Activation of the initiator caspase-9 is essential for induction of apoptosis by developmental signals, oncogenic transformation, and genotoxic stress. The c-Abl tyrosine kinase is also involved in the apoptotic response to DNA damage. The present results demonstrate that c-Abl binds directly to caspase-9. We show that c-Abl phosphorylates caspase-9 on Tyr-153 in vitro and in cells treated with DNA damaging agents. Moreover, inhibition of c-Abl with STI571 blocked DNA damage-induced autoprocessing of caspase-9 to the p35 subunit and activation of caspase-3. Caspase-9(Y153F) also attenuated DNA damage-induced processing of caspase-9 to p35, activation of caspase-3, and apoptosis. These findings indicate that caspase-9 autoprocessing is regulated by c-Abl in the apoptotic response to genotoxic stress.

Caspase-9 is the initiator caspase of the apoptosome, an oligomeric complex that controls the intrinsic apoptotic pathway. Formation of the apoptosome is induced by release of mitochondrial cytochrome c into the cytosol. Cytochrome c associates with Apaf-1 and thereby promotes its oligomerization and recruitment of caspase-9 (1–3). Binding to Apaf-1 increases activity of the caspase-9 protease and autoprocessing of the p46 pro-caspase-9 at Asp-315 to yield p35 and p12 subunits (4–6). Caspase-9 activation requires interaction with the Apaf-1 caspase recruitment domain, an increase in local concentrations of caspase-9, and the formation of caspase-9 dimers (7, 8). Following autoprocessing in the apoptosome, caspase-9 cleaves and activates caspase-3. In turn, caspase-3 directs feedback cleavage of caspase-9 at Asp-330 to generate p37 and p10 subunits (4, 9). The caspase-9 p12, and not the p10, subunit contains four N-terminal amino acids that bind to the third baculoviral repeat of the X-linked inhibitor of apoptosis (10), which maintains caspase-9 in the inactive monomer conformation (11, 12) and functions as a tether for caspase-3 (13, 14). Other studies have demonstrated that caspase-9 activity is inhibited by Akt-mediated phosphorylation on Ser-196 (15) and by extracellular signal-regulated kinase-mediated phosphorylation on Thr-125 (16). However, it is not known whether phosphorylation of caspase-9 contributes to autoprocessing of this important apoptotic initiator.

The c-Abl tyrosine kinase is activated in the response of cells to genotoxic stress (17). The product of the gene mutated in ataxia telangiectasia is responsible in part for c-Abl activation (18, 19). Other work has demonstrated that nuclear c-Abl interacts with the DNA-dependent protein kinase (DNA-PK)- Ku complex (20, 21). Phosphorylation of c-Abl by the catalytic subunit DNA-PKcs stimulates c-Abl activity (20). Activation of c-Abl by DNA damage or inhibition of DNA replication contributes to the induction of apoptosis by mechanisms in part dependent on the p53 tumor suppressor and its homolog p73 (22–26). c-Abl also contributes to DNA damage-induced activation of the mitogen-activated protein kinase/extracellular signal-regulated kinase (MEK) kinase-1, c-Jun N-terminal kinase, and p38 mitogen-activated protein kinase pathways (17, 27–29). Moreover, c-Abl interactions with Rad51, Rad9, and the hTERT telomerase catalytic subunit have been implicated in the apoptotic response to DNA damage (30–32). In concert with these studies, DNA damage-induced apoptosis is attenuated in cells that i) express a kinase-inactive, dominant-negative c-Abl(K-R) mutant, ii) are null for c-Abl (c-abl−/−), or iii) are treated with the c-Abl kinase inhibitor STI571 (23, 33, 34). Notably, however, there are no known interactions between c-Abl and the initiator or effector caspases.

The present studies demonstrate that c-Abl phosphorylates caspase-9 on Tyr-153 in vitro and in the response to DNA damage. We also show that c-Abl-mediated phosphorylation of caspase-9 contributes to DNA damage-induced autoprocessing of caspase-9, activation of caspase-3, and apoptosis.

