Role of SHP-2 Tyrosine Phosphatase in the DNA Damage-induced Cell Death Response*

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SHP-2, a ubiquitously expressed Src homology 2 (SH2) domain-containing tyrosine phosphatase, plays a critical role in the regulation of growth factor and cytokine signal transduction. Here we report a novel function of this phosphatase in DNA damage-induced cellular responses. Mutant embryonic fibroblast cells lacking functional SHP-2 showed significantly decreased apoptosis in response to DNA damage. Following cispabinet treatment, induction of p73 and its downstream effector p21<sup>WAF1/Cip1</sup> was essentially blocked in SHP-2 mutant cells. Further investigation revealed that activation of the nuclear tyrosine kinase c-Abl, an essential mediator in DNA damage induction of p73, was impaired in the mutant cells, suggesting a functional requirement of SHP-2 in c-Abl activation. Consistent with this observation, the effect of overexpression of c-Abl kinase in SHP-2 mutant cells on sensitizing the cells to DNA damage-induced death was abolished. Additionally, we found that in embryonic fibroblast cells 30–40% of SHP-2 was localized in the nuclei, and that a fraction of nuclear SHP-2 was constitutively associated with c-Abl via its SH3 domain. Phosphatase activity of nuclear but not cytoplasmic SHP-2 was significantly enhanced in response to DNA damage. These results together suggest a novel nuclear function for SHP-2 phosphatase in the regulation of DNA damage-induced apoptotic responses.

DNA damage induced by ionizing radiation, ultraviolet light, and genotoxic agents triggers intracellular signaling cascades that lead to cellular responses such as cell cycle arrest, activation of DNA repair, and apoptotic cell death (1, 2). p53, a well known tumor suppressor transcription factor, is induced and activated in response to DNA damage to mediate cell death and cell cycle arrest (3–5). However, ionizing radiation and DNA damaging agents such as cispabinet can also induce apoptosis in a p53-independent manner (6, 7). Recent studies have revealed that substantial components of the p53 independent pathway are mediated by p73 (8–10), a newly identified member of the p53 transcription factor family (11, 12). Both p53 and p73 are pro-apoptotic proteins; overexpression of p53 and p73 leads to cellular apoptosis through activation of the apoptotic machin-ery (13). They can also transactivate downstream effectors such as p21<sup>Cip1</sup>, MDM2, and GADD45 to regulate cell cycle progression. Further investigations have demonstrated that p73 but not p53, is the downstream effector and target of the nuclear tyrosine kinase c-Abl (8–10).

c-Abl, a Src homology (SH) 2 and SH3 domain-containing tyrosine kinase, shares high homology and similar overall structure with the Src family tyrosine kinases. However, a unique characteristic of c-Abl kinase is that it negatively regulates cellular growth; overexpression of c-Abl kinase induces cell growth arrest and apoptosis (14, 15). This kinase is primarily located in the nucleus and is normally repressed, but is activated upon DNA damage induced by ionizing radiation or DNA damaging drugs (16–20). c-Abl is clearly required for DNA damage-induced apoptosis, because c-Abl-deficient cells or MCF7 cells harboring dominant negative (catalytically inactive) c-Abl show defects in DNA damage-induced apoptosis (7, 21).

