Epigenetic Changes in Individuals with Arsenicosis

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 Supporting Information

ABSTRACT: Inorganic arsenic (iAs) is an environmental toxin currently poisoning millions of people worldwide, and chronically exposed individuals are susceptible to arsenicosis or arsenic poisoning. Using a state-of-the-art technique to map the methylomes of our study subjects, we identified a large interactome of hypermethylated genes that are enriched for their involvement in arsenic-associated diseases, such as cancer, heart disease, and diabetes. Notably, we have uncovered an arsenic-induced tumor suppressorome, a complex of 17 tumor suppressors known to be silenced in human cancers. This finding represents a pivotal clue in unraveling a possible epigenetic mode of arsenic-induced disease.

Inorganic arsenic (iAs) is an environmental toxicant currently poisoning tens of millions of people worldwide. Individuals chronically exposed to iAs are susceptible to arsenicosis or chronic arsenic poisoning. Heavily suffering areas such as West Bengal and Bangladesh saw a rise in incidents of arsenicosis when government officials and international aid agencies, in the hopes of mitigating waterborne diseases, introduced tube wells fed from arsenic-contaminated aquifers. Other regions, such as Mexico, are affected by both naturally occurring arsenic as well as anthropogenic sources such as smelters and ore mining operations. Chronic exposure to iAs is associated with the development of various diseases including heart disease, diabetes, and cancer, and exposed individuals often present with hallmark skin lesions. Premalignant skin lesions may indicate increased risk for arsenic-related cancer. While the precise mode of action in arsenic-induced disease is unknown, one of the proposed mechanisms is altered gene regulation via epigenetic modes of action such as DNA methylation. Supporting this is the finding that early life exposure can result in long-term health consequences, suggesting that there are heritable changes to the genome.

Previous studies highlight the association of arsenicosis with altered gene expression patterns in humans displaying the hallmark skin lesions. Moreover, gene-specific analyses suggest the role of altered DNA methylation at target sites such as tumor protein p53 (p53), cyclin-dependent kinase inhibitor 2A (CDKN2A/p16), and Ras association (RalGDS/AF-6) domain family member 1 (RASSF1A). However, it remains to be shown whether multiple genes and pathways are affected by epigenetic processes in individuals with signs of arsenicosis. Therefore, we set out to identify differentially methylated genomic regions associated with arsenicosis in humans from Zimapán, Hidalgo State, Mexico who were exposed to varying levels of iAs via their drinking water as assessed by urinary arsenic (Supporting Information, Table 1).

To our knowledge, this is the first study to examine genome-wide site-specific DNA methylation alterations due to arsenic-induced toxicity in a population with ongoing exposure. Peripheral blood lymphocyte DNA of 16 of the individuals, half with established elevated levels of iAs exposure and showing signs of arsenicosis (skin lesions), was analyzed using a methylated CpG island recovery (MIRA)-chip assay. In addition to the difference in skin lesion status, the two groups showed differential patterns, of which 182 were hypermethylated in arsenic-induced disease.

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individuals with signs of arsenicosis (Figure 1). Specifically, the identified genes showed a statistically significant (false discovery rate (FDR) q-value <0.05) difference in average DNA methylation for each CpG island. This assay allowed for interrogation of one the three genes previously identified as being hypermethylated in individuals with signs of arsenicosis, e.g., p16. While the fold change of p16 did not meet the statistical threshold for this study, here we also observed increased promoter methylation in individuals with signs of arsenicosis (fold change of 1.24).

Using a systems level approach, the 183 genes were analyzed for known molecular interactions, and a large interactome of hypermethylated genes was identified (Figure 2A). These were enriched for their involvement in cancer-associated pathways mediated by genes such as p53 (Figure 2B). Interestingly, we found that many of the proteins encoded by genes with differentially methylated CpG islands are known players in arsenic-associated disease, such as heart disease, diabetes, and cancer (Supporting Information, Table 5).

