overall survival; PFS, progression free survival.

given precedence to the data synthesis because they adjust the confounding factors and are considered to be more precise; otherwise, HRs along with 95% CIs were extracted from the univariate analysis or calculated using the Kaplan-Meier survival curves [16].

Two independent researchers (Wu JY and Wang YF) performed the quality assessment of the included studies by using the Newcastle-Ottawa Scale (NOS) [17]. The NOS scale judges the literature quality in three broad perspectives: selection of participants (four items, one star each), comparability of study groups (one item, up to two stars), and ascertainment of outcome of interest (three items, one star each). The studies were regarded as high quality if they met seven or more of the NOS scores [18].

Statistical analysis

STATA 13.1 software (STATA Corporation, College Station, TX, USA) was applied for all statistical analysis. HRs with corresponding 95% CIs was used to analyze the strength of G6PD expression with survival endpoints (OS and PFS). HR > 1 indicated a poor prognosis for patients with G6PD overexpression, and the result was considered statistically significant when the 95% CI did not overlap 1. For the pooled analysis of the correlation between G6PD expression and clinicopathological features, the odd ratio (OR) and the corresponding 95% CI was used as the summary measure to assess the effect. The heterogeneity among the included studies was both qualitatively examined through the chi-squared test based on the Q statistic, and quantitatively estimated by the I^2 metric. When significant heterogeneity was observed among the studies ($P < 0.05$ or $I^2 > 50\%$), a random-effects model was applied to syntheses the data of each study (DerSimonian and Laird method); otherwise, a fixed-effects model was performed (Mantel-Haenszel method) [19]. Sensitivity analysis was employed by sequentially removing each individual study to validate the robustness of the outcomes. Publication bias was statistically estimated by Begg's test and Egger's test [20]. If publication bias was significant ($P < 0.10$), the "trim and fill" method was used to estimate a corrected effect size after adjustment.

Results

Literature search

The details of the literature search process are shown in **Figure 1**. A total of 113 publications were initially identified from database searches

according to our search strategy, of which 65 articles were excluded due to evident irrelevance. After further screening the titles and abstracts, 24 papers were removed because of review, basic research, research on other diseases, or reports on G6PD gene expression. After viewing the full text for further selection, 13 papers were excluded due to the absence of survival analysis, insufficient data for HR calculation, reports on other survival outcomes, and having sample sizes less than 50. Finally, 11 studies with 2144 patients were regarded as eligible for our meta-analysis [21-31].

Study characteristics

The main information of the 11 included studies is summarized in **Table 1**. The 11 included studies published between 2010 and 2018 focused on several populations, including Chinese [21, 24, 27, 28, 30, 31], Korean [22, 26], American [23], Greek [25], and Italian [29]. A total of 10 studies were published in English, and one was in Chinese [31]. Various cancer types, such as hepatocellular carcinoma (HCC) [21], breast cancer (BC) [22, 26, 27], clear cell renal cell carcinoma (CCRCC) [23, 29], colorectal cancer (CRC) [24], non-small-cell lung cancer (NSCLC) [25], esophageal squamous cell carcinoma (ESCC) [28], and gastric cancer (GC) [30, 31], were covered. Among them, two studies recruited two cohorts each [24, 27]. Therefore, 13 cohorts with 2144 patients were eligible for this meta-analysis. A total of 12 cohorts reported the survival endpoint of OS [21-31] and 5 reported PFS [21, 24, 27, 29]. The HR estimates in four cohorts were directly extracted from the outcomes of multivariate analysis [23, 24, 28, 30], whereas the others were calculated from univariate analysis or Kaplan-Meier curve. The cut-off values of positive G6PD expression were extremely different among the included studies; therefore, we grouped all the patients according to their original studies (negative or positive staining).

