Mutant p53 Protein Is Targeted by Arsenic for Degradation and Plays a Role in Arsenic-mediated Growth Suppression*\textsuperscript{a,c}

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p53 is frequently mutated in tumor cells, and mutant p53 is often highly expressed due to its increased half-life. Thus, targeting mutant p53 for degradation might be explored as a therapeutic strategy to manage tumors that are addicted to mutant p53 for survival. Arsenic trioxide, a drug for patients with acute promyelocytic leukemia, is found to target and degrade a class of proteins with high levels of cysteine residues and vicinal thiol groups, such as promyelocytic leukemia protein (PML) and PML-retinoic acid receptor \(\alpha\) fusion protein. Interestingly, wild type p53 is accumulated in cells treated with arsenic compounds, presumably due to arsenic-induced oxidative stresses. In this study, we found that wild type p53 is induced by arsenic trioxide in tumor cells, consistent with published studies. In contrast, we found that arsenic compounds degrade both endogenous and ectopically expressed mutant p53 in time- and dose-dependent manners. We also found that arsenic trioxide decreases the stability of mutant p53 protein through a proteasomal pathway, and blockage of mutant p53 nuclear export can alleviate the arsenic-induced mutant p53 degradation. Furthermore, we found that knockdown of endogenous mutant p53 sensitizes, whereas ectopic expression of mutant p53 desensitizes, tumor cells to arsenic treatment. Taken together, we found that mutant p53 is a target of arsenic compounds, which provides an insight into exploring arsenic compound-based therapy for tumors harboring a mutant p53.

Mutations of the p53 tumor suppressor gene are the most common genetic alterations in human cancer (1–3). The majority of tumor-derived p53 mutations are missense point mutations and clustered within the central DNA binding domain (1), which either change wild type p53 conformation (conformation mutants, such as R175H and R249S) or abrogate its DNA contact (contact mutants, such as R248W and R273H). These mutations make p53 protein defective in sequence-specific DNA binding to p53-responsive elements in the p53 target genes and loss of tumor suppression (4, 5), which defines the classical loss of function for mutant p53. p53 mutants also acquire new oncogenic properties independent of wild type p53, termed gain of function. This notion is supported by studies with mutant p53 knock-in mice. Compared with p53-null mice, knock-in mice that carry one null allele and one mutant allele of the p53 gene develop novel tumors (6, 7), and knock-in of mutant p53 specifically promotes invasion and metastasis (8). More importantly, clinical studies have shown that a high level of mutant p53 is correlated with more aggressive tumors and poorer outcomes (8, 9). Apparently, gain of function is dependent on mutant p53 transcriptional activity. Multiple target genes of mutant p53 have been identified, including ones involved in anti-apoptotic signaling, inflammation, invasion and migration, and proliferation (4, 10–14).

One gain of function for mutant p53 is to physically interact with and inactivate p63 (15) and p73 (16). Unlike frequent mutations of the p53 gene, p63 and p73 genes are rarely mutated in human cancers (17, 18). Substantial data have supported the role of p63 and p73 in suppressing tumorigenesis (19, 20). In cultured tumor cells, mutant p53 binds to p63 and p73 and inhibits their functions. Down-modulation of p63 and p73 in p53-null cells can mimic the gain-of-function phenotypes of mutant p53 (6). Mutant p53 can also drive invasion through silencing of the p63 pathway (21). In a more physiological context, mutant p53 was found to hamper the cellular response to DNA damage through inhibiting endogenous p73 (22). Thus, it is postulated that one mode of gain of function for mutant p53 is accomplished through inactivation of p63/p73 transcriptional activities (16, 23, 24).

