The Molecular Mechanisms of Arsenic-Induced Cell Transformation and Apoptosis

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Arsenic is a well-documented human carcinogen associated with cancers of the skin, lung, liver, and bladder. Interestingly, arsenic has also been used as an effective chemotherapeutic agent in the treatment of certain human cancers. However, the mechanisms by which arsenic induces proliferation of cancer cells or cancer cell death are not well understood. We found that exposure of JB6 P+ cells to low concentrations of arsenic induces cell transformation, whereas higher concentrations of arsenic induce cell apoptosis. Arsenite induces phosphorylation of extracellular signal-regulated protein kinases (Erks) and c-Jun NH_2-terminal kinases (JNKs). Arsenite-induced Erk activation was markedly inhibited by introduction of dominant-negative Erk2 into cells, whereas expression of dominant-negative Erk2 did not inhibit JNKs or mitogen-activated protein kinase Erk kinase 1/2. Furthermore, arsenite-induced cell transformation was blocked in cells expressing dominant-negative Erk2. In contrast, overexpression of dominant-negative JNK1 increased cell transformation even though it inhibited arsenite-induced JNK activation. Arsenic also induced AP-1 and nuclear factor kappa B (NF-κB) activation. Blocking NF-κB activation by dominant-negative inhibitory kappa Bα inhibited arsenic-induced apoptosis and enhanced arsenic-induced cell transformation. Arsenic induced activation of JNKs at a similar dose range that was effective for induction of apoptosis in JB6 cells. In addition, we found that arsenic did not induce p53-dependent transactivation. Similarly, apoptosis induction was not different between p53 wild-type ($p53^{+/+}$) or p53-deficient ($p53^{-/-}$) cells. In contrast, arsenic-induced apoptosis was almost totally blocked by expression of a dominant-negative mutant of JNK. Taken together with previous findings that p53 mutations are involved in approxmiately 50% of all human cancers and nearly all chemotherapeutic agents kill cancer cells mainly by apoptotic induction, we suggest that arsenic may be a useful agent for the treatment of cancers with p53 mutations. These results suggest that the activation of Erks is required for arsenic-induced cell transformation, whereas the activation of JNKs and NF-κB is involved in arsenic-induced apoptosis of JB6 cells. *Key words:* AP-1, apoptosis, arsenic, cell transformation, MAP kinase, NF-κB, signal transduction. *Environ Health Perspect* 110(suppl 5):757–759 (2002).

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Arsenic is a well-documented human carcinogen and is associated with an increased risk of human cancers of the skin, lung, and bladder (Chen et al. 1985; Forkner and McNair-Scott 1931; Kandel and Leroy 1937; Konig et al. 1997; Nriagu 1994; Smith et al. 1992; Sommers and McManus 1953). Interestingly, arsenic-containing compounds have been used for treatment of cancer for hundreds of years in both traditional Chinese and Western medicine (Chen et al. 1996, 1997; Forkner and McNair-Scott 1931; Kandel and Leroy 1937; Konig et al. 1997). Several recent studies confirm that arsenic trioxide appears to be a valuable therapeutic tool for patients with acute promyelocytic leukemia (Chen et al. 1996, 1997).

Although arsenic and its modes of action have been the subject of reviews and symposia, little data exist regarding specific mechanism(s) differentiating its action as a carcinogen to cause cancer and as a chemotherapeutic agent used in the treatment of cancer. Recently, our laboratory findings provided mechanisms of arsenic-induced neoplastic cell transformation and arsenic-induced apoptosis in tumor cells (Bode and Dong 2000, 2002; Chen et al. 2000a, 2000b; Huang et al. 1999a, 1999b, 2001) (Figure 1).

Arsenic Induces Cell Transformation

Much of the lack of progress in determining mechanisms explaining the role of arsenic as a carcinogen or as a chemotherapeutic agent has been attributed to the availability of valid and reproducible animal models. To study whether arsenite induces cell transformation, we exposed JB6 Cl 41 cells to arsenite in soft agar. Anchorage-independent colonies were observed in the eighth week after arsenite exposure. Cell transformation can only be observed in cells exposed to 0.5–25 μM arsenite, whereas no transformed colonies were observed at higher concentrations of arsenite (50–100 μM) because of the toxicity of arsenic (Huang et al. 1999a, 1999b).

