The expression of hypoxia-inducible factor-1α and its clinical significance in lung cancer: a systematic review and meta-analysis

Weiwei Ren\textsuperscript{a,b}, Denghai Mi\textsuperscript{b,c,}, Kehu Yang\textsuperscript{a,b}, Nong Cao\textsuperscript{b}, Jinhui Tian\textsuperscript{a}, Zheng Li\textsuperscript{a,b}, Bin Ma\textsuperscript{a}

\textsuperscript{a}Evidence-Based Medicine Center of Lanzhou University, Gansu, China
\textsuperscript{b}The First Clinical Medicine College of Lanzhou University, Gansu, China
\textsuperscript{c}Gansu Provincial Second People’s Hospital, Lanzhou, Gansu, China

Summary

BACKGROUND: Hypoxia-inducible factor-1α (HIF-1α) plays an important role in tumour progression and metastasis through activation of many target genes that are especially involved in pivotal aspects of cancer biology. However, the prognostic role of HIF-1α has been controversial in primary patients with lung cancer. This meta-analysis was performed to systematically evaluate whether HIF-1α expression is associated with the clinical outcomes in lung cancer patients.

METHODS: We retrieved relevant articles from Cochrane library, PubMed, EMbase, CNKI, CBM, VIP and Wan Fang Databases from inception to May 2012. Studies were selected using specific inclusion and exclusion criteria. A systematic review and meta-analysis was performed on the association between HIF-1α expression and clinical outcomes in lung cancer patients. All analyses were performed using the Revman 5.1 software.

RESULTS: A total of 30 studies were identified as eligible for the systematic review and meta-analysis. The expression of HIF-1α was significantly higher than those in normal lung tissue; and III–IV stage, lymph node metastasis, poorly differentiation, squamous cell carcinoma and small cell lung cancer (SCLC) were significantly higher than those in I–II stage, no lymph node metastasis, well differentiation, adenocarcinomas and non small cell lung cancer (NSCLC), respectively (odds ratio (OR) = 19.00, 95% confidence interval (CI):12.12–29.78, \(p < 0.00001\); OR = 0.23, 95% CI:0.14–0.36, \(p < 0.00001\); OR = 3.72, 95% CI:2.38–5.80, \(p < 0.00001\); OR = 0.47, 95% CI:0.31–0.70, \(p < 0.00002\), OR = 0.24, 95% CI:0.07–0.77, \(p = 0.02\); OR = 0.78, 95% CI:0.63–0.98, \(p = 0.03\). VEGF and CA IX positive expression in HIF-1α positive tumour tissues were significantly higher than those in HIF-1α negative tumour tissues, respectively (OR = 3.23, 95% CI: 1.90–5.46, \(p < 0.00001\); OR = 3.84, 95% CI: 2.10–7.03, \(p < 0.00001\)). The positive HIF-1α tumour tissues of patients had lower 5-year survival rates (OR = 0.13, 95% CI: 0.03–0.47, \(p = 0.002\)) and overall survival (relative risk (RR) = 1.68, 95% CI: 1.12–2.50, \(p = 0.01\)).

CONCLUSIONS: HIF-1α is related to a differing degree of lung cancer cell, lymph node metastasis, post-operative survival time and histology (NSCLC vs. SCLC, adenocarcinomas vs. squamous cell carcinoma). HIF-1 α , which combines other proteins, such as vascular endothelial growth factor (VEGF) or CA IX, might serve as important parameters in evaluating biological behaviour and prognosis of lung cancer; it will be of benefit to clinical treatment and prognostic evaluation.

Key words: hypoxia-inducible factor 1α; clinicopathologic variables; vascular endothelial growth factor; cyclooxygenase-2; B-cell lymphoma 2; carbonic anhydrase-9; survival; lung cancer; meta-analysis

Introduction

Lung cancer is one of the most common malignancies worldwide and the paramount cause of cancer deaths in the world [1]. Lung cancer development is a multi-step process, driven by a series of genetic and environmental alterations. Neovascularisation and cellular adaptation to hypoxia have been recognised to be essential conditions for cancer progression. However the mechanisms of such cellular events have not yet been completely elucidated [2]. Semenza et al. [3] identified the hypoxia-inducible transcription factor (HIF-1) in 1992. HIF-1 is a heterodimer consisting of two sub-units, HIF-1α and HIF-1 β. HIF-1 β is constitutively expressed, unlike HIF-1α, which is rapidly degraded by proline hydroxylation. On the contrary, when cells are under hypoxic conditions, HIF-1α will accumulate and heterodimerise with HIF-1 β to form the transcription factor (HIF-1). Regions of hypoxia are known to exist within many tumours, and the extent of tumour hypoxia correlates with prognosis in number types [4–7]. In addition, enhanced levels of HIF-1α protein have been detected in the cytoplasm and nuclei of 40% to 80% of human carcinoma cases [8]. In recent years, hypoxia-inducible factor-1α (HIF-1α), which was used as the index of hypoxia, has been evaluated for many tumours.
Gradually, more detailed immunohistochemistry studies have indicated that HIF-1α may be involved in cell proliferation, invasion, angiogenesis and metastases. Over-expression of HIF-1α, which is common in lung cancer, may be correlated with poor prognosis, high metastatic risk, pathological types, pathological grade, tumour size, differentiation, smoking and the survival of patients. However, there has been lots of controversy. Meanwhile, studies have examined the association of HIF-1α expression on disease progression, such as medium microvessel density (MVD), vasculogenic mimicry (VM) and Ki-67, and have clarified the relationship between HIF-1α and the expression of other effectors of the hypoxia response element (HRE), such as vascular endothelial growth factor (VEGF), vascular endothelial growth factor receptor (VEGFR), cyclooxygenase-2 (COX-2), B-cell lymphoma 2 protein (bcl-2 protein) and carbonic anhydrase-9 (CA9).