Association between G6PD expression and clinicopathological features

The relationship of G6PD expression with clinicopathological features are illustrated in **Table 2**. Elevated level of G6PD expression was significantly associated with several phenotypes of tumor aggressiveness, such as larger tumor size (pooled OR = 1.566; 95% CI, 1.009-2.429; P = 0.045; fixed effects), worse histological grade (pooled OR = 2.260; 95% CI, 1.640-3.115; P < 0.001; fixed effects), advanced depth of invasion (pooled OR = 1.662; 95% CI, 1.008-2.739; P = 0.046; random effects), serious clinical stage (pooled OR = 2.866; 95% CI, 1.891-4.345; P < 0.001; fixed effects), positive lymph node metastasis (pooled OR = 3.328; 95% CI, 2.189-5.059; P < 0.001; fixed effects), and positive distant metastasis (pooled OR = 8.454; 95% CI, 1.930-37.036; P = 0.005; fixed effects). This finding indicated that G6PD overexpression might play a promoting role in tumor invasion and aggressiveness. However, no association existed between G6PD expression and certain factors, such as gender (pooled OR = 1.315; 95% CI, 0.938-1.845; P = 0.112; fixed effects), and age (pooled OR = 1.176; 95% CI, 0.851-1.625; P = 0.325; fixed effects).

Association between G6PD expression and survival

The main results of the analysis on the association between G6PD expression and survival are summarized in **Table 3**. A total of 12 individual cohorts comprising 2068 patients suggested that elevated G6PD expression indicated poor prognosis for OS (HR = 1.607; 95% CI, 1.448-1.784; P < 0.001; fixed effects) with moderate heterogeneity (I^2 = 26.0%, P_h = 0.189) (**Figure 2**). When the included cohorts were stratified into subgroup analyses, significant correlations were observed in the Chinese (HR = 1.645; 95% CI, 1.442-1.875; P < 0.001; fixed effects), Korean (HR = 1.467; 95% CI, 1.191-1.806; P < 0.001; fixed effects), and Caucasian (HR = 1.858; 95% CI, 1.140-1.884; P = 0.013; random effects) populations. When the subgroup analyses were conducted in terms of tumor types, G6PD overexpression was significantly associated with unfavorable survival in patients with BC (HR = 1.554; 95% CI, 1.339-1.802; P < 0.001; fixed effects), GC (HR = 1.920; 95% CI, 1.365-2.701; P < 0.001; fixed effects), CCRCC (HR = 2.377; 95% CI, 1.553-3.637; P < 0.001; fixed effects), and other cancer types (HR = 1.621; 95% CI, 1.164-2.256; P = 0.004; random effects). Similarly, positive results were observed in the subgroup analyses based on cut-off value, analysis method, and sample size. However, when the subgroup analyses were completed according to clinical stage, the role of G6PD overexpression in predicting worse prognosis was evident in patients with

Table 1. Main characteristics of the eligible studies

Study	Study population	Duration	Cancer type	Clinical stage	Primary treatment	Follow up (month)	Number	Cut off	Elevated G6PD (%)	Analysis	Outcome	Language	Quality
Lu M (2018)	Chinese	2005-2009	HCC	I-IV	Surgery	Until 2016.7	127	Score ≥ 2	68 (53.5)	Univariate	OS, PFS	English	8
Choi JJ (2018)	Korean	2001-2006	BC	I-III	Surgery	NR	348	Score ≥ 2	51 (14.7)	Univariate	OS	English	7
Zhang Q (2017)	American	NR	CCRCC	I-IV	NR	NR	149	Score ≥ 2	76 (51.0)	Multivariate	OS	English	7
Ju HQ (2017)[a]	Chinese	2010-2013	CRC	I-IV	CT	Until 2016.4	318	Score ≥ 4	96 (30.2)	Multivariate	OS	English	7
Ju HQ (2017)[b]	Chinese	2000-2007	CRC	I-IV	CT	Until 2010.9	76	Score ≥ 4	28 (36.8)	Univariate	PFS	English	7
Giatromanolaki A (2017)	Greek	2006-2010	NSCLC	I-III	Surgery	Median 46 (26-112)	98	Score ≥ 2	69 (70.4)	Univariate	OS	English	8
Cha YJ (2017)	Korean	2004-2008	BC	IV	Surgery	Until 2012.6	126	Score ≥ 3	100 (79.4)	Univariate	OS	English	8
Dong TY (2016)[a]	Chinese	2009-2014	BC	I-IV	NACT	Median 62 (16-134)	189	Score ≥ 3	99 (52.4)	Univariate	OS, PFS	English	8
Dong TY (2016)[b]	Chinese	2003-2009	BC	I-IV	NACT	Median 72 (5-124)	295	Score ≥ 3	217 (73.6)	Univariate	OS, PFS	English	7
Wang X (2015)	Chinese	NR	ESCC	I-IV	Surgery	NR	128	Score ≥ 1	95 (74.2)	Multivariate	OS	English	8
Lucarelli G (2015)	Italian	NR	CCRCC	I-IV	Surgery	Median 42.5 (38-60)	60	Score ≥ 5	36 (60.0)	Univariate	OS, PFS	English	7
Wang JX (2012)	Chinese	2003-2005	GC	I-IV	Surgery	Until 2010.3	167	Score ≥ 4	117 (70.1)	Multivariate	OS	English	8
Chen JX (2010)	Chinese	2004	GC	I-IV	Surgery	Until 2009.12	63	Score ≥ 4	43 (68.1)	Univariate	OS	Chinese	7

