Low Concentration of Arsenic-Induced Aberrant Mitosis in Keratinocytes Through E2F1 Transcriptionally Regulated Aurora-A

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Chronic exposure to low-concentration arsenic promotes cell proliferation and carcinogenesis both in vitro and in vivo. Centrosome amplification, the major cause of chromosome instability, occurs frequently in cancers. Aurora-A is a mitotic kinase and causes centrosome amplification and chromosome instability when overexpressed. Our previous study revealed that low-concentration arsenic induces Aurora-A overexpression in immortalized bladder cells. In this study, we hypothesized that low-concentration arsenic induces aberrant mitosis in keratinocytes due to Aurora-A overexpression. The specimen of Bowen’s disease (BD) and squamous cell carcinoma obtained from arseniasis-endemic areas in Taiwan showed Aurora-A overexpression. The mRNA/protein levels and kinase activity of Aurora-A were increased in immortalized keratinocyte HaCaT cells after arsenic treatment at low concentration (< 1µM). Aberrant spindles, multiple centrosomes, and multinucleated cells were detected under fluorescent microscopy in HaCaT cells after arsenic treatment. These findings were associated with increased expression of Aurora-A. We further revealed that Aurora-A was regulated by arsenic-induced transcriptional factor E2F1 as demonstrated by chromosome immunoprecipitation, promoter activity, and small interfering RNA assays. Finally, in arsenic-treated HaCaT cells and in BD, a significant increase of dysfunctional p53 was found, and this event correlated with the increase in expression of Aurora-A. Altogether, our data suggest that low concentration of arsenic induces activation of E2F1-Aurora-A axis and results in aberrant mitosis of keratinocytes. Overexpression of Aurora-A and dysfunctional p53 may act synergistically to trigger skin tumor formation. Our findings suggest that Aurora-A may be a potential target for the prevention and treatment of arsenic-related cancers. Key Words: Aurora-A; arsenic; keratinocyte; mitosis.

Skin is one of the most susceptible human organs to chronic arsenic exposure. The most common arsenic-induced skin cancers include Bowen’s disease (BD, SCC in situ), squamous cell carcinoma (SCC), and basal cell carcinoma (BCC) (Yeh et al., 1968). Many in vitro studies reveal that the biphasic effect of arsenic depends on its concentration. (Liao et al., 2011; Rossman and Klein, 2011). High concentration of arsenic induces DNA damage through generation of reactive oxygen species and AP-1-associated apoptosis in keratinocytes (Liao et al., 2011). In contrast, long-term and low-concentration arsenic exposure causes aneuploidy and induces cellular transformation in keratinocytes (Chien et al., 2004; Pi et al., 2008; Rossman and Klein, 2011; Sun et al., 2009). Due to lack of appropriate animal models to investigate arsenic carcinogenesis, in vitro studies were frequently used to investigate the molecular mechanisms involved in genotoxic effect of arsenic exposure.

Accumulating evidence reveals that arsenic exposure leads to chromosome instability, a hallmark for tumors harboring extra centrosomes (Ganem et al., 2009; Nigg, 2002). Centrosome amplification was demonstrated to associate with the aberrant expression of centrosome proteins including Aurora-A (Lukasiewicz and Lingle, 2009). Aurora-A, a member of the Aurora kinase family, is required for controlling mitotic entry, centrosome maturation, separation, bipolar spindle assembly, and chromosome segregation in mammalian cells (Marumoto et al., 2005). Aurora-A expression peaks at the M phase of the cell cycle. Autophosphorylation or protein kinase A–mediated Aurora-A phosphorylation at Thr288 is required for its activation. The activated Aurora-A phosphorylates histone H3 at serine10, which is in charge of chromosome segregation (Crosio et al., 2002). Subsequently, Aurora-A is degraded by the proteasome and the ubiquitin-dependent pathways after metaphase and anaphase transition (Dutertre et al., 2002).

