



# The Mechanism of Trivalent Inorganic Arsenic on HIF-1 $\alpha$ : a Systematic Review and Meta-analysis

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## Abstract

The purpose of our study was to investigate the role of hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) in arsenic-induced carcinogenesis. We included 39 articles for meta-analysis. The results showed that low-dose exposure to arsenic ( $\leq 10$   $\mu\text{mol/L}$ ) could promote the expression of phosphatidylinositol 3-kinase (PI3K) and phosphorylation-protein kinase B (p-AKT). High-dose arsenic exposure ( $> 10$   $\mu\text{mol/L}$ ) promoted the expression of PI3K, HIF-1 $\alpha$ , vascular endothelial growth factor (VEGF), and p38MAPK (P38). Acute arsenic exposure ( $< 24$  h) promoted the expression of PI3K, HIF-1 $\alpha$ , and VEGF. Chronic arsenic exposure ( $\geq 24$  h) promoted the expression of PI3K, p-AKT, and P38. Moreover, for normal tissue-derived cells, arsenic could induce the increased expression of PI3K, p-AKT, HIF-1 $\alpha$ , and VEGF. For tumor tissue-derived cells, arsenic could induce the expression of PI3K, p-AKT, and P38. We found that arsenic exposure could activate the PI3K/AKT pathway, further induce the high expression of HIF-1 $\alpha$ , and then upregulate the levels of miRNA-21 and VEGF, promote the expression of proliferating cell nuclear antigen (PCNA), and ultimately lead to malignant cell proliferation. Our findings indicated that arsenic could increase the expression of HIF-1 $\alpha$  by activating the PI3K/AKT pathway and eventually induce malignant cell proliferation.

**Keywords** Arsenic · HIF-1 $\alpha$  · PI3K · miRNA-21 · VEGF · Meta-analysis

## Introduction

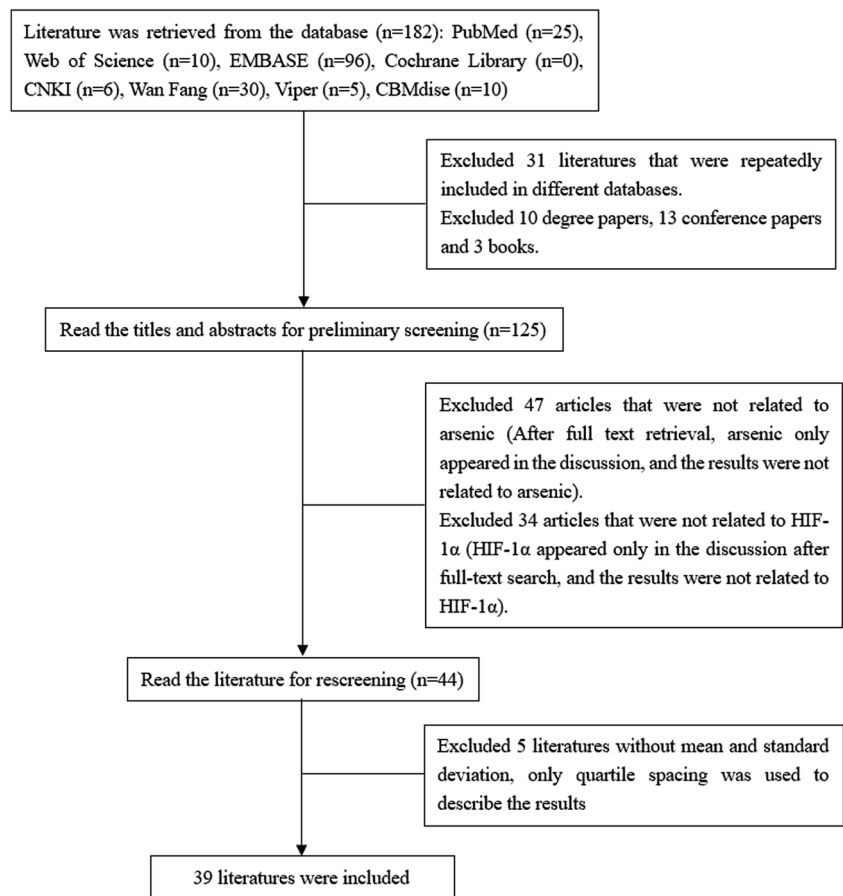
Arsenic is a kind of non-metallic element, widely found in the atmosphere, soil, water, and food [1]. Arsenic in drinking water is one of the most important forms of exposure. Arsenic exposure to drinking water is very serious in developing countries, where groundwater is the main source of drinking water supply [2]. Epidemiological studies have shown that exposure to arsenic can induce a variety of cancerous diseases, including skin, bladder, lung, and liver cancers [3]. However, although arsenic exposure is associated with the development of various diseases, the mechanism of arsenic exposure-induced carcinogenesis is not yet fully elucidated. Recent studies have found that hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) is involved in arsenic-induced cancer mechanisms. The purpose of this study is to investigate whether HIF-1 $\alpha$  plays a role in the mechanism of arsenic-induced cancer.

Previous studies have found that hypoxia-inducible factor (HIF) family protein plays an important role in the development of inflammation and tumor growth. When HIF family protein binds to the target gene, it can induce a series of compensatory and pathological reactions in the body through transcription and post-transcriptional regulation, including the occurrence of inflammation and accelerated growth of the tumor. Some researchers found that HIFs are composed of two subunits, HIF-1 $\alpha$  and HIF-1 $\beta$ , in which HIF-1 $\beta$  subunit can be expressed continuously and stably in cells, which mainly plays a structural role. HIF-1 $\alpha$  can be expressed stably under hypoxia, and then, the combination of HIF-1 $\alpha$  and HIF-1 $\beta$  can form active HIF protein, which leads to increase of HIF protein level, and then transfers to the cell to regulate the expression of various genes, and participate in the malignant transformation and proliferation of cells. Lei Wang [4] found that arsenic could activate HIF-1 $\alpha$  through PI3K/AKT pathway, then upregulate the expression of vascular endothelial growth factor (VEGF), and finally promote the proliferation of colon cancer cells. In Lu Lu's study [5], it was found that arsenic could affect the expression of HIF-1 $\alpha$  through the AKT pathway in arsenic-exposed bronchial epithelial cells and then regulate the level of miRNA-21, ultimately promoting the proliferation of bronchial epithelial cells.

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**Fig. 1** Flow chart of the methodology used to identify studies for inclusion in the meta-analysis



However, in different studies, the mechanism of arsenic on HIF-1 $\alpha$  is different. Qing Zhou [6] found that when arsenic was exposed to human urothelial cells, it could induce the high expression of HIF-1 $\alpha$ . Li Hongying [7] found that arsenic could inhibit the expression of HIF-1 $\alpha$  in human synovial fibroblasts in a long time exposure. In addition, Yuan Xu [8] found that arsenic could not only inhibit the expression of HIF-1 $\alpha$  but also increased the level of its subunit HIF-2 $\alpha$ . Therefore, in the process of arsenic-promoting malignant proliferation, the mechanism of arsenic on HIF-1 $\alpha$  is still controversial. We conducted this meta-analysis on the basis of previous studies on arsenic and HIF-1 $\alpha$ , in order to reveal the role of HIF-1 $\alpha$  in arsenic-promoting cell malignant proliferation.

## Materials and Methods

### Inclusion Criteria

Inclusion criteria were determined according to the PICO principles.

**Study Design** Experimental studies published in Chinese and English.

**Participants (P)** Cells, animals, or people.

**Intervention (I)** All the experimental groups treated with arsenic or arsenic compounds. The arsenic model group related to cell proliferation, PI3K/AKT, HIF-1 $\alpha$ , and VEGF in a dose or time relationship, and the highest dose or longest time was used in the analysis.

**Comparison (C)** The control group was a blank control group without any intervention.

**Outcome (O)** The indexes related to cell proliferation and HIF-1 $\alpha$ . PI3K, AKT, HIF-1 $\alpha$ , miRNA-21, VEGF, PCNA.

### Exclusion Criteria

1. Literature in languages other than Chinese and English.
2. The main contents of the literature were not related to arsenic or HIF-1 $\alpha$  (arsenic or HIF-1 $\alpha$  only appeared in the abstract or discussion, and there were no indicators related to arsenic or HIF-1 $\alpha$  in the results).
3. Repeated publications (published in both Chinese and English journals, repeatedly and included in different databases).

