RESEARCH ARTICLE

Prognostic Role of Hypoxic Inducible Factor Expression in Non-small Cell Lung Cancer: A Meta-analysis

Cong Li[1,3&], Hua-Jun Lu[1,3&], Fei-Fei Na[1,2,3], Lei Deng[1,2,3], Jian-Xin Xue[2], Jing-Wen Wang[1,2,3], Yu-Qing Wang[3], Qiao-Ling Li[3], You Lu[2*]

Abstract

Introduction: Reported prognostic roles of hypoxic inducible factor (HIF) expression in non-small cell lung cancer (NSCLC) have varied. This meta-analysis aimed to examine the relationship between HIF expression and clinical outcome in NSCLC patients. Methods: PubMed were used to identify relevant literature with the last report up to December 20th, 2012. After careful review, survival data were collected from eligible studies. We completed the meta-analysis using Stata statistical software (Version 11) and combined hazard ratio (HR) for overall survival (OS). Subgroup specificity, heterogeneity and publication bias were also assessed. All of the results were verified by two persons to ensure accuracy. Results: Eight studies were finally stepped into this meta-analysis in which seven had available data for HIF-1α and three for HIF-2α. Combined HRs suggested that higher expression of HIF1α had a negative impact on NSCLC patient survival (HR=1.50; 95%CI =1.07–2.10; *p*=0.019). The expression of HIF-2α was also relative to a poorer survival (HR=2.02; 95%CI =1.47–2.77; *p*=0.000). No bias existed in either of the two groups. Conclusion: This study suggests that elevations of HIF-1α and HIF-2α expression are both associated with poor outcome for patients with NSCLC. The data support further and high quality investigation of HIF expression for predicting poor outcome in patients with NSCLC.

Keywords: Hypoxic inducible factors - prognosis - non-small cell lung cancer - meta-analysis

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Introduction

Lung cancer is one of the most common human cancers and the leading cause of cancer-related deaths worldwide, and non-small cell lung cancer (NSCLC) constitutes 85% of all lung cancers (Wood et al., 2012). Despite advances have made in clinical and experimental oncology, the prognosis of lung cancer is still poor, with the five-year survival rates are only approximately 15.6%. The high mortality rate is largely due to late diagnosis, when treatment is difficult (Wood et al., 2012).

As the treatment of lung cancer is becoming more individualized, molecular targeted treatment plays an important role in cancer patients (Moldvay et al., 2012) and the finding of prognostic factor makes it possible. In addition, a good prognostic factor can predict clinical outcome and reveal therapeutic targets (Oldenhuis et al., 2008). In the resected NSCLC patients, the tumor-node-metastasis (TNM) staging system is the best prognostic factor (Chansky et al., 2009). However, each patient's outcome is different in the same TNM stage. In addition, other independent prognostic factors reported for survival in NSCLC patients have respective limitations. For example, low BMI, stage IV disease, anemia at diagnosis, and male gender are only related to poor prognosis of advanced NSCLC in young patients rather than all NSCLC patients (Hsu et al., 2012), circulating miR-125b and survivin have been identified that they are independent prognostic factor for NSCLC, but they still need further validation in a larger sample and prospective study (Ma et al., 2012; Zhang et al., 2012). Therefore, there is a need of better prognostic factors for new treatment opinions.

Hypoxia is a hallmark of solid cancer (Hanahan et al., 2012) and exists in resected NSCLC, which results from the structurally and functionally abnormal blood vessels and abnormal tumor perfusion in the tumor (Simon et al., 2007). When the hypoxic environment is induced, hypoxic inducible factors (HIFs) are activated and further activate transcription of a set of genes leading to tumor genesis and tumor aggressiveness (Harris et al., 2002; Rankin et al., 2008), including angiogenesis, proliferation, metabolism, metastasis, differentiation, and resistance to radiation therapy. The HIFs are composed of oxygen-regulated subunit HIFα and a constitutively expressed subunit HIFβ, and their regulation according to O_2 is thought of occurring on the α subunit: HIF1α, HIF2α and HIF3α (Ortiz-Barahona et al., 2010). Among them, HIF1α and HIF2α are most relevant and studied (Hu et al., 2003). A previous study of malignant and normal tissues shows that the expression of them are increased