Notes: [a]First of two cohorts in this study; [b]second of two cohorts in this study. G6PD, glucose-6-phosphate dehydrogenase; NR, none reported; HCC, hepatocellular carcinoma; BC, breast cancer; CCRCC, clear cell renal cell cancer; CRC, colorectal cancer; NSCLC, non small cell lung cancer; ESCC, esophageal squamous cell cancer; GC, gastric cancer; OS, overall survival; PFS, progression free survival; CT chemotherapy; NACT, neoadjuvant chemotherapy.

Table 2. Meta-analysis of G6PD expression and clinicopathological features in solid tumors patients

Categories	Studies (no. of patients)	OR (95% CI)	I^2 (%)	P_h	Z	P
Gender (male vs. female)	4 (507)	1.315 (0.938~1.845)	0.0%	0.841	1.59	0.112
Age (< 60 vs. ≥ 60)	6 (991)	1.176 (0.851~1.625)	0.0%	0.440	0.98	0.325
Tumor size (< 5 cm vs. ≥ 5 cm)	3 (379)	1.566 (1.009~2.429)	24.2%	0.267	2.00	0.045
Histological grade (moderate/well vs. poor)	5 (775)	2.260 (1.640~3.115)	0.0%	0.749	4..98	< 0.001
Depth of invasion (T_1+T_2 vs. T_3+T_4)	5 (863)^R	1.662 (1.008~2.739)	52.8%	0.076	1.99	0.046
Clinical stage (I+II vs. III+IV)	4 (507)	2.866 (1.891~4.345)	0.0%	0.608	4.96	< 0.001
Lymph node metastasis (negative vs. positive)	4 (653)	3.328 (2.189~5.059)	13.8%	0.323	5.63	< 0.001
Distant metastasis (negative vs. positive)	2 (316)	8.454 (1.930~37.036)	0.0%	0.867	2.83	0.005

All pooled HRs were calculated from fixed-effect model except for cells marked with (random^R). P_h denotes *P* value for heterogeneity based on *Q* test; *P* denotes *P* value for statistical significance based on *Z* test.

