

RESEARCH ARTICLE

Relative miRNA and mRNA expression involved in arsenic methylation

Huirong Cheng¹, Pei Hu¹, Weihua Wen^{1*}, Ling Liu^{2*}

1 Department of Occupational Health, Yunnan Provincial Center for Disease Control and Prevention, Kunming, Yunnan, China, **2** First Affiliated Hospital of Kunming Medical University, Kunming, People's Republic of China

* 54319937@qq.com (LL); Dongsijiehua@sina.com (WW)



Abstract

Three arsenic species in urine are measured using an atomic absorption spectrophotometer. RT-PCR is performed to detect the expression levels of AS3MT, 3 miRNAs, and 17 relative mRNAs in 43 workers producing arsenic trioxide, 36 workers who stopped exposure to arsenic for 85 days, and 24 individuals as the control group. The concentrations of urinary arsenic are very high in workers. A negative correlation between AS3MT and MiR-548c-3p is found. There exist significant changes for most selected miRNAs and mRNAs in workers. There are no significant differences between workers who stopped exposure to arsenic and the control group for most miRNAs and mRNAs, but the MiR-548c-3p levels show significant changes. Similar positive correlations between the expression of AS3MT and all selected mRNAs are found. Negative correlations between the expression of MiR-548c-3p and many relative mRNAs are found as well. AS3MT and MiR-548c-3p may regulate arsenic methylation jointly, which when involved in a group of relative mRNAs may play roles in arsenic metabolism and epigenetic changes caused by this metabolism.

OPEN ACCESS

Citation: Cheng H, Hu P, Wen W, Liu L (2018) Relative miRNA and mRNA expression involved in arsenic methylation. PLoS ONE 13(12): e0209014. <https://doi.org/10.1371/journal.pone.0209014>

Editor: Bing-Hua Jiang, Thomas Jefferson University, UNITED STATES

Received: June 5, 2018

Accepted: November 27, 2018

Published: December 13, 2018

Copyright: © 2018 Cheng et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: There are ethical or legal restrictions on sharing a de-identified data set. The data contain potentially identifying and sensitive patient information, and the government and plant has imposed them. The government department is the people's Government of Honghe Hani and Yi Autonomous Prefecture. The institutional body to which data requests may be sent is Center for Disease Control and prevention of Honghe Hani and Yi Autonomous Prefecture. The non-author institutional body to which data requests may be sent is Center for Disease Control and prevention of Honghe Hani and Yi

Introduction

Arsenic can promote cancer through epigenetic mechanisms, and AS3MT plays a role in this process [1]. Both *in vitro* and *in vivo* evidence support that inorganic arsenic acts as an epigenetic modifier of genes involved in critical cellular functions such as cellular growth and immune response [2]. AS3MT may regulated by some miRNAs. There are some mRNAs, such as lin28, Dicer, Ago2, Exportin-5, play important role in miRNA biogenesis pathways and their regulation [3]. Relative genes associated with those miRNAs and relative mRNAs are widely distributed in the genome, and most are closely involved in malignant tumor [1,4–6].

Arsenic exposure disrupts the genome-wide expression of miRNAs *in vivo*, which can lead to changes in gene expression [7]. A list of miRNAs whose expression levels are known to be affected by iAs treatment, corroborating the importance of proceeding with the hunt for specific subset of miRNAs that can serve as potential biomarkers of iAs effects and that have useful diagnostic value[8]. In this study, several gene analysis software were used to forecast what miRNAs will regulate AS3MT. It is MiR-548, MiR-129, and MiR-129 that may regulate

Autonomous Prefecture, and contact man is Liang Li. The email address is 54319937@qq.com.

Funding: The authors received no specific funding for this work.

Competing interests: The authors have declared that no competing interests exist.

AS3MT by pairing complementarity. AS3MT is probably the direct target of miR-548c-3p. The MiR-548 family has been demonstrated to be involved in the pathogenesis of several cancers; however, its role has not yet been elucidated [9]. This miRNA family is less expressed in some patients and plays an important role in some cancers [10].

The miRNA processing pathway has long been viewed as linear and universal to all mammalian miRNAs [3]. Some relative mRNAs, such as exportin-5, dicer, trbp, Argonaute (Ago2), Lin28 (also termed Lin28a), and Lin28b, play roles in this process [3,6,11]. Arsenic exposure has been shown to alter methylation levels of both global DNA and gene promoters, which may cause changes in expression for many relative mRNAs to promote cell apoptosis, cell cycle inhibition, or relative regulation of carcinogenesis [12, 13]. There are many relationships among the above mRNAs.

Although experiments in suitable model systems could complement the human studies, there may be differences in epigenetic effects between animals and humans and between various tissues and cell types [14]. Thus, studies on human populations exposed to high levels of arsenic are necessary to understand individual differences between arsenic methylation and genetic background.

