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Activation of the Mitogen- and Stress-activated Kinase 1 by Arsenic Trioxide*

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Arsenic trioxide (As_2O_3) is a potent inducer of apoptosis of leukemic cells *in vitro* and *in vivo*, but the precise mechanisms by which it mediates such effects are not well defined. We provide evidence that As_2O_3 induces activation of the mitogen- and stress-activated kinase 1 (MSK1) and downstream phosphorylation of its substrate, histone H3, in leukemia cell lines. Such activation requires upstream engagement of p38 MAPK, as demonstrated by experiments using pharmacological inhibitors of p38 or p38α knock-out cells. Arsenic-induced apoptosis was enhanced in cells in which MSK1 expression was decreased using small interfering RNA and in *Msk1* knock-out mouse embryonic fibroblasts, suggesting that this kinase is activated in a negative feedback regulatory manner to regulate As_2O_3 responses. Consistent with this, pharmacological inhibition of MSK1 enhanced the suppressive effects of As_2O_3 on the growth of primary leukemic progenitors from chronic myelogenous leukemia patients. Altogether, these findings indicate an important role for MSK1 downstream of p38 in the regulation of As_2O_3 responses.

Arsenic trioxide (As_2O_3) is a heavy metal derivative that induces apoptosis and suppresses the growth of malignant cells *in vitro* and *in vivo* (1–5). This agent has been approved for the treatment of one form of acute leukemia, acute promyelocytic leukemia, for which it is used alone or in combination with retinoids (1, 6–9). As_2O_3 is also currently under investigation for the treatment of other hematological malignancies, including chronic myelogenous leukemia (CML),[2] multiple myeloma, and myelodysplastic syndromes (1, 3, 10, 12–14). The major limiting factor for the use of As_2O_3 in the treatment of various hematological malignancies is the requirement of high cellular concentrations for the induction of antitumor effects in different malignant phenotypes. It is well established that the effects of As_2O_3 are dose-dependent, with higher concentrations ($\geq$2 μM) leading to apoptosis and lower concentrations ($\leq$0.5 μM) inducing differentiation (1–6). Thus, the potential development of future translational approaches would be facilitated by the identification of the means to enhance arsenic-dependent apoptosis at lower final concentrations of As_2O_3.

MAPKs are activated by various extracellular signals such as growth factors, stress, and cytokines to regulate downstream phosphorylation of target proteins, including transcription factors and protein kinases (15–22). There are three different major groups of MAPKs: ERKs, JNKs/SAPKs (stress-activated protein kinases), and p38 MAPKs, all of which play important roles in the regulation of cell proliferation, differentiation, survival, and apoptosis (15–22).

We have previously shown that p38 MAPK and its downstream effector, MAPKAPK2 (MAPK-activated protein kinase-2), are activated during treatment of cells with As_2O_3 (23). Such activation of p38 MAPK appears to occur in a negative feedback regulatory manner, as pharmacological inhibitors of p38 were found to enhance the generation of pro-apoptotic responses by As_2O_3 in target cells. In the present study, we sought to identify the downstream effectors of p38 that may account for the negative regulatory properties of the p38 MAPK pathway in the generation of As_2O_3 responses. We found that the nucleosomal kinase MSK1 is also activated in an As_2O_3-inducible manner. Pharmacological or small interfering RNA (siRNA)-mediated knockdown of MSK1 resulted in enhanced induction of apoptosis in leukemia cell lines and primary leukemic progenitors from the bone marrow of CML patients. Moreover As_2O_3-dependent apoptosis was enhanced in cells with targeted disruption of *Msk1* and the related *Msk2* gene. Altogether, these data identify MSK1 as a negative regulator of As_2O_3 responses.

MATERIALS AND METHODS

Cells and Reagents—The KT-1 CML and the NB-4 human acute promyelocytic leukemia cell lines were grown in RPMI 1640 medium supplemented with fetal bovine serum and antibiotics. Immortalized mouse embryonic fibroblasts (MEFs) were cultured in Dulbecco's modified Eagle's medium supplemented with fetal bovine serum and antibiotics. Immortalized MEFs from p38α knock-out mice (11) were kindly provided

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[2] The abbreviations used are: CML, chronic myelogenous leukemia; MAPKs, mitogen-activated protein kinases; ERKs, extracellular signal-regulated kinases; JNKs, c-Jun N-terminal kinases; MSK, mitogen- and stress-activated kinase; siRNA, small interfering RNA; MEFs, mouse embryonic fibroblasts; CFU-GM, colony-forming unit(s)-granulocyte/macrophage; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase; SGK, serum- and glucocorticoid-activated kinase.