However, there has not been a systematic assessment of the literature regarding the association of HIF-1α expression and clinical significance in lung cancer patients. Also, most of the individual studies had a small number of patients. We performed a meta-analysis to provide a systematic assessment of whether HIF-1α expression is associated with clinical significance in lung cancer patients. It could help diagnose and optimise selection for targeted therapy in lung cancer patients.

Methods

Search strategy

Firstly, a computer literature search was conducted in Cochrane library, PubMed and EMBase which are English Databases, and CNKI, CBM, VIP and Wan Fang which are Chinese Databases from inception to May 2012. The keywords “hypoxia-inducible factor” OR “HIF-1α” OR “HIF-1”, “lung cancer” OR “lung neoplasm” and “immunohistochemistry” OR “immunocytochemistry” were used. No language restrictions were applied. Various combinations of the keywords were applied.

Secondly, all abstracts were read by two independent reviewers (Ren WW and Li Z), and then the full-texts were independently read and checked carefully. Finally, disagreements were resolved through consensus with a third reviewer (Mi DH). For studies using the same sample in different publications, only the most complete information was included following careful and exhaustive examination. Consultation with experts in the field was performed to further identify additional published and unpublished studies.

Methodological assessment and study selection criteria

As the study design of articles which were included in the meta-analysis were not single cohort studies or case-control studies, we could not use the Newcastle-Ottawa Quality Assessment Scale [9]. To assess laboratory methodology, two reviewers (Ren WW and Mi DH) read each of the acceptable studies in duplicate independently, and performed selection criteria according to Steele’s method [10–12]. Discrepancies between the two reviewers were resolved by discussion and consensus with a third reviewer (Yang KH). The final results were reviewed by all investigators to avoid bias.

Inclusion criteria for primary studies were as follows: (1) primary lung cancer patients should be pathologically proven; (2) HIF-1α expression should be detected with immunohistochemistry (IHC); and (3) the association between clinicopathologic variables and positive HIF-1α expression; and (4) the association between HIF-1α and the expression of the other effectors; or (5) provides information on survival data; and (6) laboratory methodology of IHC: (6.1) clear and detailed description of protein (nuclear, cytoplasm or extracted from cellular components) and antibodies (type of tissue or liquid sampled); and (6.2) tissue sample conservation (fixation in formalin, alcohol or paraffin); and (6.3) description of the revelation test procedure of the biological factor with the first antibody type and clone identification, second antibody type, reaction characteristics, colouration method, epitope unmasking
method; and (6.4) description of the negative and positive control; and (6.5) test reproducibility control; and (6.6) definition of the level of positivity of the test; or (6.7) the pathologist evaluating the IHC outcome was double-blind (or random) to patient clinicopathologic data and outcome. When studies were retrospective, the pathologist blinding was simple-blind, and the studies were defined as medium quality.

Exclusion criteria for primary studies were as follows: (1) review, abstract, animal studies and cell line, or a case report; or (2) not possible to extract the exact data; or (3) patients received chemotherapy, radiotherapy, targeted therapy before operation; and (4) laboratory methodology of IHC: (4.1) the study design was not defined; or (4.2) was unclear and no detailed description of standard laboratory methodology about IHC; or (4.3) the pathologist blinding was unblinded.

Studies were considered to be of high and medium quality in this meta-analysis if they met each of inclusion criteria well, and low quality studies were excluded from further analysis.

Data extraction
To reduce the bias and to improve the reliability, two reviewers (Ren WW and Li Z) checked all relevant studies independently. Data on the following characteristics were also extracted: (1) the first author, year of publication; (2) the number of cancer cases and controls for positive HIF-1α (HIF-1α high expression, score 2+); semi-quantitatively assessing the percentage of tumour cells expressing HIF-1α, intensity of cell staining and extent of staining were included in the scoring system; (3) the number of test cases (≥60 years, male, smoking, lymph nodes metastasis) and control cases (<60 years old, female, no smoking, no lymph nodes metastasis) for positive HIF-1α; (4) the number of test cases (moderate or high differentiation) and control cases (poor differentiation); (5) the number of test cases (squamous cell carcinoma) and control cases (adenocarcinoma) for positive HIF-1α; (6) the number of test cases (non small cell lung cancer) and control cases (small cell lung cancer) for positive HIF-1α; (7) the number of test cases (I–II stage) and control cases (III–IV stage) for positive HIF-1α; (8) the hazard ratio of overall survival, 5-year survival; (9) the association of other protein positive expression (VEGF, COX-2, CA IX, bcl-2 and so on) in HIF-1α positive expression and negative expression tumour tissues (the data of dual staining of them in the same tissue section or in consecutive sections of same tumour tissue).

Statistical analysis
We estimated the odds ratio (OR) or relative risk (RR) for test cases and control cases. Statistical heterogeneity assumption among studies was checked using the X²-based Q-test [13]. When I² was no more than 50%, pooled odds ratios, relative risk and 95% confidence intervals (CIs) were calculated using Mantel-Haenszel method with fixed-effect models. Whereas significant heterogeneity (p <0.1, I² >50%) among the studies was detected, a random-effect model (Der Simonian and Laird method) was adopted. If necessary, a sensitive analysis was also performed to evaluate the influence of individual studies on the final effect. All p-values were two-sided. A p-value <0.05 was considered significant. All the statistical analyses were performed using RevMan 5.1 software (The Cochrane Collaboration, Oxford, United Kingdom, 2011).

