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Hexavalent chromium induces chromosome instability in human urothelial cells

Sandra S. Wise [a,1], Amie L. Holmes [a,b], Louis Liou [c], Rosalyn M. Adam [d], John Pierce Wise Sr. [a,*]

[a] Wise Laboratory of Environmental and Genetic Toxicology, Maine Center for Toxicology and Environmental Health, Department of Applied Medical Science, University of Southern Maine, Science Building, 96 Falmouth Street, Portland, ME 04103, USA
[b] Department of Radiation Oncology, Dana Farber Cancer Institute, 450 Brookline Ave., Boston, MA 02215, USA
[c] Department of Pathology, Boston University School of Medicine, 670 Albany St., Boston, MA 02118, USA
[d] Department of Surgery, Harvard Medical School, Boston, MA 02115, USA

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ABSTRACT

Numerous metals are well-known human bladder carcinogens. Despite the significant occupational and public health concern of metals and bladder cancer, the carcinogenic mechanisms remain largely unknown. Chromium, in particular, is a metal of concern as incidences of bladder cancer have been found elevated in chromate workers, and there is an increasing concern for patients with metal hip implants. However, the impact of hexavalent chromium (Cr(VI)) on bladder cells has not been studied. We compared chromate toxicity in two bladder cell lines; primary human urothelial cells and hTERT-immortalized human urothelial cells. Cr(VI) induced a concentration- and time-dependent increase in chromosome damage in both cell lines, with the hTERT-immortalized cells exhibiting more chromosome damage than the primary cells. Chronic exposure to Cr(VI) also induced a concentration-dependent increase in aneuploid metaphases in both cell lines which was not observed after a 24 h exposure. Aneuploidy induction was higher in the hTERT-immortalized cells. When we correct for uptake, Cr(VI) induces a similar amount of chromosome damage and aneuploidy suggesting that the differences in Cr(VI) sensitivity between the two cells lines were due to differences in uptake. The increase in chromosome instability after chronic chromate treatment suggests this may be a mechanism for chromate-induced bladder cancer, specifically, and may be a mechanism for metal-induced bladder cancer, in general.

1. Introduction

Hexavalent chromium [Cr(VI)] is a well-established human carcinogen. Most of the focus and attention has been on Cr(VI) acting as a lung carcinogen (IARC, 1990). This primary focus is based, in part, on epidemiology studies, which consistently show Cr(VI) to be a human lung carcinogen; and human pathology studies, which show lung tumors form where Cr(VI) particles impact and persist (Cohen et al., 1993; Ishikawa et al., 1994). But, this focus is also based, in part, on the toxicology of Cr(VI) which indicates that when reduced extracellularly to Cr(III) there is a loss in toxicity, especially genotoxicity (Xie et al., 2004). In other words, Cr(VI) has been thought to be a site-of-exposure carcinogen, because the expectation has been that Cr(III) cannot induce carcinogenesis as it is too poorly absorbed and any Cr(VI) that enters the body would simply be reduced after penetrating the site of exposure.

However, while epidemiology studies have focused attention on the lung, some also consistently show that metal workers with Cr(VI) exposure also have an elevated rate of bladder cancer (IARC, 1990). For example, a large study of welders showed an elevation of lung and bladder cancer, that correlated with Cr and nickel (Ni) exposure (Milham, 1983). A study of chrome tannery workers also showed an elevated bladder cancer risk (Montanaro et al., 1997). In addition, two other studies found correlations with occupations involving chromate and bladder cancer (Claude et al., 1986; Claude et al., 1988). To be clear, these studies do not definitively show Cr(VI) causes bladder cancer. There are power limitations and co-exposures to additional carcinogens that confound their full interpretation, however, at the same time, these confounders do not rule out the possibility that Cr(VI) can cause bladder cancer, and they do suggest that such an outcome is possible.

