A systematic review and meta-analysis of bidirectional effect of arsenic on ERK signaling pathway

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Abstract. Arsenic is a toxic metal, which ultimately leads to cell apoptosis. ERK is considered a key transcriptional regulator of arsenic-induced apoptosis. Due to a few controversial issues about arsenic-mediated extracellular signal-regulated MAP kinases (ERK) signaling, a meta-analysis was performed. Subgroup analyses demonstrated that high doses (≥2 μmol/l) of arsenic increased the expression of Ras, ERK, ERK1, ERK2, phosphorylated (p)-ERK, p-ERK1, and p-ERK2, while low doses (<2 μmol/l) decreased the expression of Ras, ERK1, p-ERK, and p-ERK2 when compared to control groups. Long term exposure (>24 h) to arsenic led to inhibition of expression of ERK1, p-ERK1, and p-ERK2, whereas short-term exposure (≤24 h) triggered the expression of ERK1, ERK2, p-ERK, p-ERK1, and p-ERK2. Furthermore, normal cells exposed to arsenic exhibited higher production levels of Ras and p-ERK. Conversely, exposure of cancer cells to arsenic showed a lower level of production of Ras and p-ERK as well as higher level of p-ERK1 and p-ERK2 as compared to control group. Short-term exposure of normal cells to high doses of arsenic may promote ERK signaling pathway. In contrast, long-term exposure of cancer cells to low doses of arsenic may inhibit ERK signaling pathway. This study may be helpful in providing a theoretical basis for the diverging result of arsenic adverse effects on one hand and therapeutic mechanisms on the other concerning arsenic-induced apoptosis.

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Abbreviations: MAPK, mitogen-activated protein kinases; ERK, extracellular signal-regulated MAP kinases; MEK, mitogen-induced extracellular kinase; p-ERK, phosphorylated extracellular signal-regulated kinase; caspase-3, cysteinyl aspartate-specific protease-3; Bcl-2, B-cell lymphoma/leukemia-2 protein; Bax, Bcl-associated X protein

Key words: arsenic, extracellular signal-regulated MAP kinases, bidirectional effect, apoptosis, meta-analysis

Introduction

Arsenic (As) is a naturally occurring toxic metal which was classified as potentially poisonous substance (1). Excessive exposure to arsenic damages multiple organs (2). Presently, a mounting number of studies preferably examined the molecular mechanisms of apoptosis induced by arsenic (3,4). It was believed that the mitogen-activated protein kinases (MAPK) signaling pathway was implicated in cell injury, proliferation, and apoptosis (5). The extracellular signal-regulated MAP kinases (ERK), an important member of the MAPK families, became phosphorylated and activated in response to diverse environmental stimuli (6).

It had been postulated that ERK was consequently a participant in arsenic-induced apoptosis (7,8). In all these studies, however, not all scholars were in agreement on the issue of arsenic mediating ERK signaling. Escudero-Lourdes *et al* (9) found that exposure of urothelial cells to 0.05 μmol/l of arsenic for 12 months significantly increased protein expression of p-ERK1 and p-ERK2, which indicated that the ERK signaling pathway was activated by arsenic. Conversely, Wang *et al* (10) drew a different conclusion stating that arsenic restrained the ERK signaling pathway due to the fact that inhibition and lowering of p-ERK1 and p-ERK2 levels were observed in human leukemia cell lines after being exposed to 2.5 μmol/l of arsenic for 24 h. Evidently, the effects of arsenic on ERK signaling pathway remained a debatable issue.

To probe the role of ERK signaling pathway in arsenic-induced apoptosis, a meta-analysis of experimental studies published in domestic and foreign literature was performed in our paper. The present study may be helpful in providing a theoretical basis for the diverging result of arsenic adverse effects on one hand and therapeutic mechanisms on the other concerning arsenic-induced apoptosis.

Materials and methods

Inclusion criteria. Inclusion Criteria and literature search terms were identified according to the PICO principle.

Study design. Experimental studies published in Chinese and English.

Participants (P). All cell lines and animals, disregarding age, gender and weight.

Intervention (I). All experimental groups treated with any kind of arsenic or its compounds. Arsenic model groups might show the change in indicators associated with ERK signaling pathway and apoptosis. If variable dosages of arsenic or exposure times were used in a study, the highest or longest one was chosen for this analysis.

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

Outcome (O). Following indicators in mediating of ERK signaling were used, Ras (or p21) protein, Raf protein, Mitogen-induced extracellular kinase (MEK), Total extracellular signal-regulated MAP kinases (ERK), Extracellular signal-regulated kinase 1 (ERK1), Extracellular signal-regulated kinase 2 (ERK2), Total phosphorylated extracellular signal-regulated kinase (p-ERK), Phosphorylated extracellular signal-regulated kinase 1 (p-ERK1), Phosphorylated extracellular signal-regulated kinase 2 (p-ERK2), Cysteinyl aspartate-specific protease-3 (caspase-3), Apoptotic cells (%), Pro-apoptotic protein-Bcl-associated X protein (Bax), Anti-apoptotic protein-B-cell lymphoma/leukemia-2 protein (Bcl-2).

Exclusion criteria. We excluded the studies upon following criteria: i) the papers focused only on ERK but not arsenic; ii) the papers focused on arsenic without investigating ERK; iii) no outcome indicators (as stated in '2.1.5 Outcome'); iv) duplicate publications; v) review articles; vi) inadequate information; and vii) no available data.

Search strategy. A systematic search was conducted using Cochrane Library, PubMed, Excerpta Medica database (EMBASE), Springer, Web of Science, Chinese Biomedical Literature Database (CBM), China National Knowledge Infrastructure (CNKI) and Wan Fang Data databases (last search conducted on January 24th, 2017). The key search string was 'arsenic AND (Ras OR Raf OR MEK OR ERK)'.

Quality assessment. The Cochrane collaboration's tool for bias risk assessment was used to evaluate the quality of 42 articles identified in the present study. The evaluation system consisted of seven aspects, viz. i) Random sequence generation (selection bias); ii) allocation concealment (selection bias); iii) blinding of participants and personnel (performance bias); iv) blinding of outcome assessment (detection bias); v) incomplete outcome data (attrition bias); vi) Selective reporting (reporting bias); and vii) other bias. The rating criteria were as follows, low risk of bias, unclear risk of bias and high risk of bias.