Results

Search results and characteristics
The original search identified 560 articles in PubMed, EMbase, CNKI, CBM, VIP, and Wan Fang Databases. Searching through the Cochrane database did not identify any articles. We excluded 435 studies after review of the title and abstract, because they contained duplicate documents, or were irrelevant studies and review articles. Afterwards, 125 articles were read in full, independently by two investigators, to assess their accordance with the predefined inclusion criteria. Finally, 30 articles were considered eligible for inclusion in the meta-analysis. A total of 9 articles [14–22] were published in English, and 21 articles [23–43] were published in Chinese. The study flow diagram is shown in figure 1, and the characteristics of eligible studies are summarised in table 1.

Clinicopathologic variables and HIF-1α positive expression
Table 2 showed the results of meta-analysis. Overall, there was no association between genders, age, or smoking and HIF-1α positive expression (p >0.05). The OR (95% CI) was 1.00 (0.80, 1.26) for male versus female, 1.14 (0.85, \(161\)).

Statistical analysis
We estimated the odds ratio (OR) or relative risk (RR) for test cases and control cases. Statistical heterogeneity assumption among studies was checked using the X²-based Q-test [13]. When I² was no more than 50%, pooled odds ratios, relative risk and 95% confidence intervals (CIs) were calculated using Mantel-Haenszel method with fixed-effect models. Whereas significant heterogeneity (p <0.1, I² >50%) among the studies was detected, a random-effect model (Der Simonian and Laird method) was adopted. If necessary, a sensitive analysis was also performed to evaluate the influence of individual studies on the final effect. All p-values were two-sided. A p-value <0.05 was considered significant. All the statistical analyses were performed using RevMan 5.1 software (The Cochrane Collaboration, Oxford, United Kingdom, 2011).

Results
Search results and characteristics
The original search identified 560 articles in PubMed, EMbase, CNKI, CBM, VIP, and Wan Fang Databases. Searching through the Cochrane database did not identify any articles. We excluded 435 studies after review of the title and abstract, because they contained duplicate documents, or were irrelevant studies and review articles. Afterwards, 125 articles were read in full, independently by two investigators, to assess their accordance with the predefined inclusion criteria. Finally, 30 articles were considered eligible for inclusion in the meta-analysis. A total of 9 articles [14–22] were published in English, and 21 articles [23–43] were published in Chinese. The study flow diagram is shown in figure 1, and the characteristics of eligible studies are summarised in table 1.

Clinicopathologic variables and HIF-1α positive expression
Table 2 showed the results of meta-analysis. Overall, there was no association between genders, age, or smoking and HIF-1α positive expression (p >0.05). The OR (95% CI) was 1.00 (0.80, 1.26) for male versus female, 1.14 (0.85, \(161\)).

Statistical analysis
We estimated the odds ratio (OR) or relative risk (RR) for test cases and control cases. Statistical heterogeneity assumption among studies was checked using the X²-based Q-test [13]. When I² was no more than 50%, pooled odds ratios, relative risk and 95% confidence intervals (CIs) were calculated using Mantel-Haenszel method with fixed-effect models. Whereas significant heterogeneity (p <0.1, I² >50%) among the studies was detected, a random-effect model (Der Simonian and Laird method) was adopted. If necessary, a sensitive analysis was also performed to evaluate the influence of individual studies on the final effect. All p-values were two-sided. A p-value <0.05 was considered significant. All the statistical analyses were performed using RevMan 5.1 software (The Cochrane Collaboration, Oxford, United Kingdom, 2011).

Results
Search results and characteristics
The original search identified 560 articles in PubMed, EMbase, CNKI, CBM, VIP, and Wan Fang Databases. Searching through the Cochrane database did not identify any articles. We excluded 435 studies after review of the title and abstract, because they contained duplicate documents, or were irrelevant studies and review articles. Afterwards, 125 articles were read in full, independently by two investigators, to assess their accordance with the predefined inclusion criteria. Finally, 30 articles were considered eligible for inclusion in the meta-analysis. A total of 9 articles [14–22] were published in English, and 21 articles [23–43] were published in Chinese. The study flow diagram is shown in figure 1, and the characteristics of eligible studies are summarised in table 1.

Clinicopathologic variables and HIF-1α positive expression
Table 2 showed the results of meta-analysis. Overall, there was no association between genders, age, or smoking and HIF-1α positive expression (p >0.05). The OR (95% CI) was 1.00 (0.80, 1.26) for male versus female, 1.14 (0.85, \(161\)).
1.52) for age (≥60 years vs. <60 years), 2.16 (0.77, 6.05) for smoking versus no smoking, respectively. In accordance with the \( p \) value of 0.05, we could not draw a firm conclusion regarding whether there was no association between tumour diameters and HIF-1α positive expression (\( p = 0.05 \)). However, the positive HIF-1α expression was associated with malignant tissues, tumour stage, lymph node metastasis, degrees of differentiation or histology (NSCLC vs. SCLC, adenocarcinomas vs. squamous cell carcinoma) in lung cancer patients (\( p < 0.05 \)). The OR (95% CI) was 19.00 (12.12, 29.78) for malignant tissues versus benign tissues, 3.31 (2.02, 5.44) for lymph node metastasis (yes vs. no), 0.23 (0.14, 0.36) for stage (I–II VS. III–IV), 0.47 (0.31, 0.70) for differentiation (well vs. moderately or poorly), 0.24 (0.07, 0.77) for NSCLC versus SCLC, 0.78 (0.63, 0.98) for adenocarcinomas versus squamous cell carcinoma, respectively. Interestingly, the expressions of HIF-1α was significantly higher than those in normal lung tissue; and III–IV stage, lymph node metastasis, poorly differentiation, SCLC and squamous cell carcinoma were significantly higher than those in I–II stage, no lymph node metastasis, well differentiation of lung tissues, NSCLC and adenocarcinomas, respectively.

