Arsenite Induces p53 Accumulation through an ATM-dependent Pathway in Human Fibroblasts

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ABSTRACT

Arsenic compounds are potent human carcinogens. Accumulated evidence has shown that arsenite-induced cytogenetic alterations are associated with the carcinogenicity of arsenic. Because p53 plays a guarding role in maintaining genome integrity and accuracy of chromosome segregation, the mechanistic effects of arsenite on p53 activation were analyzed. In the present study, arsenite-induced DNA strand breaks were confirmed by alkaline single-cell gel electrophoresis (comet assay) in human fibroblast (HFW) cells. Accompanying the appearance of DNA strand breaks was a significant accumulation of p53 in arsenite-treated HFW cells, as demonstrated by immunoblotting and immunofluorescence techniques. p53 downstream proteins, such as p21 and the human homologue of murine double minute-2, were also significantly induced by arsenite treatment. Cell cycle retardation and G2/M arrest were observed in 5-bromo-2'-deoxyuridine pulse-labeled HFW cells by flow cytometry. Wortmannin, an inhibitor of phosphatidylinositol 3-kinase, inhibited arsenite-induced p53 accumulation but did not alter UV irradiation- or N-acetyl-Leu-Leu-norleucinal-induced p53 accumulation. p53 phosphorylation on serine 15 was also confirmed by immunoblotting technique in arsenite- and X-ray-treated HFW cells but was not observed in UV- or N-acetyl-Leu-Leu-norleucinal-treated HFW cells. These results suggest the involvement of a phosphatidylinositol 3-kinase-related protein kinase in arsenite-induced p53 accumulation. For confirmation, we demonstrated that arsenite treatment, similar to X-ray irradiation, did not induce p53 accumulation in GM3395 fibroblasts derived from a patient with ataxia telangiectasia. In contrast, UV irradiation did cause p53 accumulation in these cells. Together, these findings infer that arsenite-induced DNA strand breaks may lead to p53 phosphorylation and accumulation through an ataxia telangiectasia mutated-dependent pathway in HFW cells.

INTRODUCTION

Arsenic compounds are ubiquitous. Arsenic is distributed naturally and is considered to be carcinogenic in humans (1). However, the molecular mechanisms of arsenic carcinogenicity remain elusive. Although arsenite by itself is thought to be inactive or typically too weak to induce gene point mutations (2, 3), increased frequencies of cytogenetic alterations, such as chromosome aberrations, sister chromatid exchanges, and micronucleus, have been found in peripheral lymphocytes of human populations with chronic exposure to arsenic through drinking water (4–6). In in vitro studies, sodium arsenite, a trivalent arsenic compound, induced similar cytogenetic alterations in a variety of cell systems (3, 7–9). At the same dose range, arsenite-induced cytogenetic alterations were closely associated with arsenite-induced morphological transformation in Syrian hamster embryo cells (10, 11). Our recent studies (12, 13) demonstrated that arsenite perturbs spindle dynamics and results in chromosome malsegregation during mitosis in cultured human cells. These investigations indicate that the genotoxicity and carcinogenicity of arsenite might be attributable to its activity on inducing cytogenetic alterations and/or genetic instability. Other mechanisms, such as modulation of DNA methylation (14) or DNA repair inhibition (15–18), are also reported to be involved in arsenic genotoxicity and carcinogenicity.

Numerous reports have shown that arsenite treatment induces DNA strand breaks and DNA-protein cross-links in a variety of cell lines (19–21). DNA strand breaks usually trigger the accumulation of p53 protein, a short half-life protein denoted as the guardian of the genome. Only a few reports have described the relationship between arsenite exposure and p53 activation and/or accumulation (22, 23). The p53 protein plays a pivotal role in maintaining genome integrity. This task is achieved through the induction of growth arrest for repair of DNA damage or apoptosis for eliminating cells with irreparable damage (24, 25). In general, p53 is involved in diverse cellular processes, including DNA repair, cell cycle checkpoints, apoptosis, cell differentiation, and a variety of stress responses induced by endogenous and exogenous sources (26, 27). In response to diverse stresses, p53 activation is tightly regulated through a complicated signaling network, including protein stability, subcellular localization, and interaction with other proteins (28). NH2-terminal phosphorylation of p53 is of importance to maintain p53 protein stability and its transactivation activities (29). Three members of the PI3-K3-related kinase family, DNA-dependent protein kinase (3), ATM, and ATR, are demonstrated to phosphorylate p53 at different sites in vitro, and two of them, ATM and ATR, possibly phosphorylate p53 on serine 15 in vivo (30–32). NH2-terminal phosphorylation of p53 hampers the association of p53 with MDM-2, the negative regulator of p53, and hence prevents p53 from proteosome-mediated degradation (29, 33–35). p53 is therefore accumulated and transactivates its downstream genes that are also involved in cellular stress responses.

