Prognostic Value of Hypoxia-Inducible Factor 1α in Solid Malignancies: A Meta-Analysis

Yuewen Qi¹, Shuai Zhang¹,², Zhe Sun¹, Xiaolu Fang¹, Hong Yu¹,²

¹Department of Pathogen Biology, Qingdao University, Qingdao 266001, Shandong, China
²Clinical Laboratory, Jinan minzu hospital, Jinan250000, Shandong, China

Abstract: Numerous studies have reported the prognostic role of Hypoxia-Inducible Factor 1α (HIF-1α) and solid malignancies, but the data of different reports remains controversial. To accurately evaluate the prognostic value of HIF-1α expression in solid malignancies, a meta-analysis based on published studies was conducted. Relevant studies from 34 observational articles with 3828 patients were collected via PubMed, Embase. These studies were chosen for the meta-analysis based on requisite criteria and the quality was evaluated using the Newcastle Ottawa Quality Assessment Scale. Hazard ratios (HRs) and 95% confidence interval (CIs) were pooled from studies on overall survival (OS) and disease-free survival (DFS) to estimate the association. The pooled HRs (95% CIs) of HIF-1α for OS and DFS were 1.94 [1.74, 2.16] and 2.16 [1.71, 2.74], respectively. The overall data of the shown meta-analysis suggested that the high expression of HIF-1α is correlated with poor survival outcome in solid malignancies.

Keywords: Hypoxia-Inducible Factor 1α, Prognostic, Solid Malignancies, Meta-Analysis

Introduction
Accumulating evidence shows that hypoxia is a common trait of solid malignancies, and tumors outgrow their own vasculature scale out a certain size could lead to hypoxia. Hypoxia-inducible factor 1 (HIF-1), a heterodimer composing of hypoxia-inducible factor 1α and hypoxia-inducible factor 1β subunits, is a major transcription factor regulating the tumorigenic response to hypoxia in micro-environmental oxygenation [1, 2]. HIF-1α has been found to be up-regulated in many types of human malignancies, leading to a more aggressive phenotype with increased proliferation, invasiveness, metastases, epithelial-mesenchymal transition and stem-cell maintenance [3-5]. The expression of HIF-1α is regulated by the oxygen level, whereas the β subunit is constitutively expressed. Under hypoxic conditions, the degradation of HIF-1α is suppressed and the expression of HIF-1α would increase [1, 6]. HIF-1α could bind to hypoxia response elements (HRE) in the promoter of target genes and activate their expressions to mediate adaptive responses to decreased oxygen concentration, such as the formation of new blood vessels via proliferation and migration of endothelial cells toward tumor developing. However, in normoxic conditions, HIF-1α would be degraded due to targeted ubiquitination and degradation by the proteasome[1, 6].

Plenty of studies revealed that elevated HIF-1α expression in solid malignancies tissue was correlated with poor survival of patients including lung cancer, colorectal cancer, hepatocellular
carcinoma and some other malignancies. However, the results are controversial or inconclusive. This discrepancy is mostly owing to the genuine heterogeneity, relatively small sample size or different detecting methods. Therefore, we conducted this meta-analysis involving available evidences to estimate the prognostic value of HIF-1α expression in solid malignancies. We also evaluated whether the relevance between HIF-1α expression and outcome of patients is different between malignancy types, thereby it’s necessary to comprise more clearly on the development of therapy and prognostic verification through biological target of HIF-1α in solid malignancies.

Materials and Methods
Search strategy and study selection
To get access to potentially eligible studies, we conducted an thorough electronic search for relevant studies using PubMed, Embase (up to August, 2017), the following terms were applied by “HIF-1α OR hypoxia-inducible factor-1α” (all fields) AND cancer OR neoplasm OR carcinoma OR malignancy” (all fields) AND “prognosis OR prognostic OR survival OR outcome” (all fields), and we initially identified 4237 studies for further examination. The reference lists of relevant articles were searched manually as well.

Publications were considered eligible in our quantitative meta-analysis when they met all of the following criteria: ① articles published before SEP. 2017; ② articles provided sufficient data to appraise the hazard ratio (HR) and 95% confidence intervals (CI); ③ a minimal follow-up duration of 3 years; ④ a minimal sample-size of 50 participants; ⑤ the diagnosis of solid malignancy was histologically and pathologically confirmed; ⑥ immunohistochemistry(IHC) method was performed to detect the expression of HIF-1α. Publications were excluded based on the following criteria: ① duplicate publications; ② studies lack of usable statistical data to calculate log hazard ratio (logHR) and SE; ③ review articles or case reports; ④ non-human studies or letters; and ⑤ articles based on the Geo database[7]. Abstracts and full texts were reviewed for all searched papers, reference list were also searched for potentially eligible studies based on the above criteria. Two independent reviewers (ZS, SZ) evaluated titles and abstracts eligibility and excluded those were considered irrelevant. Disagreements were resolved by discussion.