* Corresponding author at: Wise Laboratory of Environmental and Genetic Toxicology, Department of Pharmacology and Toxicology, University of Louisville, 505 S. Prescott St, Louisville, KY 40292, USA.
E-mail address: john.wise@louisville.edu (J.P. Wise Sr.).
[1] Present address: Wise Laboratory of Environmental and Genetic Toxicology, Department of Pharmacology and Toxicology, University of Louisville, 505 S. Prescott St, Louisville, KY 40292, USA.

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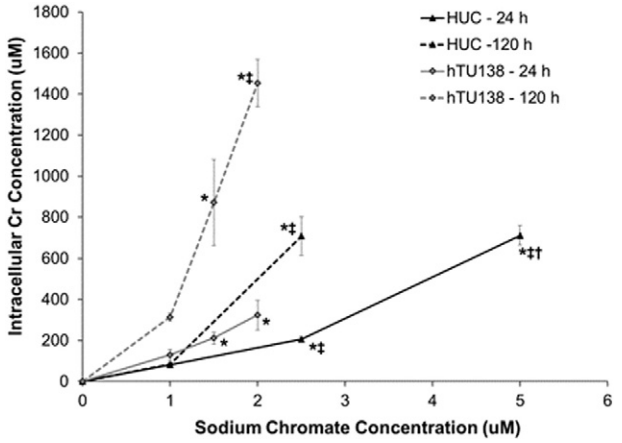

Fig. 1. hTERT-immortalized human urothelial cells take up more chromium than primary cells.

This possibility has become more urgent with the increased use and emerging health concerns of metal-on-metal hip implants. There are several types of metal-on-metal implants with cobalt chrome, titanium-based alloy or stainless steel in the stem. The epidemiology on whether a specific implant confers a greater cancer risk than another is currently poorly developed. However, concern is focused on the implants made of cobalt/chromium (CoCr) alloy, because they are extensively used and the CoCr alloy wears over time and releases a known carcinogen [chromium (Cr)] and a suspected carcinogen [cobalt (Co)] along with CoCr nanoparticles (Keegan et al., 2007, 2008; Case et al., 1994). Moreover, the new generation of these CoCr implants is failing in very high numbers, perhaps as high as 50% in some cases (Langton et al., 2010). Clinical data clearly show the bladder in these patients are directly exposed to high levels of Cr based on blood and urinary excretion levels (Keegan et al., 2007, 2008; Lhotka et al., 2003; Pilger et al., 2002). The valence of Cr released is unclear at this time, but some studies do indicate that Cr(VI) might be released (Keegan et al., 2007, 2008; Case et al., 1994).

Thus, this new public health concern combined with the previous possibility that Cr(VI) causes bladder cancer indicates a need to better understand the impact of Cr(VI) on the bladder. Moreover, our recent data showing a carcinogenic mechanism of Cr(VI)-induced lung cancer that involves chromosome instability (Xie et al., 2007, 2009; Holmes et al., 2006a, 2008, 2010; Wise and Wise, 2010, 2012) is consistent with the fact that chromosome instability is considered a key mechanistic and diagnostic event in bladder carcinogenesis (Florl and Schulz, 2008). Currently, there appears to be no data on the effects of Cr(VI) in bladder cells. Thus, the focus of this study was to investigate the ability of Cr(VI) to induce chromosomal instability in human urothelial cells.