The oncogenic properties of mutant p53 as discussed above provide a rationale for new therapeutic strategies for tumors carrying a mutant p53, including those targeting mutant p53 degradation and conversion of mutant p53 protein into wild type conformation. A recent study suggested that covalent modification of thiols in the core domain of mutant p53 by small molecule PRIMA-1 is capable of stabilizing and reactivating mutant p53 into an active conformation (25). However, there are at least 2,314 types of p53 mutants in cancers (see the World Health Organization International Agency for Research on Cancer Web site). The diverse classes of p53 mutants impose a serious challenge for the development of versatile p53-reactivating drugs. Furthermore, a number of p53 mutants, if stabilized, would associate with p63/p73 and inhibit their transcriptional activity, further contributing to the malignant phenotype of cancer cells (15, 24, 26). Thus, the therapeutic strategies targeting mutant p53 degradation may be a more feasible and logical option than making mutant p53 wild type.

Arsenic is a metalloid with paradoxical biological effects: carcinogenesis and anticancer. As a known carcinogen, arsenic can induce tumors of the skin, bladder, liver, and lung (27–30). As a meta-estrogen, arsenic promotes mammary carcinogenesis (31). However, arsenic is also a novel promising anticancer
agent for treating acute promyelocytic leukemia (APL)\(^3\) and other tumors with mild adverse effects (27–30). Studies showed that arsenic trioxide produces remissions in patients with APL at least in part through degradation of the aberrant PML-retinoic acid receptor \(\alpha\) (PML-RAR\(\alpha\)) fusion protein (32, 33). Upon arsenic trioxide exposure, PML undergoes intermolecular disulfide formation and directly binds to arsenic via cysteine residues in zinc fingers located within the RBCC domain of PML and PML-RAR\(\alpha\). Disulfide-linked PML or PML-RAR\(\alpha\) multimers form nuclear matrix-associated nuclear bodies and become sumoylated and then degraded (32, 33). In addition to PML-RAR\(\alpha\), several other proteins with a high content of cysteine residues and vicinal thiol groups are also targets of arsenic, such as AML1/MDS1/EVI1 protein produced from a fusion gene generated by a \(t(3;21)(q26;q22)\) translocation in chronic myelogenous leukemia (34), Glz2 transcriptional effector in the Hedgehog pathway (35), AKT protein (36), BCR/ABL protein produced from a fusion gene generated by a \(t(9;22)\) chromosomal translocation in chronic myelogenous leukemia (37), and survivin (38).

The success of arsenic trioxide in treating APL patients via targeted degradation of PML-RAR\(\alpha\) fusion protein prompted us to examine the potential effect of this drug on mutant p53. In this study, we explored whether arsenic compounds could target mutant p53 for degradation and inhibit the proliferation of tumor cells harboring a mutant p53.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—Human colon adenocarcinoma cell line SW480 (containing mutant p53 R273H/P309S); human pancreatic cancer cell line MIA PaCa-2 (containing mutant R248W); human keratinocyte cell line HaCaT (containing mutant H179Y); and HaCaT (containing mutant H179Y/H9251), retinoid acid receptor \(\alpha\) (RAR\(\alpha\)); MEF, mouse embryo fibroblast; PARP, poly(ADP-ribose) polymerase; PML, promyelocytic leukemia.

**FIGURE 1.** Arsenic trioxide increases wild type p53 expression in tumor cells and normal MEF cells in a dose-dependent manner. A, Western blots were prepared with extracts from MCF7 cells untreated or treated with 5–30 \(\mu\)M arsenic trioxide for 24 h and then probed with antibodies against p53, Puma, p21, PARP, and GAPDH, respectively. B and C, the experiments were performed as in A except that RKO cells (B) and HCT116 cells (C) were used. D, the experiments were performed as in A except that normal MEF cells were untreated or treated by 2.5–20 \(\mu\)M arsenic trioxide for 12 h.
cells were maintained in fresh medium for the next 12 days and then fixed with methanol/glacial acetic acid (7:1) and stained with 0.1% crystal violet. For long term treatment, the cells were untreated or treated with low doses of arsenic trioxide (0.75 μM/H9262M) or sodium arsenate (1.5 μM/H9262M) from day 3. The cells were maintained in medium with arsenic for next 12 days and then fixed and stained as above. To determine whether knockdown of endogenous mutant p53 sensitizes tumor cells to arsenic treatment in colony formation, MIA PaCa-2 (1,000 cells/well) and SW480 cells (2,000 cells/well) were uninduced or induced with tetracycline (0.1 μg/ml) to knock down endogenous mutant p53 for 3 days. The cells were then untreated or treated as above, and the dose and duration for short or long term arsenic treatment were indicated above each experiment.

