Arsenic and microRNA Expression

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Abstract

Arsenic is a naturally occurring metalloid that poses a major threat to worldwide human health. The most toxic form of arsenic is inorganic arsenic, which has been classified by the International Agency for Research on Cancer as a group 1 carcinogenic to humans. This classification is based on the increased incidence of primary skin cancer, as well as lung and urinary bladder cancer after exposure to arsenic. Exposure to arsenic typically occurs by oral consumption of contaminated drinking water, soil, and food or by inhalation in an industrial work setting. The main exposure route to inorganic arsenic remains dietary, particularly in infants. This review describes our current understanding of the molecular mechanisms through which arsenic causes harm, although the toxic effects associated with inorganic arsenic exposure are not well understood. Arsenic toxicokinetics varies depending on its form and on several factors such as life-stage, gender, nutritional status, and genetic polymorphisms. MicroRNAs play a key role in many physiological and pathological cellular processes, and they are powerful regulators of gene expression under inorganic arsenic exposure. Several *in vitro* and *in vivo* studies on the effect of inorganic arsenic exposure on the microRNA expression profile showed that microRNAs misregulation is involved in a variety of human tumors and in angiogenesis.

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Keywords

Arsenic • Inorganic arsenic • Metabolic pathway • Carcinogenesis • Human exposure • Mechanism of action • Toxicity • microRNA expression • Contaminated water • Dietary intake

List of Abbreviations

As	As
AS3MT	Human As methyltransferase
AsIII	Arsenite
AsT cell	As transformed cell
AsV	Arsenate
CCA	Chromated copper arsenate
DMAA	Dimethylarsinic acid
DNMT	DNA methyltransferase
EMT	Epithelial–mesenchymal transition
GSH	Glutathione
HBEC	Human bronchial epithelial cell
HELF	Human embryo lung fibroblast cell
iAs	Inorganic arsenic
MCL	Maximum contaminant level
mESCs	Mouse embryonic stem cells
miRNA	microRNA
MMAA	Monomethylarsonic acid
PHLPP	PH domain leucine-rich repeat protein phosphatase
ROS	Reactive oxygen species
RT-qPCR	Quantitative reverse transcription PCR
SAM	S-adenosyl-methionine
SUMO	Small ubiquitin-like modifier
VEGF	Vascular endothelial growth factor protein

Contents

Introduction

Inorganic arsenic (iAs) poses a major threat to worldwide human health. Arsenic (As) is a metalloid, occurring naturally in the Earth's crust generally as one of the several sulfides or as metal arsenates (WHO 2001). As is also a product of anthropogenic activities such as smelting, mining, or waste combustion. Both inorganic and organic species of As occur in the environment. Generally, the iAs forms are more toxic than the organic ones (oAs) for human, animal, and plant health. The chemical structure of As, its electronic status, and its bonding properties give rise to a variety of forms in the solid, aqueous, and gaseous states. Inorganic arsenic is present in different oxidation states: −3, 0, +3, and +5 but the prevalent As forms are arsenite (AsIII) and arsenate (AsV). The most common organic forms are monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMAA).

As dispersion into soil and water through the disintegration of rocks and minerals causes As to enter the food chain (Fig. 1).

Arsenic can be released in the atmosphere by natural processes, such as volcanic activity and dissolution of minerals, or anthropogenic causes such as mining, metal smelting, and pesticide use. It is mainly adsorbed on particles, which are dispersed by winds and deposited on land and water, reaching high concentration mainly in industrial areas (WHO 2001).

The mean concentration of As in soil depends on the physicochemical structure of the soil and it is higher in igneous rock compared to sedimentary rock (Smith et al. 1998). Furthermore, the interaction among soil, plant, and microorganisms may influence the As mobility and bioavailability. Under oxidizing conditions, arsenic is present in the +5 oxidation state and does not coprecipitate nor is adsorbed with the exception of the coprecipitation induced by iron hydroxides (O'Day 2006).

Arsenic water contamination is one of the major health concerns in most countries, in particular in areas, such as Bangladesh, where the major risk of contamination is represented by drinking water from tube wells (Flanagan et al. 2012). Arsenic in water exists in different oxidized or reduced forms depending on the redox potential, pH, temperature, salinity, and on the presence and distribution of microorganisms and biotic component in general (EPA 2001; EPA 2002; Wakao et al. 1988).

