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Inhibition of c-Abl with STI571 Attenuates Stress-Activated Protein Kinase Activation and Apoptosis in the Cellular Response to 1-β-D-Arabinofuranosylcytosine

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ABSTRACT

The response of myeloid leukemia cells to treatment with 1-β-D-arabinofuranosylcytosine (ara-C) includes activation of the c-Abl protein tyrosine kinase and the stress-activated protein kinase (SAPK). The present studies demonstrate that treatment of human U-937 leukemia cells with ara-C is associated with translocation of SAPK to mitochondria. STI571 (imatinib mesylate), an inhibitor of c-Abl, blocked both activation and mitochondrial targeting of SAPK in the ara-C response. In concert with these effects of STI571, similar findings were obtained in c-Abl–deficient cells. The results further show that STI571 inhibits ara-C–induced loss of mitochondrial transmembrane potential, caspase-3 activation, and apoptosis. These findings demonstrate that STI571 down-regulates c-Abl–mediated signals that target the mitochondria in the apoptotic response to ara-C.

The cellular response to 1-β-D-arabinofuranosylcytosine (ara-C) and other genotoxic agents includes activation of the stress-activated protein kinase (SAPK)/c-Jun N-terminal kinase (Saleem et al., 1995; Chen et al., 1996; Verheij et al., 1996; Zanke et al., 1996). SAPK phosphorylates and activates the c-Jun, ATF2, and Elk1 transcription factors (Derijard et al., 1994; Kyriakis et al., 1994; Gupta et al., 1995; Whitmarsh et al., 1995). These results and the demonstration that DNA damage is associated with activation of early response gene expression (Kharbanda et al., 1991, 1993; Datta et al., 1992) have suggested that SAPK regulates gene transcription as one response to genotoxic stress. Studies have also shown that SAPK activation is associated with the apoptotic response to DNA-damaging agents (Chen et al., 1996; Verheij et al., 1996; Zanke et al., 1996; Kharbanda et al., 2000b). Moreover, treatment of cells with ionizing radiation is associated with translocation of SAPK to mitochondria, interaction of SAPK with the antiapoptotic Bcl-xL protein, and thereby, induction of apoptosis (Kharbanda et al., 2000b). These findings have supported roles for SAPK in transducing both nuclear and mitochondrial signals in the cellular response to genotoxic stress.

Ara-C and other genotoxic agents activate a nuclear complex that consists in part of the c-Abl and Lyn tyrosine kinases (Kharbanda et al., 1994, 1995b; Yuan et al., 1995). c-Abl binds to the p53 tumor suppressor in the response to DNA damage (Yuan et al., 1996a,b) and regulates p53 by preventing its nuclear export (Sionov et al., 2001). c-Abl also interacts with the p73 homolog of p53 in the apoptotic response to DNA damage (Agami et al., 1999; Gong et al., 1999; Yuan et al., 1999). Activated forms of c-Abl confer induction of SAPK activity and induction of early response gene expression (Sanchez et al., 1994; Kharbanda et al., 1995a; Raitano et al., 1995). In the DNA-damage response, nuclear c-Abl activates MEK kinase 1 (MEKK-1) and thereby the SEK1 → SAPK pathway (Kharbanda et al., 1995a,b; 2000). In addition, c-Abl–deficient cells exhibit a defective SAPK response to DNA-damaging agents (Kharbanda et al., 1995a,b). Like c-Abl, nuclear Lyn activates MEKK-1 and contributes to activation of SAPK (Yoshida et al., 2000). In contrast to c-Abl, Lyn activates SAPK by an MKK7-dependent, SEK1-independent mechanism (Yoshida et al., 2000). These findings have supported a model in which activation of SAPK in the apoptotic response to DNA damage is mediated by both c-Abl and Lyn.

The present studies demonstrate that treatment of human U-937 leukemia cells with ara-C is associated with activation of the c-Abl → SAPK pathway and targeting of SAPK to

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ABBREVIATIONS: Ara-C, 1-β-D-arabinofuranosylcytosine; SAPK, stress-activated protein kinase; MEF, mouse embryo fibroblast; STI571, imatinib mesylate (Gleevec); PBS, phosphate-buffered saline; PCNA, proliferating cell nuclear antigen; HSP, heat shock protein; CCD, charge-coupled device; PAGE, polyacrylamide gel electrophoresis; GST, glutathione *S*-transferase; ΔΨm, mitochondrial transmembrane potential; MEKK-1, mitogen-activated protein kinase kinase kinase 1; ER, endoplasmic reticulum.

mitochondria. The results show that the c-Abl inhibitor, STI571, attenuates ara-C–induced SAPK activation and SAPK to mitochondria localization. STI571 treatment also attenuated loss of mitochondrial transmembrane potential, activation of caspase-3, and induction of apoptosis in the response to ara-C.

Materials and Methods

Cell Culture. Human U-937 myeloid leukemia cells (American Type Culture Collection, Manassas, VA) were grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 100 units/ml of penicillin, 100 mg/ml of streptomycin, and 2 mM L-glutamine. Human MCF-7 breast cancer cells and wild-type and c-Abl$^{-/-}$ mouse embryo fibroblasts (MEF) were maintained in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and antibiotics. Cells were treated with 10 mM ara-C (Sigma-Aldrich, St. Louis, MO) or the indicated concentrations of STI571 (Gleevec; Novartis, Basel, Switzerland).