*[1]Huaxi Student Society of Oncology Research (HASSOR), West China School of Medicine, Sichuan University, [2]Department of Thoracic Cancer, Cancer Center and State Key Laboratory of Biotherapy, West China Hospital, [3]West China School of Medicine, Sichuan University, Chengdu, Sichuan, China [&]Equal contributors *For correspondence: radyoulu@hotmail.com*

Table 1. Definitions of 18 Items of Study Reporting Quality

Study design
1. Objectives or prespecified hypothesis: state the study objectives, prespecified hypothesis or study protocol
2. Sample size: state a statistical sample size or power calculation
3. Follow-up description: state the follow-up period or the median follow-up time
4. Population source: state health care setting from which patients were recruited
5. Population selection criteria: state inclusion or exclusion
6. Population characteristics: state the population characteristics (e.g., age, gender, and disease stage)
7. Number of patients included in each stage of the analysis and reason for dropout: description of number of patients at different stage, including the number of patients who participate in the study, who met the inclusion criteria, and who followed up and reason for dropout
Assay method
1. Sample handling: state the method of storage
2. Assay method: state the type of assay method used to measure HIFs
3. Manufacturer: state the name of company which makes the assay for HIFs
4. Cutoff point determination: state methods used for cutoff point determination
Confounders
1. Conventional risk factors: state the conventional risk factors (e.g., age, gender, depth of tumor, lymph node metastasis)
2. Other biomarkers (e.g., p53, PCNA, and microvessel density): state other biologic marker relating with the disease
Outcome
1. Clinical endpoint: define the clinical endpoint
2. Validation: state the outcome events checked by independent source (e.g., medical records, outpatient visits, by letter, and by telephone)
Analysis
1. Univariate estimate: report the effect of HIFs on outcome
2. Multivariate estimate: adjusted for risk factors or other biomarkers (list above)
3. Missing value: state the number of patients with missing value for HIFs or confounders and how to deal with it

in many human tumors, including bladder, breast, colon, glial, hepatocellular, ovarian, pancreatic, prostate, and renal tumors (Talks et al., 2000). In addition, recent studies have shown overexpression of HIF-1α and HIF-2α indicates poor prognosis and implied that they are independent prognostic factor of many kinds of cancer including tumor of ovary, pancreas, head and neck, liver and so on (Giatromanolaki et al., 2001; Hui et al., 2002; Shibaji et al., 2003; Yoshimura et al., 2004; Winter et al., 2006; Bangoura et al., 2007; Osada et al., 2007).

The prognostic role of HIFs in NSCLC is variably reported (Giatromanolaki et al., 2001; Lee et al., 2003; Daniel et al., 2004; Hung et al., 2009; Park et al., 2010; Wei et al., 2011; Wu et al., 2011; Wu et al., 2011), so we systematized the available information to perform a meta-analysis of all the clinical trials about HIFs' expression of NSCLC, and further confirm HIFs' prognostic role in NSCLC.

Materials and Methods

Search strategy and eligible criteria

This is a meta-analysis of all the published cohort studies about HIFs' expression and its prognostic role in patients with NSCLC who underwent surgical resection of a tumor. We searched Pubmed for relevant literature updated to December 20th, 2012 using the strategy "hypoxic inducible factors" or "HIF" and "non-small cell lung cancer" or "NSCLC".

To complete the search, we also examined the reference lists from original and review articles. Blindness for patients was not necessary because we examined the surgery specimen and the results of study did not change even if patients knew the study. Conference Abstracts were ruled out because they lack insufficient data for meta-analysis. To avoid the duplication of data, when dealing with several publications about the same population, we selected the most recent and complete one.

To be eligible for inclusion, studies had to meet the following: (1) studied aimed at NSCLC, (2) studies measured the HIFs' expression using the immunohistochemical staining (IHC), (3) studies used surgically resected primary tumor samples but not body fluids such as sputum, pleural fluid and serum, (4) studies investigated the relationship between HIFs' expression and overall survival (OS) of patients in NSCLC, (5) case studies, review articles were excluded, (6) studies with cell lines were excluded, (7) studies with other prognosis indexes but not OS were excluded.

Data Extraction

We selected the following information from each eligible study: authors, publication year, source of patients, sample size, HIF expression evaluation, and tumor stage and survival information namely OS. OS was defined as the period from the study date to the date of death. If data from any of the above items were not given in the article, items were treated as "not given". All of these were done independently by two reviewers (HJ.L. and C.L.) and checked with each other.

