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Role of microRNAs in senescence and its contribution to peripheral neuropathy in the arsenic exposed population of West Bengal, India☆

Debmita Chatterjee [a], Apurba Bandyopadhyay [b, c], Nilendu Sarma [d], Santanu Basu [e], Tarit Roychowdhury [f], Sib Sankar Roy [g], Ashok K. Giri [a, *]

[a] Molecular Genetics Division, CSIR-Indian Institute of Chemical Biology, Kolkata 700032, India
[b] Health Point Multispeciality Hospital, Kolkata 700025, India
[c] Ramakrishna Sarada Mission Matri Bhavan, Kolkata 700 026, India
[d] Dr. B.C. Roy Post Graduate Institute of Paediatric Science, Kolkata 700054, India
[e] Department of General Medicine, Sri Aurobindo Seva Kendra, Kolkata 700068, India
[f] School of Environmental Studies, Jadavpur University, Kolkata 700032, India
[g] Cell Biology & Physiology Division, CSIR-Indian Institute of Chemical Biology, Kolkata 700032, India

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ABSTRACT

Arsenic induced senescence (AIS) has been identified in the population of West Bengal, India very recently. Also there is a high incidence of arsenic induced peripheral neuropathy (PN) throughout India. However, the epigenetic regulation of AIS and its contribution in arsenic induced PN remains unexplored. We recruited seventy two arsenic exposed and forty unexposed individuals from West Bengal to evaluate the role of senescence associated miRNAs (SA-miRs) in AIS and their involvement if any, in PN. The downstream molecules of the miRNA associated with the disease outcome, was also checked by immuoblotting. *In vitro* studies were conducted with HEK 293 cells and sodium arsenite exposure. Our results show that all the SA-miRs were upregulated in comparison to unexposed controls. miR-29a was the most significantly altered, highest expression being in the arsenic exposed group with PN, suggesting its association with the occurrence of PN. We looked for the expression of peripheral myelin protein 22 (PMP22), a specific target of miR-29a associated with myelination and found that both *in vitro* and *in vivo* results showed over-expression of the protein. Since this was quite contrary to miRNA regulation, we checked for intermediate players β-catenin and GSK-3β upon arsenic exposure which affects PMP22 expression. We found that β-catenin was upregulated *in vitro* and was also highest in the arsenic exposed group with PN while GSK-3β followed the reverse pattern. Our findings suggest that arsenic exposure alters the expression of SA-miRs and the mir-29a/beta catenin/PMP22 axis might be responsible for arsenic induced PN.

1. Introduction

Arsenic contamination through various sources is a global menace affecting at least 70 countries till date and estimated to adversely affect more than 140 million people worldwide (UNICEF, 2014). Arsenic is ranked one of the topmost toxic substances according to the U .S. Agency for Toxic Substances and Disease Registry Priority List of Hazardous Substances (Denny et al., 2013; Mazumdar, 2016). Arsenic poisoning is most prominent in the Asian countries and of grave concern in the regions of Ganga-Brahmaputra delta that includes parts of India and Bangladesh (Edmunds et al., 2015). In India, the state of West Bengal has more than 26 million individuals are facing severe arsenic contamination through drinking water. In these regions, the arsenic level- in drinking water is much higher than the WHO recommended value of 10 μg/L laid down by the WHO guidelines for safe drinking water. There are several adverse health effects for consumption of arsenic laden water of which the hallmarks are various degrees of skin pigmentation such as raindrop hypo-pigmentation, hyper-pigmentation and palmo-palmer hyper-keratosis (Paul et al., 2013). Chronic arsenic exposure leads to skin cancers as well as non-

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* Corresponding author. Molecular Genetics Division, CSIR-Indian Institute of Chemical Biology, 4 Raja S. C. Mullick Road, Jadavpur, Kolkata 700 032, India.
E-mail addresses: akgiri15@yahoo.com, akgiri@iicb.res.in (A.K. Giri).

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dermatological health effects like peripheral neuropathy, lung diseases, cardiovascular diseases and also cancers of the internal organs (Paul et al., 2013; Martinez et al., 2011). Unlike the dermatological manifestations which are reversible in terms of severity, with reduction in arsenic exposure, peripheral neuropathy once sets in can't be reverted (Paul et al., 2013). Over the years our group have identified several pathways responsible for arsenic-induced toxicity and susceptibility in humans like DNA damage, single nucleotide polymorphisms, altered methylation and senescence have been implicated in the population of West Bengal, India (Banerjee et al., 2013; Chatterjee et al., 2015; Paul and Giri, 2015). Of the several mechanisms, our group for the first time established that senescence could be a major player of arsenic induced carcinogenesis in the population of West Bengal, India (Chatterjee et al., 2015). Although, senescence is primarily a tumor suppressive mechanism, however during senescence , a cell acquires several features like senescence associated secretory phenotype (SASP) and altered telomere length. In our previous study on arsenic exposed population, we found that alteration of cytokines involved in SASP and telomere length was associated with arsenic induced carcinogenesis (Chatterjee et al., 2015)