**Table 1:** Characteristics of the 30 selected studies.

<table>
<thead>
<tr>
<th>Author (year, reference)</th>
<th>Country</th>
<th>Stage</th>
<th>Histology</th>
<th>HIF-1α positive (negative)</th>
<th>Outcome(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giromano (A 2001 [14])</td>
<td>UK</td>
<td>I–II</td>
<td>Squamous and adenocarcinomas</td>
<td>68 (40)</td>
<td>K</td>
</tr>
<tr>
<td>Giromano (A 2001 [15])</td>
<td>UK and Greece</td>
<td>I–II</td>
<td>Squamous and adenocarcinomas</td>
<td>68 (40)</td>
<td>LM</td>
</tr>
<tr>
<td>Wu XH 2011 [16]</td>
<td>China</td>
<td>I–III</td>
<td>Squamous and adenocarcinomas</td>
<td>49 (91)</td>
<td>BCFHUKNOP</td>
</tr>
<tr>
<td>Chen 2009 [17]</td>
<td>China</td>
<td>I–III</td>
<td>Squamous and adenocarcinomas</td>
<td>70 (50)</td>
<td>ABHIJ</td>
</tr>
<tr>
<td>Seok JK 2005 [18]</td>
<td>USA and Korea</td>
<td>I–II</td>
<td>Squamous, adenocarcinomas and large cell carcinoma</td>
<td>37 (37)</td>
<td>FJKL</td>
</tr>
<tr>
<td>Park SH 2011 [21]</td>
<td>Korea</td>
<td>I–IV</td>
<td>NSCLC (squamous, adenocarcinomas and other)</td>
<td>79 (74)</td>
<td>BFHIP</td>
</tr>
<tr>
<td>Swinson DE 2004 [22]</td>
<td>UK</td>
<td>I–IV</td>
<td>NSCLC (squamous, adenocarcinomas and other)</td>
<td>80 (92)</td>
<td>JUMP</td>
</tr>
<tr>
<td>Li 2011 [23]</td>
<td>China</td>
<td>I–II</td>
<td>Squamous and adenocarcinomas</td>
<td>43 (28)</td>
<td>ABCFHIK</td>
</tr>
<tr>
<td>Zhu 2007 [26]</td>
<td>China</td>
<td>I–IV</td>
<td>Squamous and adenocarcinomas</td>
<td>19 (26)</td>
<td>AFH</td>
</tr>
<tr>
<td>Wang 2011 [27]</td>
<td>China</td>
<td>I–III</td>
<td>NSCLC (squamous, adenocarcinomas and other)</td>
<td>28 (32)</td>
<td>ABCDHFUJNO</td>
</tr>
<tr>
<td>Wang 2009 [28]</td>
<td>China</td>
<td>I–IV</td>
<td>Squamous and adenocarcinomas</td>
<td>24 (21)</td>
<td>ABDHFUJ</td>
</tr>
<tr>
<td>Wu 2011 [29]</td>
<td>China</td>
<td>I–IV</td>
<td>Squamous and adenocarcinomas</td>
<td>78 (82)</td>
<td>BFHP</td>
</tr>
<tr>
<td>Liu 2011 [31]</td>
<td>China</td>
<td>I–III</td>
<td>Squamous, adenocarcinomas and large cell carcinoma</td>
<td>108 (18)</td>
<td>BCFHJU</td>
</tr>
<tr>
<td>Ding 2009 [33]</td>
<td>China</td>
<td>I–IV</td>
<td>Squamous and adenocarcinomas</td>
<td>38 (20)</td>
<td>ABCFHIJ</td>
</tr>
<tr>
<td>Deng 2010 [34]</td>
<td>China</td>
<td>I–IV</td>
<td>Squamous and adenocarcinomas</td>
<td>14 (15)</td>
<td>ABFHIJ</td>
</tr>
<tr>
<td>Han 2008 [37]</td>
<td>China</td>
<td>I–IV</td>
<td>NSCLC and SCLC</td>
<td>37 (27)</td>
<td>ABCGHJU</td>
</tr>
<tr>
<td>Zuo 2008 [38]</td>
<td>China</td>
<td>I–III</td>
<td>Squamous and adenocarcinomas</td>
<td>34 (14)</td>
<td>ABCFHIJU</td>
</tr>
<tr>
<td>Fan 2002 [39]</td>
<td>China</td>
<td>I–III</td>
<td>NSCLC and SCLC</td>
<td>17 (43)</td>
<td>GHUM</td>
</tr>
<tr>
<td>Hao 2010 [40]</td>
<td>China</td>
<td>I–IV</td>
<td>Squamous and adenocarcinomas</td>
<td>53 (30)</td>
<td>ADHFUJ</td>
</tr>
<tr>
<td>Zhao 2005 [41]</td>
<td>China</td>
<td>I–IV</td>
<td>NSCLC and SCLC</td>
<td>40 (36)</td>
<td>ABCDGHJU</td>
</tr>
<tr>
<td>Wang 2005 [42]</td>
<td>China</td>
<td>I–IV</td>
<td>Squamous, adenocarcinomas and large cell carcinoma</td>
<td>29 (39)</td>
<td>ABDHFUJK</td>
</tr>
<tr>
<td>Yuan 2010 [43]</td>
<td>China</td>
<td>I–IV</td>
<td>Adenocarcinomas</td>
<td>51 (29)</td>
<td>ABDHFUJKP</td>
</tr>
</tbody>
</table>