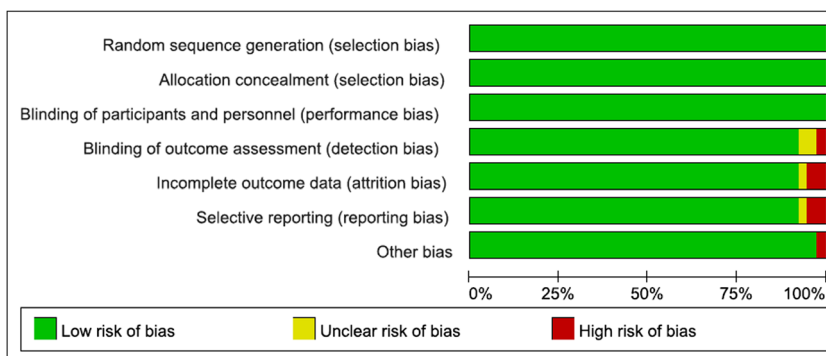
**Table 1** Basic characteristics of included literature

Author	Year	Language	<i>n</i>	Type of arsenic	Dose	Time	Type of cells	Outcome indicators
Chi Yang [10]	2019	English	3	NaAsO <sub>2</sub>	≤10	≥24	Normal Cell	1, 2, 3, 4
Qing Zhou [6]	2018	English	3	NaAsO <sub>2</sub>	≤10	≥24	Normal Cell	2, 3, 5, 6
Lu Lu [5]	2018	English	3	NaAsO <sub>2</sub>	>10	≥24	Normal Cell	2, 3, 4
Nilanjana Banerjee [11]	2017	English	3	NaAsO <sub>2</sub>	≤10	≥24	Normal Cell	1, 2, 4
Xiaoyan Jiao [12]	2017	English	3	NaAsO <sub>2</sub>	≤10	<24	Normal Cell	3, 4
Fei Luo [13]	2016	English	3	NaAsO <sub>2</sub>	≤10	≥24	Normal Cell	3
Zukaa al Taleb [14]	2016	English	3	NaAsO <sub>2</sub>	>10	<24	Cancer Cell	3
LI Hongying [7]	2016	Chinese	3	As <sub>2</sub> O <sub>3</sub>	≤10	≥24	Normal Cell	3, 5
Xiaolin Lu [15]	2016	English	3	NaAsO <sub>2</sub>	≤10	≥24	Normal Cell	2, 4
Xinlu Liu [16]	2016	English	3	NaAsO <sub>2</sub>	≤10	≥24	Normal Cell	4
Lili Song [17]	2016	English	3	NaAsO <sub>2</sub>	>10	<24	Cancer Cell	2, 3, 4
Rachel J. Person [18]	2015	English	3	NaAsO <sub>2</sub>	≤10	≥24	Cancer Cell	2, 3
Fei Luo [19]	2015	English	3	NaAsO <sub>2</sub>	≤10	≥24	Normal Cell	4
Xiao Yang [20]	2015	English	3	NaAsO <sub>2</sub>	>10	<24	Cancer Cell	1, 2, 4
Dong Zhang [21]	2014	English	3	As <sub>2</sub> O <sub>3</sub>	>10	<24	Normal Cell	2, 3
Suthakar Ganapathy [22]	2014	English	3	NaAsO <sub>2</sub>	≤10	<24	Normal Cell	3
Piyajit Watcharasit [23]	2014	English	3	NaAsO <sub>2</sub>	≤10	≥24	Cancer Cell	2, 3, 5
YingNa Li [24]	2014	English	3	NaAsO <sub>2</sub>	≤10	<24	Normal Cell	2, 3, 5
Yang Liu [25]	2014	English	3	NaAsO <sub>2</sub>	>10	<24	Cancer Cell	3, 4, 5
Yuan Xu [8]	2013	English	3	As <sub>2</sub> O <sub>3</sub>	≤10	≥24	Normal Cell	3, 5, 6, 8, 9
Fei Wang [26]	2013	English	3	NaAsO <sub>2</sub>	≤10	≥24	Normal Cell	2, 3, 5
Yue Zhao [27]	2013	English	3	NaAsO <sub>2</sub>	≤10	≥24	Normal Cell	4, 5
Lu Shen [28]	2013	English	3	NaAsO <sub>2</sub>	≤10	≥24	Normal Cell	2, 4, 7, 8
Fei Luo [29]	2013	English	3	NaAsO <sub>2</sub>	≤10	≥24	Normal Cell	4
Lei Wang [4]	2012	English	3	NaAsO <sub>2</sub>	≤10	≥24	Cancer Cell	1, 2, 3, 5
WANG Song Mei [30]	2012	Chinese	3	As <sub>2</sub> O <sub>3</sub>	≤10	≥24	Cancer Cell	3, 5
Yuan Xu [8]	2012	English	3	As <sub>2</sub> O <sub>3</sub>	≤10	≥24	Normal Cell	3
Bin Bao [31]	2012	English	3	NaAsO <sub>2</sub>	≤10	≥24	Normal Cell	3, 4, 5
LingZhi Liu [32]	2011	English	3	NaAsO <sub>2</sub>	>10	≥24	Normal Cell	2, 3, 5
Wei Guo [33]	2011	English	3	NaAsO <sub>2</sub>	>10	<24	Normal Cell	3
TANG Yinhua [34]	2009	Chinese	15	NaAsO <sub>2</sub>	>10	≥24	Cancer Cell	3, 5
Minyoung Lee [35]	2006	English	3	NaAsO <sub>2</sub>	>10	<24	Cancer Cell	3, 5, 6
Chandrashekhar D. Kamat [36]	2005	English	11	NaAsO <sub>2</sub>	≤10	≥24	Normal Cell	2, 3, 5
C. Nathaniel Roybal [37]	2005	English	3	NaAsO <sub>2</sub>	>10	<24	Cancer Cell	3, 5
Nicole V. Soucy [38]	2004	English	3	NaAsO <sub>2</sub>	>10	≥24	Normal Cell	3, 5
Ning Gao [39]	2004	English	3	NaAsO <sub>2</sub>	>10	<24	Cancer Cell	1, 2, 3, 5
Heath D. Skinner [40]	2004	English	3	As <sub>2</sub> O <sub>3</sub>	>10	<24	Cancer Cell	3
Monique C. A. Duyndam [41]	2003	English	3	NaAsO <sub>2</sub>	>10	<24	Cancer Cell	2, 3, 5
Monique C. A. Duyndam [42]	2001	English	3	NaAsO <sub>2</sub>	>10	<24	Cancer Cell	3

*n* number within the experimental group, *HIF-1 $\alpha$*  hypoxia inducible factor-1 $\alpha$ , *miRNA-21* an endogenous non-coding RNA, *PI3K* phosphatidylinositol 3-kinases, *P-AKT* phosphorylated-protein kinase B, *VEGF* vascular endothelial growth factor, *PCNA* proliferating cell nuclear antigen, *P38* p38MAPK, *Caspase-3* cysteinyl aspartate specific proteinase-3. (1) PI3K, (2) AKT, (3) HIF-1 $\alpha$ , (4) miRNA-21, (5) VEGF, (6) P38, (7) P65, (8) caspase-3, (9) PCNA

- Literature with incomplete data, data not available, or failure to provide valid data (failure to provide mean or standard deviation).
- Review literature, conference literature, degree literature, book materials (this study only included original studies, so the review literature was excluded; as the conference

Fig. 2 Quality assessment results



literature provided only the abstracts, and not the full text of the literature, so valid data could not be extracted; therefore, the conference literature was excluded; the author of the degree literature would publish the data obtained during the study period in the form of journal alone. If included, there would be the problem of repeated publication, so the degree literature would be excluded; some literature in books and works would be published separately in journals. If included, there would be problems with the repeated publication, so books would be excluded).

6. No control group

Search Strategy

The databases from which studies were retrieved include the Cochrane library, PubMed, Web of Science, Excerpta Medica database (EMBASE), China National Knowledge Infrastructure (CNKI), Wan Fang Data databases, Wiper database, and China Biology Medicine disc (CBMdisc). The retrieval period was from the beginning of the publication to October 1, 2019.

We first determine the research indicators of this study according to the PICO principle, and then, we select the keywords of the corresponding indicators according to the Mesh thesaurus.

The search terms used included arsenic, arsenite, ATO, As<sub>2</sub>O<sub>3</sub>, hypoxia-inducible factor, HIF-1, HIF-1 $\alpha$ , HIF-2 $\alpha$ , Akt, PI3K, miRNA-21, microRNA-21, miR-21, MiR-21, P38, P65, caspase-3, VEGF, and PCNA. Take PubMed database as an example: (((Arsenic) OR Arsenite) OR ATO) OR As<sub>2</sub>O<sub>3</sub>) and (((Hypoxia-inducible factor) OR HIF-1) OR HIF-1 $\alpha$ ) OR HIF-2 $\alpha$ ) and (((((((((((Akt) OR PI3K) OR miRNA-21) OR microRNA-21) OR miR-21) OR MiR-21) OR P38) OR P65) OR caspase-3) OR VEGF) OR PCNA).

