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Molecular events associated with arsenic-induced malignant transformation of human prostatic epithelial cells: aberrant genomic DNA methylation and K-ras oncogene activation

Lamia Benbrahim-Tallaa[a], Robert A. Waterland[b], Miroslav Styblo[c], William E. Achanzar[a,1], Mukta M. Webber[d], Michael P. Waalkes[a,*]

[a]*Inorganic Carcinogenesis Section, Laboratory of Comparative Carcinogenesis, NCI at NIEHS, MD F0-09, 111 Alexander Drive, Research Triangle Park, NC 27709, USA*

[b]*Departments of Pediatrics and Molecular and Human Genetics, Baylor College of Medicine, Children's Nutrition Research Center, Houston, TX 77030, USA*

[c]*Department of Pediatrics, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA*

[d]*Department of Medicine and Department of Zoology, Michigan State University, East Lansing, MI 48824, USA*

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Abstract

Numerous studies link arsenic exposure to human cancers in a variety of tissues, including the prostate. Our prior work showed that chronic arsenic exposure of the non-tumorigenic, human prostate epithelial cell line, RWPE-1, to low levels of (5 μM) sodium arsenite for 29 weeks resulted in malignant transformation and produced the tumorigenic CAsE-PE cell line. The present work focuses on the molecular events occurring during this arsenic-induced malignant transformation. Genomic DNA methylation was significantly reduced in CAsE-PE cells. A time course experiment showed that during malignant transformation DNA methyltransferase activity was markedly reduced by arsenic. However, DNA methyltransferase mRNA levels were not affected by arsenic exposure. Microarray screening showed that K-ras was highly overexpressed in CAsE-PE cells, a result further confirmed by Northern blot and Western blot analyses. Since ras activation is thought to be a critical event in prostate cancer progression, further detailed study was performed. Time course experiments also showed that increased K-ras expression preceded malignant transformation. Mutational analysis of codons 12, 13, and 61 indicated the absence of K-ras mutations. The K-ras gene can be activated by hypomethylation, but our study showed that CpG methylation in K-ras promoter region was not altered by arsenic exposure. Arsenic metabolism studies showed RWPE-1, CAsE-PE, and primary human prostate cells all had a very poor capacity for arsenic methylation. Thus, inorganic arsenic-induced transformation in human cells is associated with genomic DNA hypomethylation and K-ras overexpression. However, overexpression of K-ras occurred without mutations and through a mechanism other than promoter region hypomethylation.
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Keywords: Arsenic; Chronic exposure; DNA methylation; K-ras

Introduction

Arsenic is a naturally occurring metalloid that exists primarily in its inorganic forms in the environment. Arsenic has long been known to cause cancer in humans (Hutchinson, 1988), and inorganic arsenic exposure has been correlated with cancers of the skin, lung, liver, kidney, and urinary bladder (Chen et al., 1992; IARC, 1987; NTP, 2000). Although prostate cancer is the second most common cancer and the third most frequent cause of cancer mortality in the Western world, its molecular pathogenesis is poorly understood. Epidemiological studies suggest that exposure to inorganic arsenic may be associated with an increased risk of

* Corresponding author. Fax: +1 919 541 3970.
E-mail address: waalkes@niehs.nih.gov (M.P. Waalkes).
[1] Present address: Department of Toxicology, Bristol-Myers Squibb Pharmaceutical Research Institute, New Brunswick, NJ 08903, USA.

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prostate cancer in men (Wu et al., 1989), including American men (Lewis et al., 1999). Our recent work has shown that in vitro chronic exposure to low-level inorganic arsenic results in malignant transformation of the human prostate epithelial cell line RWPE-1 (Achanzar et al., 2002), supporting the potential for arsenic to induce carcinogenic events in this cell population. This transformed line, termed CAsE-PE cells, developed after 29 weeks of continuous low-level arsenite exposure and produced cells capable of forming tumors after inoculation into nude mice that remarkably resemble prostate epithelial carcinoma (Achanzar et al., 2002). In vitro, CAsE-PE cells show many characteristics of malignant transformation, including loss of contact inhibition, resistance to apoptosis, and secretion of matrix metalloproteinase-9 (MMP-9) (Achanzar et al., 2002). Thus, CAsE-PE cells provide a human cell model system by which the molecular events during arsenic carcinogenesis in the prostate can be studied in detail.