Isolation of Mitochondrial and Nuclear Fractions. Cells (3×10^6) were washed twice with phosphate-buffered saline (PBS), homogenized in buffer A (210 mM mannitol, 70 mM sucrose, 1 mM EGTA, and 5 mM HEPES, pH 7.4) and 110 mg/ml of digitonin in a glass homogenizer (Pyrex 7727-07; Corning, Acton, MA), and centrifuged at 5,000*g* for 20 min. Pellets were resuspended in buffer A, homogenized in a small glass homogenizer (Pyrex 7726), and centrifuged at 2,000*g* for 5 min. The supernatant was collected and centrifuged at 11,000*g* for 10 min. Mitochondrial pellets were disrupted in lysis buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 1 mM dithiothreitol, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 10 mM NaF, and 10 mg/ml each of leupeptin and aprotinin) at 4°C and then centrifuged at 15,000*g* for 15 min. Protein concentration was determined by Bio-Rad protein estimation kit (Bio-Rad, Hercules, CA). The nuclear fraction was prepared as described previously (Kharbanda et al., 1996).

Immunoblot Analysis. Cell lysates and fractions were subjected to immunoblotting with anti–c-Abl (24-11; Santa Cruz Biotechnology, Inc., Santa Cruz, CA), anti-SAPK (C-17; Santa Cruz Biotechnology, Inc.), anti-HSP60 (Stressgen Biotechnologies Corp., Victoria, BC, Canada), anti–β-actin (Sigma-Aldrich), anti-PCNA (Calbiochem, San Diego, CA), or anti-caspase-3 (Stressgen Biotechnologies Corp.). The antigen-antibody complexes were visualized by enhanced chemiluminescence (Amersham Biosciences, Piscataway, NJ).

Immunofluorescence Microscopy. Cells were plated onto poly-D-lysine–coated glass coverslips 1 day before ara-C treatment (1 h) and then fixed with 3.7% formaldehyde and PBS, pH 7.4, for 10 min. Cells were washed with PBS, permeabilized with 0.2% Triton X-100 for 10 min, washed again, and incubated for 30 min in complete medium. The coverslips were then incubated with 5 mg/ml anti–c-Abl (K-12; Santa Cruz Biotechnology, Inc.) or anti-SAPK (C-17) for 1 h followed by Texas Red goat anti-rabbit IgG (H&L regions) conjugate (Molecular Probes, Eugene, OR). Mitochondria were stained with 100 nM Mitotracker Green FM (Molecular Probes). Nuclei were stained with 4,6-diamino-2-phenylindole (1 mg/ml in PBS). Coverslips were mounted onto slides with 0.1 M Tris-HCl, pH 7.0, in 50% glycerol. Cells were visualized by digital confocal immunofluorescence, and images were captured with a CCD camera mounted on a Zeiss Axioplan 2 microscope (Zeiss, Welwyn Garden City, UK). Images were deconvolved using Slidebook software (Intelligent Imaging Innovations, Inc., Denver, CO).

Analysis of c-Abl and SAPK Activity. Cell and nuclear lysates were prepared in lysis buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 1 mM dithiothreitol, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 10 mM NaF, and 10 mg/ml each of leupeptin and aprotinin) and subjected to immunoprecipitation as described previously (Kharbanda et al., 1997) with anti–c-Abl (K-12) or anti-SAPK (C-17). The immunoprecipitates were resuspended in kinase buffer (20 mM HEPES, pH 7.4, and 10 mM each $MgCl_2$ and $MnCl_2$) containing 2.5 mCi of [γ-^{32}P]ATP, GST-Crk(120–225), GST-Crk(120–212), or GST-Jun for 20 min at 30°C. The reaction products were analyzed by SDS-PAGE and autoradiography.

Analysis of Mitochondrial Membrane Potential. Cells were incubated with 50 ng/ml of Rhodamine 123 (Molecular Probes) for 15 min at 37°C. After washing with PBS, samples were analyzed by flow cytometry using 488-nm excitation and the measurement of emission through a 575/26 (ethidium) bandpass filter.

Assessment of Apoptosis. SubG1 DNA content was assessed by staining ethanol-fixed cells with propidium iodide and monitoring by FACScan (BD Biosciences, San Jose, CA).

