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FAST TRACKS

PI-3K/Akt Signal Pathway Plays a Crucial Role in Arsenite-Induced Cell Proliferation of Human Keratinocytes Through Induction of Cyclin D1

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Abstract Exposure of arsenite can induce hyperproliferation of skin cells, which is believed to play important roles in arsenite-induced carcinogenesis by affecting both promotion and progression stages. However, the signal pathways and target genes activated by arsenite exposure responsible for the proliferation remain to be defined. In the present study, we found that: (1) exposure of human keratinocytic HaCat cells to arsenite caused an increase in cell proliferation, which was significantly inhibited by pretreatment of wortmannin, a specific chemical inhibitor of PI-3K/Akt signal pathway; (2) arsenite exposure was also able to activate PI-3K/Akt signal pathway, which thereby induced the elevation of cyclin D1 expression level in both HaCat cells and human primary keratinocytes based on that inhibition of PI-3K/Akt pathway by either pretreatment of wortmannin or the transfection of their dominant mutants, significantly inhibited cyclin D1 expression upon arsenite exposure; (3) PI-3K/Akt pathway is implicated in arsenite-induced proliferation of HaCat cells through the induction of cyclin D1 because either knockdown of cyclin D1 by its siRNA or inhibition of PI-3K/Akt signal pathway by their dominant mutants markedly impaired the proliferation of HaCat cells induced by arsenite exposure. Taken together, we provide the direct evidence that PI-3K/Akt pathway plays a role in the regulation of cell proliferation through the induction of cyclin D1 in human keratinocytes upon arsenite treatment. Given the importance of aberrant cell proliferation in cell transformation, we propose that the activation of PI-3K/Akt pathway and cyclin D1 induction may be the important mediators of human skin carcinogenic effect of arsenite. J. Cell. Biochem. 101: 969–978, 2007.
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Key words: PI-3K; AKT; cyclin D1; proliferation; arsenite

Arsenite is a well-documented carcinogen. Long-term exposure to inorganic arsenic from drinking water has been reported to induce various cancers, including skin cancer [Tseng et al., 1968; Centeno et al., 2002; Yu et al., 2006]. Arsenite tends to accumulate in the skin, which in turn causes skin hyperpigmentation and hyperkeratosis, the hallmark signs of chronic arsenite exposure [Centeno et al., 2002; Yu et al., 2006]. These skin lesions can further develop into skin cancers, including Bowen's disease (carcinoma in situ), basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) [Tseng et al., 1968; Yu et al., 2006].

The mouse multistage skin carcinogenic model has demonstrated that cancer development results from a synergism between genotoxic and nongenotoxic factors [Hecker, 1987; Zoumpourlis et al., 2003; Shi et al., 2004; Wei et al., 2005]. The former induces irreversible genetic alterations (tumor initiation), whereas the latter promotes tumor development by favoring the clonal outgrowth of the genetically altered cells (tumor promotion) through activating cell survival and proliferation signal pathways and altering the machineries controlling

Abbreviations used: DMEM, Dulbecco's Modified Eagle's Medium; FBS, Fetal bovine serum; NHEKs, normal human embryo keratinocytes; PI-3K, phosphoinositide 3-kinase.

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cell proliferation and apoptosis. It has been well accepted that aberrant cell proliferation caused by alterations in cell-cycle machinery at checkpoints is associated with tumor development and progression [Kastan and Bartek, 2004; Massague, 2004; Ohtani et al., 2004]. Arsenite has a weak mutagen effect, so it is thought that the ability to activate some signaling pathways and gene expression responsible for cell growth may play a more important role in its carcinogenetic effect. In fact, arsenite has been demonstrated to induce cell proliferation in both cell culture model and animal model [Van Wijk et al., 1993; Germolec et al., 1996; Germolec et al., 1997; Germolec et al., 1998; Simeonova et al., 2000; Hamadeh et al., 2002; Burns et al., 2004; Rossman et al., 2004]. In cell culture studies, arsenite increases cell proliferation in human keratinocytes [Germolec et al., 1996; Simeonova et al., 2000; Hamadeh et al., 2002] and enhances the mitogenic effect of suboptimal serum concentrations on quiescent C3H10T1/2 cells [Van Wijk et al., 1993]. In animal studies, low doses of arsenite cause hyperplasia in the urinary bladder epithelium and in skin [Germolec et al., 1997; Germolec et al., 1998; Simeonova et al., 2000; Burns et al., 2004], and mice exposed to arsenite in drinking water show an increased epidermal thickness and an increased fraction of epidermal cells expressing proliferating cell nuclear antigen (PCNA) as comparison with control mice [Rossman et al., 2004]. Moreover, the arsenical Bowen's disease shows a proliferating characteristic.

