EGF receptor-dependent JNK activation is involved in arsenite-induced p21^{Cip1/Waf1} upregulation and endothelial apoptosis

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Arsenic is a naturally occurring element in the earth’s crust, and environmental exposure to arsenic results primarily from consumption of contaminated drinking water and food in certain regions of the world, including areas of the southwestern United States and Asia. On the basis of numerous epidemiological studies, arsenic has been classified as a potent human carcinogen (34), and chronic arsenic intake has been associated with other adverse effects such as cardiovascular disease, peripheral vascular disease, hypertension, diabetes mellitus, and neurological effects (32).

Arsenite has been shown to cause DNA damage evident as strand breaks and DNA-protein cross-links that subsequently invoke cell cycle arrest and apoptosis (11). In association with DNA damage, the tumor suppressor protein p53 is induced, and its activation plays important roles in the regulation of cell progression, particularly in G1/S-phase transition. Furthermore, p53 protein transactivates several checkpoint key proteins regulating the cell cycle, such as p21^{Cip1/Waf1}, which is able to silence cyclin-dependent kinases essential for S-phase entry and thus inhibit cell cycle progression (30). Even though mitogen-activated protein kinases (MAPK) such as c-Jun NH2-terminal kinase (JNK) and p38 are well known to coordinate the cellular stress response, the signaling pathway leading to p21^{Cip1/Waf1} activation and cell apoptosis is not fully understood.

Emerging evidence indicates that endothelial cell dysfunction is an early, perhaps causal, component of both chronic cardiovascular (26) and neurological diseases (10). In this regard, endothelial cells long have been suspected as primary targets of arsenic toxicity (12), although the precise molecular mechanisms for these observations are not well understood. Endothelial cells exposed to arsenite (trivalent arsenic) may exhibit diverse responses ranging from increased DNA synthesis (1) to apoptosis (37) and frank toxicity (4). Sublethal concentrations of arsenite induce endothelial cell reactive oxygen species (ROS) formation and the activation of transcription factors such as NF-kB and AP-1 (1). This latter action of arsenite may protect endothelial cells against the proapoptotic action of arsenite (37). The goal of this study was to examine the implications of arsenite for endothelial cell phenotype and to identify the underlying signaling mechanisms involved.

EXPERIMENTAL PROCEDURES

Materials. Human umbilical vein endothelial cells (HUVECs) were obtained from Cambrex. The COS-7 cell line was from American Type Culture Collection (ATCC, Manassas, VA). Endothelial cell culture medium including endothelial growth medium-2 (EGM-2) kit was obtained from Cambrex, and Dulbecco’s modified Eagle’s medium (DMEM) was obtained from Invitrogen (Carlsbad, CA). Protein kinase inhibitors, including AG1478, AG1295, AG825, 4-amino-5-(4-chlorophenyl)-7-(3-chloropyrazolo[4,3-d]pyrimidine (PP2), SB-203580, and SP-600125, were obtained from Calbiochem (San Diego, CA). Primary antibodies directed against the phosphorylated (activated) forms of JNK, extracellular signal-regulated kinase (ERK1/2), p38 MAPK, and phosphotyrosine were obtained from Cell Signaling Technology (Beverly, MA). Antibody against p21^{Cip1/Waf1} was obtained from Pharmingen (San Diego, CA). Agarose-conjugated anti-ErbB1 (the EGF receptor, EGFR), anti-p53, actin antibodies, and rabbit anti-goat secondary antibody were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-EGFR and anti-ErbB2 were obtained from Neomaker. Small interfering RNA (siRNA) for EGFR, p38 MAPK, and phosphotyrosine were obtained from Cell Signaling Technology (Beverly, MA). Antibody against p21^{Cip1/Waf1} was obtained from Pharmingen (San Diego, CA). Agarose-conjugated anti-ErbB1 (the EGF receptor, EGFR), anti-p53, actin antibodies, and rabbit anti-goat secondary antibody were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-EGFR and anti-ErbB2 were obtained from Neomaker.

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ErbB2, and negative control were from Upstate Biotechnology (Waltham, MA). Lipofectamine Plus reagent was purchased from Invitrogen. The RNeasy mini kit and TransMessenger transfection reagent were obtained from Qiagen (Valencia, CA). Dominant negative mutants for the MAP kinase kinases MKK4 (MKK4-KR) (28) and MKK7 (MKK7-KE) (41) were kindly provided by Dr. Leonard I. Zon (Children’s Hospital, Boston, MA) and Dr. Tse-Hua Tan (Baylor College of Medicine, Houston, TX), respectively. All other reagents were obtained from Sigma (St. Louis, MO).

