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ROS mediated FOXO transcription factors pathway inhibition is the key event during Arsenic acid induced cellular senescence in fibroblast cell.

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ABSTRACT

Arsenic exposure through drinking water is a major public health problem. It causes a number of toxic effects on skin. Arsenic has been reported to inhibit cancer cell migration and proliferation. However, reports about the molecular effects of arsenic acid on normal skin fibroblast cells are limited. Here, we investigated the molecular effect on arsenic acid-mediated inhibition of cell migration using mouse skin fibroblast cell line (m5S). The present study found that 10 ppm arsenic acid inhibits cell migration, although it had no effect on cell death at this dose. Arsenic acid induced the generation of reactive oxygen species (ROS), resulting in oxidative stress to DNA. It also activated the mammalian Ste20-like protein kinase 1 (MST1); however the serine/threonine kinase Akt was downregulated. Forkhead box O (FOXO) transcription factors are activated by MST1 under stress conditions. They are inhibited by phosphorylation by Akt through external and internal stimuli. Activation of FOXOs assist in their nuclear localization and increased transcriptional activity. Our results showed that arsenic induced the nuclear translocation of FOXO1 and FOXO3a. Their target genes were regulated to accumulate the cell cycle in the G2/M phase. These effects caused cellular senescence. Taken together, our results indicate that arsenic acid inhibited cell migration through cellular senescence process regulated by MST1-FOXO signaling pathway.

Key words: Arsenic acid, Oxidative stress, mammalian Ste20-like protein kinase 1, Forkhead box O transcription factors, cellular senescence

DISCUSSION

Arsenic is a naturally occurring element, widely distributed in the air, water and soil. Humans can be exposed to arsenic through food, drinking water or breathing in contaminated air, with the major route of exposure being contaminated drinking water. The concentration of arsenic in natural surface and ground water is generally about 1 part in a billion parts of water (1ppb), but may exceed 1,000 ppb in contaminated areas (ATSDR, 2007). Chronic exposure to arsenic can cause skin lesions, hypertension, cardiovascular diseases, neurologic disorders, and cancer. The first signs signaling chronic exposure to arsenic include skin pigmentation / depigmentation, skin lesions and hyperkeratosis of palms and soles (Chen et al., 2007). Studies indicate that exposure to arsenic inhibits cell migration (Lin et al, 2008,

Sherwood et al., 2013). However, the molecular mechanism involved is not yet known. This study reports the mechanism of inhibition of cell proliferation and migration by arsenic.

In vitro experiments were conducted using mouse skin fibroblast cells. Cells were treated with arsenic acid for 16 hours and studied for cell death, proliferation and migration parameters. Arsenic acid was not toxic to the cells; however, it inhibited the rate of cell proliferation and migration, in a dose- and time-dependent manner. Arsenic acid also increased the intracellular levels of reactive oxygen species (ROS) in a dose-dependent manner. Excessive production of ROS can lead to oxidative stress that can further damage DNA (Storz, 2011). Oxidative damage to DNA was analysed by immunostaining the cells for 8-OHdG, a marker protein for ROS-induced-oxidative damage to DNA. Arsenic acid treated cells expressed a higher number of 8-OHdG positive cells in comparison to control cells. Further analysis revealed that arsenic acid caused an increase in the expression of phosphorylated form of mammalian ste20-like protein kinase 1 (MST1) and a decrease in the phosphorylation of Akt protein. MST1 and Akt have antagonistic action on the Forkhead box O (FOXO) transcription factors. During oxidative stress, MST1 phosphorylates FOXO1 and FOXO3a, resulting in their translocation from the cytoplasm into the nucleus and enabling them to modulate target gene expressions (Yuan et al, 2009). On the other hand, phosphorylation of FOXOs by Akt promotes their export from the nucleus besides inhibiting their nuclear import (Van Der Heide et al., 2004). Immunofluorescence studies revealed that arsenic acid caused a translocation of FOXOs into the nucleus. Since FOXOs regulate cell proliferation and survival through the expression of target genes involved in cell cycle arrest and DNA repair, we checked the cell cycle parameters by flow cytometry. Arsenic acid caused an increase in cell population at G2/M phase, implying cell cycle arrest. Cell cycle arrest can lead to senescence; we hypothesized that arsenic acid could induce senescence in the fibroblast cells. As expected, an increase in SA-β-gal activity, a standard senescence marker, was observed with arsenic treatment. Taken together, our results so far show that arsenic acid-induced ROS and oxidative stress activate MST1-FOXO signaling pathway, with consequent trigger of cellular senescence in normal fibroblast cells.

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