Results

Ara-C Targets SAPK to Mitochondria. To determine whether c-Abl localizes to mitochondria in the DNA-damage response, mitochondrial lysates from control and ara-C–treated U-937 cells were analyzed by immunoblotting with anti–c-Abl. As shown previously (Ito et al., 2001b; Kumar et al., 2001), c-Abl was detectable in mitochondria of control cells (Fig. 1A). In contrast to oxidative and endoplasmic reticulum (ER)-induced stress (Ito et al., 2001b; Kumar et al., 2001), treatment of U-937 cells with ara-C had little effect on mitochondrial c-Abl levels (Fig. 1A). Although c-Abl functions as an upstream effector of SAPK in the ara-C response (Kharbanda et al., 1995a,b, 2000), immunoblot analyses were

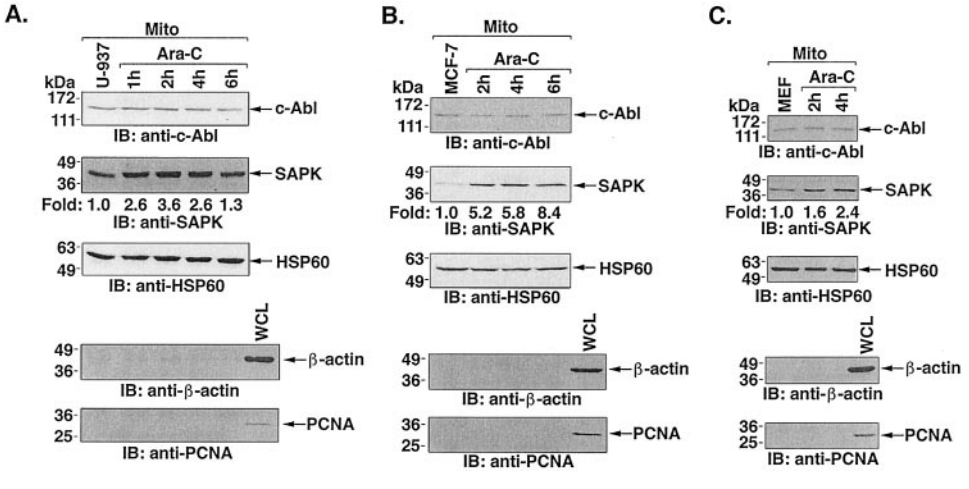

Fig. 1. Ara-C targets SAPK to mitochondria. A, U-937 myeloid leukemia cells were treated with 10 μM ara-C for the indicated times. The mitochondrial fraction (5 μg) was subjected to immunoblotting with anti–c-Abl, anti-SAPK, and anti-HSP60. As controls, the mitochondrial fractions were analyzed for the cytoplasmic β-actin protein and the nuclear PCNA. WCL, whole cell lysate. The relative levels of SAPK were determined by densitometric scanning of the signals. Mitochondrial fractions from MCF-7 cells (B) and wild-type MEFs (C) treated with ara-C for the indicated times were subjected to immunoblotting with antibodies against c-Abl, SAPK, HSP60, anti–β-actin, and PCNA.

performed to assess targeting of SAPK to mitochondria. In contrast to c-Abl, the results show that ara-C induces a 3-fold increase in mitochondrial SAPK levels at 2 h (Fig. 1A). Longer ara-C exposures (i.e., 6 h) associated with the induction of U-937 cell apoptosis resulted in a decline in SAPK signals (Fig. 1A). Equal loading of the lanes was confirmed by immunoblot analysis of the mitochondrial HSP60 (Fig. 1A). Purity of the mitochondrial fraction was confirmed by immunoblotting with antibodies against the cytoplasmic β-actin protein and the nuclear PCNA protein (Fig. 1A). There was no detectable β-actin or PCNA in the mitochondrial fraction (Fig. 1A). To assess the effects of ara-C in different cell types, studies were performed on MCF-7 breast cancer cells. The results demonstrate that ara-C induces translocation of SAPK, but not c-Abl, to mitochondria (Fig. 1B). Similar results were obtained in ara-C–treated MEFs (Fig. 1C).

To further assess the effects of ara-C on the subcellular distribution of c-Abl and SAPK, intracellular fluorescence was measured with a CCD camera and image analyzer (Intelligent Imaging Innovations). Examination of the distribution of fluorescence markers in control U-937 leukemia cells showed distinct patterns for anti–c-Abl (red signal) and a mitochondrion-selective dye (Mitotracker; green signal) (Fig. 2A). Treatment with ara-C showed no apparent targeting of c-Abl to mitochondria (Fig. 2A). Analysis of control U-937 cells also demonstrated distinct patterns for anti-SAPK (red signal) and Mitotracker (green signal) (Fig. 2B). In contrast to c-Abl, treatment with ara-C was associated with a change in fluorescence signals (red + green → yellow/orange) (Fig. 2B). These findings and those obtained by immunoblotting demonstrate that SAPK, and not c-Abl, is targeted to mitochondria in the cellular response to ara-C.