A: the control of benign tissues, B: gender, C: age (≥60 years vs. <60 years old), D: tumour diameters (≥5 cm or <5 cm), E: smoking (yes or no), F: histology (adenocarcinomas vs. squamous cell carcinoma), G: histology (NSCLC vs. SCLC), H: stage, I: differentiation, J: lymph node metastasis, K: VEGF, L: CA IX, M: Bcl-2, N: COX-2, O: 5-year’s survival rates, P: overall survival (HR), P*: overall survival (RR).
CI) was 0.31 (0.05, 1.88), and 1.72 (0.98, 3.02) for positive versus negative HIF-1α tumour tissues, respectively. Thus, VEGF or CA IX positive expression was significantly higher in HIF-1α positive tumour tissues than those in HIF-1α negative tumour tissues, respectively.

5-year survival rates, overall survival (OS) in positive and negative HIF-1α expression of tumour tissues
Significant heterogeneity existed in 4 [19, 24, 27, 29] studies when 5-year survival rates were compared in positive and negative HIF-1α tumour tissues and 7 [16, 19–22, 29, 43] studies for overall survival, too (I² = 75%, I² = 68%). The random effects model was used to pool the result (fig. 4, fig. 5). There was an association between positive and negative HIF-1α tumour tissues for 5-year survival rates (OR = 0.13, 95% CI: 0.03–0.47, p = 0.002) and overall survival (RR = 1.68, 95% CI: 1.12–2.50, p = 0.01) in lung cancer patients. Thus, a survival difference was observed in positive and negative HIF-1α tumour tissues of lung cancer patients. The patients of negative HIF-1α tumour tissues had higher 5-year survival rates and overall survival than positive HIF-1α.

Sensitivity analysis
In order to prove robust results of high heterogeneity outcomes (diameter, smoking, NSCLC vs. SCLC, stage, lymph node metastasis, differentiation, VEGF, CA IX, bcl-2, 5-year survival rates and overall survival), sensitivity analyses were conducted as mentioned above. The influence of outcome on the overall meta-analysis estimate was investigated by omitting some obviously different studies at the time (table 3). When no significant heterogeneity (p >0.1, I² <50%) among the studies was detected, the heterogeneity did not appear to impact significantly on the main outcomes of our analyses (NSCLC vs SCLC, stage, lymph node metastasis, differentiation, VEGF, CA IX, 5-year survival rates and overall survival), indicating that our results were statistically reliable. However sensitivity analyses showed that exclusion of a single study [30] considerably altered the main outcomes of our analyses (smoking), with a range from 2.16 (95% CI: 0.77–6.05, p = 0.14) to 3.52 (95% CI: 1.81–6.87, p = 0.0002). Exclusion of two studies [27, 43] considerably altered the main outcomes of our analyses (diameter), with a range from 1.84 (95% CI: 1.00–3.39, p = 0.05) to 1.30(95% CI: 0.84–2.01, p = 0.23). Exclusion of a single study [22] significantly altered the main outcomes of our analyses (bcl-2), with a range from 0.31 (95% CI: 0.05–1.88, p = 0.20) to 0.41 (95% CI: 0.02–0.83, p = 0.03), but did still not alter the significant heterogeneity (p = 0.05, I² = 74%).

Discussion
Tissue hypoxia is an essential characteristic of solid tumours and promotes biologic processes involved in tumour progression [44]. It is well known that hypoxia inducible factor-1α (HIF-1α) is the unique sub-unit that determines the HIF system activity and is a member of the basic helix-loop-helix-PAS protein family [45], is usually increased under hypoxic conditions, and can activate transcription of many genes that are critical for cellular function under hypoxic conditions. The prognostic significance of HIF-1α expression has now been evaluated in a number of solid tumours. Increased HIF-1α expression has certainly been reported to be a negative expression in benign tissues, so our results of the meta-analysis (lung cancer tissues vs. benign tissues) showed a strong association with lung cancer risk (OR = 19.00, 95% CI = 12.12–29.78). However different studies showed a different trend of HIF-1α expression in different clinicopathologic variables of tumour. Meanwhile, conflicting results had been reported in lung cancer patients. Sample size may contribute to conflicting results among original studies, and a small sample size in a single study

Table 2: Meta-analysis of association between clinicopathologic variables and positive HIF-1α expression.