Table 3. Pooled and subgroup analysis of main results for the meta-analysis

Categories	Cohort (case)	Model	HR (95% CI)	Z	P	Heterogeneity	
						I^2	P_h
Overall survival (OS)	12 (2068)	Fixed	1.607 (1.448~1.784)	8.92	< 0.001	26.0%	0.189
Study population							
Chinese	7 (1287)	Fixed	1.645 (1.442~1.875)	7.43	< 0.001	32.1%	0.183
Korean	2 (474)	Fixed	1.467 (1.191~1.806)	3.61	< 0.001	0.0%	0.621
Caucasian	3 (307)	Random	1.858 (1.140~1.884)	2.49	0.013	57.5%	0.095
Cancer type							
BC	4 (958)	Fixed	1.554 (1.339~1.802)	5.82	< 0.001	0.0%	0.809
GC	2 (230)	Fixed	1.920 (1.365~2.701)	3.75	< 0.001	0.0%	0.549
CCRCC	2 (209)	Fixed	2.377 (1.553~3.637)	3.99	< 0.001	0.0%	0.576
Others	4 (671)	Random	1.621 (1.164~2.256)	2.86	0.004	63.4%	0.042
Clinical stage							
I~IV	9 (1496)	Fixed	1.698 (1.498~1.925)	8.28	< 0.001	32.0%	0.162
I~III	2 (446)	Fixed	1.431 (1.184~1.730)	3.72	< 0.001	0.0%	0.475
IV	1 (126)	-	1.078 (0.313~3.716)	0.12	0.905	-	-
Cut-off value							
Score ≥ 2	4 (722)	Random	1.669 (1.273~2.188)	3.71	< 0.001	50.6%	0.108
Score ≥ 3	3 (610)	Fixed	1.629 (1.322~2.006)	4.59	< 0.001	0.0%	0.753
Score ≥ 4	3 (548)	Fixed	1.475 (1.212~1.795)	3.88	< 0.001	47.2%	0.151
Others	2 (188)	Fixed	2.398 (1.552~3.705)	3.94	< 0.001	0.0%	0.514
Analysis method							
Univariate	8 (1306)	Fixed	1.640 (1.451~1.854)	7.90	< 0.001	0.0%	0.433
Multivariate	4 (762)	Random	1.847 (1.241~2.749)	3.03	0.002	60.1%	0.057
Sample size							
≥ 100	9 (1847)	Fixed	1.590 (1.421~1.779)	8.10	< 0.001	26.3%	0.210
< 100	3 (221)	Fixed	1.720 (1.298~2.279)	3.77	< 0.001	46.6%	0.154
Progression free survival (PFS)	5 (747)	Fixed	1.779 (1.523~2.079)	7.25	< 0.001	0.0%	0.692

HR, hazard ratio; CI, confidence interval; BC, breast cancer; GC, gastric cancer; CCRCC, clear cell renal cell carcinoma. *P* denotes *P* value for statistical significance based on *Z* test; P_h denotes *P* value for heterogeneity based on *Q* test.

Stages I-IV (HR = 1.698; 95% CI, 1.498-1.925; *P* < 0.001; fixed effects), and Stages I-III (HR = 1.431; 95% CI, 1.184-1.730; *P* < 0.001; fixed effect), but not in Stage IV (HR = 1.078; 95% CI, 0.313-3.716; *P* = 0.905; random effects).

Five studies with 747 patients reported PFS as the primary endpoint, with the high expression of G6PD contributing to worsened PFS (HR = 1.779; 95% CI, 1.523-2.079; *P* < 0.001; fixed effects), and absence of significant heteroge-

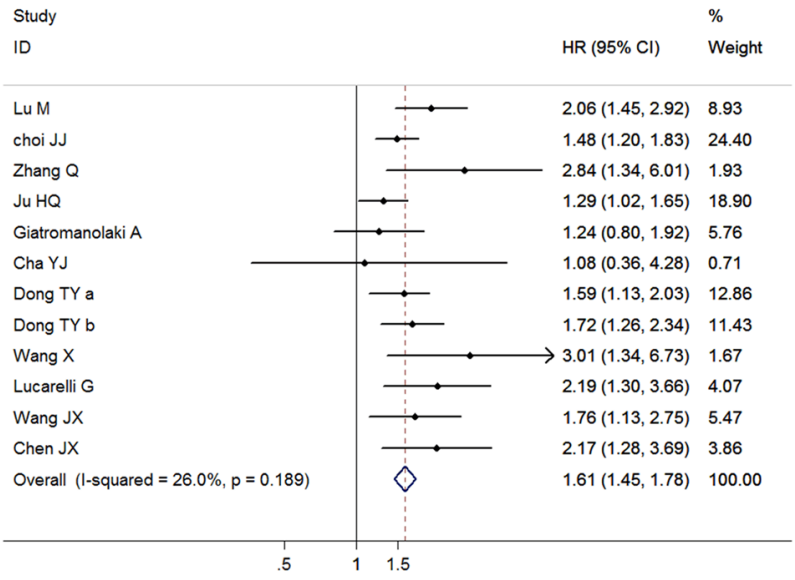

Figure 2. Forest plots of the association between G6PD expression and overall survival. Abbreviation: G6PD, Glucose-6-phosphate dehydrogenase; HR, hazard ratio; CI, confidence interval.