Because p53 reacts to different stress-promoting conditions and may serve as the center of a signal network responsible for appropriate responses to various cellular stresses, understanding how arsenite enhances p53 accumulation could help unveil cellular responses to arsenite-induced injury. HFW cells manifest normal and stable karyotypes and become senescent after several passages (12). We previously used HFW cells to investigate the effects of arsenite on cell cycle progression and cytogenetic alterations (7, 12). Our results showed that arsenite not only causes chromosome aberrations and micronuclei but also induces spindle defects and perturbed mitosis in HFW cells. Therefore, HFW cells were again used to investigate p53-dependent cellular responses to arsenite insults. In this study, we found that arsenite induces DNA strand breaks and results in p53 phosphorylation and accumulation through an ATM-dependent pathway.

MATERIALS AND METHODS

Cell Culture. HFW cells derived from newborn foreskin were kindly provided by Dr. W. N. Wen (National Taiwan University). GM3395 and GM3398 cells were obtained from Coriell Cell Repositories (Camden, NJ).

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3 The abbreviations used are: PI3-K, phosphatidylinositol 3-kinase; PI, propidium iodide; ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia-related kinase; HFW, human fibroblast; MDM-2, human homologue of murine double minute-2; Bede, 5-bromo-2’deoxyuridine; ALLN, N-acetyl-Leu-Leu-norleucinal; IR, ionizing radiation.
Cells were routinely maintained in DMEM (Life Technologies, Inc., Grand Island, NY) supplemented with 10% fetal bovine serum (HyClone Laboratories, Logan, UT), 0.37% sodium bicarbonate, 100 unit/ml penicillin, and 100 μg/ml streptomycin. The cells were cultured at 37°C in an incubator with humidity-saturated air and 10% CO₂ (36).

DNA Strand Break Analysis. DNA strand breaks were measured by single-cell alkaline gel electrophoresis (comet assay) as described by Lynn et al. (20). Briefly, HFW cells after arsenite treatment were harvested, embedded in 1% agarose gel at a density of 1 × 10⁶/ml, and spread onto a fully frosted slide. The slides were immersed in ice-cold lysis buffer (10 mM Tris-HCl, 2.5 M NaCl, 100 mM Na₂EDTA, 1% sodium N-lauryl sarcosinate, 1% Triton X-100, and 10% DMSO; pH 10) for 1 h at 4°C. Cellular DNA was denatured in electrophoresis buffer (300 mM NaOH and 1 mM Na₂EDTA) for 20 min at room temperature and then electrophoresed for 20 min at 25 V and 300 mA. Afterward, the slides were washed in distilled water, renatured in 0.4 M Tris-HCl (pH 7.5), stained with Sybr green (Molecular Probe, Eugene, OR), and examined under a fluorescence microscope (BX60; Olympus, Tokyo, Japan). Comets were classified into four groups based on the length and fluorescence intensity of the comet tail: type I, no tail (intact nuclei; Fig. 1A, A–C); type II, comet with short tail (tail length less than the head diameter; Fig. 1D–F); type III, comet with tail length longer than the head diameter and with low fluorescence intensity (Fig. 1G, G and H); and type IV, comet with tail length longer than the head diameter and with high fluorescence intensity (Fig. 1H, I; Ref. 37). Five hundred cells were examined for each treatment.

