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Mechanistic Basis of Arsenic Induced Carcinogenesis: Differential miRNA Expression

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ABSTRACT: Arsenic, a toxic metalloid, provokes many detrimental consequences to human health. It is prevalent in earth's crust and poses a major threat to humans globally. Inorganic arsenic exposure occurs mainly via drinking water or food and is metabolized in mammals to form organic metabolites/ end products. Chronic exposure to arsenic causes lung, skin and urinary bladder cancers and increases the risks of liver, kidney and prostate cancers. Arsenic-induced ROS generation, disturbances in several signaling pathways, DNA repair inhibition, chromosomal aberrations, and epigenetic changes including alterations in DNA methylation, histone modifications and differential miRNA expression profiles are involved in cancer progression, and malignant transformation. However, details of arsenic-induced carcinogenesis and molecular mechanisms involved are still remaining obscure. MicroRNAs are post-transcriptional gene expression regulators and themselves may act as oncogenes and tumor suppressor genes. Differential miRNA expression is implicated in several human cancers. This review covers general mechanistic basis of arsenic-induced carcinogenesis, explores recent *in-vitro*, *in-vivo* and cohort studies on differential miRNA expression profiles and shares associated molecular mechanistic data on miRNA dysregulation and their functional consequences leading to arsenic induced tumorigenesis, metastasis and cancer, also discusses the future directions.

KEY WORDS: Arsenic, carcinogenesis, molecular mechanisms, miRNA dysregulation, *in-vitro* and *in-vivo* studies.

INTRODUCTION

Carcinogenesis is one of the most intricate biological processes which can be induced by multiple factors such as genetic changes, epigenetic aberrations and environmental influences. Arsenic is a well-recognized environmental carcinogen in humans. Still, the detailed molecular mechanisms of arsenic-induced carcinogenesis are yet to be elucidated. Several mechanisms of action have been proposed such as epigenetic alterations including differential microRNA expression (Cardoso et al. 2018). MicroRNAs play an important role in arsenic-induced carcinogenesis and themselves may function as oncogenes and tumor suppressor genes. This review explores recent in-vitro, in-vivo, and cohort studies on differential miRNA expression profiles and shares associated molecular mechanistic data on miRNA dysregulation and their functional consequences leading to arsenic-induced tumorigenesis, metastasis, and cancer, as well as future directions.

MicroRNAs

MicroRNAs are small (~22 nucleotide long) noncoding molecules of RNA which operate as gene expression regulators post transcriptionally (Ha and Kim 2014; Hausser and Zavolan 2014). Since their first discovery in 1993 (Lee et al. 1993), they have been shown to play important roles in developmental processes, cell proliferation and differentiation, immune system functions, apoptosis, angiogenesis, stem cell functions and other cellular processes by negatively regulating expression of about two thirds of all mRNAs in humans (Bushati and Cohen 2007; Friedman et al. 2009; Humphries et al. 2016). Aberrant microRNA expressions are associated with several human diseases such as viral, cancer, neurodegenerative and immune-related disorders (Li and Kowdley 2012).

Biogenesis and mechanism of action

The microRNA biogenesis begins with transcription of their gene by RNA polymerase II, with a few exceptions that are transcribed by RNA polymerase III, generating a large primary RNA transcript (pri-mRNA) that contains a stem loop region and its size ranges from hundreds of nucleotides to several kilo bases (Bushati and Cohen 2007; Romero-Cordoba et al. 2014). Human microRNA biogenesis is a two step process involving cleavage events in both nucleus and cytoplasm (MacFarlane and R. Murphy 2010). Pri-

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mRNA is cleaved inside nucleus by a multiprotein microprocessor complex containing Drosha (a Type III RNase) and DGCR8 (an RNA-binding protein) to form precursor miRNA (Pre-miRNA) which itself is ~70 nucleotide long having ~22 nucleotides in stem and ~48 nucleotides in terminal loop region (Romero-Cordoba et al. 2014; Humphries et al. 2016). Pre-miRNA is transported from nucleus to the cytoplasm by exportin-5-RAN-GTP and is further processed in cytoplasm by Dicer (RNase III), TRBP (dsRNA binding protein) and PACT (kinase R activating protein in humans) to produce a ~22 nucleotide long miRNA duplex having one passenger strand and other mature/guide strand (Bushati and Cohen 2007; Romero-Cordoba et al. 2014). After miRNA duplex unwinding one of the strands (referred to as guide/mature strand) is loaded on the Argonaute protein containing RNA induced silencing complex (RISC) (Bushati and Cohen 2007; Romero-Cordoba et al. 2014). In activated miRNA-RISC complex, the miRNA functions as guide to recognize and form Watson-crick base pairing with its target mRNA at its 3'-UTR, mostly by the seed region of miRNA (residues 2-8 at 5' end) based on complementarity rules (Bushati and Cohen 2007; Humphries et al. 2016; Peng and Croce 2016). The mRNA silencing can occur either by cleavage (deadenylation) and degradation or by translational inhibition (Hayes et al. 2014). If the miRNA:mRNA interaction has perfect or near perfect complementarity then argonaute protein (Ago2 in mammals), having RNA endonuclease activity, cleaves the target mRNA which is later degraded and if there is imperfect base pairing then only translational inhibition occurs (MacFarlane and R. Murphy 2010; Hayes et al. 2014; Romero-Cordoba et al. 2014). Gene regulation by miRNA is a dynamic process, one miRNA can have different functions by potentially targeting multiple mRNAs and even one mRNA can be regulated simultaneously by multiple miRNAs (Zhou et al. 2017a).

Role of miRNAs in Cancer

Many studies in the recent past reported that miRNA expression is dysregulated in cancer, possibly via different underlying mechanism including deletion or amplification of miRNA genes, dysregulation of transcription factors (such as c-Myc and p53), epigenetic alterations (including aberrant DNA methylation and acetylation) or abnormalities in miRNA biosynthesis pathway (Peng and Croce 2016; Zhou et al. 2017a). Different cancer types including prostate, thyroid, breast, colon and gastric cancers are reported to have aberrant miRNA expressions (Reddy 2015). The differential miRNA expression signatures between normal and tumor tissues can be potentially used as prognostic and diagnostic biomarkers and even therapeutic targets for mitigation of cancer (Peng and Croce 2016; Zhou et al. 2017a). Dysregulated miRNAs in cancer cells enable them to resist apoptosis, evade growth suppressors, promote cell proliferation, trigger angiogenesis and activate metastasis (Peng and Croce 2016). Evidences have shown that many miRNAs possess a fundamental role in cancer development and progression by acting as oncogenes (by down-regulating mRNAs of tumor suppressor genes) or as tumor suppressor genes (by down-regulating mRNAs of oncogenes) (Lotterman et al. 2008; Romero-Cordoba et al. 2014; Zhou et al. 2017a). For instance, miR-504 over expression down-regulated p53 (a tumor suppressor gene) by binding to its 3' UTR, reducing its functions including p53-mediated apoptosis and cell cycle arrest in stress response (Hu et al. 2010). Myc oncogene was down-regulated by miRNA let-7a, thus inhibiting the cell growth induced by Myc in Burkitt lymphoma cells (Sampson et al. 2007). MiR-34a down-regulated the expression of c-Myc oncogene and transfection of miR-34a in prostate cancer (PC-3) cells in-vitro inhibited cell proliferation and invasion also promoted apoptosis (Yamamura et al. 2012). Moreover, miRNAs also play crucial roles in chemical carcinogen induced tumorigenic process and cell malignant transformation. Several studies have shown that expression of miRNAs and their functions are deregulated after chemical carcinogen exposures such as to arsenic (Humphries et al. 2016). Mixture of arsenic (As), cadmium (Cd) and lead (Pb) compounds was found to induce differential miRNA expressions, subsequently disturbing the target mRNA expression profiles, thereby enhancing the risk of cancer (Martínez-Pacheco et al. 2014; Rojas et al. 2019).

