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Arsenic and chromium in drinking water promote tumorigenesis in a mouse colitis-associated colorectal cancer model and the potential mechanism is ROS-mediated Wnt/β-catenin signaling pathway

Xin Wang [a], Ardhendu K. Mandal [a], Hiroshi Saito [b], Joseph F. Pulliam [c], Eun Y. Lee [c], Zun-Ji Ke [a], Jian Lu [a], Songze Ding [a], Li Li [d], Brent J. Shelton [e], Thomas Tucker [e], B. Mark Evers [b], Zhuo Zhang [a], Xianglin Shi [a,*]

[a] Graduate Center for Toxicology, University of Kentucky, Lexington, KY 40536 USA
[b] Department of Surgery and Physiology, Lucille P. Markey Cancer Center, University of Kentucky, Lexington, KY 40536 USA
[c] Pathology and Laboratory Medicine, University of Kentucky, Lexington, KY 40536 USA
[d] Department of Family Medicine, Case Western Reserve University, Cleveland, OH 44106 USA
[e] Markey Cancer Control Program, University of Kentucky, Lexington, KY 40504 USA

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ABSTRACT

Exposure to carcinogenic metals, such as trivalent arsenic [As(III)] and hexavalent chromium [Cr(VI)], through drinking water is a major global public health problem and is associated with various cancers. However, the mechanism of their carcinogenicity remains unclear. In this study, we used azoxymethane/dextran sodium sulfate (AOM/DSS)-induced mouse colitis-associated colorectal cancer model to investigate their tumorigenesis. Our results demonstrate that exposure to As(III) or Cr(VI), alone or in combination, together with AOM/DSS pretreatment has a promotion effect, increasing the colorectal tumor incidence, multiplicity, size, and grade, as well as cell inflammatory response. Two-dimensional differential gel electrophoresis coupled with mass spectrometry revealed that As(III) or Cr(VI) treatment alone significantly changed the density of proteins. The expression of β-catenin and phospho-GSK was increased by treatment of carcinogenic metals alone. Concomitantly, the expression of NADPH oxidase1 (NOX1) and the level of 8-OHdG were also increased by treatment of carcinogenic metals alone. Antioxidant enzymes, such as superoxide dismutase (SOD) and catalase, were decreased. Similarly, in an in vitro system, exposure of CRL-1807 to carcinogenic metals increased reactive oxygen species (ROS) generation, the expression of β-catenin, phospho-GSK, and NOX1. Inhibition of ROS generation by addition of SOD or catalase inhibited β-catenin expression and activity. Our study provides a new animal model to study the carcinogenicity of As(III) and Cr(VI) and suggests that As(III) and Cr(VI) promote colorectal cancer tumorigenesis, at least partly, through ROS-mediated Wnt/β-catenin signaling pathway.

Introduction

Trivalent arsenic [As(III)] and hexavalent chromium [Cr(VI)] are human carcinogens. Both are classified as group I carcinogens by the International Agency for Research on Cancer (IARC, 2012). Exposure to these metals occurs in both occupational and environmental settings. Acute and chronic exposure to carcinogenic metals via drinking water has been reported in many countries of the world. Drinking water contamination by carcinogenic metals remains a major public health concern and is associated with an enhanced risk of development of various cancers. Chronic As(III) exposure causes tumors of the skin, bladder, lung, liver, prostate, and colon (Tchounwou et al., 2003). Chronic Cr(VI) exposure causes tumors of the lung, gastrointestinal, and central nervous systems (Gatto et al., 2010; Stout et al., 2009).