2. Materials and methods

2.1. Chemicals and reagents

Sodium chromate, Triton X-100, demecolcine and potassium chloride (KCl) were purchased from Sigma Chemical (St. Louis, MO). Giemsa stain was purchased from Ricca Chemical Company (Arlington, TX). Crystal violet, sodium dodecyl sulfate (SDS), and acetic acid were purchased from J.T. Baker (Phillipsburg, NJ). Nitric acid and methanol were purchased from BDH Chemicals (Radnor, PA). Gurr's buffer, Keratinocyte-SFM, and 4′,6-diamidino-2-phenylindole (DAPI) were purchased from Invitrogen Corporation (Grand Island, NY). Tissue culture dishes, flasks and plasticware were purchased from BD (Franklin Lakes, NJ). Urothelial cell medium, trypsin/EDTA and trypsin neutralizing solution were purchased from ScienCell Research Laboratories (Carlsbad, CA). Penicillin/streptomycin was purchased from Mediatech (Manassas, VA). Paraformaldehyde 4% was purchased from Alfa Aesar (Ward Hill, MA). Phospho-histone H2A.X (Ser139) antibody was purchased from Cell Signaling (Beverly, MA). AlexaFluor 488-conjugated goat anti-rabbit IgG secondary antibody was purchased from ThermoScientific (Rockford, IL).

2.2. Cells and cell culture

Normal human urothelial cells (HUC) were purchased from ScienCell. These cells were maintained in urothelial cell medium supplemented

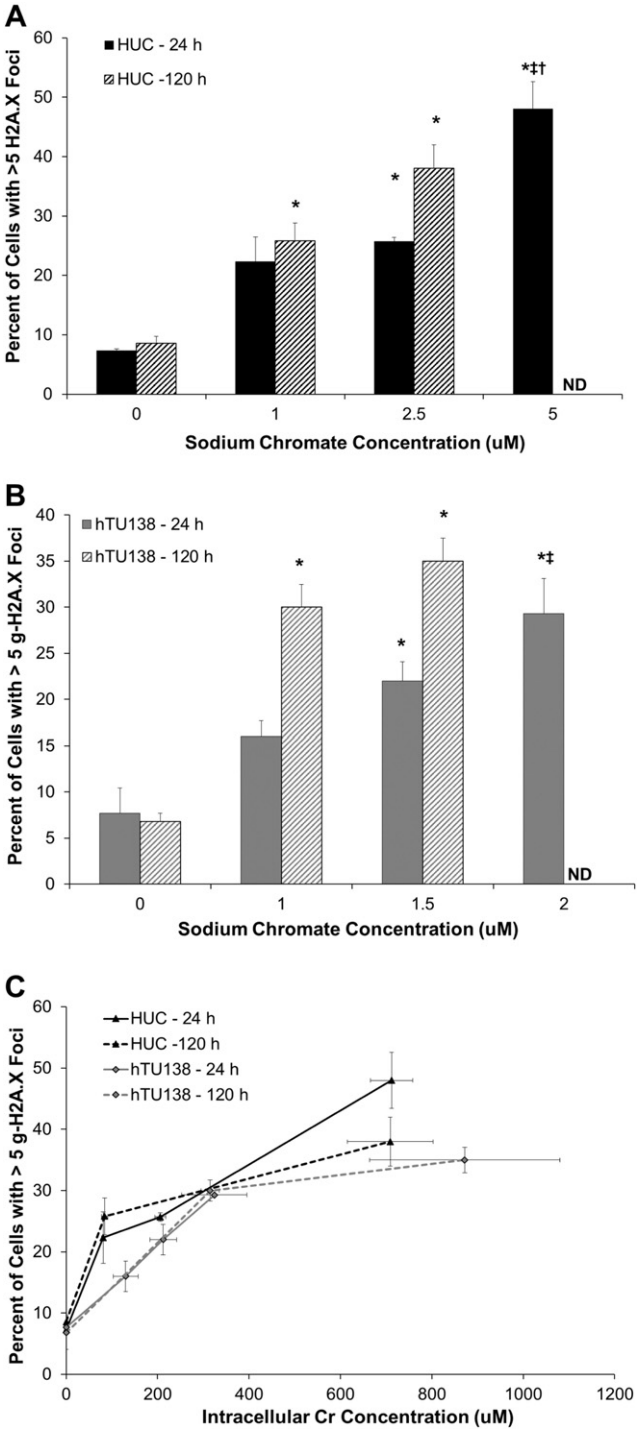

Fig. 2. Hexavalent chromium induces DNA double strand breaks in human urothelial cells.