The effect of GSK-3β in arsenic-induced apoptosis of malignant tumor cells: a systematic review and meta-analysis

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ABSTRACT

Purpose: Arsenic has been reported to induce apoptosis in malignant tumor cells. Therefore, it has been investigated as a chemotherapy. From a mechanistic standpoint, the mitochondrial apoptosis pathway, mediated by GSK-3β, plays an important role in tumor cell apoptosis. Nonetheless, the regulation of GSK-3β by arsenic remains controversial. The study aimed to clarify the mechanism of GSK-3β in arsenic-induced apoptosis of tumor cells.

Materials and methods: We included 19 articles, which conducts the role of GSK-3β in the process of arsenic-induced tumor cell apoptosis by the meta-analysis.

Results: Compared with that of control group, the expression of GSK-3β (SMD = −0.92, 95% CI (−1.78, −0.06)), p-Akt (SMD = −5.46, 95% CI (−8.67, −2.24)) were increased in the arsenic intervention group. Meanwhile, the combined treatment of arsenic and Akt agonists can inhibit p-GSK-3β. Using the dose and time subgroup analysis, it was shown that the low-dose (<5 μmol/L) and sub-chronic (>24 h) arsenic exposure could inhibit the expression of p-Akt (P < 0.05). In the subgroup analysis of GSK-3β sites, arsenic could inhibit p-Akt and GSK-3β (Ser9) (SMD = −0.95, 95% CI (−1.56, −0.33)). There was a positive dose-response relationship between arsenic and p-GSK-3β when the dose of arsenic was less than 8 μmol/L. The expression of Mcl-1 and pro-caspase-3 were decreased, while the loss of mitochondrial membrane potential and cleaved-caspase-3 increased significantly when arsenic stimulated GSK-3β (Ser9) (P < 0.05).

Conclusion: The study revealed that arsenic could induce tumor cell apoptosis, by inhibiting p-Akt/GSK-3β, and triggering the Mcl-1-dependent mitochondrial apoptosis pathway.

Introduction

Since ancient times, arsenic and its compounds have been reported to have therapeutic effects on certain diseases (Swindell et al. 2013). As an anti-angiogenesis, arsenic has been reported to induce tumor cell apoptosis, inhibit cancer stem-like cell growth, increase the sensitivity of chemotherapy and radiotherapy (Huang and Zeng 2019). Thus, Arsenic trioxide is widely used in the treatment of malignant tumors such as acute promyelocytic leukemia (Niu et al. 1999). Meanwhile, it has been included in the clinical practice guidelines as an effective agent for leukemia in 2020 (Heuser et al. 2020). However, in the treatment of other cancers, arsenic and its compounds are merely studied in vitro. In these studies, arsenic-induced tumor cell apoptosis through a variety of signaling pathways. In addition, the mechanism of arsenic-induced apoptosis of malignant tumor cells is still unclear.

Glycogen Synthase Kinase 3β (GSK-3β) is a constitutive multifunctional serine/threonine kinase that induces tumor cell apoptosis through the destruction of oncoprotein products by the proteasome destruction (Sahin et al. 2019). Therefore, GSK-3β always plays an important role in the clinical treatment of tumors as a target of therapeutic drugs. It was reported that Nerigoside induced apoptosis in colorectal cancer cells (HT29, SW620) by inhibiting the ERK/GSK-3β/β-catenin signaling pathway (Wen et al. 2019). Moreover, GSK-3β is closely related to the function of the mitochondrial apoptotic pathway. It promotes the loss of mitochondrial membrane potential (the loss of ΔΨm) and releases of Cytochrome c (Linseman et al. 2004), which may be related to the regulation of Mcl-1 (myeloid cell leukemia-1) protein degradation (Yang et al. 2017). These results suggest that the mitochondrial apoptosis pathway mediated by GSK-3β plays an important role in the apoptosis of tumor cells.

GSK-3β can be regulated by a variety of signaling pathways to mediate mitochondrial activity, including PI3K/Akt, PKA, ERK, etc. (Yang et al. 2017). GSK-3β, as a downstream target protein of Akt, participates in the PI3K/Akt signaling pathway to regulate various biological processes such as cell cycle (Liang and Slingerland 2003), cell proliferation, and apoptosis (Xie et al. 2019; Deng et al. 2019b). In addition, Gao Y H demonstrated that Akt inhibitors can significantly...
reduce GSK-3β and increase the expression of proapoptotic proteins (Bax, Bak, and caspase-3) in gastric cancer cells (SGC-7901) (Gao et al. 2014). In summary, PI3K/Akt regulates the expression of GSK-3β and induces tumor apoptosis.

