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Tam, Lok
Jiang, Ji
Wang, Pengcheng
et al.

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Arsenite Binds to the Zinc Finger Motif of TIP60 Histone Acetyltransferase and Induces Its Degradation via the 26S Proteasome

Lok Ming Tam[†], **Ji Jiang**[‡], **Pengcheng Wang**[†], **Lin Li**[§], **Weili Miao**[§], **Xuejiao Dong**[§], and **Yinsheng Wang**[*,†,‡,§,iD]

[†]Environmental Toxicology Graduate Program, University of California at Riverside, Mail Drop 027, Riverside, California 92521-0403, United States

[‡]Cell, Molecular, and Developmental Biology Graduate Program, University of California at Riverside, Mail Drop 027, Riverside, California 92521-0403, United States

[§]Department of Chemistry, University of California at Riverside, Mail Drop 027, Riverside, California 92521-0403, United States

Abstract

Arsenic is a ubiquitous environmental contaminant with widespread public health concern. Epidemiological studies have revealed that chronic human exposure to arsenic in drinking water is associated with the prevalence of skin, lung, and bladder cancers. Aberrant histone modifications (e.g., methylation, acetylation, and ubiquitination) were previously found to be accompanied by arsenic exposure; thus, perturbation of epigenetic pathways is thought to contribute to arsenic carcinogenesis. Arsenite is known to interact with zinc finger motifs of proteins, and zinc finger motif is present in and indispensable for the enzymatic activities of crucial histone-modifying enzymes especially the MYST family of histone acetyltransferases (e.g., TIP60). Hence, we reasoned that trivalent arsenic may target the zinc finger motif of these enzymes, disturb their enzymatic activities, and alter histone acetylation. Herein, we found that As^{3+} could bind directly to the zinc-finger motif of TIP60 *in vitro* and in cells. In addition, exposure to As^{3+} could lead to a dose-dependent decrease in TIP60 protein level via the ubiquitin-proteasome pathway. Thus, the results from the present study revealed, for the first time, that arsenite may target cysteine residues in the zinc-finger motif of the TIP60 histone acetyltransferase, thereby altering the H4K16Ac histone epigenetic mark. Our results also shed some new light on the mechanisms underlying the arsenic-induced epigenotoxicity and carcinogenesis in humans.

[*]**Corresponding Author**. Yinsheng.Wang@ucr.edu. Phone: (951)827-2700. Fax: (951)827-4713.

ORCID

Yinsheng Wang: 0000-0001-5565-283X

ASSOCIATED CONTENT

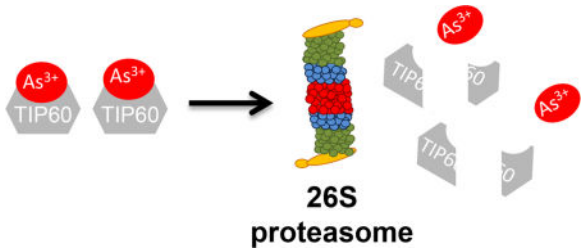
Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.chemrestox.7b00146.

MALDI-MS revealing effects of DTT on interaction between zinc finger peptide of TIP60 and As^{3+}; quantification data showing effects of arsenite treatment on levels of mutated forms of TIP60 protein; results from histone acetyltransferase assay (PDF)

The authors declare no competing financial interest.

Graphical abstract



INTRODUCTION

Being the 20th most abundant element in the Earth's crust, arsenic exists in inorganic forms as arsenate and arsenite as well as in organic forms.[1] Arsenite, the main inorganic form of arsenic present in the environment, especially in groundwater, is generally more toxic to living organisms than the organic form. Because of various natural processes such as weathering and widespread industrial use, exposure to arsenic species has become a serious public health concern, as indicated by arsenic being on the top of the Priority List of Hazardous Substances in the Agency for Toxic Substances and Disease Registry.[2] In view of the tremendous environmental impact and toxic potential of arsenic, the World Health Organization and the US Environmental Protection Agency have recommended a threshold concentration for arsenic in drinking water as 10 ppb.[3,4] Nonetheless, approximately 150 million people in more than 70 countries are exposed to excessive amounts of arsenic species through contaminated drinking water and diet.[5]

Over the last few decades, numerous epidemiological, cellular, and animal studies have provided a large body of evidence to support that chronic exposure to arsenic in drinking water is strongly associated with the increased incidence of bladder, lung, liver, skin, and kidney tumors.[6,7] Furthermore, arsenic is found to elicit other adverse human health effects including neurotoxicity, the endemic "blackfoot disease", cardiovascular disease, and childhood neurodevelopmental defects.[8] However, the mechanisms through which arsenic exposure leads to carcinogenesis remain incompletely understood.

The binding between As^{3+} and protein cysteine sulfhydryl groups is thought to play an important role in arsenic toxicity and carcinogenicity.[2,15] In this vein, As^{3+} was found to bind selectively to the C3H- and C4-types of zinc finger motifs.[16] In addition, our recently published data showed that arsenite could bind to RNF20-RNF40 and FANCL E3 ubiquitin ligases as well as ten-eleven translocation (Tet) family proteins through their RING finger (C3HC4), RING-like PHD finger, and C3H-type zinc finger, respectively.[17–19] Along this line, apart from the carcinogenic effect, As^{3+} in the form of arsenic trioxide has been approved by the Food and Drug Administration for the treatment of acute promyelocytic leukemia.[20] In this context, As^{3+} was found to bind to the RING finger domain of PML in the oncogenic PML-RARα fusion protein, which enhanced the proteasomal degradation of the fusion protein.[21]

Recently, it was argued that arsenic may induce carcinogenesis through perturbation of epigenetic pathways as global loss of acetylation and trimethylation of histone H4 is commonly found in human tumors associated with chemical exposure.[7] In this respect, acetylation of lysine 16 in histone H4 (H4K16Ac) is crucial for promoting the access of DNA repair enzymes to damaged DNA.[9–11] Although diminished H4K16Ac was observed in UTOtsa human bladder epithelial cells upon chronic arsenite exposure, the molecular mechanism contributing to reduced H4K16 acetylation remains unclear.[12,13] Additionally, previous studies revealed that many crucial enzymes involved in the deposition of histone acetylation marks harbor a zinc finger motif that is essential for their enzymatic activities.[14] TIP60, a member of the MYST family histone acetyltransferases, which also contain zinc finger motifs, was found to play an important role in H4K16 acetylation.[22] On the basis of these previous findings, we reason that As^{3+} may interact with the zinc finger motif of TIP60 histone acetyltransferase, thereby altering its conformation, stability, and activity, and resulting in H4K16 hypoacetylation. To test this hypothesis, we examined the interaction between As^{3+} and the peptide derived from the zinc finger motif of human TIP60 protein *in vitro* and in human cells. We also demonstrated that arsenite exposure led to diminished levels of TIP60 protein via the ubiquitin-proteasome pathway. Finally, we found that the arsenite-induced decrease in H4K16Ac in cultured human cells depended, in part, on TIP60. Hence, the results from this study uncovered a novel molecular mechanism underlying the arsenic-induced perturbation in epigenetic signaling, thereby broadening our understanding about the arsenic-induced carcinogenicity.