MATERIALS AND METHODS

Cell Culture—Human U-937 myeloid leukemia cells (ATCC, Manassas, VA) were cultured in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mM l-glutamine. Wild-type, c-abl−/− and c-abl+/− mouse fibroblasts (35) were grown in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum and antibiotics. Cells were treated with 10 μM 1-(β-D-arabinofuranosyl)cytosine (araC) (Sigma). Irradiation was performed with a γ-ray source (137Cs, GammaCell 1000, Atomic Energy of Canada, Ltd., Ontario, Canada) at a fixed dose of 13 grays/min.

Plasmid Construction—The vector expressing pCDNA3-caspase-9 has been described (36). The caspase-9(Y153F) mutant was generated by site-directed mutagenesis and confirmed by DNA sequencing. Caspase-9 and caspase-9(Y153F) were subcloned into pGEX4T-1 (Amersham Biosciences) at the BamH1 and EcoRI sites and into pLXIN (Clontech) at the BamH1 site.

The abbreviations used are: araC, 1-(β-D-arabinofuranosyl)cytosine; GST, glutathione S-transferase; SH3, Src homology 3; IR, ionizing radiation.
c-Abl Regulates Caspase-9 Autocleavage

RESULTS

Caspase-9(Y153F) or STI571 Treatment Attenuates DNA Damage-induced Autoprocessing of Caspase-9—Treatment of U-937 cells with araC is associated with phosphorylation of caspase-9 in the cytosolic fraction (Fig. 2D, right). Immunoblot analysis of caspase-9(Y153F) or STI571-treated U-937 cells showed reduced levels of caspase-9(Y153F) (Fig. 2C, right). In addition, treatment with STI571 (a c-Abl inhibitor) resulted in a decrease in the levels of caspase-9(Y153F) (Fig. 2D, right).

Phenylalanine (Tyr → Phe) and the mutants were analyzed as substrates for c-Abl. The results demonstrated that, in contrast to other mutants (Y251F, Y345F, Y363F, Y379F), there was no detectable phosphorylation of His-caspase-9 with the Y153F mutation (Fig. 2A, right). To determine whether c-Abl phosphorylates caspase-9 in vivo, studies were performed with wild-type and c-abl−/− mouse fibroblasts. Constitutive and araC-induced tyrosine phosphorylation of caspase-9 was detectable in wild-type, but not c-abl−/−, cells (Fig. 2B). Importantly, expression of c-Abl in the c-abl−/− cells (c-abl+/−) was also associated with constitutive and araC-induced tyrosine phosphorylation of caspase-9 (Fig. 2B). To determine whether caspase-9 is phosphorylated on Tyr-153 in cells, we generated U-937 cells stably expressing an empty vector or caspase-9(Y153F) (Fig. 2C, left). U-937 cells transfected with wild-type caspase-9 were not viable. As found for endogenous caspase-9, araC treatment was associated with coprecipitation of c-Abl and caspase-9(Y153F) (Fig. 2C, right). In addition, immunoblot analysis of caspase-9(Y153F) immunoprecipitates with anti-phospho-Tyr demonstrated that c-Abl interacts with caspase-9(Y153F) in the U-937/araC/vector cells (Fig. 2D, left). By contrast, there was little detectable tyrosine phosphorylation of caspase-9 in the araC-treated U-937/caspase-9(Y153F) cells (Fig. 2D, right). These findings indicate that c-Abl phosphorylates caspase-9 on Tyr-153 in vitro and in the response of cells to DNA damage.

