Original Article

High expression of hypoxia-inducible factor 1-alpha predicts poor prognosis in pancreatic ductal adenocarcinoma: a meta-analysis and database validation protocol

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Contributions: (I) Conception and design: A Zoa; (II) Administrative support: M Dong, Y Tian; (III) Provision of study materials or patients: None; (IV) Collection and assembly of data: A Zoa, H Wang; (V) Data analysis and interpretation: A Zoa, M Dong, Y Tian; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

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Background: Hypoxia-inducible factor 1-alpha (HIF-1α) is overexpressed in pancreatic ductal adenocarcinomas (PDACs). However, the prognosis of high expression of HIF-1α in PDACs remains controversial because of lacking a solid support. A meta-analysis may help for a better understanding of the role of HIF-1α in the prognosis of PDACs.

Methods: By using a systematic approach, we conducted a meta-analysis from current literature. We performed an advanced search in PubMed, Embase, Cochrane Library and Web of Science databases. Recorded studies were published between September 1, 2001, and February 26, 2021, in English and related to the expression of HIF-1α in PDAC. We pooled and combined hazard ratios (HRs) and 95% confidence intervals (CIs) to show the effect of HIF-1α expression on overall survival (OS). We pooled also risk ratios (RRs) and 95% CIs to assess the correlation between HIF-1α expression and clinicopathological characteristics in PDAC. We evaluated publication bias among included studies through the Begg's test and Egger's test. From "Expression Plots" modules in the Gene Expression Profiling Interactive Analysis (GEPIA) database, we showed the difference of mRNA level for HIF1A between 179 pancreatic adenocarcinomas (PAADs) and 171 normal pancreatic tissues.

Results: This meta-analysis included 11 studies and 764 patients. High expression of HIF-1α was associated with shorter OS compared to low HIF-1α expression in PDAC (HR =1.74, 95% CI: 1.49–2.04, P<0.001). Patients with high expression of HIF-1α tended to have an increased risk of earlier lymph node metastasis (LNM) (RR =1.63, 95% CI: 1.36–1.95, P<0.001), and more advanced clinical stage (RR =1.64, 95% CI: 1.38–1.97, P<0.001) compared to those with low HIF-1α expression. Expression plots from GEPIA database showed HIF1A overexpressed in PDAC tissues compared to normal tissues (Log_2FC =2, P<0.01).

Conclusions: High HIF-1α expression associated with worse prognosis of PDACs compared to low HIF-1α expression. Since HIF-1α expression is greater in PDAC than normal pancreas, it could serve as a prognostic factor and potential therapeutic target. However, due to the complex role of HIF-1α in physiology and pathology, therapeutic intervention should be considered with caution.

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Keywords: Hypoxia-inducible factor-1α (HIF-1α); pancreatic ductal adenocarcinoma (PDAC); prognosis; meta-analysis

Submitted Mar 23, 2022. Accepted for publication Jul 01, 2022.
doi: 10.21037/tcr-22-787
View this article at: https://dx.doi.org/10.21037/tcr-22-787

Introduction

Pancreatic adeno carcinoma (PDAC) is considered as the king of cancers because it is one of the most aggressive and difficult to treat cancers, with a median survival of 6 months (1). Indeed, the 5-year survival rate of pancreatic cancer at the time of diagnosis was 10% in the USA (2), and only 7.2% in China and the lowest level in all cancers (3). Surgery combined to adjuvant chemotherapy remains the best and only curative option, but most of the patients (>50%) are diagnosed when the tumor already metastasized (4). Despite the increased number of treatment options (chemotherapy, radiotherapy and chemo radiotherapy), pancreatic cancer still shows modest response to conventional cytotoxic drugs because of resistance, the lack of effective early detection methods, and the lack of tumor biomarkers. Therefore, there is a real need to identify potential biomarkers whose high expression is specific to cancers in general and to PDAC in particular.