Study Quality Control

We used Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) (Altman et al., 2012) and extract 18 items (Chen et al., 2012) (Table 1). Briefly, each item was scored by an ordinal scale

Table 2. Characteristic and Results of Eligible Prognostic Studies Evaluating NSCLC Surviving.

First author	Year	Source of patients	N. of patients	HIF expression Technique	Threshold	N. of positive	Stages	OS	HR estimate	HR	95%CIs	Study quality
HIF1a												
WU	2011	China	140	IHC	>2 vs ≤2 scores	49	I-III	U	HR	0.807	0.481-1.352	28
Park	2010	Korea	178	IHC	>10% vs ≤10% positive tumor cell	NG	I-IV	U+M	HR	1.869	0.808-4.329	32
Hung	2009	China	87	IHC	>50% vs ≤50% nuclear staining	28	I-IV	U	events	3.32	1.43-7.7	30
Swinson	2004	UK	172	IHC	>60% vs ≤60% positive tumor cell	101	I-IIIA	U+M	HR	2.05	1.23-2.44	29
Lee	2003	Korea	75	IHC	>2 vs ≤2 scores	38	I-III	U	events	0.81	0.47-1.39	29
Giatromanolaki	2001	UK	98	IHC	>2 vs ≤2 scores	63	I-II	U	events	1.64	0.94-2.87	29
WU SW	2011	China	160	IHC	>10% vs ≤10% positive tumor cell	78	I-IV	U	events	1.61	1.12-2.31	32
HIF2a												
WU	2011	China	140	IHC	>2 vs ≤2 scores	64	I-III	U+M	HR	1.714	1.036-2.836	30
Giatromanolaki	2001	UK	98	IHC	>2 vs ≤2 scores	49	I-II	U	events	2.06	1.21-3.51	29
Wei	2011	China	51	IHC	>10% vs ≤10% positive tumor cell	27	I-IV	U	events	2.55	1.35-4.82	27

N, number; HIF, hypoxic inducible factor; NSCLC, non-small cell lung cancer; IHC, immunohistochemistry; NG, not given; HR, hazard ratio; CI, confidence interval; U, univariate analysis; M, multivariate analysis

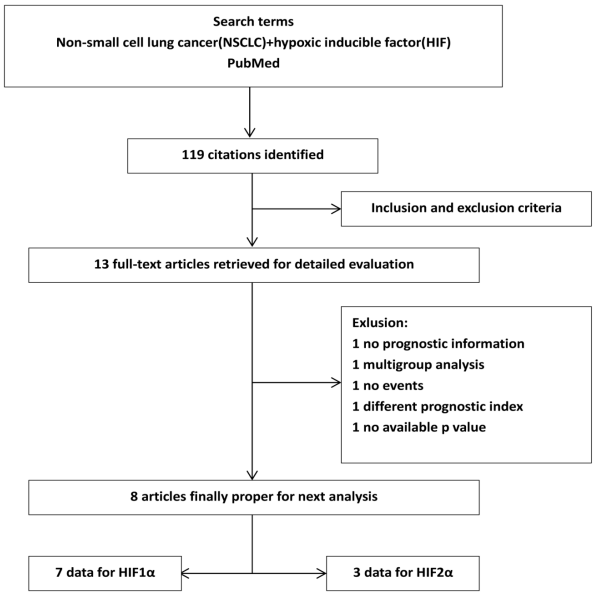

Figure 1. Flow Diagram of the Literature Search Strategy and Assessment of Studies Identified for Meta-analysis

(possible values 2, 1, and 0) : 2 represented the complete description,1 represented partly matched description, 0 represented no matched description and the maximum score was 36. The quality of studies was better with higher value.

Statistical analysis

Meta-analysis was performed with Stata statistical software Version 11.0 software (Stata Corporation, College Station, TX). Hazard ratios (HR) with 95% confidence interval (95%CI) were expressed for OS. The inter-study heterogeneity was assessed with the Cochran Q test for statistical significance and also described with I square for the amount of heterogeneity (Dinnes et al., 2005) (a=0.05). If there was no heterogeneity, fixed effects model (Mantel-Haenszel method) was used; otherwise, a random effect model was used according to the DerSimonian-Laird method (Dersimonian et al., 2007). Publishing bias was tested by using the funnel plot.

