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Arsenic exposure triggers a shift in microRNA expression

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HIGHLIGHTS

- Effects of iAs exposure on miRNAs expression profile in Jurkat cell line
- Bioinformatic techniques reconstructed iAs-relevant molecular pathways and miRNA regulatory networks
- A list of miRNAs was proposed as potential biomarkers of iAs effects
- Induction of cell cycle progression by iAs exposure

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ABSTRACT

Exposure to inorganic Arsenic (iAs) through drinking water is a major public health problem affecting most countries. iAs has been classified by the International Agency for Research on Cancer as Group 1: "Carcinogenic to humans". Although numerous studies have shown the related adverse effects of iAs, sensitive appropriate biomarkers for studies of environmental epidemiology are still required.

The present work aims at investigate the role of microRNAs (miRNAs), powerful negative regulators of gene expression, playing a key role in many physiological and pathological cellular processes, in iAs exposure. To this end, we analyzed miRNA changes in expression profile triggered by iAs exposure in Jurkat cell line.

We used microarray technology to profile the expression of miRNAs following 2 μmol/L sodium arsenite treatment at different time points. Moreover, we performed phenotypic analysis of iAs treated cells. Real Time Polymerase Chain Reaction (RT-PCR) was used to validate miRNA microarray data and to assay expression modulation of selected relevant mRNAs. Finally, bioinformatics techniques were applied to reconstruct iAs-relevant molecular pathways and miRNA regulatory networks from the expression data.

We report miRNAs modulated after iAs treatment in Jurkat cells. In particular, we highlight 36 miRNAs exhibiting consistent dysregulation and particularly a panel of 8 miRNAs which we also validated by RT-PCR analysis. Computational analysis of lists of putative target genes for these 8 miRNAs points to an involvement in arsenic-response pathways, for a subset of them, that were analyzed by RT-PCR. Furthermore, iAs exposure reveals induction of cell cycle progression and the failure of apoptosis, supporting the idea of iAs carcinogenic activity.

Our study provides a list of miRNAs whose expression levels are affected by iAs treatment, corroborating the importance of proceeding with the hunt for specific subset of miRNAs, which can serve as potential biomarkers of iAs effects with useful diagnostic value.

1. Introduction

Inorganic arsenic (iAs), a naturally occurring metalloid, is classified by the International Agency for Research on Cancer (IARC, 1987) as Group 1 or "Carcinogenic to humans" based on the iAs ability to induce primary skin, lung and urinary bladder cancer, corresponding to the category 1A of the Globally Harmonized System. Inorganic arsenic fulfils the criterion of Persistent Bioaccumulative and Toxic substance (PBT) of Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), it is not a registered substance and in reference to Biocidal Products Regulation (Regulation (EC) No 528/2012) hazard identification falls under the

Abbreviations: AP-1, activator protein-1; BACH1, BTB and CNC homology 1, basic leucine zipper transcription factor 1; CDK, cyclin-dependent kinase; CYP1A1, cytochrome P450, family 1, subfamily A, polypeptide 1; ERK, extracellular signal-regulated kinase; FACS, Fluorescence Activated Cell Sorting; HMOX, heme oxygenase 1; iAS, inorganic Arsenic; JNK, c-Jun N-terminal kinase; JUNB, Jun B proto-oncogene; MAP, mitogen-activated protein; miRNA, microRNA; NF-kB, nuclear factor-kB; NRF-1, nuclear respiratory factor-1; RNF4, Ring Finger Protein 4; ROS, Reactive Oxygen Species; RT-PCR, Real Time Polymerase Chain Reaction; SP1, transcription factor Sp1; SUMO, Small Ubiquitin-like Modifier; TGFβ1, Transforming Growth Factor Beta 1.

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exclusion and substitution criteria. iAs is the principal contaminant of soil, water and food especially in some eastern countries, such as Bangladesh, India and China. Arsenic toxicity has become a global concern owing to ever-increasing contamination of water in many regions around the world.

Exposure to arsenic typically results from either oral consumption through contaminated drinking water, soil and food or inhalation in an industrial work setting. iAs pollution is often related to mining or mining-related activities, copper smelting, coal burning and other combustion processes, as well as to volcanic eruptions, which bring iAs into the environment. Other anthropogenic sources of iAs include herbicides, insecticides, rodenticides, wood-preservatives, animal feeds, paints, dyes and semiconductors (Bohrer et al., 2006). Obviously, limitations both on natural and anthropogenic sources should be in force. To protect human health, thus also preserving the environment, restriction on the manufacture, marketing and use have been in place for about thirty years; such restrictions are now consolidated in Annex XVII of the REACH (Commission Regulation (EC) No 1907/2006 amended by Commission Regulation (EC) No 552/2009). Furthermore, regarding the use in agriculture as pesticide, according EU Pesticide Database, MSMA (methyl arsonic acid) and other Arsenic salts are out of Annex I PPPs (Commission Regulation (EC) No 1107/2009 revising the 1991 Directive 91/414/EEC on the placing of Plant Protection Products on the market).

Many different systems within the body are affected by exposure to iAs. The most damaged ones are those involved in absorption, accumulation, and/or excretion, such as gastrointestinal tract, circulatory system, skin, liver, and kidney (Abernathy et al., 2003).

Council Directive 98/83/EC (1998) and World Health Organization WHO (2008) recommended the limit of 10 μg/L of inorganic Arsenic in drinking water intended for human consumption.