Pancreatic ductal adenocarcinoma (PDAC) is a hypo vascular tumor, making hypoxia a common feature of its microenvironment (5). Hypoxia-inducible factor-1α (HIF-1α) is a transcription factor that is activated during hypoxia in a hypovascular environment (6). HIF-1α is ubiquitinated by the Von Hipple-Lindau (VHL) protein under normoxic conditions and is quickly degraded by proteasomes. However, HIF-1α expression in hypoxia can escape proteasome degradation, and HIF-1α becomes stabilized and translocate to the nuclei where it dimerize with hypoxia-inducible factor 1-beta (HIF-1β) to form a functional transcription factor capable of binding to hypoxia response elements (HREs) of deoxyribonucleic acid (DNA) and transcriptionally activating target genes (7,8). These genes include erythropoietin, glucose transporters, glycolytic enzymes, vascular endothelial growth factor (VEGF), hypoxia inducible lipid droplet associated (HILPDA), and other genes whose protein products increase oxygen delivery or facilitate metabolic adaptation to hypoxia (9). In PDAC, high HIF-1α expression associated with tumor progression, angiogenesis, cell migration, and hepatic metastasis (10-15). Despite this evidence, there is controversy regarding the association of HIF-1α expression with prognosis or survival of patients diagnosed with PDAC. Leppänen *et al.* (16), showed a significant (P=0.007 log-rank) longer mean survival time (34.2 months) in patients with high expression of HIF-1α (n=36) compared to those with lower expression (21.5 months, n=28). On the other hand, several studies indicated that a strong HIF-1α expression associated with poor overall survival (OS) of patients with PDAC (17-19). Thus, by using a search strategy in PubMed, Embase, Cochrane Library and Web of Science databases, this study first compiled all accessible existing literature on the prognostic value of HIF-1α expression in PDAC, and second, we conducted a meta-analysis of the included literature to explore the association between high HIF-1α expression and OS. We interested in the relationship between high HIF-1α expression and clinical stage, and the presence or absence of lymph node metastasis (LNM). After all, we reported positive correlation between high HIF-1α expression and poorer OS, clinical stage, and metastatic lymph nodes. At mRNA level, the differential expression of HIF1A in PAAD and normal pancreas was established using Gene Expression Profiling Interactive Analysis (GEPIA) webserver. HIF-1α could therefore be an element to consider in PDAC targeted therapies and its expression could serve as a biomarker for diagnosis. We present the following article in accordance with the PRISMA reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-787/rc) (20).

Methods

Search strategy

We performed a literature search on November 1, 2021 in PubMed, Embase, Cochrane Library and Web of Science databases. As shown in *Table 1*, we used the following key words: ("pancreatic ductal adenocarcinoma" OR "PDAC") AND ("hypoxia inducible factor OR HIF-1-alpha OR HIF-1A OR HIF-1alpha OR HIF1 OR HIF1-ALPHA OR MOP1 OR PASD8 OR bHLHe78") AND ("survival" OR

Table 1 Literature search strategy for PubMed and Web of Sciences databases

Search terms	Results	
	PubMed	Web of Sciences
#1 pancreatic ductal adenocarcinoma OR PDAC	18,276	24,110
#2 hypoxia inducible factor OR HIF-1-alpha OR HIF-1A OR HIF-1alpha OR HIF1 OR HIF1-ALPHA OR MOP1 OR PASD8 OR bHLHe78	47,445	43,526
#3 prognosis OR survival	3,788,090	2,449,847
#1AND#2AND#3	99	133

Search update: 25/03/2022. PDAC, pancreatic ductal adenocarcinomas.

"prognosis").

Inclusion and exclusion criteria

Criteria for the eligible studies included: (I) participants: PDAC patients without preoperative radiation or chemotherapy; (II) intervention: patients with high expression of HIF-1α; (III) control: patients with low expression of HIF-1α; (IV) outcomes: clinical stage, metastasis lymph nodes, and OS; (V) study design: prospective or retrospective studies; (VI) the expression level of HIF-1α in PDAC was detected by immunohistochemistry (IHC). We excluded studies according to the following criteria: (I) duplicated publications; (II) letters, reviews, case reports, communication and expert opinions; (III) animal studies, basic research studies; (IV) studies without sufficient data to estimate the risk ratios (RRs), hazard ratios (HRs) and corresponding 95% confidence intervals (CIs).

Data extraction and quality assessment

We extracted the following items: name of first author, published year, country, follow-up time, detection method, number of patients in high and low expression of HIF-1α, high expression of HIF-1α criteria, median age, quality score of the paper, clinical stage, staging system, lymph node metastasis, treatment, analysis model of OS, adjusted. For studies only reporting the survival curve of OS or RFS, the survival data was extracted from the survival curve (21). We evaluated the quality of eligible studies according to the Newcastle-Ottawa Scale (NOS), which ranged from 0 to 9. One study with an NOS score more than 5 was regarded as high quality (22). The data extraction was independently evaluated by 2 investigators (Alexis Zoa and Haibo Wang), and a consensus was reached by group discussion when the disagreement occurred.

Database validation

We compared expression of mRNA for HIF1A in pancreatic adenocarcinomas and normal pancreas using Gene Expression Profiling Interactive Analysis (GEPIA) database to emphasize the role of HIF1A in PAAD progress. GEPIA is a newly developed interactive web server for analyzing the RNA sequencing expression data of 9,736 tumors and 8,587 normal samples from The Cancer Genome Atlas (TCGA) and the genotype tissue expression (GTEx) projects, using a standard processing pipeline (23). GEPIA provides customizable functions such as tumor/normal differential expression analysis, profiling according to cancer types or pathological stages, patient survival analysis, similar gene detection, correlation analysis and dimensionality reduction analysis. This tool was developed by Zefang Tang, Chenwei Li and Boxi Kang of Zhang Lab, Peking University. TCGA and GTEx are common databases that allow cancer researchers and bio-informaticians to search and download cancer data for analysis. The difference was considered significant with a fold change cutoff equal to 2 (Log_2FC =2) and a P value less or equal to 0.05 ($P \leq 0.05$).