Analysis of Phosphorylation of p53 on Serine 15. After drug treatment, HFW cells were immediately lysed in lysis buffer (50 mM Tris-HCl, 5 mM Na₂EDTA, 150 mM NaCl, 0.5% NP40, 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride, 0.5 mM sodium vanadate, and 1 mM sodium fluoride) for 20 min on ice. To immunoprecipitate cellular p53, an aliquot of 1 mg of cellular protein was incubated with 1 μg of anti-p53 monoclonal antibody (DO-1; Santa Cruz Biotechnology) at 4°C for 1 h. The unbound antibody was removed by extensively washing with PBST. The coverslips were further incubated with FITC-conjugated secondary antibody (Organon Teknika-Cappel) in the dark for 30 min. Chromosomes were counterstained with 0.1 μg/ml 4,6-diamidino-2-phenylindole. After thoroughly rinsing with PBST, the coverslips were mounted with a 90% glycerol solution containing 1 mg/ml phe-}

**Immunoblot Analysis of p53, p21, and MDM-2.** Immunoblotting was performed as described previously (12). In brief, after drug treatment HFW cells were scraped from culture dishes with the aid of a rubber policeman, lysed immediately in electrophoretic sample buffer, and heated at 95°C for 5 min (38). Protein concentrations were determined by Bradford analysis (39). An aliquot of 10 μg of cellular protein was loaded onto a 10% SDS-PAGE. After electrophoretic separation, polypeptides were transferred onto a nitrocellulose membrane by a semidry electrotransfer system (ATTO, Tokyo, Japan). After blocking in 5% skimmed milk in PBST (PBS containing 0.2% Tween 20) for 1 h, the membranes were incubated overnight at 4°C with primary antibodies that were appropriately diluted in blocking solution. p53, p21, and MDM-2 were reacted with anti-p53 monoclonal antibody (DO-1, sc-126; Santa Cruz Biotechnology, Santa Cruz, CA), anti-p21 monoclonal antibody (Transduction Laboratories, San Diego, CA), and anti-MDM-2 monoclonal antibody (Santa Cruz Biotechnology), respectively. The membranes were then extensively washed with PBST and incubated with secondary antibodies conjugated with horseradish peroxidase (Organon Teknika-Cappel, Turnhout, Belgium). Proteins were visualized using an enhanced chemiluminescence system according to the manufacturer’s instruction (Pierce, Rockford, IL). The levels of p53 were estimated using a densitometer (Densitometer 300S; Molecular Dynamics, Sunnyvale, CA).

**Immunofluorescence Staining of Cellular p53 and Cyclin B1.** To localize the p53 in cells, HFW cells were seeded onto glass coverslips. After treatment, cells on the coverslips were washed twice with PBS and then fixed in situ with methanol:acetone (1:1) at −20°C for 10 min. The coverslips were washed twice with PBS and incubated with anti-p53 monoclonal antibody (DO-1; Santa Cruz Biotechnology) at 4°C for 1 h. The unbound antibody was removed by extensively washing with PBST. The coverslips were further incubated with FITC-conjugated secondary antibody (Organon Teknika-Cappel) in the dark for 30 min. Chromosomes were counterstained with 0.1 μg/ml 4,6-diamidino-2-phenylindole. After thoroughly rinsing with PBST, the coverslips were mounted with a 90% glycerol solution containing 1 mg/ml phe-}

**Analysis of Cell Cycle Progression.** Logarithmically growing HFW cells were treated with 20 μM BrdUrd for 30 min. BrdUrd was then removed, and the cells were further incubated in medium with or without 5 μM arsenite. At various time points, cells were harvested for analysis of BrdUrd incorporation and DNA content. In brief, total cells were collected and fixed in cold 70% ethanol. After treatment with 2 N HCl for nicking DNA, 0.1 mM Na₂B₄O₇ was added to neutralize the reaction mixture. The incorporated BrdUrd was reacted with FITC-conjugated anti-BrdUrd antibodies (Boehringer Mannheim Biochemicals, Mannheim, Germany), and cellular DNA was stained with 4 μg/ml PI in PBS containing 1% Triton X-100 and 0.1 mg/ml RNase A. The fluorescent intensities of PI and FITC of individual cells were analyzed with a fluorescence-activated cell sorter (FACStar; Becton Dickinson Immunocytometry Systems) as described previously (36). The BrdUrd level in each cell cycle phase was determined using a computer program provided by the Becton Dickinson Immunocytometry System.
time dependently increased the frequencies of type II comets from 10% to ~60% and type III and IV comets from very low to 16 and 8%, respectively (Fig. 1b). Types I–IV comets were assigned numerical scores of 0–3, respectively (40); the average scores for each time point of 5 μM arsenite treatment were 0.11 ± 0.03, 0.28 ± 0.06, 0.54 ± 0.18, 0.77 ± 0.12, 0.86 ± 0.21, 0.97 ± 0.18, and 1.15 ± 0.10. This result indicated that 5 μM arsenite significantly induced DNA strand breaks in HFW cells (P < 0.001, according to ANOVA analysis). According to dye exclusion assay, HFW cells remained viable and maintained intact membrane integrity at the time the comet assay was performed, and no apoptosis was detectable under the experimental conditions. Therefore, consistent with a previous report (20), the arsenite-induced DNA strand breaks identified through comet assay were not attributable to apoptotic or dead cells.