Search Results

According to the search terms, we examined 8 databases and found 182 kinds of literature. According to the inclusion and exclusion criteria, we screened the retrieved literature and finally included 39. Our search strategy was to screen the retrieved literature independently by two researchers using the same search terms. At first, 182 kinds of literature with proper titles and abstracts were retrieved according to the PICO principle. Then, according to the inclusion and exclusion criteria,

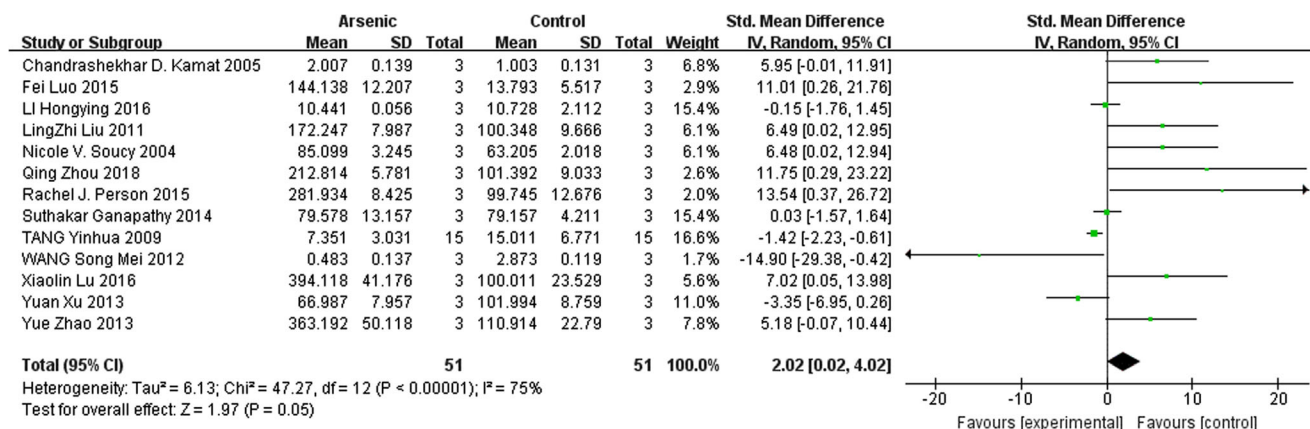
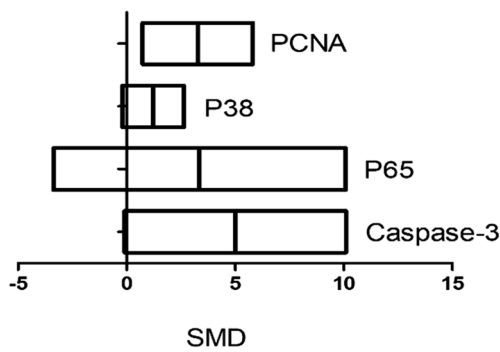


Fig. 3 Effects of arsenic on cell viability. SMD, standardized mean difference; IV, independent variable; 95% CI, 95% confidence interval; SD, standard deviation. Exposure to arsenic increased cell viability



**Fig. 4** Effects of arsenic on cell proliferation and apoptosis. SMD, standardized mean difference; PCNA, proliferating cell nuclear antigen; P38, p38MAPK; caspase-3, cysteinyl aspartate specific proteinase-3. Arsenic can promote the expression of PCNA

31 articles repeatedly collected in different databases were excluded; 10-degree papers, 13 conference papers, and 3 books were excluded (the degree paper will be published repeatedly in the form of journal, the conference paper only contains the abstract of the paper without data to be extracted, and the literature in the books will be published separately in the form of journal). The remaining 125 articles were further screened, excluding 47 more articles that were not related to arsenic (arsenic or HIF-1 $\alpha$  only appeared in the discussion section and no arsenic-related indicators were provided in the results). Furthermore, an additional 34 articles whose research content was not related to HIF-1 $\alpha$  (arsenic or HIF-1 $\alpha$  appeared only in the abstract or discussion sections, and no indicators related to HIF-1 $\alpha$  were provided in the results) were excluded. With the remaining 44 articles, a full-text rescreening was made, and the literature that did not provide a mean or standard deviation among the five results was excluded. Finally, we included 39 articles for our study. The retrieval period taken was from the beginning of the publication to October 1, 2019. The search results are shown in Fig. 1.

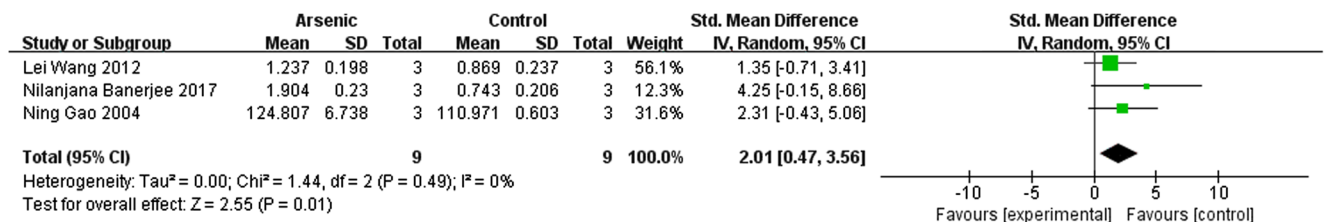
**Quality Evaluation**

The Cochrane risk deviation quality assessment tool was used to evaluate the quality of the included 39 kinds of literature. The evaluation includes seven items: (1) random sequence generation (selection), (2) allocation concealment (selection),

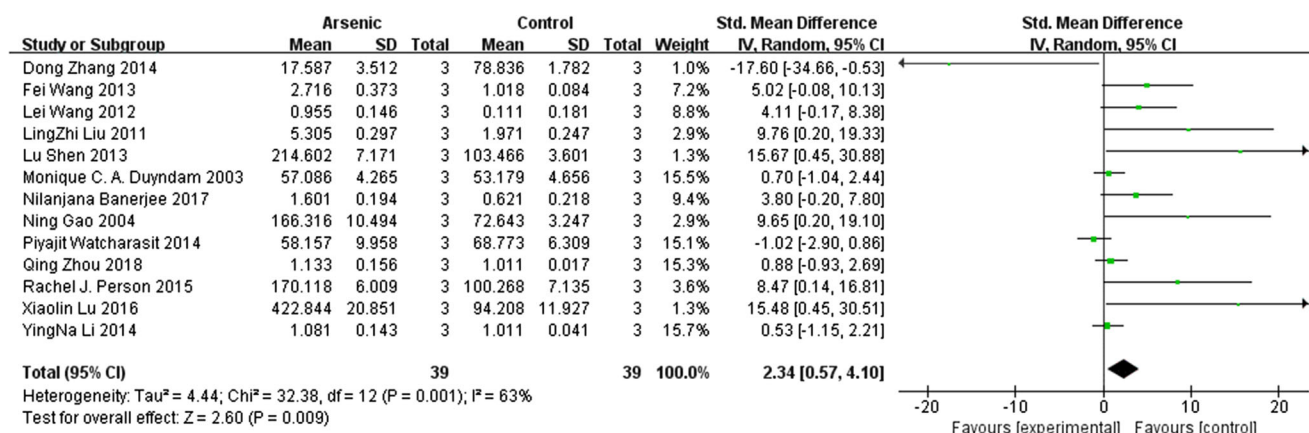
(3) blinding of participants and personnel (performance bias), (4) blinding of outcome assessment (detection bias), (5) incomplete outcome data (attrition bias), (6) selective reporting (reporting bias), and (7) other bias.

**Statistical Analysis**

Statistical analysis was performed using Review Manager 5.3 (The Nordic Cochrane Centre, The Cochrane Collaboration 2012, Portland, OR, USA) and Stata 12.0 (Stata Corp LP, College Station, TX, USA). The quality of the included studies was assessed using the scoring system in the Review Manager 5.3 software. The standard mean difference was used to compare and analyze the data. In this study, we analyzed the levels of many different indicators, including protein and mRNA levels. Since the units of these indicators were different, we used the standardized mean difference for statistical analysis so as to facilitate comparison among different articles, which avoided the problem that different indicators could not be compared due to different units. The formula for the standard mean difference was  $d_i = \frac{\bar{x}_i - \bar{x}_k}{s_d}$ ,  $i=1, 2, 3...k$ . Heterogeneity was assessed according to  $I^2$  values, which were divided into four categories according to Cochrane Handbook: 0% to 40% meant low heterogeneity; 30% to 60% moderate heterogeneity; 50% to 90% denoted a significant heterogeneity; and finally, 75% to 100% suggested a very large heterogeneity. When  $P < 0.05$  and  $I^2 > 50\%$ , the random effect model was used. When  $P > 0.05$  and  $I^2 \leq 50\%$ , the fixed effect model was used. The heterogeneous sources of 39 articles were detected according to subgroup analysis. Based on the study of the dose response relationship between Crippa and Orsini [9], we used the spline model and subgroup analysis to detect the bidirectional effect of arsenic on HIF-1 $\alpha$ . Subgroup analysis was performed according to exposure time, exposure concentration, and tissue source of the cells. The results of the experimental and control groups were expressed by standard mean differences and 95% confidence intervals. We used funnel plots to detect whether there was a publication deviation. The Chi-square test was used for hypothesis testing. When



**Fig. 5** Effects of arsenic on PI3K. SMD, standardized mean difference; IV, independent variable; 95% CI, 95% confidence interval; SD, standard deviation. Arsenic can promote the expression of PI3K



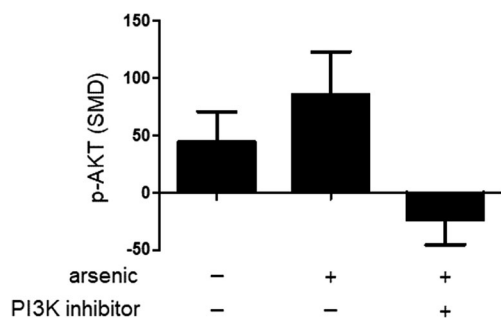
**Fig. 6** Effects of arsenic on p-AKT. SMD, standardized mean difference; IV, independent variable; 95% CI, 95% confidence interval; SD, standard deviation. Arsenic can promote the expression of P-AKT

$P < 0.05$ , there was publication deviation. Sensitivity analysis was performed using Stat 12.0 software. Bilateral test was used in all statistical analysis; when  $P < 0.05$ , there was the statistical difference.