Statistical analysis

We pooled HRs for OS and RRs for clinicopathological characteristics with corresponding 95% CIs according to the expression status of HIF-1α. We defined the OS as the time from diagnosis to death from any cause or to the date of the last follow-up. A combined HR or RR >1 reflected a shorter survival or elevated risk for patients with high expression of HIF-1α. The significance of the pooled HR and RR was determined by the Z test, and a P value <0.05 was considered statistically significant. We performed subgroup analysis to assess robustness of the synthesized results.

We evaluated heterogeneity across studies by a χ^2-based test. We also calculated the I^2 statistic, a quantitative measure of inconsistency across studies (24). When significant heterogeneity (I^2>50% or P<0.05) existed, the random effects model was used for the meta-analysis. Otherwise, a fixed effects model was adopted. The potential publication bias was investigated using inspection of the Begg's funnel plot and Egger's linear regression with P<0.1 defining publication bias (25). All analyses were conducted using Review Manager (RevMan) version 5.4. (The Cochrane Collaboration, 2020) and MedCalc - version 20.015. All statistical tests were two-sided.

Results

Study selection and characteristics

A flow diagram showing our literature search and screening strategy is presented in *Figure 1*. We initially obtained a total of 237 articles, among which 232 from databases records and 5 from citations searching (*Table 1*). After removing 80 duplicate studies, we screened 152 records by reading the abstracts. We removed 27 letters, reviews, case reports and communications, and 75 animal studies/basic research. We evaluated 55 full-text articles for eligibility, and finally, we included 11 articles with 764 patients (408 high and 356 low) in the meta-analysis (16,17,19,26-33).

The included studies were published between 2007 and 2019. 420 of 764 (54.97%) patients were from China, 180 (23.56%) from Japan, 100 (13.09%) from USA and 64 (8.38%) from Finland. Expression of HIF-1α was measured by IHC in all cohorts. The samples were tumor tissue in all the studies. Nine of eleven studies used to assess HRs and the corresponding 95% CIs of OS (17,19,27-33). Seven of eleven used to assess clinical stage (26-32), and eight of eleven used to assess the LNM status (16,26-32). The NOS scores of all these studies were between 6 and 8 points, indicating that each article was of high quality (*Table 2*). Further detailed descriptions of these eligible articles are listed in *Table 3*.