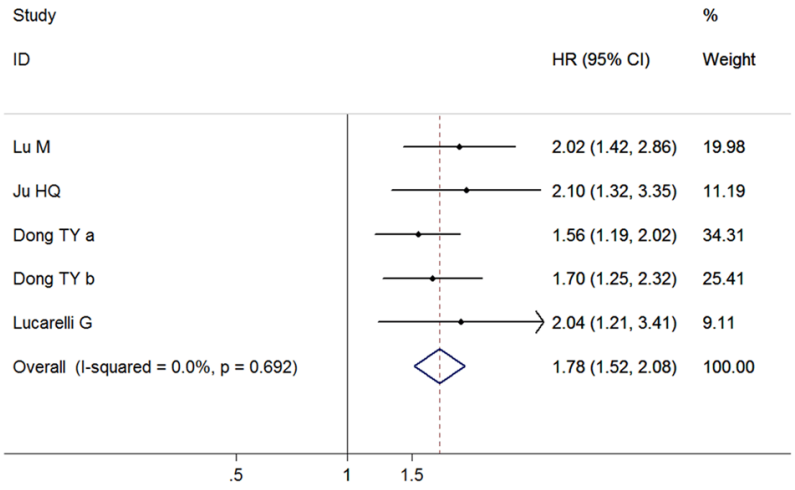

Figure 3. Forest plots of the association between G6PD expression and progression free survival. Abbreviation: G6PD, Glucose-6-phosphate dehydrogenase; HR, hazard ratio; CI, confidence interval.

neity (I^2 = 0.0%, P_h = 0.692) (**Figure 3**). Due to the limited number of included studies, we did not conduct subgroup analyses concerning this survival endpoint.

Sensitivity analysis and publication bias

The pooled results of sensitivity analysis are shown in **Figure 4**. The overall HR estimates for OS or DFS did not significantly change after the sequential omission of each single cohort, thereby indicating that individual cohorts had little substantial effect on the final results, and the outcomes of this meta-analysis were stable.

Both Begg's test and Egger' test suggested a significant publication bias with regard to the pooled outcome of OS (Begg's test, P = 0.047; Egger' test, P = 0.062), and the funnel plot also showed a certain degree of apparent asymmetry (**Figure 5A**). The trim and fill analysis indicated that three more unpublished studies were needed to balance the funnel plot (**Figure 5B**). The adjusted HR and 95% CI were slightly changed but still significant (pooled HR = 1.550; 95% CI, 1.402-1.714; P < 0.001; fixed effects). Moreover, although there was no evidence provided by Begg's test (P = 0.462), an observed publication bias was found by Egger' test (P = 0.065) and was confirmed by the funnel plot shape (**Figure 5C**). After adjusted by the trim and fill analysis, one more study had to be added into the funnel plot (**Figure 5D**), and the recalculated result remained statistically significant (pooled HR = 1.712; 95% CI, 1.485-1.973; P < 0.001; fixed effects). Therefore, the results of the meta-analysis were robust and reliable.

Discussion

Cancer cells can overcome all kinds of obstacles and rapidly proliferate mainly due to their unique growth characteristics from normal cells. As one of the 10 hallmarks of cancers summarized by Hanahan and Weinberg, reprogramming of energy metabolism reveals the