Arsenic

Arsenic (atomic number - 33, relative atomic mass – 74.92) is a metalloid prevalent in earth's crust as the 20th most common element (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 2012). It is found in air, water, soil and sediments. Arsenic can exist in oxidation states of -3, 0, +3 and +5; Most commonly present as arsenate (+5) in oxygenated environments and as arsenite (+3) in reducing or anaerobic conditions (WHO 2001).

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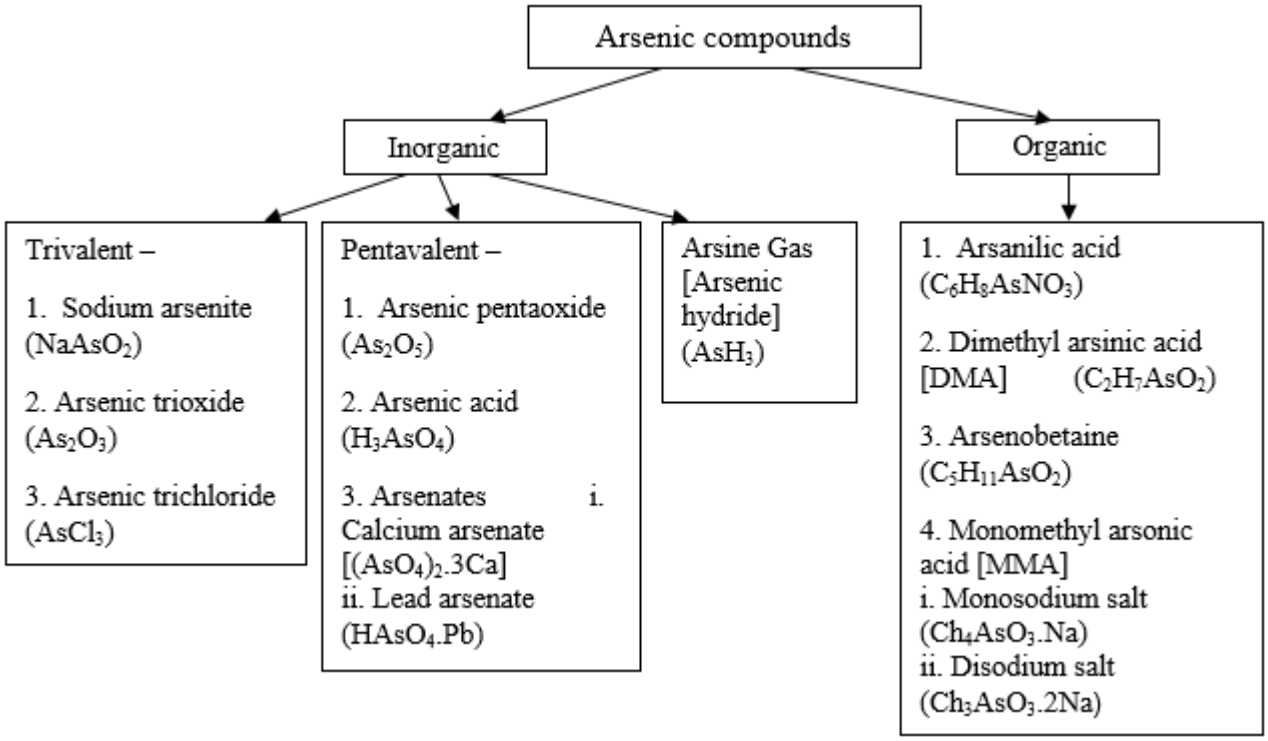

Figure 1 – Common Arsenic compounds
Ref: IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2012

Arsenic toxicity

Toxicity of arsenic is well known. Acute arsenic exposure leads to abdominal pain, vomiting and diarrhea. Arsenic poisoning for a long term leads to skin pigmentation changes, lesions, hyperkeratosis, peripheral neuropathy, destruction of erythrocytes, high blood pressure, diabetes, disturbances of cardiovascular and nervous system functions and cancer (WHO 2001). Arsenic can also cross placenta, thus prenatal arsenic exposure causes serious health impacts including cancer and other diseases (Rager et al. 2014; Liu et al. 2020). Arsenic and arsenic compounds have been classified by International agency for research on cancer (IARC) as group 1 carcinogens to humans, indicating the availability of sufficient evidence in this regard. Chronic exposure to arsenic can cause skin, lung and urinary bladder cancers and is also associated with increased risk of liver, prostate and kidney cancers (Arsenic in drinking water, WHO, 2011; IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2012; Martinez et al., 2011; WHO, 2001).

Human exposure and metabolism of arsenic

Primary arsenic exposure to humans can occur via food and water; other routes may include occupational exposure (industries) and smoking. Contamination of arsenic in drinking water is a major health concern globally, affecting more than 200 million people worldwide (Hunt et al. 2014; Sage et al. 2017). WHO has recommended 10 µg/l (10 ppb) as maximum limit of contamination for arsenic in drinking water (WHO 2011). Groundwater of several countries is known to have high levels of arsenic such as China, Argentina, Chile, United States of America, Mexico, India and Bangladesh (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 2012; Hunt et al. 2014; Karagas et al. 2015; Bjørklund et al. 2018). In India, states such as West Bengal, Bihar, Jharkhand, and regions of Gangetic flood plains of Uttar Pradesh, flood Plains of Brahmaputra and Imphal rivers in Assam and Manipur, and some districts of Rajasthan are reported to have higher than recommended levels of groundwater arsenic (Ghosh and Singh 2009; Duggal et al. 2012; IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 2012). Upon injection of inorganic arsenic (iAs) both pentavalent (As^{V}) and trivalent (As^{III}) arsenicals are absorbed from gastrointestinal tract and they are metabolized by first reduction of pentavalent arsenical into trivalent form and then oxidative methylation of

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trivalent arsenical to form monomethylated (MMA) and dimethylated (DMA) forms of organic arsenic, now these organic end products can be readily excreted out in urine (Ren et al. 2011; WHO 2011).

REDUCTION:	$As^{V}O_4^{-3} + 2e^-$ (thiol)	$\longrightarrow$ $As^{III}O_3^{-3}$
OXIDATIVE METHYLATION:	$As^{III}O_3^{-3} + Me^+$ (SAM)	$\longrightarrow$ $MMA^{V}O_3^{-2}$
REDUCTION:	$MMA^{V}O_3^{-2} + 2e^-$ (thiol)	$\longrightarrow$ $MMA^{III}O_2^{-2}$
OXIDATIVE METHYLATION:	$MMA^{III}O_2^{-2} + Me^+$ (SAM)	$\longrightarrow$ $DMA^{V}O_2^{-2}$
REDUCTION:	$DMA^{V}O_2^{-2} + 2e^-$ (thiol)	$\longrightarrow$ $DMA^{III}O^-$

Mammalian iAs metabolic pathway

AS3MT acts as catalyst for As^{III} methylation using S-adenosyl methionine (SAM) as methyl group donor and thiol as reductant (e^- donor).

MMA^{III} $\longrightarrow$ Monomethylarsonous acid
MMA^{V} $\longrightarrow$ Monomethylarsonic acid
DMA^{III} $\longrightarrow$ Dimethylarsinous acid
DMA^{V} $\longrightarrow$ Dimethylarsinic acid

Reference: IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2012; (Ren et al., 2011).