In some studies, HR and 95% CI can be obtained from papers by using multivariate survival analysis directly (Daniel et al., 2004; Park et al., 2010; Wu et al., 2011).

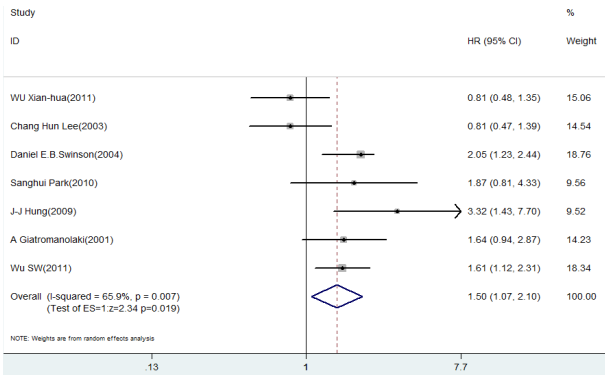

Figure 2. The Association Between HIF1α Expression and Overall Survival of NSCLC Stratified by HR Estimation

If the HR was not given directly, we calculated the HR from P-value and total events according to the methods described by Tierney et al. (2007). The final combination of HR was the effect value to show the prognostic significance.

Results

Study Identification and Quality

A total of 119 potentially relevant studies were retrieved electronically, but 106 of them were excluded from analysis after the first screening based on abstracts or titles, leaving 13 available for further full text review. After reading the full text articles carefully, 5 studies were excluded because of deficiency of sufficient data (Figure 1). As a result, 8 studies were finally stepped into this meta-analysis in which 7 had available data for HIF-1α and 3 for HIF-2α. Different subtypes of HIF existed heterogeneity in detection and expression, so we will discuss their prognostic value respectively.

The results of quality assessment of included studies are shown in the Table 2. Quality scores ranged from 27 to 32 with median value of 29, all the studies satisfied most of the items and reported total of the assay method and confounders. In addition, all of them attempted to look for other important prognostic factors which may be related to outcome of patients in NSCLC. The worst described items were validation of outcome and multivariate statistical analysis. And no studies referred to missing value.

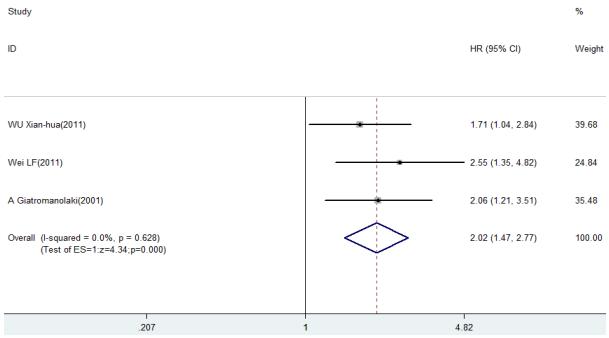

Figure 3. The Association Between HIF2α Expression and Overall Survival of NSCLC Stratified by HR Estimation

Table 3. Sub-group Analysis

Sub-group	Studies(N)	Q test *P* value	Model seclected	HR(95% CI)	*P*
Source of patients					
From Asia	5	0.011	random	1.362(0.854,2.170)	0.195
From European	2	0.504	fixed	1.929(1.440,2.582)	0
Cox model					
U	5	0.012	random	1.345(0.874,2.070)	0.177
U+M	2	0.842	fixed	2.023(1.473,2.778)	0
HR estimate					
Direct extraction	3	0.012	random	1.448(0.755,2.777)	0.265
Events	4	0.035	random	1.535(0.966,2.439)	0.07

Characteristics of the included studies

The basic characteristics of the studies are summarized in Table 2. All the studies were published from 2001 to 2011 with the study sample sizes ranging from 51 to 178. HIF-1α and HIF-2α were both detected by IHC but antibodies varied. In the 7 groups of HIF-1α, 3 of them indicated a significant positive prognostic value on OS, while the other 4 showed no statistically evidence. And in the data of HIF-2α, all 3 records had a prognostic effect of clinical outcome.