Data extraction
The primary interest data was Overall survival (OS) ratio and disease free survival (DFS) ratio of HIF-1α in solid malignancies, including hazard ratio (HR) and 95% confidence interval (CI), or the Kaplan–Meier survival curves with log-rank p value. Additional data extracted from the studies included first author, publication year, country or region of publication, cancer type, case of patients, male/female, TNM stage, number of HIF-1α over-expression patients and controls, follow-up times, outcome endpoints, analysis model, NOS score.

If a study reported both the results of univariate and multivariate analysis, only the latter was selected.

The studies enrolled in this meta-analysis were all cohort studies. Newcastle-Ottawa Scale (NOS) was applied to evaluate the quality of each included study. Scores ranged from 0 (lowest) to 9 (highest) to assess the quality of article, and studies with scores of 6 or more were regarded as high quality. A consensus NOS score for each item was achieved.

Statistical analysis
Data were extracted from the primary articles and meta-analysis was calculated with the use of RevMan5.3 software (Cochrane Collaboration, http://community.cochrane.org/tools/review-production-tools/revman-5/revman-5-download). Hazard ratios (HR) and their 95% CI were used for analysis of the effects of HIF-1α expression on prognosis. Outcome endpoints were divided into two groups, OS and DFS, according to the data acquired in the current or previous studies. We extracted the statistical variables directly when data were described...
in the study. Otherwise, calculated from available numerical data in the articles based the methods described by Tierney [8]. The data from Kaplan-Meier survival curves were read by Engauge Digitizer version 4.1, and two researchers read the curves to reduce reading variability independently.

Heterogeneity among studies was determined by Chi square-based Q test and I² statistics. P value higher than 0.05 for the Q test and I² value less than 50% were considered to be of no noteworthy heterogeneity, the fixed effects model was adopted (P > 0.05; I² < 50%); If not, the random effects model was applied. The publication bias was evaluated by funnel plot. All the statistical tests were two-sided, and P < 0.05 was considered as statistically significant publication bias.

Results

Eligible studies

The initial search yielded 4237 references were retrieved from PubMed and Embase databases using the described searching strategy after duplicates were excluded. 392 articles were review among those literatures. After screening the titles and abstracts, 3760 studies were removed. Then 96 articles were further removed after full texts reading according to the criteria. Finally, 34 eligible articles including 3828 patients containing survival outcomes were eligible for our meta-analysis. The selection flow chart was shown in Figure 1.

Figure 1: The steps for screening eligible publications for the meta-analysis.
Demographic characteristics of included studies
These enrolled studies were published from 2004 to 2015. The detailed clinical characteristics of patients and other publication information have been extracted in Table 1.