Induction of Apoptosis by Higher Concentrations of Arsenic

A higher concentration of arsenic appears to prevent cell transformation by inducing apoptosis. We showed that treatment of cells with a relatively higher concentration (200 μM) of arsenite or arsenate resulted in apoptosis by 44.5 and 61.5%, respectively (Chen et al. 2000b).

Differential Activation of Erks and JNKs by Arsenite

We found that arsenite could induce activation of both c-Jun NH_2-terminal kinases (JNKs) and extracellular signal-regulated protein kinases (Erks) (Huang et al. 1999a, 1999b). However, the activation of JNKs and Erks by arsenite differs in time course and dose response. During the time course and dose–response studies, marked Erks activation could be observed 15 min after exposure and at all dosages studied, but no significant induction of Erks by arsenite occurred after a 30-min exposure. In contrast, arsenic activation of JNKs was observed only at a relatively high dosage (>50 μM) and after 60 min of exposure.

Arsenic Induces AP-1 Activation in Cells and AP-1-Luciferase Transgenic Mice

Using JB6 cells, we found that both arsenite and arsenate could induce transactivation of AP-1 (Huang et al. 2001). This induction of AP-1 activity by arsenic appears to occur through activation of mitogen-activated protein (MAP) kinases and protein kinase C (PKC) because increased AP-1 activity by arsenite could be blocked by either treating cells with PD98059, an MAP kinase Erk kinase (MEK)1 inhibitor, or overexpression of dominant-negative PKCα (Chen et al. 2000a; Huang 2001). Furthermore, both arsenite and arsenate could induce transactivation of AP-1 in AP-1-luciferase reporter transgenic mice (Huang 2001).

Inhibition of Erks Activation Blocks Arsenic-Induced Cell Transformation

The results described above revealed that Erks activation by arsenite may be involved in its cell transformation activity. To test this possibility, we used dominant-negative

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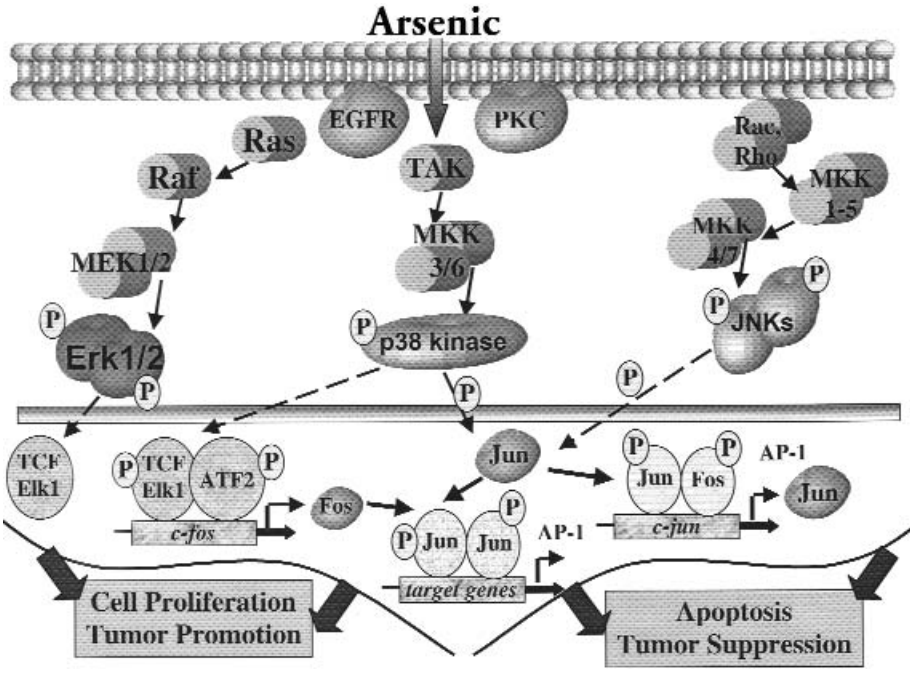

Figure 1. Arsenic-induced signal transduction pathways and their role in cell transformation and apoptosis.

Erk2-K52R stable transfectants (Chen et al. 2000b; Huang 1999a, 1999b). Our results demonstrate that Erk activation but not JNK activation is required for arsenite-induced cell transformation.