In recent years, many studies have focused on the mechanism of GSK-3β in arsenic-induced tumor cell apoptosis, whereas the GSK-3β regulatory effect on arsenic-induced cell apoptosis is paradoxical. Wang et al. (2013) demonstrated that the expression of p-GSK-3β was decreased in the apoptosis of acute myeloid leukemia cells (NB4, HL-60) induced by As₃O₃ (P < 0.05). On the contrary, Lo and Kwong (2014) found that the expression of p-GSK-3β in the arsenic intervention group was higher than that of the control group (P < 0.05), which induced MCL cells apoptosis (Jeko-1, Granta-519). For wild-type myelogenous leukemia cells (U937-AC NDRG2) (Park et al. 2019), there was no significant difference in the expression of GSK-3β and Mcl-1 between the As₃O₃ group and the control group (P > 0.05). Nowadays, the regulation of arsenic on GSK-3β is still unclear, and the systematic reviews of the relationship between arsenic and GSK-3β have not been reported. To clarify the mechanism of GSK-3β in arsenic-induced apoptosis of tumor cells, we used a meta-analysis of the literature on this topic and took advantage of existing evidence to illustrate the mechanism of GSK-3β in arsenic-induced cancer cells apoptosis, to provide a theoretical basis for elucidating the mechanism of arsenic tumor-inhibiting activity.

Materials and methods

Inclusion criteria

In this study, the inclusion criteria were formulated according to the principles of PICO; Research design: (1) Experimental research published in Chinese and English. (2) Research object (P): malignant tumor cells. (3) Intervention (I): The experimental group was exposed to arsenic or arsenic compounds. If there were time or dose-effect models related to arsenic and GSK-3β and PI3K/Akt in the study, we selected one group for analysis in each of the high-dose and low-dose groups or the acute and sub-chronic toxicity test. (4) Control (C): Blank control group without any intervention measures. (5) Outcome (O): Apoptosis-related indicators (caspase-3, caspase-9, Bax, Bak, PARP, p-PARP) and GSK-3β, p-GSK-3β, Akt, p-Akt, Mcl-1.

Exclusion criteria

(1) Non-Chinese or non-English papers. (2) The title or abstract of the paper does not contain arsenic or arsenic compounds and GSK-3β. (3) The literature does not contain clear apoptotic indicators (the rate of apoptotic changes in apoptosis-related indicators). (4) Repeated publication (published in both Chinese and English journals at the same time, the same author publishes similar articles in different magazines or the same data in articles published by the same author). (5) The data of the article is incomplete (lack of internal reference protein, the dose or time of arsenic poisoning is not clear). (6) The literature data cannot be extracted (the expression of GSK-3β or p-GSK-3β protein cannot be extracted). (7) Review articles, conference papers, or articles where only abstracts can be retrieved. 8) No control groups.

Search strategy

The literature included in this study came from PubMed, Web of Science, Cochrane Library, Excerpta Medica database (EMBASE), China National Knowledge Infrastructure (CNKI), Wan Fang Data databases, Wiper databases, and China Biology Medicine disk (CBM disk). Keywords for this search included: arsenic, arsenite, ATO, As₂O₃, NaAsO₂, Arsenic trioxide, GSK-3β, Glycogen Synthase Kinase 3 beta, apoptosis, caspase-3, caspase-9, Bax, Bcl-2, Mcl-1, Cytochrome c, PARP, Akt, and p-Akt.

Taking PubMed database as an example: (((((arsenic) OR As) OR ATO) OR Arsenic trioxide) OR NaAsO₂) OR arsenite) AND (((GSK-3β) OR Glycogen Synthase Kinase 3 beta) OR Glycogen Synthase Kinase 3 (β) AND ((((((apoptosis) OR caspase-3) OR caspase-9) OR Bax) OR Bcl-2) OR Mcl-1) OR Cytochrome c) OR PARP) OR Akt) OR p-Akt).