**Elevation of p53 Levels by Arsenite.** Because p53 is a well-documented DNA damage marker (41), the effects of arsenite on p53 protein levels were examined using immunoblotting. Treatment of HFW cells with arsenite at a dose range from 1.25 to 10 μM for 24 h dramatically increased p53 levels (Fig. 2A). In a time-dependent experiment, p53 accumulation was observed at 1 h after arsenite treatment and increased to maximum accumulation at 4–6 h (Fig. 2B). Afterward, p53 levels declined to one-third of the maximum and remained at a constant level for 24 h (Fig. 2B). Using a densitometer, the intensities of p53 induced by 5 μM arsenite (0-, 1-, 2-, 4-, and 6-h time points in Fig. 2B) were measured and plotted against the average comet scores (the same time points in Fig. 1b). As shown in Fig. 2D, the levels of p53 accumulation were linearly correlated to comet scores (r² = 0.929). These results indicated that during the first several hours, the levels of arsenite-induced p53 accumulation were parallel with the incidence of DNA strand breaks. In addition to p53 accumulation, the protein levels of p53 target genes, such as p21 and MDM-2, were concomitantly increased by arsenite treatment (Fig. 2C).

Arsenite-induced p53 accumulation was further confirmed by immunofluorescence staining. Consistent with the results of immuno-
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Fig. 4. Differential effects of arsenite on p53 and cyclin B1 accumulation. HFW cells were treated with 5 μM arsenite for 24 h. Cellular p53 protein and cyclin B1 were visualized by immunostaining techniques as described in Fig. 3 and in “Materials and Methods.” A, staining with p53 DO-1 antibody and FITC-conjugated secondary antibody. B, staining with cyclin B1 antibody and rhodamine-conjugated secondary antibody. Arrowheads, cells with p53 nuclear accumulation; arrows, cells with cyclin B1 accumulation; stars, mitotic cells.

Fig. 5. A, effects of wortmannin on p53 accumulation in arsenite-treated HFW cells. HFW cells were treated with 0–20 μM wortmannin alone or in combination with 5 μM arsenite for 6 h. Cells were then harvested for immunoblot analysis of p53 and actin. The actin levels were used as the internal control. B, effects of wortmannin on p53 accumulation by arsenite, UV, ALLN, and X-ray. HFW cells were treated singly with 5 μM arsenite, 20 J/m² UV, 50 μM ALLN, or 5 Gy X-ray, respectively, or in combination with the individual agent and 10 μM wortmannin. After a 6-h treatment, the cellular levels of p53, MDM-2, and actin were examined by immunoblot analysis as described in “Materials and Methods.”

Fig. 6. p53 phosphorylation on serine 15 of HFW cells treated with arsenite, UV, ALLN, or X-ray. HFW were treated as indicated for 6 h. p53 was immunoprecipitated with a monoclonal p53 antibody (DO-1) as described in “Materials and Methods.” The immunocomplex was then analyzed by immunoblot with a polyclonal p53 antibody (FL-393; p53, upper panel) and with an antibody specific to the phosphorylated serine 15 of p53 (p53-S15, lower panel).

blotting (Fig. 2B), p53 levels were very low in untreated HFW cells (Fig. 3, A and B). However, highly abundant p53 was frequently present in nuclei of arsenite-treated HFW (Fig. 3, C and D). The frequency of arsenite-treated cells showing bright fluorescence gradually increased from 5 to 30% during the 24-h treatment period (Fig. 3E). For comparison, we examined the kinetics of p53 nuclear accumulation in nuclei of X-ray-irradiated HFW cells. After 1 Gy of X-ray irradiation, p53 accumulation in nuclei occurred immediately and transiently, i.e., 60% of nuclei showed bright fluorescence 1 h after irradiation and the frequency rapidly declined (Fig. 3E), indicating that the kinetics of p53 accumulation in nuclei in arsenite-treated HFW cells are quite different from those in X-ray-irradiated cells. In another staining study, double staining for p53/cyclin B1 showed that arsenite-treated cells with p53 nuclear accumulation (Fig. 4A, arrowheads) were completely separable from cyclin B1-positive cells (Fig. 4B, arrows). Cyclin B1 is a marker for the G2 phase cells; hence, this finding showed that cells with p53 nuclear accumulation were not at the G2 phase.

