ARSenic trioxide inducEs Apoptosis via specific signaling pathways in HT-29 colon cancer cells

Jacqueline J. Stevens¹, Barbara Graham², Erika Dugo², Bezawit Berhaneselassie-Sumner¹, Kenneth Ndebele² and Paul B. Tchounwou²

¹Molecular and Cellular Biology Research Laboratory, NIH RCMI-Center for Environmental Health, College of Science, Engineering and Technology, Jackson State University, 1400 JR Lynch Street, Box 18540, Jackson, MS 39217, USA
²Molecular Toxicology Research Laboratory, NIH RCMI-Center for Environmental Health, College of Science, Engineering and Technology, Jackson State University, 1400 JR Lynch Street, Box 18540, Jackson, MS 39217, USA

Abstract: Arsenic trioxide (ATO) is highly effective in the treatment of patients with acute promyelocytic leukemia (APL). It is a chemotherapeutic agent that has been shown to induce apoptosis in several tumor cell lines. However, research into its effects on colon carcinoma cells is still very limited. We previously reported that ATO is cytotoxic and causes DNA damage in HT-29 human colorectal adenocarcinoma cells. In the present study we further evaluated its effect on oxidative stress (OS), and examined its apoptotic mechanisms of action on HT-29 cells. OS was assessed by spectrophotometric measurements of MDA levels while cell cycle analysis was evaluated by flow cytometry to determine whether ATO induces cell cycle arrest. Its effect on early apoptosis was also evaluated by flow cytometry using Annexin V-FITC/PI staining. Fluorescence microscopy was used to detect the morphological changes, and Western blotting was carried out to determine the expression of apoptosis-related proteins. The lipid peroxidation assay revealed a dose-dependent increase in MDA production. DAPI staining showed morphological changes in the cell's nucleus due to apoptosis. Cell cycle analysis and Annexin V-FITC assay also demonstrated a dose-dependent effect of ATO in the accumulation of cells at the sub G₁ phase, and the percentages of Annexin V-positive cells, respectively. Western blot data showed that ATO upregulated the expression of caspase 3, Bax, and cytochrome C, and down-regulated the expression of Bcl-2. Taken together, our findings indicate that ATO induces OS and cytotoxicity in HT-29 cells through the mitochondria mediated intrinsic pathway of apoptosis.

Keywords: Arsenic trioxide; apoptosis; oxidative stress; mitochondria apoptotic pathway; HT-29 human colorectal adenocarcinoma cells

Acknowledgments: This research was financially supported in part by a grant from the National Institute on Minority Health and Health Disparities (G12MD007581) from the National Institutes of Health (NIH); NIH-Minority Access to Research Careers/Undergraduate Student Training in Academic Research (MARC/U*STAR) Program Grant No. GM007672-32; and NSF Grant No. HRD-1008708, Transforming the Climate and Advancing STEM Women at Jackson State University, an HBCU in the South (JSUAdvance). We thank the Molecular and Cellular Biology Core Laboratory and the Environmental Toxicology Research Laboratory for assistance with fluorescent microscopy and flow cytometry.