Insights into how c-Abl acts came from the identification of its upstream regulatory signals and downstream targets. DNA damage activates c-Abl kinase (16–20). Activated c-Abl induces apoptosis in a p53-independent manner, as c-Abl induces apoptosis in both p53 null cells and p53 positive cells in which p53 is inactivated by expressed viral proteins (7, 8, 22, 23). Moreover, DNA damage induction of p53 is intact in c-Abl-deficient cells (7, 8). Recent studies have revealed that the c-Abl-induced p53-independent apoptotic pathway is mediated by the p73 transcription factor (8–10). Activation of p73 requires functional c-Abl; neither induction nor tyrosyl phosphorylation of p73 occurs in c-Abl-deficient cells (8–10). More importantly, it has been demonstrated that mutated in ataxia telangiectasia (ATM) kinase, the product of the gene mutated in the human genetic disorder ataxia telangiectasia and an important component of the DNA damage-induced cell cycle checkpoint, interacts and phosphorylates (activates) c-Abl at serine 465 (19, 20). In addition to regulating apoptosis, c-Abl also has an important effect on cell cycle progression; overexpression of c-Abl blocks G1/S transition, and cells with compromised c-Abl function, or cells lacking c-Abl have dysregulated cell cycles (14, 24). However, even though a critical role for c-Abl kinase in DNA damage-induced cellular responses has been well established, the precise knowledge of the molecular components involved and their working relationship in the c-Abl-mediated pathways remain unclear.

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1 The abbreviations used are: SH2, Src homology domain 2; WT, wild-type; GST, glutathione S-transferase; JNK, c-Jun N-terminal kinase; ATR, ATM-related kinase; ATM, mutated in ataxia telangiectasia; DAPI, 4',6'-diamidino-2-phenylindole.
SHP-2, an SH2 domain-containing tyrosine phosphatase, is ubiquitously expressed in a variety of tissues and cell types, and has been demonstrated to be involved in diverse signaling pathways, including those initiated by growth factors, cytokines, and insulin (25, 26). In most circumstances, SHP-2 plays a positive role in transducing the signal relay from receptor tyrosine kinases, whereby its phosphatase activity has been shown to be required (27–30), even though the biochemical significance of its catalytic activity remains ill-defined. The N-terminal SH2 domain (N-SH2) plays a critical role in mediating SHP-2 function. A targeted N-terminal deletion of SHP-2 (amino acids 46–110 including the N-SH2) results in a loss-of-function for SHP-2. As a result of this mutation, homozygous mutant (SHP-2Δ2–14) embryos die at midgestation with multiple developmental defects (31). Essential roles for SHP-2 in the regulation of a variety of signal transduction pathways and cellular processes such as cell proliferation, differentiation, adhesion, and migration have been characterized by using this SHP-2 gene knockout mouse model and its derived mutant cell lines (31–39).

Although the role for SHP-2 phosphatase in the signal transduction of growth factors and cytokines has been relatively well established, several clues from other studies in our laboratory indicated that SHP-2 might also be involved in cell death regulation. In the present studies, we examined cellular responses of SHP-2Δ2–14 mutant cells to DNA damage, and showed that SHP-2 played an important role in the genotoxic stress-induced cell death signaling.

**EXPERIMENTAL PROCEDURES**

**Cell Lines and Reagents—**Wild-type (WT) and SHP-2Δ2–14 mutant embryonic fibroblast cell lines were derived from day 9.0–9.5 embryos through SV40 T antigen immortalization (35–39). Rescued cell lines were generated by transduction of WT SHP-2 cDNA into SHP-2Δ2–14 cells through a retroviral-mediated gene transfer. All cell lines were cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum. Cisplatin and anti-tubulin antibody were purchased from Sigma. Anti-p53 (Ab-1), c-Abl (Ab-3), ATM (Ab-3), and ATR (Ab-2) antibodies and p60src enzyme were obtained from Oncogene Science. Anti-c-Abl (K-12), p73 (H-79), p21(WAF1/CIP1) (F-5), and SHP-2 (C-18) antibodies were supplied by Santa Cruz Biotechnology, Inc. Anti-phospho-SerThr-Glu motif antibodies were purchased from Cell Signaling Technology. Anti-phosphotyrosine (Tyr(P)) antibody (4G10) was obtained from Upstate Biotechnology.