Cell culture. HUVECs were grown in EGM-2 and used between passages 3 and 6. COS-7 cells were cultured in DMEM supplemented with 10% fetal bovine serum and streptomycin. Cells were grown at 37°C in a humidified, 5% CO2 atmosphere.

Cell viability assay. HUVECs were seeded at a density of 2 × 10^5 cells/ml into 96-well plates or 6-well plates, as appropriate, and treated with the indicated concentrations of sodium arsenite. Cell viability was determined by methylthiazol tetrazolium (MTT) assay or Trypan blue exclusion as described (6).

Immunoprecipitation and Western blotting. Immunoprecipitation and Western blotting procedures were performed essentially as described previously (7, 36). Briefly, after incubation, cells were washed twice with ice-cold PBS and incubated in lysis buffer containing 50 mM Tris-HCl (pH 8.0), 120 mM NaCl, 0.5% Nonidet P-40, 1% Triton X-100, 1 μg/ml leupeptin, 1 μg/ml aprotonin, 100 μg/ml phenylmethylsulfonyl fluoride, 1 mM sodium vanadate, 1 mM sodium fluoride, and 1 mM EDTA for 30 min on ice, followed by brief sonication. Cell lysates were then cleared by centrifugation (13,600 g) for 10 min and subjected to immunoprecipitation as described previously (5, 36). Precipitated proteins or cell lysates were resolved by SDS-PAGE, transferred to membranes, and subjected to immunoblotting as described previously (5, 36). Densitometric analysis of immunoblots was performed using commercially available software (PDI Imageware System, Huntington Station, NY).

Cell transfection. COS-7 cells were seeded at a density of 2 × 10^5 cells/ml into six-well plates. Transfections were carried out using Lipofectamine Plus reagent (Invitrogen) with cells at 70% confluence of a similar fashion. The RT-PCR products were resolved by electrophoresis on 1.5% agarose gels.

RNA extraction and RT-PCR. Total RNA from the cells grown in six-well plates was isolated using RNeasy mini kit (Qiagen, Valencia, CA). The forward and reverse primers corresponding to human p53 and p21(CIP1/WAF1) were 5'-ACAGCCAAGTCTGTGACTT-3' and 5'-CACCCACCTCAAAAGCCTTT-3', and 5'-GCTTGGGATGTCCTGCA-A-3' and 5'-GAGCCGAGCCACAGGTTACA-3', respectively. Constitutively expressed glyceraldehyde-3-phosphate dehydrogenase mRNA was amplified with forward (5'-ACAGTCATGCTCATACTGCCC-3') and reverse (5'-AGGAGTACGGATGGCAT-3') primers in a similar fashion. The RT-PCR products were resolved by electrophoresis on 1.5% agarose gels.

Fluorescence microscopy. After treatments, HUVECs were washed twice with PBS, fixed in 5% formaldehyde for 30 min, and washed further in PBS. Morphology was examined using phase-contrast microscopy. Cells were also stained with PI (10 μg/ml) in the dark for 20 min and visualized using fluorescence microscopy. Apoptotic cells were identified by the findings of condensation and fragmentation of chromatin.

Flow cytometric analysis of apoptosis. An annexin V-FITC kit (Oncogene, San Diego, CA) was used. Briefly, nonadherent and adherent cells, harvested by trypsinization, were pooled, washed in cold PBS, and resuspended in binding buffer (10 mM HEPES, pH 7.4, 150 mM NaCl, 2.5 mM CaCl2, 1 mM MgCl2, and 4% BSA). Annexin V-FITC (0.5 μg/ml) and PI (0.6 μg/ml) were then added to a 250-μl aliquot (~5 × 10^6 cells) of this cell suspension. After 15-min incubation in the dark at room temperature, stained cells were immediately analyzed using flow cytometry (MoFlo; Dako Cytomation, Fort Collins, CO).

Statistical analysis. All numerical data are presented as means ± SE. Western blots shown are representative of three or more independent experiments. Comparisons among treatment groups were performed with one-way analysis of variance and an appropriate post hoc comparison. Statistical significance was accepted if the null hypothesis was rejected with a P < 0.05.