Search results

In this study, 265 articles were retrieved from 8 databases. According to the inclusion and exclusion criteria, 19 papers were finally included and screened by two different researchers. According to the PICO principle, a total of 265 articles were included. There were 91 duplicate articles (same articles were retrieved in different databases), 11 conference papers, 1 review, and 1 non-Chinese non-English document. All of them were excluded and the remaining 158 articles were left. Taking the title and abstract into perspective, 118 articles and 2 additional articles (not containing arsenic and GSK-3β), and 15 articles (not related to apoptosis) were eliminated. After studying the full text and re-screening the remaining 23 articles, 4 articles with incomplete or undesirable data were eliminated, and finally, 19 articles were included. The search deadline was October 31, 2020. The search results are shown in Figure 1.

Quality evaluation

This study made use of the Cochrane risk Migration assessment tool to systematically evaluate seven aspects for the 19 included articles.

(1) Random sequence generation (selection bias). (2) Allocation concealment (selection bias). (3) Blinding of participants and personnel (performance bias). (4) Blinding of outcome assessment (measurement bias). (5) Incomplete outcome data (attrition bias). (6) Selective reporting (reporting bias). (7) Other sources of bias (other bias).
Data collection

The data of this study was collected by two reviewers independently. The collected data were cross-checked. If the literature with inconsistent results or trends is encountered, the two reviewers independently re-extracted the data from the article. The literature was included and summarized according to the following information:

1. Title of the paper, lead author, publication date.
2. Research object characteristics: cell line.
3. Intervention: type of arsenic, dose, exposure time.
4. Baseline data: site, number of groups ($n$), related proteins (mean, standard deviation (SD)).

Data analysis

The purpose of this study was to explore the effects of arsenic on Akt, GSK-3β, caspase-3, caspase-9, Bax, Bcl-2, Mcl-1, Cyt-c, PARP, and to further explore the mechanism of GSK-3β apoptosis induced by arsenic. The data analysis was performed by Review Manager 5.3 (The Nordic Cochrane Center, The Cochrane Collaboration 2012, Portland, OR, USA) and Stata 12.0 (Stata Corp LP, College Station, TX, USA). Review Manager 5.3 software was used to evaluate the quality of the included literature according to the Cochrane risk deviation assessment tool. The apoptosis-related outcome indicators were continuous variables in this study. Taking into account the different units or large mean data included in the literature, the standardized mean difference (SMD) was used to reflect their effect size. The SMD formula used is: $d_i = \frac{\bar{x}_1 - \bar{x}_2}{SE}$ ($i = 1, 2, 3 \ldots k$)

In this study, the combined effect of each indicator in the experimental group and control group was described by standardized mean difference (SMD) and its 95% confidence interval. SMD and its confidence interval were observed by drawing a forest map. If $P > 0.05$ and the confidence interval contained 0, it could not be considered a difference between the experimental and control group. If $P < 0.05$ and the confidence interval did not contain 0, it indicates a statistical difference between the experimental and control group.

Heterogeneity is assessed by calculating $I^2$. According to the Higgins JP T study (Higgins et al. 2003), 25%, 50%, and 75% of $I^2$ were defined as low, medium, and high levels. The choice of the model was determined by observing the $P$-value and $I^2$. When $P < 0.05$ and $I^2 > 50\%$, the random effect model was selected; when $P > 0.05$ and $I^2 \le 50\%$, the fixed effect model was chosen. Heterogeneity in the study was explained by $I^2$. In this study, the subgroup analysis was used to find the sources of heterogeneity in the included 19 articles. Exposure dose ($\le 5 \mu$mol/L or $>5 \mu$mol/L), exposure time ($\le 24$ h or $>24$ h), and the GSK-3 site (Ser9 or non-Ser9) were used to determine the subgroups. Hence, GSK-3β was divided into two subgroups (Ser9 or non-Ser9) in the subgroup analysis of GSK-3β sites.

Meanwhile, R 4.0.1 software was used to establish a dose-effect model of arsenic to observe the dose-effect relationship of GSK-3β and Akt during the process of arsenic-induced apoptosis. The funnel chart was drawn by Review Manager 5.3 software to evaluate publication deviation. Stata 12.0 software was used for sensitivity analysis to evaluate the stability and reliability of the results. The Chi-Square test used $\alpha = 0.05$ as the significance level; all statistical analyses are carried out on both sides. When $P < 0.05$, the difference was considered to be statistically significant.
Network construction

The STRING database version 11.5 was employed to construct a protein-protein interactions network (PPIN) for gene targets of GSK-3β based on literature sources. The interactions included for network construction in this study include Experiments, Databases, Co-expression, Neighborhood, Gene Fusion, and Co-occurrence. The primary PPINs analysis was conducted using NetworkAnalyzer in Cytoscape 3.7.2. Meanwhile, each node in the constructed network was set to different sizes based on the degree, and different species lines are set to different colors by Cytoscape 3.7.2. According to the combined-score value among nodes, the relationship of nodes was defined as the intensity.