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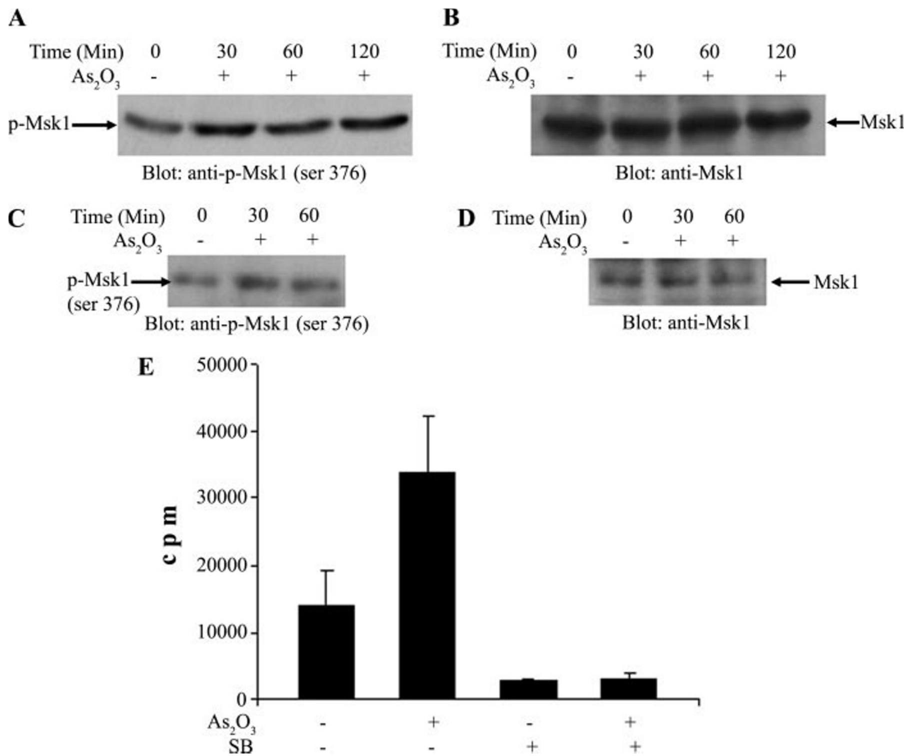

FIGURE 1. **As_2O_3-dependent phosphorylation and activation of the kinase MSK1.** *A*, KT-1 cells were treated with 2 μM As_2O_3 for the indicated times. Equal amounts of cell lysates were resolved by SDS-PAGE and immunoblotted with antibody against MSK1 phosphorylated (*p*) at Ser^{376}. *B*, the blot shown in *A* was stripped and reprobed with anti-MSK1 antibody to control for loading. *C*, NB-4 cells were treated with As_2O_3 (2 μM) for the indicated times. Equal amounts of cell lysates were resolved by SDS-PAGE and immunoblotted with antibody against MSK1 phosphorylated at Ser^{376}. *D*, the blot shown in *C* was stripped and reprobed with anti-MSK1 antibody to control for protein loading. *E*, KT-1 cells were incubated with SB 203580 (*SB*; 10 μM) for 60 min as indicated and then subsequently treated with As_2O_3 (2 μM) for 120 min. Total cell lysates were immunoprecipitated with antibody against MSK1, and *in vitro* kinase assays to detect MSK1 activation were performed using Akt/SGK as an exogenous substrate. The means ± S.D. of three experiments are shown.