STI571 Blocks Ara-C–Induced Activation of SAPK. To determine whether ara-C targets SAPK to mitochondria by a c-Abl–dependent mechanism, U-937 cells were treated with ara-C and the c-Abl inhibitor STI571 (Druker et al., 1996). Anti–c-Abl immunoprecipitates from nuclear lysates were

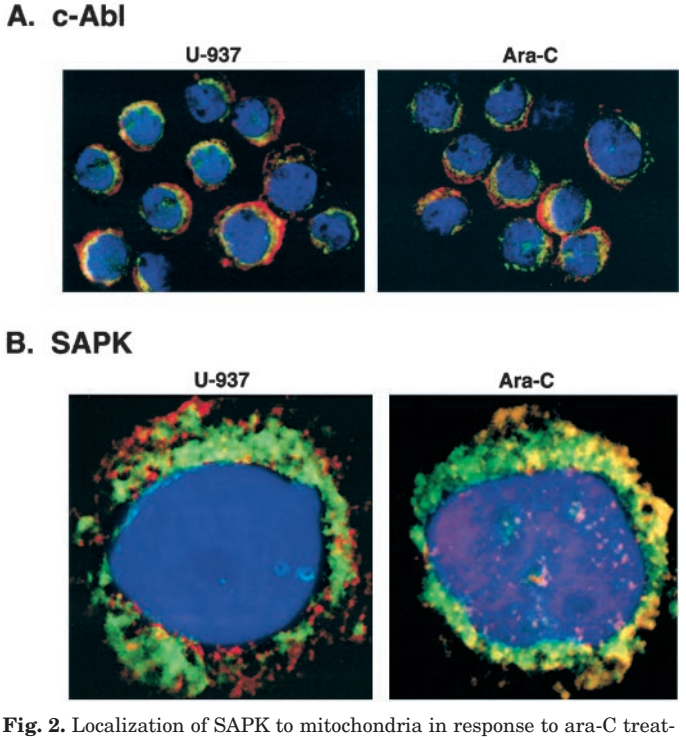

Fig. 2. Localization of SAPK to mitochondria in response to ara-C treatment. U-937 cells were left untreated or treated with ara-C for 1 h. A, after washing, the cells were fixed and incubated with anti–c-Abl, followed by Texas Red-conjugated goat anti-rabbit IgG. Mitochondria were stained with 100 nM Mitotracker Green and nuclei with 4,6-diamidino-2-phenylindole. The slides were visualized using a fluorescence microscope coupled to a high-sensitivity CCD camera and image analyzer. Red signal, c-Abl; green signal, mitochondria. The digital confocal image was set for the mitochondria. Images set at a different depth are used for visualization of nuclear c-Abl (Ito et al., 2001a). B, the cells were fixed and incubated with anti-SAPK, followed by Texas Red-conjugated goat anti-rabbit IgG. Mitochondria were stained with the Mitotracker Green and nuclei with 4,6-diamidino-2-phenylindole. Red signal, SAPK; green signal, Mitotracker; yellow/orange signals, colocalization of SAPK and Mitotracker.

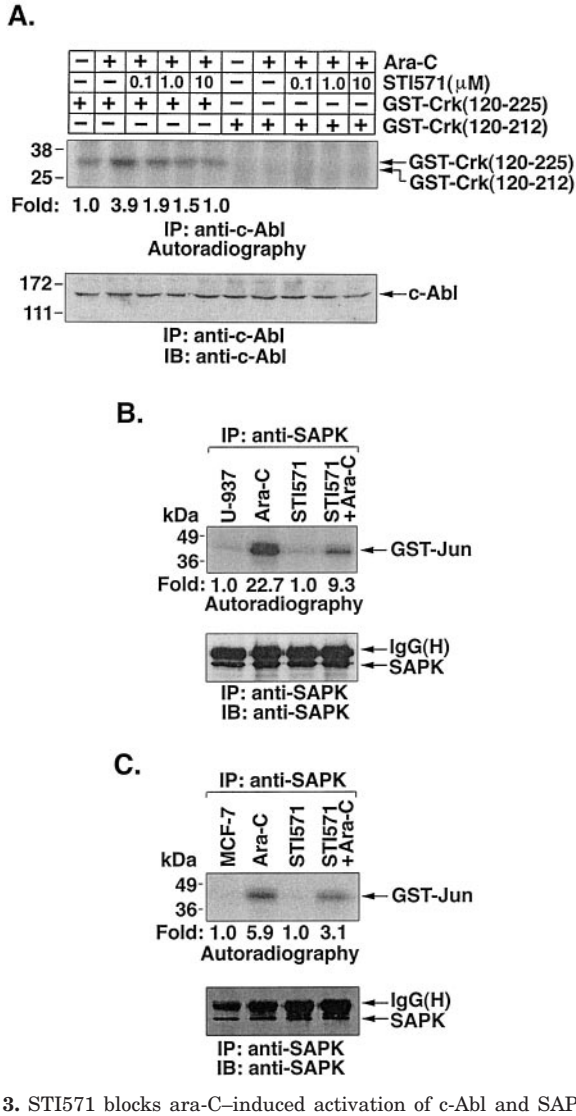

Fig. 3. STI571 blocks ara-C–induced activation of c-Abl and SAPK. A, U-937 cells were pretreated with the indicated concentrations of STI571 for 24 h and then exposed to ara-C for 1 h. Nuclear lysates were subjected to immunoprecipitation with anti–c-Abl. In vitro immune complex kinase assays were performed with GST-Crk(120–225) (first five lanes) and, as a control, GST-Crk(120–212), which lacks the critical Tyr-221 (last five lanes). The reaction products were analyzed by SDS-PAGE and autoradiography (top). The anti–c-Abl immunoprecipitates were also analyzed by immunoblotting with anti–c-Abl (bottom). U-937 (B) and MCF-7 (C) cells were treated with 10 μM STI571 for 24 h before the addition of ara-C for 1 h. Cell lysates were subjected to immunoprecipitation with anti-SAPK. In vitro immune complex kinase assays were performed with GST-Jun fusion protein as substrate. The reaction products were analyzed by SDS-PAGE and autoradiography (top). The immunoprecipitates were also analyzed by immunoblotting with anti-SAPK (bottom).