Results

The basic characteristics of included research

A total of 19 articles were included in this study, including GSK-3β site, arsenic type, dose, time, and other information, as shown in Table 1. The experimental group was treated with different types of arsenic, including 14 arsenic trioxide (As$_2$O$_3$), 3 arsenic sulfide (As$_2$S$_3$), and 2 sodium arsenite (NaAsO$_2$).

The arsenic exposure dose was divided into a low-dose group (≤5 μmol/L, n = 8) and a high-dose group (>5 μmol/L, n = 11). The exposure time was divided into acute exposure group (≤24 h, n = 17) and sub-chronic exposure group (>24 h, n = 2). According to the sites of arsenic acted on GSK-3β, they were divided into the Ser9 group (n = 10) and the non-Ser9 group (n = 8). The result variables were GSK-3β signaling pathway indicators (GSK-3β, p-GSK-3β, p-Akt, Akt, Mcl-1, p-Mcl-1) and apoptosis-related indicators (13 indicators were included).

| Authors                      | Site | Year | Language | n  | Arsenic      | Dose  | Time | Outcome | Cell line |
|------------------------------|------|------|----------|----|--------------|-------|------|---------|-----------|
| (Huang et al. 2011)          | Ser9 | 2011 | English  | 3  | As$_2$O$_3$  | >5    | ≤24  | 1,2,3   | A431, HaCaT |
| (Tan et al. 2019)            | Ser9 | 2019 | English  | 3  | As$_2$O$_3$  | >5    | ≤24  | 1,2,3,5,6,7,8,9,10 | PC12     |
| (Deng et al. 2019a)          | Total| 2019 | Chinese  | 3  | As$_2$O$_3$  | >5    | ≤24  | 1,2,3,5,6,9   | PC12     |
| (Zhai et al. 2015)           | Total| 2015 | English  | 3  | As$_2$O$_3$  | ≤5    | >24  | 1,2,3,5   | HepG2, Huh7 |
| (Lo and Kwong 2014)          | Tyr-216| 2014 | English  | 3  | As$_2$O$_3$  | ≤5    | >24  | 1,3       | Jeko-1, Granta-519 |
| (Gao et al. 2014)            | Ser9 | 2014 | English  | 3  | NaAsO$_2$    | ≤5, >5| ≤24  | 1,2,3,4,5,6,8,11 | SGC-7901 |
| (Wang et al. 2018)           | Ser9 | 2018 | English  | 3  | As$_2$O$_3$  | ≤5    | ≤24  | 1,2,5     | 12,13,16,12,13,21 | MOLM13   |
| (Hossain et al. 2003)        | Total| 2003 | English  | 3  | NaAsO$_2$    | >5    | ≤24  | 1         | Jurkat    |
| (Zheng et al. 2016)          | Ser9 | 2016 | English  | 3  | As$_2$O$_3$  | ≤5    | ≤24  | 1         | LncAP, PC3 |
| (Guo et al. 2014)            | Total| 2014 | English  | 3  | NaAsO$_2$    | >5    | ≤24  | 1         | MEFs      |
| (Wang et al. 2013)           | Ser9 | 2013 | English  | 3  | As$_2$O$_3$  | ≤5    | ≤24  | 1,2,5,12,14 | NB4, HL-60 |
| (Watcharasit et al. 2008)    | Ser9 | 2008 | Chinese  | 3  | As$_2$S$_3$  | >5    | ≤24  | 1,2,3,5,6 | SH-SYSY   |
| (You et al. 2017)            | Total| 2017 | Chinese  | 3  | As$_2$O$_3$  | >5    | ≤24  | 1,2,3,4,5,12 | HL-60   |
| (Park et al. 2019)           | Ser9 | 2019 | English  | 3  | As$_2$O$_3$  | ≤5    | ≤24  | 1,2,3,5,6,11,12 | U937   |
| (Chen et al. 2019)           | Total| 2019 | English  | 3  | As$_2$O$_3$  | >5    | ≤24  | 1,1,2      | HL-60, U937 |
| (Chen et al. 2018)           | Ser9 | 2018 | English  | 3  | As$_2$O$_3$  | ≤5    | ≤24  | 1,2,3,4,5,6,7,8,11,12,13 | U937   |
| (Huang et al. 2020)          | Ser9 | 2020 | English  | 3  | As$_2$O$_3$  | >5    | ≤24  | 1,2,3,5,6,7,8,9,12,13 | K562   |
| (Jin et al. 2016)            | Total| 2016 | Chinese  | 3  | As$_2$O$_3$  | >5    | ≤24  | 1,2,3,4,5,11,12 | U937   |