Hyperproliferation caused by arsenite exposure has been indicated to correlate with its effect on signal pathway activation. Although it was reported that arsenic-induced cell proliferation was associated with MAPKs/AP-1 and NF-κB activation [Simeonova et al., 2000; Liao et al., 2004; Luster and Simeonova, 2004], the signal pathways involved in arsenite-induced cell proliferation remain to be extensively studied. Phosphoinositide 3-kinase (PI-3K), heterodimers composed of catalytic and a regulatory subunits [Cantley, 2002; Vivanco and Sawyers, 2002], can be activated in cells exposed to diverse stimuli such as hormones, growth factors, and extracellular stressors. Upon activation, PI-3K generates phosphatidylinositol-3,4,5-trisphosphate (PIP3), a lipid second messenger essential for the translocation of Akt to the plasma membrane, where it is phosphorylated and activated by phosphoinositide-dependent kinase-1 [Alessi et al., 1997; Toker and Cantley, 1997]. Akt phosphorylates and regulates the function of many cellular proteins involved in processes of apoptosis and proliferation. PI-3K/Akt has been demonstrated to be an important signaling pathway for cell survival and growth, also play a pivotal role in tumorigenesis [Nicholson and Anderson, 2002; Samuels and Ericson, 2006]. Previous studies have indicated that activation of PI-3K/Akt can lead to transactivation of AP-1 and NF-κB through phosphorylation of MAPKs [Eder et al., 1998] and IKK [Hu et al., 1999; Li et al., 1999; Hu et al., 2001; Jeong et al., 2005; Vandermoere et al., 2005], respectively. In our previous study, we found that arsenite exposure induced NF-κB activation in mouse epidermal JB6 Cl41 cells, which was mediated by PI-3K/Akt/IKKβ cascade [Ouyang et al., 2006]. These lines of evidence suggest that PI-3K/Akt may be involved in mediating the cell proliferation upon arsenite exposure in human skin cells. To address this question in this study, we employed human keratinocytes as a cell model and demonstrated that PI-3K/Akt signal pathway was indeed implicated in arsenite-induced cell proliferation through the induction of cyclin D1. This study provides the first direct evidence that PI-3K/Akt pathway plays a role in arsenite-induced human keratinocyte proliferation by induction of cyclin D1 expression. Given the importance of aberrant cell proliferation in carcinogenesis, this study may provide molecular basis for prevention of arsenite-induced carcinogenesis by targeting PI-3K/Akt signal pathway and cyclin D1.

MATERIALS AND METHODS

Reagents, Plasmids, and Adenovirus

Fetal bovine serum (FBS) was purchased from Life Technologies, Inc. (Gaithersburg, MD); Dulbecco's modified Eagle's medium (DMEM) was from Calbiochem (San Diego, CA); and Sodium arsenite was purchased from Aldrich (Milwaukee, WI). Antibodies specific targeting phospho-Akt (Thr308), phospho-Akt (Ser473), Akt, and cyclin D1 were purchased from Cell Signaling Technology (Berverly, MA). Antibody against GAPDH was from Abcam (Cambridge, MA). The cytomegalovirus-neo vector plasmid, the DN-Akt mutant plasmid (SR-Akt-T308A/S473A) and the dominant negative mutant PI-3K plasmid (Δp85), were as described previously [Huang et al., 1996; Huang

et al., 1997; Huang et al., 2001; Li et al., 2004]. These DN-Akt and Δp85 constructs were inserted into AdEasy XL Adenoviral Vector (Stratagene, La Jolla, CA) according to the manufacturer's instruction. The adenovirus was produced and amplified by transfecting the plasmids into HEK 293 cells.

Cell Culture

Human keratinocytic HaCat cells were cultured in monolayers at 37°C, 5% CO_2 using DMEM containing 10% FBS, 2 mM L-glutamine, and 25 μg of gentamicin/ml [Ouyang et al., 2005]. The cultures were dissociated with trypsin and transferred to new 75-cm^2 culture flasks (Fisher, Pittsburgh, PA) from one to three times a week. Normal human embryo keratinocytes (NHEKs) were purchased from Cambrex (Walkersville, MA), and cultured in keratinocyte growth medium (KGM) (Cambrex, Walkersville, MA) according to the instruction provided by the manufacture.