In this study, we investigate the effects of arsenic exposure, defined by the internal biomarkers inorganic arsenic (iAs), monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) in urine, on the expression of AS3MT, 3 miRNAs and 17 relative mRNAs. We explore how miRNAs and relative mRNAs play a role in AS3MT activity and arsenic metabolism, and the relationships among the expression levels of different RNAs in workers who produce arsenic trioxide, to study the widespread roles of AS3MT and miRNAs networks in workers exposed to arsenic trioxide.

Materials and methods

Study population

Our analysis complies with the Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects (World Medical Association 1989), and the Declaration of Helsinki (1964, amended in 1975, 1983, 1989, 1996 and 2000) of the World Medical Association. Questionnaires were used to obtain information from each individual including age, sex, type of work, service length, smoking and alcohol use (current smoker who smokes more than 10 cigarettes each day and any consumption of alcohol), other potential chemical exposures (lead, carbon monoxide, silicon dioxide, and so on), and data on health status, dietary habits, history of chronic disease, family member history, place of birth, race, education. See S1 and S2 Figs. This study was approved by the Ethics Committee of the Yunnan Provincial Center for Disease Control and Prevention. Individuals were enrolled in the study after agreeing to participate and signing an Informed Consent form. Completion of the questionnaire was entirely voluntary.

The data used for our analysis were from workers in two plants. We collected the data from 43 workers in one arsenic plant that was producing arsenic trioxide (named A plant), and 36 workers from another plant in which the production had been stopped 85 days from the time biological samples were collected (named B plant). The plants chosen for our study produce arsenic trioxide using arsenic ore by reverberator smelting and charcoal deoxidizing. Based on the characteristics of the chemical components of the ore and the production technique flow adopted, there are few other occupational hazard factors except for arsenic pollution in the plant. The occupational protective measures of the plant are far from being sufficient and efficient. Additionally, the 24 individuals in the control group resided in villages that were located more than 50 km in distance from each of the two plants and had similar social economic

status to the 79 workers. The prefecture of the city to which the two plants belonged to is well known for having a high incidence of lung cancer, and arsenic may be involved in the process.

Sample collection

Written instructions regarding the hygienic conditions for collection of samples and 500 ml polyethylene containers treated with hydrochloric acid and rinsed with deionized water were provided to all participants. Subjects were asked to provide the first morning void urine. At the same time, blood samples were collected, and total RNA (1 mg) was extracted within 1 day.

Determination of As metabolisms

We determined As species (iAs, MMA, and DMA) in urine using an atomic absorption spectrophotometer (AA-6800) with an As speciation pretreatment system (ASA-2SP, Shimadzu Co., Kyoto, Japan). Speciation analysis was based on the well-established hydride generation of volatile arsines, followed by cryogenic separation in liquid nitrogen. The limit of detection of $1 \text{ ng} \pm < 5\%$ for each of the three As species was determined using hydride generation-atomic absorption spectrometry (HGAAS). Briefly, 1 ml urine that had been stored at -80°C was thawed at room temperature and digested with 2 N NaOH at 100°C for 3 hrs in a 15-ml polymethylpentene test tube, followed by dilution with Milli-Q water (Millipore, Yonezawa, Japan). This digestion procedure did not alter the distribution of iAs or methylated arsenicals (Yamauchi and Yamamura. 1984). The absorbance of As in the digested urine samples was determined at 193.7 nm.

Quantitative real-time PCR analysis

Overall, 21 RNAs including AS3MT, 3 miRNAs, 6 relative mRNAs that are important in the miRNA biogenesis pathways and their regulation, and 11 relative mRNAs that promote cell apoptosis, cell cycle inhibition, or relative regulation of carcinogenesis, as well as a β -actin sequence (control fragment), were selected in this study. Total RNA (1 mg) was extracted using Trizol reagent (Invitrogen) following manufacturer's instruction, and then transcribed into cDNA with NCodeVILOmiRNA cDNA Synthesis Kit (Invitrogen). Quantitative real-time PCR (qRT-PCR) was performed with the Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen) in ABI7900 (Applied Biosystems, America). PCR primers are designed for all RNAs. The relative expression levels of all RNAs were determined using the $2^{-\Delta\Delta C_t}$ method. All reactions were performed in triplicate.

Statistical analysis

All statistical analyses were performed using SPSS software (Version 19, Chicago, IL, USA). The concentrations of iAs, MMA and DMA were first log transformed to improve the normality of measures, then transformed back to the arithmetic scale for reporting purposes. After assessing the association among the levels of miRNAs, relative mRNAs and three arsenic species by Spearman's rank correlation analysis, covariance and independent samples *t*-test were performed for the analysis of arsenic species and all RNAs among groups under different levels of arsenic trioxide exposure. We then investigated the association of arsenic species and all RNAs among groups under different levels of arsenic trioxide exposure using multivariate linear regression models with adjustment for age, sex, smoking status, work years, and urinary creatinine. All statistical tests were two-sided, with *P* value < 0.05 considered statistically significant for any single analysis.

Table 1. Demographics of the study population.