Quality evaluation

The literature quality evaluation of the 19 documents, followed by the inclusion and exclusion criteria, found that the low-risk bias rate was greater than 75%, and the high-bias risk rate was less than 10%, as shown in the literature quality evaluation (Figure 2a).

The effect of arsenic on tumor cell apoptosis-related proteins

The expression of apoptosis-related indicators was increased in the arsenic intervention group. The apoptosis-related protein level of cleaved-caspase-3 (SMD = 7.48, 95% CI (3.35, 11.62)), cleaved-caspase-9 (SMD = 7.94, 95% CI (0.48, 15.40)), Bax (SMD = 2.87, 95% CI (0.26, 5.49)), p-PARP (SMD = 30.29, 95% CI (16.73, 43.85)) were increased and pro-caspase-3 was decreased (P = 0.002), while the expression of Bcl-2 and PARP were not statistically significant (P > 0.05, respectively; Figure 2b). Bak (SMD = −2.10, 95% CI (−3.83, −0.38)) and Mcl-1 (SMD = −2.25, 95% CI (−4.16, −0.33)) were decreased in the arsenic-exposed group (Figure 2c), the expression of Cytochrome c in the cytoplasm increased (SMD = 18.59, 95% CI (7.50, 29.69)), while the Cytochrome c in the mitochondria (SMD = −10.70, 95% CI (−18.35, −3.05)) were decreased (Figure 2d).

The effect of arsenic on the expression of GSK-3β protein in tumor cells

In the meta-analysis of arsenic on GSK-3β, the protein level of GSK-3β in the arsenic-exposed group was lower than that of the control group (SMD = −0.92, 95% CI (−1.78, −0.06); Figure 3a), and there was no statistically significant difference in the expression of p-GSK-3β (P > 0.05; Figure 3b).
The effects of arsenic on GSK-3β (Ser9) proteins in tumor cells

Compared with the control group, the expression of GSK-3β (Ser9) was decreased in the arsenic intervention group (SMD = 1.61, 95% CI (2.68, 0.55); Figure 3c). As to downstream apoptosis-related indicators of GSK-3β (Ser9) in the meta-analysis, the loss of ΔψM, the expression of cleaved-caspase-3, and cleaved-caspase-9 in the arsenic intervention group were increased, while the expression of pro-caspase-3 and pro-caspase-9 were significantly decreased (P < 0.05, respectively; Figure 3d).

The effect of arsenic on Akt-related proteins on tumor cells

The results of Figure 4 showed that there was no significant difference in the expression of Akt in the arsenic exposure group (Figure 4a), while the expression of p-Akt was significantly decreased (SMD = −5.46, 95% CI (−8.67, −2.24); Figure 4b).

The effect of arsenic on PI3K/Akt and GSK-3β signal related factors

The literature containing arsenic and Akt agonists were extracted from the 19 articles. Meanwhile, the arsenic exposed group was used as the control group, and the combined treatment with arsenic and Akt agonist was used as the experimental group. Compared with the control group (arsenic-exposed group), the expression of GSK-3β in the combined treatment group with arsenic and Akt agonist was no different (P > 0.05; Figure 4c), while the expression of p-GSK-3β was statistically decreased (SMD = −2.94, 95% CI (−5.47, −0.41); Figure 4d).

At the same time, we also included four additional pieces of literature with no arsenic intervention, taking the Akt inhibitor group as the experimental group, and the control group with no other treatment measures. The expression of p-GSK-3β in the experimental group was lower than that of the control group (SMD = −6.36, 95% CI (−8.94, −3.79); Figure 4e).