Statistics—All experiments were performed in triplicates. Two-group comparisons were analyzed by two-sided Student’s t test. p values were calculated, and p < 0.05 was considered significant.

RESULTS

Arsenic Compounds Increase Wild Type, but Inhibit Mutant, p53 Expression in Time- and Dose-dependent Manners—Arsenic trioxide is an effective agent in treating APL potentially via apoptosis, decreased proliferation, and/or differentiation. The proapoptotic role of arsenic may be in part dependent on up-regulation of wild type p53 expression (39–42). In our present study, we found that in MCF7, RKO, HCT116, and MEF cells, wild type p53 and its targets p21 and Puma were induced by treatment with arsenic trioxide along with cleavage of PARP.
indicative of cells undergoing apoptosis (Fig. 1). However, more than 50% of human tumors have p53 mutations (43). Furthermore, it is evident that in addition to loss of function for tumor suppression, mutant p53 contributes to the malignant process by acquiring additional activities, including enhanced cell proliferation, increased invasion and metastasis potential, and resistance to anticancer therapy (4, 6, 44). Thus, it is crucially important to determine whether arsenic can increase or decrease mutant p53 expression in cancer therapy. In our previous studies, we analyzed the requirement of endogenous mutant p53 in the physiological context for cell survival in the SW480 cell line, which contains mutant R273H/P309S, and the MIA PaCa-2 cell line, which contains mutant R248W. We found that mutant p53 is required for cell proliferation and resistance to DNA damage treatment in SW480 and MIA PaCa-2 cells (13, 14). Thus, both cell lines along with the HaCaT cell line were used to examine the effect of arsenic compounds on the expression of endogenous mutant p53. We found that upon arsenic treatment, the level of mutant p53 protein in HaCaT cells was markedly decreased by arsenic trioxide (Fig. 2A, top) and sodium arsenite (Fig. 2A, bottom), reaching maximum reduction within 4–6 h. The level of actin protein was not affected and thus was used as a loading control.

**FIGURE 5.** Arsenic compounds decrease the half-life of mutant p53 but have no effect on the level of p53 transcript. A, the half-life of mutant p53 protein was shortened by sodium arsenite in HaCaT cells. Western blots were prepared with extracts from HaCaT cells that were treated with cycloheximide (50 μg/ml) in the absence or presence of 10 μM sodium arsenite for 0–8 h and then probed with antibodies against p53 and actin, respectively. B, the relative levels of mutant p53 protein measured in A were normalized by levels of actin protein and then plotted versus time. C, the level of mutant p53 transcript is not altered by arsenic compounds. RT-PCR was performed with total RNAs isolated from HaCaT cells (left) and MIA PaCa-2 cells (right) untreated or treated with 10 μM arsenic trioxide or sodium arsenite. Actin mRNA was amplified as a loading control.

