Cyclin D1 Induction through IκB Kinase β/Nuclear Factor-κB Pathway Is Responsible for Arsenite-Induced Increased Cell Cycle G1-S Phase Transition in Human Keratinocytes

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Abstract

Environmental and occupational exposure to arsenite is associated with an increased risk of human cancers, including skin, urinary bladder, and respiratory tract cancers. Although much evidence suggests that alterations in cell cycle machinery are implicated in the carcinogenic effect of arsenite, the molecular mechanisms underlying the cell cycle alterations are largely unknown. In the present study, we observed that exposure of human keratinocyte HaCat cells to arsenite resulted in the promotion of cell cycle progression, especially G1-S transition. Further studies found that arsenite exposure was able to induce cyclin D1 expression. The induction of cyclin D1 by arsenite required nuclear factor-κB (NF-κB) activation, because the inhibition of IκB phosphorylation by overexpression of the dominant-negative mutant, IκKβ-KM, impaired arsenite-induced cyclin D1 expression and G1-S transition. The requirement of IκB kinase β (IKKβ) for cyclin D1 induction was further confirmed by the findings that arsenite-induced cyclin D1 expression was totally blocked in IκKβ-KM knockout (IKKβ−/−) mouse embryo fibroblasts. In addition, knockdown of cyclin D1 expression using cyclin D1–specific small interference RNA significantly blocked arsenite-induced cell cycle progression in HaCat cells. Taken together, our results show that arsenite-induced cell cycle from G1 to S phase transition is through IκKβ/NF-κB/cyclin D1–dependent pathway. (Cancer Res 2005; 65(20): 9287-93)

Introduction

Arsenite (trivalent arsenic, As3+) is introduced into the environment during energy production based on coal, oil shale, and geothermal sources. Available epidemiologic data have shown that exposure to arsenite is associated with increased risks of human cancer of the skin, respiratory tract, hematopoietic system, and urinary bladder (1-4). Many cases of skin cancer have been observed early in carcinogenesis (21-23), and overexpression of cyclin D1 was reported in several human cancers, including uterine cervix (24), ovary (25), breast (26), urinary bladder (27), endometrium (28), and skin (29) cancers. Moreover, antisense to cyclin D1 was reported to inhibit the growth and tumorigenicity of human colon cancer cells and induce apoptosis in human squamous carcinomas (30, 31). Bossman et al. reported that exposure of human fibroblasts to arsenite results in the induction of cyclin D1 expression (32). Nonetheless, the effect of arsenite on cell cycle progression remains obscure. Because human skin is a major target of arsenite, it would be interesting to know whether cyclin D1 is induced by arsenite in human keratinocytes. If it is, what are the signaling pathways responsible for this induction and what are their roles in alternations of cell cycles caused by arsenite exposure? Consequently, we here addressed these questions in human keratinocyte HaCat cells.

Materials and Methods

Cell culture and reagents. Human keratinocytic HaCat cells and their stable transfectants, wild-type (WT) and IκB kinase β (IKKβ)–deficient (IKKβ−/−) mouse embryo fibroblasts were cultured in monolayers at 37°C, 5% CO2 using DMEM containing 10% fetal bovine serum (FBS), 2 mmol/L L-glutamine, and 25 μg of gentamicin/mL. The cultures were detached with trypsin and transferred to new 75-cm² culture flasks (Fisher, Pittsburgh, PA) from one to three times per week. FBS was purchased from Life Technologies, Inc. (Gaithersburg, MD); DMEM was from Calbiochem (San Diego, CA); luciferase assay substrate was from Promega (Madison, WI). As3+ was purchased from Aldrich (Milwaukee, WI). The cyclin D1 promoter-driven luciferase reporter (cyclin D1 Luc) was from Dr. Anil Rustgi (Gastroenterology Division, University of Pennsylvania, Philadelphia, PA).