The World Health Organization (WHO) guideline value for iAs in drinking water was provisionally reduced in 1993 from 50 to 10 μg l^{-1} and the same threshold limit has been established for natural mineral waters by the Commission Directive 2003/40/EC (EFSA 2014). The new recommended value was based on the increasing awareness of the toxicity of As, particularly its carcinogenicity, and on the development of sensitive methods to measure it quantitatively. In recent years, there has been a disparity between WHO guideline values and current national standards for drinking water sources. For example, the iAs drinking water limit for Bangladesh is 50 μg l^{-1}, while the American state of New Jersey has enforced an iAs drinking water standard, *Maximum Contaminant Level* (MCL), of 5 μg l^{-1} instead of the federal MCL of 10 μg l^{-1}. Australia also has a stringent standard for iAs in drinking

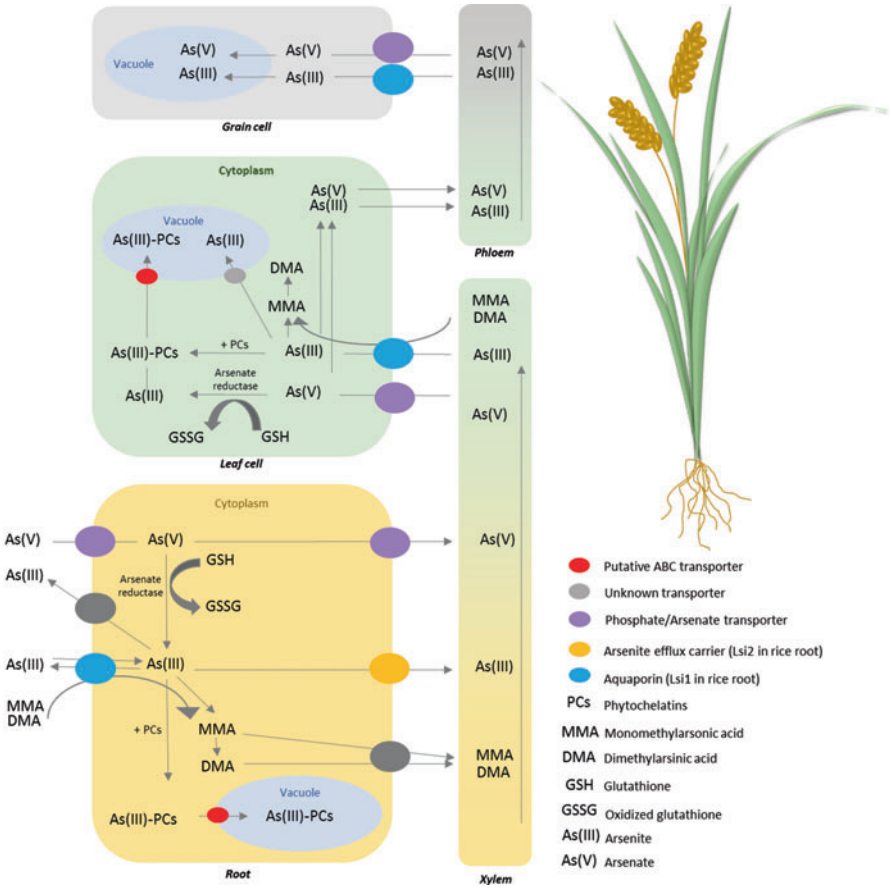

Fig. 1 **Arsenic uptake and translocation in *Oryza sativa*.** Different inorganic arsenic species uptake from contaminated soil and groundwater in a hyperaccumulating plant and their translocation from roots to grains (Modified by Zhao et al. 2010)

water. In Europe, there is no common recommended maximum level established for As in food, although some member states have their own national guidelines. In the United States, As is used in some veterinary drugs, including those used to treat animals used for commercial food products.

Human Exposure

The International Agency for Research on Cancer (IARC) classified iAs as "carcinogenic to humans" (Group 1) based on sufficient evidence of carcinogenicity in humans (IARC 2012).