Fig. 1. Sodium arsenite-induced cytotoxicity in human umbilical vein endothelial cells (HUVECs). HUVECs were treated with 50 μM sodium arsenite for the indicated time (A) or with increasing concentrations of sodium arsenite for 24 h (B). After treatment, cell viability was determined by methylthiazol tetrazolium (MTT) assay and Trypan blue exclusion as described in EXPERIMENTAL PROCEDURES. Values are means ± SE from 3–5 independent experiments. *P < 0.05 compared with respective untreated control.
Arsenite decreases endothelial cell viability. We observed both dose- and time-dependent decreases in endothelial cell viability as a function of sodium arsenite exposure (Fig. 1, A and B). These findings were evident using both the MTT assay and Trypan blue dye exclusion methods, consistent with previous reports (15, 42). Trends for an effect of arsenite were observed with concentrations as low as 5 μM, and significant effects were evident at 10 μM arsenite.

Arsenite induces both p21Cip1/Waf1 and p53 in HUVECs. Arsenite has been linked to DNA damage and p53 induction in various cell types (19, 42, 43). To probe the mechanism(s) involved in arsenite-induced endothelial cell toxicity, we examined its effect on p53 and its downstream target, p21Cip1/Waf1. We found that arsenite produced a potent induction of both p53 and p21Cip1/Waf1, and this effect was noted at arsenite concentrations as low as 1–5 μM with prolonged incubations (Fig. 2, A and B). Induction of p53 and p21Cip1/Waf1 in response to arsenite required 4–6 h, suggesting a mechanism involving gene transcription (Fig. 2C). Consistent with this notion, we found that arsenite produced an increase in the steady-state mRNA levels of p53 and p21Cip1/Waf1 (Fig. 2D). To determine whether p21Cip1/Waf1 was also regulated at the posttranslational level, we examined the half-life of the protein level of p21Cip1/Waf1 in the presence of cycloheximide. As shown in Fig. 2E, there was no difference in p21Cip1/Waf1 stability between cells with and without arsenite treatment. Thus these data indicate that arsenite-induced upregulation of p53 and p21Cip1/Waf1 is the result of increased transcription.

Arsenite-induced p21Cip1/Waf1 and p53 upregulation involve ErbB receptor activation. The activation of p53 by oxidative stress involves the platelet-derived growth factor-β (PDGF-β) receptor (5) and the EGFR (ErbB1), and the latter has been implicated in arsenite-induced MAPK activation (8). We used pharmacological probes to test the involvement of these two growth factor receptors in our system. We found that both AG1478 and AG825, inhibitors of ErbB receptors, attenuated arsenite-mediated p21Cip1/Waf1 induction, whereas inhibition of

Fig. 2. Sodium arsenite-induced expression of both p21Cip1/Waf1 and p53 in HUVECs. HUVECs were treated with increasing concentrations of sodium arsenite for 4 h (A) or 16 h (B) or with 50 μM sodium arsenite for the indicated time (C). After treatment, total cell lysates were subjected to immunoblot analysis for the levels of p21Cip1/Waf1 and p53. D: HUVECs were treated with 50 μM sodium arsenite for 4 h. Total cell RNA was obtained, and the mRNA levels of p21Cip1/Waf1, p53, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were measured using RT-PCR. E: half-life of p21Cip1/Waf1 in HUVECs was measured by cycloheximide chase. Cells were treated with or without 50 μM sodium arsenite for 4 h, and then 5 μM cycloheximide was added to all cell cultures (As+CHX or CHX alone). p21Cip1/Waf1 and actin protein levels were determined at the indicated time points after administration of cycloheximide. The p21Cip1/Waf1 protein levels were quantified as the relative (fold) change in induction after normalization to the actin level and are presented as percent changes compared with time 0 of the cycloheximide chase.
the PDGF receptor with AG1295 was ineffective (Fig. 3A). Consistent with this finding, AG1478 produced dose-dependent inhibition of both p53 and p21\(^{Cip1/Waf1}\) induction with arsenite. We also found evidence for ErbB2 involvement, because selective inhibition of this growth factor receptor impaired arsenite-mediated p53 and p21\(^{Cip1/Waf1}\) induction (Fig. 3C). We were able to confirm activation of both the EGFR (Fig. 4A) and ErbB2 (Fig. 4B) in response to arsenite by tyrosine phosphorylation. In addition, each ErbB isoform receptor tyrosine phosphorylation was dependent on its intrinsic receptor tyrosine kinase activity and the nonreceptor Src-family kinases (Fig. 4, A and B). To substantiate these results, we utilized siRNA directed against the EGFR and ErbB2. We found that EGFR siRNA produced effective gene silencing (Fig. 5A) that significantly attenuated p21\(^{Cip1/Waf1}\) induction in response to arsenite (Fig. 5B). Similarly, siRNA against ErbB2 abrogated ErbB2 expression (Fig. 5C) and abolished arsenite-induced p21\(^{Cip1/Waf1}\) induction in HUVECs (Fig. 5D). Thus arsenite-induced p21\(^{Cip1/Waf1}\) induction requires the tyrosine kinase activity of both the EGFR and ErbB2.