Several hypotheses have been proposed to describe the mechanisms of arsenite-induced carcinogenesis in the previous studies (6-12). It has been suggested that arsenic induces chromosome aberration and sister chromatid exchange may be involved in arsenite-induced carcinogenesis (9, 10). Zhao et al. (11) reported that arsenic may act as a carcinogen by inducing DNA hypomethylation, which in turn facilitates aberrant gene expression. However, more and more evidence indicates that the effect of arsenite on modulating the signaling pathways and genes expression responsible for cell cycle machinery may play a more important role in the carcinogenesis than classic genotoxic and mutagenic mechanisms, which are believed to be associated with the carcinogenesis of other metals such as cadmium or chromium (13, 14).

It is widely accepted that alterations of gene expression that drive uncontrolled cell cycle progression are requisite events during tumorigenesis (15-17). Cyclin D1, acting as a sensor in response to extracellular changes, can be induced by growth factors and stress (18-20). Aberrant cyclin D1 expression has been observed early in carcinogenesis (21-23), and overexpression of cyclin D1 was reported in several human cancers, including uterine cervix (24), ovary (25), breast (26), urinary bladder (27), endometrium (28), and skin (29) cancers. Moreover, antisense to cyclin D1 was reported to inhibit the growth and tumorigenicity of human colon cancer cells and induce apoptosis in human squamous carcinomas (30, 31). Bossman et al. reported that exposure of human fibroblasts to arsenite results in the induction of cyclin D1 expression (32). Nonetheless, the effect of arsenite on cell cycle progression remains obscure. Because human skin is a major target of arsenite, it would be interesting to know whether cyclin D1 is induced by arsenite in human keratinocytes. If it is, what are the signaling pathways responsible for this induction and what are their roles in alternations of cell cycles caused by arsenite exposure? Consequently, we here addressed these questions in human keratinocyte HaCat cells.
Cyclin D1 promoter-driven luciferase reporter was constructed by inserting a 1.23-kb EcoRI-ProII fragment of the cyclin D1 gene promoter, which contains the cyclin D1 promoter sequence from -1,095 to 135 relative to the translation initiation site, into the pA3LUC vector as described previously (33). The dominant-negative mutant of IKK\(\beta\) (IKK\(\beta\)-KM) was a gift from Dr. Hiroyasu Nakano (Juntendo University, Japan), as described in previous reports (34–36).

**Cell cycle analysis.** HaCat cells and their stable transfectants (2 \(\times\) 10\(^4\)) were cultured in each well of six-well plates to 70% to 80% confluence with normal culture medium. The cell culture medium was replaced with 0.1% FBS DMEM with 2 mmol/L l-glutamine and 25 \(\mu\)g/\(\mu\)L gentamicin and cultured for 24 hours. The cells were harvested and fixed with 3 mL of ice-cold 80% ethanol overnight. The fixed cells were then centrifuged (3,000 rpm, 3 minutes), suspended in lysis buffer (100 mmol/L sodium citrate and 0.1% Triton X-100), and incubated for 15 minutes at room temperature. Then the cells were incubated with RNase A (10 mg/mL; Sigma Chemical, St. Louis, MO) for 10 minutes at room temperature; DNA was stained with propidium iodide (50 \(\mu\)g/mL) for at least 1 hour at 4°C. The DNA content was determined by flow cytometry (Beckman Coulter, San Diego, CA) and EXPO 32 software.

**Cyclin D1 small interference RNA construction.** The specific small interference RNA (siRNA)–targeted human cyclin D1 was designed with siRNA converter on the web site of Ambion, Inc. (Austin, TX) according to the gene sequence in Genbank and guideline of siRNA and synthesized by Invitrogen, Inc. (San Diego, CA). The target sequence was 5'-GGTCAATCCGCCC-3' (bases 791-809 of NM053056.1). The siRNA sequence was confirmed by BLAST search and did not show any homology to other known human genes. The siRNA was inserted into pSuppressor Vector and verified by DNA sequencing.