Signalings Involved in Arsenite-induced p53 Accumulation. Numerous reports have shown that p53 is phosphorylated by ATM kinase (30, 31), a member of the PI3-K-related protein kinase family, and hence is prevented from degradation and subsequently results in p53 accumulation (33, 42). To test whether ATM kinase is involved in the signaling of arsenite-induced p53 accumulation, HFW cells were cotreated with arsenite and wortmannin, an inhibitor of the PI3-K family (43). Wortmannin by itself did not affect the levels of p53 but effectively inhibited arsenite-induced p53 accumulation (Fig. 5A). For comparison, wortmannin also inhibited X-ray-induced p53 accumulation (Fig. 5B). However, wortmannin did not abrogate p53 accumulation in UV-irradiated or ALLN-treated cells (Fig. 5B). ALLN is a calpain/proteosome inhibitor that induces p53 stabilization by inhibiting its degradation instead of altering its phosphorylation level (33). Similarly, wortmannin reduced MDM-2 levels in arsenite- or X-ray-treated HFW cells but had no effect in UV-irradiated and ALLN-treated cells (Fig. 5B). Because ATM kinase phosphorylates p53 on serine 15 (30, 31), a p53-S15 antibody that specifically recognizes p53 with phosphorylated serine 15 was used to examine the phosphorylation status of arsenite-accumulated p53. As shown in Fig. 6, p53 that accumulated in arsenite-treated and X-ray-irradiated HFW cells strongly reacted to the p53-S15 antibody, whereas p53 in ALLN-treated and UV-irradiated HFW cells was much less reactive to the antibody. These results demonstrate that arsenite, similar to X-ray, induces phosphorylation of p53 on serine 15 but ALLN and UV do not. Accordingly, arsenite mimics X-ray in causing DNA strand breaks and activating ATM kinase, which phosphorylates p53 on serine 15 but follows different kinetics to produce these DNA strand breaks and p53 accumulation.

No Arsenite-induced p53 Accumulation in ATM−/− Fibroblasts. To further confirm the requirement of ATM for induction of p53 accumulation in arsenite-treated HFW cells, we examined the
effects of arsenite on p53 accumulation in ATM−/− cells. GM3395 cells are fibroblasts derived from an ataxia telangietasia patient in which both alleles of the ataxia telangietasia gene are mutated (atm−/−) and extremely sensitive to X-ray-irradiation (44). GM3395(atm−/−) cells, derived from the healthy brother of the same ataxia telangietasia patient, were included for comparison. The results shown in Fig. 7, A and B, demonstrate that neither arsenite nor X-ray irradiation were able to induce p53 accumulation in GM3395(atm−/−) cells, whereas the same treatment induced significant p53 accumulation in GM3398(atm+/+) cells. Because a different pathway is involved in UV irradiation-induced p53 accumulation (45), p53 accumulation was also examined in UV-irradiated-GM3395 cells. UV irradiation unexpectedly and significantly enhanced p53 accumulation in GM3395(atm−/−) cells (Fig. 7C).

Induction of Cell Death and Cell Cycle Arrest by Arsenite. Induction of p53 accumulation often leads to cell cycle arrest and/or apoptosis. It is very difficult to induce apoptosis in HFW cells. By colony-forming assay, treatment of HFW cells with 5 μM arsenite for 24 h resulted in a 25% survival rate (12); however, no apoptosis was observed immediately after treatment. Even 24 h after arsenite was withdrawn from the medium, the incidence of nucleus-fragmented cells, a manifestation of apoptosis, was 1.2 ± 0.3% (average ± SD from four independent experiments). These results indicated that arsenite-treated HFW cells did not die through apoptosis.