Notably, we have also identified an arsenic-methylated tumor suppressorome (Figure 2C), a pivotal clue in unravelling a possible epigenetic mode of arsenic-induced disease. The tumor suppressorome is a complex of 17 known or putative tumor suppressors silenced in human cancers. It comprises the following hypermethylated genes: C11orf70 (chromosome 11 open reading frame 70), CENPE (centromere protein E, 312 kDa), EEF1E1 (eukaryotic translation elongation factor 1 epsilon 1, also known as p18), ENDOG (endonuclease G), FOXF1 (forkhead box F1), HOXB5 (homeobox B5), HOXB9 (homeobox B9), hsa-mir-126 (human microRNA 126), MMP15 (matrix metalloproteinase 15 (membrane inserted)), MSX1 (msh homeobox 1, also known as HOX7), POLD4 (polymerase (DNA-directed), delta-4, also known as p12), PRDM2 (PR domain containing 2, with ZNF domain, also known as RIZ), RNF20 (ring finger protein 20), SMARCD2 (SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 2), SUFU (suppressor of fused homologue (Drosophila)), TBR1 (T-box, brain, 1), and TSC22D3 (TSC22 domain family, member 3).

Among the members of the tumor suppressorome, of particular interest are those with known associations to arsenic-induced diseases such as cancer of the bladder, kidney, lung, liver, and prostate, as well as cardiovascular disease and diabetes mellitus (Figure 2C, Supporting Information, Table 5). Interestingly, the expression levels of specific members of the tumor suppressorome have previously been shown to be altered via iAs exposure. For example, iAs exposure in vitro results in the downregulation of both MSX1 and CENPE. In this study, we find that the CpG islands within the promoter regions of the identified genes are hypermethylated in individuals with skin lesions. As mentioned, DNA hypermethylation of the promoter regions of three genes has been reported in arsenic-induced disease. Notably, the results from our study vastly increase this list of gene targets. Examination of these gene targets would be the next step in understanding how epigenetic changes regulate gene expression and, subsequently, cause dysregulation leading to disease.

In this study we have analyzed epigenetic changes in the peripheral blood lymphocyte DNA from iAs exposed and diseased individuals and do not directly measure alterations in target organs. Recent studies support the utilization of lymphocyte
DNA to detect genomic and epigenetic biomarkers of organ-specific disease. In future research, it will be possible to compare the epigenetic alterations of the tumor suppressorome from tissue samples of arsenic-exposed individuals.

In conclusion, these results demonstrate that a large number of genes are epigenetically modified in the lymphocyte DNA of individuals exposed to iAs with related arsenicosis. It is likely that the pathways we have identified here are influenced at the transcriptional level resulting in the repression of their activity in exposed individuals. Our findings demonstrate the significant effects of iAs on the epigenome. The identified methylation sites and differential DNA methylation patterns may serve as biomarkers of adverse health effects associated with iAs exposure. Through the identification of differential patterns of methylation, we hope to detail arsenic effects in humans in order to understand arsenic-induced disease and to identify potential methods for disease prevention.

ASSOCIATED CONTENT

Supporting Information. Experimental methods and detailed gene lists. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
Microarray data have been submitted to NCBI’s Gene Expression Omnibus (GEO) repository (http://www.ncbi.nlm.nih.gov/geo/) and are available under accession number GSE26073.