Construction of siRNA Vector

The specific small interference RNA (siRNA) targeted human cyclin D1 was designed with a 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 (San Diego, CA). The target sequence was 5′-GTTCATTTCCAATCCGCCC-3′ (bases 791–809 of NM053056.1). The siRNA sequence was controlled via 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. The siRNA vectors were designated as sicyclin D1.

Stable Transfection

HaCat cells were transfected with Δp85, DN-Akt, and sicyclin D1 plasmids by nucleofection with the Nucleofector™ II and Nucleofector Kit V (Amaxa Biosystems, Gaithersburg, MD) according to the manufacturer's instruction. Briefly, 1×10^6 of HaCat cells were resuspended in 100 μl of Nucleofector™ Solution V, and then mixed together with 1 μg of CMV-neo vector and 10 μg of plasmids of interest. The mixture was electroporated with a program specifically modified for HaCat cells. After the nucleofection, the cells were immediately transferred from the cuvette to a 6-well plate with prewarmed medium. Twenty-four hours after the nucleofection, the cells were cultured in complete DMEM medium containing 1 mg/ml of G418 for 24–28 days. The stable transfectants were identified by either measuring the blocking activity of the relative kinase activation or by the inhibitory effect of siRNA on the expression of its target gene. The stable transfectants, HaCat-Δp85, HaCat-DN-Akt, and HaCat-sicyclin D1 were established and cultured in G418-free DMEM for at least two passages before each experiment.

Phosphorylation Assay for Akt

Cells (2×10^5) were cultured in each well of 6-well plates to 70–80% confluence with normal culture medium. The cell culture medium was replaced with 0.1% FBS DMEM with 2 mM L-glutamine and 25 μg of gentamicin and cultured for 45 h. The cells were incubated in serum-free DMEM for 3–4 h at 37°C. After arsenite exposure, the cells were washed once with ice-cold PBS and then extracted with SDS-sample buffer. The cell extracts were separated on polyacrylamide-SDS gels, transferred, and probed with one of the following antibodies, including phospho-specific Akt (Thr308) antibody, phospho-specific Akt (Ser473) antibody, and total Akt antibody. The 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) [Ouyang et al., 2005; Ouyang et al., 2006].

Cyclin D1 Expression Assay

Cells (2×10^5) were cultured in each well of 6-well plates to 90% confluence. After exposure of cells to arsenite with or without the pretreatment of wortmannin 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 separated on polyacrylamide-SDS gels, transferred, and probed with specific antibodies against cyclin D1 or β-actin, which was used as a protein loading control. The protein bands specifically bound to primary antibodies were detected as described above.

Cell Proliferation Assay

Confluent monolayers of HaCat cells were trypsinized, and 1×10^3 of viable cells suspended in 100 μl 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. Twelve hours later, the cells were pretreated with or without wortmannin, and then exposed to arsenite for the times as indicated. The exposed cells were lysed with a 50 μl lysis buffer, and the proliferation of the cells was measured using CellTiter-Glo® Luminescent Cell Viability Assay kit (Promega, Madison WI) with a luminometer (Wallac 1420 Victor2 multipliable counter system) as described previously [Ouyang et al., 2006]. The results are expressed as luciferase activity relative to control medium (proliferation index).

RESULTS

Inhibition of PI-3K/Akt Pathway Down-Regulates Arsenite-Induced Proliferation of HaCat Cells

Arsenite has been reported to be able to induce cell proliferation both in cell culture model and animal model [Van Wijk et al., 1993; Germolec et al., 1996; Germolec et al., 1997; Germolec et al., 1998; Simeonova et al., 2000; Hamadeh et al., 2002; Burns et al., 2004; Rossman et al., 2004]. Here, we found that exposure of HaCat cell, a nontumorigenesis human keratinocyte cell line, to low concentrations of arsenite also caused a significant increase in cell proliferation (Fig. 1a). Previous studies have also indicated that PI-3K/Akt signal pathway plays a crucial role in eukaryotic cells in the modulation of the expression of genes involved in cell proliferation, survival, and malignant transformation by activating a set of transcription factors, including CREB and NFκB [Krasilnikov, 2000; Brunet et al., 2001]. In this study, we tested whether PI-3K/Akt was also required for arsenite-induced proliferation of HaCat cells by pretreatment of the HaCat cells with wortmannin, a specific chemical PI-3K/Akt inhibitor. As shown in Figure 1b, the pretreatment of cells with wortmannin did markedly inhibit the cell proliferation induced by arsenite exposure, indicating that low concentration of arsenite is able to induce human keratinocyte proliferation through PI-3K/Akt signal pathway.