Data collection. Two reviewers (Dongjie Li and Yutao Wei) independently extracted data which was then cross-checked before putting the results into a collective spreadsheet. If the results seem to be inconsistent, Dr. Shugang Li and Mingxia Jing were asked to verify before final confirmation. The following information was documented meticulously out of completed manuscripts from each qualified study: i) information about the paper including title, first author, publication date and the name of the journal where published; ii) characteristics of the research object including the type and source of cell lines and breed of animals; iii) type, dosage and exposure time of arsenic; iv) outcome indicators; and v) baseline data for experimental and control groups, viz. number of groups (n), mean and standard deviation (SD).

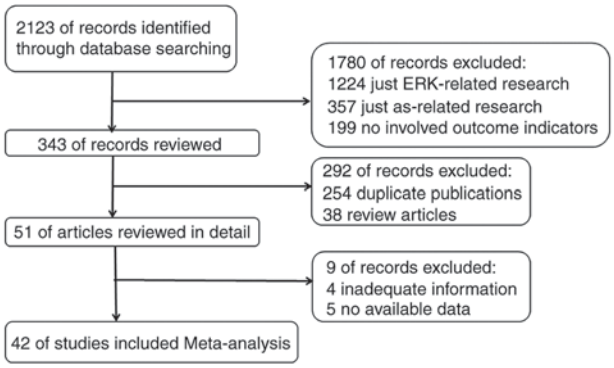

Figure 1. Flow chart of identifying and including studies. ERK, extracellular signal-regulated MAP kinases.

Data analysis. Forty-two articles were analyzed using Review Manager Version 5.2 (The Nordic Cochrane Centre, The Cochrane Collaboration 2012, Portland, OR, USA) and Stata 12.0 (StataCorp LP, College Station, TX, USA). Standardized mean difference (SMD) was chosen for consolidating statistical data. Heterogeneity was detected by calculating the I^2 index. I^2 ≤50% and >50% represented low and high levels of heterogeneity, respectively. Random effects model was chosen when $P<0.05$ and I^2 >50%, and fixed effects model was used when $P>0.05$ and I^2 ≤50%. Subgroup analyses and meta-regression analyses (including univariate and multivariate meta-regression analyses) were conducted to examine sources of heterogeneity among 42 studies. Subgroup analyses were performed on the basis of the source (normal and cancer cells), exposure time (>24 h and ≤24 h) and dosage of arsenic (≥2 and <2 μmol/l). The combined effect was estimated as SMD with 95% confidence interval (95% CI) between arsenic model and control group. All reported P-values were two-sided and $P<0.05$ was considered to indicate a statistically significant difference. Small-study effects were assessed by using funnel plots. Egger's tests and sensitivity analyses were conducted using Stata 12.0.

Results

Search results. A total of 2,123 articles were initially identified by search criteria. Utilizing our inclusion and exclusion criteria, 42 of those articles were qualified for meta-analysis (Fig. 1).

Basic characteristics of included studies. Characteristics of the studies included in this meta-analysis were listed in Table I. In the present study, the effects of arsenic on ERK signaling pathway was assessed. Arsenic model groups encompasses

Table I. Characteristics of the studies included in the meta-analysis.

Author (Refs.)	Year	Language	n	Type of arsenical compounds	Dosage of arsenic, μmol/l	Time of exposure, h	Type of cells	Outcome indicators
Yen *et al* (2)	2011	English	12	As_2O_3	<2	>24	Normal cells	5, 6, 8, 9, 11, 12
Eguchi *et al* (3)	2011	English	3	As_2O_3	≥2	≤24	Cancer cells	5, 6, 8, 9, 11
Ray *et al* (4)	2013	English	3	As_2O_3	≥2	≤24	Normal cells	4, 7, 11
Lau *et al* (5)	2004	English	3	$NaAsO_2$	≥2	≤24	Normal cells	5, 6, 8, 9
Li *et al* (6)	2006	English	3	$NaAsO_2$	≥2	≤24	Cancer cells	6, 8, 9
Lozano-Santos *et al* (7)	2015	English	3	As_2O_3	<2	>24	Cancer cells	7, 12, 13
Ge *et al* (8)	2005	Chinese	3	AA	≥2	>24	Cancer cells	10
Escudero-Lourdes *et al* (9)	2010	English	3	MMA	<2	>24	Normal cells	4, 5, 6, 8, 9
Wang *et al* (10)	2012	Chinese	3	As_2O_3	≥2	≤24	Cancer cells	8, 9, 11
Daum *et al* (11)	2001	English	3	$NaAsO_2$	≥2	≤24	Normal cells	8, 9
Benbrahim-Tallaa *et al* (12)	2005	English	3	$NaAsO_2$	≥2	>24	Normal cells	1
Chowdhury *et al* (13)	2010	English	3	$NaAsO_2$	≥2	≤24	Normal cells	5, 6, 8, 9
Li *et al* (14)	2010	Chinese	3	$NaAsO_2$	≥2	≤24	Normal cells	7
Suzuki *et al* (15)	2011	English	3	As_2O_3	≥2	≤24	Cancer cells	5, 6, 8, 9
Guilbert *et al* (16)	2013	English	3	As2O3	≥2	≤24	Cancer cells	4, 8, 9
Huff *et al* (17)	2016	English	3	$NaAsO_2$	<2	≤24	Cancer cells	5, 6, 8, 9
Wang *et al* (18)	2012	English	3	As_2O_3	≥2	≤24	Normal cells	5, 6, 8, 9(18)
Aodengqimuge *et al* (19)	2014	English	3	$NaAsO_2$	≥2	≤24	Normal cells	4, 7, 10
Gong *et al* (20)	2016	English	3	$NaAsO_2$	≥2	≤24	Normal cells	5, 6, 8, 9, 10, 12
Person *et al* (21)	2015	English	3	$NaAsO_2$	≥2	>24	Normal cells	1, 4, 7
Huang *et al* (22)	1999	English	3	$NaAsO_2$	≥2	≤24	Cancer cells	5, 6, 8, 9
Martinez-Finley *et al* (23)	2011	English	4	$NaAsO_2$	<2	>24	Normal cells	1, 2, 5, 6, 8, 9
Estañ *et al* (24)	2012	English	3	As_2O_3	≥2	≤24	Cancer cells	5, 6, 8, 9, 10, 11,12
Zheng *et al* (25)	2006	Chinese	3	As_2O_3	≥2	≤24	Cancer cells	4, 10, 11
Zhang *et al* (26)	2015	Chinese	3	$NaAsO_2$	≥2	>24	Normal cells	1, 2, 3, 5, 6, 7
Luo *et al* (27)	2012	Chinese	3	$NaAsO_2$	≥2	≤24	Normal cells	5, 6, 8, 9, 10, 11, 12, 13
Banerjee *et al* (28)	2011	English	3	As2O3	<2	≤24	Normal cells	4, 7, 11
Wu *et al* (29)	2008	Chinese	3	As_2O_3	≥2	>24	Cancer cells	8, 9, 10, 13
Li *et al* (30)	2016	Chinese	8	As_2O_3	<2	≤24	Cancer cells	1, 2, 3, 4, 11, 12, 13
Ye (31)	2006	Chinese	3	As_2O_3	<2	≤24	Cancer cells	5, 6, 10
Iwama *et al* (32)	2001	English	3	As_2O_3	≥2	≤24	Cancer cells	3, 5, 6, 8, 9, 10, 11, 13
Calviño *et al* (33)	2011	English	3	As_2O_3	≥2	≤24	Cancer cells	4, 7, 10, 11, 12
Liu *et al* (34)	2006	English	3	As_2O_3	≥2	≤24	Cancer cells	1, 6, 8, 9
Huang *et al* (35)	2006	English	3	As_2O_3	≥2	≤24	Cancer cells	1, 4, 5, 6, 7
Liao *et al* (36)	2015	English	3	$NaAsO_2$	≥2	≤24	Normal cells	5, 6,7
Wang (37)	2012	Chinese	3	$NaAsO_2$	≥2	≤24	Normal cells	8, 9
Ngalame *et al* (38)	2014	English	3	$NaAsO_2$	≥2	>24	Normal cells	1, 7
Ju (39)	2007	Chinese	3	As_2O_3	≥2	≤24	Cancer cells	8, 9, 10, 11
Petit *et al* (40)	2013	English	3	As_2O_3	≥2	≤24	Cancer cells	5, 6, 8, 9