Association between HIF-1α expression and OS

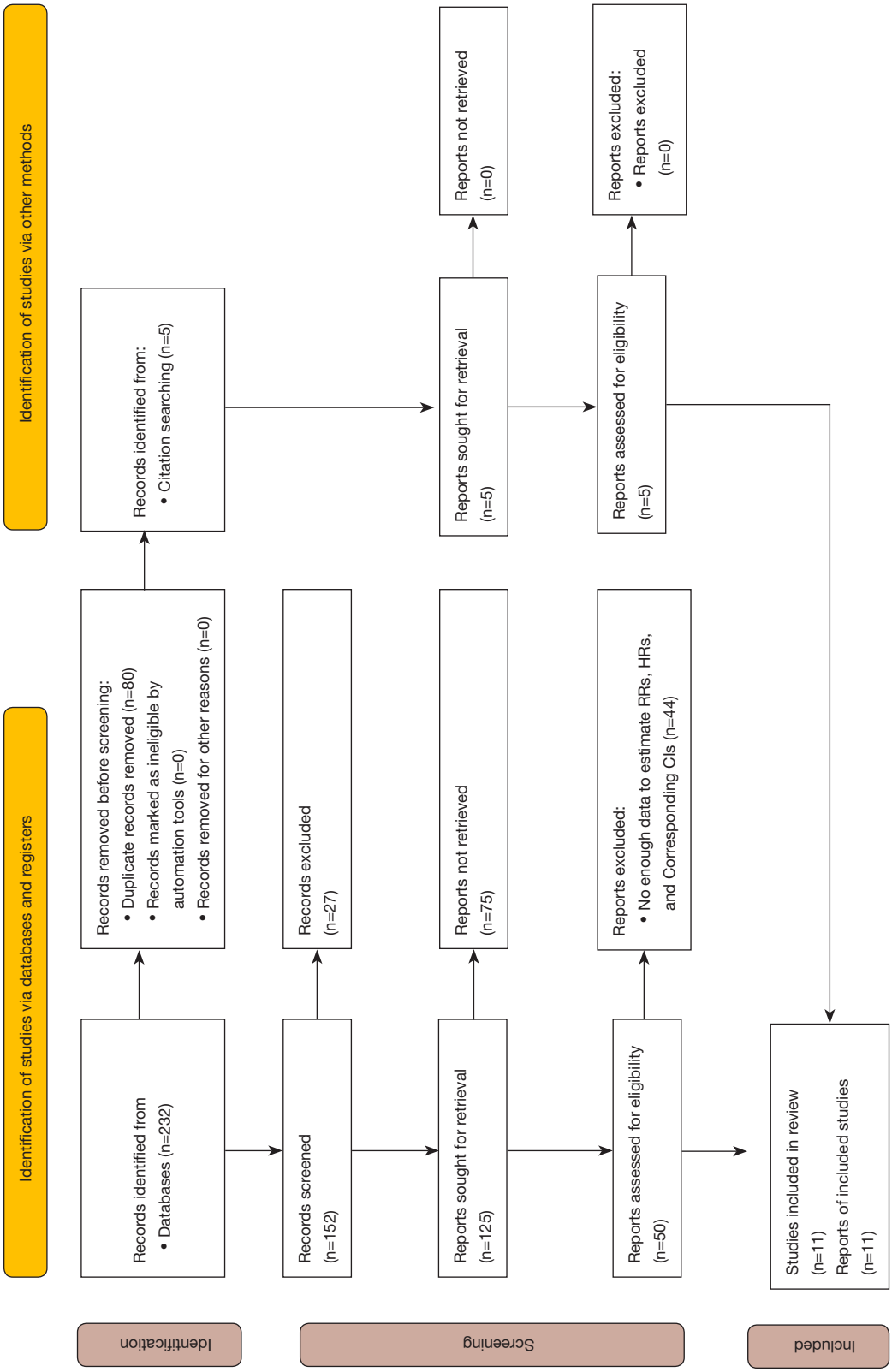
Ten of the eleven studies included in this quantitative analysis had enough data to estimate the HRs (16,17,19,27-33). We used the techniques described by Tierney *et al.* to extract data from survival curves and estimate HRs and 95% CIs corresponding (21). We used fixed effect model to calculate the HR and 95% CI because of lower statistical heterogeneity (I^2=28.0%, P=0.19). The results indicated that high HIF-1α was associated with poor OS compared to low HIF-1α (HR =1.74, 95% CI: 1.49–2.04, P<0.001; *Figure 2*). Although the heterogeneity was not significant, we took the liberty of doing a subgroup analysis (by country). The results showed that six studies were conducted in China (26,28,30-32), and the meta-analysis of these studies revealed zero heterogeneity (I^2=0%, HR =1.97, 95% CI: 1.58–2.47, P<0.001; *Figure 2*). Three studies were conducted in Japan (27,29,33), and the meta-analysis showed zero heterogeneity (I^2=0%, HR =1.77, 95 % CI: 1.29–2.42, P<0.001; *Figure 2*).

Association between HIF-1α expression and clinicopathological parameters

Six studies reported the staging of tumor according to the American Joint Committee on Cancer (AJCC) staging manual (5th & 6th edition) (16,26,28,30-32), and two studies reported according to the Classification of Pancreatic Carcinoma (2nd English edition) proposed by the Japan Pancreas Society (JPS) (27,29). One study did not mention a staging system (19). We excluded two studies in this part because they did not classified clinical stage according to the level of HIF-1α expression (17,33). The results showed that PDAC patients with advanced tumor stage (III-IV according to the AJCC 6th edition, and IV according to JPS 2nd English edition) have 164% times more risk (RR =1.64, 95% CI: 1.38–1.96, P<0.001; *Figure 3*) of high HIF-1α expression.

Eight studies reported the LNM status according to HIF-1α expression (16,26-32). Six of them reported a significant difference (P<0.05) in the number of metastatic lymph nodes between the high expression of HIF-1α and the low expression groups (26-28,30-32). In the two other studies, the number of metastatic lymph nodes was still higher in the high expression group than in the lower, but the difference was not statistically significant (16,29). The meta-analysis results showed more metastatic lymph nodes in the high HIF-1α groups. So, PDAC patients with more metastatic lymph nodes number have 163% times more risk (RR =1.63, 95% CI: 1.36–1.95, P<0.001; *Figure 3*) of high expression of HIF-1α.

We also evaluated the prognostic power of high expression of HIF-1α in patients at advance clinical stage



From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, *et al.* The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71. For more information, visit: http://www.prisma-statement.org/

Figure 1 Flow diagram of the screening and selection process for the included studies.