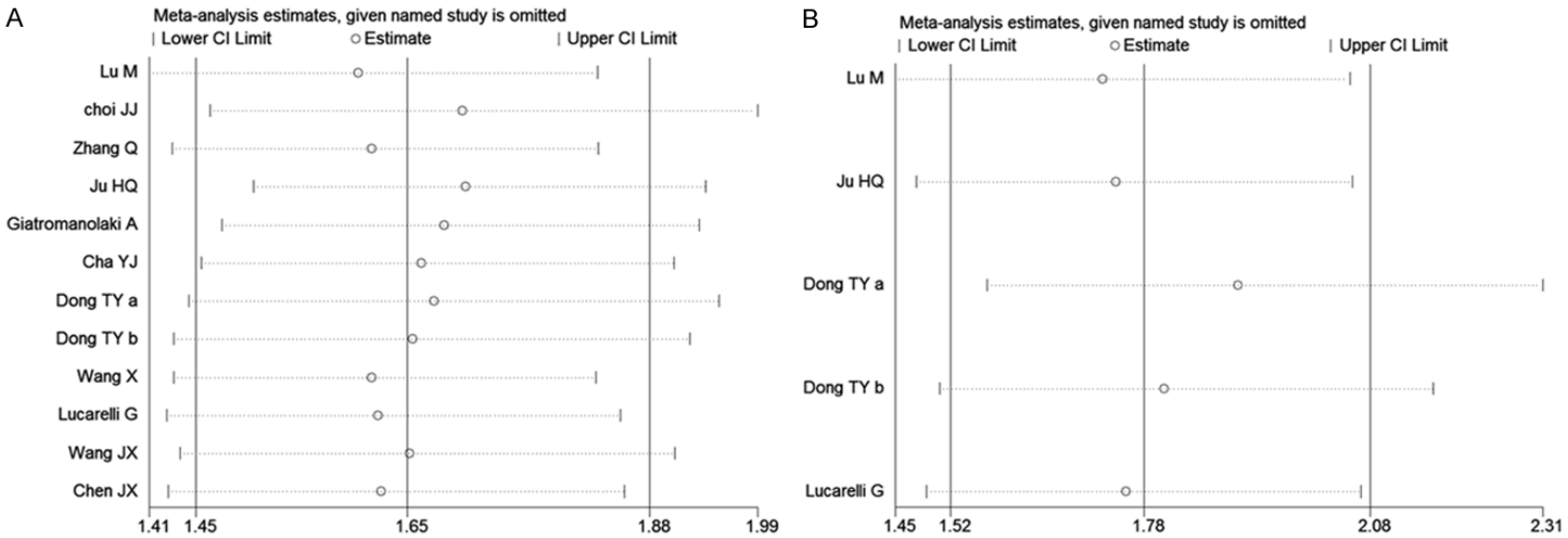

Figure 4. Sensitivity analysis of this meta-analysis. A. Sensitivity analysis for overall survival. B. Sensitivity analysis for progression free survival.