**FIGURE 6.** Arsenic mediates proteasomal degradation of mutant p53 primarily in the cytoplasm. A, arsenic-induced degradation of mutant p53 is inhibited in HaCaT cells treated with MG132, a 26 S proteasome inhibitor. Western blots were prepared with extracts from HaCaT cells that were untreated or pretreated with 5 μM MG132 for 4 h and then untreated or treated with arsenic trioxide for 3 h. B and C, the experiments were performed as in A except that SW480 cells (B) and MIA PaCa-2 cells (C) were treated by arsenic trioxide for 5 and 7 h, respectively. D and E, arsenic-induced degradation of mutant p53 in HaCaT cells (D) and SW480 cells (E) is inhibited upon treatment with leptomycin B, an inhibitor of nuclear export factor CRM1. Western blots were performed with extracts from HaCaT and SW480 cells, which were untreated or pretreated with 25 ng/ml leptomycin B for 5 h and then untreated or treated with arsenic trioxide for 3 h.
Mutant p53, a Novel Target of Arsenic

A. HCT116(p53-/-)-R273H and HCT116(p53-/-)-R175H

|       | HCT116(p53-/-)-R273H | HCT116(p53-/-)-R175H |
|-------|----------------------|----------------------|
| #7    | -        +           | -        +           |
| #8    | -        +           | -        +           |
|       | Induction           | Induction           |
| p53   | -        +           | -        +           |
| Actin | -        +           | -        +           |

B. HCT116(p53-/-)-R273H #7

- No treatment
- Treated for 24 h at day 3
- NaAsO2 (20 μM)
- As2O3 (7.5 μM)
- Treated from day 3 to 15
- NaAsO2 (1.5 μM)
- As2O3 (0.75 μM)

C. HCT116(p53-/-)-R175H #33

- No treatment
- Treated for 24 h at day 3
- NaAsO2 (20 μM)
- As2O3 (7.5 μM)
- Treated from day 3 to 15
- NaAsO2 (1.5 μM)
- As2O3 (0.75 μM)
(Fig. 2A, actin panels). We also found that upon exposure to arsenic trioxide and sodium arsenite, the level of mutant p53 showed a marked decrease as early as 2 h in SW480 cells (Fig. 2B) and 4 h in MIA PaCa-2 cells (Fig. 2C), reaching maximum reduction within 4–6 h.

p63 and p73 are members of the p53 family and are tumor suppressors (19, 20). In cultured tumor cells, mutant p53 binds to p63 and p73 and inhibits their functions. Down-modulation of p63 and p73 in p53-null cells can mimic the gain-of-function phenotypes of mutant p53 (6). Thus, the effect of arsenic on the expression of p63 and p73 was examined in HaCaT and SW480 cells, respectively. We found that TAp73 expression of p63 and p73 was examined in HaCaT and SW480 cells. The effect of arsenic on the expression of mutant p53 was investigated in HaCaT cells (Fig. 3, A and B). We also showed that arsenic reduces p63 and p73 expression as early as 2 h in SW480 cells (Fig. 2C). However, upon treatment with sodium arsenite, the half-life of mutant p53 protein was shortened to 2.3 h (Fig. 5, A and B).

Next, we examined whether arsenic compounds have an impact on arsenic-mediated down-regulation of mutant p53 transcription. These results suggest that arsenic compounds do not down-regulate mutant p53 transcription but rather target the stability of mutant p53 protein.

**Arsenic Compounds Decrease the Half-life of Mutant p53 Protein but Have No Effect on the Level of p53 Transcript**—To examine whether down-regulation of mutant p53 by arsenic is through a post-translational mechanism, HaCaT cells were treated with cycloheximide (50 μg/ml) in the absence or presence of sodium arsenite for 0–8 h. The relative levels of mutant p53 protein were quantified by Western blotting and normalized by levels of actin protein, which were then plotted versus time (h) to calculate the half-life of mutant p53. We found that the half-life for mutant p53 protein in HaCaT cells was about 6 h (Fig. 5, A and B). However, upon treatment with sodium arsenic, the half-life of mutant p53 protein was shortened to 2.3 h (Fig. 5, A and B).