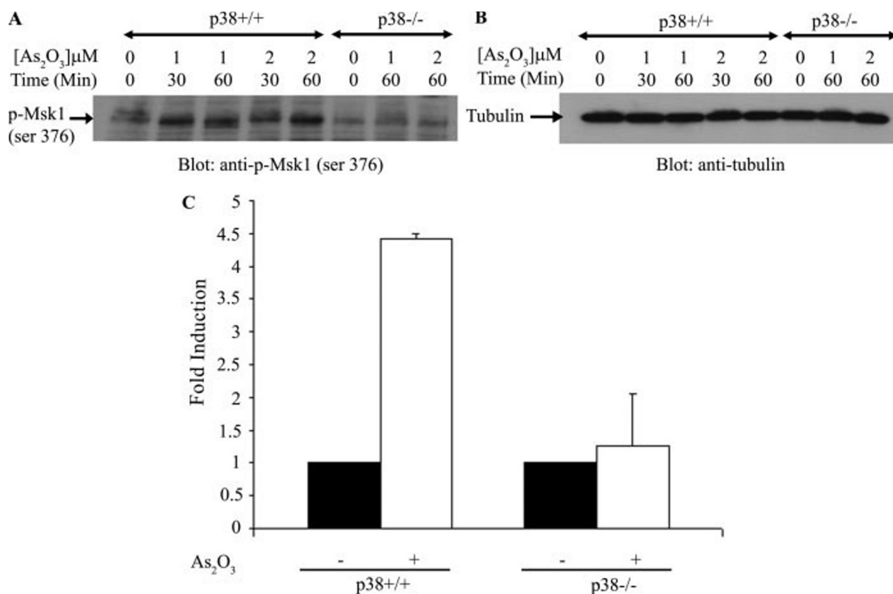

FIGURE 2. **As_2O_3-induced activation of the kinase MSK1 is p38-dependent.** *A*, $p38\alpha^{+/+}$ and $p38\alpha^{-/-}$ MEFs were incubated with As_2O_3 for the indicated times. Equal amounts of total cell lysates were analyzed by SDS-PAGE and immunoblotted with antibody against MSK1 phosphorylated (*p*) at Ser^{376}. *B*, the blot shown in *A* was stripped and reprobed with anti-tubulin antibody to control for protein loading. *C*, $p38\alpha^{+/+}$ and $p38\alpha^{-/-}$ MEFs were incubated with As_2O_3 for 120 min. Total cell lysates were immunoprecipitated with antibody against MSK1, and *in vitro* kinase assays to detect MSK1 activation were carried out on the immunoprecipitates. The means ± S.D. of four experiments are shown.

from Dr. Angel Nebreda (Centro Nacional de Investigaciones Oncológicas, Madrid, Spain). As_2O_3 was purchased from Sigma. The inhibitors SB 203580 and H-89 were obtained from Calbiochem and Alexis Biochemicals (San Diego, CA), respectively. Polyclonal antibodies against phosphorylated MSK1 and cleaved poly(ADP-ribose) polymerase were obtained from Cell Signaling Technology, Inc. (Beverly, MA). Antibodies against histone H3 phosphorylated at Ser^{10} and histone H3 were purchased from Upstate Biotechnology, Inc. Antibody against MSK1 was obtained from Zymed Laboratories Inc.. Antibodies against tubulin and glyceraldehyde-3-phosphate dehydrogenase were purchased from Abcam Inc. (Cambridge, MA) and Chemicon International, Inc. (Temecula, CA), respectively.

Cell Lysis, Immunoprecipitation, and Immunoblotting—Cells were incubated with As_2O_3 for the indicated times and lysed in phosphorylation lysis buffer as described previously (17, 18). In experiments in which pharmacological inhibitors were used, SB 203580 (10 μM) or H-89 (10 μM) was added 1 h prior to treatment with As_2O_3. Immunoprecipitations and immunoblotting using enhanced chemiluminescence method were performed as described previously (24, 25).

Kinase Assays—Cells were incubated with As_2O_3 for the indicted times. Total cell lysates were immunoprecipitated with antibody against MSK1 or non-immune rabbit IgG, which was used as a control. *In vitro* kinase assays were performed as described previously (26–29). For the MSK1 kinase assay, the values were calculated by subtracting the activity in the rabbit IgG immunoprecipitates from the kinase activity in the anti-MSK1 immunoprecipitates.

Evaluation of Apoptosis—Evaluation of apoptosis by annexin V/propidium iodide staining was performed using an apoptosis detection kit (Pharmingen) as described previously (23, 26). Briefly, cells were plated in 100-mm plates and treated

for 48 h with the indicated concentrations of As_2O_3. The cells were harvested, washed with cold phosphate-buffered saline, and then incubated for 15 min with fluorescein isothiocyanate-conjugated annexin V and propidium iodide prior to flow cytometric analysis. In the experiments in which the effects of siRNA-mediated targeting of MSK1 were evaluated, KT-1 cells were electroporated with an MSK1 siRNA duplex or control mixture siRNA (New England Biolabs, Ipswich, MA) as recommended by the manufacturer. Twenty-four hours later, the cells were incubated with the indicated concentrations of As_2O_3 for 48 h and analyzed by flow cytometry.

Hematopoietic Cell Progenitor Assays—Bone marrow and peripheral blood from CML patients were collected. The effects of As_2O_3 on the growth of hematopoietic progenitors from CML patients were determined in clonogenic assays in methylcellulose as described previously (26, 30). Briefly, mononuclear cells were separated by Ficoll-Hypaque sedimentation, and cells were cultured in a methylcellulose mixture containing hematopoietic growth factors (26, 30) in the presence or absence of As_2O_3 (1 μM) and H-89 (10 μM). Colony forming units-granulocyte/macrophage (CFU-GM) from the leukemic bone marrow were scored on day 14 of culture.

Acid-soluble Protein Extraction—KT-1 cells were treated with H-89 for 1 h prior to treatment with As_2O_3 (2 μM) for 20 min. Extraction of proteins was performed as described previously (31, 32). Briefly, acid-soluble proteins were extracted with lysis buffer (10 mM HEPES (pH 7.9), 1.5 mM $MgCl_2$, 10 mM KCl, and 0.5 mM dithiothreitol), and 0.2 M sulfuric acid was added, followed by incubation on ice for 30 min. The acid-soluble proteins contained in the supernatant were retained after centrifugation at 11,000 × *g* for 10 min at 4 °C and precipitated on ice for 30 min with a final concentration of 25% trichloroacetic acid. The proteins were pelleted at 12,000 × *g* for 10 min and washed once with 100% acetone and 0.05 M HCl and once with 100% acetone. The dried pellets were resuspended in acetic acid/urea buffer and resolved by SDS-PAGE.