Arsenite-induced p21\(^{Cip1/Waf1}\) and p53 upregulation involve distinct MAPks. Because growth factor receptors often are upstream components of MAPK activation, we probed p38 MAPK, JNK, and ERK1/2 in HUVECs exposed to arsenite. We found that arsenite induced activation of p38 MAPK and JNK, but not ERK1/2, in our system (Fig. 6A). Moreover, inhibition of p38 MAPK with SB-203580 inhibited both p21\(^{Cip1/Waf1}\) and p53 induction (Fig. 6B), whereas the selective JNK inhibitor SP-600125 did not inhibit arsenite-mediated p53 induction (Fig. 6C). These data are most consistent with a p53-independent signaling pathway directly between JNK and p21\(^{Cip1/Waf1}\) that is distinct from p38 MAPK-mediated activation of p53. To assess this hypothesis directly, we transfected cells with dominant negative isoforms of MKK4 or MKK7 and examined the implications for arsenite-induced p21\(^{Cip1/Waf1}\) upregulation. It is known that MKK4 activates both JNK and p38 MAPK, whereas MKK7 serves as a specific activator of JNK. As shown in Fig. 6D, inhibition of MKK4 or MKK7 significantly attenuated arsenite-induced p21\(^{Cip1/Waf1}\) induction with distinct effect on p53. Inhibition of MKK4 inhibited both p21\(^{Cip1/Waf1}\) and p53 induction, whereas inhibition of MKK7 significantly attenuated arsenite-mediated p21\(^{Cip1/Waf1}\) induction without any material effect on p53. Thus arsenite-induced p21\(^{Cip1/Waf1}\) upregulation involves a unique role for EGFR/JNK that is independent of p53 (Fig. 6E).

Arsenite-induced endothelial cell apoptosis requires JNK-mediated p21\(^{Cip1/Waf1}\) upregulation. To determine the functional consequences of this pathway, we manipulated arsenite-induced p21\(^{Cip1/Waf1}\) upregulation and determined the implications for cell survival and apoptosis. Arsenite exposure produced a significant decrease in cell viability, an effect that was substantially reversed with inhibition of p21\(^{Cip1/Waf1}\) upregulation by interruption of arsenite signaling using AG1478, SP-600125, and SB-203580 (Fig. 7, A and B). PI staining of pyknotic nuclei suggested apoptosis as a mechanism of arse-
ite-induced cell death (Fig. 7C). We also used flow cytometric analysis of externalized phosphatidylserine to quantify early apoptotic cells and measured PI uptake to assess cells in the later stages of apoptosis or cells that sustained direct plasma membrane damage (necrosis). As shown in Fig. 7, D and E, arsenite treatment of HUVECs (50 μM) for 16 h increased the number of both early apoptotic cells and late apoptotic/necrotic cells. Moreover, these phenomena were significantly attenuated by inhibitors of p21Cip1/Waf1 induction. Similar results were observed in cells using EGFR gene silencing by siRNA (Fig. 7F). Together, these data are consistent with the notion that arsenite upregulates p21Cip1/Waf1 through multiple pathways resulting in cell apoptosis.