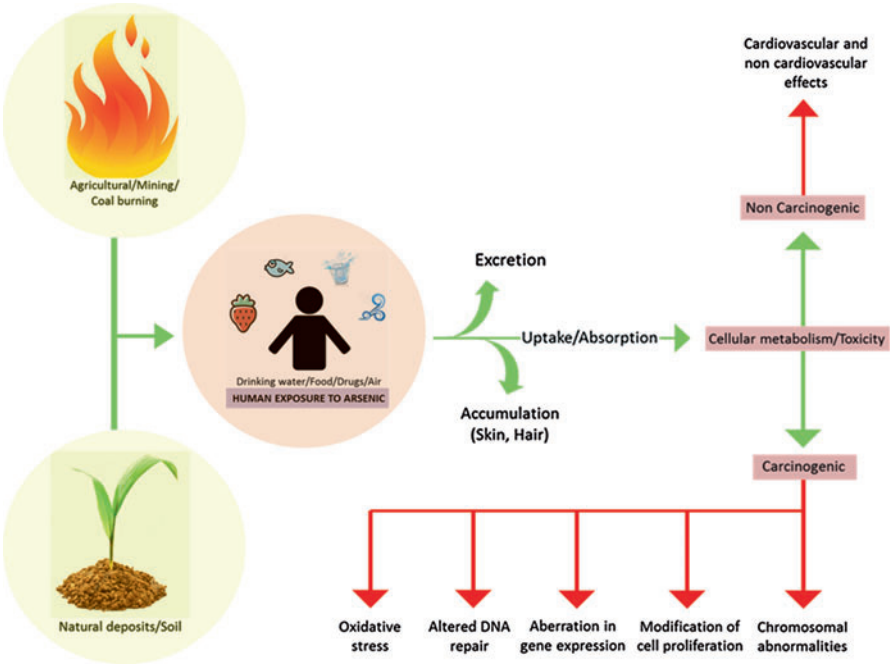

Fig. 2 Source and effects of arsenic human exposure. Human health effects of As are directly correlated with contaminated drinking water consumption and consist of cancer and several nonmalignant diseases

Worldwide the most common route of iAs exposure is through the consumption of drinking water contaminated with natural sources of this metalloid.

Human exposure to As typically results from either oral consumption through contaminated drinking water, soil, and food or As inhalation and skin contact in industrial work-settings. Many industrial processes involve the use of arsenic. For instance, chromated copper arsenate (CCA) was intensively used to treat wood as a preservative agent and represents an important source of exposure to arsenic, in particular in the United States. Arsenic contamination may also occur in soil surrounding glass and pharmaceutical industries or metal smelters and mining activities (Ramirez-Andreotta et al. 2013) (Fig. 2). One of the main nonoccupational sources of iAs for human exposure in most European and non-European countries is represented by drinking water and by food, especially cereals, milk, and seafood. For instance, in the Bengal Delta Plain of Bangladesh and West Bengal, India, As in groundwater has emerged as the largest environmental health disaster, putting at least 100 million people at risk of cancer and other As-related diseases (Smith et al. 2000). In Northern Chile, the limited drinking water and irrigation water sources are contaminated by As leading to chronic arsenicosis in resident population. Dietary intake is another common route of exposure to iAs, especially for rice consumers belonging to specific ethnic groups.

In Italy, high As concentrations were determined on water, marine and river sediments, and on soils only in Lazio and Campania regions. In hydrothermal conditions, As can be selectively mobilized reaching concentrations of the order of mg/l.

In 2009, the EFSA highlighted the toxicological risk associated with young children eating food contaminated with high levels of iAs, exacerbated by the fact that infants consume a large amount of food compared to their body mass. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established a limit of between 0.3 and 8 μg/kg b.w. per day for an increased risk of skin lesions and cancer of the lung, skin, and bladder (European Food Safety Authority EFSA 2009), and introduced a provisional tolerable weekly intake (PTWI) of 15 μg/kg b.w.

Most of the occurrence data for As in food collected for official food control are reported as total As (tAs) without differentiating between the various As species. The need for speciation data is evident, especially in food associated with emerging risks, such as rice-based infant food. The food subclasses of cereal grains and cereal-based products, followed by food for special dietary uses, bottled water, coffee and beer, rice grains and rice-based products, fish, and vegetables were identified as the main contributors to iAs exposure in the general European population. Meharg et al. (2008) reported that the risk for adverse health effects is higher for exposure occurring during childhood compared to adulthood.

Arsenic toxicity has been well characterized and the cancerogenic effect of human exposure to As has been demonstrated for cancer of skin, lungs, bladder, kidney, and liver (Argos 2015). The molecular mechanisms associated with these effects have been hypotesized to be epigenetic processes. The polymorphism of the genotype may affect the individual response to iAs toxicity and its metabolism within the population (Tantry et al. 2015). Exposure to As has been correlated to epigenetic changes, such as DNA methylation, hypo- and hypermethylation at different loci, and changes in microRNA (miRNA) expression profiling (Reichard and Puga 2010), although *in vivo* studies in human populations exposed to As are necessary for better understanding these correlations (Ren et al. 2011). Arsenic can cause suppression of specific genes involved in the DNA repair pathway and it can interfere with the control of the cell cycle. Furthermore, As is a powerful endocrine disruptor: it can affect gene regulation by interacting with steroid hormones receptors that can result in adverse effects on human development (Bodwell 2006) with different modulations on gene transcription at low or high doses.

The effects of high-dose exposure to As on human health include severe consequences such as neurological disorders; cancer; and liver, renal, fertility, and cardiovascular diseases, including systemic toxicity and death. Chronic exposure to a low dose of As can result in cardiovascular diseases and other disorders such as skin changes and skin cancer, peripheral sensorimotor neuropathy, and diabetes mellitus (Naujokas et al. 2013). Results have been reported that evidence a correlation between exposure to a low level of As in utero and a reduced birth weight, and other authors have linked exposure to As to spontaneous or neonatal death (Marsit 2015). A study by Rebuzzini et al. (2015) regarding the understanding of specific human cardiac diseases caused by in utero exposure to As demonstrated a disruption

at each step of developmental cellular process by As trioxide. Such work is the first example of *in vitro* model study performed using the whole 15 days of *in vitro* differentiated mouse embryonic stem cells (mESCs) into cardiomyocytes.

