Environmental Research 163 (2018) 289–296

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Epigenetic alteration of mismatch repair genes in the population chronically exposed to arsenic in West Bengal, India

Pritha Bhattacharjee[a,c], Tamalika Sanyal[a,c], Sandip Bhattacharjee[b], Pritha Bhattacharjee[c,*]

[a] Department of Zoology, University of Calcutta, Kolkata 700019, India
[b] Health-Management in Occupational Health, Siemens, India
[c] Department of Environmental Science, University of Calcutta, Kolkata 700019, India

ARTICLE INFO

Keywords:
Arsenic exposure
DNA damage
Mismatch repair pathway
Epigenetic modifications

ABSTRACT

Introduction: Arsenic exposure and its adverse health outcome, including the association with cancer risk are well established from several studies across the globe. The present study aims to analyze the epigenetic regulation of key mismatch repair (MMR) genes in the arsenic-exposed population.
Method: A case-control study was conducted involving two hundred twenty four (N = 224) arsenic exposed [with skin lesion (WSL = 110) and without skin lesion (WOSL = 114)] and one hundred and two (N = 102) unexposed individuals. The methylation status of key MMR genes *i.e. MLH1*, *MSH2,* and *PMS2* were analyzed using methylation-specific PCR (MSP). The gene expression was studied by qRTPCR. The expression of H3K36me3, which was earlier reported to be an important regulator of MMR pathway, was assessed using ELISA.
Results: Arsenic-exposed individuals showed significant promoter hypermethylation ($p < 0.0001$) of *MLH1* and *MSH2* compared to those unexposed with consequent down-regulation in their gene expression [*MLH1* ($p = 0.001$) and *MSH2* ($p < 0.05$)]. However, no significant association was found in expression and methylation of *PMS2* with arsenic exposure. We found significant down-regulation of H3K36me3 in the arsenic-exposed group, most significantly in the WSL group ($p < 0.0001$). The expression of SETD2, the methyltransferase of an H3K36me3 moiety was found to be unaltered in arsenic exposure, suggesting the involvement of other regulatory factors yet to be identified.
Discussion: In summary, the epigenetic repression of DNA damage repair genes due to promoter hypermethylation of *MLH1* and *MSH2* and inefficient recruitment of MMR complex at the site of DNA damage owing to the reduced level of H3K36me3 impairs the mismatch repair pathway that might render the arsenic-exposed individuals more susceptible towards DNA damage and associated cancer risk.

1. Introduction

Chronic exposure to arsenic and its role in contribution to multi-organ cancer is well-known. Skin has been considered to be the hallmark of arsenic exposure, including noncancerous (raindrop pigmentation and hyperpigmentation), precancerous (palmer, planter, and palmo-planter hyperkeratosis), and cancerous skin lesions (basal cell carcinoma [BCC], squamous cell carcinoma [SCC], and Bowen's diseases [BD]) (Banerjee et al., 2013). Despite of exposure at similar extent, exposed individuals show differential susceptibility. It has been earlier reported that only 15–20% of the exposed individuals show arsenic-induced characteristic skin lesions (Ghosh et al., 2006). Epidemiological studies across the globe have also reported higher incidence of bladder, liver, lung, colorectal (Bhattacharjee et al., 2013a, 2013b; Yang et al., 2008), pancreatic (Liu-Mares et al., 2013), breast (Khanjani et al., 2017), prostate (Benbrahim-Tallaa and Waalkes, 2008), and skin cancer (Mayer and Goldman, 2016) in populations chronically exposed to arsenic (Bardach et al., 2015; Gamboa-Loira et al., 2017; Liu and Waalkes, 2008; Putila and Guo, 2011; Rao et al., 2017). Additionally, individuals suffer from various adverse health effects including peripheral neuropathy, lung diseases, cardiovascular problems and many more (Bhattacharjee et al., 2013b). Understanding the mode of arsenic exposure and differential disease pathophysiological outcome is complex and has been studied for years (Bhattacharjee et al., 2016, 2013b). Among the major pathways identified, oxidative stress-induced DNA damage has been reported to be the most widely accepted cause of arsenic toxicity (Ercal et al., 2001). Upon exposure, arsenic is readily absorbed in the gastrointestinal tract and biotransformed to its