Workers	Control group	Workers in B plant	Workers in A plant
Number of subjects	24	36	43
Sex			
Male	13	21	25
Female	11	15	18
Age(years)	32.9±3.8	36.5±6.9	35.9±8.2
service length(months)	-	22.9±8.5	20.7±9.9
Type of work			
Blast furnace		12	13
Reverberatory furnace		3	11
Rotary house		4	5
Machine repair		3	4
On-site inspection		9	5
Administration and test		5	5
Race			
Han	16	22	27
Hani	5	9	11
Miao	3	5	5
Other exposures			
Lead	0	6	7
High temperature	0	1	2
Other exposures	0	1	3
Education(years)			
≤5	8	12	16
5~10	7	11	17
≥10	9	13	10
Workers who smoking	13	21	26
Workers who drinking	11	15	17

Workers who smelt arsenic are in A plant; workers who stopped exposure to arsenic about 85 days are in B plant.

<https://doi.org/10.1371/journal.pone.0209014.t001>

Results

There are no significant differences in sex, smoking, alcohol consumption, and so on among the exposed workers in the two plants and the control group. For the demographics of the study population, see [Table 1](#).

Changes in iAs species and selected RNAs

The concentrations of iAs, MMA and DMA in urine are shown in [Fig 1](#). Compared to the control group, the increases in the levels of iAs, MMA, and DMA in urine of workers exposed to arsenic are all statistically significant ($P < 0.01$).

The expression levels of 21 RNAs are shown in [Figs 2–4](#). Compared to that in the control group, *AS3MT* expression shows significant changes for workers producing arsenic ($P < 0.01$), but not for workers who stopped exposure to arsenic for 85 days. Based on genome informatics analysis, it is *MiR-548c-3p* that may regulate *AS3MT* expression. Compared with that in the control group, the decreases in *MiR-548c-3p* expression in workers from the two plants are statistically significant ($P < 0.01$).

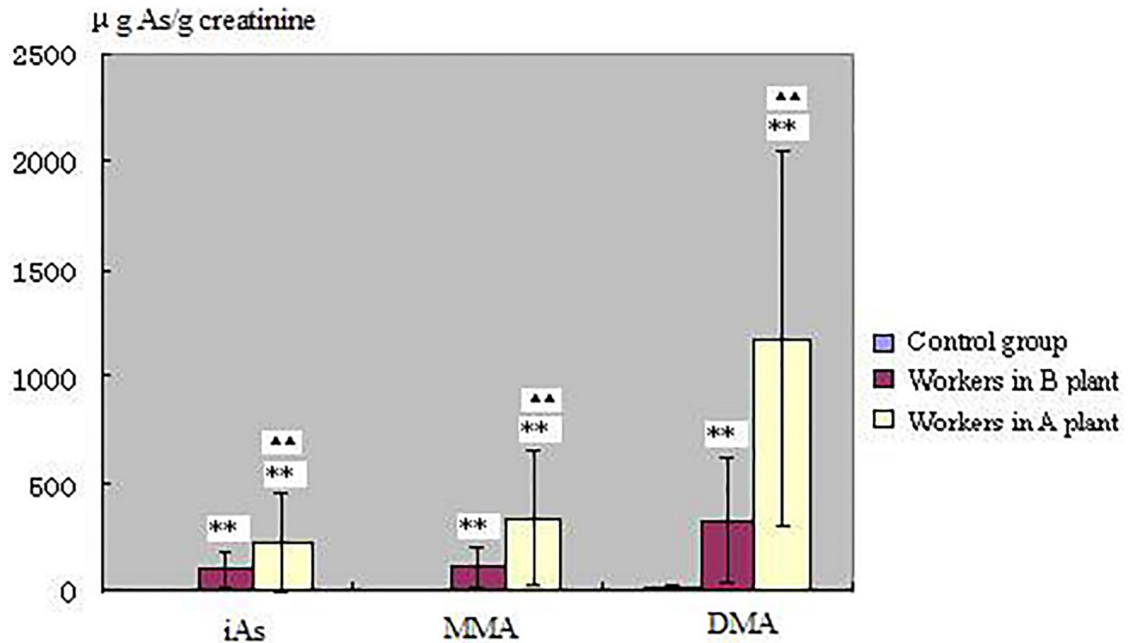


Fig 1. Urinary inorganic arsenic (iAs), monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) of subjects. Double stars(**) indicate that the difference from control group is statistically significant($P < 0.01$), double stars(▲▲) indicate that the difference from workers who stopped exposure to arsenic about 85 days (in B plant) is statistically significant($P < 0.01$). Workers who smelt arsenic are in A plant.

<https://doi.org/10.1371/journal.pone.0209014.g001>

Lin28, Lin28b, Dicer, Exptothin-5, Trbp and Ago2 are mRNAs that are involved in the generation and maturation of miRNAs. Compared to those in the control group, the relative mRNA levels increase significantly for workers producing arsenic ($P < 0.05$), but not for workers who stopped exposure to arsenic for 85 days.