RESULTS

We initially determined whether the kinase MSK1 is activated during treatment of malignant hematopoietic cell lines with As_2O_3. The derived KT-1 CML cell line and the NB-4 acute promyelocytic leukemia cell line were studied. Cells were incubated in the presence or absence of As_2O_3, and after cell lysis, equal amounts of lysates were analyzed by SDS-PAGE and immunoblotted with antibody against MSK1 phosphorylated at Ser^{376}. As_2O_3 induced strong phosphorylation of MSK1 in both KT-1 cells (Fig. 1, *A* and *B*) and NB-4 cells (Fig. 1, *C* and *D*). We subsequently directly examined whether As_2O_3 treatment of cells results in activation of the kinase domain of MSK1. KT-1 cells were incubated in the presence or absence of As_2O_3; cell lysates were immunoprecipitated with anti-MSK1 antibody; and *in vitro* kinase assays were performed on the immunoprecipitates. As shown in Fig. 1*E*, treatment of KT-1 cells with As_2O_3 resulted in activation of MSK1, whereas such activation was blocked by pretreatment of the cells with the p38 pharmacological inhibitor SB 203580. On the other hand, in similar experiments in which KT-1 cells were pretreated with the MEK/ERK inhibitor PD 98059, we found no significant inhibition of such activation (data not shown), suggesting that p38 is the major kinase that regulates activation of MSK1 by As_2O_3 in these cells. Previous studies established that activation of MSK1 is regulated by both the p38 and ERK MAPK signaling pathways in different systems (33–36). Inhibition of MSK1 activity by the p38 inhibitor strongly suggested that p38 plays an important regulatory role in activation of MSK1 by As_2O_3. To further explore the requirement of the p38 kinase for activation of MSK1 by As_2O_3, MEFs with targeted disruption of the p38α gene (68) were used. As expected, As_2O_3 treatment induced phosphorylation of MSK1 in wild-type MEFs, whereas such phosphorylation was defective in p38$\alpha^{-/-}$ cells (Fig. 2, *A* and *B*). Consistent with this, the induction of MSK1 kinase activity was defective in cells lacking p38α compared with parental cells (Fig. 2*C*). Altogether, these findings established that phosphorylation/activation of MSK1 by As_2O_3 is p38-dependent.

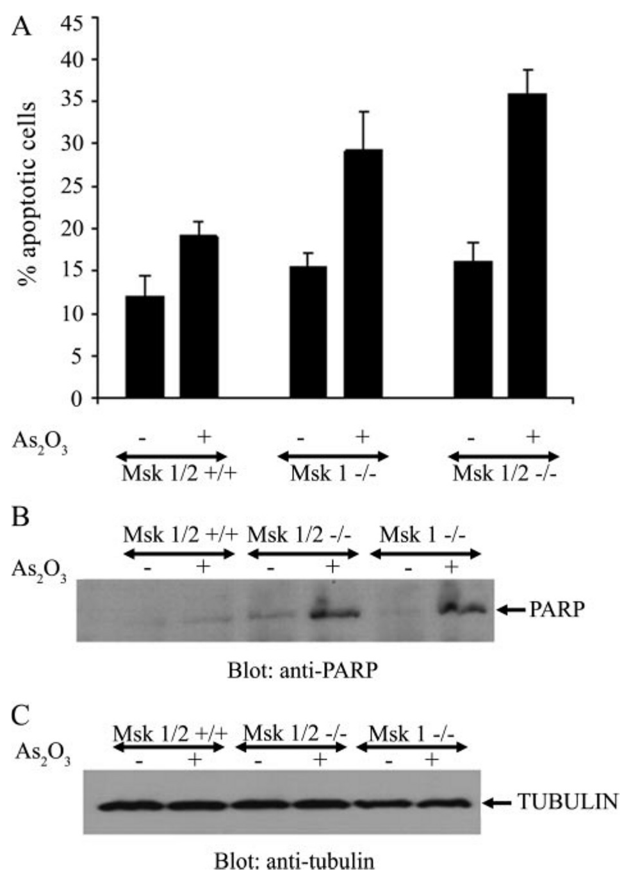

FIGURE 3. **As_2O_3-induced apoptosis is enhanced in *Msk1* knock-out cells.** *A*, *Msk1/2*$^{+/+}$, *Msk1/2*$^{-/-}$, and *Msk1*$^{-/-}$*/2*$^{+/+}$ MEFs were treated with As_2O_3 for 48 h. The cells were subsequently stained with fluorescein isothiocyanate-conjugated annexin V and propidium iodide and analyzed by flow cytometry. The means ± S.D. of four experiments are shown. *B*, *Msk1/2*$^{+/+}$, *Msk1/2*$^{-/-}$, and *Msk1*$^{-/-}$*/2*$^{+/+}$ MEFs were incubated with As_2O_3 for 48 h. Equal amounts of protein were loaded onto SDS-polyacrylamide and immunoblotted with polyclonal antibody to poly(ADP-ribose) polymerase (*PARP*). *C*, the same blot shown in *B* was subsequently stripped and reprobed with anti-tubulin antibody to control for protein loading.