PI-3K-Akt Pathway is Involved in Cyclin D1 Induction in Human Keratinocytes upon Arsenite Exposure

The cell proliferation is stringently controlled by a set of proteins including cyclins. It has been demonstrated that cyclin D1 plays a crucial role in controlling cell proliferation. Our previous

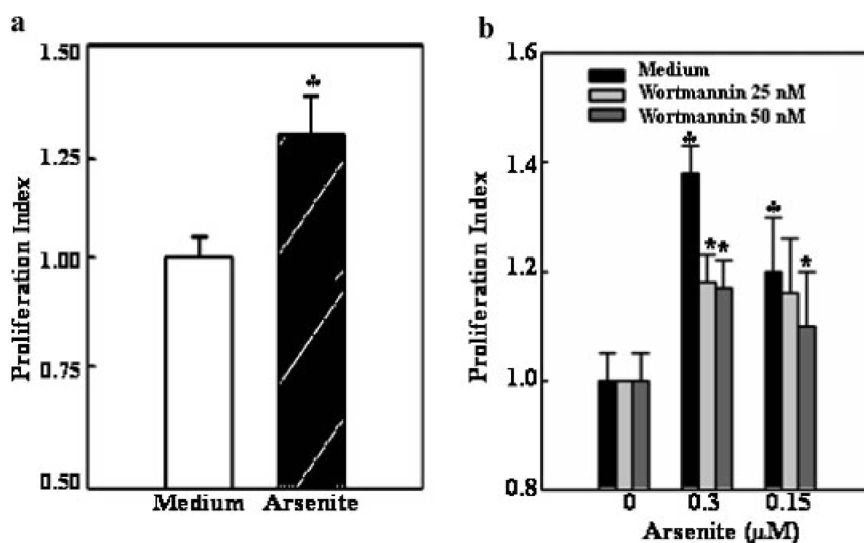

Fig. 1. Pretreatment of wortmannin inhibited arsenite-induced human HaCat keratinocyte proliferation. Viable cells (1×10^3) were seeded into each well of 96-well plates. After being cultured at 37°C in a humidified atmosphere of 5% CO_2 for 12 h, the cells were exposed to 0.15 μM of arsenite for 72 h (**a**), or pretreated with wortmannin for 1 h, and then exposed to arsenite for 72 h (**b**). The proliferative rate of the cells was measured using CellTiter-Glo® Luminescent Cell Viability Assay kit with a luminometer. The results are expressed as luciferase activity relative to control medium (proliferation index). The symbol (♣) indicates a significant increase from medium control ($P < 0.01$), and the symbol (*) indicates a significant decrease from arsenite-treated cells ($P < 0.01$).

study indicated that arsenite can induces cyclin D1 in HaCat cell [Ouyang et al., 2005], and that PI-3K/Akt pathway is also reported to regulate cyclin D1 level in mouse epidermal JB6 cells [Ouyang et al., 2006]. Thus, we examined the induction of cyclin D1 and the potential contribution of PI-3K/Akt to its induction in human keratinocytes exposed to low concentration of arsenite. Consistent with our previous results, low concentration of arsenite was able to induce cyclin D1 expression (Fig. 2a). Further, pretreatment of wortmannin could markedly inhibit the cyclin D1 induction by arsenite in HaCat cells (Fig. 2b), suggesting that cyclin D1 induction requires PI-3K/Akt activation. To further provide direct evidence for the contribution of PI-3K/Akt to cyclin D1 induction in arsenite exposure, we established HaCat cells stably transfected with dominant negative mutant of PI-3K (Δp85) and dominant negative mutant of Akt (DN-Akt), and then examined the phosphorylation of Akt. As shown in Figure 2c, the exposure of HaCat cells to low concentrations of arsenite was able to induce Akt phosphorylation, which was inhibited by the transfection of Δp85, the dominant negative mutant of regulating subunit of PI-3K. More importantly, the induction of cyclin D1 by arsenite was also significantly impaired in both Δp85- and DN-Akt-transfected HaCat cells (Fig. 2d), which is consistent with their inhibitory effect on arsenite-induced Akt phosphorylation (Fig. 2c).