Table I. Continued.

Author/(Refs.)	Year	Language	n	Type of arsenical compounds	Dosage of arsenic, μmol/l	Time of exposure, h	Type of cells	Outcome indicators
Lu *et al* (41)	2014	English	3	As_2O_3	≥2	≤24	Cancer cells	5, 6, 8, 9, 11, 12, 13
Liu *et al* (42)	2013	Chinese	3	As_2O_3	<2	>24	Cancer cells	1, 7
Zhao *et al* (43)	2015	English	3	As_2O_3	<2	>24	Cancer cells	1

n, number of experimental group; N, normal cells; C, cancer cells; ERK, extracellular signal-regulated MAP kinases; MEK, mitogen-induced extracellular kinase; p-ERK, phosphorylated extracellular signal-regulated kinase; Raf, serine/threonine-specific protein kinases; Bcl-2, B-cell lymphoma/leukemia-2 protein; Bax, Bcl-associated X protein; caspase-3, cysteinyl aspartate-specific protease-3; RTKs, receptor tyrosine kinases. 1, Ras; 2, Raf; 3, MEK; 4, ERK; 5, ERK1; 6, ERK2; 7, p-ERK; 8, p-ERK1; 9, p-ERK2; 10, Apoptotic cells; 11, caspase-3; 12, Bax; 13, Bcl-2.

those cell lines which were treated with various forms of arsenic including sodium arsenite ($NaAsO_2$), arsenic trioxide (As_2O_3), monomethyl arsenous acid (MMA) and arsenious acid (AA). The control models were blank controls without any exposure to arsenic. In subgroup analyses, arsenic exposure time varied among the studies and hence was stratified into ≤24 h (n=30) and >24 h (n=12). The dosage of arsenic was also variable and thus was differentiated into ≥2 μmol/l (n=32) and <2 μmol/l (n=10) groups. Likewise, different cell lines were separated into normal cells (n=19) and cancer cells (n=23). In this review, cancer cells included the following ones, U937 cells (human leukemia cell line), A431 cells (human epidermoid carcinoma cells), CL3 cell line (non-small-cell lung carcinoma cell line), Flt3-ITD cells (acute myeloid leukemia cells), JB6 Cl 41 mass cells, NCI-H2052 cells (human mesothelioma cells), HL-60 cells (human leukemia cell line), SGC7901/ADM (human gastric cancer cell line), MDA-MB-468 (breast cancer cells), SH-SY5Y (human neuroblastoma cells), Neuro-2a cells (murine neuroblastoma cell line), CLL cells (chronic lymphocytic leukemia cell line, but not the WSU-CLL cell line), SGC7901/S (human gastric cancer cell line), NCI-H1793 (non-small-cell lung carcinoma cell line), U-251 MG cells (human glioma cells), NCI-H157 (non-small-cell lung carcinoma cell line), BEL-7402 cells (human hepatocarcinoma cells), FRO (anaplastic thyroid cancer cell line) and Hela cells (cervical cancer cells). NCI-H157 was a misidentified cell line according to http://iclac.org/wp-content/uploads/Cross-Contaminations-v8_0.pdf.Taking all these factors into consideration, we have arrived at the conclusion that time (P=0.012) and dosage (P=0.037) were statistically significant in the univariate meta-regression analysis. Outcome variables were assessed for any association with apoptosis (including apoptotic cells, caspase-3, Bax, and Bcl-2) and ERK signaling pathway (i.e., Ras, Raf, MEK, ERK, ERK1, ERK2, p-ERK, p-ERK1, and p-ERK2).

Quality assessment of included studies. The quality of the 42 articles identified in the study was evaluated (Table II) and the proportion of low risk was accounted for more than 75% (Fig. 2).