Overexpressed Aurora-A is detected in various cancers. Ectopic Aurora-A expression induces centrosome amplification,
cytokinesis failure, aneuploidy formation, and malignant transformation in immortalized rodent fibroblasts and colon carcinoma cells, indicating its involvement in tumorigenesis (Lassus et al., 2011; Lei et al., 2011). Aurora-A gene was located in chromosome 20q13.2–13.3, which shows frequently copy number gains in ovarian cancers. About 10–25% of ovarian cancer cell line and primary tumors contain the amplification of Aurora-A gene. Furthermore, increased Aurora-A protein levels are observed in 57% ovarian specimens (Gritsko et al., 2003). The alteration of Aurora-A at mRNA and protein levels is more frequently observed than gene amplification in ovarian and breast cancers (He et al., 2008). Therefore, Aurora-A overexpression seems to be regulated by gene amplification or other mechanism such as transcriptional activation.

Aurora-A is transcriptionally regulated by E2F3 and E4TF1 (a member of the Ets family) during G2/M phase of cell cycle (He et al., 2008; Tanaka et al., 2002). However, the precise mechanism of tumor-associated transcriptional regulation of Aurora-A in cancer cells is not well understood. Previous studies reveal that exposure to low concentration (1µM) of arsenic trioxide results in cell proliferation, which is associated with the regulation of c-myc gene through transcriptional regulation of E2F1 in HaCaT cells and CSH 10T1/2 in fibroblasts (Bi et al., 2010; Trouba et al., 2000). In mammalian, the E2F family of transcription factor contains eight E2F and two DP members. More specifically, the E2F1-3 members are transcriptional activators that regulate cell-cycle progression from G1 to S phase (Ivanova et al., 2005).

It has been reported that Aurora-A phosphorylates p53 at Ser215 to block p53-dependent apoptosis, and p53 interacts with Aurora-A to suppress its oncogenic activity (Marumoto et al., 2005). In hepatocellular carcinoma, Aurora-A overexpression combined with p53 mutation is associated with the worst survival rate (Jeng et al., 2004). In addition, higher level of Aurora-A staining has been detected in SCCs with p53 (p53R172) mutation or deletion (p53−/−) compared with the SCCs (Ivanova et al., 2000). In mammals, the E2F family of transcription factor contains eight E2F and two DP members. More specifically, the E2F1-3 members are transcriptional activators that regulate cell-cycle progression from G1 to S phase (Ivanova et al., 2005).

Based on these studies, we hypothesized that E2F1 activation and Aurora-A overexpression may be involved in arsenic-related carcinogenesis of the skin. Using arsenic-related BD and SCC specimens and immortalized HaCaT cells that harbor two mutant alleles of p53 (Lehman et al., 1993), this study was performed to validate our hypothesis.

**MATERIALS AND METHODS**

**Reverse transcription PCR.** The Aurora-A mRNA in arsenic-treated cells and clinical tissues were detected by reverse transcription PCR (RT-PCR). Total RNA was extracted from arsenic-treated and untreated cells using the TRIzol reagent (Invitrogen, Carlsbad, CA). One microgram of total RNA was reverse transcribed using the Superscript II RNase H Reverse Transcriptase (Invitrogen). PCR was carried out using GoTaq DNA master mixes (Promega, Madison, WI). Used primer names and sequences were as follows: Aurora-A sense: 5′-aattcagatttggtg, antisense: 5′-aacctcagtacaagctcttgt, crossing exon 9 to exon 11 of Homo sapiens aurora kinase A mRNA; β-actin sense: 5′-aggcggaaacttggtg, antisense: 5′-caggtaatggcttggtgc.