Compared to those in the control group, there are significant changes for 9 mRNAs that promote cell apoptosis, cell cycle inhibition, or relative regulation of carcinogenesis for

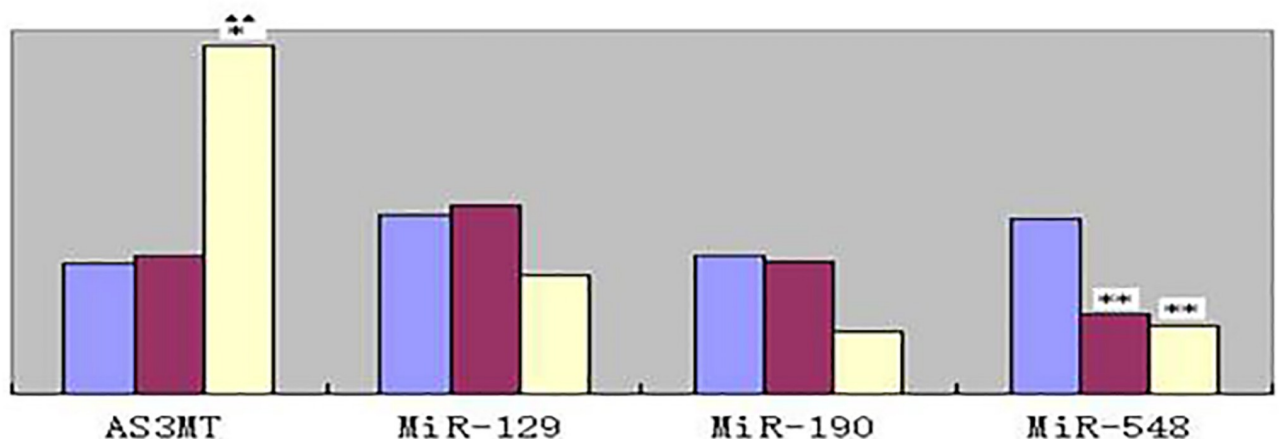


Fig 2. Relative levels of AS3MT RNA and 3miRNA selected in this study. All RNAs were selected with adjustment for age, gender, smoking status, work years, and urinary creatinine, respectively. Double stars(**) indicate that the difference from control group is statistically significant($P < 0.01$), star(*) indicate that the difference from control group is statistically significant($P < 0.05$), double stars(▲▲) indicate that the difference from workers who stopped exposure to arsenic about 85 days (in B plant) is statistically significant($P < 0.01$).

<https://doi.org/10.1371/journal.pone.0209014.g002>

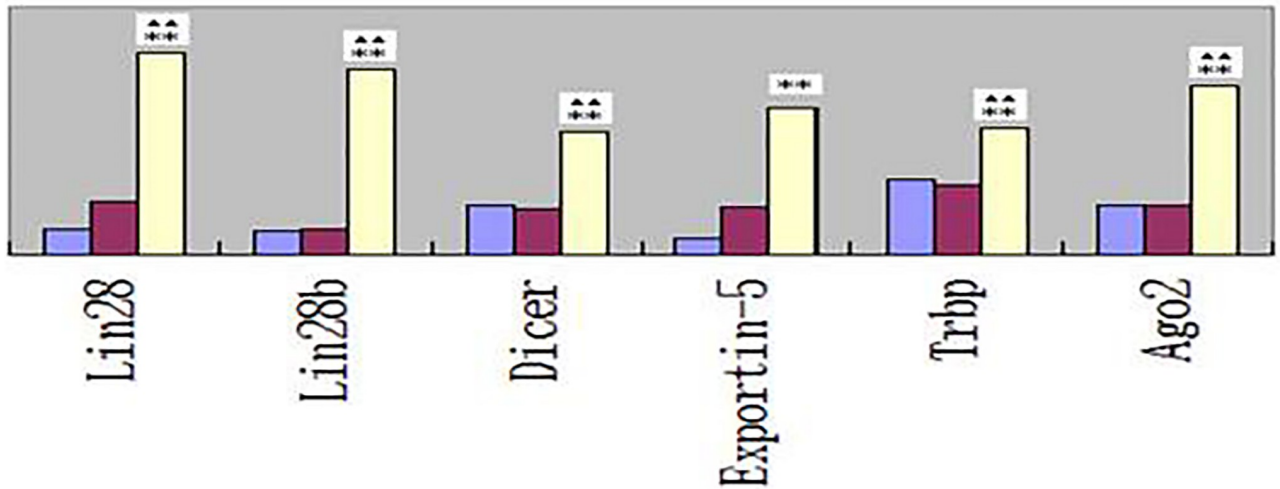


Fig 3. Relative mRNAs levels of selected genes that are important in the miRNA biogenesis pathways and their regulation. Double stars(**) and stars(▲▲) are same as Fig 2.

<https://doi.org/10.1371/journal.pone.0209014.g003>

workers producing arsenic ($P < 0.01$), but only changes in *p15* and *Puma* expression for workers who stopped exposure to arsenic for 85 days ($P < 0.05$). It is worth noting that the levels of *noxa* expression in workers who stopped exposure to arsenic for 85 days are lower than that of the control group ($P < 0.01$).

