Report as of FY2007 for 2007DC91B: "Molecular Signaling by Environmental Arsenicals in Mammalian Cells"

Publications

Project 2007DC91B has resulted in no reported publications as of FY2007.

Report Follows

Molecular Signaling by Environmental Arsenicals in Mammalian Cells

Annual Progress Report for FY2007

Principal Investigator: Deepak Kumar, Ph.D.
Assistant Professor
University of the District of Columbia
Email: dkumar@udc.edu

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Molecular Signaling by Environmental Arsenicals in Mammalian Cells

Arsenic is a major contaminant in drinking water that is associated with various cancers, skin lesions, peripheral vascular disease and hypertension. Additionally, humans are exposed to organic arsenicals when used as pesticides and herbicides (e.g., monomethylarsonic acid, dimethylarsinic acid (DMAV) also known as cacodylic acid) (ref). The main sources of arsenic in drinking water are erosion of natural deposits; runoff from orchards, runoff from glass and electronics production wastes. On January 22, 2001 EPA adopted a new standard for arsenic in drinking water at 10 parts per billion (ppb), replacing the old standard of 50 ppb. The rule became effective on February 22, 2002. The date by which systems must have complied with the new 10 ppb standard was January 23, 2006. According to EPA estimates about 3,000 (or 5.5 percent) of the nation's 54,000 Community Water systems (CWSs) and 1,100 (or 5.5 percent) of the 20,000 non-community water systems (NTNCWSs) will need to take measures to lower arsenic in their drinking water. Although, arsenic is a known human carcinogen *in vitro*, carcinogenicity of arsenic *in vivo* has not been established. Unlike many other carcinogens, arsenical compounds do not directly induce gene mutations. However, they do potentiate the genotoxic effects of other mutagens and are associated with chromosomal abnormalities. These properties indicate that arsenic has a mode of action different from other well-characterized environmental carcinogens whose actions are mediated by DNA damage and warrants investigation into the molecular signaling modulated by environmental arsenicals.

Long-term exposure to inorganic arsenic (As) from drinking water has been documented to induce cancers in lung, urinary bladder, kidney, liver and skin in a dose-response relationship (1) Arsenic accumulates in the skin. A well-established exposure-response relationship exists between arsenic level of drinking water and skin lesions. There are significant associations between these dermatological lesions and risk of skin cancer (2). Arsenic is a well-established human carcinogen (3-5); however, the exact mechanism by which it causes cancer is not known. Most of the effects of arsenic on human diseases have been established on the basis of epidemiologic studies, which have shown a significant association between the consumption of arsenic through drinking water and cancers of the skin, lung, bladder, liver, and kidney (6), neurologic disease (7), cardiovascular disease and other nonmalignant diseases (8). Premalignant skin lesions (melanosis, leucomelanosis, and keratosis) are the hallmark of arsenic toxicity. However, the molecular basis of arsenic-induced skin lesions and its progression to cancer is poorly understood.

Normal eukaryotic cells progress through a well-defined cell cycle consisting of four distinct stages, G_1, S, G_2 and M. The cell cycle progression is mainly controlled by several key checkpoints, including G_1/S restriction/checkpoint, S-phase DNA damage checkpoint and G_2/M spindle integrity checkpoint. Faulty G_1/S control of cell cycle enabling cells to pass through restriction point is caused by the activation of many oncogenes such as cyclin D1 or inactivation of tumor suppressor genes such as Rb and play critical roles in tumorigenesis.

Materials and Methods

Cell Culture and Media

Human MCF-7 breast cancer cells were grown as a monolayer in Dulbecco's modified Eagle medium (DMEM) (Invitrogen Life Technologies, Inc., Carlsbad, CA, USA) supplemented with 10% heat-inactivated fetal bovine serum and 25 μg/ml gentamicin. The cells were grown and maintained in 75-cm^2 tissue culture flasks in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C. The cells were detached by 0.2% trypsin containing 0.025% ethylene diamide tetra acetic acid (EDTA) dissolved in phosphate buffer saline (PBS, pH 7.4) (Invitrogen) and the cells were counted by trypan blue dye exclusion method (MP Biomedical, Aurora, OH).

Chemicals, Reagents and Antibodies

The following antibodies, reagents, and chemicals were obtained commercially: Arsenic as sodium meta arsenite (Sigma-Aldrich); monoclonal antibodies for P53, P21, cleaved PARP and actin (Cell Signaling, Danvers, MA); horseradish peroxidase-conjugated secondary antibody (Jackson Immunoresearch Laboratories, West Grove, PA)WST-1 reagent used for cytotoxicity assay and proteinase inhibitor cocktail tablets (Roche); ECL Plus Western blotting detection system (GE-Healthcare); and Coomassie protein assay reagent (Pierce).

Cell Viability Assay

Effects of Arsenic on cell viability and proliferation of MCF-7 breast cancer cells were determined using a cell viability detection kit (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate, WST-1) according to the manufacturer's instructions (Roche Applied Science, Indianapolis, IN) Briefly, cancer cells were seeded onto 96-well plates at density of 3,000 cells/well in media and maintained overnight in 100 ul of 10% fetal bovine serum-containing medium. The following day, cells were

treated for various times with sodium arsenite made in complete medium using six wells per treatment condition. At the end of treatment, 10 µl of the WST-1 reagent was added to each well. Plates were incubated for 2 h at 37 °C and analyzed at A = 450/600 using a Bio-Rad Model 680 micro plate reader.