**Immunohistochemical staining.** The protein expression level of Aurora-A in arsenic-related skin tumors was detected by immunohistochemical staining (IHC). The normal skins on the sun-protected areas were obtained from the peripheral uninvolved skin of a follicular cyst or nevus during surgical excision. Consecutive cases of cutaneous BD (38) and SCC (20) samples were retrospectively retrieved from the tissue bank of the Department of Dermatology, National Cheng Kung University Hospital, Taiwan. Ethics approval was obtained from the Human Experiment and Ethics Committee (HEEC) of National Cheng Kung University Hospital and Department of Executive Yuan, R.O.C., Taiwan (Protocol no./HEEC no.: 95WFA001035/HR-95-47). All samples were obtained from sun-protected areas of patients from endemic areas of chronic arsenicism located in the southern region of Taiwan. The 5-µm thick specimens underwent deparaffinization and rehydration in serial concentrations of ethanol. After retrieval with Tris-EDTA buffer (pH 9.0), the endogenous peroxidase was blocked with 0.5% H2O2. The skin sections were incubated with mouse anti-Aurora-A (1:50, Novocasta, Newcastle, UK) and rabbit anti-p53 (1:200, DO-1, Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4°C and incubated with biotinylated secondary antibody (LSAB2+, DakoCytomation, Carpinteria, CA) for 30 min at room temperature. After incubation with the streptavidin-HRP reagent (LSAB2+, DakoCytomation) for 30 min, the skin sections were incubated with aminochrome/biotinylazide solution (AEC, DakoCytomation) for 30 min at room temperature. An Olympus DP50 light microscope (Olympus, Tokyo, Japan) was used to examine the skin sections. Blind reading of immunohistochemistry tissue sections was performed by Dr Tsu-Kai Lin (Dermatologist) and Dr Chien-Huen Huang (Dermatologist and Pathologist) at the National Cheng Kung University Hospital. Quantification of the Aurora-A and p53 protein intensities in each tissue section was performed as follows: −, negative (0%); +, focal positive (< 5%; ±, moderate positive (5–50% tumor areas); ++, diffuse positive (> 50% tumor areas).

**Cell line and treatment.** The HaCaT cell line, an immortalized keratinocyte, was maintained in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen) and antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin) at 37°C in 5% CO2 humidified atmosphere. Both p53 alleles of HaCaT cells contain mutations. The codon 174 mutation is present on one allele and alters a histidine residue to a tyrosine residue, whereas 281 and 282 mutations are on the other allele and change an arginine to a tryptophan residue (Lehman et al., 1993). Sodium arsenite (NaAsO2, Sigma-Aldrich, St Louis, MO) was dissolved in double distilled water, and stock solution was frozen at −80°C. The culture medium was changed every 2 days, and indicated concentration of sodium arsenite was adjusted using stock solution.

**Immunofluorescent assay.** The distribution of Aurora-A in arsenic-treated cells was detected by immunofluorescent staining. After incubation with arsenic for the indicated time, cells were fixed with 4% paraformaldehyde and incubated with mouse anti-Aurora-A (1:100, Novocasta) antibody overnight at 4°C. Cells were then washed with cold PBS and incubated with Alexa488-conjugated anti-mouse IgG (Invitrogen) at room temperature for 30 min. The mouse anti-c-tubulin was pretreated with Alexa Fluor 594 Monoclonal Antibody Labeling Kit (Invitrogen) to detect tubulin distribution in HaCaT cells. The cell nuclei were stained with 0.1 µg/ml of Hoechst 33342 (Promega). An Olympus DP50 light microscope (Olympus) and a multichannel confocal microscopy (Olympus) were used to examine the fluorescence expression.