Table 2 Quality assessment of eligible studies

First author, year	Representativeness of exposed cohort	Selection of non-exposed cohort	Ascertainment of exposure	Demonstration that outcome was not present at start of study	Comparability based on the design or analysis	Assessment of outcome	Follow-up long enough for outcomes to occur	Adequacy of follow-up of cohorts	Total NOS score
Ide *et al.*, 2007	*	*		*	*	*	*	*	7
Sun *et al.*, 2007	*	*	*	*	*	*	*	*	8
Miyake *et al.* 2008	*	*	*	*	*		*	*	7
Zhang *et al.*, 2010	*	*		*	*	*	*	*	7
Zhao *et al.*, 2012	*	*	*	*			*	*	6
Zhu *et al.*, 2013	*	*	*	*	*	*	*	*	8
Matsuo *et al.*, 2014	*	*	*	*			*	*	6
Qin *et al.*, 2015	*	*	*	*	*	*	*	*	8
Leppanen *et al.*, 2018	*	*	*	*	*		*	*	7
Wang *et al.*, 2019	*	*	*	*	*		*	*	7
Wang *et al.*, 2019	*	*	*	*	*			*	6

A study can be awarded a maximum of one star for each numbered item within the selection and outcome categories. A maximum of two stars can be given for comparability. NOS, New Ottawa Scale.

or with positive LNM status. The results showed that high expression of HIF-1α was associated with poor OS compared to low HIF-1α (HR =1.82, 95% CI: 1.48–2.25, P<0.001; *Figure 4*) when the patients is at advance clinical stage or when the patients have positive LNM status (N1).

Assessment of publication bias

We evaluated publication bias among included studies through the Begg's test and Egger's test. We found no obvious publication bias for the meta-analysis of the association between expression of HIF-1α and OS (Begg test, P=0.1035, *Figure 5*); Egger test, P=0.568, 95% CI: −3.0177 to 5.1352).

Differential expression of HIF1A between pancreatic adenocarcinomas and normal pancreas

We analyzed the differential expression of HIF1A between PAAD and normal pancreas through GEPIA webserver. We generated boxplots distribution of the expression of mRNA for HIF1A in 179 pancreatic adenocarcinomas (PAADs) and 171 normal pancreatic tissues. According to *Figure 6*, the expression of HIF1A is significantly higher in PAADs than in normal pancreatic (Log_2FC =2, P<0.01).

Discussion

We conducted a meta-analysis of 764 patients included

Table 3 Characteristics of included studies

First author, year	Country	Follow-up	Detection method	Patients HIF-1α high)/(low)	High HIF-1α definition	Mean/ median age	Quality score	HR	Survival analysis	Staging system
Ide, 2007	Japan	1994/1–2005/4	IHC	15/26	>10%	63	7	Estimated	DFS	JPS (2nd edition)
Sun, 2007	China	1993/4–2005/2	IHC	26/32	4–7	60.66	8	Reported in text	OS	AJCC (5th edition)
Miyake, 2008	Japan	1994/3–2004/3	IHC	16/23	>10	64.4	7	Reported in text	OS	JPS (2nd edition)
Zhang, 2010	China	2007/1–2008/12	IHC	46/19	4–7	52	7	Estimated	OS	AJCC (6th edition)
Zhao, 2012	China	1997/7–2010/4	IHC	60/35	4–9	Not stated	6	Estimated	OS	AJCC (6th edition)
Zhu, 2013	China	2000/1–2009/12	IHC	27/36	4–7	Not stated	8	Reported in text	OS	AJCC (6th edition)
Matsuo, 2014	Japan	Not stated	IHC	55/45	2–4	65.1	7	Estimated	OS	AJCC (6th edition)
Qin, 2015	USA	1985–2001	IHC	45/55	>40%	63.1	8	Reported in text	OS	AJCC (6th edition)
Leppanen, 2018	Finland	1993–2011	IHC	28/36	>2.167	66	7	Reported in text	OS	AJCC (6th edition)
Wang, 2019	China	2010–2013	IHC	31/38	4–9	Not stated	7	Estimated	OS	AJCC (6th edition)
Wang, 2019	China	Not stated	IHC	43/27	4–9	59	6	Not stated	Not stated	AJCC (6th edition)

HR, hazard ratio, DFS, disease free survival; OS, overall survival; IHC, immunohistochemistry; JPS, Japan Pancreas Society; AJCC, American Joint Committee on Cancer.

in 11 studies (16,17,19,26-33), and found that PDAC patients with high expression of HIF-1α had significantly poorer survival than those with low expression. In global, the probability of death of patients with high expression of HIF-1α is 1.74 times more than patients with low expression of HIF-1α. From the 11 studies, one did not have sufficient data to estimate the HR (26), and only one had a HR less than 1 (16), thus generating a slight heterogeneity. The retrospective nature of that study, the sample size, and low number of T4 (clinical stage IV) cases may have contributed to this discrepancy (16). It is important to mention that, the study (16), was done on the European continent. This leads us to wonder about the influence that race could have on HIF-1α expression. Indeed, the level of HIF-1α expression differs among individuals according to the presence or absence of C1772T (C→T mutation) or G1790A (G→A mutation), the region of transcriptional activity and of the binding site to VHL protein (34). Subgroup analyses showed that HIF-1α expression was an unfavorable prognostic factor in PDAC patients from China (26,28,30-32), and Japan (27,29,33) with zero heterogeneity.