EXPERIMENTAL PROCEDURES

Cell Culture

HEK293T cells (ATCC) were cultured in Dulbecco's modified Eagle's medium (DMEM, Thermo Fisher Scientific). All culture media except those used for transfection were supplemented with 10% fetal bovine serum (FBS, Thermo Fisher Scientific) and 1% penicillin/streptomycin solution (Millipore). The cells were maintained in a humidified atmosphere with 5% CO_2 at 37 °C, with medium renewal once in every 2 days depending on cell density. For plasmid transfection, cells were cultured in the same media as mentioned above except that no penicillin/streptomycin solution was added.

In Vitro Arsenite Binding Assay

The zinc finger peptide of TIP60 (with amino acid residues 261–286 a.a., i.e., LYLCEFCLKYGRSLKCLQRHLTKCDL) was obtained from ChinaPeptides (Shanghai, China), purified by HPLC, and used for *in vitro* binding assays. Arsenite binding to the zinc finger peptide was monitored by matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) mass spectrometry in the linear, positive-ion mode on a TOF-TOF 5800 instrument (Sciex, Framingham, MA). Peptides were dissolved at a concentration of 1 mg/mL in sterilized deionized water and diluted to a concentration of 100 μM in a buffer containing 20 mM Tris-HCl (pH 6.8) and 1 mM dithiothreitol. Aliquots of 100 μM peptides were incubated with 200 μM $NaAsO_2$ at room temperature for 1 h. The resultant solution was diluted by 100-fold and mixed with an equal volume of 2,5-dihydroxybenzoic acid matrix solution before spotting onto a sample plate. The mass spectrometer was equipped

with a pulsed nitrogen laser operating at 337 nm with a pulse duration of 3 ns. The acceleration voltage, grid voltage, and delayed extraction time were set at 20 kV, 65%, and 190 ns, respectively. Each mass spectrum was acquired from an average of signal from 100 laser shots.

Plasmid Construction

The expression plasmids for TIP60 were pcDNA3.1-HA-TIP60 (from Dr. Yingli Sun)[31] and pRK7-TIP60–3Flag. The expression plasmids of HA-TIP60 harboring the C263A, or C266A, or C283A mutation were obtained by site-directed mutagenesis, and the successful construction for the mutated plasmids was verified by sequencing analysis.

Streptavidin Agarose Affinity Assay and Western Blot

The biotin-As probe was synthesized previously.[17] HEK293T cells were transfected with wild-type or mutant form of HA-TIP60. At 24 h after the transfection, the cells were exposed with 5 μM biotin-As for 2 h and lysed in CelLytic M lysis buffer supplemented with a protease inhibitor cocktail (Sigma-Aldrich). The cell lysates were incubated with high-capacity streptavidin agarose beads for overnight. Streptavidin agarose beads were subsequently washed with 1× PBS, resuspended in SDS-PAGE loading buffer and separated by SDS-PAGE.

After SDS-PAGE separation, proteins were transferred to a nitrocellulose membrane using a transfer buffer containing Tris base, methanol, glycine, and water. The membrane was blocked with 5% BSA in PBST buffer for 2 h and incubated with rabbit anti-HA antibody at 4 °C overnight (1:10 000 dilution, Sigma-Aldrich). The membranes were washed with fresh PBST buffer at room temperature six times (5–10 min each). After washing, the membranes were incubated with rabbit secondary antibody at room temperature for 1 h. The membranes were subsequently washed with PBST six times. The secondary antibody was detected by using Amersham ECL Select Western blotting Detection Kit Reagent (GE Healthcare) and visualized with Hyblot CL autoradiography film (Denville Scientific, Inc., Metuchen, NJ). Similar experiments were also conducted by pretreatment of cells with 10 μM $ZnCl_2$, PAPAO, or $NaAsO_2$ for 1 h prior to the biotin-As treatment. Similarly, the pull-down experiments for mutant HA-TIP60 were conducted with the same biotin-As pull-down assay except that the cells were transfected with plasmids for expressing HA-TIP60 with specific Cys → Ala mutations.

For monitoring the effect of arsenite or MG132 on the protein level of HA-TIP60, HEK293T cells were transfected with HA-tagged TIP60 plasmids for 24 h and exposed to several different concentrations of $NaAsO_2$ (i.e., 0, 1, 2, and 5 μM) with or without cotreatment with 4 μM MG132 (Sigma-Aldrich) for another 24 h, followed by cell harvesting, protein extraction, and Western blot analysis for HA-TIP60, as described above.

Histone Acetyltransferase Assay

HEK293T cells were first transfected with FLAG-tagged TIP60 plasmid constructs for 24 h, followed by cell harvesting and protein extraction as described above. The FLAG-TIP60 protein was subsequently precipitated from protein lysates and quantified with Bradford

assay. After that, 50 μg of protein lysate in 800 μL of lysis buffer including protease inhibitor was incubated overnight with anti-FLAG M2 affinity gel (Sigma-Aldrich), followed by washing the beads with HAT buffer. Next, the beads with bound FLAG-TIP60 were suspended in a HAT reaction mixture, which was composed of the same amount of FLAG-TIP60, 3 μg of histone H4, and 100 μM acetyl-coenzyme A in 60 μL of HAT buffer containing 50 mM Tris HCl, 0.1 mM EDTA, 1 mM DTT, and 10% glycerol (pH 8.0), with or without 5 μM $NaAsO_2$. The HAT reaction was performed by incubating the reaction mixture at 37 °C. After a 24-h incubation, the reaction mixture was analyzed by Western blot as described above using rabbit anti-H4K16Ac (1:50 000 dilution, Millipore) and rabbit anti-histone H4 antibodies (1:5000 dilution, Cell Signaling Technology).