Micro RNAs (miRNA) are small 19–25 nucleotides long non-coding RNA molecules that functions in controlling gene expression post-transcriptionally by destabilizing the transcribed mRNA or translational repression (Filipowicz et al., 2008). A spectrum of miRNAs have been implicated to be an integral part of the senescence process as evident from their involvement in liver aging, endothelial cell senescence and neurodegenerative diseases like Alzheimer's disease and neuropathy. miRNAs are also known to induce senescence by modulating the different aspects of senescence like telomeric and telomerase regulation, senescence associated secretory phenotype and epithelial to mesenchymal transition (Luo et al., 2015; Olivieri et al., 2013). Both senescence and peripheral neuropathy are prominent features of the adverse outcomes of arsenic exposure through drinking water (Chatterjee et al., 2015; Mochizuki et al., 2016). Although, we have identified that peripheral neuropathy cannot be reverted back by reducing arsenic contamination through drinking water, the mechanism of arsenic induced peripheral neuropathy is the most unattended of all the diseases (Paul et al., 2013).

Arsenic induced peripheral neuropathy is characterized by symptoms like muscle cramps, extremity fatigue, numbness, weakness, pain as well as paraesthesias in stocking and glove distributions (Paul et al., 2013; Sińczuk-Walczak et al., 2014). Arsenic induced peripheral neuropathy have been reported worldwide extensively in humans (Mochizuki et al., 2016). In a very recent report by Chakraborti et al. (2016) in the Patna district of Bihar, India, 40.5% of arsenicosis patients had peripheral neuropathy suggesting the high incidence of peripheral neuropathy in chronic arsenic toxicity. Several studies on cell lines or animal models have attempted to elaborate on the mechanistics involved including oxidative damage to neurons, hampered neurofilament transport, axonal degeneration, morphological alterations in axons of peripheral nerves and severe demyelination (García-Chávez et al., 2007; Windebank, 1986). Nowadays miRNA have emerged as crucial regulators of myelination in the peripheral nervous system. Since arsenic has been associated with senescence as well as peripheral neuropathy, we hypothesize that either of them might be inter-related upon arsenic exposure in humans. Of the several miRNAs we shortlisted five miRNAs, namely miR-34, miR-29, 126, 141 and 424 which have been implicated in different aspects of senescence (Al-Khalaf and Aboussekhra, 2017; Li et al., 2016). Hence in this study we have attempted to evaluate the alteration in the miRNAs associated with senescence in the arsenic exposed population of West Bengal, India. Considering the severe incidence of neuropathy in the arsenic exposed population, we recognized the need to assess the contribution of the differentially expressed senescence related miRNAs in arsenic associated peripheral neuropathy and unravel the downstream probable pathway of the disease outcome.

2. Materials and methods

2.1. Study site and participants

In this study, a total of seventy two arsenic exposed study participants were recruited from the highly arsenic-affected district of Murshidabad where the arsenic content in drinking water was much above the recommended maximum permissible limit (MPL) of 10 μg/L (Arsenic fact sheet No 372; http://www.who.int/mediacentre/factsheets/fs372/en/;). Forty arsenic unexposed controls were selected from arsenic unaffected districts of East Midnapur, West Bengal where the arsenic content in drinking water was within permissible limits. The procedure of field survey, strategy for genetically unrelated case-control selection, and sample collection have been described in detail in our previous studies (Paul et al., 2013; Ghosh et al., 2007). Care was taken that none of the individuals had any habit of consuming tobacco in the form of smoking or chewing. For the exposed individuals, selection criteria were such that they resided in the same arsenic affected area for at least 10 years. Demographic details of the recruited individuals were recorded. Expert clinicians screened individuals with As-specific skin lesions and also identified them for non-dermatological health effects like respiratory problems, eye problems and peripheral neuropathy. The arsenic exposed individuals were further subdivided into two groups based on the occurrence of peripheral neuropathy. Thirty two arsenic exposed individuals were with peripheral neuropathy and forty exposed individuals were without peripheral neuropathy. Both the arsenic exposed group i.e with peripheral neuropathy and without peripheral neuropathy had similar arsenic exposure and was residing in the same area. Drinking water, urine and blood samples were collected from the study participants after a well informed written consent. This study was in accordance to the Declaration of Helsinki II and approved by the Institutional Human Ethical Committee of CSIR-Indian Institute of Chemical Biology.

2.2. Identification of neurological symptoms

Arsenic-induced neuronal problems were associated with symptoms like muscle cramps, numbness, weakness, pain as well as paraesthesias in stocking and glove distributions. The neurologist recorded the various types of clinical manifestations including the power and deep tendon reflexes, calf tenderness, pressure and pain as detailed previously (Paul et al., 2013). Initially, the study participants who were clinically confirmed to have peripheral neuropathy by the neurologist, were brought for confirmatory electrophysiological studies such as nerve conduction velocity test (NCV) and electromyography (EMG) test. Only those participants were selected who were both clinically and diagnostically positive as arsenic exposed individuals with peripheral neuropathy (PN). The arsenic exposed individuals without PN and the unexposed referents did not have detectable symptoms of PN and hence were not subjected to NCV or EMG analysis. In case of the arsenic exposed individuals without PN and the unexposed referents, those who had probable symptoms were subjected to NCV and EMG tests and excluded from the study.