**Stable transfection.** HaCat cells were transfected with cyclin D1 Luc, nuclear factor-\(\kappa\)B (NF-\(\kappa\)B)-luciferase reporter, KK\(\kappa\)B-KM, siCyclin D1, or vector control plasmids according to the manual of LipofectAMINE 2000 reagent. Briefly, HaCat cells were cultured in a six-well plate to 85% to 90% confluence. Five micrograms of plasmid DNA alone or in combination with CMV-neo vector for cotransfection were mixed with 10 \(\mu\)L of LipofectAMINE 2000 reagent and used to transfect each well in serum-free DMEM for 3 hours at 37°C. After 2 days, the medium was replaced with 10% FBS DMEM. Approximately 30 to 36 hours after the beginning of the transfection, the cells were trypsinized with 0.033% trypsin, and cell suspensions were plated onto 75-cm\(^2\) culture flasks and cultured for 24 to 28 days with G418 selection (800 \(\mu\)g/mL). Stable transfectant was established and cultured in G418-free DMEM for at least two passages before each experiment.

**Gene reporter assay.** Confluent monolayers of HaCat cyclin D1 Luc mass1 and HaCat NF-\(\kappa\)B Luc mass1 cells were trypsinized, and 8 \(\times\) 10\(^4\) viable cells suspended in 100 \(\mu\)L DMEM supplemented with 10% FBS were added to each well of 96-well plates. The plates were incubated at 37°C in a humidified atmosphere of 5% CO\(_2\). After the cell density reached 80% to 90%, the cell culture medium was replaced with an equal volume of DMEM supplemented with 0.1% FBS and 2 mmol/L l-glutamine. Twelve hours later, cells were exposed to arsenic for cyclin D1 or NF-\(\kappa\)B induction. After 12-hour culture, the cells were lysed with 50 \(\mu\)L of lysis buffer, and the luciferase activity was measured using Promega Luciferase assay reagent with a luminometer (Wallac 1420 Victor2 multiplicable counter system). The results are expressed as cyclin D1 induction (relative cyclin D1 induction) or NF-\(\kappa\)B activity (relative NF-\(\kappa\)B activity) relative to medium control (37).

**Phosphorylation assay for I\(\kappa\)B kinase \(\beta\) and I\(\kappa\)B\(\alpha\).** HaCat cells and HaCat-IKK\(\beta\)-KM cells (2 \(\times\) 10\(^4\)) were cultured in each well of six-well plates to 70% to 80% confluence with normal culture medium. The cell culture medium was replaced with 0.1% FBS DMEM with 2 mmol/L l-glutamine and 25 \(\mu\)g of gentamicin and incubated for 45 hours. Cells were incubated in serum-free DMEM for 3 hours at 37°C and then exposed to arsenite for the time as indicated in the figure legends. After exposure to arsenite, the cells were washed once with ice-cold PBS and then extracted with SDS sample buffer. The cell extracts were quantified with a detergent-compatible (DC) protein assay kit (Bio-Rad, Hercules, CA), separated on polyacrylamide-SDS gels, transferred, and probed with one of the antibodies, including rabbit p-IKK\(\alpha\)/\(\kappa\), IKK\(\alpha\), IKK\(\beta\), I\(\kappa\)B\(\alpha\), and p-I\(\kappa\)B\(\alpha\). The IKK\(\alpha\)/\(\kappa\)B and I\(\kappa\)B protein bands specifically bound to primary antibodies were detected using an anti-rabbit IgG alkaline phosphatase–linked secondary antibody and an ECF Western blotting system (Amersham, Piscataway, NJ; ref. 38).

**Cyclin D1 expression assay.** HaCat cells and their transfectants, WT and IKK\(\beta\)-/– mouse embryo fibroblasts (2 \(\times\) 10\(^5\)), were cultured in each well of six-well plates to 80% confluence. After exposure to arsenite for different times as indicated in the figure legends, the cells were washed once with ice-cold PBS and then extracted with SDS sample buffer. The cell extracts were quantified with a DC protein assay kit (Bio-Rad), separated on polyacrylamide-SDS gels, transferred, and probed with rabbit-specific antibody against cyclin D1. The protein band specifically bound to the primary antibody was detected as mentioned above.