The presence of arsenic and its various metabolites in urine, blood, hair and nails can be utilized as preliminary biomarker of arsenic exposure. Previously it was believed that inorganic arsenicals are more toxic than organic forms of arsenic because organic arsenicals are readily eliminated via kidney in urine but inorganic arsenic compounds are found to be accumulated in various tissues like muscles, liver, kidney, skin and bones, and within these, trivalent arsenicals show higher level of accumulation than pentavalent arsenicals (Arsenic in drinking water, WHO, 2011; WHO, 2001). However recent studies indicate that methylated arsenites including MMA^{III} and DMA^{III} are more toxic than inorganic arsenicals, probably due to their direct inhibitory effects on enzymes involved in oxidative metabolism (Ren et al. 2011; Hunt et al. 2014).

Mechanism of Cancer induction by arsenic

Arsenic induced carcinogenesis is proposed to have several mechanisms of action including increased oxidative stress, inhibition of DNA repair process, disarray of cellular signaling cascades, chromosomal aberrations such as aneuploidy and co-mutagenicity and epigenetic alterations particularly the DNA methylation changes, histone modifications and miRNA dysregulation (Ren et al. 2011; Bailey and Fry 2014; Hunt et al. 2014; States 2015; Li and Chen 2016; Sage et al. 2017; Bjørklund et al. 2018). In this review we will generally cover these mechanisms involved in arsenic induced carcinogenesis and explore miRNA dysregulation in detail.

Oxidative Stress

Arsenic heightens oxidative stress by stimulating production of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide anion radicals ($O2^{o-}$), peroxyl radicals (ROO^o) and hydroxyl radicals (^oOH) (Li and Chen 2016; Sage et al. 2017). During metabolism of arsenic, oxidative conversion of arsenite to arsenate produces two electrons leading to hydrogen peroxide formation which further produces hydroxyl radical. Arsenic also up-regulates membrane-bound NADPH oxidase enzyme complex, specifically its p22phox protein subunit that produces superoxide anion radicals ($O2^{o-}$), hence contributing to ROS generation. Arsenic induces activation of NADPH oxidase enzyme by translocating Rac1 (a GTP binding protein, NADPHO activator) and enhances its function by activating CDC42 (a GTPase) (Hunt et al. 2014; Li and Chen 2016).

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Arsenic causes changes in mitochondrial membrane potential and integrity, which leads to the discharge of reactive oxygen species from mitochondria to cytoplasm (Hunt et al. 2014; Li and Chen 2016). This excessive generation of ROS is responsible for arsenic-induced oxidative DNA damage, generating both single and double stranded DNA breaks, and also activating various cell proliferation signaling pathways (Martinez et al. 2011; Li and Chen 2016; Sage et al. 2017). Arsenic also depletes natural antioxidant pools including nitric oxide (NO) and glutathione. Arsenic induced NO depletion occurs by binding of MMA^{III} to thiol moiety of NO synthase enzyme, thereby inhibiting it, and systemic NO depletion by its reaction with excessive arsenic generated ROS (Hunt et al. 2014). Arsenic induced excessive ROS generation also activates cell proliferative pathways, affects DNA repair systems negatively and damages lipid and protein structures (Hunt et al. 2014; Sun et al. 2014).

Disarray of cellular signaling cascades

Arsenic causes cell proliferation by affecting several signaling pathways such as PI3K/AKT, Wnt, MAPK, Ras and JNK-STAT3 (Hunt et al. 2014; Sage et al. 2017; Cardoso et al. 2018). Disruption in most of these pathways may lead to multiple oncogene activations, tumor suppressor gene inhibitions and further cell proliferative upregulation.

Arsenic blocks JNK phosphatase by binding to its sulfhydryl group which in turn causes irreversible activation of JNK signaling leading to downstream activation of c-Jun (a proto-oncogene) (Yu et al. 2006). Arsenic induced the expression of mineral dust-induced gene (mdig), an oncogene associated with lung cancer, by upregulating JNK and STAT3 signaling pathways (Sun et al. 2014). Arsenic can induce skin cell proliferation via activation of canonical Hippo signaling pathway by upregulating its various components also it can do the same by upregulating Yes associated protein (Yap) dephosphporylation independent of hippo activation pathway. Dephophorylated Yap is known to target GLI2 gene (a downstream effector of sonic hedgehog signaling pathway) (Hunt et al. 2014). Arsenic also upregulates various inflammatory pathways such as AP-1 activation in MAPK signaling and release of various inflammatory cytokines such as IL-6, IL-8, GM-CSF and TGF-α (Yu et al. 2006; Hunt et al. 2014). All of these dysregulated pathways result in inflammatory and proliferative responses in skin cells.

Arsenic also inhibits the activation of tumor suppressor protein p53 and the subsequent expression of p21, which is responsible for disturbed p53-dependent apoptotic response and allows the survival of cells with DNA damage thus, facilitates cell proliferation and often leads to tumorigenesis (Taylor et al. 2006; IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 2012; Hunt et al. 2014).

DNA repair and co-mutagenicity

Arsenic negatively impacts various DNA repair mechanisms such as nucleotide excision repair (NER), base excision repair and mismatch repair (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 2012; Hunt et al. 2014; Sage et al. 2017). It works by negatively affecting several DNA repair associated genes such as inhibiting Excision Repair Cross-Complementing rodent repair, complementation group 2 (ERCC2) which results in impaired transcriptional proofreading (Hunt et al. 2014; Sage et al. 2017). Arsenic is also known to deregulate several regulatory proteins involved in DNA repair mechanism such as DNA ligase I–III, DNA polymerase β, PARP and O^6-methyl-guanine-DNA methyltransferase (Yu et al. 2006). Thus genomic stability of the cell is compromised when arsenic interferes with these proteins. This also explains the co-mutagenicity of arsenic. Arsenic induces genotoxicity and interferes with DNA damage response mechanisms, thereby enhancing the mutagenicity of other carcinogens, for instance UV radiation and chemical agents (Yu et al. 2006; Hunt et al. 2014; Sage et al. 2017).

Chromosomal aberrations

Arsenic causes genomic instability which is associated with arsenic induced ROS generation and oxidative DNA damage and is further responsible for chromosomal aberrations caused by disruptions in microtubule assembly, increased micronuclei formation and aneuploidy which may activate proto-oncogenes and/or inactivation of tumor suppressor genes leading to increased risk of cancer development (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 2012; Bhattacharjee et al. 2013; States 2015; Sage et al. 2017). Both in vitro and in vivo chronic arsenic exposure may lead to impaired regulation of microtubule formation by mitotic spindle checkpoint, cell cycle checkpoints, chromosomal condensation and sister chromatid cohesion, which results in structural alterations in chromosomes including end to end fusion, aberrant segregation, abnormal separation of sister chromatids, aneuploidy and polyploidy (Bhattacharjee et al. 2013).

Arsenic induces the formation of micronuclei, at low dose it interferes with function of spindle fibers resulting in formation of micronuclei with centromeres, thus acting as an aneugen, but its chronic exposure leads to formation of micronuclei those are

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without centromeres, hence acting as a clastogen (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2012, Yu et al., 2006).

Epigenetic alterations

Epigenetic alterations are genome modifications without changing DNA sequences. Arsenic induced epigenetic alterations include histone modification, DNA methylation changes, and microRNA dysregulation.

DNA methylation and histone modification

Arsenic can cause differential DNA methylation patterns by depletion of S-adenosylmethionine (SAM). S-adenosylmethionine (SAM) cofactor is necessary for both the methylation of DNA by DNA methyltransferase at cytosine of CpG islands and biotransformation of inorganic arsenic. Arsenic-induced global hypomethylation activates multiple oncogenes, whereas specific gene promoter hypermethylation of DNA silences tumour suppressor genes such cyclin-dependent kinase inhibitor 2A (CDKN2A/p16) or tumour protein 53 (P53), and so plays a crucial role in carcinogenesis. (Martinez et al. 2011; Ren et al. 2011; IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 2012; Bailey and Fry 2014; Li and Chen 2016; Sage et al. 2017; Bjørklund et al. 2018; Liu et al. 2020).