**Arsenic Mediates Proteasomal Degradation of Mutant p53**—In the above study, we showed that arsenic decreases the stability of mutant p53 protein. Thus, we examined whether mutant p53 is degraded by the proteasomal pathway. HaCaT cells were untreated or treated with MG132, a 26 S proteasome inhibitor, in the absence or presence of arsenic trioxide. We found that arsenic-induced mutant p53 degradation was almost abrogated by MG132 (Fig. 6A). To confirm this, similar experiments were performed with SW480 (Fig. 6B) and MIA PaCa-2 (Fig. 6C) cells. Again, we showed that arsenic-mediated degradation of mutant p53 was nearly abolished by MG132.

p53, a nuclear protein, is known to shuttle between the nucleus and the cytoplasm. It has been reported previously that leptomycin B inhibits nuclear export of wild type p53, leading to p53 accumulation in the nucleus (47). Thus, we examined whether leptomycin B has an impact on arsenic-induced mutant p53 degradation. We found that after pre-treatment with leptomycin B, arsenic-induced mutant p53 degradation was examined in HaCaT (Fig. 6C) and SW480 (Fig. 6D) cells was obviously inhibited. Taken together, these results suggest that arsenic inhibits mutant p53 expression at least in part via nuclear export and the proteasome degradation pathway.

In addition to directly targeting PML-RARα fusion protein for degradation via the sumoylation pathway (32, 33), arsenic trioxide also induces APL cell apoptosis by disrupting mitochondrial respiration and promoting generation of oxygen free radicals, such as hydrogen peroxide (H2O2) (48, 49). Thus, the potential effect of H2O2 on wild type p53 expression in HCT116 cells was measured as a control. We found that wild type p53 expression in HCT116 cells increased by H2O2 in a dose-dependent manner (Fig. 7A, actin panels). We also found that upon exposure to arsenic trioxide and sodium arsenite, the level of mutant p53 showed a marked decrease as early as 2 h in HCT116 cells (Fig. 7B) and 4 h in MIA PaCa-2 cells (Fig. 7C), reaching maximum reduction within 4–6 h. We showed that the level of mutant p53 protein in HaCaT cells was markedly decreased by sodium arsenite (supplemental Fig. S1).

Next, we determined how much arsenic is required for inhibiting mutant p53 expression. To test this, HaCaT cells were treated with various doses of sodium arsenite (Fig. 3A, top) or arsenic trioxide (Fig. 3A, bottom) for 3 h. We showed that the level of mutant p53 protein in HaCaT cells was markedly decreased at a dose as low as 2.5 μM. Thus, the arsenic concentration for mutant p53 degradation in cultured solid tumor cells is close to that for activation of wild type p53 in MEF cells (Fig. 1D). The concentration is also close to a clinically relevant dose (0.15–0.3 mg/kg, equal to 0.75–1.5 μM) of arsenic trioxide for myeloma patients (45) and the plasma peak values of 1.535–3.424 μM in APL patients treated with arsenic trioxide (46). As the concentration was increased to 5–10 μM, arsenic further decreased the level of mutant p53 in HaCaT cells (Fig. 3A). Interestingly, the effect of arsenic on mutant p53 down-regulation was not further increased at concentrations of more than 10 μM (Fig. 3A). A similar result was seen in SW480 cells and p53<sup>R270H/-</sup>-MEF cells (Fig. 3, B and C).

We then asked whether arsenic compounds can decrease ectopically expressed mutant p53. For this purpose, p53-null HCT116 cells were induced to express R273H or R175H for 24 h and then mock-treated or treated with various concentrations of arsenic trioxide or sodium arsenite for 2 h. We found that the levels of R273H and R175H proteins were decreased by 2.5 μM arsenic trioxide (Fig. 4, A and B, left panels) and 5 μM sodium arsenite (Fig. 4, A and B, right panels). Taken together, the immediate effect of arsenic compounds on mutant p53 points to a post-translational mechanism by which mutant p53 expression is suppressed by arsenic.