Although epidemiological studies have documented the global impact of carcinogenic metal contamination, the mechanisms of their carcinogenicity remain unclear. One problem in establishing their carcinogenic activity is that the animal models are very limited. It is difficult to provide experimental evidence of the carcinogenicity of As(III) or Cr(VI) in laboratory animals (Tokar et al., 2010). A recent National Toxicology Program study showed an increased rates of

Abbreviations: AOM, Azoxymethane; DSS, Dextran sodium sulfate; DMEM, Dulbecco's modified Eagle's medium; FBS, Fetal bovine serum; H&E, Hematoxylin & Eosin; Cr(VI), Hexavalent chromium; 8-OHdG, 8-Hydroxydeoxyguanosine; IARC, International Agency for Research on Cancer; IEF, Isoelectric focusing; NOX1, NADPH oxidase 1; NTP, National Toxicology Program; MTT, 3-(4,5-dime-thylthiazol-2yl)-2,5-diphenyl tetrazolium bromide; ROS, Reactive oxygen species; SDS-PAGE, SDS polyacrylamide gel electrophoresis; SDD, Sodium dichromate dehydrate; As(III), Trivalent arsenic; 2D-DIGE, Two-dimensional differential gel electrophoresis.

* Corresponding author at: Graduate Center for Toxicology, 1095 V.A. Drive, 306 Health Sciences Research Building, Lexington, KY 40536, USA. Fax: +1 859 323 1059.
E-mail address: xshi5@uky.edu (X. Shi).

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oral-cavity tumors in rats and small intestine tumors in mice administrated Cr(VI) in drinking water for 2 years (Stout et al., 2009). More recent work with oral sodium arsenate in drinking water for 18 months showed an increase of lung tumor multiplicity and size in male strain A/J mice (Cui et al., 2006; Ding et al., 2009; Tokar et al., 2010). Although As(III) and Cr(VI) are believed to act through very different mechanisms (Hamilton et al., 1998; Huff et al., 2000; Zhang et al., 2011), they share several properties in regard to their carcinogenicity. Both can activate NADPH oxidase, which is a major source of cellular reactive oxygen species (ROS) and is able to induce oxidative stress (Qian et al., 2005; Wang et al., 2011; Zhang et al., 2011). Oxidative stress plays an important role in both the initiation and the progression of various types of cancer.

Colorectal cancer is one of the most common neoplasias in western countries and the second leading cause of cancer-related death (Jemal et al., 2010). The most current five years of data from the United States Cancer Statistics show that Kentucky has the second highest colorectal cancer incidence in the U.S. compared to other states, especially in the Appalachian region (USCS, 2011). In this area, the concentrations of carcinogenic metal reported in drinking water are relatively high compared with EPA standards (Johnson et al., 2011). These ecological studies suggest that there is a correlation between metal level in the environment and colorectal cancer incidence. However, the exact molecular mechanism is still unknown.

The Wnt/β-catenin signal pathway has a critical role in carcinogensis (Polakis, 2000, 2007). Abnormal subcellular localization and aberrant accumulation of β-catenin are often observed in human cancers, including colorectal cancer (Polakis, 2000). The cellular levels of β-catenin protein are regulated by the ubiquitin-proteasome system (Peifer and Polakis, 2000; Polakis, 2000, 2007). Phosphorylation of β-catenin by GSK3β is essential for the ubiquitination of β-catenin. Initiation of Wnt signaling leads to inhibition of GSK3β-dependent phosphorylation and degradation of β-catenin, activating the β-catenin transcriptional pathway (Peifer and Polakis, 2000; Polakis, 2000, 2007). Aberrant activation of Wnt signaling is common in colorectal cancer (Barker and Clevers, 2006; Segditsas and Tomlinson, 2006). A Recent study has shown that NADPH oxidase 1 (NOX1) modulates Wnt and NOTCH1 signaling to control the fate of proliferative cells in the colon (Coant et al., 2010).

We hypothesized that carcinogenic metals play an important role in colorectal tumor development. We used the azoxymethane/dextran sodium sulfate (AOM/DSS) murine colitis-associated colorectal cancer model, which is a well established model for studying colon carcinogenesis. Animals treated with AOM and DSS developed colitis-associated colorectal tumors (De Robertis et al., 2011; Greten et al., 2004). We found that the carcinogenic metals As(III) or Cr(VI), alone or in combination, in drinking water promote tumorigenesis in the murine AOM/DSS colitis-associated colorectal cancer model.