Inhibition of JNKs Blocks Arsenic-Induced Apoptosis

To investigate the role of activation of JNKs in arsenic-induced apoptosis, we used JB6 cells and a dominant-negative mutant of JNK1 to test the effects on arsenic-induced apoptosis (Huang et al. 1999a). Expression of the dominant-negative mutant JNK1 blocked induction of apoptosis by arsenite (4%) or arsenate (7%) compared with vector-transfected control cells (31.5 and 40.5% for arsenite and arsenate, respectively) (Sommers and McManus 1953).

p53 Is Not Involved in Induction of Apoptosis by Arsenic

Arsenic had no effect on p53-dependent transcription activation in Cl 41 p53 cells treated with a wide dose range of arsenic (Huang et al. 1999a). This suggested that p53 may not be involved in arsenic-induced apoptosis. This hypothesis was further tested by studying the effects of arsenic on two fibroblast cell lines derived from mouse embryos either containing wild-type p53 ($p53^{+/+}$) or deficient in p53 ($p53^{-/-}$). Results showed that treatment with arsenite or arsenate results in apoptosis in both cell lines (Huang et al. 1999a). Therefore, arsenic may be effective in counteracting drug resistance in p53-deficient cancers because arsenic appears to be able to induce apoptosis in tumor cells independently of p53 activation and thus could be specifically directed against p53-defective cancer cells.

Involvement of PKC in Arsenic-Induced Signal Transduction

Our recent data show that PKC, upstream from the MAP kinases, may be involved in mediating arsenite-induced signal transduction (Chen et al. 2000a). Translocation of PKC from the cytosol to the membrane is a critical step for activation of this enzyme, and treatment of JB6 cells with arsenite resulted in an increased translocation of PKC within 15 min. Inhibition of activation of PKC blocked both arsenite-induced AP-1 activity and arsenite-induced phosphorylation of Erks, JNKs, and p38 kinase, suggesting that PKC is required for arsenite-induced activation of MAP kinases.

Tea Polyphenols Block Arsenic-Induced Signal Transduction and Cytotoxicity

Arsenite-induced apoptosis appears to be important in accounting for its toxicity. Green tea has been used as a traditional Chinese remedy for detoxification of arsenite-caused toxicity. In recent work, we found that tea polyphenols, (–)-epigallocatechin-3-gallate (EGCG) and theaflavins, effectively blocked arsenite-induced apoptosis in JB6 cells and inhibited arsenite-induced AP-1 transcriptional activation and AP-1 DNA-binding activity (Chen et al. 2000b). EGCG and theaflavins potently inhibited arsenite-induced Erk activity but not p38 kinase activity. PD98059, an inhibitor of Erks, and dominant-negative mutant (DNM)-JNK1 blocked arsenite-induced apoptosis, whereas DNM-p38 kinase or SB2020190, an inhibitor of p38 kinase, did not. We conclude that Erks and JNKs may be involved in arsenite-induced apoptosis, and the inhibition of arsenite-induced apoptosis by EGCG and theaflavins may be mediated by a decreased phosphorylation of Erks and JNKs. Furthermore, these results provide a possible mechanism explaining the detoxification effect of tea on arsenite-induced toxicity (Chen et al. 2000b).

Blocking NF-κB Activation Inhibits Arsenic-Induced Apoptosis

Nuclear factor kappa B (NF-κB) is a rapidly induced stress-responsive transcription factor that may play an important role in arsenic-induced signal transduction, cell transformation, and apoptosis (Bode and Dong 2000, 2002). Barchowsky et al. (1996,1999) reported that arsenic-induced oxidant stress, H_2O_2, and superoxide are the predominant reactive species in endothelia cells and may be the mediators for the activation of the NF-κB pathway. We showed that arsenic induced activation of NF-κB in different cell culture models. Expression of a dominant-negative inhibitory kappa Bα blocked arsenic-induced activation of NF-κB and apoptosis.

Conclusions

Arsenic is clearly a human carcinogen, but also acts as a beneficial chemotherapeutic agent. The lack of information regarding its mechanisms of action has been attributed to the availability of appropriate animal models. In the past few years we have established cell culture models to study arsenic-induced neoplastic cell transformation and apoptosis. Others have also reported that exposure of arsenic could induce cell transformation in a rat liver epithelial cell line (TRL 1215) (Meng and Meng 2000) or in blast transformation and DNA synthesis in human lymphocytes (Zhao et al. 1997). We found that arsenic significantly affects specific signal transduction pathways (Figure 1). Our data provided a model for the role of signaling molecules including MAP kinases, p53, AP-1, and NF-κB in arsenic-induced cell transformation and apoptosis. More detailed studies are needed to determine precise mechanisms of the effects of arsenic on animals and humans.