analyzed for phosphorylation of GST-Crk(120–225) and, as a control, GST-Crk(120–212), which lacks the c-Abl phosphorylation site (Kharbanda et al., 1995b). As shown previously (Kharbanda et al., 1995b), ara-C treatment is associated with induction of nuclear c-Abl activity (Fig. 3A). Pretreatment of the cells with 0.1 or 1.0 μM STI571 resulted in only partial inhibition of c-Abl activation in ara-C–treated cells (Fig. 3A). In contrast, treatment with 10 μM STI571 blocked ara-C–induced nuclear c-Abl activity (Fig. 3A). To assess the effects of STI571 on ara-C–induced activation of SAPK, anti-SAPK immunoprecipitates were analyzed for phosphorylation of c-Jun. The results demonstrate that, in contrast to ara-C, treatment with STI571 alone has no apparent effect on SAPK activity (Fig. 3B). Moreover, STI571 attenuated the induction of SAPK activity by over 50% in ara-C–treated U-937 cells (Fig. 3B). Similar findings were obtained in MCF-7 cells that

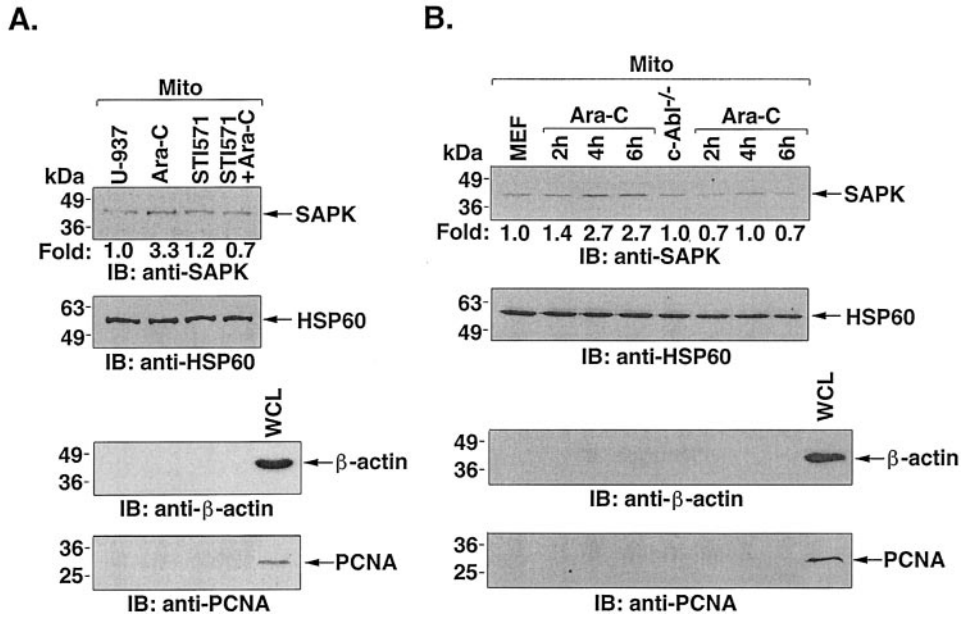

Fig. 4. STI571 inhibits ara-C–induced targeting of SAPK to mitochondria. A, U-937 cells were treated with 10 μM STI571 for 24 h before addition of ara-C for 1 h. Mitochondrial fractions were subjected to immunoblotting with the indicated antibodies. B, wild-type and Abl$^{-/-}$ MEFs were treated with ara-C for the indicated times. Mitochondrial fractions were subjected to immunoblotting with anti-SAPK, anti-HSP60, anti–β-actin, and anti-PCNA.