Meta-analysis of arsenic-related apoptosis. A pooled analysis showed that apoptotic cell levels were 3.84-fold higher in arsenic exposed group compared to those of control (95% CI (1.44, 6.24)). Levels of caspase-3 were 11.67 times higher in the exposed group than in control group (95% CI (7.06, 16.28)). Bax levels were 5.27-fold higher in the exposed group compared to control group (95% CI (2.18, 8.36)). Levels of Bcl-2 were 2.08 times lower in the exposed group than in control group (95% CI (-2.96, -1.21)) (Fig. 3).

Meta-analysis regarding arsenic and level of ERK. Levels of p-ERK1 were 3.59 times higher in exposed group as compared to control group (95% CI (0.45, 6.74)). The levels of p-ERK2 were comparatively 4.39 times higher in exposed than in control group (95% CI (0.12, 8.67)). Raf levels were 1.78-fold lower in exposed than in control group (95% CI (-2.72, -0.85)). Similarly, MEK levels were 1.61 times lower in exposed group as compared to control group (95% CI (-2.59, -0.63)). There was no statistical difference in Ras, ERK, ERK1, ERK2, and p-ERK levels (P>0.05) (Fig. 4).

Subgroup analyses of arsenic exposure effects

Subgroup analyses based on sources of arsenic. The analysis had demonstrated that arsenic promoted the expressions of Ras and p-ERK (P<0.05) in normal cells. Though, in cancer cells, arsenic decreased the expressions of Ras and p-ERK as well as caused an increase in p-ERK1 and p-ERK2 levels (P<0.05) (Fig. 5).

Subgroup analyses based on exposure time of arsenic. Our results showed that arsenic exposure time of >24 h had suppressed the levels of ERK1, p-ERK1, and p-ERK2 (P<0.05), conversely arsenic exposure time of ≤24 h promoted the levels of ERK1, ERK2, p-ERK, p-ERK1, and p-ERK2 (P<0.05) (Figs. 6, 7).

Subgroup analyses based on arsenic dose. Subgroup analyses exhibited increased expressions of Ras (SMD=7.29, 95% CI (0.90, 13.68)), ERK (SMD=4.62, 95% CI (0.17, 9.07)), ERK1 (SMD=5.28, 95% CI (1.02, 9.54)), ERK2 (SMD=8.17, 95% CI (2.73, 13.62)), p-ERK (SMD=6.15, 95% CI (0.20, 12.11)), p-ERK1 (SMD=4.48, 95% CI (1.67, 7.30)), p-ERK2 (SMD=7.28, 95% CI (2.87, 11.70)) with high doses of arsenic

Table II. Quality assessment of included studies.

Author/(Refs.)	Year	1	2	3	4	5	6	7
Huang *et al* (22)	1999	L	L	L	L	L	L	L
Iwama *et al* (32)	2001	L	L	L	L	L	L	L
Daum *et al* (11)	2001	L	U	L	L	L	L	L
Benbrahim-Tallaa *et al* (12)	2005	L	L	L	L	L	L	L
Liu *et al* (34)	2006	L	L	L	L	L	L	L
Huang *et al* (35)	2006	L	L	L	L	L	L	L
Li *et al* (6)	2006	L	L	L	L	L	L	L
Chowdhury *et al* (13)	2010	L	L	H	L	L	U	L
Li *et al* (14)	2010	L	L	L	L	L	L	L
Calviño *et al* (33)	2011	L	L	L	L	L	L	L
Suzuki *et al* (15)	2011	L	L	L	L	L	L	L
Banerjee *et al* (28)	2011	L	L	L	L	L	L	L
Eguchi *et al* (3)	2011	L	L	L	L	L	L	L
Martinez-Finley *et al* (23)	2011	L	L	L	L	L	L	L
Estañ *et al* (24)	2012	L	L	L	L	L	L	L
Wang *et al* (10)	2012	L	U	L	L	L	L	L
Liu *et al* (42)	2013	L	L	L	L	L	L	L
Guilbert *et al* (16)	2013	L	L	L	L	L	L	L
Ray *et al* (4)	2013	L	L	L	L	L	L	L
Ngalame *et al* (38)	2014	L	L	L	L	L	L	L
Lu *et al* (41)	2014	L	L	L	L	L	L	L
Lozano-Santos *et al* (7)	2015	L	L	H	L	L	L	L
Zhao *et al* (43)	2015	L	L	L	L	L	L	L
Huff *et al* (17)	2016	L	L	L	L	L	L	L
Zheng *et al* (25)	2006	L	U	L	L	L	L	L
Liao *et al* (36)	2015	L	L	L	L	L	L	L
Wu *et al* (29)	2008	L	L	L	L	L	L	L
Zhang (26)	2015	L	L	L	L	L	L	L
Escudero-Lourdes *et al* (9)	2010	L	L	L	L	L	L	L
Yen *et al* (2)	2011	L	L	L	L	L	L	L
Wang *et al* (18)	2012	L	L	L	L	L	L	L
Aodengqimuge *et al* (19)	2014	L	L	L	L	L	L	L
Gong *et al* (20)	2016	L	L	L	L	L	L	L
Ju (39)	2007	L	L	L	L	L	L	L
Ge *et al* (8)	2005	L	U	L	L	L	L	L
Luo (27)	2012	L	U	L	L	L	L	L
Li *et al* (30)	2016	L	L	L	L	L	L	L
Wang *et al* (10)	2012	L	L	L	L	L	L	L
Ye (31)	2006	L	L	L	L	L	L	L
Person *et al* (21)	2015	L	L	L	L	L	L	L
Lau *et al* (5)	2004	L	L	L	L	L	L	L

1, Random sequence generation (selection bias); 2, Allocation concealment (selection bias); 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); 7, Other bias; L, low risk of bias; U, unclear risk of bias; H, high risk of bias.