**Cell Survival Assay—**WT, SHP-2Δ2–14, and rescued SHP-2Δ2–14 cells were seeded in 96-well plates (8000 cells/well). Cells were treated with freshly prepared cisplatin in 0.9% saline solution 24 h later. Forty-eight hours after treatment, the media was decanted, and the plates were submerged in 0.4% crystal violet in 50% methanol for 30 min, and then rinsed with water. The plates were dried and the bound dye was solubilized by incubation at 37 °C for 1 h with 200 μl of 0.5% SDS in 50% ethanol. The plates were read at 595 nm, and the cell survival rate was then determined by comparing the OD value of the treated to the untreated control cells (37).

4′,6′-Diamidino-2-phenylindole Staining—Cells grown in slide chambers were treated with various concentrations of cisplatin. Twenty-four hours later, the cells were fixed with methanol for 15 min, washed with distilled water, and then stained with 4′,6′-diamidino-2-phenylindole (DAPI) (1 μg/ml) for 30 min. Nuclei were visualized under a fluorescence microscope. Apoptotic cells were identified by condensation and fragmentation of nuclei, and the percentage of apoptotic cells was calculated as the ratio of apoptotic cells to total cells in randomly selected fields (40, 41). A minimum of 300 cells was counted.

**Preparation of Cytosolic and Nuclear Extracts—**Cytosolic and nuclear extracts were prepared essentially as described (42). Cells were lysed on ice for 15 min with the buffer containing 10 mM Hepes, pH 7.5, 2 mM MgCl2, 1 mM EDTA, 1 mM EGTA, 10 mM KCl, 1 mM dithiothreitol, 10 mM NaF, 0.1 mM Na3VO4, and protease inhibitor mixture (Roche Molecular Biochemicals). Nonidet P-40 was then added to 0.5%. After further incubation on ice for 5 min, samples were vortexed for 20 s and then spun at 13,000 rpm for 30 s. Supernatant was collected as cytosolic extracts. Nuclear pellets were washed once with the aforementioned buffer and then suspended and sonicated in nuclear extraction buffer (25 mM Hepes, pH 7.5, 500 mM NaCl, 1 mM dithiothreitol, 10 mM NaF, 10% glycerol, 0.2% Nonidet P-40, 5 mM MgCl2). Supernatant containing nuclear extracts was finally collected after centrifugation at 13,000 rpm for 30 min. The concentration of tyrosyl phosphorylation of nuclear SHP-2, nuclear pellets were homogenized and lysed in nuclear extraction buffer for 40 min instead of being sonicated.

**In Vitro Fusion Protein Binding Assay—**GST, GST-Ab1 SH2, and GST-Ab3 SH3 fusion proteins were purified by glutathione-Sepharose 4B beads and the immobilized proteins on beads were equilibrated in RIPA buffer. Cell lysates (500 μg) were incubated with 2 μg of immobilized GST fusion proteins at 4 °C for 2 h. The protein complexes were washed three times with RIPA buffer, resolved by SDS-PAGE, and then subjected to immunoblotting with anti-SHP-2 antibody as reported (20, 43).

**In Vitro Kinase Assays—**The c-Abl kinase assay was performed as reported (16, 17). Briefly, whole cell lysates (500 μg) were immunoprecipitated with anti-c-Abl antibody (2 μg). Immunoprecipitates were washed three times with RIPA buffer, one time with kinase buffer (50 mM Tris-HCl, pH 7.5, 10 mM MnCl2, and 0.1 mM sodium orthovanadate), and were finally resuspended in 30 μl of kinase buffer containing GST-Crk (5 μg), 10 μM ATP, and 5 μCi of [γ-32P]ATP. The reaction was incubated at room temperature for 30 min. Proteins in the whole reaction system were resolved by SDS-PAGE, and phosphorylated GST-Crk was visualized by autoradiography. For the JNK kinase assay, cell lysates (500 μg) were incubated with 5 μg of immobilized GST-c-Jun protein at 4 °C for 3 h. After the beads were washed three times with RIPA buffer and once with kinase buffer (20 mM Hepes, pH 7.8, 20 mM KCl, 2 mM MgCl2, 0.5 mM Na3VO4, 0.1 mM NaF, 0.05% Nonidet P-40, 10 μM NaF, 1 mM dithiothreitol), 10 μM of kinase buffer containing 10 μCi of [γ-32P]ATP was then added into each reaction system. The reaction was incubated at 30 °C for 20 min, and phosphorylation of GST-c-Jun was examined by SDS-PAGE and autoradiography.