Histone Extraction

HEK293T cells were exposed to 0, 2, and 5 μM $NaAsO_2$ for 24 h and then harvested. The cell pellets were washed with 1× PBS and resuspended in Triton X-100 sucrose buffer containing 0.25 M sucrose, 0.01 M $MgCl_2$, 0.5 mM PMSF, 0.05 M Tris-HCl (pH 7.4), and 0.5% v/v Triton X-100. The resulting pellet was incubated overnight, with vortexing, in Triton X-100 sucrose buffer supplemented with protease inhibitor cocktail. The suspension was subsequently centrifuged, and the pellet was washed with the above-described sucrose buffer without Triton X-100. The histone proteins in the cell pellet were then extracted using 0.4 N sulfuric acid with vortexing at 4 °C for 4 h. The supernatant was subsequently collected and mixed with 10 volumes of cold acetone to precipitate histone proteins from the mixture at −20 °C overnight. Finally, the pellet of the precipitate was washed with acetone, dried by Speedvac, and redissolved in water for subsequent Western blot analysis using rabbit anti-H4K16Ac and rabbit anti-histone H4 antibodies as described above. The same experiment was conducted using *TIP60*$^{-/-}$ HEK293T cells, which were generated from CRISPR-Cas9 knockout technology, as described previously.[23]

Statistical Analysis

The quantification data represent the mean ± S.D. of results obtained from at least three independent experiments. The *p* values were calculated using unpaired two-tailed Student's *t*-test: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

RESULTS

Arsenite Exposure Leads to Diminished Level of TIP60, but not hMOF Protein

Jo et al.[12] showed that arsenite exposure could lead to H4K16 hypoacetylation in bladder epithelial cells. To explore the role of TIP60 in arsenite-induced alteration in H4K16Ac, we first asked whether arsenite exposure could alter the protein levels of the histone acetyltransferase. In this vein, it is worth noting that we attempted, but failed to detect endogenous TIP60 using Western blot analysis with TIP60 antibody from several commercial sources, which was also noted by others.[24,25] We were also not able to detect endogenous TIP60 by using a proteomic approach, where we fractionated the whole cell lysate of HEK293T cells using SDS-PAGE, cut the gel band from the region where TIP60 migrates, digested the proteins in-gel with trypsin, and subjected the digestion mixture to LC–MS/MS analysis on a Q Exactive Plus quadrupole-Orbitrap mass spectrometer. The

failure in detecting endogenous TIP60 is likely attributed to the relatively low level of expression of this protein in HEK293T cells. Thus, we treated HEK293T cells, which express HA-tagged TIP60, with increasing concentrations (i.e., 0, 1, 2, and 5 μM) of $NaAsO_2$ for 24 h and assessed the levels of HA-TIP60 by Western blot analysis. Our results revealed a dose-dependent decrease in the TIP60 protein level (Figure 1A,B). In contrast, similar Western blot analysis of hMOF, the other member of MYST family of histone acetyltransferase, revealed no significant alteration in the level of this protein in cells upon treatment with As^{3+} (Figure 1C,D).

As^{3+} Binds to the Cysteine Residues in the Zinc Finger Motif of TIP60 Protein *in Vitro* and in Cells

To explore the mechanism through which As^{3+} induces the decrease in TIP60 protein level, we examined whether $NaAsO_2$ could bind directly to the zinc finger motif of the protein by employing a mass spectrometry (MS)-based assay. We employed a synthetic peptide derived from the zinc finger motif of human TIP60, which carries a.a. 261–286. Positive-ion MALDI-TOF MS revealed the $[M + H]^+$ ion of the apopeptide at m/z 3146.79 (Figure 2A). Incubation of the apopeptide with As^{3+} leads to a +72 Da mass shift (Figure 2B), which emanates from the binding of the peptide with one As^{3+} after the release of three protons from the side chains of the three Cys residues in the peptide. In accordance with the previous findings about the interaction between As^{3+} and other C3H-type zinc finger proteins,[16,19] the MS result showed that arsenite could bind to the peptide derived from the Zn^{2+}-binding site of TIP60.

We also assessed how dithiothreitol (DTT) modulates the interaction between As^{3+} and the apopeptide of TIP60 by conducting the MALDI-TOF MS experiments with the use of different molar ratios of As^{3+}/DTT (i.e., 1:1, 1:5, and 1:25). Our results showed that the presence of an equal concentration of DTT did not affect the binding between As^{3+} and apopeptide (Figure S1A). Likewise, addition of a five-fold excess of DTT (As^{3+}/DTT = 1:5) did not abolish the interaction, though the relative intensity of the peak for As^{3+}-bound peptide was weakened (Figure S1B). The presence of DTT in 25-fold excess, however, led to the loss of signal for the As^{3+}-bound peptide (Figure S1C). Thus, As^{3+} binds more strongly with the C3H-type zinc finger peptide than DTT, which is not surprising considering that these bindings involve the formation of three and two As–S bonds, respectively.

We next asked whether the binding of arsenite with TIP60 occurs in mammalian cells. To this end, we treated HEK293T cells with a *p*-aminophenylarsenoxide (PAPAO)-conjugated biotin probe (Figure 3A) and assessed its interaction with ectopically expressed HA-TIP60 by streptavidin agarose affinity assay.[17] The Western blot result revealed that the biotin-As probe could facilitate the pull-down of HA-TIP60 protein in human cells, whereas we failed to pull down HA-TIP60 in the control experiment without the use of the biotin-As probe (Figure 3B). This result suggested that As^{3+} could bind to TIP60 in human cells. We next pretreated HA-TIP60-expressing HEK293T cells individually with 5 μM $ZnCl_2$, PAPAO, and $NaAsO_2$ and assessed the interaction between As^{3+} and HA-TIP60 by conducting the same pull-down assay. The result showed that pretreatment with $NaAsO_2$ or PAPAO could

suppress markedly the pull-down of HA-TIP60, though a slight decrease in pull-down was also observed for pretreatment with Zn^{2+} (Figure 3C,D). This result suggests that As^{3+} can displace Zn^{2+} bound to the zinc finger motif of HA-TIP60. To further substantiate the involvement of zinc finger cysteine residues of TIP60 in its binding toward As^{3+}, we performed site-directed mutagenesis to substitute individually the three Cys residues with Ala (i.e., C263A, C266A, and C283A) in the zinc finger motif of HA-TIP60, and conducted the same pull-down assays. Our results showed that all three mutant forms of HA-TIP60 exhibited significantly attenuated interaction with the biotin-As probe (Figure 3E,F), indicating that the biotin-As probe interacts with the cysteine residues in the zinc finger motif of the TIP60.

It is worth noting that the use of the biotin-As probe may not fully recapitulate the interaction between arsenite and the zinc finger motif of TIP60 because As^{3+} in the probe and arsenite can bind two and three cysteine residues, respectively. Hence, the binding affinity of biotin-As toward the zinc finger of TIP60 protein is expected to be weaker than that of arsenite. The observation that TIP60 can interact with the biotin-As probe in cells strongly suggests that the protein can also interact with arsenite in cells.

Arsenite Induces the Degradation of TIP60 Protein through the Ubiquitin–Proteasome Pathway and Inhibits TIP60-Mediated H4K16 Acetylation in Mammalian Cells

We next investigated whether the ubiquitin-proteasome pathway contributes to the As^{3+}-induced decrease of HA-tagged TIP60 protein. We repeated the dose-dependent experiment by cotreating HA-TIP60-expressing HEK293T cells with 4 μM MG132 (with DMSO as control) in conjunction with 0, 2, and 5 μM of $NaAsO_2$, where MG132 is a peptide aldehyde, carbobenzoxy-Leu-Leu-leucinal, and serves as a proteasomal inhibitor. As displayed in Figure 4, we found that the addition of 4 μM MG132 could augment the HA-TIP60 protein level when compared with the DMSO control group. Importantly, the arsenite-induced decrease in HA-TIP60 protein was abolished in cells cotreated with MG132 (Figure 4B). Thus, As^{3+} induces decrease in HA-TIP60 protein by promoting its degradation via the ubiquitin-proteasome pathway.