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**Table 1. Effect of arsenite on cell cycle progression**

<table>
<thead>
<tr>
<th>Arsenite ((\mu)mol/L)</th>
<th>Phase (time)</th>
<th>Glycosylation of cell surface protein</th>
<th>Glycosylation of cell surface protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G0-G1 (24 h)</td>
<td>S (24 h)</td>
<td>G2-M (24 h)</td>
</tr>
<tr>
<td>0</td>
<td>13.6 ± 1.0</td>
<td>56.3 ± 1.5</td>
<td>24.9 ± 1.2</td>
</tr>
<tr>
<td>1.25</td>
<td>14.4 ± 1.1</td>
<td>47.7 ± 1.2</td>
<td>29.8 ± 1.4*</td>
</tr>
<tr>
<td>2.5</td>
<td>16.9 ± 0.4</td>
<td>39.6 ± 2.6</td>
<td>33.8 ± 1.6†</td>
</tr>
<tr>
<td>5</td>
<td>22.0 ± 0.4</td>
<td>31.6 ± 1.4</td>
<td>38.7 ± 1.4†</td>
</tr>
</tbody>
</table>

NOTE: Data are means ± SE (\(n = 3\)). HaCat cells (2 \(\times\) 10\(^4\)) were seeded into each well of a six-well plate and cultured with DMEM containing 10% FBS at 37°C overnight. After being cultured in DMEM containing 0.1% FBS for 24 h, cells were treated with various concentrations of arsenite, as indicated, for 24 and 48 h and were fixed with 3 mL of ice-cold ethanol 80% overnight. The fixed cells were suspended in lysis buffer (100 mmol/L sodium citrate and 0.1% Triton X-100) and incubated for 15 min at room temperature. The cells were incubated with RNase A (10 mg/mL; Sigma Chemical) for 10 min at room temperature and DNA was stained with propidium iodide (50 \(\mu\)g/mL) for at least 1 h at 4°C. The cell cycle distribution was determined by flow cytometry using the Epics XL FACS (Beckman Coulter) and EXPO 32 software. The experiment was done at least thrice with triple wells for each assay. Data were expressed as mean ± SE of one representative experiment with triple wells for each assay.

\(P < 0.05\), significantly different from medium control.

\(P < 0.005\), significantly different from medium control.
Statistical analysis. The significance of the difference between the treated and untreated groups was determined with the Student’s t test. The results are expressed as mean ± SD.

Results

Cell cycle progression induced by arsenite in HaCat cells. To analyze the effects of arsenite on cell cycle, we exposed sub-confluent HaCat cells to 0, 1.25, 2.5, and 5 μmol/L of arsenite for 24 and 48 hours. As shown in Table 1 and Fig. 1, exposure of cells to arsenite resulted in dose- and time-dependent increases in the proportion of cells in S phase and G2-M phase. The highest proportion of cells in the S and G2-M phases was 40.11% and 28.49%, respectively, which occurred after 48 hours of 5 μmol/L arsenite treatment. This increase in the S phase may be due to the promotion of G1-S phase transition rather than the blockage of S to G2-M phase transition, because the proportion of cells in G2-M phase also significantly increased after arsenite treatment. This data show that arsenite exposure is able to promote the cell cycle transition from G1 to S phase, and in turn, may lead to G2-M increase.

Cyclin D1 induction by arsenite in HaCat cells. It has been reported that arsenite exposure was able to induce cyclin D1 expression in human fibroblasts (32). The above results showed that arsenite treatment led to an increase in G1-S phase transition. To elucidate the molecular mechanisms underlying the effect of arsenite on cell cycle progression, we first focused on the alteration in cyclin D1 expression, based on the knowledge that cyclin D1 plays a crucial role in controlling cell cycle progression from G0-G1 to S phase and acts as a sensor for cell cycle machinery in response to stress, cytokine, or growth factor signaling (18–20). The results showed that exposure of HaCat cells to arsenite resulted in a marked increase in cyclin D1, at both transcription and protein levels (Fig. 2).