<table>
<thead>
<tr>
<th>Clinicopathologic variables</th>
<th>Included studies</th>
<th>Test cases</th>
<th>Control cases</th>
<th>Heterogeneity</th>
<th>Meta-analysis model</th>
<th>Outcome(s)</th>
<th>OR (95%CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor vs. benign tissues</td>
<td>16 [17, 23, 26–28, 30, 32–35, 37–38, 40–43]</td>
<td>579 1085</td>
<td>21 385</td>
<td>0%</td>
<td>0.91</td>
<td>Fixed</td>
<td>19.00 (12,12, 29.78)</td>
<td>0.00001</td>
</tr>
<tr>
<td>Male vs. female</td>
<td>20 [16–17, 20–21, 23–24, 27–31, 33–38, 41–43]</td>
<td>621 1173</td>
<td>236 438</td>
<td>0%</td>
<td>0.50</td>
<td>Fixed</td>
<td>1.00 (0.80, 1.26)</td>
<td>0.99</td>
</tr>
<tr>
<td>Age (≥60 years vs. &lt;60 years)</td>
<td>12 [23, 27, 29–33, 35–38, 41]</td>
<td>253 428</td>
<td>249 438</td>
<td>0%</td>
<td>0.91</td>
<td>Fixed</td>
<td>1.14 (0.85, 1.52)</td>
<td>0.38</td>
</tr>
<tr>
<td>Diameter (≥25 cm vs. &lt;5 cm)</td>
<td>7 [24–27, 28–40–43]</td>
<td>125 205</td>
<td>132 282</td>
<td>57%</td>
<td>0.03</td>
<td>Random</td>
<td>1.84 (1.00, 3.39)</td>
<td>0.05</td>
</tr>
<tr>
<td>Smoking vs. no smoking</td>
<td>4 [27–28, 30, 35]</td>
<td>66 111</td>
<td>41 99</td>
<td>68%</td>
<td>0.03</td>
<td>Random</td>
<td>2.16 (0.77, 6.05)</td>
<td>0.14</td>
</tr>
<tr>
<td>AD vs. SCC</td>
<td>16 [16, 18, 20–21, 23, 26–29, 32–36, 38, 40, 42–43]</td>
<td>309 611</td>
<td>440 795</td>
<td>20%</td>
<td>0.22</td>
<td>Fixed</td>
<td>0.78 (0.63, 0.98)</td>
<td>0.03</td>
</tr>
<tr>
<td>NSCLC vs. SCLC</td>
<td>4 [24, 37, 39, 41]</td>
<td>91 233</td>
<td>30 42</td>
<td>54%</td>
<td>0.09</td>
<td>Random</td>
<td>0.24 (0.07, 0.77)</td>
<td>0.02</td>
</tr>
<tr>
<td>Stage (I - II vs. III-IV)</td>
<td>21 [16–17, 21, 23–24, 26–34, 37–43]</td>
<td>412 991</td>
<td>466 655</td>
<td>67%</td>
<td>0.00001</td>
<td>Random</td>
<td>0.23 (0.14, 0.36)</td>
<td>0.00001</td>
</tr>
<tr>
<td>Lymph node metastasis (yes vs. no)</td>
<td>22 [17–18, 20–22, 24, 27–33, 35–43]</td>
<td>601 901</td>
<td>374 886</td>
<td>72%</td>
<td>0.00001</td>
<td>Random</td>
<td>3.72 (2.38, 5.80)</td>
<td>0.00001</td>
</tr>
<tr>
<td>Differentiation (well vs. poorly)</td>
<td>16 [17, 20, 23, 27, 29–39, 41–43]</td>
<td>352 757</td>
<td>321 524</td>
<td>54%</td>
<td>0.003</td>
<td>Random</td>
<td>0.47 (0.31, 0.70)</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

may be under statistical power and incapable to draw a reliable conclusion [46]. Meta-analysis as an important statistical method for medical research can extremely improve statistical power by enlarging sample size, and afterwards a more reliable conclusion can be drawn [47]. The results of the meta-analyses showed there was association between positive HIF-1α expression and tumour stage, lymph node metastasis, histology (NSCLC vs. SCLC, adenocarcinomas vs. squamous cell carcinoma) or degrees of differentiation in lung cancer patients. Thus the process of HIF-1α expression may be controlled by different mechanisms between histology [48], but the precise mechanism in the hypoxia-sensitive pathway of different histology is still not clear. In those studies [16, 20, 22, 24, 29, 31, 33–34, 37–39, 42], the choice of the cutoff value may be the main reason of heterogeneity. When there were no significant heterogeneity ($p > 0.1, I^2 = 0\%$) by omitting those obviously different studies, the heterogeneity did not appear to impact significantly on the main outcomes of our analyses. Based on a meta-analysis of data obtained from 7 [24, 27–28, 40–43] studies, this review showed no evidence of difference in HIF-1α expression for tumour diameter ($p = 0.05$). As we know, the size of tumour does not necessarily predict a benign or malignant tumour, meanwhile sensitivity analyses showed that there was no evidence of difference in HIF-1α expression for tumour diameter ($p = 0.23$). Therefore, we are drawn to the conclusion that there was no relationship between the size of tumour and HIF-1α expression, but the measurement method of tumour diameter still has influence on the heterogeneity of the main outcomes of our analyses. Sensitivity analyses showed that exclusion of a single study considerably altered the main outcomes of our analyses (smoking). The detailed histories of smoking were not assessed in these studies [27–28, 30, 35]; therefore, smoking exposure variables (quantity and time) may have significant influence on the heterogeneity of the main outcomes of our analyses (smoking). In a word, these conclusions are needed to develop the further verification.

It is well known that hypoxia inducible factor-1α (HIF-1α) can regulate more than 40 downstream genes, which are involved in adaptive responses to hypoxia, and regulate many biological behaviours of cells, such as tumour metabolism, growth and angiogenesis [49]. The results of the meta-analyses showed there was association between VEGF or CA IX positive expression and HIF-1α positive tumour tissues. Given that these studies [18, 22, 25, 30, 36, 39, 42–43] have analysed the markers separately but on sequential tissue sections from one tumour, and used different methods of the immunohistochemistry (SP, PV9000, MaxVision™, EnVision™, ABC, the catalysed signal amplification kit), these may be the main reason of heterogeneity. When there were no significant heterogeneity ($p > 0.1, I^2 = 0\%$) by omitting those obviously different studies [18, 39, 42] (Seok JK 2005 for CA IX; Cheng 2005 and Wang 2005 for VEGF), the heterogeneity did not appear to impact significantly on the main outcomes of our analyses. Sensitivity analyses showed that exclusion of a single study considerably altered the main outcomes of our analyses (bcl-2). Two studies [14, 39] have shown that a significant inverse association of the HIF1α with bcl-2(cytoplasm) expression, but no association was found between HIF-1α and Bcl-2 (unclear) expression [22]; therefore, the difference of protein accumulation region (cytoplasm, nuclear) may have significant influence on the heterogeneity of the main outcomes of our analyses (bcl-2). However, maybe due to including a small sample size, there was no association