## Results

### Basic Characteristics

The basic characteristics of the 39 articles included in this analysis are shown in Table 1. The experimental groups were treated with arsenic and arsenic compounds ( $\text{NaAsO}_2$ ,  $\text{As}_2\text{O}_3$ ), the control groups were blank controls without arsenic exposure. According to the effect of different doses of arsenic on cell activity in the study, we conducted a dose-response analysis, and then according to the results of the dose-response relationship analysis of arsenic on cells, it was found that when the arsenic concentration was less than  $10 \mu\text{mol/L}$ , the cell activity increased with the increase in arsenic concentration. When the concentration of arsenic is higher than  $10 \mu\text{mol/L}$ , the



**Fig. 7** Effects of arsenic on the PI3K/AKT pathway. SMD, standardized mean difference. Arsenic plus PI3K inhibitor group inhibited the level of intracellular p-AKT

cell activity decreases with the increase in arsenic concentration, so we use  $10 \mu\text{mol/L}$  as the threshold of high or low dose. In the subgroup analysis, the arsenic exposure was divided into a low-dose group ( $\leq 10 \mu\text{mol/L}$ ,  $n = 23$ ) and a high-dose group ( $> 10 \mu\text{mol/L}$ ,  $n = 16$ ). According to the exposure time of arsenic, it was divided into a short time group ( $< 24 \text{ h}$ ,  $n = 15$ ) and a long-time group ( $\geq 24 \text{ h}$ ,  $n = 24$ ). The subjects were divided into normal tissue source group ( $n = 24$ ) and tumor tissue source group ( $n = 15$ ). Outcome variables were cell proliferation and apoptosis indicators (P38, P65, caspase-3, VEGF, and PCNA expression) and HIF-1 $\alpha$  pathway-related (HIF-1 $\alpha$ , AKT, PI3K, and miRNA-21 expression).

### Quality Assessment

Quality assessment of the 39 articles included in the analysis and the low-risk rate was more than 75% (Fig. 2).

### A Meta-analysis of Arsenic on Cell Viability, Proliferation, and Apoptosis of Cells

The cell viability in arsenic exposure groups was higher than those produced by the normal control groups (SMD = 2.02, 95% CI (0.02, 4.02)) (Fig. 3). PCNA levels in the arsenic-exposed group were 3.28 times higher than those in the control groups (95% CI (0.74, 5.81)), while there were no statistically significant differences in P38 (SMD = 1.22, 95% CI (-0.19, 2.64)), P65 (SMD = 3.36, 95% CI (-3.37, 10.09)), and caspase-3 (SMD = 5.01, 95% CI (-0.09, 10.10)) ( $P > 0.05$ ) expression in the arsenic exposure groups and normal control groups (Fig. 4). These results suggest that arsenic could increase the cell viability and upregulate the level of intracellular tumor proliferation index PCNA, leading to the malignant proliferation of cells.

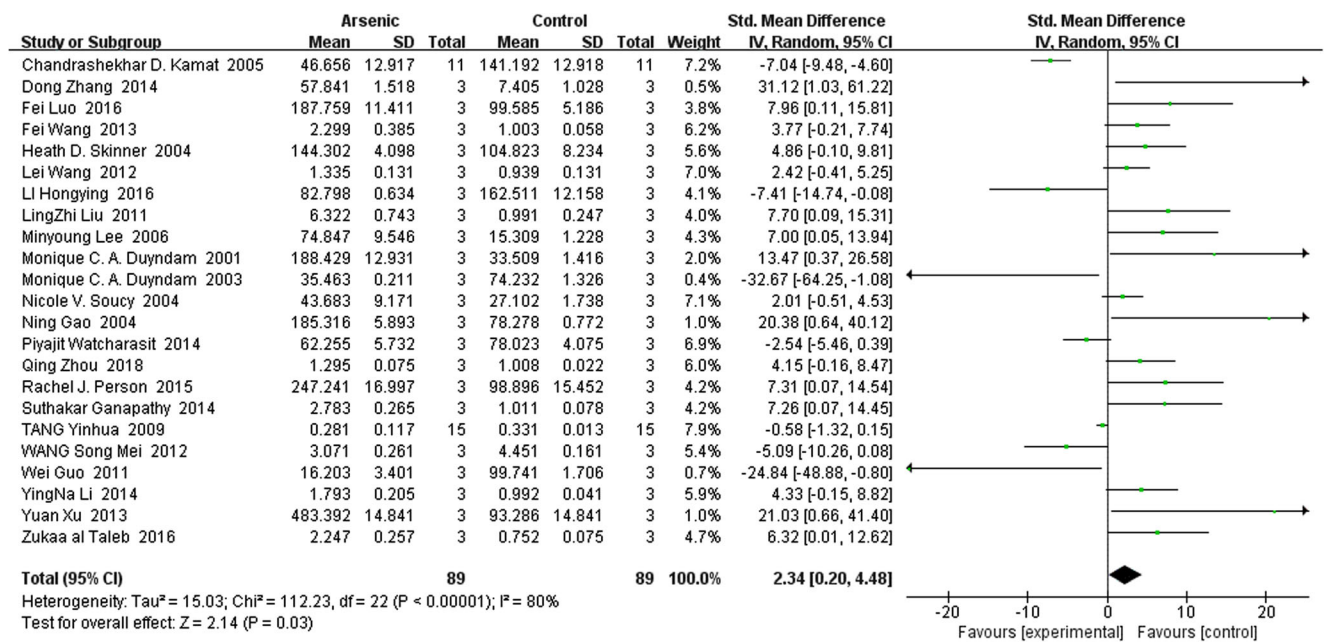


Fig. 8 Effect of arsenic on HIF-1 $\alpha$ . SMD, standardized mean difference; IV, independent variable; 95% CI, 95% confidence interval; SD, standard deviation. Arsenic can promote the expression of HIF-1 $\alpha$

### A Meta-analysis of Arsenic on PI3K/AKT Pathway

The results showed that the level of PI3K in the arsenic-exposed groups was 2.01 times higher than in normal control group (95% CI (0.47, 3.56)) (Fig. 5). The level of p-AKT was 2.34 times higher than that of the normal control group (95% CI (0.57, 4.10)) (Fig. 6). In the study of Chi Yang,<sup>[10]</sup> AKT was found to be a downstream indicator of PI3K. In order to reveal the role of arsenic in the PI3K/AKT pathway, we observed the effect of arsenic on the PI3K/AKT pathway by taking the arsenic exposure group as the control group and the arsenic combined with PI3K inhibitor groups as experimental groups. The results showed that arsenic combined with PI3K inhibitor groups inhibited the level of intracellular p-AKT

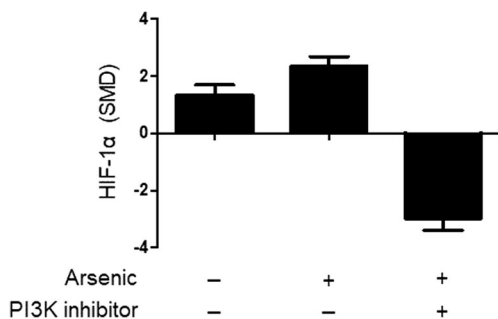


Fig. 9 Effect of PI3K/AKT pathway on HIF-1 $\alpha$ . SMD, standardized mean difference. Arsenic plus PI3K inhibitor group inhibited the level of intracellular HIF-1 $\alpha$

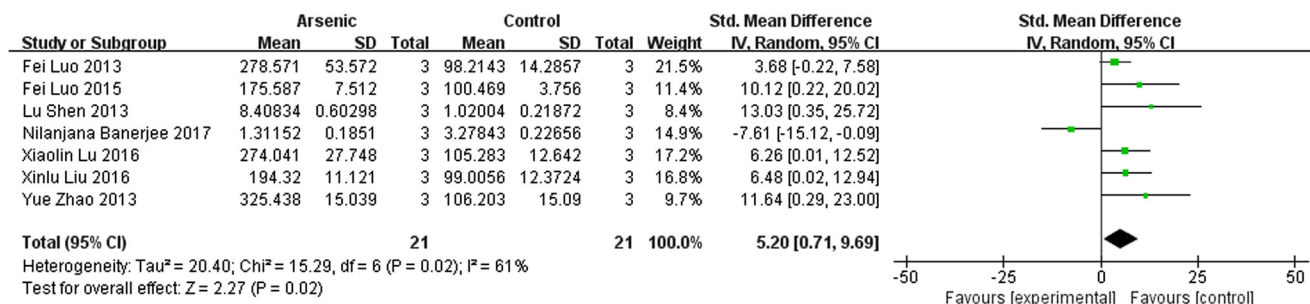
(SMD = -22.11, 95% CI (-43.51, -0.70)) (Fig. 7). These results suggested that arsenic can activate the PI3K/AKT pathway and affect the expression of p-AKT by regulating PI3K.