In subsequent experiments, we sought to determine the functional role of MSK1 in the generation of As_2O_3-mediated apoptosis. In a previous study, we showed that pharmacological inhibition of p38 results in enhanced induction of apoptosis by As_2O_3 (23), but the downstream effectors that mediate such anti-apoptotic effects have not been identified to date. When

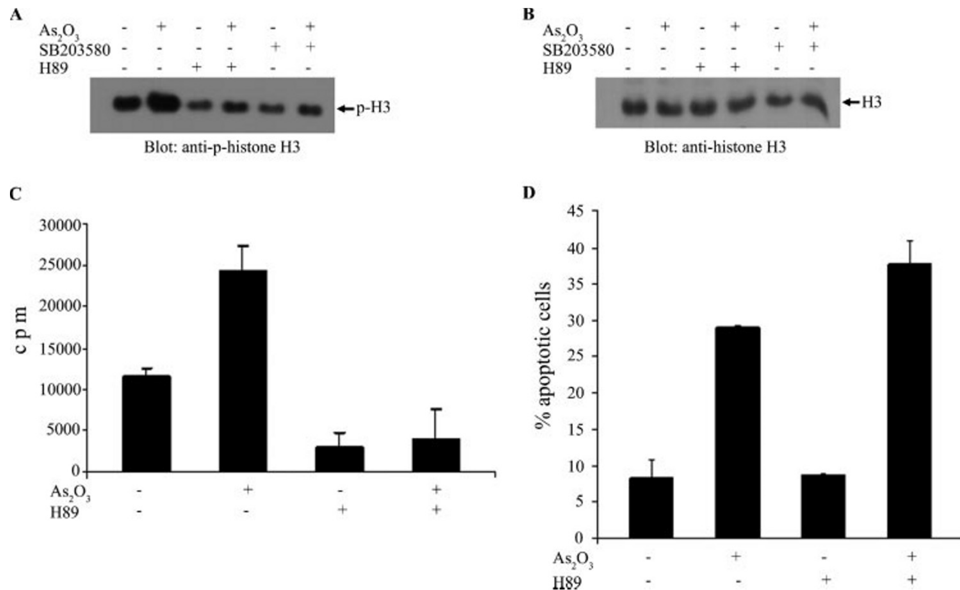

FIGURE 4. **H-89 enhances arsenic-induced apoptosis.** *A*, KT-1 cells were treated with H-89 or SB 203580 for 60 min and subsequently incubated with As_2O_3 (2 μM) for 20 min in the continuous presence or absence of H-89. Acid-soluble proteins were subsequently isolated, analyzed by SDS-PAGE, and immunoblotted with antibody against histone H3 phosphorylated (*p*) at Ser^{10}. *B*, equal amounts of protein from the same extracts as in the experiment shown in *A* were analyzed separately by SDS-PAGE and immunoblotted with anti-histone H3 antibody. *C*, KT-1 cells were treated with H-89 (10 μM) for 60 min prior to treatment with As_2O_3 (2 μM) for 120 min. Total cell lysates were immunoprecipitated with antibody against MSK1, and *in vitro* kinase assays to detect MSK1 activation were performed using Akt/SGK as an exogenous substrate. The means ± S.D. of three independent experiments are shown. *D*, KT-1 cells were pretreated with H-89 for 60 min prior to the addition of As_2O_3 (2 μM) to the cultures for 48 h. The cells were then stained with fluorescein isothiocyanate-conjugated annexin V as well as propidium iodide and analyzed by flow cytometry. The means ± S.D. of three independent experiments are shown.

the induction of apoptosis by As_2O_3 was determined in immortalized MEFs with targeted disruption of the *Msk1* gene, we found enhanced As_2O_3-induced apoptosis in cells lacking *Msk1* or in double knockout MEFs for *Msk1* and the related *Msk2* gene compared with parental cells (Fig. 3*A*). Consistent with this, poly(ADP-ribose) polymerase cleavage was enhanced in $Msk1^{-/-}$ and $Msk1/2^{-/-}$ MEFs compared with wild-type MEFs (Fig. 3, *B* and *C*). Thus, it appears that MSK1 is a key mediator of the anti-apoptotic effects of p38 MAPK during its activation in response to As_2O_3 treatment of cells.