Requirement of IκB kinase β/nuclear factor-κB pathway for cyclin D1 induction and G1-S phase transition by arsenite. The level of cyclin D1 protein is largely controlled by the rate of cyclin D1 gene transcription, which is regulated by multiple transcription factors (39–43). The cyclin D1 promoter region includes multiple binding sites for NF-κB (39, 40), E2F/DP (41), sp-1/sp-3 (42), activator protein (AP-1; ref. 43), signal transducers and activators of transcription (44), and cyclic AMP–responsive element binding protein (45). It has been reported that different transcription factors play the key roles in the regulation of cyclin D1 expression.

Figure 1. Promotion of cell cycle progression induced by arsenite in human keratinocyte HaCat cells. HaCat cells (2 × 10^4) were seeded into each well of a six-well plate and cultured with DMEM containing 10% FBS at 37°C overnight. After being cultured in DMEM containing 0.1% FBS for 24 hours, the cells were treated with various concentrations of arsenite, as indicated, for 24 and 48 hours, and then were fixed and stained with propidium iodide as described in Materials and Methods. Cell cycle distribution was determined by flow cytometry. The experiment was done at least thrice with triple wells for each assay. The results were from one representative data from three independent experiments.

Figure 2. Induction of cyclin D1 by arsenite. A, HaCat cyclin D1 luc mass1 cells (8 × 10^3) were seeded into each well of a 96-well plate. After being cultured at 37°C overnight, the cells were exposed to various concentrations of arsenite as indicated for 12 hours. The luciferase activity was then measured, and the results are presented as cyclin D1 induction relative to control. Columns, means of triplicate assay wells; bars, SD. *, P < 0.05, significant increase from medium control. B, HaCat cells (2 × 10^5) were seeded into each well of a six-well plate and cultured with DMEM containing 10% FBS at 37°C overnight. After being cultured in DMEM containing 0.1% FBS for 24 hours, the cells were exposed to various concentrations of arsenite, as indicated, for 12 and 24 hours. The cells were then washed once with ice-cold PBS and extracted with SDS sample buffer. The cell extracts were separated on polyacrylamide-SDS gels, transferred, and probed with specific antibody as indicated. The protein band specifically bound with primary antibodies was detected by using anti-rabbit IgG–AP-linked secondary antibody and an ECF Western blotting system (32). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a control for protein loading.
D1 expression in a cell type- and stimulus-dependent manner (39–45). Our previous studies have shown that arsenite exposure is able to activate NF-κB and AP-1 transcription in mouse epidermal CH1 cells (46). To elucidate the signaling pathways leading to cyclin D1 induction by arsenite in human HaCat cells, we investigated the potential role of NF-κB in the cyclin D1 induction by arsenite, because it is believed that NF-κB transactivation is associated with arsenite-induced tumor promotion (47). NF-κB is bound to an inhibitory protein, IκBα in an inactive form in most cells, and composed of NF-κB p50 and Rel A p65 subunits (48). In response to stress, cytokine, or growth factor, IκBα is phosphorylated by IKKα/β and then ubiquitinated and degraded by the proteasome, enabling nuclear translocation and binding of p50/p65 to the promoter region of target genes (49). Thus, we measured the phosphorylation of IKKα/β and NFκB-dependent transactivation activity after exposure of HaCat cells to arsenite. The data indicated that arsenite treatment resulted in obvious increases in IKKα/β phosphorylation and NFκB-dependent transactivation (Fig. 3A and B). To further unravel the potential role of IKK/NFκB pathway in cyclin D1 induction by arsenite, we established stable pCR-FLAG-IKKβ-KM-transfected HaCat cells and verified the expression of IKKβ-KM by Western blot with specific antibody against the FLAG (M2, Sigma Chemical) as previously described (refs. 34–36; Fig. 3C). We found that overexpression of IKKβ-KM was able to inhibit IκBα degradation (Fig. 3D). Moreover, overexpression of IKKβ-KM dramatically reduced cyclin D1 expression induced by arsenite (Fig. 3E). Furthermore, impairment of IKKα/β-IκBα-b-cyclin D1 pathway subsequently resulted in inhibition of arsenite-induced alternations of cell cycle G1-S phase transition (Fig. 4A and B). Strong direct evidence for involvement of IKKβ/NFκB in arsenite-induced cyclin D1 expression was further provided by the studies using Iκκβ-deficient cells. As Fig. 3F