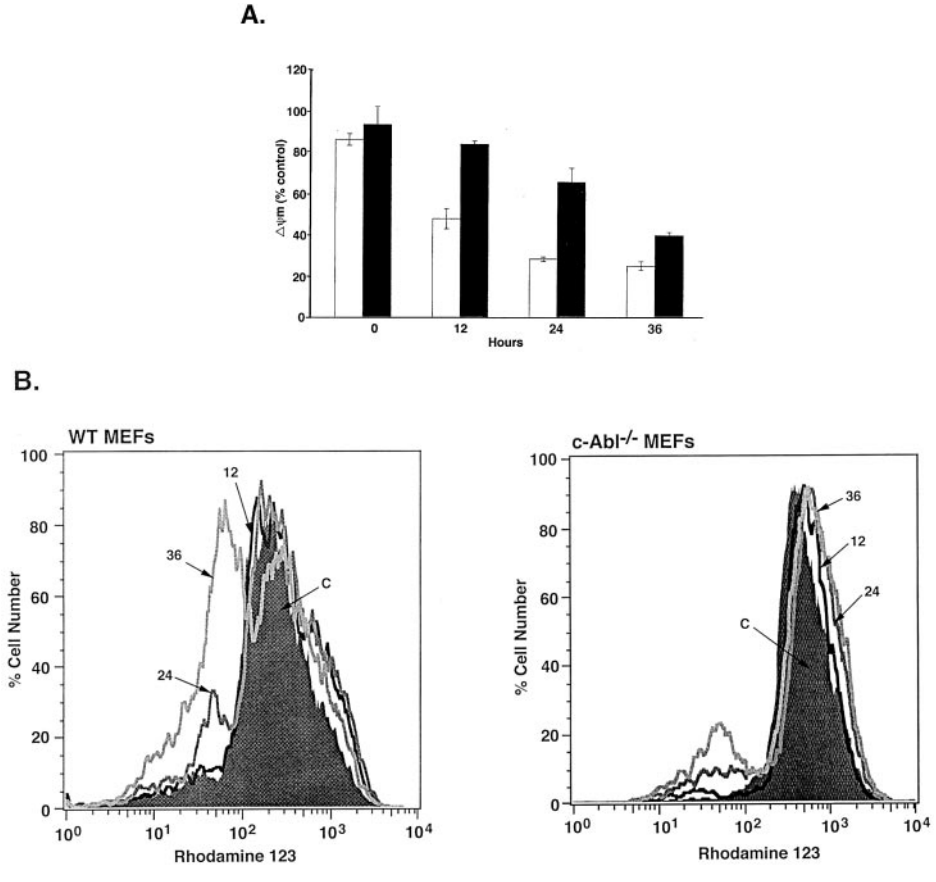

Fig. 5. STI571 blocks ara-C–induced loss of $\Delta\Psi$m. A, wild-type MEFs were treated with ara-C (□) for the indicated times and/or 10 μM STI571 for 24 h before the addition of ara-C (■). Cells were stained with Rhodamine 123 and analyzed by flow cytometry. The results are expressed as the percentage (mean ± S.D.) of control $\Delta\Psi$m from three different experiments. B, wild type (left) and Abl$^{-/-}$ (right) MEFs were treated with ara-C for 12, 24, or 36 h, stained with Rhodamine 123, and analyzed by flow cytometry.