**Arsenic Compounds Decrease the Half-life of Mutant p53 Protein but Have No Effect on the Level of p53 Transcript**—To examine whether down-regulation of mutant p53 by arsenic is through a post-translational mechanism, HaCaT cells were treated with cycloheximide (50 μg/ml) in the absence or presence of sodium arsenite for 0–8 h. The relative levels of mutant p53 protein were quantified by Western blotting and normalized by levels of actin protein, which were then plotted versus time (h) to calculate the half-life of mutant p53. We found that the half-life for mutant p53 protein in HaCaT cells was about 6 h (Fig. 5, A and B). However, upon treatment with sodium arsenic acid, the half-life of mutant p53 protein was shortened to 2.3 h (Fig. 5, A and B).

Next, we examined the possibility that mutant p53 transcription is suppressed by arsenic. RT-PCR was performed to measure the level of mutant p53 transcript in HaCaT and MIA PaCa-2 cells, which were untreated or treated with 10 μM arsenic trioxide or sodium arsenite as indicated. The actin mRNA was also measured and used as a control. We found that the level of mutant p53 mRNA in HaCaT cells (Fig. 5C, left) and MIA PaCa-2 cells (Fig. 5C, right) was not obviously altered by arsenic. These results suggest that arsenic compounds do not down-regulate mutant p53 transcription but rather target the stability of mutant p53 protein.
manner (supplemental Fig. S2A), consistent with our previous results (50). However, we found that the levels of mutant p53 in SW480 cells were not obviously altered by H$_2$O$_2$ (supplemental Fig. S2B). Thus, we conclude that arsenic-induced H$_2$O$_2$ does not play a major role in arsenic-mediated degradation of mutant p53.
Ectopic Expression of Mutant p53 Desensitizes, whereas Knockdown of Endogenous Mutant p53 Sensitizes, Tumor Cells to Arsenic Treatment—Because mutant p53 has been implicated in promoting cell survival and conferring cell resistance to DNA damage (14, 51), we tested whether ectopic expression of mutant p53 is capable of making cells resistant to arsenic treatment. For this purpose, a colony formation assay was performed with p53-null HCT116 cells, which were uninduced or induced to express R273H or R175H for 3 days (Fig. 7A) and then untreated or treated with sodium arsenite or arsenic trioxide as indicated (Fig. 7, B and C). We found that ectopic expression of R273H (Fig. 7B) or R175H (Fig. 7C) conferred p53-null HCT116 cells resistance to both short term high doses of arsenic treatment (Fig. 7, B and C, middle panels) and long term low doses of arsenic treatment (Fig. 7, B and C, right panels). Also, R273H and R175H alone had little effect on cell proliferation in p53-null HCT116 cells (Fig. 7, B and C, left panels).

To further examine the role of mutant p53 in promoting the survival and proliferation of arsenic-treated cells, a colony formation assay was performed with MIA PaCa-2 and SW480 cells, in which endogenous mutant p53 can be inducibly knocked down (14). Consistent with previous reports (14), cell proliferation was inhibited by knockdown of mutant p53 in both MIA PaCa-2 and SW480 cells (Fig. 8, A and B, left panels). Moreover, we found that, compared with control cells, cells with mutant p53-KD were highly sensitive to both short term and long term arsenic treatments because the number of colonies in the p53-KD cells was reduced from 58.2% to 32.2% of the control cells (Fig. 8, A and B, middle and right panels). Together, these results suggest that mutant p53 plays a role in arsenic-induced growth suppression, and knockdown of mutant p53 further sensitizes tumor cells to arsenic.

DISCUSSION

Mutant p53 is often highly expressed in tumor cells due to its increased half-life. Thus, targeting mutant p53 for degradation is a novel therapeutic strategy to manage tumors that are addicted to mutant p53 for survival. However, the signaling pathways through which mutant p53 protein escapes degradation present difficult challenges in the drug discovery.