The underlying mechanism by which HIF-1A could lead to poor prognosis in PDAC patients may be through transcription of genes involved in invasion, angiogenesis and metastasis (35). Hoffmann *et al.* demonstrated a positive correlation between expression for HIF-1α with platelet derived growth factor A (PDGFA), VEGF, and beta fibroblast growth factor (bFGF) to promote proliferation, invasion, angiogenesis and metastasis in PDAC (36). Hence, the results of the meta-analysis we conducted on 474 patients included in 8 studies confirmed a positive correlation between expression of HIF-1α and the presence of metastatic lymph nodes in PDAC. It could be understood since increased HIF-1α can promote tumor growth and enhance the migration capacity of tumor cells by the activation of small GTPases and the induction of fibroblast phenotypes (37). The positive correlation of the expression of HIF-1α with clinical stage means that of HIF-1α antagonists could be used to prevent metastasis and progression to advanced stages of cancer. Indeed, the 8th AJCC staging manual states that expression of HIF-1α and clinical stage in cancer are closely related (38). To evaluate

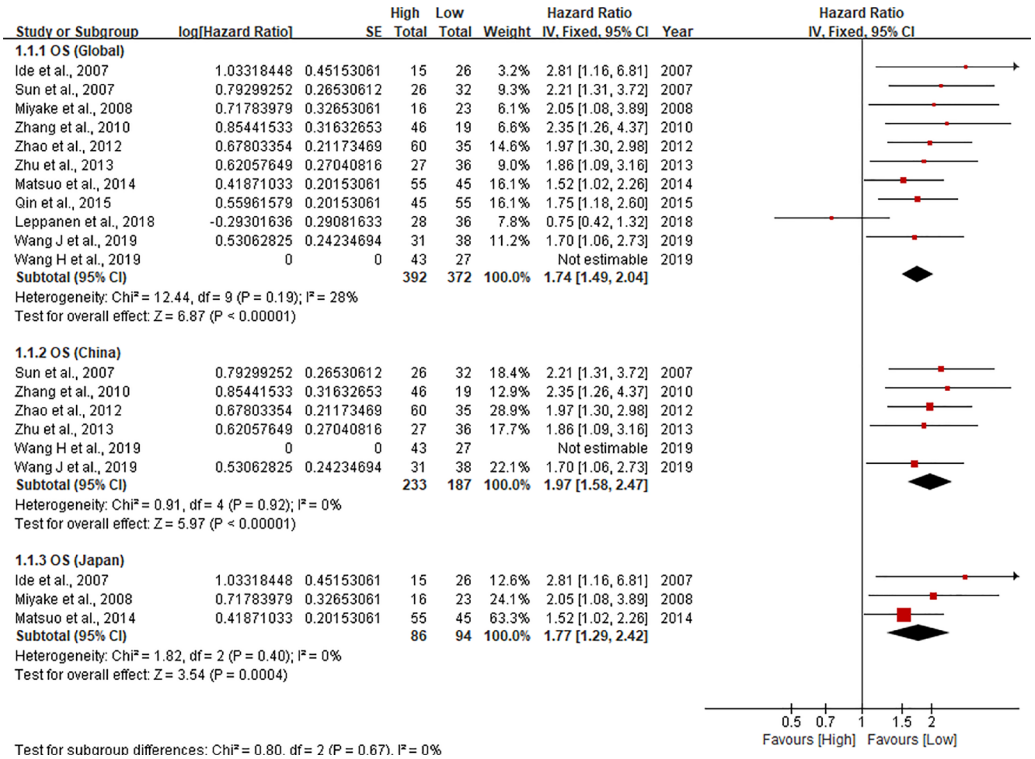


Figure 2 Forest plot of comparison: high expression of HIF-1α *vs.* low expression of HIF-1α in PDAC, outcome: OS. HIF-1α, hypoxia-inducible factor 1-alpha; PDAC, pancreatic ductal adenocarcinomas; OS, overall survival.