### A Meta-analysis of the Effects of Arsenic and PI3K/AKT Pathways on HIF-1 $\alpha$

The results showed that the level of HIF-1 $\alpha$  in arsenic-exposed groups was higher than in the normal control groups (SMD = 6.32, 95% CI (0.01, 12.62)) (Fig. 8). We took an arsenic exposure group as a control group and arsenic combined with PI3K inhibitor groups as an experimental group to observe the effect of the PI3K/AKT pathway on HIF-1 $\alpha$ . The results showed that the level of HIF-1 $\alpha$  in arsenic combined with PI3K inhibitor groups was inhibited compared with arsenic exposure groups (SMD = -3.27, 95% CI (-5.55, -0.99)) (Fig. 9). These results indicated that arsenic could activate the expression of HIF-1 $\alpha$  and regulate the level of HIF-1 $\alpha$  through PI3K/AKT pathway.

### A Meta-analysis of Arsenic and HIF-1 $\alpha$ on miRNA-21 and VEGF

The results showed that the levels of miRNA-21 (SMD = 5.20, 95% CI (0.71, 9.69)) (Fig. 10) and VEGF (SMD = 2.20, 95% CI (0.05, 4.35)) (Fig. 11) in the arsenic exposure groups were higher than those in the normal control group. We



**Fig. 10** Effect of arsenic on miRNA-21. SMD, standardized mean difference; IV, independent variable; 95% CI, 95% confidence interval; SD, standard deviation. Arsenic can promote the expression of miRNA-21

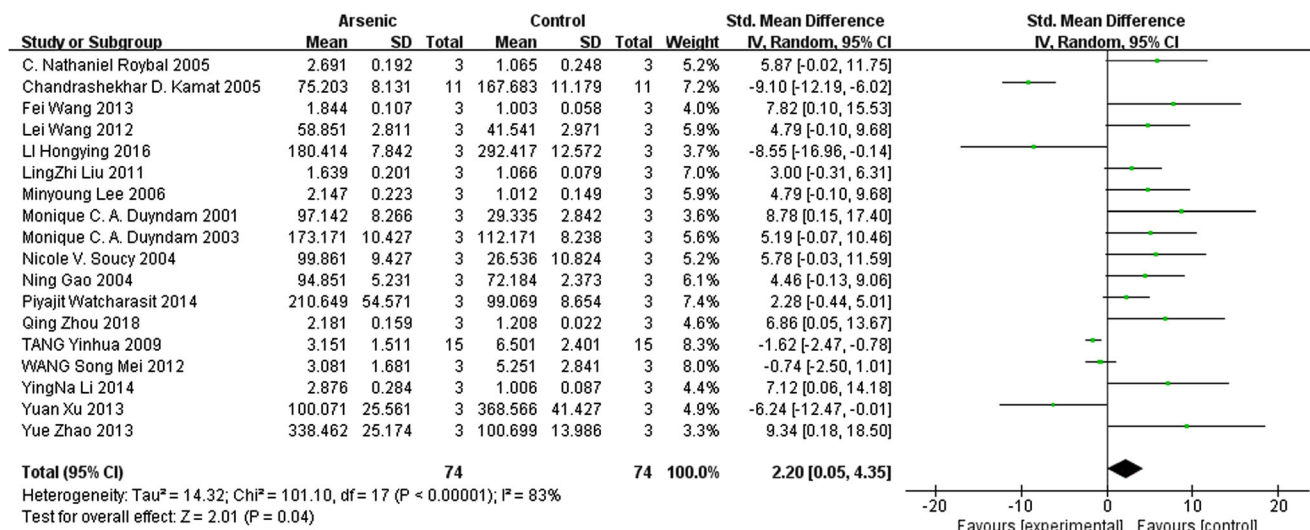
used the arsenic exposure group as a control group, arsenic combined with HIF-1 $\alpha$  inhibitor groups and arsenic combined with HIF-1 $\alpha$  activity groups as an experimental group to observe the effect of HIF-1 $\alpha$  on the expression of miRNA-21. The results showed that arsenic combined with HIF-1 $\alpha$  activity could upregulate the level of miRNA-21 (SMD = 7.64, 95% CI (0.09, 15.19)), arsenic plus HIF-1 $\alpha$  inhibitor could down-regulate the level of miRNA-21 (SMD = -9.42, 95% CI (-18.66, -0.18)). We took the arsenic exposure group as the control group, arsenic combined with HIF-1 $\alpha$  inhibitor groups, and arsenic combined with miRNA-21 inhibitor groups as the experimental group to observe the effects of HIF-1 $\alpha$  and miRNA-21 on VEGF. The results showed that arsenic combined with HIF-1 $\alpha$  inhibitor groups could down-regulate the expression of VEGF (SMD = -5.42, 95% CI (-10.90, 0.05)), arsenic combined with miRNA-21 inhibitor groups could also down-regulate the expression of VEGF (SMD = -7.89, 95% CI (-17.74, 1.96)) (Fig. 12). These results suggested that arsenic could upregulate the levels of miRNA-21 and VEGF by HIF-1 $\alpha$ .

### The Role of HIF-1 $\alpha$ in the Process of Arsenic-Promoting Malignant Cell Proliferation

We synthesized the results of the study on the effect of HIF-1 $\alpha$  inhibitor combined with arsenic treatment on cell viability and further revealed the effect of HIF-1 $\alpha$  on cell viability in the process of arsenic-promoting cell malignant proliferation. The results showed that the effect of HIF-1 $\alpha$  inhibitor combined with arsenic treatment on cell viability was lower than that of arsenic treatment alone (SMD = -1.74, 95% (-3.78, 0.29)). The results showed that HIF-1 $\alpha$  inhibitor could inhibit the promoting effect of arsenic on cell activity, that is, HIF-1 $\alpha$  inhibitor inhibited cell malignant proliferation.

### Dose-Response of Arsenic to HIF-1 $\alpha$

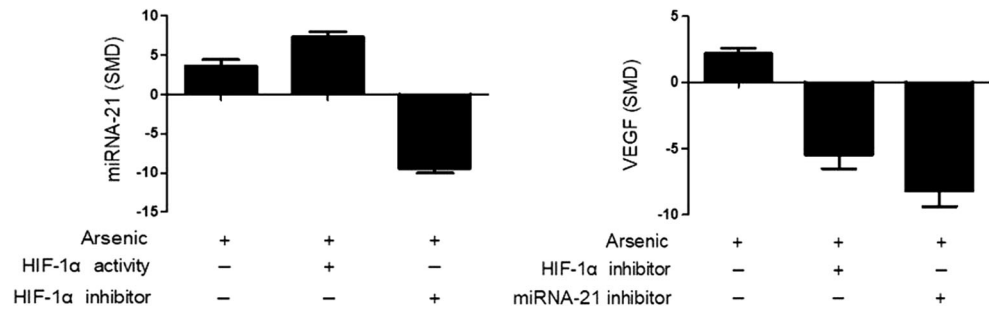
We used the spline model to quantitatively analyze the included literature to demonstrate the bidirectional effect of arsenic on HIF-1 $\alpha$ . We examined some of the data included in the study, and the basic information is shown in Table 2. The



**Fig. 11** Effect of arsenic on VEGF. SMD, standardized mean difference; IV, independent variable; 95% CI, 95% confidence interval; SD, standard deviation. Arsenic can promote the expression of VEGF



**Fig. 12** Effect of HIF-1 $\alpha$  on miRNA-21 and VEGF. SMD, standardized mean difference. Arsenic plus HIF-1 $\alpha$  inhibitor group could down-regulate the expression of VEGF, arsenic plus miRNA-21 inhibitor group could also down-regulate the expression of VEGF



results showed that the mean difference at diverse doses was 5  $\mu$ mol/L (SMD = 6.109, 95% CI (3.405, 8.918)), 10  $\mu$ mol/L (SMD = 10.092, 95% CI (5.486, 14.921)), and 15  $\mu$ mol/L (SMD = 7.725, 95% CI (3.36, 12.019)). The results showed that when the dose of arsenic exposure was less than 10  $\mu$ mol/L, the dose-response relationship between arsenic and HIF-1 $\alpha$  was positive, and when the dose of arsenic exposure was more than 10  $\mu$ mol/L, the dose-response relationship between arsenic and HIF-1 $\alpha$  was negative (Fig. 13). These results suggested that arsenic has a bidirectional effect on HIF-1 $\alpha$  regulation.