**DISCUSSION**

In the present report, we provide evidence that arsenite triggers membrane receptor-dependent signaling pathways that ultimately lead to endothelial cell toxicity. As an initial step of our study, we observed an evident toxicity in HUVECs at 10 μM arsenite after 24 h of treatment. This toxic effect is in agreement with that previously reported by Kao et al. (15) in HUVECs and appears to be cell type dependent with more resistance in porcine endothelial cells (42). The discrepancies with regard to species could be explained by differences in the genetic background. Although this article focuses on elucidating the signaling pathways by arsenite at toxic levels, it is important to note that arsenite upregulates p21Cip1/Waf1 through multiple pathways resulting in cell apoptosis.

Arsenic, a pervasive carcinogen in the environment, is known to promote DNA damage. Indeed, very low physiologically relevant doses of arsenite induce DNA strand breaks and DNA-protein cross-links in a variety of cell types (2, 29). Increasing evidence now links oncogenes and tumor suppressors such as Ras and p53, typically associated with carcinogenesis, with vascular disease (13, 23). We recently demonstrated that oxidative DNA damage induces p53 expression through protein stabilization via a PDGF-β receptor-mediated process (5). Despite the similar propensity for DNA damage, however, it appears that arsenite and oxidative stress act on p53 via distinct mechanisms. In particular, we found in the present study that arsenite-induced upregulation was largely a consequence of increased transcription (Fig. 2) that was independent of the PDGF-β receptor (Fig. 3A) and appeared to involve ErbB receptors instead. Thus arsenite and oxidative stress facilitate increases in cellular p53 levels via different mechanisms.
Although distinct with regard to p53 activation, arsenite and oxidative stress do share some common features pertaining to ErbB receptors. In this study, we found that arsenite induced EGF and ErbB2 receptor activation in a manner dependent on both receptor kinase activity and Src-family kinases (Fig. 4). This finding is reminiscent of previously reported data on H2O2-mediated EGFR transactivation (7). Investigators in our laboratory (7) previously demonstrated that endothelial cells treated with H2O2 exhibit activation of the EGFR in a Src-dependent manner that is distinct from EGFR autophosphorylation. This "transactivation" of the EGFR has been described with respect to a number of diverse stimuli, including G protein-coupled receptors, cytokines, and cellular stress (45). This similarity between the response to H2O2 and arsenite is consistent with other data showing that arsenite exposure leads to the intracellular generation of ROS (39) and participation of the EGFR in redox-sensitive signal transduction (8, 21). Thus arsenite and H2O2 share common features with regard to the EGFR transactivation reported presently.

The similarities between the responses to arsenite and H2O2 may be consistent with putative mechanisms of signal transduction. For example, arsenite is a well-recognized sulphydryl reactant that modifies cysteiny1 residues of many cellular proteins, including the EGFR and protein tyrosine phosphatase (PTPase) (3). The EGFR contains an extracellular cysteine-rich domain that has proven important with regard to receptor dimerization (14). A similar scenario has been proposed in which arsenite, via reaction with vicinal dithiols, alters the conformation of the EGFR and produces an increase in its intrinsic tyrosine kinase activity (35). Upstream kinases also are targets of arsenite, as demonstrated by Simeonova et al. (31), who found that activation of Src was induced by arsenite in epithelial cells and that this process produced EGFR transactivation and stimulation of ERK. In that study, PP2 significantly inhibited arsenite-induced EGFR phosphorylation, consistent with both the present results reported and those of a previous study (7) demonstrating H2O2 transactivation of the EGFR in a Src-dependent manner. Finally, PTPase activity is subject to modulation by arsenite, and inhibition of PTPases has received considerable attention as a mechanism for signal transduction with ligand-induced growth factor activation (20, 25, 27, 33).

The link between arsenite and activation of the MAPK pathway has been studied in some detail. Arsenite has been