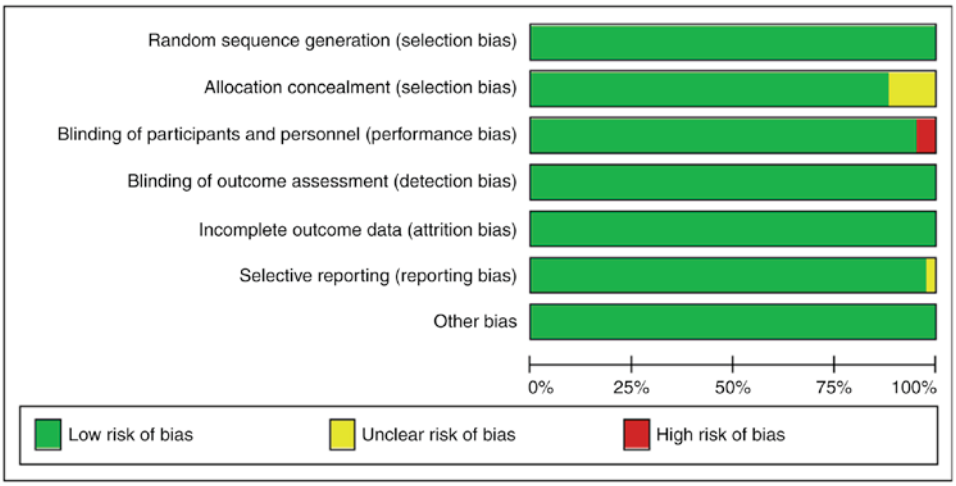

Figure 2. Risk of bias graph.

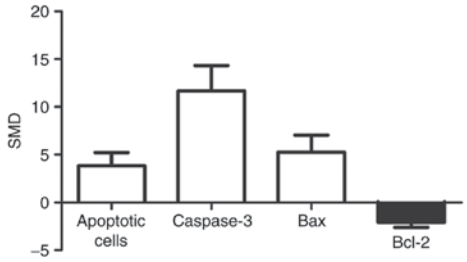

Figure 3. Effects of arsenic on apoptosis. SMD, standardized mean difference; caspase-3, cysteinyl aspartate-specific protease-3; Bcl-2, B-cell lymphoma/leukemia-2 protein; Bax, Bcl-associated X protein.

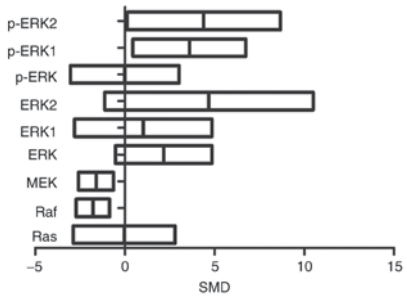

Figure 4. Effects of arsenic exposure on ERK. SMD, standardized mean difference; ERK, extracellular signal-regulated MAP kinases; MEK, mitogen-induced extracellular kinase; p-ERK, phosphorylated extracellular signal-regulated kinase; Raf, serine/threonine-specific protein kinases.

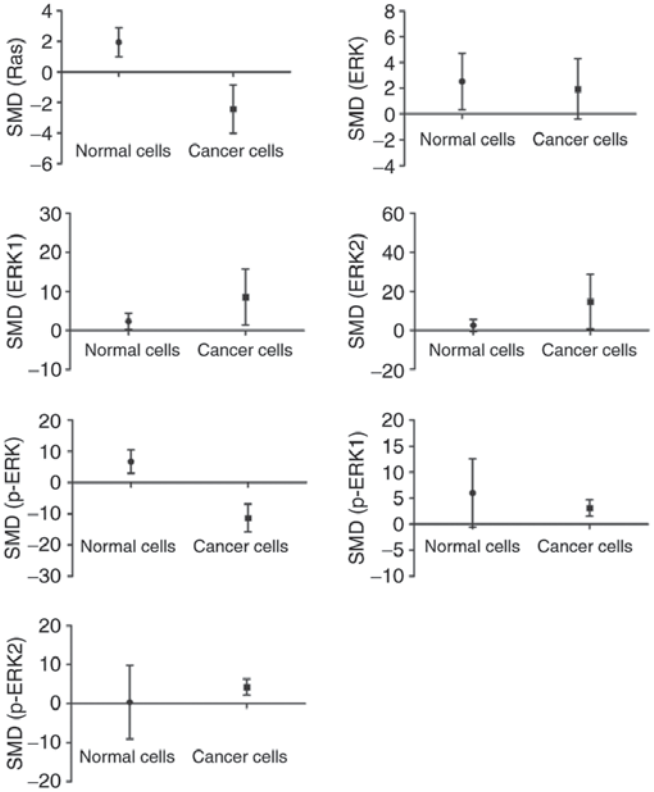

Figure 5. Subgroup analyses to determine the effects of arsenic on ERK based on source. SMD, standardized mean difference; ERK, extracellular signal-regulated MAP kinases; MEK, mitogen-induced extracellular kinase; p-ERK, phosphorylated extracellular signal-regulated kinase.

(≥2 μmol/l). Conversely decreased expressions of Ras (SMD=-3.96, 95% CI (-5.36, -2.56)), ERK1 (SMD=-10.11, 95% CI (-18.40, -1.81)), p-ERK (SMD=-6.07, 95% CI (-11.89, -0.26)), p-ERK2 (SMD=-20.34, 95% CI (-39.58, -1.11)) were seen with low doses (<2 μmol/l) (Fig. 8).

Small-study effect evaluation. The funnel plot (Fig. 9) shows that there was a symmetrical distribution of all the studies, suggesting no significant small-study effects.

Sensitivity analysis. A sensitivity analysis was performed for p-ERK. The results of all the studies were distributed evenly from the center line and no significant deviation was seen. Thus, there seems to be no individual study affecting the combined results (Fig. 10).