**Subgroup Analysis Based on Exposure Dose of Arsenic**

The results showed that the levels of PI3K (SMD = 1.70, 95% CI (0.05, 3.35)), p-AKT (SMD = 2.31, 95% CI (0.37, 4.26)), and cell viability (SMD = 2.33, 95% CI (0.32, 4.98)) were increased when the arsenic exposure dose was less than 10  $\mu$ mol/L. When the dose of arsenic exposure was more than 10  $\mu$ mol/L, the levels of intracellular PI3K (SMD = 2.31, 95% CI (0.43, 5.06)), HIF-1 $\alpha$  (SMD = 4.16, 95% CI (0.63, 7.70)), VEGF (SMD = 3.87, 95% CI (0.05, 7.74)), and P38 (SMD = 8.04, 95% CI (0.11, 15.96)) were increased, but there was no significant difference in cell viability (SMD = 3.21, 95% CI (-3.18, 9.59)) (Fig. 14). These results suggested that a low

arsenic dose could promote the expression of PI3K, p-AKT and increase cell viability and promote cell proliferation. High-dose arsenic could promote the expression of PI3K, HIF-1 $\alpha$  and VEGF, P38, but had no significant difference in cell activity.

**Subgroup Analysis Based on the Exposure Time of Arsenic**

The results showed that when the arsenic exposure time was less than 24 h, the levels of intracellular PI3K (SMD = 2.31, 95% CI (0.43, 5.06)), HIF-1 $\alpha$  (SMD = 4.53, 95% CI (0.04, 9.10)), and VEGF (SMD = 5.29, 95% CI (2.85, 7.73)) were increased, but the cell viability (SMD = 0.03, 95% CI (-1.57, 1.64)) had no significant difference. When the arsenic exposure duration was more than 24 h, the levels of intracellular PI3K (SMD = 2.12, 95% CI (0.40, 4.64)), p-AKT (SMD = 3.67, 95% CI (1.07, 6.26)), and P38 (SMD = 8.04, 95% CI (0.11, 15.96)) were upregulated, and the cell viability (SMD = 2.77, 95% CI (0.29, 5.25)) was significantly increased (Fig. 15). These results suggested that acute arsenic exposure could promote PI3K, HIF-1 $\alpha$ , and VEGF expression, but could not induce malignant proliferation of cells. Chronic arsenic exposure could promote PI3K, p-AKT, P38 expression and induced malignant proliferation of cells.

**Table 2** Dose response of arsenic to HIF-1 $\alpha$

ID	Author	Year	Dose [mean (SD)]				
			0	5	10	15	20
1	Qing Zhou	2018	1.008 (0.022)	1.187 (0.077)	1.295 (0.076)	-	-
2	Fei Luo	2016	0.996 (0.051)	1.878 (0.114)	2.268 (0.041)	-	-
3	Zokan al Taleb	2016	0.739 (0.166)	0.954 (0.062)	1.532 (0.065)	0.832 (0.158)	0.664 (0.136)
4	Dong Zhang	2014	0.988 (0.155)	1.215 (0.023)	1.851 (0.123)	2.122 (0.249)	2.472 (0.169)
5	Ling Zhi Liu	2016	0.992 (0.245)	1.251 (0.235)	1.521 (0.023)	1.123 (0.124)	0.632 (0.074)
6	Yuan Xu	2013	0.932 (0.148)	1.061 (0.148)	4.834 (0.148)	-	-
7	Fei Wang	2013	1.004 (0.058)	1.576 (0.265)	2.299 (0.385)	-	-

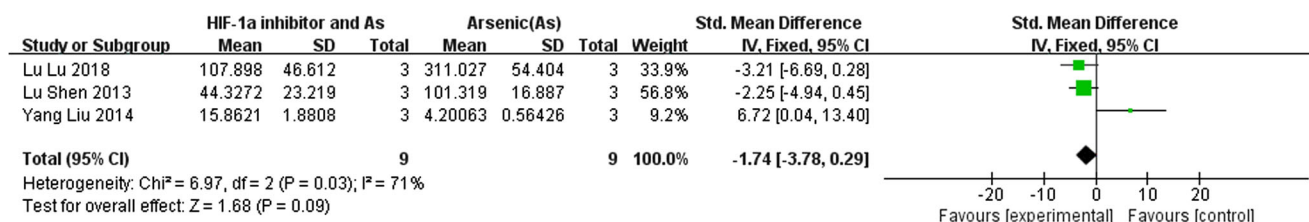


Fig. 13 Effect of HIF-1 $\alpha$  on cell viability

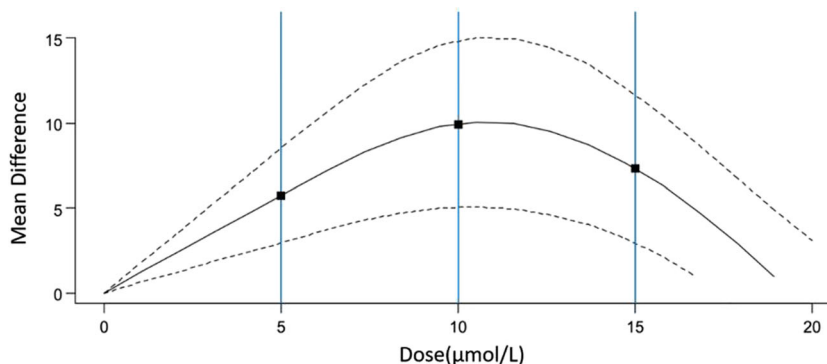
## Subgroup Analysis of Different Tissue Sources

According to different tissue sources, the subjects were divided into normal tissue and tumor tissue sources for subgroup analysis. The results showed that the levels of PI3K (SMD = 4.25, 95% CI (0.15, 8.66)), p-AKT (SMD = 2.80, 95% CI (0.33, 5.92)), HIF-1 $\alpha$  (SMD = 3.79, 95% CI (0.74, 6.84)), and VEGF (SMD = 3.42, 95% CI (0.16, 7.00)) were upregulated and the cell viability (SMD = 2.64, 95% CI (0.19, 5.09)) was significantly increased in normal tissues. In the tumor tissue source, the levels of intracellular PI3K (SMD = 1.70, 95% CI (0.05, 3.35)), p-AKT (SMD = 2.37, 95% CI (0.11, 4.85)), and P38 (SMD = 8.04, 95% CI (0.11, 15.96)) were increased, but the cell viability (SMD = 1.29, 95% CI (-6.10, 8.68)) was not significantly changed (Fig. 16). These results suggested that for cells from normal tissues, arsenic exposure could upregulate the expression of PI3K, p-AKT, HIF-1 $\alpha$ , and VEGF and promote the malignant proliferation of cells. While, for cells from tumor tissue, arsenic exposure could increase the expression of PI3K, p-AKT, P38, but had no significant effect on cell viability.

## Publication Bias Analysis

The results of the funnel plot showed that all the studies were evenly distributed on both sides of the ineffective line, indicating that the literature included in this study was stable and there was no publication bias (Fig. 17).

Fig. 14 Dose-response of arsenic to HIF-1 $\alpha$ . Analysis of the relationship between arsenic and HIF-1 $\alpha$  by spline model in the random-effects model. The dotted line indicates a 95% confidence interval of the spline model. The solid line indicates the mean difference of the spline model

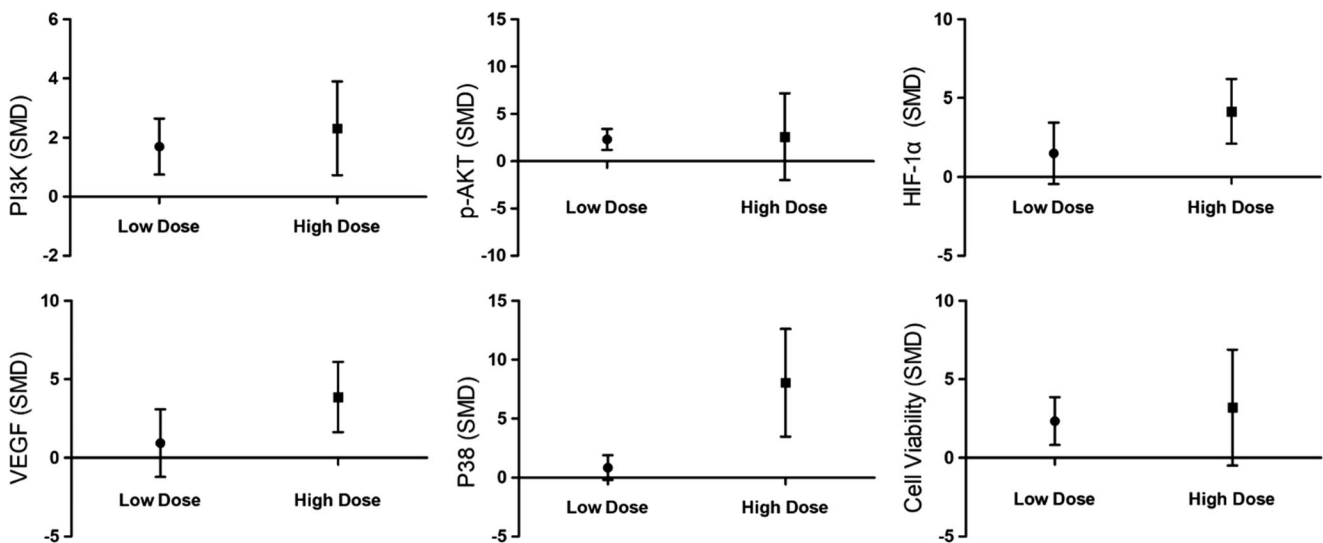


## Sensitivity Analysis

Taking the sensitivity analysis of arsenic to HIF-1 $\alpha$  as an example, we found that all the studies were evenly distributed on both sides of the midline, with no obvious deviation. It showed that the data included in this study were more robust, and there were no individual studies affecting the overall results (Figs. 18 and 19).