Spearman’s rank correlation analysis among arsenic species, relative mRNAs and miRNAs networks

The concentrations of iAs, MMA and DMA in urine are positively correlated with the expression levels of *AS3MT* RNA ($P < 0.05$) and negatively correlated with those of *MiR-548c-3p*

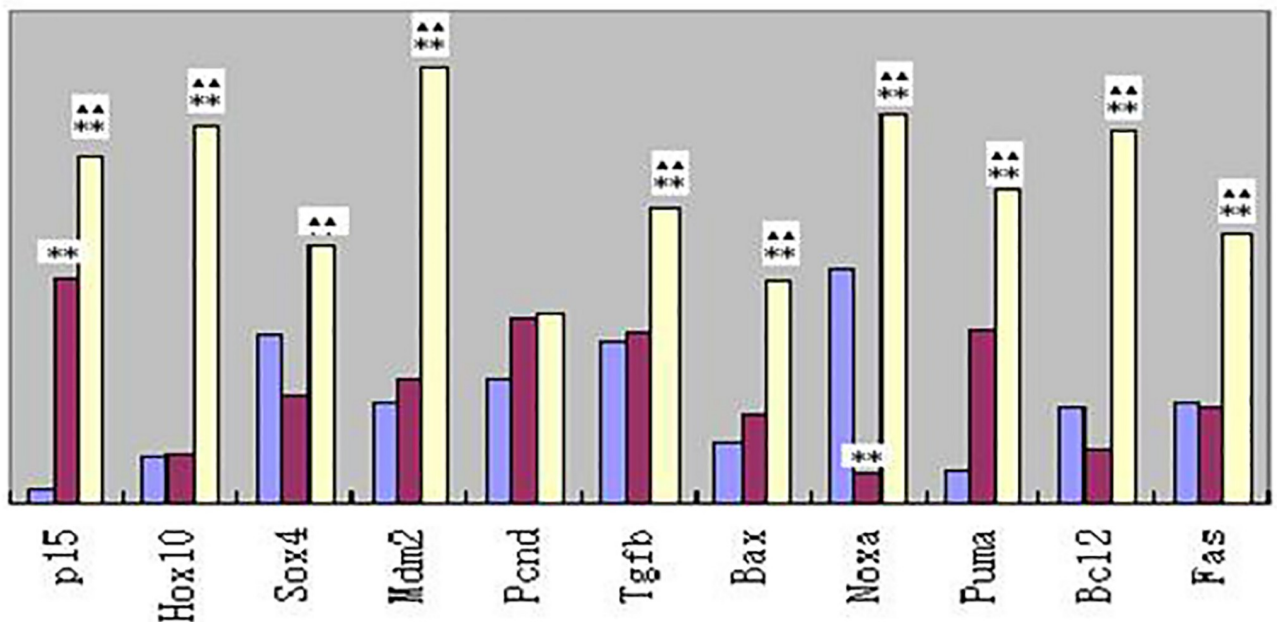


Fig 4. Relative mRNAs levels of 11 selected genes that promote cell apoptosis, cell cycle inhibition, or relative regulation of carcinogenesis. Double stars(**) and stars(▲▲) are same as Fig 2.

<https://doi.org/10.1371/journal.pone.0209014.g004>

Table 2. Spearman’s rank correlation analysis along arsenic species, MiR-548c-3p, AS3MT and relative mRNAs.