Subgroup analysis of arsenic exposure dose

The results of subgroup analysis showed that the expression of GSK-3β (Ser9) and pro-caspase-3 in the high-dose and low-dose arsenic intervention groups were decreased, and the expression of Bax and the loss of ΔψM were increased (P < 0.05, respectively). After a low-dose arsenic intervention, the expression of p-Akt was decreased (SMD = −4.17, 95% CI (−6.77, −1.57)), while the expression of cleaved-caspase-3 increased in the high-dose arsenic-exposed group (SMD = 12.70, 95% CI (5.21, 20.20), and the expression of GSK-3β, p-GSK-3β, Akt, p-GSK-3β (Ser9) showed no statistically difference (P > 0.05, respectively; Table 2).

Subgroup analysis of arsenic exposure time

There was no significant difference in the expression of GSK-3β, p-GSK-3β, and Akt in the acute exposure group (≤24 h) and sub-chronic exposure group (>24 h). The expression of p-Akt, GSK-3β (Ser9), and p-GSK-3β (Ser9) showed no significant differences in the acute arsenic exposure group.
Figure 3. The effect of arsenic on GSK-3β and p-GSK-3β. The forest plot shows the difference in the expression of GSK-3β and p-GSK-3β between the control group and the arsenic-exposed group. SMD, standardized mean difference; 95% CI, 95% confidence interval; GSK-3β, Glycogen Synthase Kinase 3β; (a) GSK-3β expression between the control group and the arsenic-exposed group. (b) The expression of p-GSK-3β between the control group and the arsenic-exposed group. (c) The effect of arsenic on GSK-3β (Ser9). (d) The effect of arsenic on GSK-3β (Ser9) downstream apoptotic factors; A, Mcl-1; B, the loss of ΔψM; C, cleaved-caspase-3; D, pro-caspase-3; E, cleaved-caspase-9; F, pro-caspase-9.

Figure 4. The effect of arsenic on Akt and p-Akt. The forest plot shows the difference between Akt and p-Akt expression between the control group and the arsenic-exposed group. SMD, standardized mean difference; 95% CI, 95% confidence interval; Akt, protein kinase B; (a) the expression of Akt between the control group and the arsenic-exposed group. (b) the expression of p-Akt between the control group and the arsenic-exposed group. (c) The GSK-3β expression between the arsenic-exposed group and the combined treatment group with arsenic and Akt agonist. (d) The expression of p-GSK-3β between the arsenic-exposed group and the combined treatment group with arsenic and Akt agonist. (e) The forest plot shows the difference in GSK-3β expression between the control group and the Akt inhibitor treatment group.
The loss of GSK-3β Cleaved-caspase-3 2.10 Bax 3.93 0.35 7.50 6.36 1.95 10.76 0.40 Pro-caspase-3 Mcl-1 p-Akt 

respectively; Figure 5b). 

changes in the expression of p-GSK-3β (ser9), p-Akt, Mcl-1, pro-caspase-3, cleaved-caspase-3, and the effect of the dose of arsenic on GSK-3β. 

In this study, the Spline model was used to explore the effect of the dose of arsenic on GSK-3β, p-GSK-3β, Akt, and p-Akt (Figure 6). The results showed that the content of p-GSK-3β existed a dose-response relationship with the arsenic exposure when the dose of arsenic was less than 8 μmol/L. It had also shown a downward trend when the dose of arsenic was more than 8 μmol/L (Figure 6b). In the dose-effect analysis of arsenic and Akt, as the dose of arsenic was less than 9 μmol/L, the expression of p-Akt showed a decreasing trend. The content of p-Akt decreased with the increase in the arsenic exposure dose when the dose of arsenic was more than 9 μmol/L (Figure 6d). There was no dose-response relation between GSK-3β and Akt (Figure 6a,c).

### Sensitivity analysis

Taking the sensitivity analysis of arsenic and GSK-3β as an instance, the points of all results were distributed on both sides of the midline, and the results of 19 references did not exceed the midline with 95% CI. After being excluded, the results did not change significantly, which revealed that the results of this study were relatively stable (Figure 7a).

### Publication bias

Taking arsenic and p-GSK-3β as an example to explore whether there is a publication bias, the funnel chart showed that the results of all the included literature were arranged symmetrically around the centerline, indicating that the publication offset was not significant (Figure 7b).