Arsenic Mechanism of Action

The molecular mechanisms underlying the toxic effects associated with iAs exposure are not well understood. Arsenic toxicokinetics varies depending on the As form and on different factors such as life stage, gender, nutritional status, and genetic polymorphisms.

Oral absorption through drinking water and food is the main form of exposure to iAs, with a high rate of absorption in the gastrointestinal tract (typically >70%). Absorption via inhalation is relatively limited and via dermal exposure is even more limited (NRC 1999; WHO 2001).

The absorption of iAs is influenced by the solubility of the arsenic compound, the presence of other food constituents and nutrients in the gastrointestinal tract, and by the food matrix itself [As(III) and As(V) in drinking water are almost completely and rapidly absorbed] (EFSA CONTAM Panel 2009).

The metabolic pathway of iAs is a complex process that gives rise to different organic species.

In humans, the product of the first methylation is monomethylarsonic acid (MMA) and the product of the second and final methylation is dimethylarsinic acid (DMA). DMA and MMA were classified as potentially carcinogenic (IARC Group 2B). Arsenobetaine and other organic As compounds not metabolized in humans are "not classifiable as to their carcinogenicity to humans" (Group 3) (IARC 2012).

After oral ingestion, iAs is absorbed in the intestine, primarily methylated in the liver, and excreted in urine by most species, mainly as DMA and smaller amounts of inorganic and organic AsV and AsIII species.

The metabolic pathway of iAs consists of two sequential reactions: first the inorganic pentavalent As (AsV) is converted to trivalent As (AsIII), which is then methylated to monomethylated and dimethylated arsenicals (MMA, DMA, respectively) (Fig. 3).

Another recently discovered pathway is based on the formations of As-GSH complexes (Hayakawa 2005), which are methylated by AS3MT via transfer of the methyl group from S-adenosyl-methionine (SAM).

Metabolism of inorganic As varies between human populations (Hughes 2006). Such variability is due to genetic polymorphisms in the regulation of the enzyme(s) that metabolize arsenic, which may lead to differences in the toxicity related to As exposure (De Chaudhuri 2008; Schläwicke Engström et al. 2008).

Our understanding of As metabolism and kinetics in infants is very limited, although studies have been conducted on infants exposed to iAs through drinking water in South America and Asia (Vahter 2009). Vahter and coworkers were among the first to raise concerns that children may be more vulnerable to iAs exposure

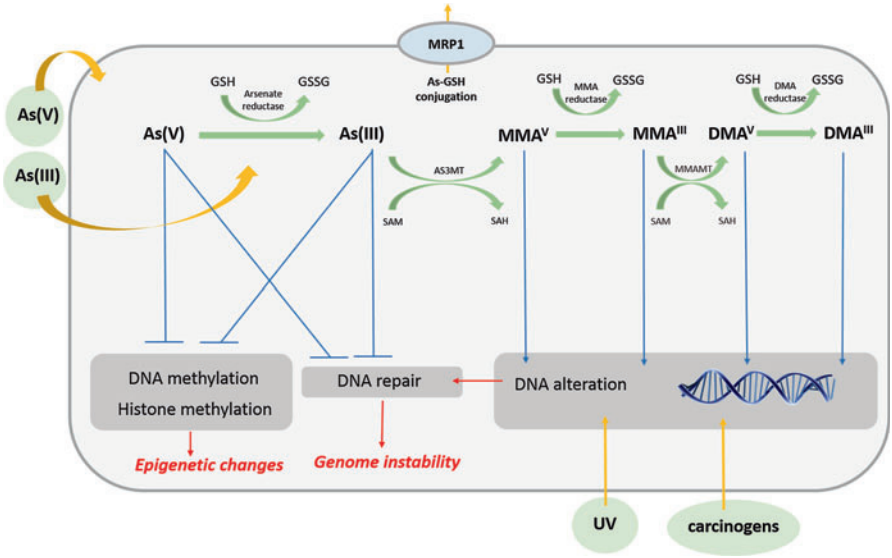

Fig. 3 Metabolic pathway of inorganic arsenic in the liver cell. The main processes, altered after exposure to As, affect the DNA repair and epigenetic changes

compared to adults. In 2009, Fängström et al. demonstrated that the urinary metabolite pattern of children (18 months of age) in Bangladesh is indicative of a decreased As methylation efficiency during weaning. Similar studies that take into account infant exposure to iAs through consumption of rice-based products or drinking water are not available within the EU or any other part of the world. The most important conclusion of the EFSA Scientific Opinion on As in Food (2009) was that the dietary exposure to iAs should be reduced. It is necessary to produce speciation data for different food commodities to obtain dietary exposure assessment and dose-response data for the possible health effects.