Figure 3. Requirement of IKKβ/NFκB activation by arsenite for cyclin D1 induction in HaCat cells. A, HaCat NFκB-Luc mass1 cells (8 x 10⁶) were seeded into each well of a 96-well plate. After being cultured at 37°C overnight, the cells were exposed to various concentrations of arsenite as indicated for 12 hours. The luciferase activity was then measured, and the results are presented as NFκB luciferase activity relative to control. Columns, means of triplicate assay wells; bars, SD. *, $P < 0.05$, significant increase from medium control. B, HaCat cells (2 x 10⁵) were seeded into each well six-well plate and cultured in 10% FBS DMEM at 37°C. When the cell density reached 70% to 80%, the culture medium was replaced with 0.1% FBS DMEM. After being cultured for 48 hours, the cells were exposed to various concentrations of arsenite for the time points as indicated. The cells were then washed once with ice-cold PBS and extracted with SDS sample buffer. The cell extracts were quantified with DC protein assay kit (Bio-Rad), separated on polyacrylamide-SDS gels, transferred, and probed with specific antibody against cyclin D1 and the ECF Western blotting system. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a protein loading control.
These data showed that cyclin D1 is at least a major player in cell cycle G1-S phase transition.

Essential role of cyclin D1 in arsenite-induced cell cycle progression in HaCat cells. To directly assess the role of cyclin D1 induction in arsenite-induced increase in G1-S transition, we established stable cyclin D1 siRNA-transfected HaCat cells and verified the silencing effect of the siRNA. As shown in Fig. 5A, cyclin D1 siRNA was able to specifically knockdown not only the basal level of cyclin D1 but also the arsenite-induced cyclin D1 expression (Fig. 5A). The specificity for cyclin D1 siRNA on cyclin D1 expression was verified by the data that the siRNA did not show any inhibitory effect on cyclin D2 expression (Fig. 5A). More importantly, arsenite-induced increase in the proportion of cells in the S phase was dramatically inhibited in the stable cyclin D1 siRNA-transfected HaCat cells compared with that in vector control transfectant (Fig. 5B and C).

This data showed that cyclin D1 is at least a major player in arsenite-induced increase in G1-S phase transition.

Discussion

Arsenite is a well-documented human skin carcinogen (3, 4). However, the molecular mechanisms underlying arsenite-induced carcinogenesis are still largely unknown. Growing evidence indicates that cell cycle is regulated by oncogenes and tumor suppressor genes, and cell cycle alterations occurred in the response of cells to various carcinogens (50, 51), suggesting that cell cycle regulation is implicated in carcinogenesis (52). Normal eukaryotic cells progress through a well-defined cell cycle consisting of four distinct stages: G1, S, G2, and M. G1 and G2 phases are gaps installed into M-S phase and S-M transitions, respectively. These gaps allow for the repair of DNA damage and replication errors. Moreover, G1 is a period when many signals intervene to influence cell fate (15). Extracellular changes, such as diverse metabolic, hypoxia, and stress, are integrated and interpreted during this period. Faulty G1 control, caused by activation of many oncogenes or inactivation of tumor suppressor genes, plays a critical role in tumorigenesis (15). Cyclin D1 is one of the key regulators of G1-S transition (28). Inducible cyclin D1 forms a complex with CDK4/6, which phosphorylates the retinoblastoma tumor suppressor protein, helping to sequester its growth-inhibitory effects and enabling E2F transcription factors to activate genes required for entry into the DNA synthetic phase (S) of the cell division cycle. Among the E2F-responsive genes are cyclin E and cyclin A, which combine with and activate CDK2 to facilitate S phase entry and progression. In the present study, we showed that exposure of human keratinocyte HaCat cells to arsenite resulted in the increase in the proportion of cells in S phase, which was at least partially associated with cyclin D1 induction. In addition, the increase in proportion of cells in G2-M phase was also observed after arsenite exposure, which may be a mere consequence of the increase in the S phase. However, we cannot exclude the possibility that the increase in G2-M phase was directly caused by arsenite exposure. Actually, it has been reported that arsenite is able to arrest cell cycle at G2-M phase by down-regulation of Cdc25C activity in the G2-M phase of the cell cycle through inducing Cdc25C degradation via ubiquitin-proteasome pathways (53).