Meta-analysis of HIF for NSCLC

The analysis results of HIF-1α and HIF-2α are shown respectively in Figure 2 and Figure 3. HIF-1α overexpression was shown to be significantly associated with a poor outcome of NSCLC (HR=1.50; 95%CI =1.07-2.10; p=0.019). We used random-effect model to adjust it due to the extensive heterogeneity ($P_{heterogeneity}$ =0.007). The expression of HIF-2α was also relative to a poorer survival (HR=2.02; 95%CI =1.47-2.77; p=0.000) and showed a well homogeneity ($P_{heterogeneity}$ =0.628).

Due to the heterogeneity among the eligible studies about HIF-1α, we conducted the subgroup analyses stratified by source of patients, Cox model and HR estimate (Table 3). Only the group with patients from European (p=0.000) or Cox model of univariable and multivariable statistical analysis (p=0.000) was statistically significant and with well homogeneity.

Publication bias

Publication bias was assessed by funnel plot and Egger's test. The funnel plots for overexpression and low expression of HIF-1α were basically (Figure 4A) and Egger's test did not indicate asymmetry of the plot (P = 0.905). HIF-2α in the same bias tests also showed

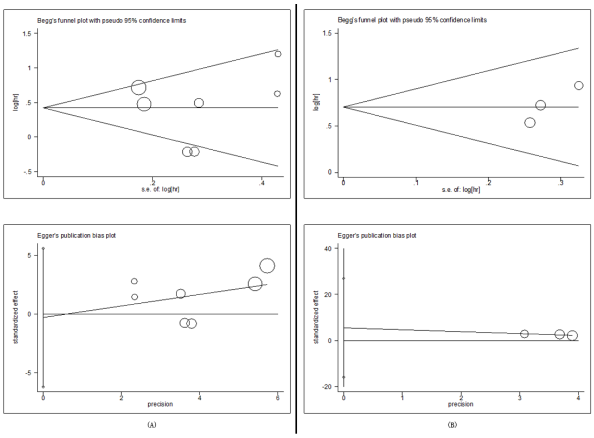

Figure 4. Funnel Plots of Begg's and Egger's were Used to Detect Publication Bias on Overall Estimate. Studies are distributed symmetrically above and below the horizontal line, and suggest that the meta-analysis is absence of publication bias. (A)This figure is about HIF1α. (B) This figure is about HIF2α

symmetric characteristic (Figure 4B). These results showed the meta-analysis was absence of publication bias in both groups.

Discussion

HIFs commonly exist in tumors and play a role in the tumor formation, progression and metastasis by activating genes which are related to regulation of angiogenesis (Giatromanolaki et al., 2003), cell survival and metabolism (Koppenol et al., 2011). Therefore, HIFs may be related to prognosis of cancer patients.

Previous studies shows that overexpression of HIF-1α is related to poor outcome in head and neck cancer, nasopharyngeal carcinoma, colorectal, pancreatic cancer, NSCLC (Giatromanolaki et al., 2001; Hui et al., 2002; Shibaji et al., 2003; Yoshimura et al., 2004; Winter et al., 2006; Osada et al., 2007) and so on. Meanwhile, overexpression of HIF-2α is related to poor outcome in colorectal carcinoma, hepatocellular, melanoma, and NSCLC (Giatromanolaki et al., 2001; Giatromanolaki et al., 2003; Yoshimura et al., 2004; Bangoura et al., 2007). A meta-analysis of HIF-1α gene polymorphisms and cancer risk has been conducted (Mottet et al., 2003), but no meta-analysis has been conducted for HIF-1α and HIF-2α for their prognostic role in patients with NSCLC. Therefore, our study aimed at finding the relationship with HIFs expression with NSCLC patients' outcome. We found that elevated HIFs expression including HIF-1α or HIF-2α correlate with poor patient outcome in NSCLC, which provide evidence for generating new treatment plan on NSCLC.

In our study, we find elevated HIF-1α and HIF-2α expression correlate with a poor outcome on NSCLC. But comparing with HIF-2α, HIF-1α's prognostic role seems a little disputable because of heterogeneity. In the groups of HIF-1α, 4 of them provide negative outcome that is to say the HRs of these studies correlating with p value lager than 0.05. However, all eligible studies in the group of HIF2α offer positive outcome with the same trend of risk. Although both of HIF-1α and HIF-2α are induced