According to several toxicological studies, As exposure leads to global changes in gene expression, in particular in epigenetic processes. Epigenetic alterations do not cause a genotoxic effect as they do not involve modification of the DNA sequence, but they can lead to heritable changes in the regulation of gene expression (Feinberg and Tycko 2004). Arsenic induces cancer and changes in gene expression, though maintaining genome stability; changes occur predominantly in CpG islands that are cytosine-rich gene regions (Yoder et al. 1997).

Arsenic exposure has been associated to global genomic DNA hypomethylation that causes illegitimate recombination events and transcriptional deregulation of affected genes. The hypomethylation results from insufficiency of the unique methyl group donor in each conversion step of biomethylation of arsenic, the SAM and from gene expression reduction of the enzyme that catalyzes the As methylation, the DNA methyltransferase (DNMT). Arsenic can affect both people directly exposed and those of future generations in a heritable manner (Ren et al. 2011).

Different epigenetic mechanisms, such as altered DNA methylation, histone modification, and miRNAs expression, have been proposed to play a role in arsenic-induced carcinogenesis. In mammals, DNA methylation is the basic process of gene expression regulation and may cause a reduction of mRNA levels and DNMTs activities.

Arsenic and miRNA Expression Profiles

MiRNAs are endogenous, single-stranded, noncoding RNA molecules ~ 21 nucleotides long which act as negative regulators of gene expression at the post-transcriptional level (Bartel 2009). They exert modulatory roles in crucial cellular processes such as development, differentiation, and apoptosis.

Due to their broad impact on gene regulation, individual miRNAs, or a combination of them, have a potential involvement in a wide range of human diseases such as inflammatory, autoimmune, and metabolic diseases (Sonkoly and Pivarcsi 2009).

It is widely recognized that miRNAs are misregulated in a variety of human tumors, and they act as either oncogenes or tumor suppressors (Yin et al. 2012). In 2013, Xu et al. demonstrated the biphasic effects of arsenite, showing that levels of arsenite in the range of 1.0–2.5 μM induce cell proliferation, while higher levels of arsenite (10–100 μM) induce DNA damage and apoptosis (Xu et al. 2013). In the last years, several experiments based on microarray and RT-qPCR analysis have been conducted both *in vivo* and *in vitro* in order to study miRNA expression changes triggered by iAs exposure.

Despite the significant progress made toward understanding the mechanisms of action of miRNAs, much less is known about the role played by each individual miRNA, and there is a lack of information regarding the effects of chronic As exposure on miRNA expression *in vivo*.

According to Ren et al. (2015), miRNAs expression in rat liver was altered by As exposure in a concentration-dependent manner. Among the identified arsenic-responsive miRNAs, miR-151 and miR-183 were significantly upregulated. miR-151 has been suggested to play a role in breast cancer metastasis and tumor cell proliferation. miR-183 upregulation was observed in lung, breast, prostate, and cervical cancers, and it seems to be associated with tumor cell migration, metastasis, and invasion (Ren et al. 2015). Furthermore, in human urine, miR-200c and miR-205 are inversely associated with As exposure (Michailidi et al. 2015).

These results suggest that the biologic consequences of altered miRNAs induced by As are complex. Further studies are required in order to understand how changes in individual miRNA contribute to arsenic-induced toxicity.

Specific miRNAs Are Implicated in iAs-Mediated Carcinogenesis

Induction of reactive oxygen species (ROS) in cells is closely related with exposure to heavy metals including arsenic, chromium, and cadmium (Wang et al. 2012). In 2012, Ling et al. highlighted that the upregulation of **miR-21** is an important event in the arsenite-induced malignant transformation of human embryo lung fibroblast cells (HELF) mediated by ROS activation of the ERK/NF-κB pathway. It was demonstrated that miR-21 upregulation induces angiogenesis and enhances the invasive potential of transformed cells. Subsequent studies in 2014 by Luo et al. showed that miR-21 acts on PDCD4, an inhibitor of neoplastic transformation, contributing to the EMT (epithelial–mesenchymal transition) induced by arsenite. Wang et al. (2011) treated immortalized p53-knocked down human bronchial epithelial cells ($p53^{low}$HBECs) with low levels of arsenite for 16 weeks ($NaAsO_2$, 2.5 μM). They reported for the first time a causal role for reduction of **miR-200b** expression in human cell malignant transformation and tumor formation resulting from As exposure. Chronic arsenite exposure causes downregulation of miR-200b. This phenomenon can trigger the expression of the EMT-inducing transcription factors ZEB1 and ZEB2, two direct targets of this miRNA. Moreover, the authors highlighted a potential interplay between multiple forms of epigenetic regulations: ZEB1 and ZEB2 are not only targets of miR-200, but they also repress the expression of the miR-200 genes by increasing the methylation of its promoter (Vrba et al. 2010), resulting in a double-negative feedback loop (Bracken et al. 2008; Burk et al. 2008). iAs was also found to trigger a fivefold induction of **miR-190** in a dose-dependent manner in human lung epithelial (BEAS-2B) cells exposed to 20 μM $As3^+$ (Beezhold et al. 2011). miR-190 overexpression triggers the downregulation of its target gene PHLPP (PH domain leucine-rich repeat protein phosphatase), which in turns results in an enhanced activation of Akt signaling and in an increased expression of vascular endothelial growth factor protein (VEGF). These events result in enhanced cell proliferation and carcinogenic transformation (Table 1).