A major substrate for the MSK1 kinase is histone H3 (36), the phosphorylation of which is associated with immediate-early gene induction (37). We examined whether As_2O_3 induces phosphorylation of histone H3 in KT-1 cells. As shown in Fig. 4 (*A* and *B*), treatment of KT-1 cells with As_2O_3 induced phosphorylation of histone H3 at Ser^{10}, and such phosphorylation was inhibited by concomitant treatment of the cells with either SB 203580 or H-89, a pharmacological inhibitor to which MSK1 is known to exhibit high sensitivity (37). Pretreatment of KT-1 cells with H-89 also inhibited the arsenic-dependent induction of MSK1 kinase activity (Fig. 4*C*) and further enhanced the induction of apoptosis by As_2O_3 in KT-1 cells (Fig. 4*D*).

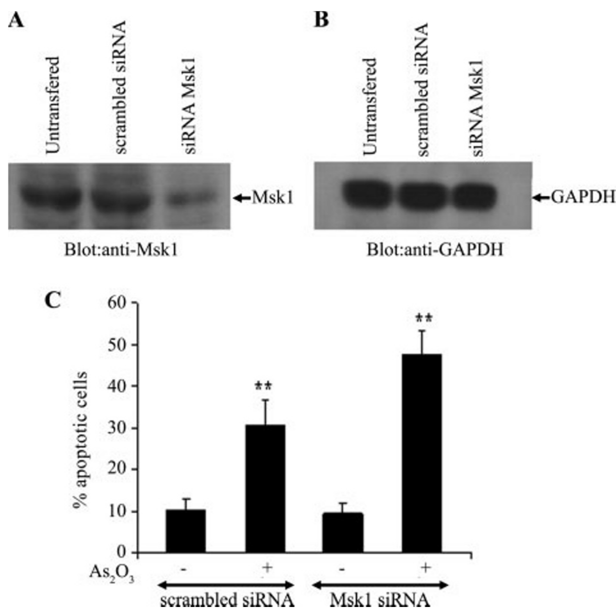

FIGURE 5. **Enhancement of As_2O_3-induced apoptosis by siRNA-mediated targeting of MSK1.** *A*, KT-1 cells were left untransfected or were transfected with scrambled siRNA or MSK1-specific siRNA for 72 h as indicated. Cell lysates were resolved by SDS-PAGE and immunoblotted with anti-MSK1 antibody. *B*, the blot shown in *A* was stripped and reprobed with anti-glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) antibody to control for protein loading. *C*, KT-1 cells were transfected with scrambled siRNA or MSK1-specific siRNA for 72 h and concurrently stimulated with 2 μM As_2O_3 for 48 h. Apoptosis was analyzed by flow cytometry with annexin V/propidium iodide staining. The means ± S.D. of six experiments are shown. **, $p = 0.001744$ (paired *t* test analysis of MSK1 siRNA-transfected cells treated with As_2O_3 compared with scrambled siRNA-transfected cells treated with As_2O_3).

To further establish the relevance of MSK1 in the negative regulation of As_2O_3-induced apoptosis, we used siRNA interference to block MSK1 expression in cells of hematopoietic origin. KT-1 cells were transfected with an MSK1-specific siRNA, and after 72 h, cell extracts were prepared and immunoblotted with anti-MSK1 antibody. As in our previous study (38), transfection of cells with the MSK1-specific siRNA resulted in knockdown of MSK1 (Fig. 5, *A* and *B*). The induction of cell death by As_2O_3 was subsequently assessed after 48 h of treatment. As illustrated in Fig. 5*C*, knockdown of MSK1 in the presence of As_2O_3 further potentiated the induction of apoptosis compared with As_2O_3 alone.

Altogether, these data established that pharmacological or molecular inhibition of MSK1 expression potentiates the generation of the inhibitory effects of As_2O_3 on leukemic cells. To further explore the role of MSK1 in a more physiologically relevant system, we evaluated the effects of pharmacological inhibition of MSK1 on the induction of the suppressive effects of As_2O_3 on primary leukemic progenitors from CML patients. Bone marrow and peripheral blood mononuclear cells from six patients with CML were isolated, and leukemic CFU-GM progenitor colony formation was