\begin{table}[h]
\centering
\caption{Sensitivity analyses of high heterogeneity outcomes in meta-analysis.}
\begin{tabu}{|c|c|c|c|c|c|c|}
\hline
Heterogeneity outcomes & Omitted(excluded) studies & Test cases & Control cases & Heterogeneity & Meta-analysis model & Outcome(s) \\
\hline
Diameter (≥25 cm vs. <5 cm) & 2 [27, 43] & 90 & 160 & 89 & 187 & 0.37 & Fixed \\
\hline
Smoking vs. no smoking & 1 [30] & 56 & 91 & 24 & 74 & 0.56 & Fixed \\
\hline
NSCLC vs. SCLC & 1 [37] & 62 & 180 & 24 & 31 & 0.75 & Fixed \\
\hline
Stage (I – II vs. III – IV) & 2 [16, 29] & 366 & 606 & 385 & 540 & 0.63 & Fixed \\
\hline
Lymph node metastasis (Yes vs. No) & 6 [22, 24, 29, 31, 33, 34, 42] & 369 & 567 & 240 & 571 & 0.48 & Fixed \\
\hline
Differentiation (well vs. moderately or poorly) & 6 [20, 29, 31, 34, 38, 39] & 220 & 504 & 199 & 296 & 0.59 & Fixed \\
\hline
VEGF & 2 [36, 42] & 176 & 246 & 101 & 231 & 0.65 & Fixed \\
\hline
CA IX & 1 [18] & 109 & 225 & 56 & 227 & 0.65 & Fixed \\
\hline
bcl-2 & 1 [22] & 11 & 81 & 49 & 83 & 74% & Random \\
\hline
5-year survival rates & 2 [19, 27] & 4 & 111 & 62 & 124 & 0.75 & Fixed \\
\hline
5-year survival rates & 2 [29, 24] & 11 & 52 & 193 & 336 & 0.34 & Fixed \\
\hline
Overall survival & 2 [16, 21] & – & – & – & – & 0.75 & Fixed \\
\hline
\end{tabu}
\end{table}

between positive and negative HIF-1α tumour tissues for Bcl-2 or COX-2 positive expression. Meanwhile, owing to including few (less than three) studies about other proteins and variables, such as p53 protein, BNIP3, survivin protein, VEGFR, MMP-9, MVD, VM and Ki-67, we could not perform a meta-analysis of those variables. Moreover, some studies [30–51] reported the relationship between other proteins and HIF-1α expression, but there was limited data of HIF-1α to perform a meta-analysis and therefore they could not be included in our meta-analysis. Furthermore, from a clinical point of view it would be interesting to know something about the role of HIF-1α in molecular (KRAS wt/mut, EGFR wt/mut, ALK rearranged) sub-types of NSCLC, but there were the absences of actual data of molecular subtypes in our included studies. All of these will be needed to provide sufficient data to calculate the effect sizes in future research.

All of the survival data was confounded by variable use of postoperative adjuvant therapy, such as adjuvant chemotherapy and adjuvant radiotherapy, or no treatment after surgery. This may be the main reason of heterogeneity, and undoubtedly affected patient’s survival. Only one study [19] reported postoperative adjuvant radiotherapy, three studies [24, 27, 29] did not report postoperative therapy (5-year survival rates); three studies [16, 29, 43] did not report postoperative therapy, and four studies [19–22] reported postoperative adjuvant radiotherapy, chemotherapy or radiotherapy with chemotherapy (overall survival). When there were no significant heterogeneity ($p > 0.1$, $I^2 = 0\%$) by omitting those obviously different studies, the heterogeneity did not appear to impact significantly on the main outcomes of our analyses. Adjuvant treatments after surgery are unavoidable, taking into account the issues of ethics and patient interests. So combined with the results of our study about association between positive HIF-1α expression and clinicopathologic variables, we could draw the firm conclusion that HIF-1α expression has an influence on the survival of lung cancer patients.

Some limitations of this systematic review and meta-analysis are as follows: First, this meta-analysis had to address heterogeneity issues. We found significant heterogeneity among these studies. The heterogeneity could be explained by immunohistochemistry techniques used to detect protein expression (including antigen retrieval methods, choice of antibody, tumour specimens and the choice of the cutoff value), methods of the immunohistochemistry (SP, PV9000, MaxVision™, EnVision™, ABC and the catalysed signal amplification kit) and postoperative adjuvant treatment have been mentioned above. Specifically, there were 15 studies [14–15, 17, 23, 26–27, 31, 34–41] which used SP, 5 studies [24–25, 30, 32–33] which used PV9000, 4 studies [16, 20, 29, 43] used EnVision™, 3 studies [18, 19, 42] used ABC, 2 studies [21–22] used the catalysed signal amplification kit, and 1 study [28] used the MaxVision™ method of the immunohistochemistry, but the sensitivity and specificity of these methods are differences in the immunohistochemistry. However, average immunohistochemistry measurements are more reproducible, stable, and less affected by bias [52]. Nonetheless, the potential limitations of this study should be considered. We also conducted sensitivity analyses to assess the accuracy and reliability of our results by the removal of obviously different studies. When no significant heterogeneity ($p > 0.1$, $I^2 = 0\%$) among the studies was detected, the heterogeneity did not appear to impact significantly on the main outcomes of our analyses (NSCLC vs. SCLC, stage, lymph node metastasis, differentiation, VEGF, CA IX, 5-year survival rates and overall survival) (table 3). Secondly, publication bias is a wide phenomenon for all forms of meta-analysis, such as positive results easily published by journals, including double articles published in Chinese. Therefore, the test for publication bias was not performed and possible bias still could not be ruled out. Third, some studies which were included in our study used continuous variables and r value of statistical methods, but due to the limitation of quantity (less than three studies) they could not be used to develop a meta-analysis, too. These could affect the comprehensive and integrity of our research data.