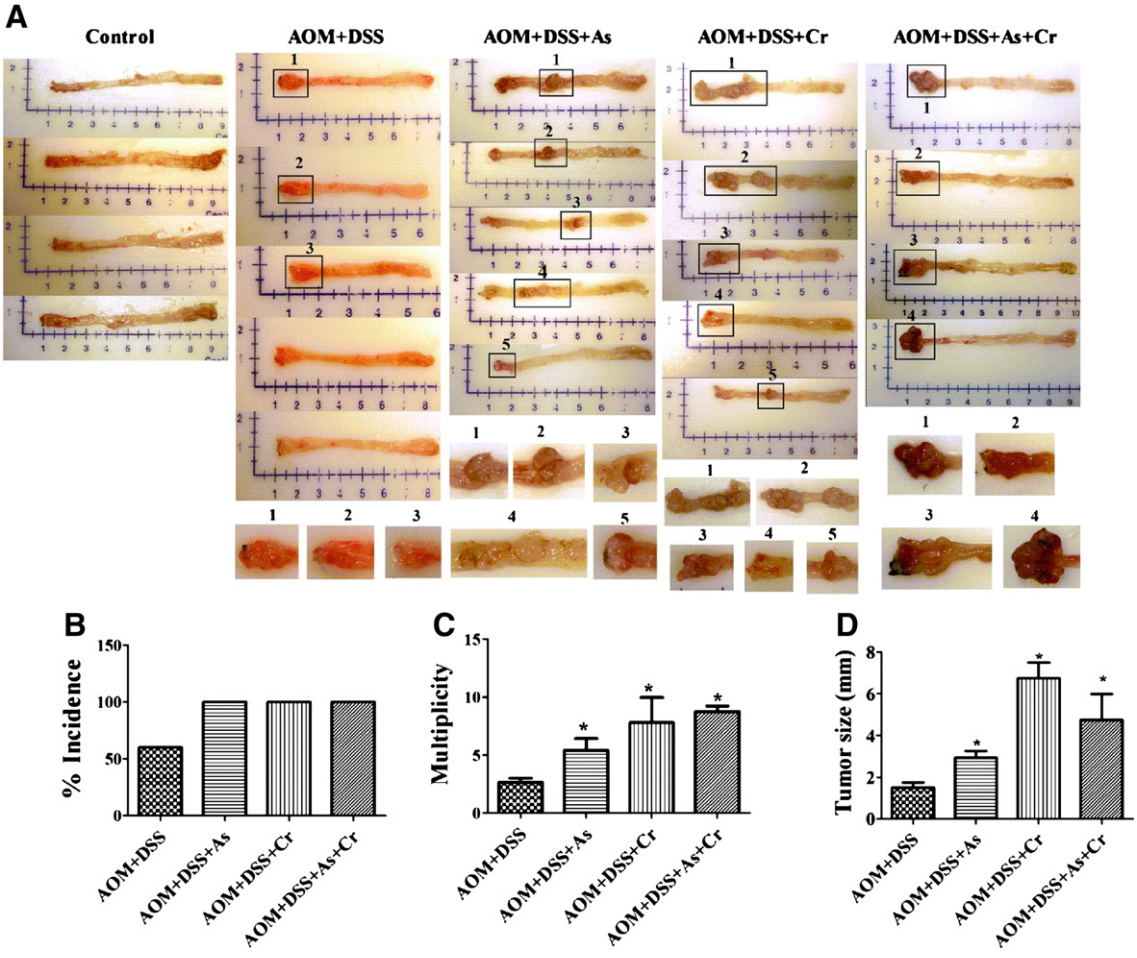

Fig. 1. Carcinogenic metals in drinking water increased the incidence and multiplicity of colorectal tumors in AOM/DSS-induced mouse colitis-associated colorectal cancer model. (A) Representative gross specimens from indicated treatments with longitudinally opened colons. Incidence (B), multiplicity (C), and average tumor size (D) at weeks 27 in carcinogenic metals in combination with AOM/DSS pretreated mice. Treatment with carcinogenic metals alone or in combination with either AOM or DSS did not produce colorectal tumors in the observation period. For graph (B–D), the data are expressed as the mean ± S.E. (n = 4 or 5). $^{*}p<0.05$, statistically significant difference from AOM/DSS pretreated alone group.