Table 1. Baseline characteristics of all enrolled studies in the meta-analysis

| Reference | Country | Cancer type | cases | male/female | TNM stage | HIF-1α (+/-)N.O. | Follow-up times | survival outcome | Multivariate analysis | NO S score |
|-----------|---------|-------------|-------|-------------|-----------|------------------|----------------|------------------|---------------------|------------|
| Malfftone 2012 [9] | Italy | Breast cancer | 156 | All female | NA | 58/98 | NA | OS | Yes | 7 |
| Isobe 2013 [10] | Japan | Gastric cancer | 128 | 91/39 | NA | 84/44 | 60 | OS | Yes | 8 |
| Zhan 2013 [11] | China | Gastric cancer | 60 | 38/22 | 1-IV | 35/25 | 100 | OS | No | 8 |
| Lu 2013 [12] | China | Gastric cancer | 68 | 43/25 | 1-IV | 36/22 | 80 | OS | Yes | 6 |
| Kolev 2008 [13] | Japan | Gastric cancer | 152 | 110/42 | 1-IV | 95/57 | 60 | OS+DFS | Yes | 7 |
| Qiu 2010 [14] | China | Gastric cancer | 188 | 127/61 | 1-IV | 78/110 | 120 | OS | Yes | 8 |
| Kim 2013 [15] | Korea | Cervical cancer | 151 | All female | NA | 60/91 | 60 | OS+DFS | Yes | 6 |
| Yu 2012 [16] | China | Ovarian cancer | 117 | All female | I-IV | 59/58 | 100 | OS | Yes | 7 |
| Shimogai 2008 [17] | Japan | Ovarian cancer | 66 | All female | I-IV | 11/55 | 120 | OS+DFS | Yes | 7 |
| Huang 2014 [18] | China | Cervical cancer | 74 | All female | NA | 16/58 | 68 | OS+DFS | Yes | 7 |
| Ogane 2010 [19] | Japan | ESCC | 96 | 89/7 | NA | 65/31 | 180 | OS+DFS | Yes | 7 |
| Chen 2008 [20] | China | ESCC | 54 | 38/16 | 1-IV | 25/29 | 60 | OS | No | 7 |
| Tzao 2008 [21] | China | ESCC | 85 | 80/5 | 1-IV | 52/33 | 70 | OS | Yes | 7 |
| Lee 2012 [22] | Korea | SCLC | 111 | 93/18 | NA | 71/40 | 60 | OS | Yes | 6 |
| Hung 2009 [23] | China | NSCLC | 87 | 69/18 | 1-IV | 28/59 | 50 | OS | Yes | 7 |
| Kaira 2011 [24] | Japan | NSCLC | 126 | 81/45 | 1-III | 101/25 | 60 | OS | Yes | 7 |
| Park 2011 | Korea | NSCLC | 178 | 130/48 | 1-IV | 80/98 | 100 | OS | Yes | 6 |
In the stratified survival outcomes

Thirty-three studies, including 3778 patients, provided eligible data for OS analysis. Figure 2 showed that the pooled HR (95% CI) of these studies for OS was 1.94 [1.74, 2.16] (P < 0.00001; P = 32%). In the stratified analysis of malignancy type, high levels of HIF-1α was an unfavorable predictor of OS with Gastric cancer (pooled HR = 1.61, 95%CI = 1.34-1.95; P < 0.00001; P = 0%), HCC (pooled HR = 1.61, 95%CI = 1.34-1.95; P < 0.00001; P = 0%), lung cancer (pooled HR = 2.63, 95%CI = 1.78-3.89; P < 0.00001; P = 0%), Pancreatic Cancer (pooled HR = 1.95; P < 0.00001; P = 0%), and Nasopharyngeal cancer (pooled HR = 1.95; P < 0.00001; P = 0%).
1.77, 95%CI = 1.03-3.06; P = 0.04; P = 0%), colorectal cancer (pooled HR = 1.83, 95%CI = 1.10-3.06; P = 0.02; P = 0%), ESCC (pooled HR = 1.95, 95%CI = 1.27-2.97; P = 0.002; P = 0%), cervical cancer (pooled HR = 2.41, 95%CI = 1.25-4.63; P = 0.009; P = 0%), ovarian cancer (pooled HR = 3.91, 95%CI = 2.68-5.69; P < 0.00001; P = 0%), and other malignancies (pooled HR = 2.18, 95%CI = 1.52-3.14; P < 0.00001; P = 32%) including breast cancer, tongue Carcinoma, Osteosarcoma, Soft tissue Sarcoma, Gallbladder Carcinoma, Oral squamous cell carcinoma and Oropharyngeal Cancer. However, over-expression of HIF-1α in gastric cancer (pooled HR = 1.73, 95%CI = 0.99-3.02; P = 0.05; P = 64%) failed to affected OS for patients statistically. All above meta-analysis results were reviewed in Table 2

Nine studies, including 874 patients, provided eligible data for DFS analysis. Figure3 showed that the pooled HR (95% CI) of these studies for DFS was 2.16 [1.71, 2.74] (P < 0.00001; P = 4%).