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Fig. 6. Differential involvement of JNK and p38 kinases in sodium arsenite-induced p21<sup>Cip1/Waf1</sup> and p53 expression. A: HUVECs were treated with 50 μM sodium arsenite for the indicated time. Cell lysates were subjected to immunoblotting with phospho-specific antibodies for p38, JNK, and ERK1/2. B and C: HUVECs were pretreated with increasing concentrations of SB-203580 and SP-600125, respectively, and p21<sup>Cip1/Waf1</sup> and p53 induction was determined as described in A. D: COS-7 cells were transfected with the dominant negative MAPK kinase MKK7 or the dominant negative MKK4 before exposure to 50 μM sodium arsenite for 14 h. A green fluorescent protein (GFP) vector transfection served as a control. All blots were reprobed for actin to ensure equal protein loading. DN, dominant negative. E: HUVECs were pretreated with inhibitors for EGFR (AG1478, 40 μM), ErbB2 (AG825, 40 μM), p38 (SB-203580, 20 μM), and JNK (SP-600125, 40 μM) for 30 min before sodium arsenite treatment (50 μM, 4 h). Immunoblot analysis was performed as described above. Data are representative of 3 independent experiments.
Fig. 7. Role of sodium arsenite-induced p21Cip1/Waf1 in apoptosis. HUVECs were incubated with 100 μM sodium arsenite (As) for 8 h in the presence or absence of AG1478 (40 μM), SB-203580 (20 μM), or SP-600125 (40 μM). After treatment, cell morphology was observed using phase-contrast microscopy (A) and cell viability was measured using MTT assay (B). Ctl, control. *P < 0.05 compared with other groups. C: cells were fixed and stained with 10 μg/ml propidium iodide (PI) to detect pyknotic nuclei (arrows) with the use of a fluorescent microscope. D and E: HUVECs were treated with 50 μM sodium arsenite for 16 h in the presence or absence of AG1478 (20 μM), SB-203580 (10 μM), or SP-600125 (20 μM), followed by flow cytometric analysis of apoptosis: R3, viable or undamaged cells (annexin V−, PI−); R2, cells undergoing early apoptosis (annexin V+, PI−); R4, necrotic or late apoptotic cells (annexin V+, PI+). F: HUVECs were transfected with EGFR siRNA or control siRNA. After 48 h, cells were treated with arsenite and stained with PI as in C.
shown to induce ERK, JNK, and p38 kinase components of the MAPK cascade in a number of human cell lines (24, 33, 35). For example, arsenite-treated human bronchial epithelial cells exhibit EGFR tyrosine phosphorylation, MEK1/2 activation, ERK1/2 phosphorylation, and enhanced transcriptional activity of Elk-1 (40). With regard to the current study, the p38 kinase and JNK components of the MAPK pathway have been linked to cell cycle regulation, particularly in response to external cellular stress (9, 18, 38). Treatment of NIH/3T3 cells with sodium arsenite induced growth inhibition through a mechanism that involved p38 kinase-mediated induction of p21cip1/waf1 (17). Our data are in general agreement with this report, given that we found both JNK and p38 kinase activation in response to arsenite and that inhibition of p38 kinase attenuated arsenite-induced p21cip1/waf1 induction (Fig. 6). However, our data add new information in that we found distinctions between p38 kinase and JNK activation. For example, activation of JNK required arsenite-induced ErbB receptor activation, whereas p38 MAPK did not. In addition, we found no role for p53 in the linear pathway of p21cip1/waf1 induction mediated by EGFR and JNK in response to arsenite. This latter finding is consistent with published reports that growth factors such as PDGF-α and basic fibroblast growth factor also have been shown to induce p38-independent transcription of p21cip1/waf1 (22, 44). With regard to PDGF-α, the mechanism of p21cip1/waf1 induction appears to involve JNK-1-responsive cis-acting regulatory elements residing in the p21 promoter (44). There is precedent for this mechanism, because c-Jun, a substrate of JNK1, mediates p53-independent p21cip1/waf1 promoter activation via physical interaction with the Sp1 protein (16). Thus our results are consistent with the model depicted in Fig. 8, which indicates that arsenite induces p21cip1/waf1 through a pathway that involves EGF-mediated JNK activation. Our data also are consistent with the pathways involving ErbB2 and p38 kinase-mediated p38 induction that contributes to the p21cip1/waf1 upregulation in response to arsenite. The precise nature of the coordination between these pathways is not yet known. Given the important role of both p53 and p21 in regulating cell growth and apoptosis, however, one might submit that such coordination would be expected.

In summary, the data presented here indicate that arsenite induces endothelial cell death via a mechanism that involves p21cip1/waf1 induction. These data are in keeping with a large body of literature indicating that arsenite produces abnormalities in endothelial function. In this study, we have linked the mechanism of arsenite-induced injury, in part, to ErbB receptor activation and coordinated activation of the MAPK cascade. The data presented here suggest that p21cip1/waf1 may represent an attractive target to ameliorate arsenite-induced endothelial cell dysfunction.

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