by hypoxia, the condition of hypoxia they response to is different. Firstly, HIF-1α responses to acute and severe hypoxia while HIF-2α responses to chronic and moderate hypoxia (Uchida et al., 2004; Dersimonian et al., 2007). Destabilization of Hif-1α mRNA as a result of special antisense transcripts from the Hif1a (but not Epas1 which is related to HIF-2α) may explain the gradual reduction of HIF1α protein (Jackson et al., 2010). Therefore, the different results of HIF-1α's prognostic role could be explained by HIF-1α's down-regulation during tumor growth, whereas HIF-2α may gradually accumulate and increase (Löfstedt et al., 2007; Zhao et al., 2009). Moreover, in the hypoxia-regulated pathway HIF-1α has pro-tumorigenic and anti-tumorigenic properties contradictorily. On the one hand, HIF-1α directly or indirectly activates many target locus including vascular endothelial growth factor (VEGF), which promotes angiogenesis; glucose transporter 1 (GLUT1), which activates glucose transport; lactate dehydrogenase (LDH-A), which is involved in the glycolytic pathway; and erythropoietin (EPO), which induces erythropoiesis. All of above contribute to proliferation and regulation of tumor (Harris et al., 2002; Rankin et al., 2008). On the other hand, HIF-1α activates transcription of many pro-apoptotic proteins such as NIX and NIP3 and also promotes p53-dependent apoptosis (Harris et al., 2002). So the interaction of the conflicting effect may make HIF1-α's prognostic role be more uncertain and worth to discuss. In contrast, HIF-2α expression is more restricted, and particularly abundant in blood vessels. Despite the more prominent role in neovascularization, it regulates hypoxia-regulated genes such as c-Myc and p53, similarly to HIF1, but differs in detailed mechanism (Uchida et al., 2004). Previous in vitro and in vivo data implicating HIF-2α as an important pro-tumor factor is abundant with no controversy (Jackson et al., 2010; McKee et al., 2012).

Furthermore, according to the statistics knowledge, we find the heterogeneity might focus on these two studies made by Wu et al. (2011) and Lee et al. (2003). The results of these two studies showed a different trend of risk but not statistical significance. This difference may be explained by complex role of HIF-1α in tumorigenesis, the different method used to estimate the results and the different cut-off levels used. In addition, the method used by these two studies to estimate the results was univariable statistic analysis; Study of Lee et al. (2003) used *P*-value and total events to calculate HR, which can make some error due to variation of calculation model.

Nowadays, many studies about HIFs mechanism in tumor have already been conducted and their relationship has been confirmed. The critical role of the hypoxia response network and HIF has resulted in it being viewed as an ideal target for small molecule intervention. In recent years, small molecule inhibitors of HIF-1α are widely studied and highly desirable because of is central role in tumorigenesis. Generally, the inhibitors may inhibit HIF-1α by decreasing its protein levels, DNA-binding, or transactivation (Tang et al., 2013). For example, PX-478 (Koh et al., 2008) which can decrease HIF-1α protein stabilization, EZN-2968 (Greenberger et al., 2008) which inhibit expression of HIF-1α mRNA and echinomycin (Kong et al., 2005) which directly focus on DNA-HIF obstruction to decrease DNA binding are all reported in development. In addition, HIF-2α also gradually comes into people's sight as a considerable therapeutic target. McKee et al. (2012) and his partners tried to separate and identify small molecule inhibitors of HIF-2 gene expression through a high throughput screen (HTS), but the final compounds they identified are only discussed in vitro evaluation so that further development need to be conducted. Our data can provide a more sufficient evidence to ensure practical value of HIF inhibitors and promote relevant industries especially of HIF2.

However, the extent of these drugs acting in the patients and whether they affect both HIFs subunits are unknown (Uchida et al., 2004), which needs further investigation. Therefore, our results can confirm the meaning of HIFs inhibitors using in patients with NSCLC.

Certainly, this meta-analysis also has several limitations. First, the number of included studies and sample size are small. Second, most of studies are based on univariable statistical analysis, if the extracted information is from multivariable statistical analysis, this meta-analysis would be more precise. Third, because of data limitation, we can't conduct the subgroup analyses stratified by age, gender, smoking or other variables, which leads to lack of heterogeneity resource. All the limitations mentioned should affect our results. Therefore, more high-quality studies with sufficient information needs to be conducted, and lead to a more significant meta-analysis.

In conclusion, elevated HIF-1α and HIF-2α are both associated with poor outcome for patients with NSCLC. And it supports further and high quality investigations of HIFs expression for predicting poor outcome in patients with NSCLC.

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