Similar experiments with the aforementioned mutant forms of TIP60 protein (i.e., C263A, C266A, and C283A) showed that the substitution of any of zinc finger cysteines to an alanine abrogated the arsenite-induced proteasomal degradation of the TIP60 protein (Figure S2). This result indicates that the arsenite-induced decrease in HA-TIP60 protein is due to the selective and strong binding of arsenite to zinc-binding cysteine residues within the zinc finger motifs of TIP60.

Having demonstrated that As^{3+} induces the degradation of TIP60 protein via the ubiquitin-proteasome pathway, we next investigated, by using histone acetyltransferase (HAT) assay, whether the As^{3+}-induced hypoacetylation of H4K16 is attributed, in part, to the arsenite-induced decrease in enzymatic activity of HA-TIP60. After exposure to 5 μM arsenite for 24 h, we precipitated Flag-TIP60 from HEK293T cells using anti- FLAG M2 affinity gel and employed the immunoprecipitated protein for the HAT assay. Our result revealed that arsenite treatment did not result in any significant alteration in the HAT activity of TIP60 (Figure S3).

Finally, we examined whether endogenous TIP60 plays a significant role in As^{3+}-induced decrease in H4K16Ac. Thus, we employed the CRISPR-Cas9 genome-editing method to generate *TIP60*$^{-/-}$ HEK293T cells.[23] Our Western blot result indicated that a 24-h treatment with 2 and 5 μM of arsenite led to significantly decreased levels of H4K16Ac in both wild-type and *TIP60*$^{-/-}$ cells (Figure 5A). However, the magnitude of decrease in the level of H4K16Ac in *TIP60*$^{-/-}$ cells was significantly lower than that in the HEK293T cells upon exposure with 5 μM As^{3+} (Figure 5B), thereby demonstrating that As^{3+}-induced decrease in H4K16Ac involves endogenous TIP60.

DISCUSSION

Role of Zinc Fingers in the Toxicity of Arsenic and Different Mechanisms Involving the MYST Family of Histone Acetyltransferases

As^{3+} can interact with zinc finger proteins,[2,15] particularly with the C3H- and C4-types of zinc finger motifs,[16] and the interaction between As^{3+} and zinc finger domains of crucial DNA repair enzymes is thought to contribute to arsenic toxicity and carcinogenicity.[24] In this vein, As^{3+} is known to bind to and inhibit the activities of several enzymes that function in DNA repair including poly(ADP-ribose) polymerase-1 (PARP-1), XPA, and bacterial formamidopyrimidine-DNA glycosylase.[16] The results from our streptavidin agarose pull-down assay and MALDI-TOF-MS measurements demonstrated that the cysteine residues in the zinc-finger motif of TIP60 can bind directly with As^{3+}. In addition, our Western blot results revealed that As^{3+} binding could stimulate the degradation of TIP60 via the 26S proteasome, a finding that is reminiscent of the As^{3+}-induced proteasomal degradation of the PML-RARα oncoprotein.[21] Interestingly, Legube et al.[25] found that TIP60 protein undergoes ubiquitination-mediated degradation, where Mdm2 serves as the E3 ubiquitin ligase. Furthermore, arsenite was found to induce the overexpression of Mdm2 protein[26] without increasing its mRNA expression level,[27] though the mechanism underlying the arsenite-induced Mdm2 protein overexpression remains unknown.

Given that zinc finger motif within the catalytic MYST domain of TIP60 is indispensable for its acetyltransferase activity and protein–protein interaction,[28] the arsenic-induced structural alteration of TIP60 could modify the catalytic core of TIP60, thereby affecting its acetyltransferase activity and its interaction with regulatory proteins such as the aforementioned Mdm2. However, our *in vitro* HAT assay indicated that arsenic could not significantly diminish the histone acetyltransferase activity of TIP60, indicating that the catalytic core of TIP60 is not affected by the potential conformational change conferred by As^{3+} binding. Combining our result and the previous findings, two possible mechanisms for arsenic-induced proteasomal degradation of TIP60 can be deduced. In this respect, arsenite may elicit the overexpression of Mdm2 protein, which may stimulate the ubiquitination and proteasomal degradation of the TIP60 protein. Alternatively, As^{3+} may displace the Zn^{2+} ion in the zinc-finger domain of TIP60, thereby altering the conformation of the MYST domain of TIP60 and enhancing its proteasomal degradation. The relative contributions of these two mechanisms in arsenite-induced degradation of TIP60 remains unknown.

TIP60 and hMOF both regulate the level of H4K16Ac. Liu et al.[29] previously reported that arsenite induces global H4K16 hypoacetylation through binding directly to hMOF, another

member of the MYST-family histone acetyltransferases. Different from our findings made for TIP60, Liu et al.[29] showed that arsenite does not cause significant dose-dependent alterations in the protein level of hMOF, which is in keeping with our Western blot results. Therefore, arsenite targets hMOF and TIP60 both through binding directly to their zinc-finger motifs. The arsenic-induced conformational change of catalytic MYST domains of these two HATs, however, elicits H4K16 hypoacetylation via distinct mechanisms, namely through enhanced degradation of TIP60 protein and diminished catalytic activities of hMOF. This observation unveiled the complexity in the mechanisms of MYST-family of histone acetyltransferase in arsenic epigenotoxicity.

Implications in DNA Repair from Arsenite-Induced Perturbations in Epigenetic Signaling

Recently, epigenetic dysregulation is thought to play a central role in the mechanisms of arsenic carcinogenicity.[30] Previous research revealed the importance of TIP60-mediated acetylation in the sensing and repair of DNA double strand breaks (DSBs).[31] In particular, the TIP60-catalyzed H4K16 acetylation is responsible for the decompaction of the 30 nm chromatin fiber,[10] where efficient DNA repair necessitates chromatin modification/remodeling to allow DNA repair machinery to access DNA damage sites in chromatin.[32] In addition, TIP60 functions in DNA damage response signaling by directly acetylating ATM.[22] We found that arsenite enhanced the proteasomal degradation of TIP60, thereby reducing the overall activities of TIP60. Thus, our work suggests that arsenite may trigger carcinogenesis by binding to TIP60 protein and triggering its degradation, thereby compromising DNA damage response signaling and DNA repair (Figure 6). In this context, it is worth noting that, owing to the lack of a suitable antibody for detecting endogenous TIP60 protein, most of our cellular experiments were conducted with the use of ectopically expressed TIP60. This may not fully reflect the interaction between As^{3+} and endogenous TIP60. Nevertheless, the finding that the arsenite-induced decrease in H4K16Ac in TIP60-depleted cells is not as pronounced as in the isogenic wild-type cells (Figure 5) suggests that the findings made with the ectopically expressed TIP60 are relevant to what may occur with the endogenous protein upon arsenite exposure.