**In Vitro Phosphatase Assay—**Phosphatase activity of SHP-2 was measured as we and others previously described (32, 44). Briefly, myelin basic protein was labeled with [γ-32P]ATP upon phosphorylation by p60src kinase. Whole cell lysates, cytosolic, or nuclear extracts (250 μg) of WT and SHP-2 mutant cells treated with cisplatin (25 μM, 2 h) were immunoprecipitated with anti-SHP-2 antibody. Immunoprecipitates were then mixed with the [γ-32P]-labeled myelin basic protein in the phosphatase assay buffer for 30 min. Then free [γ-32P]ATP released into the supernatant was measured after trichloroacetic acid precipitation. SHP-2 phosphatase activities in WT and mutant cells following cisplatin treatment were determined by the extent of myelin basic protein dephosphorylation in vitro.

**RESULTS**

To define new potential functions of SHP-2 phosphatase, we recently tested for its role in DNA damage-induced signaling by examining cell death sensitivity to DNA damage in WT and mutant embryonic fibroblast cells carrying an amino acid 46–110 deletion mutation of SHP-2 (SHP-2Δ2–14). Cell survival assays demonstrated that mutant cells lacking functional SHP-2 were much more resistant to the DNA damaging chemotherapeutic drug cisplatin than their WT counterparts (Fig. 1A). This appears to be attributed to their differential apoptotic responses to DNA damage, as chromatin DAPI staining showed that the percentage of cells with typical morphological changes associated with apoptosis, including nuclear blebbing and heterochromatin aggregation, was significantly decreased in SHP-2Δ2–14 mutant cells following DNA damage (Fig. 1B). The DNA fragmentation assay, a characteristic analysis for apoptotic cells, also showed that significantly reduced DNA fragmentation was observed in SHP-2 mutant cells treated with cisplatin (data not shown). Moreover, the percentage of hypodiploid cells with sub-G1 DNA content, known to be apoptotic, was signifi-
cantly decreased in SHP-2\(\Delta\) mutant cells following either cisplatin treatment (Fig. 1C) or \(\gamma\)-irradiation (Fig. 1D). Notably, re-introduction of functional WT SHP-2 into mutant cells (rescued cell line) partially restored their sensitivity to DNA damage (Fig. 1), suggesting that the decrease in apoptosis in mutant cells resulted from loss-of-function of SHP-2 rather than gain-of-function of the truncated SHP-2 expressed in the mutant cells.

Previous studies have demonstrated that p53 tumor suppressor and its homologue p73 are major mediators in cellular death responses to DNA damage (45, 46). The observation that DNA damage gave rise to different cell death sensitivities between WT and SHP-2\(\Delta\) mutant cells prompted us to examine p53 and p73 induction in SHP-2\(\Delta\) mutant cells. Consistent with previous reports (8), p73 induction in SHP-2\(\Delta\) mutant cells was completely blocked. Protracted incubation and increased dosages of cisplatin did not increase the expression of p73 in these mutant cells (data not shown). As p73 can transactivate the downstream effector p21\(^{Cip1}\), the induction of p21\(^{Cip1}\) was next analyzed. Consistent with the expression of p73, p21\(^{Cip1}\) induction was also diminished in SHP-2\(\Delta\) mutant cells (Fig. 2). Notably, p73 and p21\(^{Cip1}\) induction was partially restored in the rescued cell line, further confirming a role for SHP-2 tyrosine phosphatase in DNA damage-induced intracellular responses.
In response to cisplatin treatment, SHP-2 phosphorylation was significantly increased shortly after cisplatin treatment. However, phosphorylation of the truncated form of SHP-2 (SHP-2Δ) expressed in SHP-2Δ mutant cells was unchanged at all time points examined (Fig. 3A). Subsequent in vitro phosphatase assays demonstrated that catalytic activity of SHP-2 was doubled in the WT cells following cisplatin treatment (Fig. 3B). By contrast, although SHP-2Δ in the untreated mutant cells had 2 times higher basal activity as we previously reported (32), its phosphatase activity remained unchanged after DNA damage (Fig. 3B). Together, these results suggest that SHP-2 tyrosine phosphatase is involved in DNA damage-induced signaling and that the truncated form of SHP-2 expressed in the mutant cells might not be functioning in response to DNA damage.