Arsenic exposure alters post-translational histone modification by increasing H3K9 dimethylation and H3K4 trimethylation, decreasing H3K27 trimethylation, producing changes in H3 and H4 histone acetylation and up-regulating H2AX and H3 phosphorylation (Ren et al. 2011; Bailey and Fry 2014; Bjørklund et al. 2018). These histone modifications are also associated with tumorigenesis by deregulating oncogenes and tumor suppressor genes.

miRNA dysregulation

An increasing number of both in vitro and in vivo studies in the past decade show that arsenic dysregulates various microRNA expression levels, which plays a crucial role in arsenic-induced carcinogenesis process (Cardoso et al. 2018). Here, we will now discuss these available studies and their associated miRNA dysregulation data and effects of their dysregulation.

Differential miRNA expressions triggered by arsenic and their carcinogenic effects

Discovery of microRNAs (Lee et al. 1993) also brought out questions about their potential roles in several cellular processes and consequences of their dysregulation. The first work showing that miRNAs are dysregulated in cancer was published in 2002 (Calin et al. 2002). Several studies were performed for evaluating the role of environmental carcinogens in producing differential microRNA expression profiles. One such study, published in 2006, for the first time demonstrated that 2 µM sodium arsenite exposure for 6 days to human lymphoblastoid cells (TK-6) led to altered miRNA expression profiles (Marsit et al. 2006). Recently, various cohort studies have determined alterations in miRNA expression levels in circulating fluids of human populations exposed to significantly high levels of arsenic (Sun et al., 2017; Beck et al., 2018; Ruíz-Vera et al., 2019; Zeng et al., 2019). Some works have focused on miRNA mediated growth inhibiting and apoptosis-inducing properties of arsenic trioxide (As_2O_3) and its use as therapeutic agent in treatment of leukemia (Gao et al. 2010; Li et al. 2010). Several other studies have evaluated the role of miRNAs in arsenic-induced carcinogenesis and metastasis using various in vitro and in vivo models. For instance, in a study on jurkat cell line, sodium arsenite exposure induced cell cycle progression, apoptosis failure and cell proliferation and also caused differential miRNA expression (Sturchio et al. 2014). In the past decade many studies have advanced towards mechanistic basis of miRNA expression changes triggered by arsenic and their functional consequences.

Lungs

Studies on chronic arsenic exposure to human bronchial epithelial (BEAS-2B) cells up-regulated miR-21 (an oncomiR) levels and decreased the expression of its target protein programmed cell death 4 (PDCD4, a tumor suppressor) in a time and dose dependent manner causing malignant cell transformation and tumorigenesis (Pratheeshkumar et al. 2016). Furthermore, arsenic exposure induced ROS through $p47^{phox}$, which is a NADPH Oxidase subunit, a key source of arsenic-induced ROS generation. It also resulted in transcriptional activation of signal transducer and activator of transcription 3 (STAT3) by interleukin-6 (IL-6, a pro inflammatory cytokine) and STAT3 phosphorylation. MiR-21 up-regulation and PDCD4 suppression was found to be dependent upon ROS generation and IL-6 mediated STAT3 activation and phosphorylation, facilitating its binding to miR-21 promoter. These results suggest that arsenic-induced activation of ROS-STAT3-miR21-PDCD4 signaling pathway is responsible for malignant transformation of BEAS-2B cells (Pratheeshkumar et al. 2016). Interestingly, similar results were obtained when human bronchial epithelial (HBE) cells were exposed to 1 µM sodium arsenite (NaAsO2) resulting in increased expression of miR- 21 in a time of exposure dependent manner (Luo et al. 2013). Arsenic induced the secretion of IL-6, which in turn activated pSTAT3, leading to

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the up-regulation of miR-21 in an autocrine manner (Luo et al. 2013). Furthermore, miR-21 was found to target PDCD4 with suppressed Twist1 (a transcription factor involved in cell differentiation), leading to Twist1 over-expression, finally causing epithelial to mesenchymal transition (EMT) in arsenite transformed HBE cells (Luo et al. 2015). In a meta-analysis study, arsenic was found to increase the levels of IL-6 which transmitted inflammatory signals by phosphorylating STAT3. pSTAT3 promoted miR-21 up-regulation which lead to reduction in expression levels of its target tumor suppressor proteins PDCD4, PTEN and SPRY1 (sprouty RTK signaling antagonist 1), and subsequent decrease in E-cadherin and N-cadherin protein expression, ultimately causing malignant transformation and cell proliferation (LIU et al. 2018). MicroRNA-21 inhibitors were found to enhance the expression of PDCD4, PTEN and SPRY1 and suppress the cell malignant transformation, implying their potential use in cancer treatment caused by arsenic (Luo et al. 2015; LIU et al. 2018). In another study on BEAS-2B cells, arsenic induced the expression of MDIG (mineral dust-induced gene), an oncogene associated with occupational lung diseases and lung cancer (Sun et al. 2014). Activation of JNK-STAT3 signaling and up-regulation of miR-21 were essential for induction of MDIG expression by arsenic (Sun et al. 2014).

Arsenic has been reported to down-regulate the expression of miRNA-31 resulting in over expression of its downstream target SATB2 (Special AT rich sequence binding protein 2) in a dose-dependent manner, where BEAS-2B cells were exposed to 1.25, 2.5, 5 and 10 µM of sodium arsenite for 48 hours (Chen et al. 2018b). Also, SATB2 levels were increased in a time-dependent manner when BEAS-2B cells were given 2 µM NaASO2 exposure for 1, 2, 4 and 6 weeks, and after 6 weeks anchorage independent growth was observed (Chen et al. 2018b). SATB2 protein acts as a transcription factor and has an important role in several diseases such as lung cancer (Chen et al. 2018b). BEAS-2B cells after chronic exposure to arsenic (1 µM for 26 weeks) showed up-regulation of miR-222, which acts as an oncogene by inhibiting expression of phosphatases and tension homologue (PTEN) and ARID1A tumor suppressor proteins (Wang et al. 2016). PTEN regulates AKT activation by PI3Kinase, hence inhibiting cell proliferation, migration and angiogenesis and ARID1A is associated with PI3K/AKT pathway and apoptosis induction. Furthermore, treatment by anti-miR-222 inhibitor decreased cell proliferation, migration and induced apoptosis in-vitro and decreased tumor growth in-vivo (Wang et al. 2016). 2.5 µM sodium arsenite exposure to p53 knocked down human bronchial epithelial cells ($p53^{low}$HBECs) for 16 weeks down-regulated miR-200 family members by inducing zinc-finger E-box binding homeobox factor (ZEB1) and ZEB2 and increasing the methylation of promoters of miR-200 family (Wang et al. 2011). ZEB1 and ZEB2 are EMT inducing transcription factors and the EMT process can be reversed by miR-200 family members, hence arsenic-induced increase in ZEB1 and ZEB2 and down-regulation of miR-200 family resulted in EMT, malignant transformation and tumor formation (Wang et al. 2011). MiR-200 family also targets ZEB1 and ZEB2 mRNAs indicating that ZEB1 and ZEB2, and miR-200 family regulate each other via reciprocal feedback loop mechanism (Wellner et al. 2009). Re-expression of miR-200b in $p53^{low}$HBECs transformed by arsenic resulted in reversal of their transformed phenotypes in-vitro and also prevented in-vivo xenograft transformation in nude mice (Wang et al. 2011). MiR-200b was found to target protein kinase Cα (PKCα) and WNT-5b-PKCα positive feedback loop, subsequently suppressing Rac1 (a Rho GTPase) activation and actin cytoskeleton reorganization, thus finally inhibiting cell migration and metastasis (Wang et al. 2014). So, down-regulation of miR-200b, leading to over expression of PKCα and activation of Rac1 was found to be responsible for arsenic transformed cell migration and metastasis in $p53l^{ow}$HBECs. Human bronchial epithelial (16-HBE) cells when exposed to 2.5 µM arsenite continuously for 13 weeks underwent malignant transformation (Chen et al. 2017). In arsenic transformed 16-HBE cells, expression of miR-155 was increased, resulting in subsequent decrease of Nrf2 (nuclear factor (erythroid derived 2)-like 2) expression, but there was no observable change in the expression of NF-KB (Chen et al. 2017). Inhibition of miR-155 in arsenic transformed cells reduced the malignant phenotype and decreased colony formation. Together, these results suggest that arsenite induced malignant transformation might be regulated by miR-155 by targeting Nrf2 signaling pathway (Chen et al. 2017). Chronic arsenic exposure (0.25 µM for 6 months led to malignant transformation of BEAS-2B cells and miR-301a (an oncomiR) was found to be highly expressed in transformed cells (Zhong et al. 2018). Also, with increase in concentrations of arsenic exposure (2.5, 5 and 10 µM for 12 hours) or increasing the time of exposure (1 µM for 10, 20 and 30 passages), both resulted in significantly greater expression of miR-301a (Zhong et al. 2018). Arsenic induced miR-301a up-regulation was found to be dependent upon IL-6/STAT3 signaling pathway. MiR-301a was found to target SMAD4, a tumor suppressor involved in impeding cell growth and migration (Zhong et al. 2018). These findings suggest that IL-6/STAT3/miR-301a/SMAD4 signaling pathway is involved in arsenic induced malignant transformation of BEAS-2B cells (Zhong et al. 2018).