### Network analysis and PPIN characteristics

The PPINs were constructed for each GSK-3β separately using STRING to highlight the relationship between the target genes (Figure 8a). The networks were comprised of 10 nodes and 42 edges for targets of GSK-3β. GSK-3β was strongly correlated with Akt and CTNNB (interaction scores were 0.999, respectively).
Arsenic and its compounds have been widely used in the treatment of leukemia and other tumors, while there were only in vitro studies in the treatment of other tumors. At the same time, there were contradictions in the process of an arsenic agent inducing tumor cell apoptosis through the GSK-3β signaling pathway in vitro. In this study, the mechanism of arsenic-induced tumor cell apoptosis was explored through meta-analysis, and it provided a reference for perfecting the anti-tumor mechanism of arsenic. We found that arsenic could inhibit the expression of p-Akt to inhibit GSK-3β, and then down-regulate the expression of Mcl-1, finally mediate the mitochondrial apoptosis pathway. At the same time, we analyzed the relationship among the signaling molecules in the articles by string (Figure 8b). The results showed that GSK-3β was strongly correlated with a variety of molecules in this signaling pathway (Akt, Mcl-1, caspase-3, caspase-9, Cytochrome c). The results of this study provided a theoretical basis for the molecular mechanism of arsenic inhibitor tumor growth.

It was reported that arsenic could down-regulate the expression of GSK-3β (Ser9) and induce tumor cell apoptosis. Tan et al. (2019) showed that arsenic significantly decreased the expression of Akt and GSK-3β on PC12 cells. This study found that arsenic inhibited the expression of GSK-3β, p-Akt, and Mcl-1 protein to induce tumor cell apoptosis. Moreover, the combined treatment of arsenic and Akt agonists inhibited the expression of p-GSK-3β. In addition, GSK-3β was
regulated by multiple pathways in arsenic-induced tumor cell apoptosis, and the mechanism might be related to cross-talk between various signaling pathways. In the apoptosis of myelogenous leukemia induced by As$_2$O$_3$ (Park et al. 2019), NDRG2, as a carrier between PP2A and GSK-3β, promoted the dephosphorylation of GSK-3β and reduced the expression of its downstream Mcl-1 protein, and finally induced tumor cell apoptosis through mitochondrial apoptosis pathway (Figure 9). Similarly, As$_2$O$_3$ induces acute promyelocytic leukemia (NB4) apoptosis by down-regulating the expression of IL-3Rα and inhibiting PI3K/Akt signaling pathway. Meanwhile, the expression of p-GSK-3β was significantly reduced (Chen et al. 2014), suggesting that arsenic might inhibit IL-3Rα by GSK-3β (Figure 9). Furthermore, arsenic could down-regulate the expression of GSK-3β through activating NF-κB (Zhong et al. 2018) and IKKβ kinase (Guo et al. 2014) (Figure 9). The above studies indicated that arsenic can regulate GSK-3β in a variety of ways, thereby inducing apoptosis of malignant tumors. Furthermore, there are two sites for GSK-3β (Ser9 and Tyr-216). The subgroup analysis results showed that high-dose and low-dose arsenic could inhibit the expression of p-Akt, down-regulate GSK-3β (Ser9), and ultimately induce tumor cell apoptosis. Similarly, we have analyzed the expression of GSK-3β and p-GSK-3β according to different types of arsenic by subgroup analysis. The results showed that there was no significant difference
in the expression of GSK-3β in As$_2$O$_3$ and NaAsO$_2$ exposure groups. While the expression of p-GSK-3β was statistically decreased treatment by As$_2$S$_3$ (SMD = −3.09, 95% CI (−4.85, −1.32)). Due to the literature of As$_2$S$_3$ being insufficient (n = 3), the results were included in the supplementary materials rather than the results. Meanwhile, there was no difference in the expression of p-GSK-3β by different types of arsenic exposure. Therefore, the types of arsenic are not the source of its heterogeneity (Supplementary Figures 1, 2).