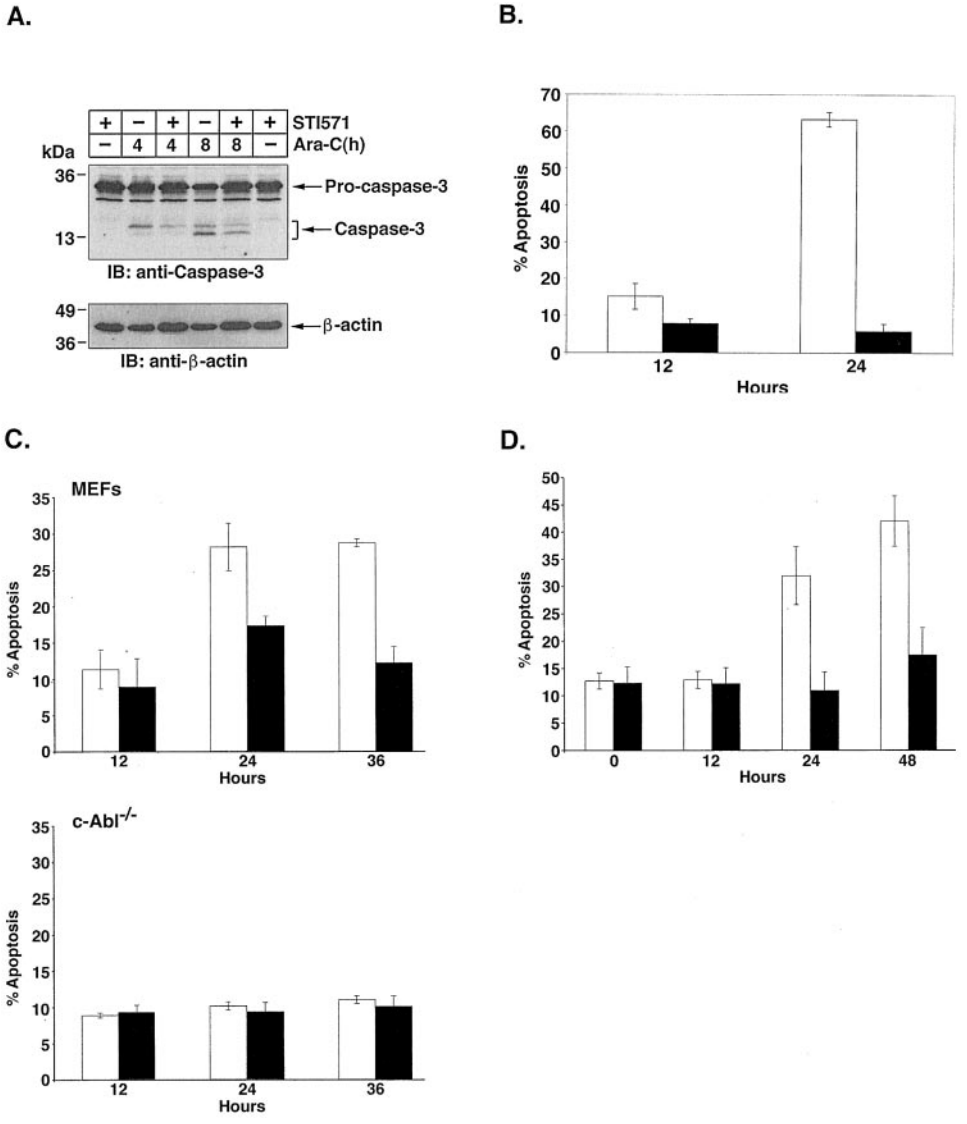

Fig. 6. STI571 blocks ara-C induced caspase-3 cleavage and apoptosis. A, U-937 cells were treated with 10 μM STI571 for 24 h before the addition of ara-C for 4 or 8 h. Lysates were subjected to immunoblotting with anti-caspase-3 (top) and anti–β-actin (bottom). B, U-937 cells were treated with ara-C for the indicated times with (■) or without (□) 10 μM STI571 pretreatment for 24 h. The cells were analyzed for subG1 DNA content. The results are expressed as percentage apoptosis (mean ± S.D.) from three separate experiments. C, wild-type (top) and c-Abl$^{-/-}$ (bottom) MEFs were treated with ara-C for the indicated times without (□) or with (■) 10 μM STI571 pretreatment for 24 h. The results are expressed as the percentage apoptosis (mean ± S.D.) from three separate experiments. Using trypan blue exclusion, the percentage of positive c-Abl$^{-/-}$ cells treated with 1) ara-C alone for 36 h was 3.9 ± 0.9% or 2) ara-C + STI571 was 3.8 ± 0.4%. D, wild-type (□) and Abl$^{-/-}$ (■) MEFs were treated with Ara-C for the indicated times and then analyzed for subG1 DNA content. The results are expressed as percentage apoptosis (mean ± S.D.) from three separate experiments.

were pretreated with STI571 and then exposed to ara-C (Fig. 3C).

STI571 Inhibits Ara-C–Induced Targeting of SAPK to Mitochondria. To determine whether STI571 affects mitochondrial targeting of SAPK, studies were performed on STI571- and STI571 + ara-C–treated U-937 cells (Fig. 4A). The results demonstrate that STI571 alone has no apparent effect on the mitochondrial distribution of SAPK compared with that in untreated cells (Fig. 4A). Moreover, in contrast to ara-C–treated cells, targeting of SAPK to mitochondria was attenuated by STI571 treatment (Fig. 4A). As a control, similar studies were performed on MEFs that were deficient in c-Abl expression. Compared with that in wild-type MEFs, ara-C–induced targeting of SAPK to mitochondria was attenuated in c-Abl$^{-/-}$ MEFs (Fig. 4B). These results indicate that STI571 blocks c-Abl–dependent mitochondrial targeting of SAPK in the ara-C response.

STI571 Attenuates Ara-C–Induced Loss of Mitochondrial Transmembrane Potential, Caspase-3 Activation, and Apoptosis. To determine whether activation of the c-Abl → SAPK pathway contributes to loss of mitochondrial transmembrane potential ($\Delta\Psi$m), wild-type MEFs were treated with ara-C and then stained with Rhodamine 123. The results demonstrate that ara-C treatment is associated with a decrease in $\Delta\Psi$m (Fig. 5A). Moreover, although STI571 alone had no effect on $\Delta\Psi$m, STI571 pretreatment attenuated this response to ara-C (Fig. 5A). As a control, the response of c-Abl$^{-/-}$ MEFs to ara-C was associated with an attenuated loss of $\Delta\Psi$m compared with that found with wild-type MEFs (Fig. 5B).

The response of cells to ara-C also includes cleavage of pro-caspase-3 and induction of apoptosis (Datta et al., 1996). To assess the involvement of c-Abl signaling in these responses to ara-C, U-937 cells were treated with STI571 and then assayed for cleavage of pro-caspase-3. The results demonstrate that STI571 treatment attenuates ara-C–induced caspase-3 activation (Fig. 6A). In concert with these findings, STI571 blocked the apoptotic response of U-937 cells to ara-C (Fig. 6B). Thus, although 60% of the ara-C–treated cells exhibited sub-G_1 DNA at 24 h, less than 10% apoptosis was found when the cells were pretreated with STI571 (Fig. 6B). STI571 also blocked ara-C–induced apoptosis of wild-type MEFs (Fig. 6C, top) but had little effect on apoptosis of c-Abl$^{-/-}$ cells treated with ara-C (Fig. 6C, bottom). In concert

with these results, ara-C–induced apoptosis was attenuated in c-Abl$^{-/-}$ MEFs (Fig. 6D). These results demonstrate that inhibition of c-Abl → SAPK signaling with STI571 attenuates ara-C–induced loss of $\Delta\Psi m$, activation of caspase-3, and apoptosis.