iAS-Induced miRNA Dysregulation Promotes Angiogenesis

The process of angiogenesis normally occurs in the embryo, in the placenta, during the menstrual cycle, and during wound-healing (Patella and Rainaldi 2012). Under pathological conditions, such as cancer, angiogenic signaling pathways are induced in order to form new blood vessels from existing vasculatures and this induces tumor growth. A number of miRNAs have been reported to be involved in blood vessel development and angiogenesis by directly or indirectly regulating proangiogenic factors or antiangiogenic factors (Urbich et al. 2008). Some studies have indicated that abnormal angiogenesis is fundamental in the pathogenesis of a number of diseases caused by environmental As exposure (Navas-Acien et al. 2005; Straub et al. 2009). In 2012, an *in vivo* experiment by Cui Y. and colleagues demonstrated that **miR-9 and miR-181b** exhibited a massive decrease of expression upon sodium arsenite injection and that this reduction was reflected in increased angiogenesis

Table 1 MicroRNAs implicated in inorganic arsenic-mediated carcinogenesis

Carcinogenesis				
MicroRNA	Up/down-regulated	Cell type	Effects	References
miR-21	Up	Human embryo lung fibroblast cells (HELF)	ROS activation of the ERK/NF-κB path way	Ling et al. 2012
	Up	Human bronchial epithelial (HBE) cells	Contributes to the epithelial-mesenchymal transition acting on PDCD4	Luo et al. 2014
miR-200b	Down	Immortalized p5 3-knocked down human bronchial epithelial cells	Expression of the EMT-inducing transcription factors ZEB1 and ZEB2	Wang et al. 2011
miR-190	Up	Human lung epithelial (BEAS-2B) cells	Downregulation of its target gene PHLPP, activation of Akt signaling and increased expression of vascular endothelial growth factor protein (VEGF)	Beezhold et al. 2011

Specific miRNAs are up- or downregulated in the arsenite-induced malignant transformation

levels. The experiment was performed by injecting fertilized eggs with 100 nM sodium arsenite at Hamburger–Hamilton (HH) stages 6, 9, and 12, and harvesting them at HH stage 18. miR-9 and miR-181b are implicated in promoting abnormal angiogenesis in iAs-exposed chick embryos by targeting NRP1, a transmembrane receptor implicated in vascular development (Bielenberg and Klagsbrun 2007). Cui et al (Cui et al. 2012) also investigated the role of miR-9 and miR-181b in sodium arsenite-mediated angiogenesis in the human umbilical vein endothelial cell line EA hy926. Their results indicated that overexpression of miR-9 or miR-181b decreased NRP1 expression, cell migration, and tube formation, supporting involvement of miR-9 and miR-181b in arsenite-induced NRP1 expression and angiogenesis *in vitro* in human. In 2011, Carpenter et al. (2011) established an *in vitro* model of arsenic-induced carcinogenesis by transforming immortalized human lung epithelial BEAS-2B cells via chronic exposure to 1 μM sodium As for 26 weeks (Arsenic-transformed BEAS-2B, AsT). They observed that AsT cells produced higher levels of ROS (Carpenter et al. 2011). In 2014, He et al. observed that basal levels of HIF-1α and COX-2 under normoxia were markedly upregulated in AsT cells. A miRNA microarray experiment analysis was performed to compare the miRNA profiles between BEAS-2B and AsT cells, and **miR-199a-5p** resulted to be the most downregulated miRNA. Previously, He et al. (2012) demonstrated that ROS inhibit miR-199a expression through increase of the promoter methylation of miR-199a gene by DNA methyltransferase-1 (He et al. 2012). Taqman RT-qPCR analysis showed MiR-199a-5p expression level was 100-fold lower in AsT cells which, accordingly, brought to overexpression of HIF-1α, a known direct target of