The requirement of PI-3K/Akt pathway for arsenite-induced cyclin D1 expression was further verified in human primary keratinocytes (NHEKs). As shown in Figure 3a, exposure of NHEKs to low concentrations of arsenite also induce cyclin D1 accumulation, which was markedly impaired in NHEKs infected with adenovirus expressing either DN-PI-3K (Δp85) (Fig. 3b and d) or DN-Akt (Fig. 3c and d). Taken together, these results demonstrate that activation of PI-3K/Akt pathway is required for cyclin D1 induction by arsenite in human keratinocytes.

PI-3K/Akt Regulates Arsenite-Induced Cell Proliferation of HaCat Cells Through the Induction of Cyclin D1

Although numerous studies indicate that cyclin D1 plays an essential role in controlling cell proliferation by promoting G1/S cell cycle

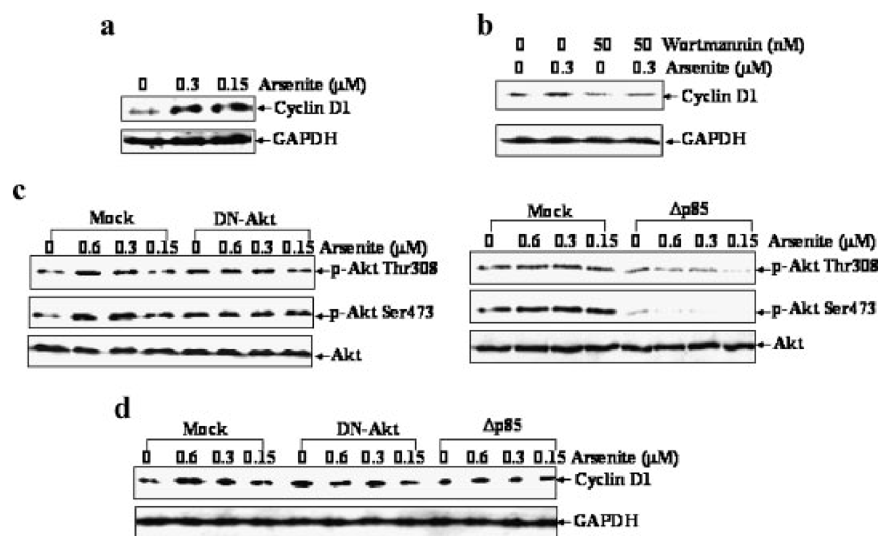

Fig. 2. Requirement of PI-3K/Akt signal pathway for the induction of cyclin D1 by arsenite in HaCat cells. (**a**, **b**) HaCat cells were seeded into each well of 6-well plates. After being cultured at 37°C overnight, the cells were treated with various concentrations of arsenite as indicated for 24 h (a), or pretreated with 50 nM of wortmannin for 1 h, and then exposed to 0.3 μM of arsenite for 24 h (b). (**c**, **d**) HaCat-mock, HaCat-DN-Akt, and HaCat-Δp85 cells were seeded into each well of 6-well plates and cultured at 37°C overnight. (c and d) The cell culture medium was replaced with 0.1% FBS DMEM. Forty-five hours later, the cells were incubated in serum-free DMEM for 3–4 h, and then exposed to various concentrations of arsenite as indicated for 12 h (c). The cells were exposed to various concentrations of arsenite as indicated for 24 h (d). The cells were 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 antibodies as indicated. The protein band specifically bound with the primary antibody was detected by using anti-rabbit IgG–AP-linked secondary antibody and an ECF Western blot system. GAPDH was used as a control for protein loading.