		iAs	MMA	DMA	AS3MT	MiR-548c-3p
iAs	<i>r</i> value (<i>p</i> value)		0.923(0.000)	0.874(0.000)	0.216(0.042)	-0.344(0.000)
MMA	<i>r</i> value (<i>p</i> value)	0.932(0.000)		0.968(0.000)	0.260(0.014)	-0.334(0.001)
DMA	<i>r</i> value (<i>p</i> value)	0.874(0.000)	0.968(0.000)		0.316(0.003)	-0.308(0.002)
AS3MT	<i>r</i> value (<i>p</i> value)	0.216(0.042)	0.260(0.014)	0.316(0.003)		-0.212(0.049)
MiR-548c-3p	<i>r</i> value (<i>p</i> value)	-0.344(0.000)	-0.334(0.001)	-0.308(0.002)	-0.212(0.049)	
Lin28	<i>r</i> value (<i>p</i> value)	0.356(0.000)	0.404(0.000)	0.454(0.000)	0.665(0.000)	-0.391(0.000)
Lin28b	<i>r</i> value (<i>p</i> value)	0.283(0.019)	0.314(0.009)	0.404(0.001)	0.461(0.000)	-0.157(0.204)
Dicer	<i>r</i> value (<i>p</i> value)	0.310(0.002)	0.257(0.009)	0.445(0.000)	0.552(0.000)	-0.226(0.024)
Exportin-5	<i>r</i> value (<i>p</i> value)	0.389(0.000)	0.428(0.000)	0.473(0.000)	0.655(0.000)	-0.398(0.000)
Trbp	<i>r</i> value (<i>p</i> value)	0.307(0.004)	0.353(0.002)	0.454(0.000)	0.285(0.011)	-0.225(0.037)
Ago2	<i>r</i> value (<i>p</i> value)	0.383(0.000)	0.423(0.000)	0.533(0.000)	0.533(0.000)	-0.352(0.000)
p15	<i>r</i> value (<i>p</i> value)	0.417(0.000)	0.497(0.000)	0.494(0.000)	0.447(0.000)	-0.291(0.003)
Hox10	<i>r</i> value (<i>p</i> value)	0.163(0.158)	0.187(0.105)	0.242(0.035)	0.595(0.000)	-0.040(0.733)
Sox4	<i>r</i> value (<i>p</i> value)	0.184(0.087)	0.211(0.049)	0.308(0.008)	0.272(0.010)	-0.188(0.069)
Mdm2	<i>r</i> value (<i>p</i> value)	0.288(0.003)	0.355(0.000)	0.432(0.000)	0.673(0.000)	-0.232(0.020)
Pcnd	<i>r</i> value (<i>p</i> value)	0.179(0.101)	0.245(0.021)	0.321(0.001)	0.289(0.007)	-0.199(0.050)
Tgfb	<i>r</i> value (<i>p</i> value)	0.158(0.110)	0.184(0.063)	0.276(0.014)	0.575(0.000)	-0.176(0.079)
Bax	<i>r</i> value (<i>p</i> value)	0.229(0.020)	0.240(0.015)	0.235(0.017)	0.639(0.000)	-0.204(0.041)
Noxa	<i>r</i> value (<i>p</i> value)	0.228(0.021)	0.239(0.031)	0.283(0.004)	0.247(0.018)	-0.187(0.052)
Puma	<i>r</i> value (<i>p</i> value)	0.295(0.003)	0.364(0.000)	0.355(0.000)	0.496(0.000)	-0.241(0.016)
Bcl2	<i>r</i> value (<i>p</i> value)	0.237(0.016)	0.307(0.002)	0.393(0.000)	0.626(0.000)	-0.286(0.004)
Fas	<i>r</i> value (<i>p</i> value)	0.137(0.171)	0.157(0.117)	0.231(0.020)	0.508(0.000)	-0.219(0.029)

<https://doi.org/10.1371/journal.pone.0209014.t002>

($P < 0.01$). The expression levels of AS3MT RNA are negatively correlated with those of MiR-548c-3p ($P < 0.05$). The expression levels of AS3MT RNA are positively correlated with those of all relative mRNAs ($P < 0.05$), while the levels of MiR-548c-3p are negatively correlated with those of many relative mRNAs ($P < 0.05$). With the increase in the content of iAs, MMA and DMA in urine, an increase in the expression levels for many relative mRNAs is observed ($P < 0.05$). See Table 2.

Discussion

There are many data show that biomethylation plays a role in activating cancer and inducing toxicity for inorganic arsenic. The methylation of iAs yields methylated metabolites in which arsenic is present in both pentavalent and trivalent forms [15]. Studies show that arsenic exposure and the incomplete methylation capacity of arsenic are adversely associated with cancer and other diseases [16–18]. AS3MT is the key enzyme in the biotransformation pathway, catalyzing the methylation of inorganic arsenic and playing an important role in the metabolism of this metalloid [3,4,19].

Our previous study showed that arsenic levels at 7 posts in the plant producing arsenic trioxide were very high. The workers had been exposed to high arsenic levels for long periods of time [16]. Relative concentrations and proportions of arsenic metabolites in urine have been identified as potential biomarkers of susceptibility to iAs toxicity [17,20].

Acute arsenic exposure at the levels observed in poisoned Japanese patients has been associated with a high concentration of 8-OHdG, which may result from DNA damage caused by arsenic metabolism [21]. The data indicate that some aspects of chronic and acute arsenic poisoning may be reversible with the cessation of exposure. Consistent with Yamauchi et al., our

results show that there are substantial changes in the expression of many relative mRNAs that are important in the miRNA biogenesis pathways and their regulation and that promote cell apoptosis, cell cycle inhibition, or relative regulation of carcinogenesis. These RNAs may play a role in promoting cancer and inducing toxicity, which are probably involved in arsenic metabolism, just like AS3MT.

There exist aberrant changes for many relative mRNAs from workers in the two plants, and these changes can not be explained using differences in arsenic concentration only. *AS3MT* and MiR-548-3p may play roles in arsenic metabolism, promoting cancer and inducing toxicity by multiple pathways.

MiRNAs are involved in temporal and tissue-specific eukaryotic gene regulation, either by translational inhibition or exonucleolytic mRNA decay [22]. Altered miRNA expression may lead to widespread gene expression changes [23]. Although the mode of action of miRNAs has attracted great attention, the principles governing their expression and activity are only beginning to emerge. There are many branches, crossroads and detours in miRNA processing, according to recent studies [1]. In this study, the expression levels of selected relative mRNAs are likely under the control of transcription factors, for example, *p53* [24, 25]. Relative mRNAs, such as *Lin28* and *Dicer*, which are important in the generation and maturation of miRNAs, may be involved in the metabolism of arsenic by ASMT, MiR-548c-3p, *p53*, and so on.