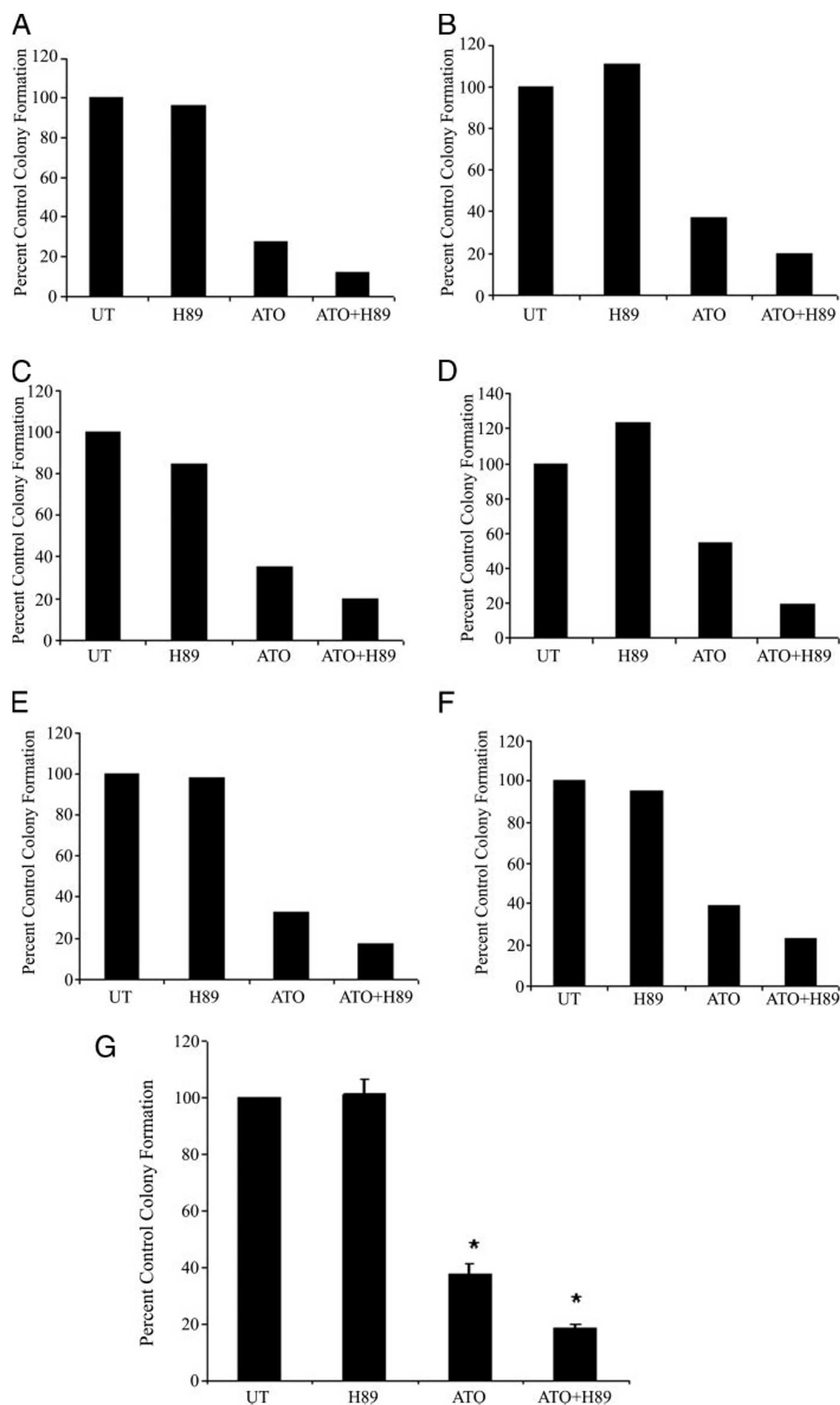

FIGURE 6. **Pharmacological inhibition of MSK1 enhances the anti-leukemic properties of As_2O_3.** *A–F*, bone marrow or peripheral blood mononuclear cells from six different CML patients were plated in a methylcellulose assay system with 1 μM As_2O_3 (*ATO*) in the presence or absence of 10 μM H-89 as indicated. The data are expressed as percent control CFU-GM colony numbers for untreated (*UT*) cells. *G*, the means ± S.E. of the values from the experiments using different patient samples (*A–F*) are shown. *, $p = 0.001974$ (paired *t* test analysis of the combination of As_2O_3 plus H-89 compared with As_2O_3 alone).

determined by clonogenic assays in methylcellulose. As expected, addition of As_2O_3 to the cultures suppressed leukemic CFU-GM progenitor growth (Fig. 6, *A–F*). On the other hand, addition of H-89 to the cultures alone had no significant effects. However, concomitant addition of H-89 to the cultures strongly enhanced (two-tailed *p* value = 0.001974) the suppressive effects of As_2O_3 on leukemic CFU-GM progenitor growth.

DISCUSSION

Over the last few years, extensive efforts have been undertaken to understand the mechanisms by which As_2O_3 induces apoptosis of target cells and exhibits antitumor properties. Treatment of malignant cells with As_2O_3 is known to result in elevation of reactive oxygen species, loss of mitochondrial membrane potential, and release of cytochrome *c* (39–42). These events are followed by activation of the caspase cascade and programmed cell death (39–42). There is also evidence that As_2O_3 can influence cell death through activation of JNK (43, 44) and inhibition of the transcription factor NF-κB (45–47). It should also be noted that the generation of reactive oxygen species depends on cellular glutathione stores (48) and that a decrease in reduced cellular GSH levels by pretreatment of arsenic-sensitive cells with ascorbic acid (48) or buthionine sulfoximine (49) enhances their sensitivity to arsenic-dependent apoptosis. On the other hand, increased cellular GSH levels result in attenuation of the cytotoxic effects of As_2O_3 (49).