Implications in Environmental Exposure to Arsenic

There are about 150 million people living in the environment containing amounts of inorganic arsenic in drinking water that are much higher than the recommended standard of 10 ppb set by the World Health Organization and U.S. Environmental Protection Agency.[5] Early life exposure to arsenic was found to confer elevated incidence and severity of cancer in childhood and adulthood as supported by a latency period that is decades long.[33] Arsenic-induced epigenetic dysregulation not only gives rise to DNA repair inhibition and cancer progression partly through disrupted gene imprinting, but also leads to the impairment in learning and memory as well as cognitive functions in children and adults after arsenic exposure.[33,34] Furthermore, histone acetylation was found to regulate cognitive gene expression by chromatin remodeling in neurons, similar to chromatin decompaction during DNA damage response.[34] Given that TIP60 is abundant in the learning and memory center in the Drosophila brain, and is necessary for mediating axonal outgrowth,[34] our work sets the stage for future studies on the roles of arsenic binding with TIP60 in perturbing the epigenetic patterns in cognitive impairment in human brain after chronic arsenic exposure.

Combining all these profound implications together, we should protect the living environments of the populations especially infants and children to prevent chronic arsenic exposure via contaminated drinking water so as to alleviate the life-long risk of developing cancers and neurocognitive impairment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

PAPAO	*p*-aminophenylarsenoxide
DTT	dithiothreitol
MALDI-TOF MS	matrix-assisted laser desorption ionization-time-of-flight mass spectrometry
PARP-1	poly(ADP-ribose)-polymerase-1

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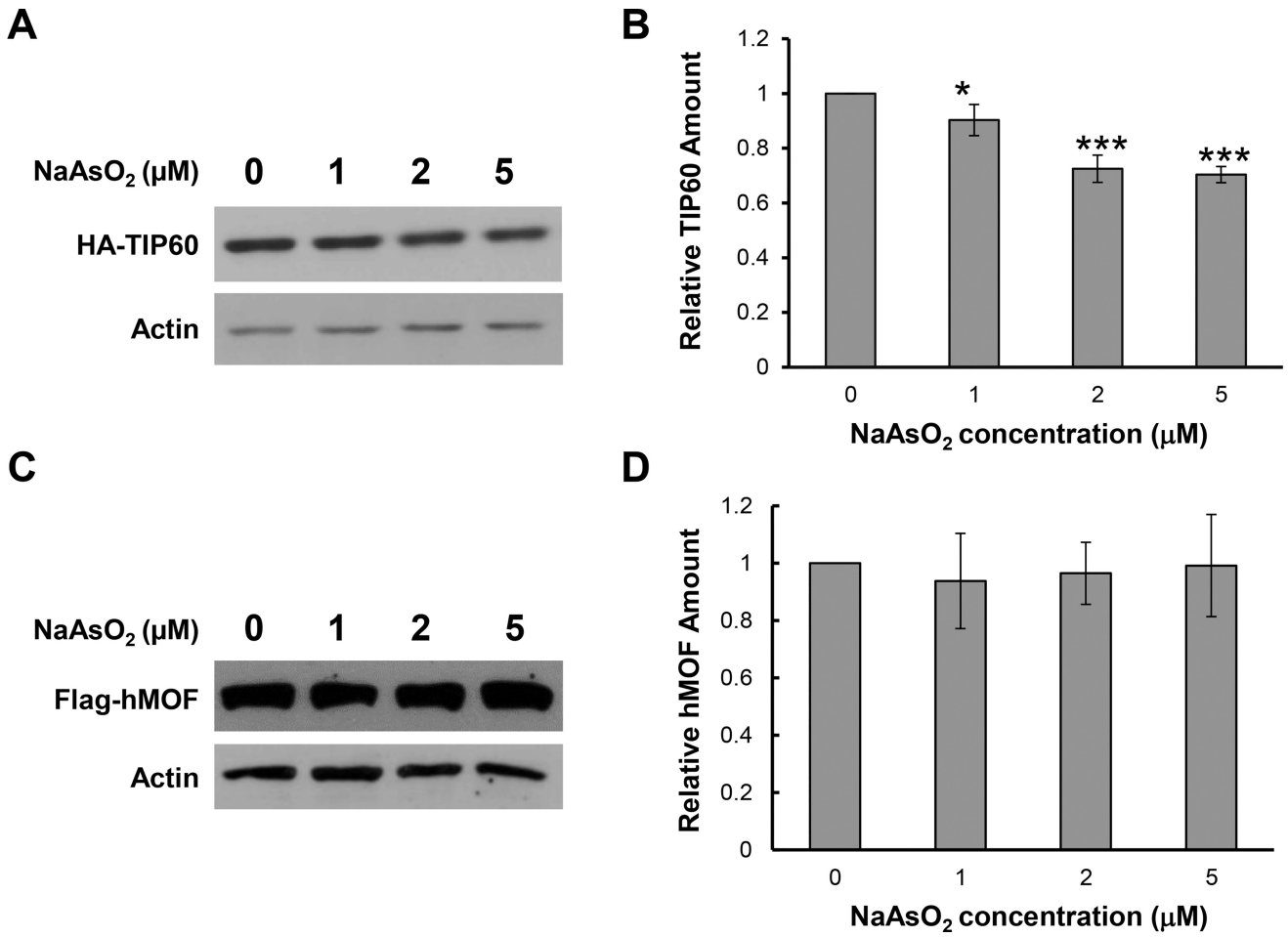

Figure 1.

Arsenite treatment results in a dose-dependent decrease in TIP60, but not hMOF protein. Western blot images showing the effect of treatment with different doses of arsenite (0, 1, 2, and 5 μM) on the protein levels of (A) HA-TIP60 and (C) hMOF. Quantification results of the relative level of (B) TIP60 and (D) hMOF proteins following exposure to different doses of $NaAsO_2$ ($n = 3$). Error bars represent standard deviations. The p values were calculated using unpaired two-tailed student's t-test, and the asterisks designate significant differences between arsenite-treated samples and untreated control (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