Based on genome informatics analysis, it is MiR-548c-3p that very likely regulates AS3MT. Our preinvestigation shows that there are obvious changes in the expression levels of MiR-548c-3p in workers exposed to arsenic, and we plan to explore the expression rules of this miRNA and the role it plays in cancers associated in arsenic exposure. The current results show that there is lower expression of mir548 and higher expression of AS3MT in workers exposed to arsenic. The miRNA processing pathway may be closely involved in this process.

Both AS3MT and MiR-548c-3p may be highly involved in metabolizing arsenic, activating cancer and inducing toxicity, but they may play their roles in different ways. Most likely, AS3MT has close relations with many relative mRNAs, including all mRNAs selected in this study. At the same time, MiR-548c-3p is closely related to some mRNAs that may be closely involved in AS3MT functions, such as *Lin28*, *Exportin-5* and *Ago2*. AS3MT function may be influenced by not only MiR-548c-3p, but also many other RNAs. The biogenesis pathways of MiR-548c-3p and other miRNAs, as well as their generation and maturation, are involved in many pathways that may play an important role in arsenic metabolism and epigenetic changes.

Conclusions

Our data and relative analysis suggest potentially widespread rules about the expression levels of *AS3MT*, miRNAs and relative mRNAs in workers exposed to arsenic, which may be caused by arsenic metabolism. *AS3MT* and MiR-548c-3p likely play important roles in arsenic metabolism and epigenetic changes, but in different ways. AS3MT is probably the direct target of miR-548c-3p, and a group of relative mRNAs which selected in this study take part in this process.

Supporting information

S1 Fig. Questionnaire used in arsenic smelting plant.
(DOCX)

S2 Fig. Questionnaire used in arsenic smelting plant(in Chinese).
(DOCX)

S1 File.
(DOC)

Acknowledgments

We thank all individuals who volunteered to participate in this study.

Author Contributions

Conceptualization: Huirong Cheng, Weihua Wen, Ling Liu.

Data curation: Huirong Cheng, Pei Hu, Weihua Wen, Ling Liu.

Formal analysis: Huirong Cheng, Pei Hu, Weihua Wen, Ling Liu.

Funding acquisition: Huirong Cheng, Pei Hu, Weihua Wen, Ling Liu.

Investigation: Huirong Cheng, Pei Hu, Weihua Wen, Ling Liu.

Methodology: Huirong Cheng, Weihua Wen, Ling Liu.

Project administration: Huirong Cheng, Weihua Wen, Ling Liu.

Resources: Huirong Cheng, Weihua Wen, Ling Liu.

Software: Huirong Cheng, Weihua Wen, Ling Liu.

Supervision: Huirong Cheng, Weihua Wen, Ling Liu.

Validation: Huirong Cheng, Weihua Wen, Ling Liu.

Visualization: Huirong Cheng, Weihua Wen, Ling Liu.

Writing – original draft: Huirong Cheng, Weihua Wen, Ling Liu.

Writing – review & editing: Huirong Cheng, Pei Hu, Weihua Wen, Ling Liu.

References

1. Engström K, Vahter M, Mlakar SJ, Concha G, Nermell B, Raqib R, et al. Polymorphisms in Arsenic(+III oxidationState) Methyltransferase (AS3MT) Predict Gene Expression of AS3MT as Well as Arsenic Metabolism. *Environ Health Persp*. 2011; 119: 182–188.
2. Meakin CJ, Martin EM, Fry RC. Epigenetic mechanisms underlying arsenic-induced toxicity. *Current Opinion in Toxicology*, 2017, 6:1–9.
3. Winter J, Jung S, Keller S, Gregory RI, Diederichs S. Many roads to maturity: microRNA biogenesis pathways and their regulation. *Nat. cell biol.* 2009; 11:228–234. <https://doi.org/10.1038/ncb0309-228> PMID: 19255566
4. Fujihara J, Soejima M, Yasuda T, Koda Y, Agusa T, Kunito T, et al. Global analysis of genetic variation in human arsenic (+3 oxidation state) methyltransferase (AS3MT). *Toxicol Appl Pharm.* 2010; 243:292–299.
5. Chitkara D, Mittal A, Mahato RI. miRNAs in pancreatic cancer: Therapeutic potential, delivery challenges and strategies, *Adv Drug Deliv Rev.* 2015; 81:75–93.
6. Valenzuela-Miranda D, Nuñez-Acuña G, Valenzuela-Muñoz V, Asgari S, Gallardo-Escárate C. MicroRNA biogenesis pathway from the salmon louse (*Caligus rogercresseyi*): Emerging role in delousing drug response, *gene*, 2015; 555(2):231–241. <https://doi.org/10.1016/j.gene.2014.11.008> PMID: 25447902
7. Ren X, Gaile DP, Gong Z, Qiu W, Ge Y, Zhang C, et al. Arsenic responsive microRNAs in vivo and their potential involvement in arsenic-induced oxidative stress *Toxicol Appl Pharmacol.* 2015; 283(3):198–209. <https://doi.org/10.1016/j.taap.2015.01.014> PMID: 25625412