A study using mutant p53 knock-in mice showed that in normal tissues, the murine mutant p53 protein is sensitive to MDM2-dependent degradation (52). However, mutant p53 in tumor cells derived from such mice is resistant to MDM2-mediated degradation, leading to mutant p53 stabilization (52). Although MDM2 can drive the degradation of both mutant and wild type p53, the ability of MDM2 to function as a ubiquitin ligase is less important in the degradation of mutant p53, which is heavily ubiquitinated in an MDM2-independent manner (53). Interestingly, the increase in ubiquitination of mutant p53 proteins does not lead to increased degradation (53). Furthermore, MDM2 functions as an oncogene in a p53-independent manner: MDM2 is often amplified in many cancers (54); high levels of MDM2 are found in many tumor cell lines that express high levels of mutant p53 (55); and targeted expression of MDM2 in the mammary gland of p53 knock-out mice causes polyplody and mammary tumor development (56). Thus, targeted expression or activation of MDM2 is not feasible to degrade mutant p53 as a therapeutic strategy for tumors carrying a mutant p53.

Arsenic trioxide, an effective chemical drug for the treatment of patients with APL, was found to target and degrade proteins with a high content of cysteines and vicinal thiol groups, such as PML and PML-RARα (32). A recent study showed that PML protein interacts with, and enhances the transcriptional activity of, mutant p53 (57). Thus, we hypothesized that arsenic compounds may have a promising role in targeting mutant p53 for degradation. In this study, we found that in tumor cells, wild type p53 is induced by arsenic trioxide. In contrast, we found that arsenic compounds degrade endogenous and ectopically expressed mutant p53 in time- and dose-dependent manners in solid tumor cell lines. In addition, we found that arsenic compounds decrease the half-life of mutant p53 protein through the proteasome degradation pathway, and blockage of mutant p53 nuclear export alleviates arsenic-induced mutant p53 degradation. Furthermore, we found that ectopic expression of mutant p53 desensitizes, whereas knockdown of endogenous mutant p53 sensitizes, tumor cells to arsenic-induced growth suppression.

The arsenic-mediated selective degradation for mutant p53 and stability for wild type p53 is probably due to activation of different signal pathways. Arsenic trioxide can disrupt mitochondrial respiration and promote generation of oxygen free radicals, such as H₂O₂ (48, 49). It was reported that in response to hydrogen peroxide, p53 stability is increased via post-translational modifications (58). However, it is not clear whether and how mutant p53 protein is modified under a stress condition. Thus, we postulate that, like PML-RARα, mutant p53 might be subject to ubiquitination and subsequently proteasomal degradation upon exposure to arsenic.

Most proteins degraded by the 20 S/26 S proteasome are modified by ubiquitination. However, arsenic trioxide exerts its therapeutic effect in patients with APL at least in part by triggering degradation of PML-RARα oncogenic protein via sumoylation, which requires RNF4, the human orthologue of the yeast SUMO-dependent E3 ubiquitin ligase (59). In this study, although mutant p53 was found to be degraded by arsenic via the 26 S proteasome pathway, it is still unclear whether the degradation is triggered by sumoylation and what residues in mutant p53 are involved in this post-translational modification. Several studies showed that wild type p53 protein is modified by SUMO. Topors SUMO-1 E3 ligase enhances the conjugation of SUMO-1 to p53 at lysine 386 in vivo (60). SUMO ligases PIAS1 and PIASxβ together with Ubc9-conjugating enzyme modify p53 at lysine 386 and repress the transcriptional activity of p53 (61). In addition, SUMO E3 ligase PIASγ can interact with and sumoylate wild type p53 and enhance the localization of p53 to the cytoplasm (62). Nevertheless, it is also possible that other modifications may elicit arsenic-induced mutant p53 degradation. Thus, future studies are warranted to determine whether mutant p53 is sumoylated and to identify SUMO E3 ligase for mutant p53. These studies would provide an insight into developing novel strategies for tumors harboring a mutant p53.