Caspase-9(Y153F) or STI571 treatment attenuated DNA damage-induced autophosphorylation of caspase-9 (Fig. 2C and 2D). The results demonstrated that, in contrast to other mutants (Y251F, Y345F, Y363F, Y379F), there was no detectable phosphorylation of His-caspase-9 with the Y153F mutation (Fig. 2A, right). To determine whether c-Abl phosphorylates caspase-9 in vivo, studies were performed with wild-type and c-abl−/− mouse fibroblasts. Constitutive and araC-induced tyrosine phosphorylation of caspase-9 was detectable in wild-type, but not c-abl−/−, cells (Fig. 2B). Importantly, expression of c-Abl in the c-abl−/− cells (c-abl+/−) was also associated with constitutive and araC-induced tyrosine phosphorylation of caspase-9 (Fig. 2B). To determine whether caspase-9 is phosphorylated on Tyr-153 in cells, we generated U-937 cells stably expressing an empty vector or caspase-9(Y153F) (Fig. 2C, left). U-937 cells transfected with wild-type caspase-9 were not viable. As found for endogenous caspase-9, araC treatment was associated with coprecipitation of c-Abl and caspase-9(Y153F) (Fig. 2C, right). In addition, immunoblot analysis of caspase-9(Y153F) immunoprecipitates with anti-phospho-Tyr demonstrated that c-Abl interacts with caspase-9(Y153F) in the U-937/araC/vector cells (Fig. 2D, left). By contrast, there was little detectable tyrosine phosphorylation of caspase-9 in the araC-treated U-937/caspase-9(Y153F) cells (Fig. 2D, right). These findings indicate that c-Abl phosphorylates caspase-9 on Tyr-153 in vitro and in the response of cells to DNA damage.

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treated U-937/caspase-9(Y153F) cells (Fig. 3A). Moreover, araC-induced activation of caspase-3 was attenuated in U-937/caspase-9(Y153F) cells compared with that in U-937/vector cells (Fig. 3B). Caspase-3 induces cleavage of caspase-9 to the p37 subunit (4, 9). Thus, cleavage of caspase-9(Y153F) to p37 in the absence of caspase-3 activation indicates that low levels of caspase-3 activity or other proteases, perhaps even caspase-9 itself, may induce cleavage at Asp-330 in the presence of the Y153F mutation. To determine whether inhibition of c-Abl activity has similar effects on caspase-9 and caspase-3, U-937 cells were treated with the c-Abl inhibitor, STI571. Exposure to STI571 had no apparent effect on araC-induced release of cytochrome c (Fig. 3C). However, STI571 treatment was associated with attenuation of araC-induced cleavage of caspase-9 to the p35 subunit (Fig. 3C). STI571 also attenuated araC-induced activation of caspase-3 (data not shown). Consistent with
Caspase-9(Y153F) attenuates DNA damage-induced apoptosis. A, U-937/vector (open bars) and U-937/caspase-9(Y153F) (solid bars) cells were treated with 10 μM araC for the indicated times. The results are expressed as the percentage (mean ± S.D. of three independent experiments) of apoptotic cells with sub-G1 DNA content, and this response was attenuated in the caspase-9 large subunit near the caspase recruitment domain region. B, U-937/caspase-9(Y153F) cells were treated with 20 grays of IR and harvested at the indicated times. The percentage of apoptotic cells with sub-G1 DNA is expressed as the percentage of cells with sub-G1 DNA. araC treatment of U-937/vector and U-937/caspase-9(Y153F) cells was treated with araC for 18 h or with 20 grays of IR and harvested at 24 h. The cells were then assayed for DNA fragmentation by agarose gel electrophoresis.

discussion

Caspase-9(Y153F) attenuates DNA damage-induced apoptosis. As an additional assay for apoptotic cells, araC-induced DNA laddering was attenuated in U-937 cells expressing caspase-9(Y153F) (Fig. 4C). Similar results were obtained with IR-treated U-937/vector and U-937/caspase-9(Y153F) cells (Fig. 4C). These findings indicate that caspase-9(Y153F) attenuates DNA damage-induced apoptosis.

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references

1. Adrain, C., Slee, E., Harte, M., and Martin, S. (1999) J. Biol. Chem. 274, 11549–11556
2. Zou, H., Li, X., Liu, X., and Wang, X. (1999) J. Biol. Chem. 274, 11549–11556
3. Jiang, X., and Wang, X. (2000) J. Biol. Chem. 275, 31199–31203
4. Srivivasalu, S., Ahmad, M., Alhemri, T., and Alhemri, E. (1998) Mol. Cell 1, 949–957
5. Stennicke, H., Deveraux, Q., Homcke, E., Reed, J., Dixit, V., and Salvesen, G. (1999) J. Biol. Chem. 26, 8359–8362
6. Rodriguez, J., and Lasek, Y. (1999) Genes Dev. 13, 3179–3184
c-Abl Tyrosine Kinase Regulates Caspase-9 Autocleavage in the Apoptotic Response to DNA Damage

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