## Discussion

Arsenic exposure can induce a variety of cancers, such as liver, lung, and bladder cancers. It has been found that HIF-1 $\alpha$  played an important role in carcinomatous diseases induced by arsenic. Especially in malignant cell proliferation, HIF-1 $\alpha$  can promote malignant transformation and malignant proliferation by regulating its upstream and downstream proteins. In the study of Lili Song et al. [17], it was found that arsenic could upregulate the level of HIF-1 $\alpha$  by activating the AKT signal pathway and further promoting the malignant proliferation of cells. However, in the study of WANG Song Mei et al. [30], it was found that arsenic could down-regulate the level of HIF-1 $\alpha$  through the AKT signaling pathway, but had no significant effect on malignant proliferation of cells. To sum up, the regulatory effects of arsenic on HIF-1 $\alpha$  are different, so the regulatory effect of arsenic on HIF-1 $\alpha$  is not yet fully elucidated [43]. Therefore, this study systematically evaluated the relationship between arsenic and HIF-1 $\alpha$ . The



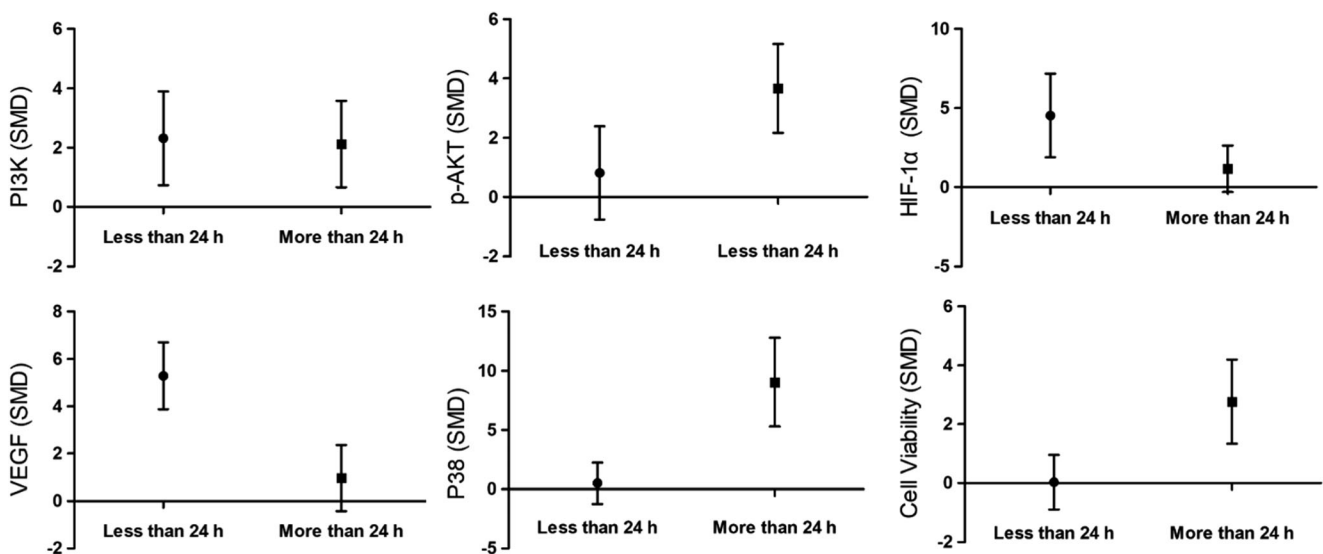
**Fig. 15** Subgroup analysis of arsenic exposure dose. SMD, standardized mean difference. Low-dose exposure to arsenic ( $\leq 10$   $\mu\text{mol/L}$ ) can promote the expression of PI3K and p-AKT, increase cell activity, and

promote cell proliferation. High-dose exposure to arsenic ( $> 10$   $\mu\text{mol/L}$ ) promoted the expression of PI3K, HIF-1 $\alpha$ , VEGF, and P38, but had no significant effect on cell activity

results showed that exposure to arsenic could activate the PI3K/AKT pathway, induce the high expression of HIF-1 $\alpha$ , and then upregulate the levels of miRNA-21 and VEGF through HIF-1 $\alpha$ . When miRNA-21 and VEGF were upregulated, the expression of cell proliferation protein PCNA was increased, and eventually leading to the malignant proliferation of cells. The results provide a theoretical basis for the molecular mechanism of arsenic carcinogenesis.

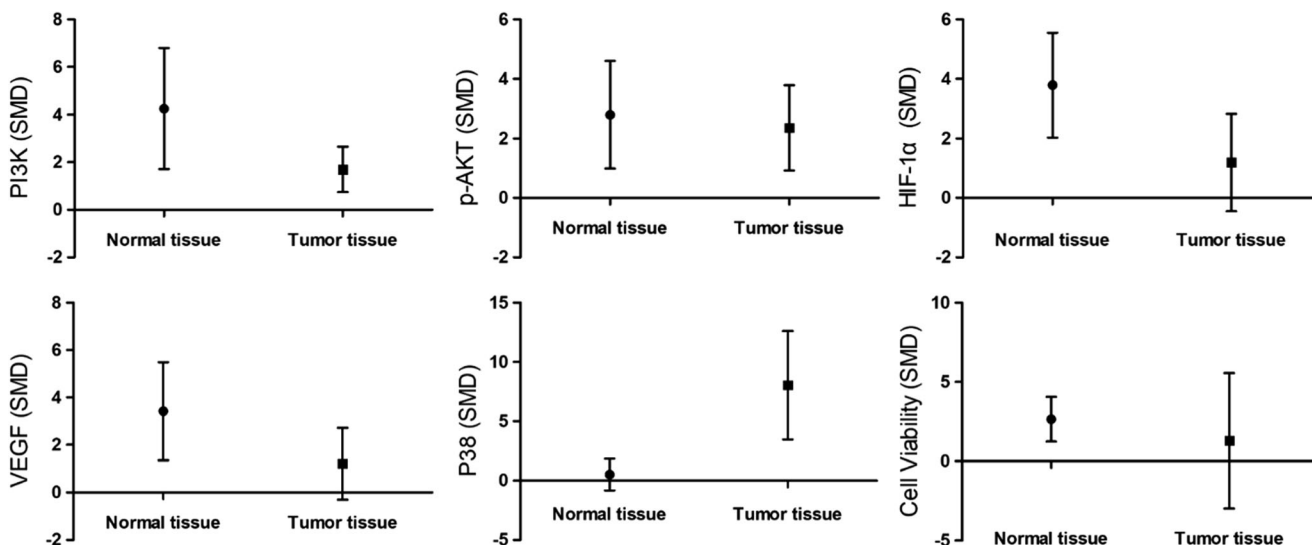
In this study, we focused on the role of HIF-1 $\alpha$  in the malignant proliferation induced by arsenic. In the study of Zukaa al Taleb [14], it was found that exposure to arsenic could increase the expression of HIF-1 $\alpha$  in cells, and a variety of cell signaling pathways involved in regulating the

expression of HIF-1 $\alpha$ , the PI3K/AKT pathway is one of the known pathways involved in cell proliferation, differentiation, and apoptosis and related to cancer. In the study of Dong Zhang [21], it was found that high-dose and short time arsenic exposure could inhibit PI3K/AKT pathway and cell viability; Fei Wang [26] found that low-dose and long-term arsenic exposure could activate PI3K/AKT pathway and increase cell viability. Based on previous studies, this study found that exposure to arsenic can increase the expression of PI3K and p-AKT. In the subgroup analysis, we found that low-dose, long-term arsenic exposure had a stronger activation effect on the PI3K/AKT pathway, in which cells from normal tissue sources were more obvious. Then, we analyzed the group of



**Fig. 16** Subgroup analysis of arsenic exposure time. SMD, standardized mean difference. Acute arsenic exposure ( $< 24$  h) promoted the expression of PI3K, HIF-1 $\alpha$ , and VEGF, but did not induce malignant cell

proliferation. Chronic arsenic exposure ( $\geq 24$  h) can promote the expression of PI3K, p-AKT, and P38, significantly increase cell activity, and promote malignant cell proliferation



**Fig. 17** Subgroup analysis of different tissue sources. SMD, standardized mean difference. For normal tissue-derived cells, arsenic exposure can induce the increased expression of PI3K, p-AKT, HIF-1α, and VEGF and