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Table 1: Differentially expressed miRNAs in Lung targeted models of arsenic-induced carcinogenesis

Type of Study	Cell type analyzed	Arsenic exposure Dose	Duration	miRNA	Expression Change	Putative targets	References
In vitro	BEAS-2B	2.5, 5 and 10µM	24 hours	miR-21	Upregulation	PDCD4	Pratheeshkumar et al., 2016)
	BEAS-2B	0.1, 0.25 and 0.5µM	2, 4 and 6 months	miR-21	Upregulation	PDCD4	Pratheeshkumar et al., 2016
	HBE	1 µM	30 passages	miR-21	Upregulation	PDCD4	Luo et al., 2013 Luo et al., 2015
	BEAS-2B	1.25, 2.5, 5 and 10µM	48 hours	miR-31	Downregulation	SATB2	Chen et al., 2018
	BEAS-2B	1 µM	26 weeks	miR-222	Upregulation	PTEN and ARID1A	Wang et al., 2016
	$p53^{low}$HBEC	2.5µM	16 weeks	miR-200 family	Downregulation	ZEB1, ZEB2 and PKCα	Wang et al., 2011; Wang et al. 2014
	16-HBE	2.5µM	13 weeks	miR-155	Upregulation	Nrf2	Chen et al., 2017
	BEAS-2B	0.25µM	6 months	miR-301a	Upregulation	SMAD4	Zhong et al., 2018
	BEAS-2B	2.5, 5 and 10 µM	12 hours	miR-301a	Upregulation	SMAD4	Zhong et al., 2018
	BEAS-2B	1µM	26 weeks	miR-199a	Downregulation	HIF-1α and Cox2	He et al., 2014
	HBE	1µM	24 hours	miR-191	Upregulation	BASP1	Xu et al., 2015

Table 1: Differentially expressed miRNAs in Lung targeted models of arsenic-induced carcinogenesis

Another work on BEAS-2B cells demonstrated that chronic arsenic exposure combined with the inactivation of miR-100 significantly promoted metastasis, proliferation, viability and migration in-vitro and tumorigenesis in-vivo (Yang et al. 2017).

Hypoxia-inducible factors (HIFs) are transcription factors which are heterodimers of an O_2-labile α-subunit (1α, 2α or 3α) and a stable β-subunit (1β). HIF-1α and HIF-2α are found to be over expressed in many cancer cells and are involved in tumor progression, angiogenesis and metastasis (Xu et al. 2015). HIFs are regulated by miRNAs and they themselves can also affect the expression of miRNAs (Xu et al. 2015). Excessive generation of ROS is a characteristic of arsenic-induced carcinogenesis (Li and Chen 2016). ROS has been reported to down-regulate miR-199a, a tumor suppressor (He et al. 2012), and up-regulate HIF-1α and cyclooxygenase-2 (COX-2), an angiogenesis activator protein (Chen et al. 2012). Chronic exposure to 1 µM arsenite for 26 weeks transformed immortalized human lung epithelial (BEAS-2B) cells (He et al. 2014). Comparative miRNA microarray profiles showed about a 100 fold decrease in miRNA-199a levels in As-T BEAS-2B cells (He et al. 2014). Arsenic-induced ROS was found to down regulate miR-199a expression through methylating miR-199a gene promoted by DNA methyltransferase 1 (He et al. 2014). Down-regulation of miR-199a resulted in increased expression of its direct targets HIF-1α and COX-2 (He et al. 2014). Furthermore, HIF1α and COX-2 formed a bidirectional positive feedback loop and promoted angiogenesis and tumor growth (He et al. 2014). Up-regulation of miR-191 in arsenite-transformed human bronchial epithelial (HBE) cells was found to be involved in neoplastic and metastatic progression (Xu et al. 2015). HIF-2α over expression increased the transcription of miR-191 by binding to hypoxia response element (HRE) in its promoter region in arsenic transformed HBE (Xu et al. 2015). MiR-191 up-regulation reduced the expression of its target brain acid soluble protein 1 (BASP1) and resulted in increased expression of matrix-metalloproteinase 9 (MMP-9), Wilm's tumor 1 protein (WT-1, a transcription factor involved in angiogenesis and metastasis), and vascular endothelial growth factor (VEGF, a secreted protein involved in angiogenesis) (Xu et al. 2015). Blocking of HIF-2α by siRNA inhibited miR-191 up-regulation, stopped angiogenesis and reduced metastasis and neoplasticity (Xu et al. 2015).

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Skin

Low concentration (1.0 µM) of sodium arsenite ($NaAsO_2$) exposure to human keratinocyte (HaCaT) cells for about 15 weeks (30 passages) led to malignant transformation and resulted in decreased expression levels of let-7a, let-7b and let-7c tumor suppressor microRNAs (Jiang et al. 2014). Pre-treatment of HaCaT cells with 5-AZA (a methyltransferase inhibitor) prevented down-regulation of let-7c, implying that epigenetic silencing via hypermethylation of let-7c promoter was responsible for arsenite induced down-regulation let-7c. Let-7 family members target Ras, an oncogene which is upstream regulator of NF-kB (nuclear factor kappa-light-chain-enhancer of activated B cells) signaling pathway, thus arsenic was found to activate Ras/NF-kB signaling pathway in HaCaT cells leading to acquisition of cancer stem cell (CSC) like properties and neoplastic transformation (Jiang et al. 2014). A study by Y. Zhou et al. (2017), combined microRNAome, proteomic and metabolomic approaches to get insights into arsenic-induced skin cancer. Exposure of HaCaT cells to 200 nM of sodium arsenide for 28 weeks led to malignant transformation and resulted in dysregulation of 26 miRNAs, out of which, expression of 6 miRNAs was confirmed using Q-RT-PCR, showing up-regulation of miR-6739-5p, miR-4521, miR-181b-5p, miR-100-5p and hmiR-3919, and down-regulation of hsa-miR-513a-5p (Zhou et al. 2017b). Twelve proteins were differentially expressed in arsenite transformed HaCaT cells which were potentially responsible for oxidative stress induced carcinogenesis, including up-regulated heat shock protein beta-1 (HSPB1), peroxiredoxin-2 (PRDX-2) and adenosyl homocystinase (SAHH), and down-regulated leukocyte elastase inhibitor (ILEU) (Zhou et al. 2017b). Also, 68 metabolites were found to be altered following arsenic exposure leading to disruptions in amino acid biosynthesis and metabolism, glutathione and redox metabolism pathways, which can serve as potential biomarkers for arsenic-induced carcinogenesis (Zhou et al. 2017b). Chronic arsenite exposure (100 nM for 3 and 7 weeks) to HaCaT cells induced differential small-RNA (including miRNAs) and mRNA expression profiles (Al-Eryani et al. 2018b). After both 3 and 7 weeks of exposure time 19 small-RNAs were found to be aberrantly expressed, out of which 8 were snoRNAs, 5 stem-loop miRNAs including up-regulated miR-339, miR-4309 and down-regulated miR-1228, miR-4692, miR-548au, and 6 mature miRNAs including up-regulated miR-645, miR-2682-5p and down-regulated miR-548a-3p, miR-1254, miR-3618 and miR-8083 (Al-Eryani et al. 2018b). Out of 323 differentially expressed mRNAs at both time points, dysregulated miRNAs were predicted to potentially target 38 mRNAs, which were found to be associated with carcinogenic pathways. Such as, TCF7L2 mRNA, a target of miR-548-3p, was increased and found to play role in inducing cytoskeleton remodeling, wnt signaling and suppressing PTEN pathways (Al-Eryani et al. 2018b). Similarly, expression levels of mouse double minute 2 homolog (MDM2, a target of miR-548-3p) and high mobility group box 1 (HMGB1, a target of multiple dysregulated miRNAs), were found to be increased, both were involved in TP53 regulation (Al-Eryani et al. 2018b).