In conclusion, despite the limitations of this meta-analysis, our study confirmed that HIF-1α is associated with differentiation degree of lung cancer cell, lymph node metastasis, postoperative survival time and histology (NSCLC vs. SCLC, adenocarcinomas vs. squamous cell carcinoma). HIF-1α which combined other proteins, such as VEGF or CA IX, might serve as important parameters in evaluating biological behaviour and prognosis of lung cancer; it will be benefit to clinical treatment and prognostic evaluation.

Funding / potential competing interests: This study was supported by the Research Programme for health of Gansu Province of China (No. GSWST09-06), the Natural Science Foundation of Gansu Province of China (No. 1010RJA162) and the Postgraduate Business Programme of Evidence-Based Medicine Center of Lanzhou University (No. 2010LDEBM-A).

None of the authors has any conflict of interest.

Correspondence: Denghai Mi, Evidence-Based Medicine Center of Lanzhou University, 199 West Donggang Road, Chengguan District, CN-Lanzhou 730000, Gansu, China, mi.dh(at)163.com

References


Figure 1
Flow chart of article selection in the systematic review and meta-analysis.
**Figure 2**
Forest plot of VEGF, CA IX and bcl-2 positive expression in positive and negative HIF-1α tumour tissues, respectively (Giatromanolaki A 01 2001: Giatromanolaki A 2001 [14], Giatromanolaki A 2001: Giatromanolaki A 2001 [15], Swinson 01 2004: pCA IX, Swinson 02 2004: mCA IX).

**Figure 3**
Forest plot of COX-2 positive expression in positive and negative HIF-1α tumour tissues.
**Figure 4**
Forest plot of association between HIF-1α expression and 5-year survival rates.

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Experimental Events</th>
<th>Total Events</th>
<th>Weight</th>
<th>Odds Ratio</th>
<th>M-H, Random 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sigve 2011</td>
<td>7</td>
<td>25</td>
<td>185</td>
<td>303</td>
<td>0.25 [0.0, 0.61]</td>
</tr>
<tr>
<td>Wang 2011</td>
<td>4</td>
<td>27</td>
<td>8</td>
<td>33</td>
<td>0.54 [0.14, 2.05]</td>
</tr>
<tr>
<td>Wu 2011</td>
<td>3</td>
<td>78</td>
<td>40</td>
<td>82</td>
<td>0.64 [0.01, 0.14]</td>
</tr>
<tr>
<td>Xiang 2008</td>
<td>1</td>
<td>33</td>
<td>22</td>
<td>42</td>
<td>0.03 [0.00, 0.23]</td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td><strong>163</strong></td>
<td><strong>460</strong></td>
<td><strong>100.0%</strong></td>
<td><strong>0.13 [0.03, 0.47]</strong></td>
<td></td>
</tr>
<tr>
<td>Total events</td>
<td>15</td>
<td>295</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: Tau² = 1.26; Chi² = 11.82, df = 3 (P = 0.008), I² = 75%
Test for overall effect: Z = 3.12 (P = 0.002)

**Figure 5**
Forest plot of association between HIF-1α expression and overall survival.

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>log(Risk Ratio)</th>
<th>SE</th>
<th>Weight</th>
<th>IV, Random 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wu 2011</td>
<td>-0.2144</td>
<td>0.284</td>
<td>15.9%</td>
<td>0.81 [0.48, 1.35]</td>
</tr>
<tr>
<td>Park 2011</td>
<td>0.0315</td>
<td>0.265</td>
<td>15.9%</td>
<td>1.03 [0.61, 1.74]</td>
</tr>
<tr>
<td>Hirami 2004</td>
<td>0.4187</td>
<td>0.500</td>
<td>9.4%</td>
<td>1.52 [0.57, 4.06]</td>
</tr>
<tr>
<td>Swinson 2004</td>
<td>0.5196</td>
<td>0.249</td>
<td>16.4%</td>
<td>1.68 [1.03, 2.74]</td>
</tr>
<tr>
<td>Sigve 2011</td>
<td>0.8329</td>
<td>0.291</td>
<td>15.1%</td>
<td>2.30 [1.50, 4.07]</td>
</tr>
<tr>
<td>Wu 2011</td>
<td>0.8424</td>
<td>0.325</td>
<td>14.1%</td>
<td>2.32 [1.23, 4.37]</td>
</tr>
<tr>
<td>Yuan 2010</td>
<td>1.3396</td>
<td>0.352</td>
<td>13.2%</td>
<td>3.82 [1.91, 7.61]</td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td><strong>100.0%</strong></td>
<td><strong>1.60 [1.12, 2.50]</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: Tau² = 0.19; Chi² = 18.48, df = 6 (P = 0.005), I² = 56%
Test for overall effect: Z = 2.54 (P = 0.01)