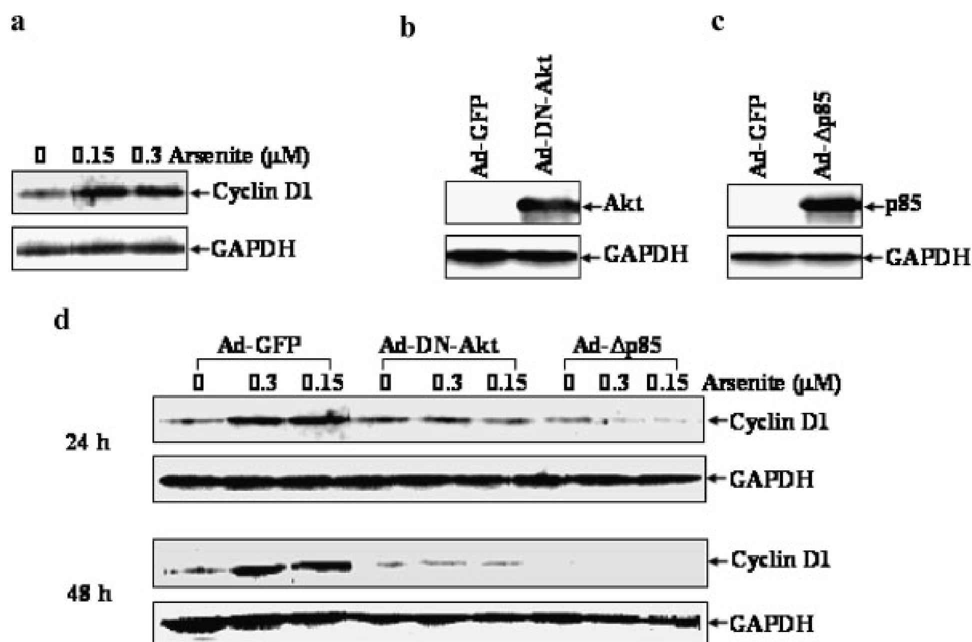

Fig. 3. Requirement of PI-3K/Akt signal pathway for the induction of cyclin D1 by arsenite in NHEKs. (**a**) NHEKs (2×10^5) were seeded into each well of 6-well plates. After being cultured at 37°C overnight, the cells were treated with various concentrations of arsenite as indicated for 24 h. (**b–d**) NHEKs (2×10^5) were seeded into each well of 6-well plates, cultured at 37°C overnight, and then infected by adenovirus expressing DN-Akt or Δp85 for 36 h. The infected cells were extracted with SDS-sample buffer for Western blot assay as described in "Materials and Methods" to detect the expression of DN-Akt (b) or Δp85 (**c**), or exposed to various concentrations of arsenite as indicated for 24 or 48 h (d). The cells were then washed once with ice-cold PBS and extracted with SDS-sample buffer. The cell extracts were analyzed by Western blot to detect cyclin D1 expression level. GAPDH was used as a control for protein loading.

progression, there is still no direct evidence to show the involvement of cyclin D1 in arsenite-induced cell proliferation. To examine whether the induction of cyclin D1 is responsible for PI-3K/Akt-mediated HaCat cell proliferation by low concentrations of arsenite, we established cyclin D1 specific small interference RNA-transfected HaCat cells. Western blot assay indicated that the siRNA markedly impaired cyclin D1 expression in HaCat cells (Fig. 4a). Moreover, the transfection of cyclin D1 siRNA dramatically inhibited HaCat cell proliferation induced by arenite (Fig. 4b). These results provided the direct evidence to indicate the importance of cyclin D1 in arsenite-induced proliferation of HaCat cells. Consistent with the role of PI-3K/Akt in the induction of cyclin D1 in HaCat cells exposed to arsenite, the HaCat cell proliferation induced by arenite was also blocked by transfection of either Δp85 or DN-Akt (Fig. 4c and d). Collectively, these results demonstrate that PI-3K/Akt pathway plays a pivotal role in arsenite-induced proliferation of human keratinotyes through the induction of cyclin D1.

DISCUSSION

Since arsenite, a well-documented human carcinogen, has only a weak mutagen effect, it is believed that its ability to activate some signaling pathways and gene expression responsible for cell growth may play an important role in mediating its carcinogenetic effect. In the present study, we demonstrate that the exposure of human keratinocytes to low concentration of arsenite leads to the activation of PI-3K/Akt, which thereby plays a critical role in arsenite-induced cell proliferation through inducing cyclin D1 expression. Inhibition of PI-3K/Akt dramatically blocked arsenite-induced cyclin D1 induction and cell proliferation in human keratinocytes.

PI-3K/Akt signaling pathway has been demonstrated to play a role in cell survival and growth, and be involved in the tumorigenesis under some context. One means by which PI-3K/Akt regulates the cell proliferation is to upregulate the level of cyclin D1 at both transcription and protein levels [Cappellini et al., 2003; Liang and Slingerland, 2003].