* Correspondence to: Environmental Epigenomics Lab, Department of Environmental Science, University of Calcutta, 35, Ballygunge Circular Road, Kolkata 700019, West Bengal, India.
E-mail address: 777.pritha@gmail.com (P. Bhattacharjee).

https://doi.org/10.1016/j.envres.2018.01.002
Received 9 October 2017; Received in revised form 4 January 2018; Accepted 6 January 2018
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methylated derivatives and gets excreted from the human body mostly in the form of dimethylarsenic (DMA) (Steinmaus et al., 2010). The primary and secondary methylation index is an important parameter to determine the methylation capacity of individuals and the risk of toxicity that again depends on several other factors including gender age, body mass index (BMI), personal lifestyle, as well as dietary factors and nutritional status (Zhang et al., 2014). The trimethylated highly reactive arsenicals give rise to oxidative damage to cellular macromolecules by formation of free radicals that overcome the body's antioxidation barrier (Bhattacharjee et al., 2013a; Chervona and Costa, 2012; Howe and Gamble, 2016; Paul et al., 2017, 2014). As previously reported, the methylated arsenicals are not classical point mutagens, but clastogenic and epimutagenic in their nature (Banerjee et al., 2013; Bhattacharjee et al., 2016, 2018; Chervona and Costa, 2012; Klein et al., 2007; Rossman, 2003). They have been associated with genomic instability at either the chromosomal level (chromosomal instability) or at the nuclear level (microsatellite instability) (Bhattacharjee et al., 2013a). The first line of defense against genotoxic damage is presented by the group of DNA repair enzymes that sustain the cellular integrity through successful correction of the damage. Previous publications by our group elaborated on the role of persistent DNA damage and inefficacy of DNA repair mechanisms as a potential factor for inter-individual variations in arsenic susceptibility (Bhattacharjee et al., 2016, 2013a; Paul et al., 2014). Recent trends in research identified epigenetic regulations of genome beyond the DNA sequence and found this to be one of the critical regulators in carcinogenic manifestations. Arsenic-induced epigenetic alteration of key DNA damage repair genes might lead to accumulation of excessive DNA damage giving rise to carcinogenic manifestation (Bhattacharjee et al., 2016; Paul et al., 2014). It is important to mention here that arsenic-induced DNA damage is widely studied (Nollen et al., 2009; Tong et al., 2015; Wnek et al., 2011). It is noteworthy to mention here that higher levels of γH2AX, a known marker of DSBs were reported in WSL individuals (Bhattacharjee et al., 2018). However, among the repair mechanisms, how MMR system functions at population level, is yet unknown. The mechanism although is understood at different cell line/animal model, further studies are required at population level (Mauro et al., 2016; Tong et al., 2015; Treas et al., 2013; Zhang et al., 2007, 2008).