References

Barchowsky A, Dudek EJ, Treadwell MD, Wetterharn KE. 1996. Arsenic induces oxidant stress and NF-kappa-B activation in cultured aortic endothelial cells. Free Radic Biol Med 21:783–790.

Barchowsky A, Klei LR, Dudek EJ, Swartz HM, James PE. 1999. Stimulation of reactive oxygen, but not reactive nitrogen species, in vascular endothelial cells exposed to low levels of arsenic. Free Radic Biol Med 27:1405–1412.

Bode A, Dong Z. 2000. Apoptosis induction by arsenic: mechanisms of actions and possible clinical applications for treating therapy-resistant cancers. J Drug Resist Updat 3:21–29.

Bode AM, Dong Z. 2002. The paradox of arsenic: molecular mechanisms of cell transformation and chemotherapeutic effects. Crit Rev Oncol/Hematol 42:5–24.

Chen C-J, Chuang Y-C, Lin T-M, Wu H-Y. 1985. Malignant neoplasms among residents of a Blackfoot disease-endemic-area in Taiwan: high-arsenic artesian well water and cancers. Cancer Res 45:5895–5899.

Chen G-Q, Zhu J, Shi X-G, Ni J-H, Zhong H-J, Si G-Y et al. 1996. *In vitro* studies on cellular and molecular mechanisms of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia: As_2O_3 induces NB_4 cell apoptosis with downregulation of Bcl-2 expression and modulation of PML-Raα/PML proteins. Blood 88:1052–1061.

Chen G-Q, Shi X-G, Tang W, Xiong S-M, Zhu J, Cai X, et al. 1997. Use of arsenic oxide (As_2O_3) in the treatment of acute promyelocytic leukemia (APL) I. As_2O_3 exerts dose-dependent dual effects on APL cells. Blood 89:3345–3353.

Chen N-Y, Ma W-Y, Huang C, Ding M, Dong Z. 2000a. Activation of PKC is required for arsenite-induced signal transduction. J Environ Pathol Toxicol Oncol 19(3):297–305.

Chen N-Y, Ma W-Y, Yang CS, Dong Z. 2000b. Inhibition of arsenite-induced apoptosis and AP-1 activity by epigallocatechin-3-gallate and theaflavins. J Environ Pathol Toxicol Oncol 19(3):287–295.

Forkner C, McNair-Scott TF. 1931. Arsenic as a therapeutic agent in chronic myeloid leukemia. JAMA 97:305.

Huang C, Bode AM, Chen N-Y, Ma W-Y, Li J, Nomura M, Dong Z. 2001. Transactivation of AP-1 in AP-1-luciferase reporter transgenic mice by arsenite and arsenate. Anticancer Res 21:261–268.

Huang C, Ma W-Y, Li J, Dong Z. 1999a. Arsenic induces apoptosis through a c-Jun NH_2-terminal kinase-dependent, p53-independent pathway. Cancer Res 59:3053–3058.

Huang C, Ma W-Y, Li J, Goranson A, Dong Z. 1999b. Requirement of Erk, but not JNK, for arsenite-induced cell transformation. J Biol Chem 274:14595–14601.

Kandel EV, Leroy GV. 1937. Chronic arsenical poisoning during the treatment of chronic myeloid leukemia. Arch Intern Med 60:846–866.

Konig A, Wrazel L, Warrel RP, Jr, Rivi R, Pandolfi PP, Jakubowski A, Gabrilove JL. 1997. Comparative activity of melarsoprol and arsenic trioxide in chronic B-cell leukemia lines. Blood 90:562–570.

Meng ZQ, Meng NY. 2000. Effects of arsenic on blast transformation and DNA synthesis of human blood lymphocytes. Chemosphere 41:115–119.

Nriagu JO. 1994. Arsenic in the Environment, Part II. Human Health and Ecosystem Effects. New York:Wiley & Sons.

Smith AH, Hopenhayn-Rich C, Bates MN, Goeden HM, Hertz-Picciotto P, Duggan HM, et al. 1992. Cancer risks from arsenic in drinking water. Environ Health Perspect 97:259–267.

Sommers SC, McManus RG. 1953. Multiple arsenical cancers of skin and internal organs. Cancer 6:347–359.

Zhao CQ, Young MR, Diwan BA, Coogan TP, Waalkes MP. 1997. Association of arsenic-induced malignant transformation with DNA hypomethylation and aberrant gene expression. Proc Natl Acad Sci USA 94:10907–10912.