A cohort study by Banerjee et al. (2019), focused on the role of miRNAs in causing susceptibility to arsenic in individuals of West Bengal, chronically exposed via contaminated drinking water, based on the fact that even with comparable levels of exposure, skin lesions caused by arsenic were found in only 15-20% of the total population. Using miRNA profiling of plasma samples, the study demonstrated that 202 miRNAs were dysregulated in group of individuals having skin lesions (Banerjee et al. 2019). Furthermore, using Q-RT-PCR it was proved that in the group with skin lesions miR-21, miR-23a, miR-126, miR-619 and miR-3613 were up-regulated, and miR-4530 and miR-1282 were down-regulated, while there was no significant change in miR-124, when compared with no skin lesion group (Banerjee et al. 2019). These differentially expressed miRNAs were found to play roles in glycerophospholipid metabolism, glycosphingolipid biosynthesis, T-cell receptor signaling, colorectal cancer, neutrophil and insulin signaling pathways (Banerjee et al. 2019). In another study by Al-Eryani et al. (2018a), samples of pre- malignant hyperkeratosis (HK), malignant squamous cell carcinoma (SCC) and basal cell carcinoma (BCC) from affected individuals of West Bengal were examined and 35 miRNAs were found to be expressed differentially. Expression of miRNAs was specific to the phenotype or stage as, in comparison to HK, miR-425-5p and miR-433 both were up-regulated in BCC and SCC, suggesting their role in malignancy (Al-Eryani et al. 2018a). MiR-184 and miR-576-3p were up regulated in SCC in contrast to both BCC and HK, indicating their role in induction of metastasis (Al-Eryani et al. 2018a). And, miR-590-5p, miR-494, miR-487b, miR-452, miR-29c and miR-381 were down-regulated only in BCC, suggesting their stage specificity (Al-Eryani et al. 2018a). Exposure of 500 nM sodium arsenite for 4 weeks to HaCaT cells resulted in dysregulation of 30 miRNAs, as shown by microarray analysis (Gonzalez et al. 2015). Further qPCR analysis confirmed the significant up-regulation of 3 miRNAs, miR-21, miR-200a and miR-141, that were involved in regulation of several signaling pathways associated with carcinogenesis such as MAPK, JAK-STAT, Wnt and KEGG melanoma pathways (Gonzalez et al. 2015). To study the role of miR-21 in arsenic induced skin cancers, Banerjee et al. (2017), comparatively analyzed blood samples from individuals of West Bengal grouped into control or unexposed group and two arsenic

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exposed groups, one with skin lesions and the other without skin lesions. MiR-21 expression levels were found to be highly up-regulated in arsenic exposed group with skin lesions, whereas, miR-21 levels were not significantly different between unexposed group and exposed group without skin lesions (Banerjee et al. 2017). Also, the expression levels of tumor suppressor proteins PTEN and PDCD4, which are downstream targets of miR-21, were found to be decreased leading to the up-regulation of PI3K and pAKT finally resulting in increased cell survival (Banerjee et al. 2017). In-vitro studies with HaCaT cells exposed to 0.05 ppm of sodium arsenite for 60 days (15 passages) reported similar outcomes, thereby, confirming the role of miR-21 up-regulation in arsenic-induced skin cancer (Banerjee et al. 2017). HaCaT cells were exposed to 100 nM sodium arsenite for up to 28 weeks in order to obtain a detailed molecular picture of changes that occur during arsenic-induced transformation in human keratinocytes. It was observed that exposed cells grew slower at first, reached growth similar to unexposed cells by 19 weeks, and subsequently grew faster later on. EMT was observed at 28 weeks transformation in arsenic exposed cells (Banerjee et al. 2021).

Table 2: Differentially expressed miRNAs in Skin targeted models of arsenic-induced carcinogenesis.

Type of Study	Cell type analyzed	Arsenic exposure Dose	Duration	miRNA	Expression Change	Putative targets	References
In vitro	HaCaT	1µM	15 weeks	Let-7a, let-7b, let-7c	Downregulation	Ras/NF-ΚB	Jiang et al., 2014
	HaCaT	100nM	28 weeks	miR-6739-5p, miR-4521, miR-181b-5p, miR-100-5p, miR-3919 has-miR-513-a-5p	Upregulation Downregulation	12 dysregulated mRNAs With downregulation of ILEU and upregulation of HSPB1, PRDX2 and SAHH	Zhou et al., 2017
	HaCaT	100nM	3 and 7 weeks	miR – 645, 2682-5p, 339, 4309 548a-3p, 1254, 3618, 8083, 1228, 4692, 548au	Upregulation Downregulation	Multiple target mRNAs including several yet unknown.	Al-Eryani et. al., 2018
	HaCaT	500nM	4 weeks	miR-21, miR-200a, miR- 141	Upregulation	Targets in many signaling pathways including MAPK, Wnt, Jak-STAT and EMT	Gonzalez et. al., 2015
	HaCaT	0.05ppm	60 days	miR-21	Upregulation	PTEN and PDCD4	Banerjee et al., 2017

Liver

Liu et al. (2016) reported that human hepatic epithelial (L-02) cells exposed to sodium arsenite at various concentrations (1, 2, 4 and 8 µM) for 24 hours showed autophagic activity. Also, exposure of arsenite resulted in increased expression levels of miR-21, leading to decreased expression of its target proteins PTEN, PDCD4 and SPRY1, in a concentration-dependent manner (Liu et al. 2016). Furthermore, miR-21 induced down-regulation of PTEN was found to activate ERK (extracellular-signal-regulated-kinase), which promoted autophagy in arsenite exposed L-02 cells (Liu et al. 2016). Induction of autophagy may also play important role in arsenic-induced carcinogenesis (Liu et al. 2016). When pregnant C3H mice were given sodium arsenite at doses of 42.5 and 85 ppm in their drinking water from gestation day 8 to 19, and the livers of male foetal mice were examined, global DNA hypomethylation and a variety of gene-specific DNA methylation changes were observed, including hypomethylation of Cyclin D1 and hypermethylation of Tp53. There were 260 methylation changes, with 143 microRNAs affected. Out of 718 miRNAs, 140 were found to be abnormally expressed on the microRNA array. qPCR corroborated the increased expression of miR-205, miR-203, miR-