Discussion

Eukaryotic cells respond to DNA damage with cell cycle arrest, activation of DNA repair, and, in the event of irreparable damage, induction of apoptosis. The signals that determine cell fate (that is, repair of DNA damage and survival versus activation of cell death mechanisms) remain unclear. Exposure of diverse types of mammalian cells to ara-C and other DNA-damaging agents is associated with the induction of SAPK activity (Kharbanda et al., 1995a,b; Chen et al., 1996; Verheij et al., 1996; Zanke et al., 1996). Induction of SAPK in the response to genotoxic stress is associated with activation of c-Jun and transcription of the c-jun gene (Kharbanda et al., 1990; Sherman et al., 1990). The functional significance of c-jun transcription is presumably related to the activation of later response genes that determine cell fate. In this context, activation of SAPK in the response to DNA damage has been associated with induction of apoptosis (Chen et al., 1996; Verheij et al., 1996; Zanke et al., 1996). Moreover, direct evidence for involvement of SAPK in the induction of apoptosis has been obtained from studies in SAPK-deficient MEFs (Tournier et al., 2000). SAPK is required for UV-induced apoptosis, and the absence of SAPK causes a defect in the mitochondrial death signaling pathway (Tournier et al., 2000). These findings indicate that, in addition to activation of early response genes, the SAPK pathway confers proapoptotic signals to mitochondria.

Certain insights into how DNA damage is converted into intracellular signals that induce an apoptotic response have been obtained from the finding that a nuclear complex of the c-Abl and Lyn tyrosine kinases is activated by ara-C and other genotoxic agents (Kharbanda et al., 1994, 1995a,b; Yuan et al., 1995). Activation of the Lyn → MEKK-1 → MKK7 → SAPK pathway contributes to the induction of apoptosis by DNA-damaging agents (Yoshida et al., 2000). c-Abl also functions in DNA-damage–induced activation of SAPK but by a mechanism involving the c-Abl → MEKK1 → SEK1→ SAPK pathway (Kharbanda et al., 1995a, 2000a). In contrast, other studies have suggested that SAPK functions upstream to c-Abl activation (Dan et al., 1999). Although the basis for a SAPK → → c-Abl signaling cascade is not clear (Dan et al., 1999), we asked whether activation of the c-Abl → SAPK pathway transduces proapoptotic signals in the response to DNA damage. The present studies demonstrate that treatment of U-937 leukemia cells with ara-C is associated with targeting of SAPK, and not c-Abl, to mitochondria. Similar results were obtained in other cell types, suggesting that the findings are broadly applicable to the genotoxic stress response. In this context, treatment of cells with ionizing radiation is also associated with mitochondrial targeting of SAPK and not c-Abl (Kharbanda et al., 2000b). These findings are in contrast to the demonstration that cytoplasmic c-Abl localizes to mitochondria in the cell death response of cells to oxidative stress (Sun et al., 2000; Kumar et al., 2001). Moreover, ER-associated c-Abl is targeted to mitochondria in the apoptotic response of cells to ER stress (Ito et al., 2001b). Thus, signaling of nuclear c-Abl in the DNA-damage response differs from that found for targeting of c-Abl to mitochondria with other types of stress.

Experiments were performed with the c-Abl inhibitor, STI571 (Druker et al., 1996), to assess the role of c-Abl in DNA-damage-induced targeting of SAPK to mitochondria. STI571 has exceptionally high affinity and specificity for Bcr-Abl and c-Abl (Buchdunger et al., 1996). Activation of nuclear c-Abl by ara-C was completely inhibited with 10 mM STI571. These findings are in concert with the demonstration that STI571 exhibits a K_i value of 7 mM for the tyrosine-phosphorylated or -activated form of c-Abl (Schindler et al., 2000). Other studies have shown that STI571 inhibits c-Abl activation in cells treated with the genotoxic agent etoposide (Dan et al., 1999). Our findings with ara-C differ, however, in that STI571 has no effect on SAPK activation in the response of U-937 cells to etoposide (Dan et al., 1999). In the present studies, STI571 inhibited ara-C–induced SAPK activation, but, consistent with involvement of both c-Abl and Lyn, failed to completely abrogate this response. STI571 also attenuated targeting of SAPK to mitochondria in the response to ara-C. In concert with the finding that SAPK transduces mitochondrial death signals (Tournier et al., 2000), STI571 blocked ara-C–induced loss of mitochondrial transmembrane potential. Although other studies have shown that loss of $\Delta\Psi m$ is not required for activation of caspases (Li et al., 2000), our results demonstrate that STI571 also inhibits ara-C–induced activation of caspase-3 and apoptosis. STI571 has also been shown to inhibit the platelet-derived growth factor receptor and c-kit (proto-oncogene tyrosine-protein kinase kit) (Carroll et al., 1997). Consequently, we performed studies with c-Abl–deficient MEFs to confirm whether c-Abl activation is responsible for transducing ara-C–induced proapoptotic signals to mitochondria. Taken together with the results from c-Abl$^{-/-}$ cells, the findings with STI571 support a model in which activation of c-Abl contributes, at least in part, to targeting proapoptotic signals to mitochondria in genotoxic stress.

Acknowledgments

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