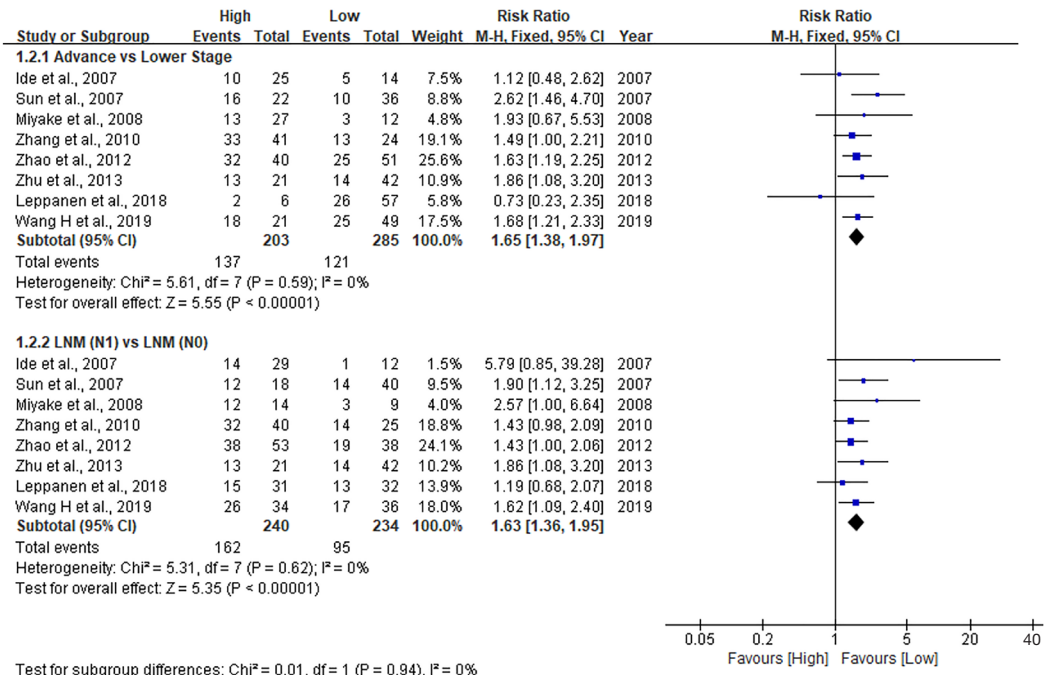


Figure 3 Forest plot of comparison: association of high expression of HIF-1α with clinical stage and LNM in PDAC. N1 = Presence of LNM, N0 = absence of LNM. HIF-1α, hypoxia-inducible factor 1-alpha; LNM, lymph node metastasis; PDAC, pancreatic ductal adenocarcinomas.

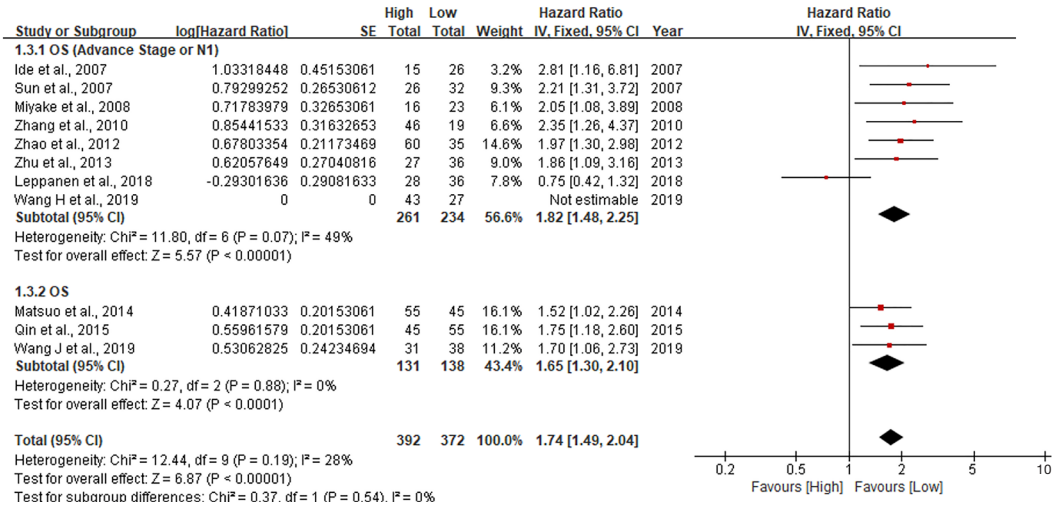


Figure 4 Forest plot of comparison: prognostic power of high expression of HIF-1α in PDAC patients with advance stage or positive metastatic lymph nodes (N1), outcome: OS. HIF-1α, hypoxia-inducible factor 1-alpha; PDAC, pancreatic ductal adenocarcinomas; OS, overall survival.