To examine the effect of arsenite on cell cycle progression, HFW cells were first pulse-labeled with 20 μM BrdUrd for 30 min and then chased for 0–24 h in the presence or absence of 5 μM arsenite. The levels of BrdUrd were flow cytomterically detected after immunostaining with FITC-conjugated anti-BrdUrd antibodies, and cell cycle stages G1, S, and G2-M were concurrently analyzed by staining the cells with PI. Because the cell population with or without FITC could be easily distinguished and gated by the analysis software of FACStar, we could chase the effects of arsenite on the cell cycle progression of two cell populations with (S-phase cells) or without (non-S-phase cells) BrdUrd labeling. In untreated cultures, we observed a rapid decline of BrdUrd-labeled S-phase cells (Fig. 8B), subsequently followed by an increase of BrdUrd-labeled G2-M cells (Fig. 8C) and G1 cells (Fig. 8A). A reappearance of BrdUrd-labeled S-phase cells occurred at 16 h after BrdUrd pulse labeling (Fig. 8B), indicating that untreated cells normally and actively underwent their cell cycle. In arsenite-treated culture, BrdUrd-labeled S-phase cells declined as did untreated cells but at a slower rate (delay for at least 7 h; Fig. 8B). However, the G2-M cells continued to accumulate during arsenite treatment, indicating a G2-M arrest. Furthermore, the appearance of BrdUrd-labeled G1 cells was first observed after a 16-h chase, at least 8 h later than in untreated cells (Fig. 8A). These results support that arsenite treatment results in S-phase retardation and G2-M arrest.

The kinetics of the increase in non-BrdUrd-labeled S phase in arsenite-treated HFW cells were similar to those of untreated cells (Fig. 8E), indicating that arsenite did not affect the progression of non-BrdUrd-labeled G1 cells to S-phase. However, the decline of non-BrdUrd-labeled G2-M phase cells was apparently slower in arsenite-treated cells than in untreated cells (Fig. 8F). These results indicate that arsenite treatment immediately affects the progression of G2-M cells. The delay of G2-M progression apparently results in a decrease in cell division (Fig. 8D).

DISCUSSION

In the present study, using comet assay, BrdUrd labeling, and flow cytometry, we found that arsenite induces DNA strand breaks and G2-M arrest. Concomitantly with DNA strand breaks, we observed p53 accumulation in HFW cells. Our results also showed that arsenite-and X-ray-induced p53 accumulation was inhibited by wortmannin, an inhibitor of the PI3-K family. In contrast, UV- or ALLN-induced p53 accumulation was insensitive to the inhibitory effects of wortmannin. We further confirmed that arsenite- and X-ray-induced p53 is phosphorylated at serine 15. Furthermore, both arsenite and X-ray irradiation, but not UV irradiation, failed to induce p53 accumulation in ATM−/− fibroblasts, GM3395. These results support that arsenite causes DNA strand breaks in HFW cells and mimics X-ray irradiation to induce p53 phosphorylation and accumulation through an ATM-dependent pathway.

Nevertheless, arsenite and X-ray irradiation obviously follow dif-
ferent kinetics in terms of DNA strand break induction and p53 accumulation. In general, IR immediately causes DNA strand breaks and hence induces early and transient accumulation of p53 to nuclei (46). Alternatively, arsenite is gradually taken up by cells. The induction of DNA strand breaks and p53 accumulation by arsenite apparently follows a time-dependent course. The slow kinetics of DNA strand breaks and p53 accumulation are also manifested by a slow transport of p53 to nuclei in arsenite-treated cultures. Although arsenite showed different kinetics of DNA strand break induction and p53 accumulation from those of X-ray irradiation, other evidence indicated that arsenite and X-ray treatment of HFW cells share a similar signaling pathway for p53 accumulation, i.e., an ATM-dependent pathway. Our present results showing that p53 nuclear-accumulated cells are distinguishable from cyclin B-positive cells were similar to a previous report (47) that irradiation induces p53 nuclear accumulation in human fibroblasts, predominantly during the G1 phase and at the beginning of the S-phase of the cell cycle. Whether these cells lost their proliferation activity is an interesting question that remains to be answered.

Phosphorylation of p53 is a pivotal modification that regulates p53 stability and transactivation activities (48). Numerous studies have demonstrated that phosphorylation at serine 15 is required for p53 protein stabilization in response to DNA damage (32, 33). Furthermore, recent reports have also shown that phosphorylation of serine 15 of p53 is possibly involved in enhancing the p53s transactivation activities (49). At least two distinct signal transduction pathways can lead to p53 activation. One of these pathways involves ATM, the product of the gene mutated in patients with the cancer-predisposing disorder ataxia telangiectasia (50). ATM is a member of the PI3-K-related protein kinase family because its COOH terminus shows high similarities to the catalytic domain of PI3-K (51). The ATM pathway is activated upon exposure to IR and chemical compounds that induce DNA strand breaks (52). The other pathway, the ATM-independent pathway, is activated upon exposure to UV irradiation and cisplatin, which produce bulky DNA lesions and induce DNA strand breaks during repair processes (28, 53). Our present results demonstrate that the ATM-dependent pathway is involved in arsenite-induced p53 accumulation, supporting that arsenite exposure is indeed a genotoxic stress.

