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Persistent dysregulation of DNA methylation in cells with arsenic-induced genomic instability

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Abstract:

The mechanisms by which arsenic-induced genomic instability is initiated and maintained are poorly understood. In previous studies long-term progression of chromosomal instability was typified by increasing aneuploidy in Chinese hamster V79 and human keratinocyte cells treated with arsenite for a 24 hr exposure period followed by growth in arsenic-free medium for 40-120 cell generations. In the current study the role of progressive DNA methylation changes was evaluated in long-term cell cultures after brief arsenite treatments as above. We have found altered genomic methylation patterns in cells that were briefly exposed to arsenic with evidence for widespread dysregulation of CpG methylation that persists for many population doublings after the treatment.

In V79 cell populations, progressive aneuploidy increases were notable by 50 cell generations after a 24 hr exposure to 1-10 uM arsenite. Dicentric chromosomes and/or telomeric associations, as well as complex chromosome rearrangements, occurred by 90 cells generations post treatment; and mutator and transformed phenotypes began to appear thereafter. This increasing genomic instability correlated with modifications of global DNA methylation patterns in V79 cells evaluated by 5-methylcytosine antibody binding and MeSAP-PCR. The results show that short-term exposure to arsenite induced an apparent genome hypomethylating effect within a short time after exposure.

In identical protocols using human HaCaT keratinocytes exposed to low doses of arsenite (0.05-0.1 μM) for 24 hr, genomewide methylation levels were measured by LINE1 pyrosequencing and gene-specific methylation status was assessed by Methylation-Specific-PCR for up to 40 generations post exposure. Global demethylation following treatment was supported by preliminary LINE-1 studies. Moreover, the study of gene-specific MSP and determination of expression levels by RT-PCR of several genes (p16, hMLH1, hMSH2, DNMT1, DNMT3a and DNMT3b) demonstrated that hMSH2 gene was epigenetically regulated by arsenite and that down regulation of DNMT3a and DNMT3b genes occurred in an arsenite dose-dependent manner.

The results reported here demonstrate that acute 24 hr arsenic exposure promptly induces genome wide DNA hypomethylation, and support the hypothesis that the cells continue to undergo epigenetic reprogramming both at the gene and genomic levels in the absence of further arsenite treatment; thus likely contributing to long-lasting genomic instability that manifests as aberrant chromosomal, mutator and cell transformation effects.