Despite the advances in our understanding of the mechanisms by which As_2O_3 induces apoptosis, little is known about the role of pathways that negatively regulate the generation of the anti-leukemic properties of As_2O_3. There is evidence that the phosphatidylinositol 3-kinase/Akt pathway mediates As_2O_3 resistance in human leukemic cells (50, 51) and that pharmacological inhibitors of phosphatidylinositol 3-kinase potentiate As_2O_3-induced apoptosis via glutathione depletion and increased peroxide accumulation in myeloid leukemia cells (51). Thus, one pathway that appears to negatively regulate the generation of the anti-leuke-

mic properties of As_2O_3 is the phosphatidylinositol 3-kinase pathway.

We have also previously shown that activation of the p38 MAPK pathway in response to As_2O_3 treatment of cells exhibits negative regulatory effects on the induction of arsenic-dependent apoptosis (23). This was evidenced by experiments demonstrating that pharmacological inhibitors of p38 or overexpression of a p38 dominant-negative mutant promotes the effects of As_2O_3. These findings have suggested that the p38 pathway is activated in a negative feedback regulatory manner to regulate arsenic-induced apoptosis, but the downstream p38 effectors that mediate such effects are not known. Interestingly, p38 MAPK appears to be also activated in a negative feedback regulatory manner in response to all-*trans*-retinoic acid in acute promyelocytic leukemia cells and to negatively regulate leukemic cell differentiation (52).

In this study, we have provided the first evidence that the kinase MSK1 is activated by As_2O_3 in leukemia cell lines. Such activation requires upstream engagement of the p38 MAPK pathway, as evidenced in studies using pharmacological inhibitors of p38 or p38α knock-out cells. We have also demonstrated that pharmacological inhibition of MSK1 or siRNA-mediated disruption of its expression results in enhanced arsenic-induced apoptosis, suggesting that this p38 effector is a primary mediator of the anti-apoptotic effects of the p38 MAPK pathway on leukemic cells. Similar effects were also seen in cells with targeted disruption of the *Msk1* gene or double knock-outs for both *Msk1* and the related *Msk2* gene. Notably, pharmacological inhibition of MSK1 activity was found to enhance the suppressive effects of As_2O_3 on primary leukemic CFU-GM progenitors from CML patients, indicating that such effects occur in physiologically relevant systems.

MSK1 and MSK2 are serine kinases that are activated downstream of the p38 and MEK/ERK signaling cascades (36, 37, 53–55). Studies using *Msk1* and *Msk2* knock-out MEFs have demonstrated that these kinases regulate phosphorylation of histone H3 at Ser^{10} and Ser^{28} as well as phosphorylation of HMGNI (HMG-14) at Ser^{6}, Ser^{20}, and Ser^{24} (36, 53), but they are not required for acetylation of histone H3 (36). Such functions of MSK1 and MSK2 are critical for stress-induced chromatin remodeling and immediate-early gene transcription of a variety of genes (36, 37). Notably, phosphorylation of histone H3 at Ser^{10} is elevated in oncogene-transformed fibroblasts, suggesting that MSKs are involved in aberrant gene expression observed in oncogene-transformed cells (56). In addition to histone H3 and HMGNI, there are additional targets for the activity of MSKs (53). In response to activation by certain stress signals, MSK1 and MSK2 mediate phosphorylation of cAMP-responsive element-binding protein and ATF1 (57–59), which results in the regulation of c-*fos* and *junB* transcription. MSK1 also phosphorylates NF-κB and the ER81 transcription factor, which is involved in oncogenesis (53, 60, 61), whereas its function is critical for interleukin-1-induced c-*fos* gene expression in keratinocytes and promotes the growth of both keratinocyte and human epidermoid carcinoma cell lines (62). Recent evidence also suggests important roles for MSK1/2 in epidermal growth factor (63) and vascular endothelial growth factor (64) signaling. Moreover, MSK1 and MSK2 regulate the transcription of the *Nur77*, *Nurr1*, and *Nor1* nuclear orphan receptor genes of the NR4A subfamily (65), the up-regulation of which has been implicated in cellular transformation (66). Thus, it appears that MSK1 and MSK2 regulate engagement of multiple downstream signals to mediate anti-apoptotic and mitogenic responses. Our study has established that As_2O_3 phosphorylates histone H3 at Ser^{10} in an MSK1-dependent manner, suggesting that one mechanism by which this kinase ameliorates As_2O_3-induced apoptosis may involve the regulation of early expression of anti-apoptotic genes. Altogether, our data strongly suggest that the kinase MSK1 (alone or in combination with As_2O_3) is a highly attractive target for the design of novel therapeutic approaches for the treatment of leukemias.

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