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# Aberrant DNA methylation and gene expression in livers of newborn mice transplacentally exposed to a hepatocarcinogenic dose of inorganic arsenic

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**Abstract**

Our prior work showed that brief exposure of pregnant C3H mice to inorganic arsenic-induced hepatocellular carcinoma (HCC) formation in adult male offspring. The current study examined the early hepatic events associated with this oncogenic transformation. Pregnant mice were exposed to a known carcinogenic dose of arsenic (85 ppm) in the drinking water from gestation days 8 to 18. The dams were allowed to give birth and liver samples from newborn males were analyzed for arsenic content, global DNA methylation and aberrant expression of genes relevant to the carcinogenic process. Arsenic content in newborn liver reached 57 ng/g wet weight, indicating arsenic had crossed the placenta, reached the fetal liver and that significant amounts remained after birth. Global methylation status of hepatic DNA was not altered by arsenic in the newborn. However, a significant reduction in methylation occurred globally in GC-rich regions. Microarray and real-time RT-PCR analysis showed that arsenic exposure enhanced expression of genes encoding for glutathione production and caused aberrant expression of genes related to insulin growth factor signaling pathways and cytochrome P450 enzymes. Other expression alterations observed in the arsenic-treated male mouse newborn liver included the overexpression of cdk-inhibitors and stress response genes including increased expression of metallothionein-1 and decreased expression of betaine-homocysteine methyltransferase and thioether *S*-methyltransferase. Thus, transplacental exposure to arsenic at a hepatocarcinogenic dose induces alterations in DNA methylation and a complex set of aberrant gene expressions in the newborn liver, a target of arsenic carcinogenesis.


*Keywords:* Arsenic; Transplacental exposure; Newborn mouse liver; DNA methylation; Gene expression

## 1. Introduction

Inorganic arsenic is a human carcinogen, producing tumors of the skin, lung, liver, urinary bladder, prostate, kidney, and possibly other sites (NRC, 2001; Centeno et al., 2002; IARC, 2004). Inorganic arsenic

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can readily cross the rodent and human placenta and enters the fetal system (NRC, 2001; Devesa et al., 2006). We have shown that short-term exposure to inorganic arsenic *in utero* produces a variety of internal tumors (liver and adrenal tumors in males, tumors of the lung, ovary and the urogenital system in females) in the offspring when mice reached adulthood (Waalkes et al., 2003, 2004a, 2006a,b). Gestation is a period of high sensitivity to chemical carcinogenesis in rodents and probably in humans (Anderson et al., 2000), and the transplacental carcinogenic risks observed in rodents could also occur in humans. To define early events associated with transplacental arsenic carcinogenesis should enhance our understanding of the mechanisms of arsenic carcinogenesis.

Inorganic arsenic is enzymatically methylated to mono- and di-methylated species in most mammals and occurs at a high level in the liver (Aposhian and Aposhian, 2006; Thomas et al., 2007). Arsenite is sequentially methylated to form methylarsonate ($MMA^{5+}$) and dimethylarsinic acid ($DMA^{5+}$) by arsenic methyltransferase (AS3MT or Cyt19) using *S*-adenosylmethionine (SAM) as a methyl group donor (Thomas et al., 2007). SAM is also required for most other cellular methylation reactions, including DNA methylation (Baylin et al., 1998). Chronic exposure of rat liver epithelial cells to inorganic arsenic induces SAM depletion in rodent liver cells, causing a global loss of DNA methylation during malignant transformation (Zhao et al., 1997). Chronic exposure of intact animals to inorganic arsenic also produces hepatic DNA hypomethylation (Chen et al., 2004; Xie et al., 2004). Hypomethylation of DNA is thought to be a nongenotoxic mechanism of carcinogenesis that acts by facilitating aberrant gene expression, and can be a causative factor in hepatocarcinogenesis (Goodman and Watson, 2002), especially during a critical period of genetic programming (Anderson et al., 2000).

The liver is clearly a potential target of arsenic in humans and arsenic exposure has been associated with development of hepatocellular carcinomas as well as other hepatic lesions (Chen et al., 1997; Lu et al., 2001; Centeno et al., 2002; Zhou et al., 2002; Mazumder, 2005). In accord with human data, transplacental exposure to inorganic arsenic induced a marked, dose-related increase in hepatocellular tumors including carcinoma, in adult male mice (Waalkes et al., 2003, 2004a, 2006b). Hypomethylation of the promoter region of the estrogen receptor-α (*ER-α*) is thought to be responsible for aberrant estrogen signaling and may play a role in the formation of HCC during carcinogenesis induced by *in utero* arsenic exposure (Waalkes et al., 2004b). Thus, the present study was designed to investigate genome-wide and site-specific DNA methylation in newborn mouse liver following *in utero* exposure to a carcinogenic dose of inorganic arsenic. In addition, microarray and real-time RT-PCR were used to profile gene expression changes. The results indicate that a brief exposure to inorganic arsenic during gestation induced DNA hypomethylation changes at the GC-rich regions and was associated with aberrant gene expressions in newborn mouse liver. These could be key early events leading to arsenic-induced hepatocarcinogenesis later in life.

## 2. Materials and methods

### 2.1. Chemicals

Sodium arsenite ($NaAsO_2$) was obtained from Sigma Chemical Co. (St. Louis, MO) and dissolved in the drinking water at 85 mg arsenic/L (85 ppm). Customer-designed cDNA microarrays (600 genes) were purchased from BD Biosciences Clontech, Inc. (Palo Atlo, CA). [α-$^{32}$P]-dATP was purchased from Perkin-Elmer, Inc. (Boston, MA), and $^{3}$H labeled *S*-adenosyl-methionine ([$^{3}$H]-SAM) was from Amersham (Arlington Heights, IL). All other reagents are of reagent grade.

### 2.2. Animal treatment and sample collection

Timed pregnant C3H mice were given drinking water containing 85 ppm arsenic as sodium arsenite or unaltered water *ad libitum* from days 8 to 18 of gestation. At day 21 of gestation, dams were allowed to give birth, and the newborn mice were killed by $CO_2$ asphyxiation and livers removed. Animal care was provided in accordance with the US Public Health Policy on the Care and Use of Animals, and the study protocol was approved by the Institutional Animal Care and Use Committee of National Cancer Institute at Frederick.

### 2.3. Hepatic arsenic levels

A portion of the frozen liver (120–150 mg) was digested in nitric acid. Total arsenic, which would include inorganic and organic forms, was determined using graphic furnace atomic absorption spectrometry (Perkin-Elmer AAanalst100, Norwalk, CT). Results were expressed as μg arsenic/g wet weight liver, as shown in previous publications (Xie et al., 2004).

### 2.4. Global DNA methylation assay

Genomic DNA was extracted from liver tissue and purified using DNeasy Kits (Qiagen, Valencia, CA). Global DNA methylation status was assessed by methyl acceptance assay (Chen et al., 2004). Briefly, 1 μg DNA was incubated at 37 °C for 2 h in a 30 μl mixture containing 1.25 μM (3 μCi) [$^{3}$H]-SAM, 4 units of CpG methylase (M. Sss I) (New England