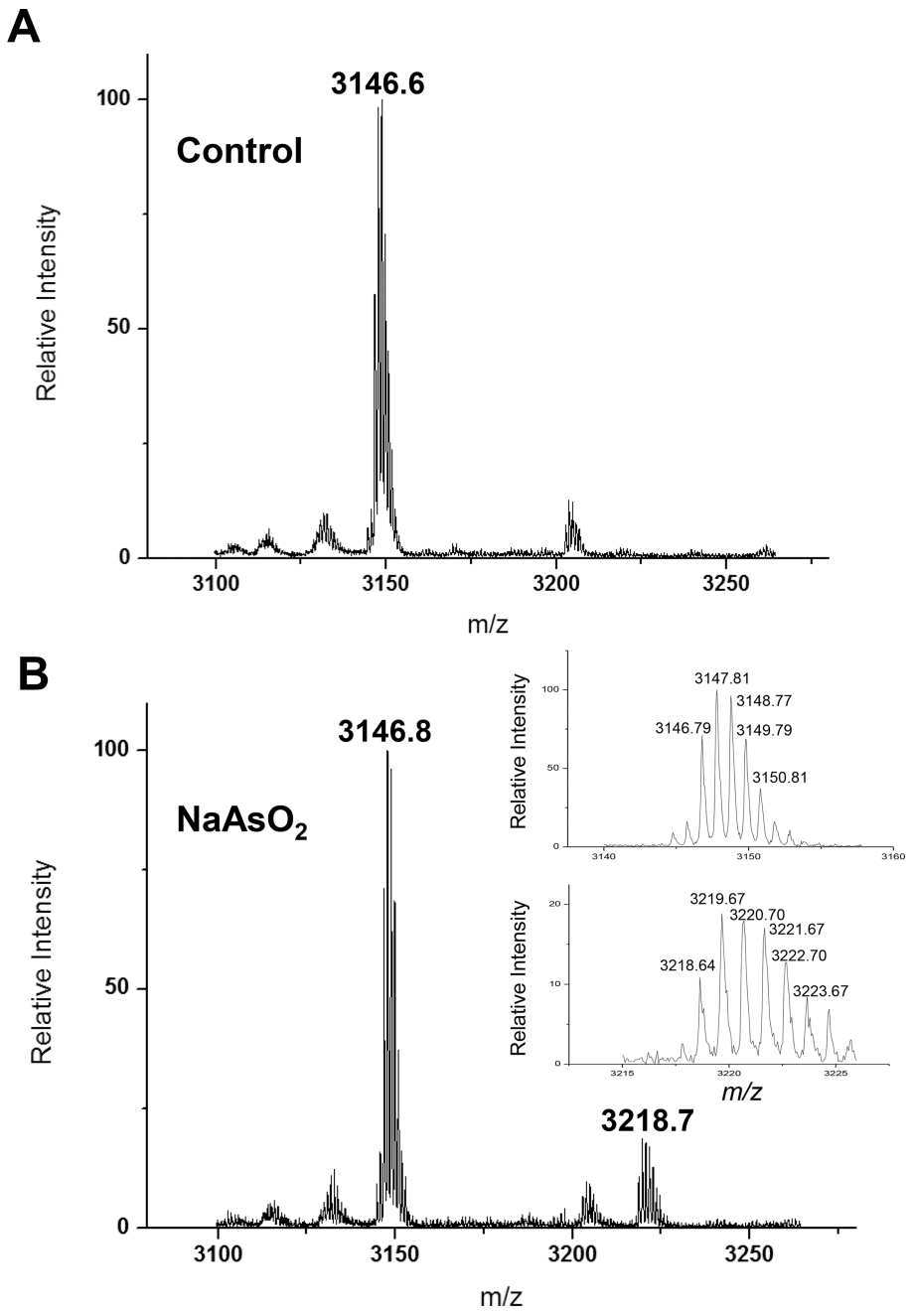

Figure 2.

In vitro binding between arsenite and zinc finger peptide of TIP60. Shown are the MALDI-TOF MS results for monitoring the zinc finger peptide of (A) TIP60 and (B) its interaction with As^{3+}.

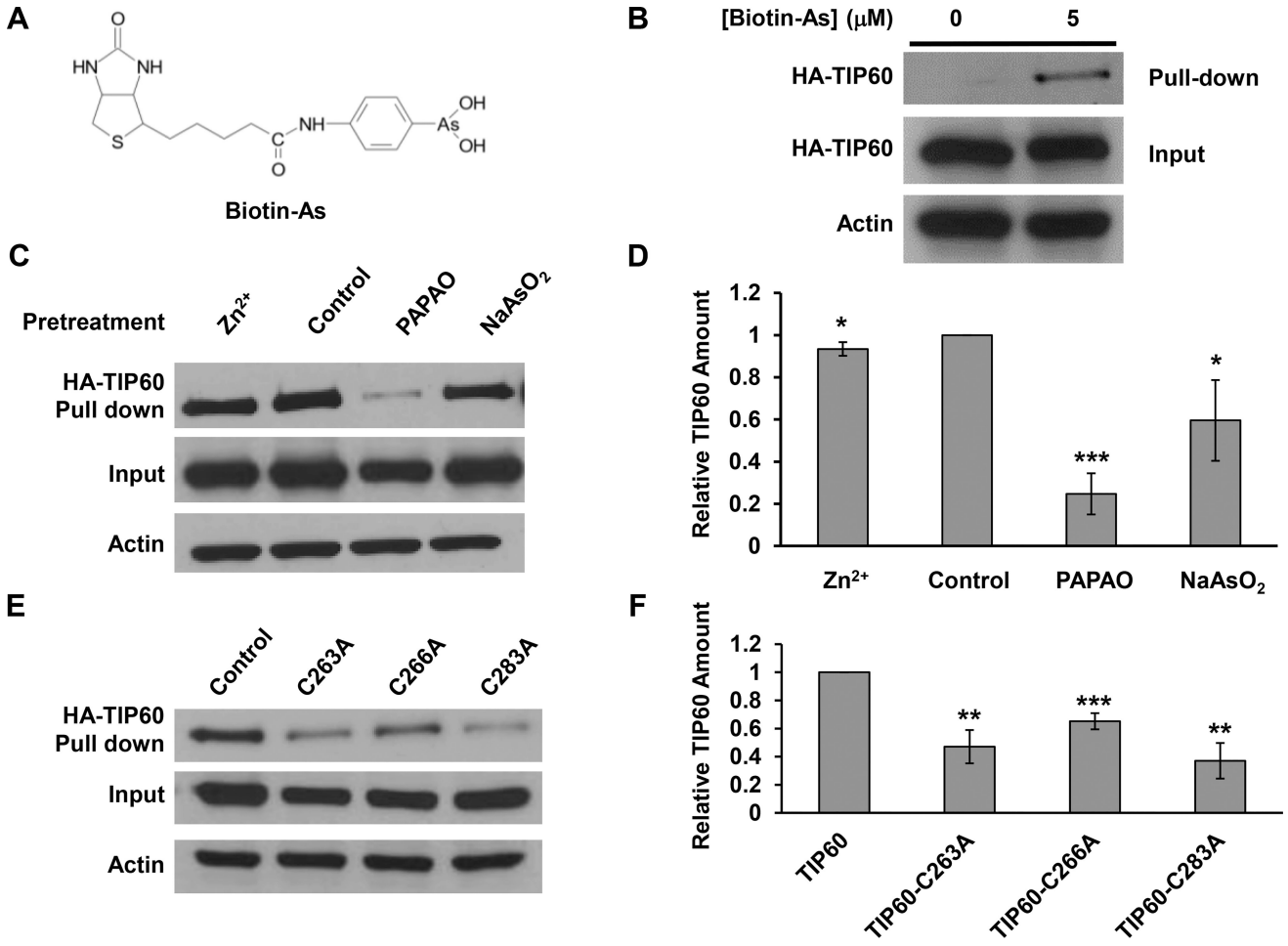

Figure 3.