Discussion

Arsenic contributes to cell apoptosis (3) leading to serious damage (23). However, arsenic has recently been explored for its anti-tumor ability in leukemia and other malignant tumors

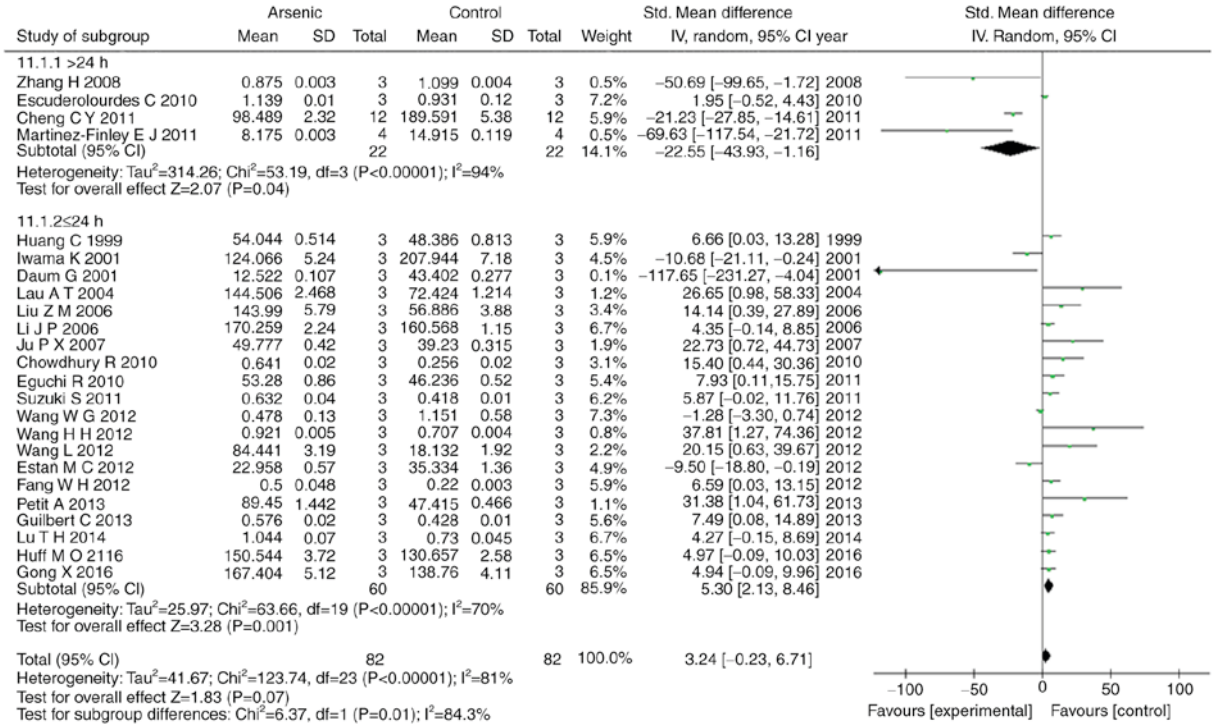

Figure 6. Subgroup analyses to determine the effects of arsenic on p-ERK1 based on exposure time. Forest plot showing the impact of arsenic treatment on p-ERK1 compared to controls. Total column represents total number of studies performed. SMD, standardized mean difference; IV, independent variable; 95% CI, 95% confidence interval; SD, standard deviation.

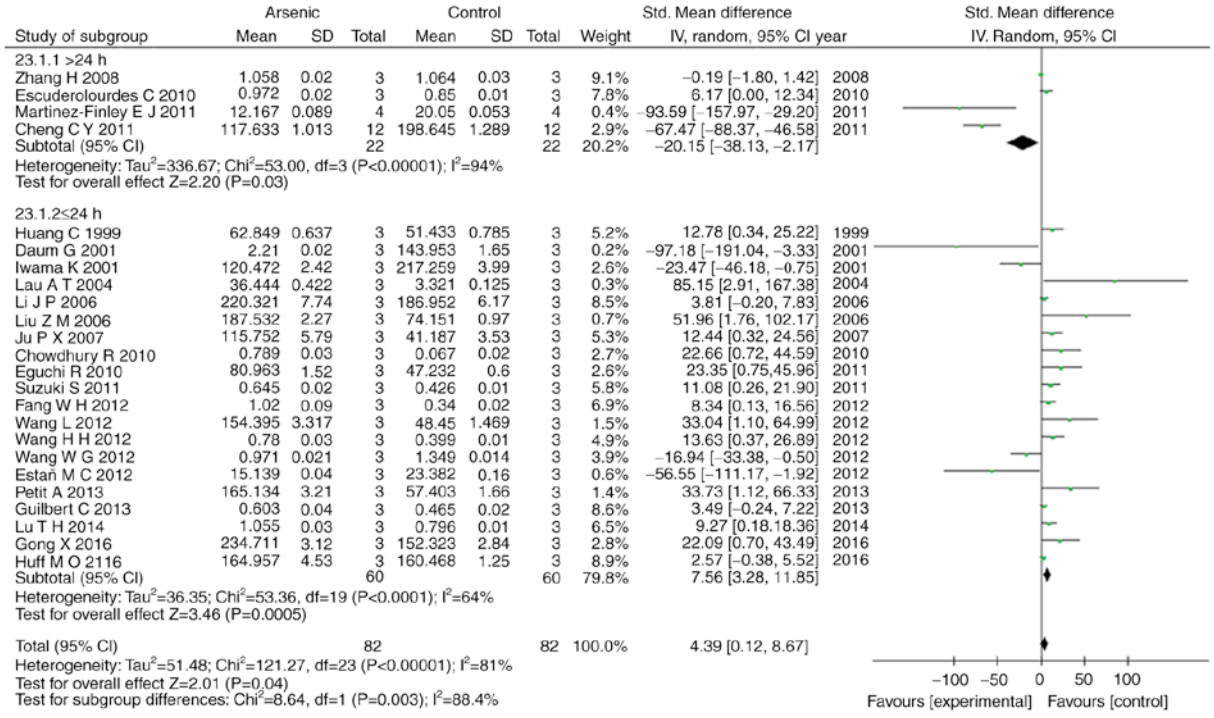

Figure 7. Subgroup analyses to determine the effects of arsenic on p-ERK2 based on exposure time. Forest plot showing the impact of arsenic treatment on p-ERK2 compared to controls. Total column represents total number of studies performed. SMD, standardized mean difference; IV, independent variable; 95% CI, 95% confidence interval; SD, standard deviation.

using its induction of apoptosis (24). ERK had been reported to participate in arsenic-induced apoptosis (25), but reports on the interaction between arsenic and ERK signaling pathway were inconsistent. In our meta-analysis, we found that arsenic had a bidirectional effect on ERK signaling pathway. Arsenic could activate it in the normal cell, but inhibit ERK pathway in cancer cell line, which was also related to dosage and exposure time. These findings provided a divergent theoretical basis