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215, miR-34a, and reduced expression of miR-217. qPCR validated the increased expression of Xist, Prrc2, Krit1, Nish, and the reduced expression of Prss2, Spp1, Col1a2, and Lox (Liu et al. 2020). Chronic exposure to 2µM sodium arsenite for upto 15 weeks (30 passages) induced EMT and up-regulated miR-191, an oncomiR (Nagpal et al. 2013), in a time dependent manner (Chen et al. 2018a). MiR-191 was associated with induction of EMT markers, such as increased expression of WT-1, N-cadherin and α-SMA, and decreased expression of E-cadherin and BASP-1 (Chen et al. 2018a). It also promoted up-regulation of liver cancer stem cell (CSC) markers EpCAM and CD90 (Chen et al. 2018a). Further analysis reported that HIF-2α was responsible for arsenite induced up-regulation of miR-191 by binding to hypoxia-response element (HRE) in the promoter region of miR-191 (Chen et al. 2018a). These results suggest that arsenic induces epithelial-mesenchymal transition and acquisition of CSC properties via up-regulation of miR-191, mediated by HIF-2α (Chen et al. 2018a).

In a study on individuals exposed to toxic levels of arsenic caused by coal burning in Guizhou province of China, miR-145 was found to be up-regulated with increase in the extent of poisoning (Wei et al. 2018). Further mechanistic analysis using human L-02 cells reported that miR-145 expression levels were increased after arsenite exposure in a concentration and time dependent manner (Wei et al. 2018). Additionally, miR-145 was found to decrease the levels of ERCC2 by targeting its 3'-UTR (Wei et al. 2018). ERCC2 is known to participate in nucleotides excision repair pathway, thus inhibition of ERCC2 negatively impacts DNA repair process leading to DNA damage (Wei et al. 2018). Therefore, the proposed mechanism of arsenite induced DNA damage might be involved in increasing the co-mutagenicity of other carcinogens. Ren et al. (2015), performed an in vivo study where Sprague-Dawley (SD) rats were exposed to various concentrations of sodium arsenite (0.1, 1, 10 and 100 mg/l) in drinking water for 60 days after which miRNA expression profiles of liver tissues were analyzed. After arsenic exposure miR-423, miR-26a and miR-148b were found to be down-regulated while miR-151 and miR-183 were up-regulated significantly (Ren et al. 2015). Further studies are required for finding out the roles of these miRNAs in arsenic induced carcinogenesis.

Table 3: Differentially expressed miRNAs in Liver targeted models of arsenic-induced carcinogenesis.

Type of Study	Cell type/ Animal strain	Arsenic exposure Dose	Duration	miRNA	Expression Change	Putative targets	References
In vitro	L-02 Cells	1, 2, 4 and 8 µM	24 hours	miR-21	Upregulation	PTEN, PDCD4, SPRY1	Liu et al., 2016
	L-02 Cells	2 µM	15 weeks	miR-191	Upregulation	BASP -1	Chen et al., 2018
	L-02 Cells	2.5, 5, 10, 20, 40 µM	24 hours	miR-145	Upregulation	ERCC2	Wei et al., 2018
	L-02 Cells	20 µM	12, 24, 48 hours	miR-145	Upregulation	ERCC2	Wei et al., 2018
In vivo	Sprague Dawley Rats	0.1, 1, 10, 100 mg/l	60 days	miR-151, 183 423, 26a, 148b	Upregulation Downregulation	-	Ren et al., 2015

Prostate

Immortalized human prostate epithelial cells (RWPE-1) when exposed to 5µM sodium arsenite for 29 weeks transformed malignantly into CAsE-PE cells (Ngalame et al. 2014). Similarly, WPE-stem cells when exposed to 5µM arsenite for 18 weeks transformed malignantly into arsenic cancer stem cells (AS-CSCs) (Ngalame et al. 2014). MiRNA expression profiling demonstrated 29 dysregulated miRNAs in CAsE-PE cells and 13 dysregulated miRNAs in As-CSC cells, many of them were involved in various signaling processes including oncogenesis, proliferation, metastasis and invasion pathways (Ngalame et al. 2014). Ras oncogenes, such as, RAN, RAB27A, RAB22A mRNAs and KRAS protein, were found to be up-regulated in CAsE-PE cells due to down-regulation of miR-134, miR-373, miR-155, miR-138, miR-205, miR-181d, miR-181c and let-7 (Ngalame et al.

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2014). Similarly, RAN mRNA and KRAS, RRAS and NRAS proteins were up-regulated in As-CSC cells due to down-regulation of miR-143, miR-34c-5p and miR-205 (Ngalame et al. 2014). Furthermore, expression levels of ERK kinase, BCL2, BCL-XL and VEGF were increased while those of PTEN were decreased in both transformants, suggesting that RAS/ERK and PI3K/PTEN/AKT pathways are involved in miRNA mediated arsenic-induced prostate cancer (Ngalame et al. 2014). MiR-143 was one of the down-regulated miRNAs in As-CSCs (Ngalame et al. 2014), so another study by Ngalame et al. (2016) was focused upon the role of miR-143 in arsenic-induced carcinogenesis. MiR-143 was over expressed in As-CSCs by lentivirus-mediated tranduction method, which resulted in down-regulation of matrix metalloproteinases (MMP-2 and MMP-9), BCL-2 and BCL-XL proteins leading to decreased cell proliferation and increased apoptosis of As-CSCs (Ngalame et al. 2016). In addition, miR-143 was found to target LIMK1 protein expression which led to inhibition of phosphorylation of cofilin, a substrate of LIMK1 (Ngalame et al. 2016).

Table 4: Differentially expressed miRNAs in Prostate targeted models of arsenic-induced carcinogenesis.

Urinary bladder

Type of Study	Cell type/ Animal strain	Arsenic exposure Dose	Duration	miRNA	Expression Change	Putative targets	References
In vitro	L-02 Cells	1, 2, 4 and 8 µM	24 hours	miR-21	Upregulation	PTEN, PDCD4, SPRY1	Liu et al., 2016
	L-02 Cells	2 µM	15 weeks	miR-191	Upregulation	BASP -1	Chen et al., 2018
	L-02 Cells	2.5, 5, 10, 20, 40 µM	24 hours	miR-145	Upregulation	ERCC2	Wei et al., 2018
	L-02 Cells	20 µM	12, 24, 48 hours	miR-145	Upregulation	ERCC2	Wei et al., 2018
In vivo	Sprague Dawley Rats	0.1, 1, 10, 100 mg/l	60 days	miR-151, 183 423, 26a, 148b	Upregulation Downregulation	-	Ren et al., 2015

Chronic arsenic exposure to immortalized normal human bladder cell line (HUC1) resulted in increased cell growth in a time dependent manner, induced EMT and cell invasive properties (Michailidi et al. 2015). Expression levels of miR-200a, miR-200b and miR-200c were also found to be down-regulated in arsenic exposed HUC1 cells. In arsenic exposed human population, low levels of miR-200a, miR-200b, miR-200c and miR-205 were observed in urine samples (Michailidi et al. 2015). Since miR-200 family members are involved in regulation of EMT, they may play important role in arsenic-induced carcinogenesis of urinary bladder (Michailidi et al. 2015).

Kidney

Human renal epithelial (HK-2) cells underwent malignant transformation after chronic exposure to arsenic, characterized by increased rate of proliferation, colony formation and activity of MMP9 protein (Fang et al. 2018). In arsenite transformed cells HIF-2α was over expressed which stimulated malignant phenotype. Also, levels of miR-182-5p and miR-802 were down-regulated. Furthermore, miR-182-5p was found to regulate HIF-2α expression. Collectively, these results suggests that miR-182-5p/HIF-2α pathway is involved in arsenic-induced renal cell carcinoma (Fang et al. 2018).