Table 2 Inorganic arsenic-induced microRNA dysregulation promotes angiogenesis

Angiogenesis				
MicroRNA	Up/down-regulated	Cell type	Effects	References
miR-9	Down	*in vivo* on fertilized eggs	Increased angiogenesis levels by targeting NRP1, a transmembrane receptor implicated in vascular development	Cui et al. 2012; Bielenberg et al. 2007; Staton et al. 2007
miR-181b	Down	*in vivo* on fertilized eggs	Increased angiogenesis levels by targeting NRP1, a transmembrane receptor implicated in vascular development	Cui et al. 2012; Bielenberg et al. 2007; Staton et al. 2007
miR-199a-5p	Down	Arsenic-transformed BEAS-2B cells (As T cells)	Up-regulation of HIF-1α and COX-2, two angiogenic activators	He et al. 2014

The downregulation of different miRNAs cause abnormal angiogenesis, a fundamental process in the pathogenesis of a number of diseases caused by environmental As exposure

mir-199a (Rane et al. 2009). HIF-1α is one of the major proangiogenic factors through inducing transcriptional activation of vascular endothelial growth factor (VEGF) (Semenza 2000). The authors also validated that COX-2, another potent angiogenic activator for tumor angiogenesis (Xue and Shah 2013), is a novel target of miR-199a. Taken together these data indicate that miRNA dysregulation upon As exposure promotes tumor growth and angiogenesis both *in vitro* and *in vivo* (Table 2).

Arsenic Exposure Impairs DNA Damage Repair and Inhibits Apoptosis Through Specific miRNA Dysregulation

MiR-222, a known oncogene, can increase migration and proliferation of hepatocellular carcinoma (Zhang et al. 2015) and inhibit apoptosis by regulating different targets such as PTEN (Chun-Zhi et al. 2010; Yang et al. 2014), and TIMP3 (Lu et al. 2011). A microarray experiment analysis, also confirmed using RT-PCR and RT-qPCR, showed that miR-222 expression is upregulated the most in arsenic-transformed BEAS-2B cells (As-T cells) (Carpenter et al. 2011), as evidenced by a fourfold higher levels of miR-222 in As-T cells compared to the levels present in B2B cells (Wang et al. 2016). The authors demonstrated that miR-222 directly targets PTEN for inducing the activation of its downstream molecules AKT and ERK in arsenic-transformed cells, inhibiting apoptosis. It is known that upregulation of PI3K/AKT/mTOR pathway is involved in the tumorigenesis of several cancers, particularly through mutations and inactivation of PTEN (Jin et al. 2014; Lavorato-Rocha et al. 2015). In this study, ARID1A was demonstrated to be a new direct target

of miR-222. ARID1A, a subunit of the SWItch/sucrose nonfermentable (SWI/SNF) chromatin remodeling complex, has been found to be expressed at low levels in many cancers (Nagymanyoki et al. 2015). An overexpression of miR-222 (twofold), **miR-221** (threefold), and **miR-638** (2.6 fold) was reported by Sturchio et al. (2014) in Jurkat cell line upon 144h iAs exposure. Accordingly, the overexpression of these miRNAS corresponded to the decreasing expression of their putative target gene, the ring finger protein 4 (RNF4). RNF4 is a member of the family of SUMO targeted ubiquitin ligases, and it has a role in active DNA demethylation involving enzymes implicated in DNA repair (Hu et al. 2010). Previously, Marsit and colleagues (Marsit et al. 2006) evidenced the same trend in human immortalized lymphoblast cell line TK-6 and hypothesized that this upregulation could be due to a change in As methylation patterns as a result of reduced levels of S-adenosylmethionine. Moreover, miR-638 is known to be upregulated in oxidative stress conditions, which suggests that miR-638 may play a role in the generalized cellular response to iAs-induced oxidative stress (Simone et al. 2009). Sturchio et al. also showed a time-dependent upregulation of the miR-663 gene expression and at the same time a downregulation of TGFβ1 expression, a validated target of **miR-663** (Tili et al. 2010) and one of the most commonly altered cellular signaling pathways in human cancers. In physiological conditions, TGFβ1 inhibits cell growth, migration, differentiation, apoptosis, and matrix organization (Inman and Allday 2000). Taken together, these results indicate that miRNA dysregulation upon iAs exposure triggers pathways which bring to inhibited cell apoptosis and impaired DNA damage repair, prompting tumor growth (Table 3).

Conclusion

This review evidences that several recommendations regarding As exposure (including knowledge and data gaps) are still to be addressed:

1. Dietary exposure to iAs should be reduced.
2. In order to refine risk assessment of iAs, there is a need to produce speciation data for different food to support dietary exposure assessment and dose-response data for the possible health effects.
3. Future epidemiological studies should incorporate better characterization of exposure to iAs, including food sources.
4. There is a need for more information on critical age periods of As exposure, in particular in early life.
5. There is a need for improved understanding of the human metabolism of organoarsenicals in foods (arsenosugars, arsenolipids, etc.) and the corresponding human health implications.