In addition to p53, a variety of targets have been identified for ATM catalytic activity, such as c-Abl, 1xB, Chk2, and replication protein A (52), which are involved in ATM-mediated multiple responses to genotoxic stress including stress gene expression, DNA repair, and activation of cell cycle checkpoints (54, 55). ATM thus serves a surveillance role in maintaining genomic integrity. Elucidating the mode of transmission of ATM-mediated responses to arsenite-induced stress may help us better understand arsenite carcinogenesis and to develop chemopreventive strategies.

In the present study, arsenite treatment induced immediate G2-M arrest. In response to DNA damages, p53 transcriptionally activates p21, the inhibitor of cyclin-dependent kinases, and leads to G1 and G2 arrest (56). Disruption of either the p53 or p21 gene abrogates the G2 arrest induced by IR (57). Arsenite-induced G2-M arrest might be a consequence mediated by p53-dependent p21 induction. However, the involvement of p21 in G2-M arrest is controversial (58). A strong p53-dependent induction of the 14-3-3σ gene was observed in IR-induced G2 arrest cells (59). The mammalian cell cycle checkpoint protein kinase Chk-2 is phosphorylated and activated in response to DNA damages in an ATM-dependent manner (55). Activated Chk-2 phosphorylates Cdc25C on serine 216. This phosphorylation promotes the binding of 14-3-3σ to Cdc25C, thereby negatively regulating Cdc25C phosphatase activities and hence preventing the onset of mitosis, which results in G2 arrest (60, 61). Because both 14-3-3σ and Chk are downstream transducers of the ATM-dependent DNA damage checkpoint pathway, their involvement in arsenite-induced G2-M arrest requires further investigation.

It has been reported that fibroblasts with wild-type p53 fail to enter into mitosis when DNA synthesis is blocked (62). The delayed progression of S-phase HFW cells probably resulted from retarded DNA polymerization in the presence of arsenite-induced DNA damages. Numerous reports have shown that ATM is also involved in the activation of S-phase checkpoints, such as the down-regulation of cyclinA/Cdk2 activity (63) and interference with replication protein A (64), and consequently reduces the rate of DNA synthesis.

Arsenite induces G2-M arrest but does not trigger apoptosis in HFW cells. According to colony-forming assay, 75% of HFW cells lost their proliferation ability after treatment with 5 μM arsenite for 24 h (12), indicating that arsenite-induced injury eventually kills HFW cells. Nevertheless, we have demonstrated previously that arsenite at the same dose used in this study induces aneuploidy and other cytogenetic alterations in HFW colonies that survived (7, 12). Because the roles of p53 in triggering apoptosis are complex and not fully understood, the reason for the lack of apoptosis in arsenite-treated HFW cells is unclear. Arsenite is known to exert at least some of its toxic effects through interaction with sulfhydryl groups, and the nonprotein sulfhydryl glutathione appears to play an important role in the detoxification of arsenite (65). Disturbances in intracellular calcium homeostasis is also involved in arsenite-induced cytotoxicity (66). A cathepsin-like protease activity may also be involved in the killing effects of arsenite (67). These studies and speculation suggest that arsenite may induce other cellular injuries besides DNA damage to cause cell death.

Numerous studies have shown that p53 activation is signaled via distinct transduction pathways in response to different stresses and results in various cellular responses (28). Arsenite has been reported to induce DNA damage (19–21), oxidative stress (68, 69), oncogene expression (70–72), and gene amplification (73). In the present study, using a highly sensitive comet assay, we confirmed that arsenite treatment results in DNA strand breaks in HFW cells. At the same dose used in this study, we demonstrated previously that arsenite not only induces chromosome aberrations and micronuclei but also induces aneuploidy in HFW cells that survived (7, 12). DNA strand breaks are potential lesions that can be converted into such cytogenetic alterations. Although arsenite-induced oxidative stress is thought to play a crucial role in the induction of DNA strand breaks (20, 74), the exact mechanism of arsenite-induced DNA strand breaks and the roles of ATM pathway activation warrant our further concern.

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