Table 5: Differentially expressed miRNAs in Urinary bladder and Kidney targeted models of arsenic-induced carcinogenesis.

Prenatal studies

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A latest in vivo study by Liu et al. (2020), utilized CH-3 mice model to test epigenetic effects of in-utero arsenic exposure and its

Type of Study	Cell type analyzed	Arsenic exposure Dose	Duration	miRNA	Expression Change	Putative targets	References
In vitro	RWPE-1 (CAsE-PE)	5 µM	29 weeks	miR – 134, 127-5p, 373, 34c-5p, 146b-5p, 135b, 222, 155, 138, 205, 218, 10b, 181d, 125a-5p, 181b, 98, 34a, 196a, 181a, 181c, 125b, 126, let-7b, let-7i, let-7e, let-7c	Downregulation	Multiple mRNAs* - RAN, KRAS, RRAS, NANOG, RAB27A, RAB22A, VEGFA, EREG, BCL2, MAP4K4, BCL2L2, DICER1, RAS, BCL2L1, MAPK1, ITGA10, CTNND1, MAPK1	Ngalame et al., 2014
				miR-9, miR-96, miR-183	Upregulation	DICER1, MTSS1, FOXO4, ITGB1, RRAS	
	WPE (As-CSC)	5 µM	18 weeks	miR – 34c-5p, 135-b, 138, 205, 218, 143, 9, 355, 148a	Downregulation	BCL2, MAPK4K4, RRAS, BCL2L2, RAB22A, VEGFA, RAN, KRAS, MAPK1, NRAS, DICER1	
				miR – 34a, 193b, 7, let-29b	Upregulation	PTEN, ITGB1, ITGA6, CTNND1	

postnatal consequences. 42.5 and 85 ppm doses of sodium arsenite were given to pregnant CH3 mice for 12 days during gestation period and fetal livers were subjected to further analysis. Global DNA hypomethylation and gene specific hypermethylation such as TP53 gene were reported in fetal liver after arsenic exposure (Liu et al. 2020). Furthermore, 140 miRNAs were dysregulated including up-regulated miR-205, miR-203, miR-215 and miR-34a and down-regulated miR-217(Liu et al. 2020). Therefore, in-utero arsenic exposure can modify DNA methylation patterns leading to microRNA dysegulation and altered gene expression with may enhance postnatal susceptibility to cancer (Liu et al. 2020). A cohort study by Rager et al. (2014), was performed to assess miRNA dysregulation after prenatal arsenic exposure in pregnant women of Gomez Palacio, Mexico. Twelve microRNAs were found to be up-regulated cord blood of newborns associated with arsenic in maternal urine (Rager et al. 2014). Many among the dysregulated miRNAs including let-7a, miR-16 and miR-20b are known to be involved in carcinogenesis (Rager et al. 2014). Chronic arsenic exposure to human lung embryo fibroblast (HELF) cells induced malignant transformation, up-regulation of miR-21 and activation of ERK/NF-kB (extracellular signal-regulated kinase/ nuclear factor kB) signaling pathway (Ling et al. 2012). Generation of ROS (including superoxides and hydrogen peroxides) induced the activation of ERK/NF-kB, which further increased miR-21 expression in arsenite transformed HELF cells (Ling et al. 2012). Up-regulation of miR-21 led to subsequent decrease of its downstream targets PTEN, PDCD4 and SPRY1. MiR-21 directed inhibition of SPRY1 (an inhibitor of Ras/MEK/ERK pathways) activated ERK/NF-kB (positive feedback loop), and promoted arsenic-induced neoplastic transformation of HELF cells (Ling et al. 2012).

Table 6: Differentially expressed miRNAs involved in arsenic-induced carcinogenesis during prenatal exposure.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

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In light of the aforementioned studies, it is a well established fact that miRNA dysregulation has a pivotal role in arsenic-induced

Type of Study	Cell type/ Animal strain	Arsenic exposure Dose	Arsenic exposure Duration	miRNA	Expression Change	Putative targets	References
In vivo	CH3 Mice	42.5 and 85 ppm	12 days during gestation period	miR-205, 203, 215, 34a miR-217	Upregulation Downregulation	-	Liu et. al., 2019
In vitro	HELF Cells	1 µM	15 weeks (30 passages)	miR-21	Upregulation	PTEN, PDCD4 and SPRY1	Ling et. al., 2012

carcinogenesis. Thus, miRNAs can serve as potential biomarkers and therapeutic agents or targets for mitigation of cancer induced by arsenic. Also, these studies suggests that various modes of action of arsenic-induced carcinogenesis such as ROS generation, aberrant DNA methylation, histone modification, dysregulation of DNA repair, differential microRNA expression and many signaling pathways are all interconnected, possibly having various feedback loops, profoundly regulating each other, thus making the process more in intricate. Therefore, further mechanistic studies are required in order to gain detailed insights into the process. There are some important aspects and missing links that need to be considered for future studies. Expression levels of various miRNAs and their downstream targets are dependent upon multiple factors such as cell type or animal strain analyzed, dosing and time of exposure. For instance, miR-200 family members (tumor suppressor miRNAs), were down-regulated in both HBECs exposed to 2.5µM sodium arsenite for 16 weeks (Wang et al. 2011) and HUC1 cells exposed to 1µM of arsenic trioxide for 10 months (Michailidi et al. 2015). On the contrary, miR-200b and miR-200c were up-regulated in CH3 mice exposed to 85ppm sodium arsenite for 12 days during gestation period (Liu et al. 2020). Interestingly, arsenic trioxide induces apoptosis in cancer cells, so it is also employed in treatment of promyelocytic leukemia (Cai et al. 2003; Yedjou et al. 2010; Sun et al. 2018). Expression levels of tumor protein 53 (TP53) also vary after arsenic exposure according to cell type, dosing and exposure time (Huang et al. 2008). Most of the in vitro studies have used higher concentrations of arsenic than those available internally at physiological levels and may not exactly represent the effects of long term, lower dose arsenic exposure experienced by most of the human population (Cardoso et al. 2018). Therefore, more focused studies are required to elucidate effects of varying degrees of arsenic exposure to different cell types, throughout the transformation process. Another problem is the unavailability of an in vivo animal model that has almost identical response to arsenic as humans. Currently available studies indicate that arsenic itself is insufficient to cause skin cancer in mice, rather, it acts as a co-carcinogen in mice (Rossman et al. 2001; Burns et al. 2004). So most of the studies available on arsenic-induced carcinogenesis process are in vitro, but these studies predominantly utilize immortalized cell lines which may yield different results than primary cells due to possible attainment of variations and culture heterogeneity over a long period of time (Kaur and Dufour 2012; Cardoso et al. 2018). Many miRNAs can target a single mRNA, also one miRNA can target multiple mRNAs, but their specificity may differ based upon their seed sequences at 5' end (Hayes et al. 2014). But the role of many miRNAs which have been reported to be dysregulated during arsenic-induced carcinogenesis process is not completely known yet. So, upcoming research may focus upon elucidating the role of these miRNAs in transformation process induced by arsenic. This will not only provide us more significant insight into the mechanism involved behind the process but will also help us in establishing new potential biomarkers and therapeutic targets for treatment of arsenic induced cancer.

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DISCLOSURES & DECLARATIONS

Ethical Approval

Not applicable

Consent to Participate

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Authors Contributions:

All authors contributed to the study conception and design. Literature search, data analysis and preparation of first draft was performed by Navneet Kumar. Dr. P. J. John provided his guidance during data collection, analysis, formatting and performed critical revision of the work. All authors read and approved the final manuscript.

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Competing Interests:

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Availability of data and materials

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