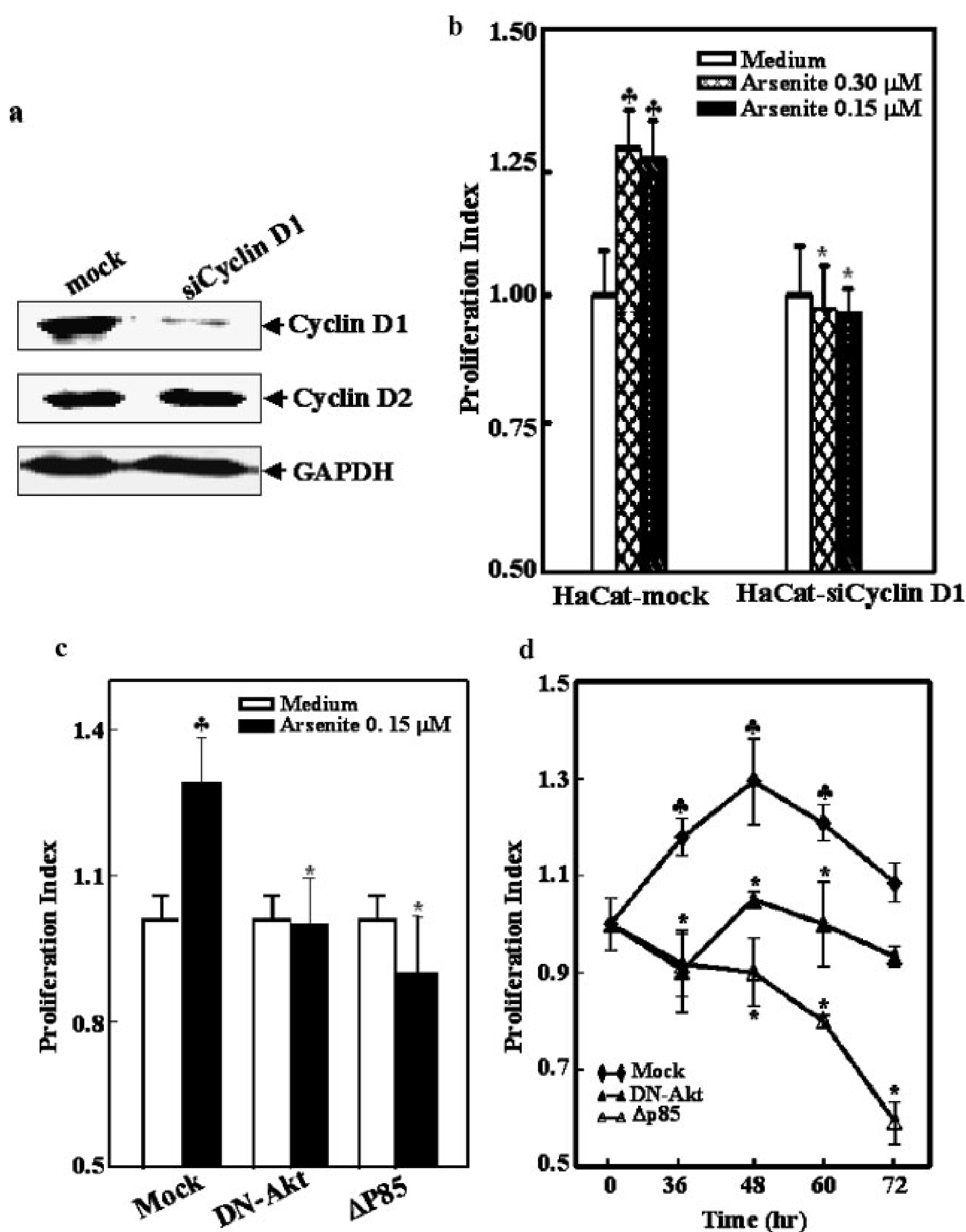

Fig. 4. The essential role of PI-3K/Akt and cyclin D1 in arsenite-induced cell proliferation. (**a**) HaCat-mock and HaCat-siCyclin D1 cells were extracted and analyzed by Western blot to detect cyclin D1 expression level as described above. Cyclin D2 was used as a control to indicate the specificity of the effect of cyclin D1 siRNA on silencing cyclin D1 expression. GAPDH was used as a protein loading control. (**b**) HaCat-mock and HaCat-siCyclin D1 cells (1×10^3) were seeded into each well of 96-well plates. After being cultured for 12 h, the cells were exposed to various concentrations of arsenite for 48 h. The proliferation of the cells was measured using CellTiter-Glo® Luminescent Cell Viability Assay kit with a luminometer. The results are expressed as luciferase activity relative to control medium (proliferation index). (**c**, **d**) HaCat-mock, HaCat-DN-Akt, and HaCat-Δp85 cells (1×10^3) were seeded into each well of 96-well plates. After being cultured for 12 h, the cells were exposed to 0.15 μM of arsenite for 48 h (c) or different times as indicated (d). The proliferation of the cells was measured as described above. The results are expressed as luciferase activity relative to control medium (proliferation index). The symbol (♣) indicates a significant increase from medium control ($P < 0.01$), and the symbol (*) indicates a significant decrease from vector control cells ($P < 0.01$).

The transcription of cyclin D1 is regulated by multiple transcription factors including AP-1 [Watanabe et al., 1996; Lee et al., 1999] and NF-κB [Guttridge et al., 1999; Hinz et al., 1999; Joyce et al., 1999; Krasilnikov, 2000; See et al., 2004]. AP-1 and NF-κB are activated by MAPKs and IKK signal cascades, respectively. Interestingly, both MAPKs [Eder et al., 1998] and IKK [Hu et al., 1999; Li et al., 1999; Hu et al., 2001; Jeong et al., 2005; Vandermoere et al., 2005] were reported to be activated by arsenite, and to be the downstream of PI-3K/Akt signal cascade under certain context. Presumably, activation of these transcription factors through MAPKs and IKK may be partially responsible for the induction of cyclin D1 and proliferation of human keratinocytes exposed to low concentrations of arsenite.