Previous studies have clearly demonstrated that induction and stabilization of p73 but not p53 in response to DNA damage is completely dependent on nuclear c-Abl kinase activity (8–10). The blockade of p73 induction in SHP-2Δ mutant cells indicated that c-Abl activation following cisplatin treatment in these cells might be impaired. To test this hypothesis, tyrosyl phosphorylation response of c-Abl kinase was first examined to determine its activation status. As shown in Fig. 4A, c-Abl became phosphorylated in WT cells 2–3 h after DNA damage. In contrast, phosphorylation of c-Abl in SHP-2 mutant cells was significantly reduced. Several c-Abl antibodies from various manufacturers were used for this experiment, producing similar results. We also checked longer time points following genotoxic insult (4, 8, and 24 h) in which no obvious phosphorylation of c-Abl was observed in mutant cells (data not shown). Subsequent examination of the tyrosyl phosphorylation response of baseline p73 through anti-p73 immunoprecipitation followed by anti-phosphotyrosine immunoblotting revealed that its phosphorylation in WT cells was rapidly induced after DNA damage as previously reported (9, 10). Interestingly, this response in SHP-2Δ mutant cells was undetectable (Fig. 4B). Tyrosyl phosphorylation of p73 in response to DNA damage is fully attributed to c-Abl kinase activity (9, 10), these results further suggested that c-Abl activation in mutant cells was compromised. Subsequently, this notion was supported by in vitro c-Abl kinase assays. Using GST-Crk as the substrate, we showed that in response to cisplatin-induced DNA damage, activation of c-Abl kinase was barely detectable in the SHP-2 mutant cells, but was partially restored in the rescued cell line following reintroduction of WT SHP-2 (Fig. 4C), confirming a requirement of SHP-2 for c-Abl activation.

Interestingly, activation of JNK kinase, known to be implicated in mediating stress-induced apoptosis (50), was dramatically enhanced in SHP-2Δ mutant cells (Fig. 4D), suggesting that decreased sensitivity of SHP-2Δ mutant cells to DNA damage-induced apoptosis was not related to JNK activity. This result additionally indicates that SHP-2 tyrosine phosphatase negatively regulates DNA damage-induced JNK kinase activation.

c-Abl kinase has been previously demonstrated to negatively regulate cellular growth. Overexpression of WT c-Abl leads to cell growth arrest and sensitizes cellular apoptotic responses to DNA damage, whereas interference of endogenous c-Abl function by overexpression of kinase inactive c-Abl (c-Abl K/R) reduces DNA damage-induced cell death (14–16). To further confirm the functional requirement of SHP-2 for c-Abl activation in response to DNA damage, we transfected WT and c-Abl K/R into WT and SHP-2Δ mutant cells and assessed the effects of WT c-Abl and c-Abl K/R in these cells. As shown in Fig. 5, the sensitivity of WT cells to cisplatin was enhanced by transfection of the WT c-Abl as compared to the pSR vector
control, whereas expression of the c-Abl K/R mutant in WT cells significantly reduced cell death. Interestingly, although the transfected WT c-Abl was equally overexpressed in SHP-2<sup>2Δ/Δ</sup> mutant cells, the effect of overexpression of c-Abl in these mutant cells was abrogated, suggesting that the c-Abl kinase did not function in mutant cells without WT SHP-2. Because endogenous c-Abl in SHP-2<sup>2Δ/Δ</sup> cells was disabled as described above (Fig. 4), the dominant negative effect of catalytically inactive c-Abl K/R in mutant cells was not observed. Together, these results further confirm a requirement of SHP-2 for c-Abl activation and thereby cell death responses to DNA damage.