Among the major excision repair proteins, the mismatch repair system is one of the highly conserved biological pathways that maintain the genomic integrity during replication and recombination. The replication fidelity is primarily maintained by the DNA polymerase (exonuclease domain) and the MMR system. In eukaryotic MMR system, MLH1, MSH2, MSH6 and PMS2 are the key component of the mismatch repair pathway (Hinrichsen et al., 2014; Li, 2008; Yamamoto and Imai, 2015). Both MSH2 and MSH6 belong to MutS homolog that recognizes the mismatches and small insertions/deletions. MutSα (MSH2-MSH6 complex) has the major activity than the MutSβ. MSH6 is unstable in the absence of MSH2 and studies regarding MSH6 are somewhat less than the other subunits (Edelbrock et al., 2013). *MLH1* is one of the important genes for all MutL homologs that have some important endonuclease activity. MutLα, the major heterodimer contains about 90% of the MLH1 complexed with PMS2 and plays a significant role in mismatch repair (Yamamoto and Imai, 2015). Defects in MMR are associated with genomic instability (microsatellite instability), leading to its association with a number of cancers (Dogan et al., 2017; Gelsomino et al., 2016; Kurzius-Spencer et al., 2017; Zarour et al., 2017). Epimutations of the MMR genes were identified as a prognostic marker for numerous types of cancer including glioblastoma, colorectal, ovarian, and gastric cancers (Cheyuo et al., 2017; Ma et al., 2017; McFaline-Figueroa et al., 2015; Wu et al., 2017). Whereas, epimutation in *MLH1* and *MSH2* reported frequently, no reports have been found on *MSH6* and *PMS2*. Emerging evidence suggests the role of arsenic in epigenetic reprogramming, including the alteration in DNA methylation status (Argos, 2015; Banerjee et al., 2013; Chanda et al., 2013; Gribble et al., 2014; Hossain et al., 2012; Mass and Wang, 1997; Mauro et al., 2016; Paul et al., 2014; Seow et al., 2014). Considering the scenario, we have selected the three most important component of MMR pathway i.e. *MLH1*, *MSH2* and *PMS2* gene in particular and tried to correlate the promoter methylation status of these gene with their expression in peripheral blood of arsenic exposed individuals. We adapted the concept of surrogate tissue analysis (STA) for our study which is a convenient way of assessing "exposure to, and effect of, genotoxic agents on internal, inaccessible tissues and organs" (Rockett et al., 2004). Peripheral blood sample of individuals chronically exposed to arsenic was assessed to study the epigenetic alteration that might give us a reflection of possible toxic endpoints and changes occurring in the surrogate tissue/tissues due to arsenic exposure.

Recently, the regulation of MMR pathway through chromatin-mediated recruitment has been unveiled with the discovery of its association with a well-known histone mark- trimethylation of histone H3 on Lys 36 (H3K36me3) (Li, 2013). H3K36me3 has been reported to recruit *MSH6* (a component of MutSα) onto the chromatin and served as a critical regulator of the genome maintenance system in MMR (Li et al., 2016a, 2016b). Trimethylation of H3K36 position is primarily mediated by SETD2, a histone methyltransferase (Li, 2013). The expression of H3K36me3 and *SETD2* was also analyzed in response to arsenic exposure. Overall, the present study is an attempt to explore the epigenetic regulation of key MMR genes in population chronically exposed to arsenic.

2. Methodology

2.1. Study area and sample selection

The arsenic-exposed study participants were recruited from Murshidabad, one of the highly affected districts of West Bengal. The Indo-Gangetic Basin is severely affected by arsenic contamination and apart from arsenic, fluoride contamination also poses a great threat for a number of districts in West Bengal. To avoid the risk of arsenic and fluoride contamination, we have selected the unexposed population from East Midnapore district (SOES, 2006; CGWB, 2007; Supplementary Fig S1). Though the two districts are physically apart, the socio-economic, occupational status, population statistics were well matched between the study groups. Initially, a door-to-door survey was conducted to find out the presence of skin lesions among the villagers and requested to attain the medical camp. The procedure for site selection, clinical survey and sample collection has been done following standard methodology published earlier (Banerjee et al., 2013; Chatterjee et al., 2015; Ghosh et al., 2006; Paul et al., 2014). A total of 110 exposed individuals with arsenic-induced skin lesions (WSL) and 114 without skin lesions (WOSL) (aged 40 ± 10 years) residing in the same area for over 10 years (self-reported) comprised the exposed group, and were recruited during 2015–2017 through cross-sectional survey. The unexposed population comprised of 102 individuals of similar age (37 ± 15 years), gender distribution, and socioeconomic status as in the exposed population from Murshidabad. Following the structured questionnaire, patient information regarding lifestyle and sources of drinking water were recorded (Ghosh et al., 2006). Drinking water samples and biological samples, including blood and urine, were collected with written consent. Exposure assessment from water and urine was performed by Flow injection-hydride generation-atomic absorption spectrometry (FI-HG-AAS) using a Perkin Elmer Spectrophotometer, Model Analyst 700. The study was conducted in accordance with the Helsinki II Declaration and institutional ethical guidelines.

2.2. Promoter methylation analysis

Genomic DNA from patient blood samples was isolated using the DNA Isolation kit (Qiagen, GmBH, Germany). The purified DNA (1.5 μg) was bisulfite treated using EpiTect Bisulfite Modification Kit

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