Figure 2: Meta-analysis of overall survival of HIF-1α expression in solid malignancies.
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Table 2: Pooled hazard ratios for OS based on malignancy type

| Malignancy type      | number | HR(95% CI)     | P value | heterogeneity (I^2 %) |
|----------------------|--------|----------------|---------|-----------------------|
| Overall survival     | 33     | 1.94 [1.74, 2.16] | < 0.00001 | 32%                   |
| Gastric cancer       | 5      | 1.70 [1.27, 2.28] | 0.05    | 64%                   |
| HCC                  | 4      | 1.61 [1.34, 1.95] | < 0.00001 | 0%                    |
| Lung Cancer          | 4      | 2.63 [1.78, 3.89] | < 0.00001 | 0%                    |
| Pancreatic Cancer    | 3      | 1.77 [1.03, 3.06] | 0.04    | 0%                    |
| Colorectal Cancer    | 3      | 1.83 [1.10, 3.06] | 0.02    | 0%                    |
| ESCC                 | 3      | 1.95 [1.29, 2.97] | 0.002   | 0%                    |
| Cervical cancer      | 2      | 2.41 [1.25, 4.63] | 0.009   | 0%                    |
| Ovarian cancer       | 2      | 3.91 [2.68, 5.69] | < 0.00001 | 0%                    |
| Others               | 7      | 1.90 [1.47, 2.46] | < 0.00001 | 27%                   |

Sensitivity analysis

Eliminating studies about gastric cancer (n = 29, pooled HR = 1.98, 95%CI = 1.76-2.23; P < 0.00001; I^2 =23%) had no substantial effect on the outcome of overall survival, and a lower heterogeneity was consistently observed.

Removal of studies that scored 6 on the NOS scale (n =29, pooled HR = 1.96, 95%CI = 1.74-2.21; P < 0.00001; I^2 =23%) did not change the unfavorable prognostic effect of HIF-1α over-expression on the overall survival in patients with solid malignancies.

Potential publication bias

Funnel plots was adopted to visualize the publication bias. (Figure 4)
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Figure 4: Funnel plot to assess the potential publication bias in the impact of HIF-1α on overall survival (A) and disease-free survival (B)

Discussion
HIF1α plays a critical role in hypoxia to improve glycolysis, oxygen delivery, and angiogenesis for tumor cells [43]. Recent studies have demonstrated that HIF1α is over-expressed in various human malignancies and intimately correlates with tumorigenesis, tumor progression, metastasis potential, treatment failure and increased mortality in many malignancies [44-46]. Dysregulation of HIF1α expression and HIF1α signal pathway in tumor microenvironment may serve as a key factor in human cancer development [46, 47]. In view of its role in regulating tumor pathophysiology, evaluating its prognostic value in malignancies is of great clinical importance, which may lead to better patient stratification and targeted therapies in the future.

The prognostic role of HIF-1α expression has been proven in the recent meta-analysis in gastric cancer [48], hepatocellular carcinoma [49], lung cancer [50] and some other tumors. As far as we know, this study was the most well-rounded assessment of the studies towards HIF-1α expression and malignancies prognosis to date. Systematically, we evaluated survival data for 3778 patients included in 34 studies containing 15 different solid malignancies. In this meta analysis, the pooled HRs (95% CI) of HIF-1α expression for OS and DFS were 1.94 [1.74, 2.16] and 2.16 [1.71, 2.74] respectively. The combined results of OS and DFS suggested that high expressions of HIF-1α were obviously related with poor survival outcome, which indicates that that the expression of HIF-1α could be deemed as a potential biomarker of poor prognosis in most patients with solid malignancies. As for malignancy types, the high expression of HIF-1α in malignancy tissues were associated with worse OS of most human solid malignancies, such as HCC, lung cancer, ovarian cancer, pancreatic cancer and some other malignancies. Statistically, we failed to draw the correlation between HIF-1α expression and overall survival of gastric cancer.

This meta-analysis also have some limitations. Firstly, we could only extract the data of summarized-population level rather than individual-patient level. Secondly, the HRs of some studies was calculate based on the Kaplan-Meier survival curve and p-value, these data were less receivable compared to those from the original article directly. Besides, we used the software designed by Matthew Sydes and Jayne Tierney [8] to calculate the logHR and SE, which reserved only percentile. Thirdly, we barely analyzed the relation between HIF-1α over-expression and patient survival in the respect of clinical parameters. Fourthly, the vast majority of included studies was Asian ethnicity, thus the prognosis role of HIF-1α in Caucasians might be
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less convincible. Lastly, there were too many literatures about the prognosis of HIF-1α and human malignancies, we failed to spread them all, which may lead to publication bias or even inaccurate results.

In conclusion, over-expression of HIF-1α was correlated with poor survival in patients with solid malignancies. These findings suggested that HIF-1α inhibition therapy could be an important method in malignancies, and that HIF-1α might be routinely detected to predict prognosis in solid malignancies. However, more literatures need to be adopted for further verification of our results.

Disclosure of conflict of interest
None

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