Streptavidin agarose affinity pull-down assay using biotin-As as a probe reveals the binding between As^{3+} and the C3H-type zinc finger domain of TIP60. (A) Structure of the biotin-As probe. (B) Treatment of HEK293T cells expressing HA-TIP60 with 5 μM biotin-As probe could pull down HA-TIP60 by using streptavidin agarose. (C) Pretreatment of cells with 10 μM $ZnCl_2$, $NaAsO_2$, or PAPAO for 2 h attenuates the binding between biotin-As and TIP60. (D) Relative protein level of TIP60 pulled down by streptavidin agarose using biotin-As probe upon pretreatment with 10 μM $ZnCl_2$ or $NaAsO_2$ or PAPAO when compared with untreated control ($n = 3$). The p values were calculated using unpaired two-tailed student's t-test, and the asterisks designate significant differences between different pretreatments and control without pretreatment (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). (E) Mutations of cysteine residues (Cys→ Ala) in the zinc finger motif of TIP60 weakened the pull-down of TIP60 with the biotin-As probe. (F) Relative levels of different forms of TIP60 protein pulled down by streptavidin agarose using the biotin-As probe. Specific Cys → Ala mutations within the zinc finger domain of TIP60 led to compromised interaction between TIP60 and biotin-As ($n = 3$). The p values were calculated using unpaired two-tailed student's t-test, and the asterisks designate significant differences between mutant forms of HA-TIP60 and the wild-type control (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). Error bars in panels D and F indicate standard deviations.

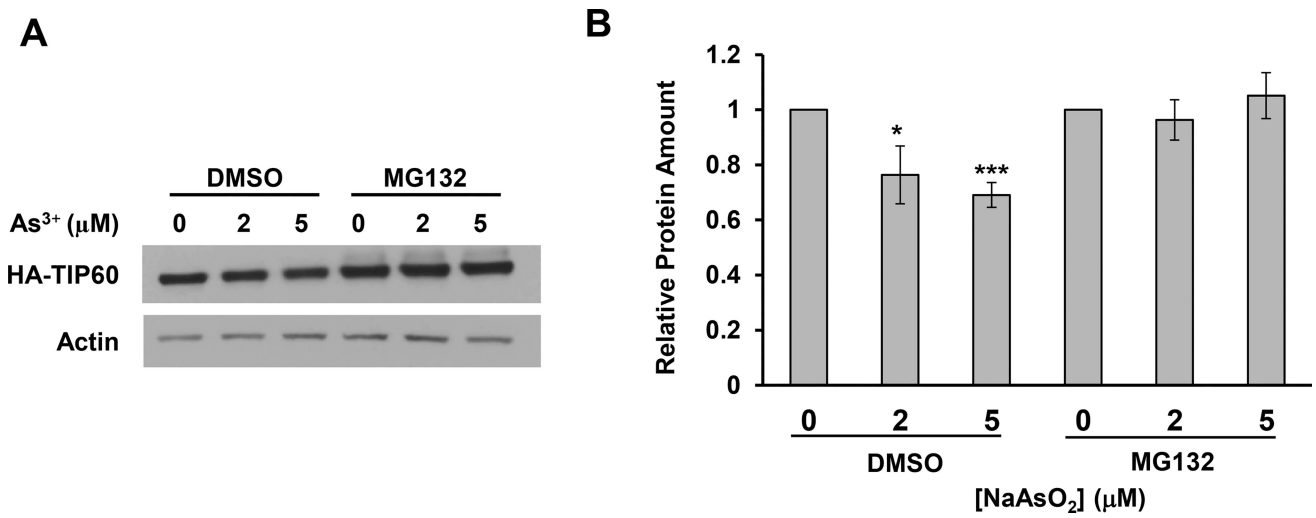

Figure 4.

Exposure to arsenite induces proteasomal degradation of TIP60. (A) Co-treatment of HEK293T cells with 4 μM MG132 together with different doses of $NaAsO_2$ (0, 2, and 5 μM) could rescue the dose-dependent decrease of TIP60 protein induced by arsenite exposure. (B) Relative protein level of TIP60 upon treatment with different doses of $NaAsO_2$ together with MG132 or DMSO control ($n = 3$). The p values were calculated using unpaired two-tailed student's t-test, and the asterisks designate significant differences between the arsenite-treated samples and untreated control (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). Error bars in panel B represent standard deviations.