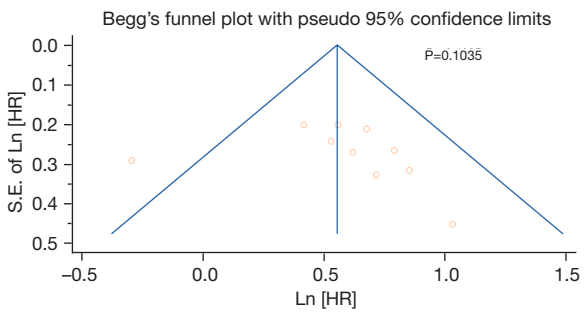


Figure 5 Publication bias among studies: association between expression of HIF-1α and global OS. HIF-1α, hypoxia-inducible factor 1-alpha; HR, hazard ratio, S.E, Standard Error of HR; OS, overall survival.

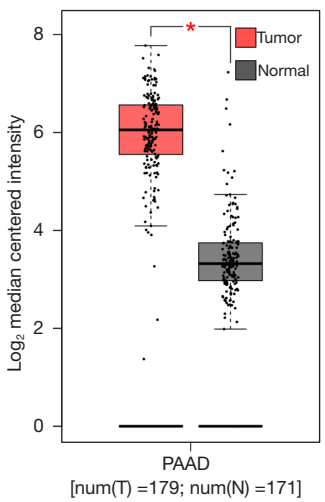


Figure 6 Box plots distribution of the expression of mRNA for HIF1A in 179 PAADs and 171 normal pancreatic tissues. Graph production: GEPIA (http://gepia.cancer-pku.cn); Log_2FC =2, *, P<0.01. PAAD, pancreatic adenocarcinomas.

the prognostic power of clinicopathological parameters (clinical stage and metastatic lymph nodes) on survival, we performed subgroup analysis on patients with high expression of HIF-1α and advance clinical stage. The results showed that (*Figure 4*), the probability of death in patients with advance clinical stage or patients with metastatic lymph node (N1) is 1.82 times more than patients at lower stage or without LNM (N0). No significant association of expression of HIF-1α with tumor size (14,26-28,30). This could be due to the fact that each study defined its own limits of dimension. For some it was two centimeters (cm), for others three or even four. Therefore, it was difficult to group them together.

Chemotherapy and immunotherapy have poor success in PDAC because of tumor microenvironment, and hypoxia is a common feature in solid cancer microenvironment like PDAC (5). We did the meta-analysis using protein expression data; then we also need to verify at mRNA level the differential expression of HIF1A in PDAC and normal pancreas despite the fact that, there is somehow an intermediate correlation (medium consistency) between

mRNA levels of HIF-1α and protein levels in PDAC (39). For the purpose, GEPIA database is a famous webserver used for gene profiling and integrative analysis, and cited in high quality papers (40-42). The differential analysis of HIF1A expression through GEPIA webserver showed a significant difference in the expression of HIF1A between PDAC and normal pancreas. High expression of HIF1A in PDAC emphasizes the role of HIF1A in PDAC development and progress. Indeed, HIF-1α is considered as the main activator of VEGF under hypoxia (6), and angiogenesis is a preliminary step for metastasis and cancer progress (43). Leppänen *et al.* also demonstrated high HIF-1α expression in 35 of 64 (54.68%) normal pancreatic tissues compared to 61 of 65 (93.84%) PDACs cases (16).

In conclusion, the present study suggested that high expression of HIF-1α may be considered as a predictive biomarker for advance clinical stage, positive metastatic lymph nodes and poor OS in PDACs. Although no significant evidence of publication bias was detected from the funnel plots, this work may have limitations like: continent and country under-representativeness, inter-study heterogeneity as well as limited number of patients, community-based studies. Also, it is a literature-based analysis. Publication bias may result in as predominantly positive results were reported. However, we anticipate more studies from other regions such as Africa, Europe and America to assess the generalizability of our conclusions.

Acknowledgments

Funding: This work was supported by grants from the National Natural Science Foundation of China (No. 81560422), the Natural Science Foundation of Jiangxi of China (No. 20151BAB205016), the Key Project of Science and Technology from Department of Education of Jiangxi Province (No. GJJ190787) and the Innovation Project of Gannan Medical University (No. TD2-1703).

Footnote

Reporting Checklist: The authors have completed the PRISMA reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-787/rc

Peer Review File: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-787/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-787/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Cite this article as: Zoa A, Yang Y, Huang W, Yang J, Wang J, Wang H, Dong M, Tian Y. High expression of hypoxia-inducible factor 1-alpha predicts poor prognosis in pancreatic ductal adenocarcinoma: a meta-analysis and database validation protocol. Transl Cancer Res 2022;11(9):3080-3091. doi: 10.21037/tcr-22-787