In the multistage carcinogenesis mouse skin model, cell proliferation has been indicated to promote tumor development by favoring the clonal outgrowth of the genetically altered cells (tumor promotion) [Kastan and Bartek, 2004; Massague, 2004; Ohtani et al., 2004]. In this study, we found that the exposure of low concentration of arsenite-induced proliferation of human keratinocyte, and that PI-3K/Akt pathway is involved in the promotion of the proliferation through the induction of cyclin D1. Both PI-3K/Akt pathway chemical inhibitor, and dominant negative mutants of PI-3K and Akt, markedly inhibited cyclin D1 induction in human keratinocytes exposed to arsenite. Subsequently, the proliferation of human keratinocyte induced by arsenite was also impaired. These results demonstrate that PI-3K/Akt pathway is implicated in arsenite-induced cell proliferation through the induction of cyclin D1.

As an important signal pathway for cell survival and growth, PI-3K/Akt has been demonstrated to associate with the tumorigenesis [Nicholson and Anderson, 2002; Samuels and Ericson, 2006]. More than 30% of various solid tumor types were recently found to contain mutations in PIK3CA, the catalytic subunit of PI-3K [Samuels and Ericson, 2006]. In addition, several studies have shown gene amplification of PIK3C in human ovarian cancer, cervical cancer, head and neck cancer, gastric cancer, and glioblastoma [Osaki et al., 2004]. The PI-3K/Akt pathway has also been found dysregulated in skin related cancers such as melanomas. Moreover, it has been reported that the region of chromosome 3q26 containing the p110 catalytic subunit of PI3K is frequently amplified in cancer of the ovary and cervix, leading to overexpression and enhanced PI3K catalytic activity [Gavin, 2005]. Considering the carcinogenic activity of arsenite, our present study demonstrates that PI-3K/Akt pathway contributes to arsenite-induced cell proliferation in human keratinocytes. We therefore anticipate that PI-3K/Akt pathway may be involved in arsenite human skin carcinogenic effect.

In summary, our studies demonstrate that PI-3K/Akt pathway plays a role in the arsenite-induced proliferation of human keratinocytes through the induction of cyclin D1. In light of the important role of cell proliferation in carcinogenesis, the PI-3K/Akt may contribute to arsenite-induced carcinogenesis through the induction of cyclin D1. These results provide novel information for understanding the molecular mechanisms underlying the carcinogenic effect of arsenite on its major target tissue of skin, which also suggest that PI-3K/Akt pathway may be a target for chemoprevention of arsenite-induced carcinogenesis.

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