8. Sturchio E, Colombo T, Boccia P, Carucci N, Meconi C, Minoia C, Macino G. Arsenic exposure triggers a shift in microRNA expression. *Sci Total Environ*. 2013; 472:672–80. <https://doi.org/10.1016/j.scitotenv.2013.11.092> PMID: 24317173
9. Shi YF, Qiu M, Wu Y, Hai L. MiR-548-3p functions as an anti-oncogenic regulator in breast cancer. *Bio-medicine & Pharmacotherapy*, 2015; 75:111–116.
10. Tahiri A, Leivonen SK, Lüders T, Steinfeld I, Aure MR, Geisler J, et al. Deregulation of cancer-related miRNAs is a common event in both benign and malignant human breast tumors. *Carcinogenesis* 2014; 35 (1):76–85. <https://doi.org/10.1093/carcin/bgt333> PMID: 24104550
11. Zhou JB, Ng SB, Chng WJ. LIN28/LIN28B: An emerging oncogenic driver in cancer stem cells, *Inter. J. Biochem. Cell B*. 2013; 45(5):973–978.
12. Yang TY, Hsu LI, Chiu AW, Pu YC, Wang SH, Liao YT, et al. Comparison of genome-wide DNA methylation in urothelial carcinomas of patients with and without arsenic exposure, *Environ. Res*. 2014; 128 (1):57–63.
13. Reichard JF, enburger MS, Puga A. Long term low-dose arsenic exposure induces loss of DNA methylation. *Biochem Biophys Res Commun*. 2007; 352: 188–192. <https://doi.org/10.1016/j.bbrc.2006.11.001> PMID: 17107663
14. Kille P, Andre J, Anderson C, Ang HN, Bruford MW, Bundy JG, et al. DNA sequence variation and methylation in an arsenic tolerant earthworm population. *Soil. Biol. Biochem*. 2013; 57:524–532.
15. Styblo M, Del Razo LM Vega L, Germolec DR, LeCluyse EL, Hamilton GA, Reed W, et al. Comparative toxicity of trivalent and pentavalent inorganic and methylated arsenicals in rat and human cells. *Arch Toxicol*. 2000; 74(6):289–99. PMID: 11005674
16. Yu RC, Hsu KH, Chen CJ, Froines JR. Arsenic methylation capacity and skin cancer. *Cancer Epidemiol Biomarkers Prev*. 2000; 9(11):1259–62. PMID: 11097236
17. Wen JH, Wen WH, Li L, Liu H. Methylation capacity of arsenic and skin lesions in smelterplant workers, *Environ. Appl. Pharmacol*. 2012; 34:624–630.
18. Wen WH, Wen JH, Lu L, Liu H, Yang J, Cheng HR, et al. Metabolites of arsenic and increased DNA damage of p53 gene in arsenicplant workers, *Toxicol. Appl. Pharmacol*. 2011; 254, 41–47. <https://doi.org/10.1016/j.taap.2011.04.013> PMID: 21550362
19. Song XL, Geng ZR, Li XL, Zhao Q, Hu X, Zhang XR, et al. Functional and structural evaluation of cysteine residues in the human arsenic (3 oxidation state) methyltransferase (hAS3MT). *Biochimie* 2011; 93:369–375. <https://doi.org/10.1016/j.biochi.2010.10.010> PMID: 20971157
20. Antonelli R, Shao K, Thomas DJ, S R, II, Cowden J. AS3MT, GSTO, and PNP polymorphisms: Impact on arsenic methylation and implications for disease susceptibility. *Environ. Res*. 2014; 132:156–167. <https://doi.org/10.1016/j.envres.2014.03.012> PMID: 24792412
21. Yamauchi H, Aminaka Y, Yoshida K, Sun GF, Pi JB, Waalkes MP. Evaluation of DNA damage in patients with arsenic poisoning: urinary 8-hydroxydeoxyguanine. *Toxicol. Appl. Pharmacol*. 2004; 198:291–296. <https://doi.org/10.1016/j.taap.2003.10.021> PMID: 15276408
22. Iorio MV, Casalini P, Piovan C, Braccioli L, Tagliabue E.)Breast cancer and microRNAs: therapeutic impact. *The Breast* 2011; 20:63–70.
23. Sonkoly E, Pivarcsi E. MicroRNAs in inflammation and response to injuries induced by environmental pollution. *Mut Res*. 2011; 717:46–53.
24. O'Donnell KA, Wentzel EA, Zeller KI, Dang CV, Mendell JT. c-Myc-regulated microRNAs modulate E2F1 expression. *Nature* 2005; 435, 839–843. <https://doi.org/10.1038/nature03677> PMID: 15944709
25. He L, He XY, Lim LP, Stanchina ED, Xuan ZY. A microRNA component of the p53 tumour suppressor network. *Nature* 2007; 447:1130–1134. <https://doi.org/10.1038/nature05939> PMID: 17554337