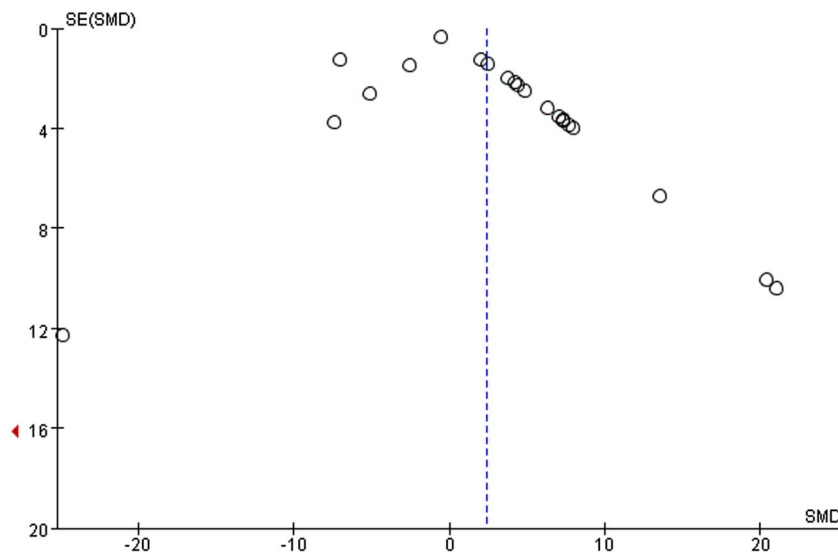
promote the malignant proliferation of cells. For tumor tissue-derived cells, arsenic exposure can induce the increased expression of PI3K, p-AKT, and P38 expression, but has no significant effect on cell activity

PI3K inhibitor combined with arsenic, further analyzed the interaction in the PI3K/AKT pathway; the result showed that PI3K inhibitor could inhibit the activation of p-AKT induced by arsenic. These results suggest that exposure to arsenic up-regulated the expression of PI3K and activated the AKT pathway to promote the expression of p-AKT. In addition, the promoting effect of high dose and short time arsenic exposure on HIF-1α was more obvious in normal tissues cells; the PI3K inhibitor could inhibit the expression of HIF-1α. These results suggested that arsenic could increase the expression of HIF-1α and induce malignant proliferation of cells by activating the PI3K/AKT pathway.

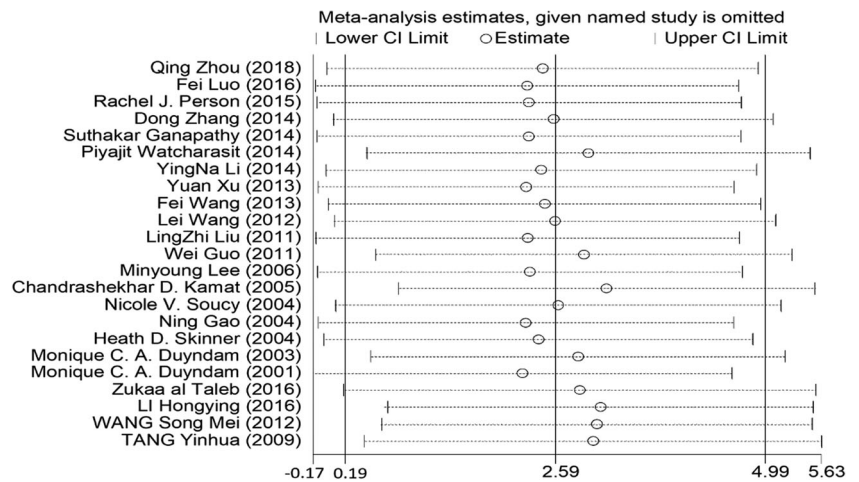
In addition to the PI3K/AKT pathway, miRNA-21 and VEGF can also interact with HIF-1α in arsenic-induced malignant cell proliferation and play an important role in the

process of malignant cell proliferation (Fig. 20). miRNA-21 is one of the most important oncogenes in the miRNA family. In Xiao Yang’s study [20], the results showed that miRNA-21 could be regulated by HIF-1α as a downstream indicator. When the level of miRNA-21 was upregulated, it could increase cell activity and promote cell proliferation. In this study, it was also found that arsenic could increase the expression of miRNA-21. We also compared the effect of HIF-1α activity groups and HIF-1α inhibitor groups to observe the effect of HIF-1α on miRNA-21. The results showed that HIF-1α activity groups could increase the expression of miRNA-21, and HIF-1α inhibitor groups could inhibit the expression of miRNA-21. The results suggested that arsenic could regulate the expression of miRNA-21 through HIF-1α and affect the proliferation of cells. VEGF mainly exists on the

**Fig. 18** The Funnel plot of HIF-1α. The blue dotted line shows the overall estimated standard mean difference. Evidence for publication bias was not found ( $P = 2.37$ ). SMD, standard mean difference; SE, standard error



**Fig. 19** Sensitivity analysis for HIF-1 $\alpha$ . Stable results were observed for all included studies, indicating that no individual study influenced the combined results. CI, confidence interval

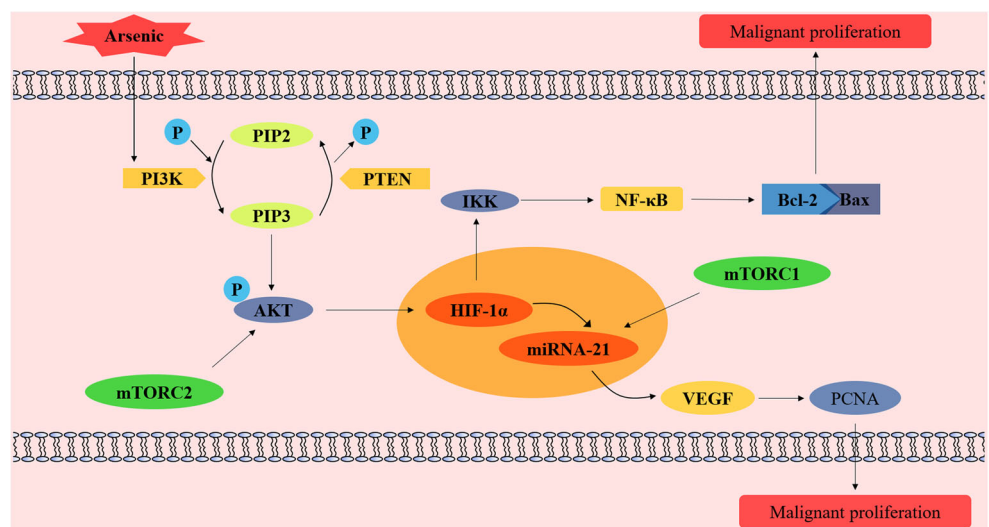


surface of vascular endothelial cells. VEGF plays an important role in promoting the growth of endothelial cells, inflammatory response, growth, and metastasis of tumor cells. In the study of LingZhi Liu [32] and Minyoung Lee et al. [35], the results showed that HIF-1 $\alpha$  could regulate the expression of VEGF in both humans immortalized lung epithelial cells and human prostate cancer cells. These results indicated that VEGF was likely to be the downstream target protein of HIF-1 $\alpha$ . We found that arsenic could increase the expression of VEGF. In normal tissues, high dose and short time arsenic exposure had a more obvious effect on the activation of VEGF. Then, we analyzed the effect of HIF-1 $\alpha$  inhibitor group to observe the effect of HIF-1 $\alpha$  on VEGF. The results showed that HIF-1 $\alpha$  inhibitor could inhibit the expression of VEGF, which indicated that arsenic could regulate the expression of VEGF through HIF-1 $\alpha$ . In order to demonstrate the interaction between miRNA-21 and VEGF, we found that miRNA-21 inhibitor could inhibit the expression of VEGF.

The above results revealed that arsenic could promote the expression of miRNA-21 through HIF-1 $\alpha$ . Furthermore, the level of VEGF was increased, and finally caused the malignant proliferation of cells.

There were still high levels of heterogeneity in this study; we analyzed the source of heterogeneity by subgroup analysis. The results showed that arsenic exposure dose and exposure time had great heterogeneity in the expression of HIF-1 $\alpha$  and VEGF. It showed that dose and time were the main sources of heterogeneity. In the published migration, we found that the literature was not completely evenly distributed on both sides of the ineffective line, and in the sensitivity analysis, we still found that the effect values of some literature deviated from the overall results. Therefore, the results of this study still need to be further confirmed by the experimental results. Moreover, a large number of studies have found that the effect of arsenic on HIF-1 $\alpha$  is not only related to PI3K/AKT pathway but also affected by

**Fig. 20** Mechanism of arsenic on HIF-1 $\alpha$ . Arsenic exposure could activate the PI3K/AKT pathway, further induce the high expression of HIF-1 $\alpha$ , and then upregulate the levels of miRNA-21 and VEGF through HIF-1 $\alpha$ , promote the expression of cell proliferation protein PCNA, and ultimately lead to malignant cell proliferation



NF- $\kappa$ B, Nrf2, mTOR, and other signal pathways [44–46]. We also need to further explore the relationship between other signaling pathways and HIF-1 $\alpha$  and further clarify the regulation mechanism of arsenic on HIF-1 $\alpha$ .

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