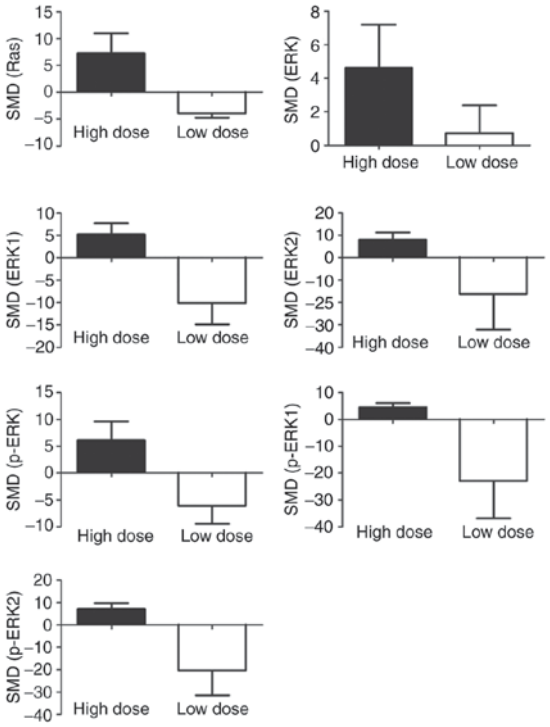

Figure 8. Subgroup analysis based on dosage of arsenic. SMD, standardized mean difference. ERK, extracellular signal-regulated MAP kinases; MEK, mitogen-induced extracellular kinase; p-ERK, phosphorylated extracellular signal-regulated kinase.

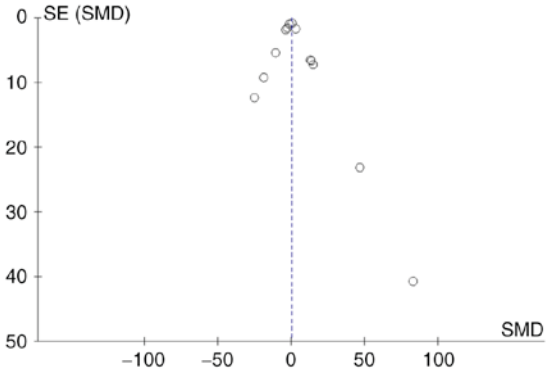

Figure 9. Funnel Plot for p-ERK. Blue-dotted line shows overall estimated standard mean difference. Evidence for publication bias was not found (P=0.490). SMD, standard mean difference; SE, standard error; p-ERK, phosphorylated extracellular signal-regulated kinase.

of injurious as well as beneficial therapeutic mechanisms of arsenic.

Apoptosis is of great significance in maintaining normal development and homeostasis (26). As shown in Fig. 3, apoptotic cells, pro-apoptotic protein (Bax) and activity of caspase-3 had increased while anti-apoptotic protein (Bcl-2) had decreased suggesting an undoubted proof of arsenic-induced apoptosis.

Present results suggested that ERK plays an opposing role in normal and cancer cells. Luo (27) and Banerjee *et al* (28) had reported that in normal cells, arsenic-induced apoptosis was brought about by activation of ERK signaling pathway. As shown in Fig. 5, it can be seen that arsenic increased levels of Ras and p-ERK in normal cells indicating that arsenic led to ERK signaling pathway activation. As for cancer cells, induction of apoptosis is one of the most efficient approaches for the clinical treatment of cancer. It had been reported that arsenic-induced apoptosis of cancer cells was correlated with inhibition of ERK (29-32). Furthermore, arsenic inhibition of ERK signaling pathway in human leukemia cells was also verified as a fact (7,10,24,33). Likewise, decreased levels of both Ras and p-ERK were shown in cancer cells (Fig. 5) along with restraint of ERK signaling. Obviously, the mechanism of arsenic-induced apoptosis is different between normal cells and cancer cells.

ERK was also considered an important mechanism of arsenic causing toxic injury (34,35). Our results showed that arsenic increased the levels of Ras and p-ERK in normal cells (Fig. 5), suggesting that arsenic may activate ERK signaling pathway via Ras/Raf/MEK/ERK pathways. Some studies stated that the activation of ERK signaling pathway leads to DNA damage steering genetic toxicity (36,37). These results also demonstrated that arsenic, through activation of ERK signaling pathway in normal cells, causes toxicity. Activated ERK had been reported to be involved in pathogenesis and development of tumor and cancer (30,38). In this study, cancer cells exposed to arsenic had decreased levels of Ras and p-ERK (Fig. 5) and thus induced suppression of ERK signaling pathway. Therefore, arsenic could inhibit the development of tumor by restraining ERK signaling.

A difference in arsenic doses and exposure time could account for opposite effects caused by arsenic on ERK activity. Ju (39) found out that high doses and short exposure time of arsenic led to activation of ERK. This point was also testified by other studies (40,41). However, some studies (42,43) showed that low doses and long exposure time of arsenic contributed to inactivation of ERK signaling pathway. These discoveries were consistent with the results of this meta-analysis, suggesting that high dose and short exposure time may lead to ERK signaling pathway activation, while low dose and long exposure time depress the activation of ERK signaling.

From what has been discussed above, we may reasonably conclude that ERK signaling pathway was activated when normal cells were exposed to high doses of arsenic for a short period of time, contributing to cell apoptosis, explaining the toxic injury caused by arsenic (Fig. 11A). As for cancer cells, low dose arsenic intervention for long period of time may play a role in promoting cell apoptosis by inhibiting ERK signaling pathway thus suppressing the growth of the tumor (Fig. 11B). These findings not only contributed to a potential approach for seeking ERK inhibitors which work against toxic injury but also provided a reference for a long-term treatment of cancer with low doses of arsenic.

The literature incorporated in this study exhibit heterogeneity. It may also be related to certain factors such as strains of objects, the method of arsenic exposure and possibly others in addition to the factors shown in subgroup analysis. The existing literature did not provide a detailed description of the said factors. Moreover, the data in the selected papers do not support our comparison of time and dose together. Arsenic also affects JNK, p38 and other MAPK signaling pathways and whether ERK interacts with all these may be regarded as a new direction for future research.