In order to protect public health, a number of maximum tolerances for specific contaminants are currently laid down in the Commission Regulation (EC) No. 1881/2006 (2006), where the maximum levels for certain contaminants in foodstuffs are outlined. While the maximum levels for the elements lead, cadmium, mercury and inorganic tin are set for a number of food commodities, arsenic is not under this regulation yet. However, the European Food Safety Authority warned that levels of iAs should be reduced and indicated children as subjects of primary concern in that they are the most exposed ones to iAs (EFSA, 2009). According to WHO arsenic guidelines 2010 (WHO technical report series; no. 960, 2011) the lower limit of iAs was determined on the benchmark dose for a 0.5% increased incidence of lung cancer ($BMDL_{0.5}$) from epidemiological data to be 3.0 μg/kg body weight per day (2–7 μg/kg body weight per day based on the range of estimated total dietary exposure).

Another iAs exposure route of interest in Public Heath other than nutrition is occupational exposure. The European Commission's third Directive on Indicative Occupational Exposure Limit Values (Commission Directive 2009/161/EU) was implemented by the Health and Safety Executive (2011) that approved new and revised workplace exposure limits (WELs). The long-term exposure limit (8-hour time-weighted average (TWA) reference period) for arsenic and arsenic compounds except arsine (as As) is 0.1 mg/m^{-3}.

Several mechanisms by which arsenical compounds may induce tumorigenesis have been proposed. These mechanisms include oxidative stress, genotoxic damage and chromosomal abnormalities, co-carcinogenesis with other environmental toxicants as well as alteration of DNA methylation (epigenetic mechanisms) that can alter multiple biological processes (Ren et al., 2011).

iAs increases production of Reactive Oxygen Species (ROS) and can thus result in oxidative DNA damage. This in turn can interfere with the ability of methyltransferases to properly interact with the DNA, yielding widespread alterated methylation patterns.

At the cellular level, iAs can cause impaired cell function and apoptotic cell death. iAs is a potent inducer of mitogen-activated protein (MAP) kinase signal transduction pathways, including the extracellular signal-regulated kinase (ERK1/2), the c-Jun N-terminal kinase (JNK1/2), and the p38 kinase pathways. Lau et al. (2004) reported that differential activation of MAP kinase pathways may contribute to cell growth regulation and cell death in response to diverse doses of iAs. These findings indicate that exposure to a high iAs concentration (40 μM) may lead to apoptosis, whereas exposure to a low iAs concentration (2 μM) may be carcinogenic and result in enhanced cell proliferation.

In light of the hazardous potential of iAs to humans, it is a priority issue to develop effective markers for its risk assessment. Here we propose to use miRNAs as a powerful molecular-based approach to biomarker development.

miRNAs are endogenous, single-stranded, non-coding RNA molecules ~21 nucleotides long which act as negative regulators of gene expression at the post-transcriptional level (Bartel, 2009). Despite the relatively short time since their first discovery in *Caenorhabditis elegans* (Lee et al., 1993), miRNAs have gained significant attention in these years due to appreciation of their important modulatory role in crucial cellular processes, such as development, differentiation and apoptosis.

To date, about 2000 different miRNAs have been identified in human (Griffiths-Jones et al., 2006) and it has been predicted that each of them can regulate hundreds of target genes (Lewis et al., 2005). Due to their broad impact on gene regulation, individual miRNAs or a combination of them have the potential to take part in the regulation of complex diseases. A large body of evidence supports the miRNA leading involvement in a wide range of human diseases such as inflammatory, autoimmune and metabolic diseases (Sonkoly and Pivarcsi, 2009). In line with the finding that the majority of miRNA genes are located at cancer-associated or fragile genomic regions, it is now recognized that miRNAs are mis-regulated in a variety of human tumors and that they can function as both oncogenes or tumor suppressors (Yin et al., 2012). Utterly relevant to human health, miRNAs have been proven powerful diagnostic and prognostic biomarkers for several human tumors and diseases (Zhi et al., 2010) and represent a promising therapeutic target for many human conditions. Furthermore, miRNAs are attractive candidate biomarkers for their ease of access since they can be sampled non-invasively from serum and blood (Mitchell et al., 2008) as well as from formalin-fixed tissue biopsies routinely available from clinical pathology laboratories (Hasemeier et al., 2008).

It is well demonstrated that iAs can alter the physiology of various immune cells (Soto-Pena et al., 2006). Prompted on the one hand by the bio-medical relevance of miRNAs and on the other by the iAs toxicity potential, we monitored miRNA expression changes triggered by iAs exposure at different time points in Jurkat cell line, an immortalized line of T lymphocytes.

2. Materials and methods

2.1. Cell cultures and treatment

Human Jurkat leukemic T cell (DSMZ, Braunschweig, Germany) were grown in Dulbecco Modified Eagle medium (Gibco) supplemented with 10% Fetal Bovine Serum, penicillin/streptomycin, and L-glutamine at 37 °C in 5% CO_2. Jurkat cells were exposed to 2 μmol/l sodium arsenite (SIGMA) and incubated for 0, 24 and 144 hours, in triplicate. At the end of the different time points of iAs exposure, the cells were collected. We investigated if there was evidence for an apoptotic effect at the work concentration of 2 μM, a phenotype that has been linked to iAs exposure. The rationale for the applied 2 μM sodium arsenite concentration comes from a preliminary study where we examined the effects of sodium arsenite on cell proliferation and identified the above concentration as the most appropriate in order to analyze changes of miRNA expression profiles in As-induced Jurkat proliferation. This value is in agreement with other reports (Lau et al., 2004). For sodium arsenite treatment, Jurkat cells were seeded at the density of 5×10^5 cells/ml and iAs was added to the culture medium. The cells were splitted every 3 days and iAs was newly added to the medium after reseeding.