Western Blotting

Immunoblotting was performed essentially as described previously (9). Adherent and floating cells were collected. Whole cell extracts (total cell homogenates) were prepared by cell lysis in radioimmune precipitation assay buffer and proteins were separated on a 4-12% gradient SDS gel (Pierce). Following SDS-PAGE, proteins were transferred to polyvinylidene difluoride membranes (0.45 µm) immunoblotted with the appropriate primary antibody and peroxidase-conjugated secondary antibody. The antigen-antibody complex was determined using the ECL detection assay (GE-Healthcare).

Statistical Analyses

Student's t test was used to analyze treated vs. untreated cells. Results were expressed as averages ± SD. $P<0.005$ was considered significant.

Results

Arsenic inhibit the proliferation of MCF-7 cells. We examined the cytotoxic effects of arsenic on MCF-7 human breast cancer cell line. The cells were treated with various concentrations of sodium arsenite ranging from 0.5µg/ml to 6 µg/ml for 24h. Sodium Arsenite inhibited the growth of MCF-7 cells (Figure 1). We further studied if the arsenic induced growth inhibition is due to induction of apoptosis in these cells and whether arsenic induces the DNA damage response pathway genes P53 and P21.

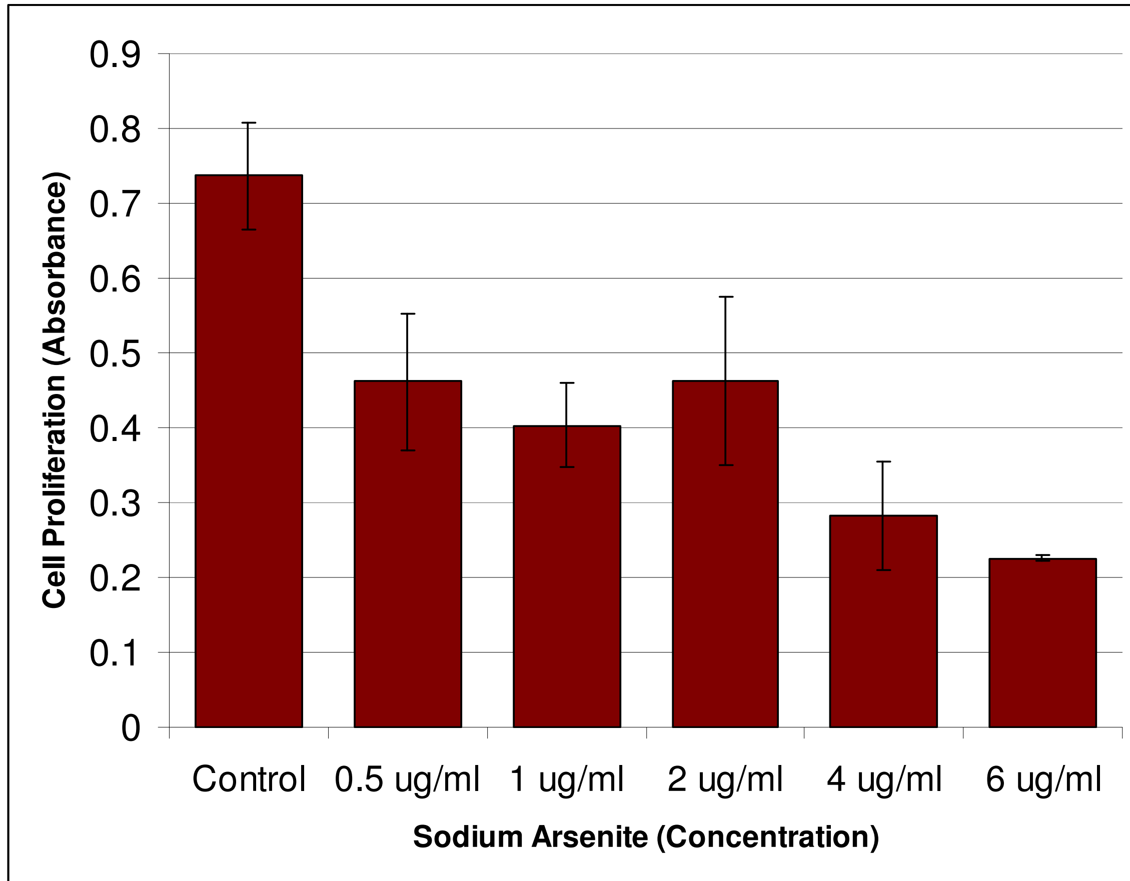

Figure 1. Sodium Arsenite inhibits the proliferation of MCF-7 breast cancer cells.

<u>Sodium Arsenite treatment induces the expression of DNA Damage response genes in MCF-7 breast cancer cells.</u> We studied the effect of Arsenic on the expression of P53 and its target gene P21. There was a marked increase in P53 and its target P21 protein expression after treatment with sodium arsenite (Figure 2.).

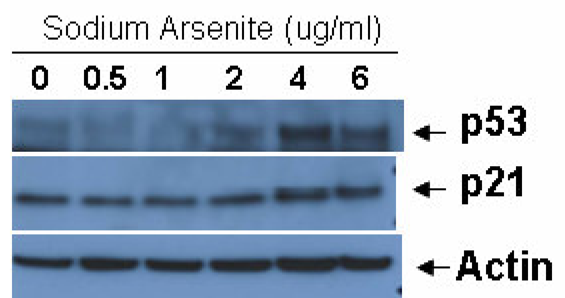

Figure 2. Sodium Arsenite induces the expression of P53 and its target gene P21.

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