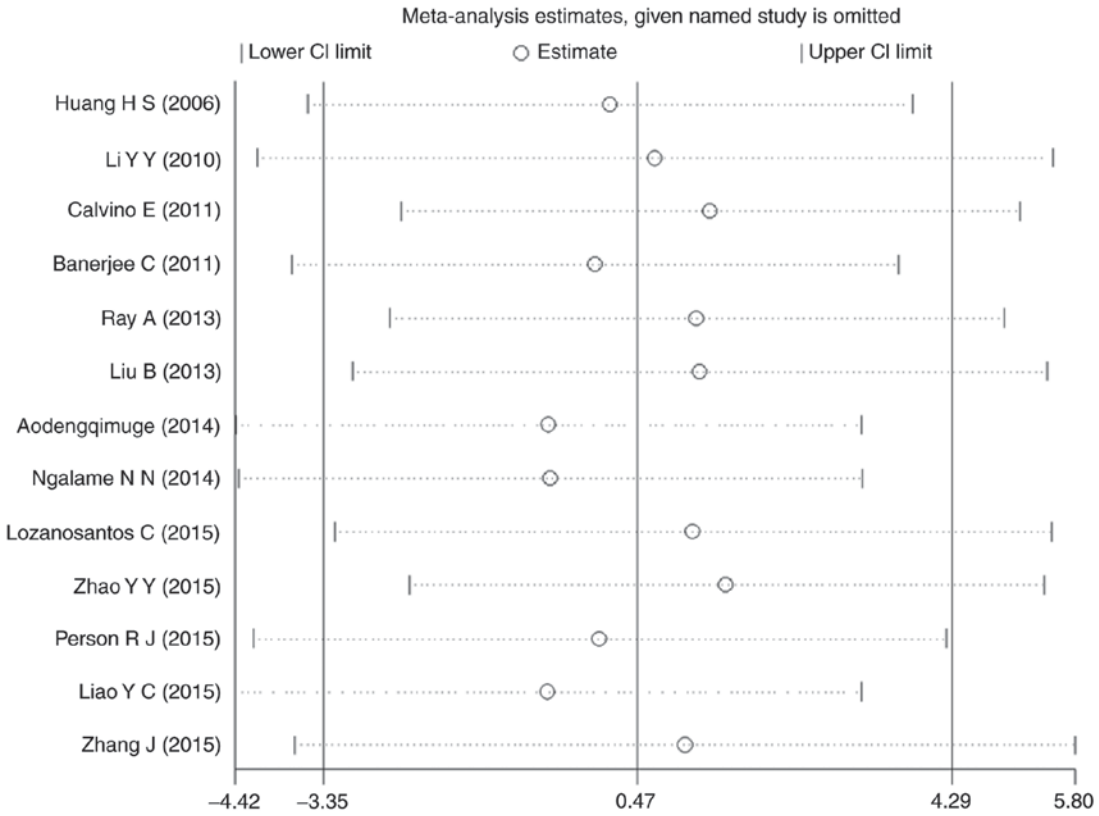

Figure 10. Sensitivity analysis for p-ERK. Stable results were observed for all the studies, indicating no individual study influencing the combined results. CI, confidence interval; p-ERK, phosphorylated extracellular signal-regulated kinase.

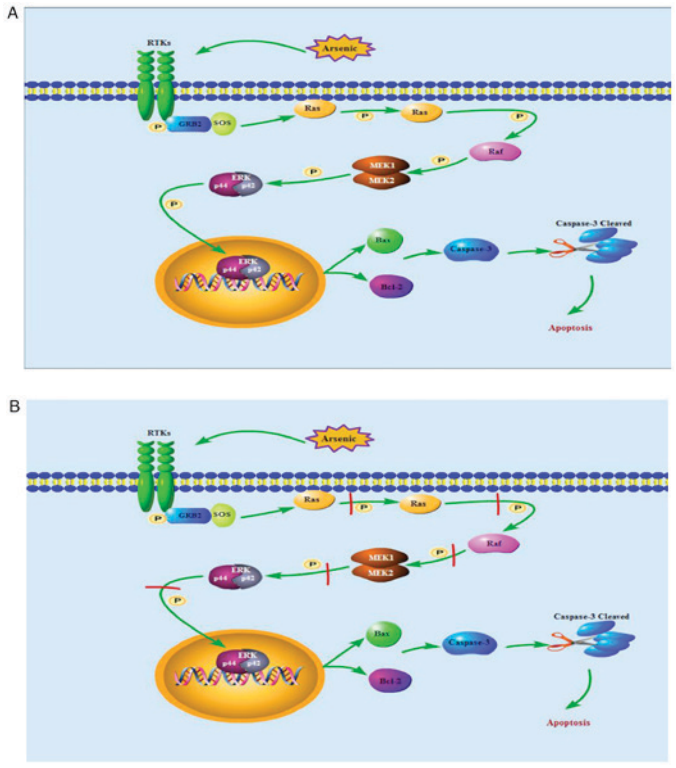

Figure 11. The ERK signaling pathway. (A) shows that high doses of arsenic for a short period of time enhances Ras, Raf, MEK and ERK phosphorylation in normal cells, thereby the activated ERK translocates from cytoplasm into nucleus, increases levels of Bax protein, decreases levels of Bcl-2 protein and cleaves caspase-3, contributing to cell apoptosis. (B) indicates that, in cancer cells, low dose arsenic intervention for long period of time suppresses phosphorylation of Ras, Raf, MEK and ERK, blocking ERK translocation from cytoplasm into nucleus, thereby increases levels of Bax protein, decreases levels of Bcl-2 protein and cleaves caspase-3, contributing to cell apoptosis. ERK, extracellular signal-regulated MAP kinases; MEK, mitogen-induced extracellular kinase; p-ERK, phosphorylated extracellular signal-regulated kinase; Raf, serine/threonine-specific protein kinases; Bcl-2, B-cell lymphoma/leukemia-2 protein; Bax, Bcl-associated X protein; caspase-3, cysteinyl aspartate-specific protease-3; RTKs, receptor tyrosine kinases.

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