**Western blot analysis.** The protein expression level and kinase activity of Aurora-A in arsenic-treated cells were detected by Western blot analysis. Cells were lysed using T-PER Tissue Extraction Reagent (Pierce, Rockford, IL) containing protease inhibitors (Roche, Mannheim, Germany). The cell lysates (50µg) were subjected to a 10% SDS-PAGE and subsequently transferred to a PVDF membrane (Millipore, Billerica, MA). After blocking with 5% nonfat milk, the membranes were incubated with Aurora-A (Cell Signaling, Danvers, MA), phosphor-Aurora-A (Thr288, Cell Signaling), phosphor-Histone3 serine
Promoter activity assay. The promoter activity of Aurora-A after arsenic or other inducer treatment was assessed by the Aurora-A reporter plasmid pGL2-AAP (containing firefly luciferase gene). HaCaT cells (1 × 10^4) were cotransfected with pGL2-AAP and pRLTK (Renilla luciferase, as an internal control) at ratio 1:0.1 using LipoGold 2000 (Invitrogen). Cells were treated with indicated concentration of arsenic for another 24 and 48h after transfection. In addition, pGL2-AAP, pRLTK, and indicated amount of pCMV-E2F1 (E2F1 cDNA) were cotransfected into HaCaT cells for 24 and 48h. Thereafter, the cell lysate was collected in 1× passive lysis buffer. The supernatant of the centrifuged cell lysate (20 μl) and 50 μl of LAR II were added to the luminometer tubes. The tubes were gently shaken to measure the firefly luciferase activity (representing Aurora-A luciferase activity). To measure Renilla luciferase activity, 50 μl of Stop and Glo Reagent (SGR) and 1 μl of substrate (50x) were added into the tubes (the same tubes used to measure firefly luciferase activity) and mixed by shaking. The luciferase activity of Aurora-A was normalized by the activity of Renilla luciferase to represent equal loading of protein samples and to ensure that the number of the cells of each treatment is the same.

Chromatin immunoprecipitation assay. To determine whether arsenic-induced E2F1 regulates Aurora-A by directly binding at the promoter region, the chromatin immunoprecipitation (ChIP) assay was conducted. ChIP assay was performed with the Chip-IT express enzymatic kit (Active Motif, Carlsbad, CA) according to the manufacturer’s instructions. After arsenic treatment, 5 × 10^4 cells were fixed with 1% formaldehyde at room temperature for 5 min, nuclei were lysed using a Dounce homogenizer (Kimble-Kontes, Vineland, NJ), and chromatin was enzymatically digested at 37°C for 30 min. ChIP was performed over 1% of a 10 μl with 7 μg of DNA, 3 μg of control IgG, or E2F1 (C-20, Santa Cruz Biotechnology) and captured using protein G magnetic beads (Active Motif). After reversion of cross links for 15 min at 95°C, protein was digested with proteinase K. The isolated DNA was employed as a template for PCR amplifications using GoTaq DNA master mixes (Promega). The primer set used to predict the putative E2F1 binding site (from −104 to +106) within Aurora-A promoter was as follows: sense 5′-cagaccgctgtgcttcaacctgccc and antisense 5′-ccagagctcgactgcctggaa (He et al., 2008). Ten microliters of each PCR reaction product were separated on a 2% agarose gel containing 0.1% SYBR Safe DNA gel stain (Invitrogen) and visualized by ultraviolet illumination.

Suppression of E2F1 by shRNA. The endogenous E2F1 was suppressed to determine whether arsenic-induced Aurora-A expression is related to E2F1 expression. Recombinant lentivirus carrying a short hairpin (sh) RNA against E2F1 (target sequence: 5′-cagaccgctgtgcttcaacctgccc) was obtained from the National RNAi Core Facility of Academia Sinica (Taipei, Taiwan). HaCaT cells were infected with lentivirus for 1 day, and stable cell lines were stably selected with 1 μg/ml puromycin for 1 week. After arsenic treatment, we confirmed the knockdown of E2F1 and activated Aurora-A by Western blotting.

Statistical analysis. Data were analyzed using GraphPad Prism 4 software (GraphPad, Inc., San Diego, CA). The experimental results were presented as mean ± SD. Statistical significance in the analysis of clinical SCC samples was determined by the paired Student’s t-test and in the analysis of time- or dose-dependent experiments by one-way ANOVA with Dunnett’s post hoc test. Difference is considered significant when p value is < 0.05.