Cyclin D1, acting as a sensor in response to extracellular changes, can be induced by growth factors and stress through activation of various signaling pathways including ras/mitogen-activated protein kinases (MAPK; refs. 54–56), phosphatidylinositol 3-kinase (PI-3K)/Akt (57, 58), IKK/NF-κB (39, 40, 57), and GSK3β/β-catenin (45, 59, 60). Here, we showed that activation of IKKβ/NF-κB contributed to arsenite-induced cyclin D1 expression. IKKβ-KM almost totally blocked cyclin D1 induction after exposure of HaCat cells to arsenite for 12 hours. The contribution of IKKβ-KM/NF-κB to arsenite-induced cyclin D1 expression was further confirmed by the findings that cyclin D1 induction by arsenite was impaired in IKKβ−/− mouse embryonic fibroblasts. However, arsenite-induced cyclin D1 induction in IKKβ-KM-transfected HaCat cells was partially recovered when the cells were exposed to arsenite for 48 hours, although the basal level of cyclin D1 was still obviously lower than that in the control cells. Consistent with this result, IKKβ-KM was able to impair G1-S transition when IKKβ-KM-transfected HaCat cells were exposed to arsenite for 24 hours but only showing a slight effect at 48 hours after arsenite exposure (data not shown). These results suggest that the effect of dominant-negative IKKβ-KM may be overcome after long-term exposure of cells to arsenite, or there may be some other pathways involved in the late phase of cyclin D1 induction by arsenite. Recently, we showed that that arsenite-induced cyclin D1 expression requires activation of PI-3K/Akt, because inhibition of PI-3K dramatically impairs cyclin D1 induction by arsenite in mouse epidermal CH1 cells.1

1 W. Ouyang et al, unpublished data.
shown that the downstream of PI-3K/Akt cascade includes IKK/ NF-κB, MAPK kinase/extracellular signal-regulated kinases/ERK, and GSK3β/β-catenin pathways (61). GSK3β has been reported to play a very important role in the negative regulation of cyclin D1 level at both transcription and protein levels (53–55). It has been shown that GSK3β can phosphorylate cyclin D1 and thereby facilitate its degradation (60, 62). In addition, GSK3β is able to phosphorylate β-catenin and retain it in the cytoplasm in conjunction with adenosomatous polyposis coli. Phosphorylation of GSK3β by Akt leads to its inactivation, and in turn, dephosphorylation of β-catenin, which enables its translocation into the nucleus and activation of its target gene expression including cyclin D1 (60).

Actually, we have observed that β-catenin can be activated after arsenite exposure. Its role and the crosstalks among those different signaling pathways in cyclin D1 induction by arsenite are currently being investigated in our laboratory.

In addition to the induction of cyclin D1/pRb, p21WAF1-cyclin E and ARF-MDM2-p53 could also be involved in controlling G1-S transition (16, 28). p21WAF1 is a cyclin kinase inhibitor that induces growth arrest by preventing phosphorylation of pRb in the G1-S transition, being able to silence cyclin-dependent kinases essential for S-phase entry including cyclin E thus inhibiting cell cycle progression. Previous studies showed that exposure to arsenite was able to induce p21WAF1 expression, which was believed to be implicated in growth arrest caused by a high dose of arsenite (30, 63). Nonetheless, the contribution of p21WAF1 to the effect of arsenite on cell cycle progression remains to be explored. The final output may depend on the balance between cyclin D1 and p21WAF1.

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References