Inorganic arsenic undergoes complex cellular metabolism. Arsenite is methylated in the liver and other tissues to monomethylarsenic acid (MMA) and dimethylarsenic acid (DMA) that contain either pentavalent or trivalent arsenic (Thomas et al., 2001). The methylation of arsenic requires S-adenosyl-methionine (SAM) and is carried out by methyltransferases isoenzymes (Lin et al., 2002). Methylation of inorganic arsenic has been proposed as a detoxification process. However, recent studies indicate trivalent methylated arsenicals may be genotoxic in vitro (Mass et al., 2001). Furthermore, exposure to DMA can induce urinary bladder tumors in rats (Wei et al., 2002) and acts as a tumor promoter in several tissues (Yamamoto et al., 1997). However, epidemiological studies in villages of Mexico with endemic arsenicalism indicate that increased risk for skin cancer is associated with high urinary inorganic arsenic and low urinary DMA (Yu et al., 2000). So the role of arsenic methylation in arsenic carcinogenesis is as yet incompletely defined.

DNA methylation in mammals primarily occurs at cytosine residues in 5′-CpG-3′ base sequences (Bird, 1986). DNA methylation appears to be an important controlling factor in gene expression particularly when found in CpG islands in promoter regions (Stirzaker et al., 2004). Cytosine methylation is carried out by DNA methyltransferases that require SAM in a manner similar to arsenic methyltransferases (Martin and McMillan, 2002). In general, loss of DNA methylation can lead to gene activation, whereas inactive genes are often methylated (Jaenisch and Bird, 2003). Hypomethylation of DNA is a common alteration of the genome during carcinogenesis (Laird and Jaenisch, 1994). DNA hypomethylation is viewed as a nongenotoxic mechanism facilitating aberrant gene expression (Chen et al., 2004; Vorce and Goodman, 1989). For example, expression of the ras gene can be controlled by promoter methylation and excessive expression of the normal (unmutated) ras can, by itself, transform cells (Counts and Goodman, 1994; Goodman and Counts, 1993; Pulciani et al., 1985). Arsenic can impact DNA methylation status as, for example, during malignant transformation of rat liver cells where chronic exposure to arsenic is associated with genomic DNA hypomethylation, possibly due to SAM depletion by continuous arsenic methylation (Zhao et al., 1997). In addition, chronic exposure to arsenic in vivo causes genomic DNA hypomethylation and aberrant gene expression in mouse liver (Chen et al., 2004). Furthermore, transplacental exposure to inorganic arsenic at levels that induce hepatocellular carcinoma is associated with aberrant gene expression that is caused, at least in part, by loss of DNA methylation (Waalkes et al., 2003, 2004).

Thus, in the present study, we examined molecular events associated with arsenic-induced malignant transformation in CAsE-PE cells. Specifically, DNA methylation, DNA methyltransferase activity, K-ras oncogene expression and arsenic methylation capacity were examined. Results showed that chronic exposure of human prostate epithelial cells to arsenic induced genomic DNA hypomethylation and a marked over-expression of unmutated K-ras, which occurred in the absence of changes in methylation in its promoter region. Thus, multiple factors likely contributed to arsenic-induced malignant transformation of these cells.

Material and methods

Chemicals and reagents. Sodium arsenite ($NaAsO_2$) (Sigma Company, St. Louis, MO); keratinocyte serum-free medium (K-SFM), epidermal growth factor (EGF), bovine pituitary extract (BPE), antibiotic–antimycotic mixture, and TRIzolW Reagent (Life Technologies, Inc., Grand Island, NY); [α-^{32}P] dATP and [^{3}H] S-adenosylmethionine (SAM) (Amersham Biosciences, Inc., Piscataway, NJ); 18S riboprobe template (Ambion, Inc., Austin, TX); K-ras Oligoprobe template, the mouse monoclonal anti-K-ras and the mouse monoclonal anti-actin (Oncogene Research Products, Cambridge, MA); Horseradish peroxidase-conjugated secondary antibody (Amersham Life Science, Piscataway, NJ); The Quick Start Bradford Protein Assay (Bio-Rad Laboratories, Hercules, CA).

Cells and cell culture. Control (untransformed) RWPE-1 cells (Bello et al., 1997; Webber et al., 1997) were originally derived from normal human prostate epithelial cells and are immortalized with human papillomavirus 18 (HPV 18) but are non-tumorigenic (Bello et al., 1997). Cells were grown in K-SFM containing 50 μg/ml bovine pituitary extract, 5 ng/ml epidermal growth factor, supplemented with antibiotic–antimycotic mixture (Achanzar et al., 2002; Bello et al., 1997). Cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2 and passaged weekly. Cells were exposed continuously to 5 μM arsenite (as sodium arsenite) for up to 37 weeks. The