RESULTS

Aurora-A Expression in Arsenic-Related Skin Cancers

To clarify the relationship between Aurora-A expression level and skin cancer tumorigenesis, skin cancer specimens at different stages were analyzed. The Aurora-A mRNA expression was significantly increased (p < 0.05) in arsenic-related SCCs compared with perilesional nontumor skin by RT-PCR (Fig. 1A). Similar result was observed in arsenic-related BD (Supplementary data 1). More specimens were required to increase the significance between BD and the perilesional nontumor part. In addition, IHC staining results showed that Aurora-A was barely detectable in the normal epidermis. Only the proliferating basal cells undergoing mitosis showed strong Aurora-A staining (Fig. 1B, arrowhead). In contrast, Aurora-A expression was slightly increased in perilesional epidermis obtained from arsenic-related cancers. Furthermore, Aurora-A expression was obviously increased in the nuclei and cytoplasm of the tumor lesions of BD and SCCs (Fig. 1B, arrow). We further classified the expression level of Aurora-A into three classes in the tumor lesions of 38 patients with BD and 20 with SCC (see Materials and Methods section). The protein intensity of Aurora-A was significantly increased in the SCC lesions (Fig. 1C). Altogether, our results indicate that the expression level of Aurora-A correlated with the tumor malignancy in arsenic-related skin cancers.

Morphological Alteration and Aberrant Distribution of Aurora-A in Arsenic-Treated HaCaT Cells

At first, we clarified the dosage effect of arsenic on the viability in HaCaT cells (Supplementary data 2). Low concentration (≤ 1 μM) of arsenic promoted cell proliferation, whereas high concentration (≥ 10 μM) inhibited cell proliferation. For long-term treatment, low concentration of arsenic was used to treat HaCaT cells for 1 to 4 weeks. The number of multinucleated giant cells increased in a time-dependent manner after HaCaT cells were subjected to treatment with 1 μM of arsenic (Fig. 2A). Furthermore, aberrant chromosome segregation in the arsenic-treated cells was detected during mitosis (Fig. 2B, arrowhead). It is well known that Aurora-A is responsible for centrosome maturation and segregation. In arsenic-treated HaCaT cells, Aurora-A expression in the cytoplasm was significantly increased compared with the untreated control cells (Fig. 2C; green fluorescence). The results of fluorescence staining at high magnification showed that Aurora-A was originally localized at the centrosome, spindle, and spindle poles during early mitosis (Fig. 2D, control). However, two types of aberrant distribution of Aurora-A (green fluorescence) accompanied with abnormal spindle formation (α-tubulin, red fluorescence) were detected in arsenic-treated HaCaT cells (Fig. 2D, arsenic-treated cells). The number of cells with multiple centrosomes (more than two centrosomes during mitosis) was also increased in a time-dependent manner after arsenic treatment (Fig. 2E). The IHC staining results of BD and SCC (Fig. 1B) showed small number of abnormal Aurora-A staining, which was observed at high magnification (Fig. 2F). In these cancerous keratinocytes, condensed chromosomes and multiple centrosomes were displayed in the in vitro study described above. Taken together, we demonstrated that arsenic-induced centrosome amplification is associated with aberrant distribution and location of Aurora-A in HaCaT cells and in cancer specimens.
Effect of Low-Concentration Arsenic on Aurora-A Expression

To clarify whether Aurora-A overexpression is associated with arsenic treatment, Aurora-A mRNA and protein were determined after treatment with low concentrations (≤ 1 μM) of arsenic for 7 days. Aurora-A mRNA expression level increased in a dose-dependent manner (Fig. 3A). Consistent with the result of mRNA detection, a significant increase (p < 0.05) of Aurora-A protein expression was detected after 1 μM arsenic treatment (Fig. 3B). In addition, the phosphorylated Aurora-A (p-Aurora-A) and Histone 3 (p-Histone 3, the substrate of Aurora-A), which represents the kinase activity of Aurora-A, were also significantly increased after 1 μM arsenic treatment (Fig. 3B). In summary, low concentration of arsenic treatment induces Aurora-A overexpression and its kinase activity in HaCaT cells.

Aurora-A Was Transcriptionally Upregulated by E2F1 After Arsenic Treatment

Neither DNA methylation nor gene amplification was involved in Aurora-A overexpression in arsenic-treated HaCaT cells (Supplementary data 3). To clarify whether Aurora-A promoter is activated by E2F1, the correlation between Aurora-A and E2F1 in arsenic-treated cells was investigated.