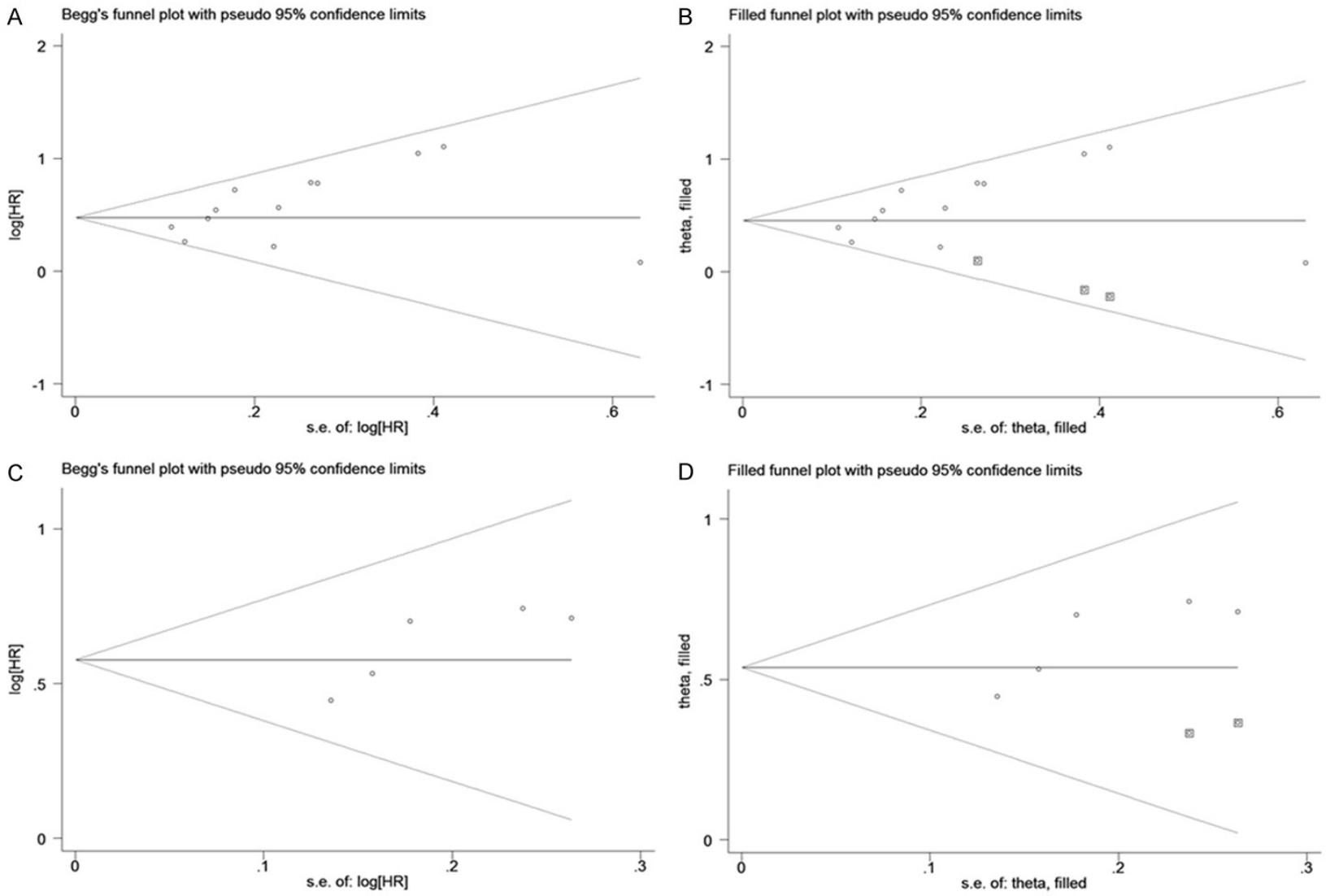

Figure 5. Funnel plots assessing the potential publication bias of the included studies. A. Funnel plot of publication bias for overall survival. B. Funnel plot adjusted by trim and fill analysis for overall survival. C. Funnel plot of publication bias for progression free survival. D. Funnel plot adjusted by trim and fill analysis for progression free survival.

potential mechanism of tumor formation and progression from the metabolic standpoint [32]. In the past decade, studies have mainly focused on aerobic glycolysis; moreover, the key enzymes of glycolysis, such as glucose transporter [33], hexokinase [34], pyruvate kinase [35], and lactate dehydrogenase [36], all have been proven to be involved in tumor invasion. However, with the comprehensive understanding of the abnormal metabolism of tumor cells, researchers have realized that the Warburg effect was only "the tip of the iceberg" [37]. Recently, the effect of the PPP pathway in cancer cell metabolism has gained increasing attention. Owing to the reversible nature of the nonoxidative branch, the interaction of PPP with glycolysis can control the production of NADPH and R5P by regulating the glucose flux from glycolysis to the PPP pathway, thus allowing PPP to adapt to the metabolic demands of cancer cells operating in different patterns [38]. The upregulation of PPP in cancer cells generates high levels of NADPH to reduce ROS, while simultaneously producing sufficient nucleotides for DNA synthesis and repair. Therefore, elevated PPP is critical for cancer cell proliferation and survival.

G6PD usually works at 1%-2% of its maximal potential in healthy people due to the high level of NADPH in resting states. The level and activity of G6PD are higher in cancer tissues rather than in normal tissues [39]. G6PD activity often loses its original regulation during the transformation of normal cells into malignant cells, and acts as an oncogene. The activation of G6PD has been involved in tumor origination and progression, but the prognostic role of G6PD on solid tumors remains uncertain. A meta-analysis can provide an overall and precise evaluation of individual studies for a specified outcome; thus, this first meta-analysis examines the association between G6PD and prognosis in solid tumors. The combined outcomes based on 11 studies with 2144 patients showed that the high density of G6PD expression is considerably associated with worsened OS and PFS in solid tumors; hence, G6PD overexpression can be an independent predictor for unfavorable all-cause mortality and cancer progression in patients with solid malignancies. Notably, when subgroup analysis was performed based on clinical stage, the overexpression of G6PD was associated with worsened OS in patients with Stages I-IV and Stages I-III, but not in Stage IV, and this finding indicated that G6PD might function differently at different phases of tumor growth. However, given the limited study number (1 study) and small sample size (126 patients), the prognostic value of G6PD in cancer patients with Stage IV is not strongly evidenced-based. Thus, more studies are necessary to explore the realistic prognostic effect of G6PD at this tumor stage. Moreover, despite the broadly searched criteria, evidence of publication bias remained possible among the studies concerning OS and PFS, and this finding might have inflated the pooled outcomes. Trim-and-fill analysis was then applied to recalculate the pooled effect size, and the adjusted results remained statistically significant. This finding indicated that the publication bias has slight systematic influence on the overall outcomes, and further confirms the reliability of our results.