Author Contributions
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ABBREVIATIONS

iAs, inorganic arsenic; C11orf70, chromosome 11 open reading frame 70; CDKN2A/p16, cyclin-dependent kinase inhibitor 2A; CENPE, centromere protein E; 312 kDa; EEF1E1, eukaryotic translation elongation factor 1 epsilon 1, also known as p18; ENDOG, endonuclease G; FOXF1, forkhead box F1; FDR, false discovery rate; HOXB5, homeobox B5; HOXB9, homeobox B9; hsa-mir-126, human microRNA 126; MIRA-Chip, methylated CpG Island Recovery-Chip assay; MMP15, matrix metallopeptidase 15 (membrane inserted); MSX1, msh homeobox 1, also known as HOX7; p53, tumor protein p53; POLD4, polymerase (DNA-directed), delta-4, also known as p12; PRDM2, PR

REFERENCES

(1) Bhattacharjee, Y. (2007) Toxicology: a sluggish response to humanity’s biggest mass poisoning. Science 315, 1659–1661.
(2) Yoshida, T., Yamauchi, H., and Fan Sun, G. (2004) Chronic health effects in people exposed to arsenic via the drinking water: dose-response relationships in review. Toxicol. Appl. Pharmacol. 198, 243–252.
(3) NRC (2001) Arsenic in Drinking Water, 2001 Update, National Academy Press, Washington, DC.
(4) Ren, X., McHale, C. M., Skibola, C. F., Smith, A. H., Smith, M. T., and Zhang, L. (2011) An emerging role for epigenetic dysregulation in arsenic toxicity and carcinogenesis. Environ. Health Perspect. 119, 11–19.
(5) Waalkes, M. P., and Liu, J. (2008) Early-life arsenic exposure: methylation capacity and beyond. Environ. Health Perspect. 116 (3), A104.
(6) Yuan, Y., Marshall, G., Ferreccio, C., Steinmaus, C., Liaw, J., Bates, M., and Smith, A. H. (2010) Kidney cancer mortality: fifty-year latency patterns related to arsenic exposure. Epidemiology 21, 103–108.
(7) Argos, M., Kibiya, M. G., Parvez, F., Jasmine, F., Rakhub-Zaman, M., and Ahsan, H. (2006) Gene expression profiles in peripheral lymphocytes by arsenic exposure and skin lesion status in a Bangladeshi population. Cancer Epidemiol. Biomarkers Prev. 15, 1367–1375.
(8) Chan, M. W. Y., Chan, L. W., Tang, N. L. S., Lo, K. W., Tong, J. H. M., Chan, A. W. H., Cheung, H. Y., Wong, W. S., Chan, P. S. F., Lai, F. M. M., and To, K. F. (2003) Frequent hypermethylation of promoter region of RASSF1A in tumor tissues and voided urine of urinary bladder cancer patients. Int. J. Cancer 104, 611–616.
(9) Chanda, S., Dasgupta, U. B., GoharAzmudner, D., Gupta, M., Chaudhuri, U., Lahiri, S., Das, S., Ghosh, N., and Chatterjee, D. (2006) DNA hypermethylation of promoter of gene p53 and p16 in arsenic-exposed people with and without malignancy. Toxicol. Sci. 89, 431–437.
(10) Flora, S. J. S., and Mehta, A. (2009) Monoisoamyl dimercaptosuccinic acid abrogates arsenic-induced developmental toxicity in human embryonic stem cell-derived embryoid bodies: comparison with in vivo studies. Biochem. Pharmacol. 78, 1340–1349.
(11) Zheng, X. H., Watts, G. S., Vaugh, S., and Gandolfi, A. J. (2003) Low-level arsenite induced gene expression in HEK293 cells. Toxicology 187, 39–48.
(12) Anglim, P. P., Alonzo, T. A., and Laird-Offringa, I. A. (2008) DNA methylation-based biomarkers for early detection of non-small cell lung cancer: an update. Mol. Cancer 7, 81.
(13) Sinnaeve, P. R., Donahue, M. P., Grass, P., Seo, D., Vonderscher, J., Chibout, S. D., Kraus, W. E., Sketch, M., Jr., Nelson, C., Ginsburg, G. S., Goldschmidt-Clermont, P. J., and Granger, C. B. (2009) Gene expression patterns in peripheral blood correlate with the extent of coronary artery disease. PLoS One 4, e7037.