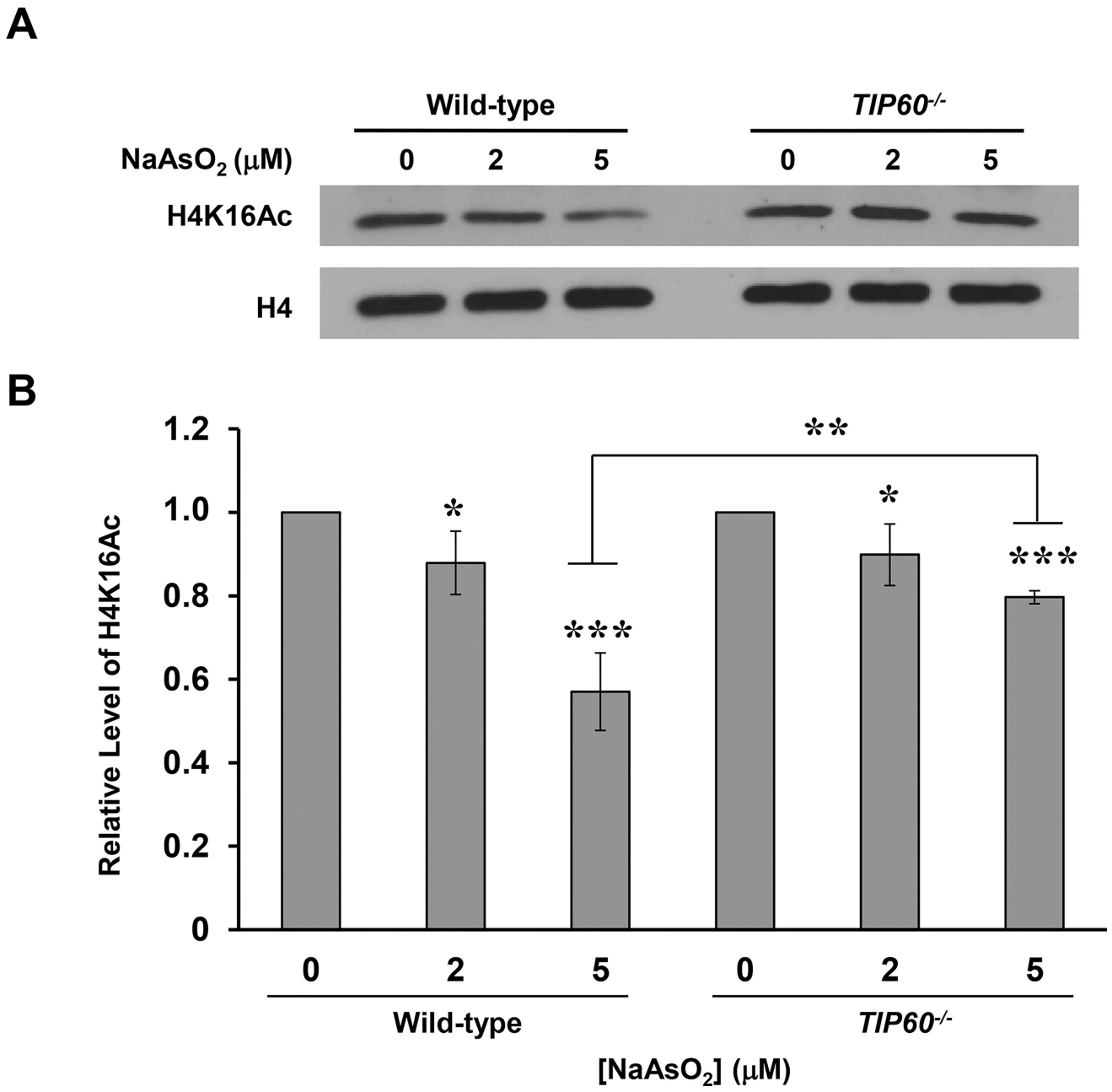

Figure 5.

Arsenite exposure led to diminished H4K16Ac in both wild-type and *TIP60*$^{-/-}$ HEK293T cells, and a higher concentration of arsenite (i.e., 5 μM) gave rise to a more pronounced decrease in H4K16Ac level in wild-type cells than *TIP60*$^{-/-}$ cells. (A) Western blot image showing the effect of different concentrations (0, 2, and 5 μM) of $NaAsO_2$ on the level of H4K16Ac in wild-type and *TIP60*$^{-/-}$ HEK293T cells. (B) Relative levels of H4K16Ac in wild-type and *TIP60* knockout cells upon treatment with different concentrations of arsenite ($n = 4$). Error bars represent standard deviations. The p values were calculated using unpaired two-tailed student's t-test, and the asterisks designate significant differences between arsenite-treated cells and untreated control as well as the significant difference upon

the same arsenite treatment between wild-type and *TIP60*$^{-/-}$ cell lines (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

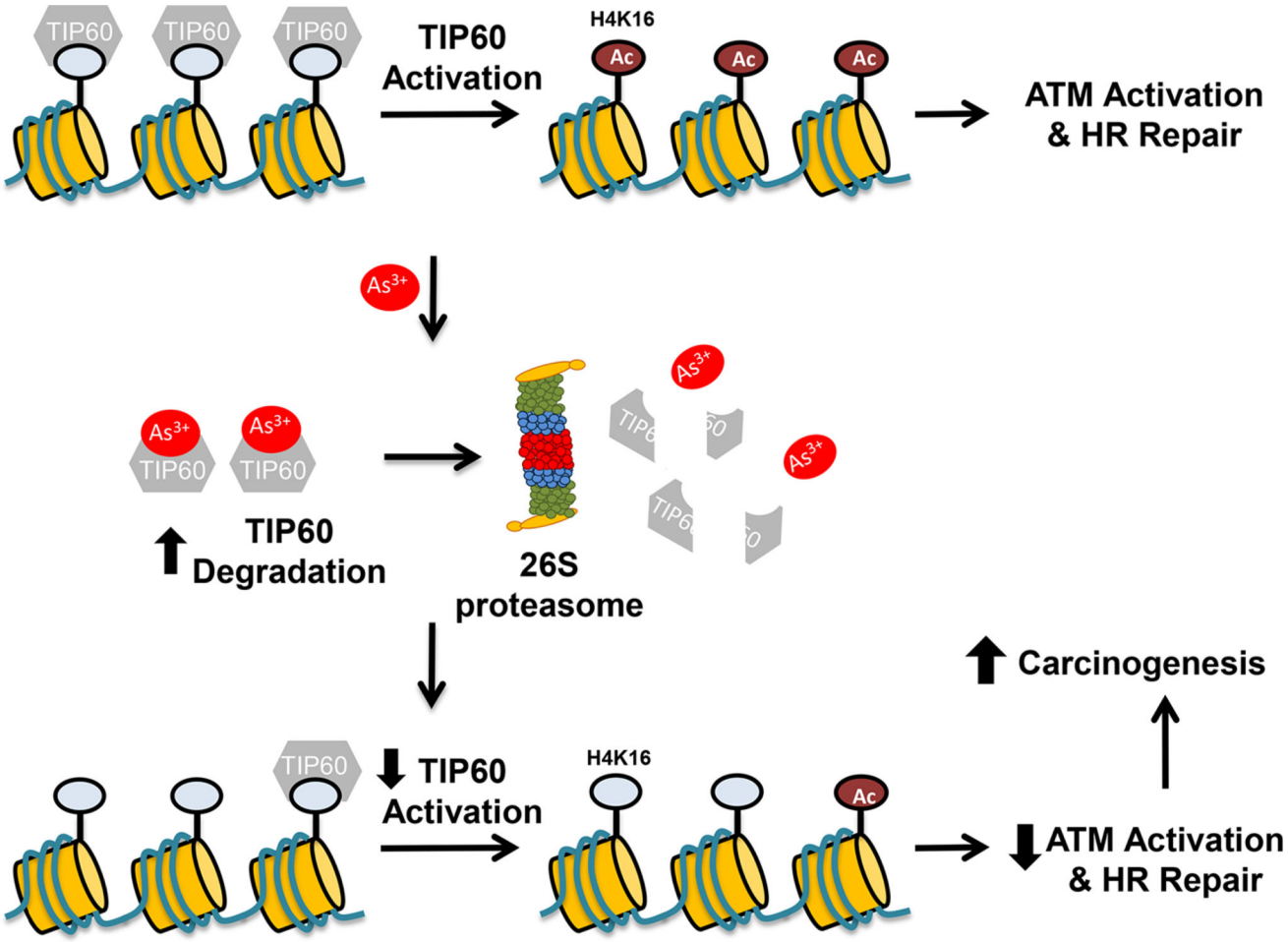

Figure 6.

Proposed role of TIP60 in arsenic-induced carcinogenesis. As^{3+} interacts with cysteine residues in the zinc finger motifs of TIP60, promoting its degradation via the ubiquitin-proteasomal pathway. Diminished level of TIP60 protein results in lower level of H4K16Ac. This in turn generates a biochemically less accessible chromatin state, which may compromise DNA repair.