We further analyzed the relationship between G6PD and clinicopathological parameters, and the synthesized outcomes suggested that increased levels of G6PD was significantly associated with some phenotypes of tumor progression, including poor histological grade, advanced tumor depth, serious clinical stage, positive lymph node metastasis, and positive distant metastasis. These results strongly support the predictive value of G6PD overexpression on poor prognosis in malignancies. Additionally, elevated G6PD is closely related to aggressive tumor behavior. Therefore, cancer patients with the preceding clinicopathological features might benefit most from G6PD estimation for clinical-decision making.

Elevated G6PD activity might affect the prognosis of solid malignancies through the following mechanisms. First, malignant cells with high levels of G6PD favor proliferation and survival, and strongly support oncogenic transformation by benefiting from the sufficient supply of R5P and NADPH [40]. Second, overexpression of G6PD in tumor cells can provide additional NADPH and eventually prevent cell death from oxidative damage [41]. Inhibition of G6PD considerably increases H_2O_2-mediated cell death, whereas elevated G6PD expression leads to resistance to apoptosis [42-44]. Third, G6PD activity has a pro-angiogenic role in promoting proliferation and invasion of tumor cells [45].

Knockdown of the G6PD *in vitro* reduces endothelial cell proliferation, migration, and phosphorylation of the vascular endothelial growth factor receptor, whereas G6PD overexpression exhibits a pro-angiogenic phenotype [46. 47]. Fourth, G6PD modulates Nox-derived ROS production and subsequently promotes cancer cell metastasis [48, 49]. Fifth, elevated G6PD activity protects tumor cells from oxidative stress-induced apoptosis by enriching the NADPH-reducing power and glutathione, thus reducing sensitivity to some anticancer agents [50].

Given the unique role of the PPP in cancer metabolism, targeting G6PD, which is a gatekeeper of the PPP, has been identified as a relevant and challenging therapeutic option [51]. Knockdown of G6PD slows down tumor cell proliferation and increases susceptibility to oxidative stress. Thus, inhibition of G6PD will attenuate tumor growth by limiting the capacity of cells to activate carcinogens, produce ROS, and provide intermediates for cell reproduction [52]. At present, many PPP inhibitors have been discussed in experimental works, but their clinical values remain uncertain. Among these inhibitors, a competitive G6PD inhibitor, known as 6-aminonicotinamide (6-AN), involves the biosynthesis of 6-aminonicotinamide adenine dinucleotide phosphate and subsequently inhibits PPP at the level of 6PGD; this leads to reduced NADPH production and increased sensitivity to cytotoxic drugs [53]. Furthermore, a noncompetitive one, dehydroepiandrosterone (DHEA), has been shown to block the development of methylnitrosourea-induced breast cancer [54] and prostate cancer [55] in animal models due to the binding of DHEA to the ternary enzyme-coenzyme-substrate complexes. Administration of DHEA restricted the growth of early preneoplastic liver lesions and delayed of HCC progression in persistent liver nodules in rats [56].

Some limitations of our meta-analysis should be acknowledged. First, most of the individual HR values were calculated from survival curves or univariate analysis, and this step may lead to some minor differences from actual HRs. Second, most included studies originated from East Asia, and thus, some limitations on racial representation may be observed. Therefore, the conclusions should be cautiously taken for other ethnic populations. Third, our meta-analysis focused on the clinical significance of pretreatment G6PD level, but not on posttreatment G6PD changes; hence, the dynamic alteration of G6PD after completion of therapy remains unclear. Fourth, although the same G6PD protein detection of the included studies was all based on IHC, the difference between experimental factors, such as experimental design, specimen preparation, choice of antibody, dilution of antibodies, and other relevant information, may have confounded the pooled outcomes. Finally, the only evidence for the correlation study was provided by this meta-analysis, which cannot be simply interpreted as a causal relationship.

In conclusion, G6PD overexpression is markedly associated with poor survival and some progressive phenotypes in solid tumors. Therefore, G6PD might be a useful prognostic biomarker for solid tumors. However, due to the limitations of our work, the results should be cautiously interpreted, and further high-quality studies with increased samples are required to validate our results.

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Disclosure of conflict of interest

None.

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