6. It should be necessary to grow knowledge about coexposure to different factors (i.e., As and uv) on human to develop putative therapeutics for arsenic-induced cancer in occupational and non occupational exposure.
7. Studies regarding miRNA expression profiling highlighted that iAs induced miRNA dysregulation triggers pathways which bring to inhibited cell apoptosis and impaired DNA damage repair, prompting tumor growth.

Table 3 Inorganic arsenic-induced microRNA dysregulation impairs DNA damage repair and inhibits apoptosis

Impairs DNA damage repair and inhibits apoptosis				
MicroRNA	Up/down-regulated	Cell type	Effects	References
miR 222	Up	Hepatocellular carcinoma	Inhibit apoptosis by regulating different targets such as FTEN	Chun-Zhi et al. 2010
	Up	Human hepatocellular carcinoma HepG2 cells	Inhibit apoptosis by regulating different targets such as p27	Yang et al. 2014
	Up	Tamoxifen-resistant MCF-7 (OHT(R)) cells and Her2-positive human breast tumors	Inhibit apoptosis by regulating different targets such as TIMFE	Lu et al. 2011
miR-222	Up	Ars enic-transformed BEAS-2B (As-T cells)	Inhibit apoptosis by regulating different targets such as FTEN	Carpenter et al. 2011; Wang et al. 2016
	Up	Jurkat cell line	Targets the Ring Finger Protein 4 (RNF4), that has a role in DNA repair	Sturchio et al. 2014
	Up	Human immortalized lymphoblast cell line TK-6	Targets the Ring Finger Protein 4 (RNF4), that has a role in DNA repair	Marsit et al. 2006
miR-221	Up	Jurkat cell line	Targets the Ring Finger Protein 4 (RNF4), that has a role in DNA repair	Sturchio et al. 2014
miR-638	Up	Jurkat cell line	Targets the Ring Finger Protein 4 (RNF4), that has a role in DNA repair	Sturchio et al. 2014
miR-663	Up	Jurkat cell line	Down-regulation of TGFβl expression	Sturchio et al. 2014

miRNAs dysregulation upon iAs exposure triggers pathways which bring to inhibited cell apoptosis and impaired DNA damage repair, thus prompting tumor growth

Future research should focus on the development of new miRNA profile for preventive or therapeutic use.

Key Facts of Arsenic Adverse Effects on Health

- Inorganic arsenic poses a major threat to worldwide human health.
- International Agency for Research on Cancer classified arsenic and inorganic arsenic compounds as "carcinogenic to humans" (Group 1) based on sufficient evidence of carcinogenicity in humans.
- One of the major routes of human exposure is represented by dietary intake in most European and non-European countries.
- The molecular mechanisms underlying the toxic effects of inorganic arsenic exposure are not well understood.
- A chronic arsenic exposure may alter miRNAs expression profiling.

Dictionary of Terms

- **DNA repair pathway** – Set of chemical reactions involved in the processes to correct DNA damage.
- **Endocrine disruptor** – A molecule that mimes a natural hormone, interfering with hormonal equilibrium.
- **Metabolic pathway of inorganic arsenic** – Cellular detoxification process under inorganic arsenic exposure.
- **Reactive oxygen species** – Chemical species containing oxygen that have a crucial role in cell signaling.
- **Apoptosis** – Programmed cellular death.
- **Angiogenesis** – Normal physiological process in growth and development for granulation tissue formation.

Summary Points

- This chapter focuses on inorganic arsenic and its effects on human health.
- Arsenic is a ubiquitous metalloid, widely distributed in the environment.
- Exposure to inorganic arsenic typically results from either oral arsenic consumption through contaminated drinking water, soil, and food or arsenic inhalation in an industrial work setting.
- The data show that the main exposure route to inorganic arsenic remains dietary, particularly for young infants. Therefore, the human exposure to food and drinking water As contamination is a real concern.
- International Agency for Research on Cancer (IARC) classified inorganic arsenic as "carcinogenic to humans" (Group 1) based on sufficient evidence of carcinogenicity in humans.

- Many of the human health effects of inorganic arsenic may be considered: cancers of the skin, lung, bladder, liver, and kidney, neurologic disease, cardiovascular disease, as well as other nonmalignant diseases.
- We examined the effects of inorganic arsenic exposure on miRNAs expression profile through *in vitro* and *in vivo* studies.
- It is widely recognized that miRNAs are misregulated in a variety of human tumors acting as both oncogenes and tumor suppressors.
- Several authors showed that inorganic arsenic-induced microRNA dysregulation promotes angiogenesis and carcinogenesis.
- However, we argue that a list of miRNAs may be considered as potential biomarkers of inorganic arsenic effects.

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