GSK-3β, which is closely related to mitochondrial function, can activate the apoptotic pathway of mitochondrial damage. In recent years, some studies have shown that GSK-3β participated in the opening of mitochondrial permeability transition pore (mPTP) and phosphorylation of GSK-3β at Ser9, which could increase the threshold of mPTP opening. The results suggested that the loss of ΔψM was significantly increased in the arsenic-exposed group, which further confirmed this apoptosis pathway. GSK-3β participated in regulating glycogen synthesis to change mitochondrial permeability, and further promoting the release of Cytochrome c in mitochondria (Vidri and Fitzgerald 2020). At the same time, the study found that the expression of Mcl-1 in the arsenic treatment group was decreased, and the expression of Bax, Bak, and caspase-3 was increased. It may be due to As$_2$O$_3$ induced phosphorylation of Bcl-2 which triggered the Caspase cascade reaction, and finally promoted the release of Cytochrome c (Watcharasil et al. 2008). This further implied that arsenic may trigger the mitochondrial damage pathway through GSK-3β. The intracellular GAPDH reaction where As$_3^+$ replaces Pi has remarkably similar kinetic constants to the Pi-dependent reaction after being exposed to arsenic (Finnegan and Chen 2012). Indeed, the different forms of arsenic, by antagonizing Pi’s action on ATP synthase, hinder the phosphorylation of ADP to ATP, reduce ATP production and the metabolism of cells. Mitochondria are the most important organelles for ATP production and metabolism. When ATP synthesis becomes inhibited or dysfunctional, it could lead to the dysregulation of mitochondrial Ca$^{2+}$ and excessive ROS production which triggers the early events of apoptosis (Brookes et al. 2004). In addition, most GSK-3β inhibitors have been co-crystallized with GSK-3β and all localize within the ATP-binding pocket of the enzyme (Meijer et al. 2004). What’s more, Mito-TEMPO, a mitochondrial-targeted antioxidant, has been reported to alleviate the down-regulation of the Akt/GSK3β/β-catenin pathway and the increase of mitochondrial damage after exposed to cadmium (Luo et al. 2021). The above studies suggest that arsenic may induce mitochondrial apoptosis by affecting ATP synthesis and metabolism, and subsequently activating the Akt/GSK-3β signaling pathway.

In the sensitivity analysis, the stability of the included literature was sound. The points of all the results were distributed on both sides of the midline, and the results did not change significantly after being excluded. Based on the funnel chart, the results of the included literature were arranged symmetrically around the centerline, and the publication bias was not significant. However, there were several limitations in this study. Non-English and non-Chinese literature were not included in the search, which might result in insufficient literature. At the same time, there were few pieces of literature involved in experiments in vivo (n = 2) (Zhai et al. 2015; Miltonprabu et al. 2017), which were not included in this study. p-GSK-3β had high heterogeneity in our research. Although we did subgroup analysis of time, dose, and GSK-3β sites, it might also be affected by other factors such as cell lines. Due to the number of included literature, the subgroup analysis of cell lines was not carried out in this study.

In summary, arsenic can inhibit the expression of PI3K/Akt and GSK-3β(Ser9), down-regulate the expression of Mcl-1 protein, and trigger apoptosis mediated by the mitochondrial pathway. The role of NDRG2, IL-3Rx, NF-kB, and other molecular relationships in the regulation of GSK-3β by arsenic should be further explored in the future, to clarify the molecular mechanism of arsenic regulating GSK-3β.

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**Author contributions**

Xin Gao contributed significantly to analysis and manuscript preparation, extract data from the literature, performed the data analyses, and wrote the manuscript; Bin Deng and Shanshan Ran contributed to the conception of the study; Shugang Li helped perform the analysis with constructive discussions.

**Consent for publication**

This paper is approved by all authors for publication.

**Disclosure statement**

The authors declare no conflicts of interest.

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**Data availability statement**

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

In this study, the datasets came from PubMed, Web of Science, Cochrane Library, Excerpta Medica database (EMBASE), China National Knowledge Infrastructure (CNKI), Wan Fang Data databases, Wiper databases, and China Biology Medicine disk (CBM disk).

1) PubMed (https://pubmed.ncbi.nlm.nih.gov/), 2) EMBASE (https://www.embase.com/), 3) Web of Science (http://isiknowledge.com/wos), 4) Cochrane Library (https://www.cochrane.org/library), 5) CNKI (https://www.cnki.net/), 6) Wan Fang Data databases (http://www.wanfangdata.com.cn/index.html), 7) Wiper databases (http://www.cqvip.com), 8) CBM disk (http://www.sinomed.ac.cn/).