After transfection with the Aurora-A reporter plasmid and arsenic treatment for additional 24 and 48 h, the luciferase activity of Aurora-A was increased (Fig. 4A). Aurora-A reporter plasmid and E2F1 plasmid were then cotransfected into HaCaT cells, in which the luciferase activity of Aurora-A was significantly elevated with increased amount of E2F1 at 48 h (Fig. 4B). Furthermore, the protein level of E2F1 significantly increased after 1 μM arsenic treatment (Fig. 4C). Therefore, we suggest that E2F1 may be involved in arsenic-induced Aurora-A activation.

Thereafter, the predicted E2F1 binding site of Aurora-A promoter was detected by PCR amplification in the immunoprecipitate captured by E2F1 antibody after 1 μM arsenic treatment (Fig. 4D). Furthermore, after transfection with small interfering RNA of E2F1, the protein level of E2F1 was greatly reduced with or without arsenic treatment (Fig. 4E, upper panel). Interestingly, siRNA-mediated silencing of E2F1 only blocked arsenic-induced Aurora-A overexpression as reflected by the decreased fold of change (Fig. 4E, lower panel). E2F1 was also induced by arsenic; however, the underlying mechanism remains unclear. Our results indicate that Aurora-A expression is transcriptionally regulated by E2F1 under low concentration of arsenic treatment.
Increased Expression of p53 in Arsenic-Treated Cells and Skin Cancers

Synergistic action of Aurora-A with dysfunctional or inactive p53 was important in tumorigenesis. In this study, we detected a marked increase of p53 after 0.5 and 1μM arsenic treatment (Fig. 5A). After IHC staining, strong nuclear staining of p53 was diffusively observed in the epidermis of the perilesional skin but was widely detected in the tumor lesions of arsenic-related BD and SCC (Fig. 5B). We further classified the expression levels of p53 in the tumor lesions of BD...
(n = 38) and SCC (n = 20). The expression of p53 was significantly increased in the SCC lesions (Fig. 5C). Furthermore, we analyzed the correlation between Aurora-A and p53 protein intensity of arsenic-related BD and SCC by linear regression analysis. The coefficients of determination were 0.1129 (p = 0.0451) and 0.04318 (p = 0.5247) in arsenic-related BD and SCC, respectively (Fig. 5D). In summary, our results indicate that Aurora-A activation combined with dysfunctional p53 accumulation may play an important role in arsenic-induced skin cancer progression.

**DISCUSSION**

Arsenic is a common environmental pollutant and has been classified as a human carcinogen by the International Agency for Research on Cancer (IARC). It has been well documented that chronic low-dose arsenic exposure increases proliferation and transformation of human and rodent cells (Yu et al., 2006). In cell culture, low-dose arsenic-induced cell proliferation is associated with activation of nuclear transcription factors, such as AP-1 and NFkB (Liao et al., 2004). In this study, we revealed that proliferation induced by low-dose (≤ 1μM) arsenic is related to the activation of the transcriptional factor E2F1, which transcriptionally regulates Aurora-A activation and subsequently results in aberrant mitosis in keratinocytes. Our results revealed that the E2F1-Aurora-A pathway, induced by low concentration of arsenic, may be involved in arsenic-related carcinogenesis as cells accumulate dysfunctional p53 (Fig. 6). This is the first report to provide a link between the proliferation regulator (E2F1 and p53) and the activation of oncogene Aurora-A in arsenic-induced carcinogenesis.

The proposed carcinogenic effects of arsenic include oxidative DNA damage, genomic instability, aneuploidy, gene amplification, DNA repair inhibition, and epigenetic dysregulation (Ren et al., 2011; Straif et al., 2009). Treatment of hamster fibroblast cells with inorganic arsenic induces the formation of multiple spindles, resulting in unequal segregation of chromosomes during the mitosis process, and produces multinucleated cells (Ochi et al., 1999). A similar phenomenon has been observed in colon carcinoma cells with expression of ectopic Aurora-A (Lentini et al., 2007). The Aurora kinase family includes Aurora-A, -B, and -C that function differently and are located in different subcellular areas. Aurora-B and -C
are associated with chromosome segregation and are localized at the centrosome during prophase to metaphase (Lukasiewicz and Lingle, 2009). Aurora-A is localized at the centrosome from the beginning of centrosome duplication and during the entire process of mitosis (Kollareddy et al., 2012). It is possible that arsenic-induced centrosome amplification is associated with Aurora-A overexpression. Our results demonstrated that low-concentration arsenic induces Aurora-A overexpression in HaCaT cells and in immortalized bladder E7 cells (Tseng et al., 2006). In addition, dimethylarsenic-induced histone H3 phosphorylation results in the abnormal distribution of Aurora-B and further cytokinesis failure in HepG2 cells (Suzuki et al., 2009). Collectively, arsenic-induced mitotic abnormality seems to be related to the increase of component proteins of centrosome.

In this study, overexpressed Aurora-A protein was detected in skin cancers of BD and SCC, and this phenomenon correlated with tumor progression. Aurora-A overexpression is associated with its gene amplification in esophageal SCC (Yang et al., 2007). Our in vitro study revealed that genomic modifications, including promoter methylation and gene amplification of Aurora-A, were not detected in HaCaT cells that received low-concentration arsenic treatment (Supplementary data 3A and B). Further study revealed that arsenic-induced E2F1 binds to and activates Aurora-A promoter. This activation can be silenced by E2F1 shRNA. Similar results were observed in arsenic-treated immortalized bladder E7 cells (unpublished data). Transcriptional factor E2F3 was also induced by low concentration of arsenic. However, it was not involved in the regulation of Aurora-A promoter (data not shown). Increased E2F1 expression is associated with poor prognosis in head and neck cancers, which is consistent with the correlation between abnormal E2F1 regulation and tumorigenesis (Kwong et al., 2003). However, whether E2F1-Aurora-A axis is specific for arsenic-related signaling requires further confirmation.

Excessive Aurora-A expression in keratinocytes results in cell death, suggesting that deregulation of Aurora-A may trigger...
cell death caused by delayed mitosis and cell-cycle arrest at the G1 phase through activation of the postmitotic G1 checkpoint (Torchia et al., 2009). However, if G1 checkpoint including p53 is dysfunctional, the oncogenic effect of Aurora-A would be enhanced. Liao et al. (2007) demonstrated that arsenic exposure promotes centrosome amplification in p53-compromised (p53 dysfunction or inhibited) lung cells. These results suggest that arsenic-induced tumorigenesis frequently accompanies dysfunction of p53. In addition, arsenic-induced BD has a higher p53 mutation rate and is prone to malignant transformation (i.e., invasive skin cancers) compared with non-arsenic–induced BD (Chang et al., 1998; Hsu et al., 1999). Our study revealed that arsenic exposure promotes Aurora-A overexpression and p53 dysfunction of keratinocytes both in vitro and in vivo. These results suggest that Aurora-A and p53 dysregulation may enhance cancer progression through a synergic effect during the progression stage of arsenic carcinogenesis. However, how Aurora-A interacts with dysfunctional p53 in the progression of arsenic-related carcinogenesis needs further investigation.

Recent studies showed that Aurora kinases are one of the major targets in anticancer therapy. There are about 30 Aurora
kinase inhibitors in different stages of clinical trials. The small molecules inhibit Aurora-A kinase activity through interaction with Aurora-A by binding with hydrogen or covalent bonds (Kollareddy et al., 2012). In addition, we previously revealed that curcumin exhibits the anticancer effect through inhibition of Aurora-A (Liu et al., 2011). Collectively, the inhibition of Aurora-A overexpression may be a new strategy in cancer prevention.

In summary, we reveal that E2F1-Aurora-A signaling pathway together with accumulation of dysfunctional p53 may act synergistically in the progression of arsenic-induced carcinogenesis. Inhibition of Aurora-A may disrupt this balance and thus may have therapeutic potential for prevention and/or treatment of arsenic-induced skin cancer.

**SUPPLEMENTARY DATA**

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

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**REFERENCES**


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