Co-immunoprecipitation experiments showed that SHP-2 protein was detected in the anti-c-Abl immunocomplex, suggesting direct or indirect interactions between SHP-2 and c-Abl. Interestingly, their association was constitutive, being independent on DNA damage treatment (Fig. 6A). Extension of cisplatin treatment to 4, 8, 12, and 24 h did not increase their physical association (data not shown). In addition, SHP-2 was also detected in the immunocomplex pulled down with anti-ATM antibody. Likewise, their association was found to be constitutive. By contrast, the truncated form of SHP-2 expressed in mutant cells was dramatically reduced in these immunocomplexes. To determine whether the association between c-Abl and SHP-2 is direct, in vitro GST fusion protein binding assays were performed. As shown in Fig. 6B, the GST-SH3 domain but not the GST-SH2 domain of c-Abl pulled down WT SHP-2, suggesting a direct interaction between these two proteins.

**Fig. 4.** DNA damage-induced activation of the c-Abl kinase is compromised in SHP-2<sup>2Δ/Δ</sup> mutant cells. WT and mutant cells were treated with 25 μM cisplatin for various time periods. Whole cell lysates were prepared and subjected to anti-c-Abl (A) and anti-p73 (B) immunoprecipitation followed by anti-Tyr(P) immunoblotting. Cell lysates (500 μg) were also used for the in vitro c-Abl kinase assay (C) and the JNK kinase assay (D) as described under “Experimental Procedures.” Shown are representatives of two to three independent experiments.

**Fig. 5.** The effect of overexpression of c-Abl kinase on DNA damage-induced apoptosis is abolished in SHP-2<sup>2Δ/Δ</sup> mutant cells. WT and SHP-2<sup>2Δ/Δ</sup> mutant cells were transfected with the indicated plasmids by calcium phosphate precipitation. Forty-eight hours after transfection, the cells were treated with 15 μM cisplatin, and the cell survival rate was determined using the One Solution Proliferation Assay Kit (Promega) 48 h later. The upper panel is the expression of c-Abl kinase or c-Abl (K/R) in the transfected cells. Three independent experiments were performed, similar results were obtained. Results shown are the mean ± S.E. of triplicates from one experiment.
signaling proteins, with their interaction being mediated by the c-Abl SH3 domain. By contrast, binding of the truncated form of SHP-2 expressed in the mutant cells with GST-Abl SH3 was greatly reduced.

Because c-Abl is localized both in the nucleus and the cytoplasm of fibroblast cells, we wanted to determine which fraction of c-Abl associated with SHP-2. Cytosolic (C) and nuclear (N) extracts prepared from untreated and cisplatin-treated (25 μM, for 2 h) WT embryonic fibroblast cells were immunoblotted with anti-SHP-2, anti-c-Abl, and anti-tubulin Abs. Cytosolic and nuclear extracts (500 μg) were also subjected to anti-c-Abl immunoprecipitation followed by anti-SHP-2 immunoblotting. Additional methanol-fixed WT cells were double-immuno-stained with anti-SHP-2 (rabbit IgG) and anti-tubulin (mouse IgG) antibodies according to standard procedures. Normal rabbit IgG and mouse IgG were included as negative controls. Goat anti-rabbit IgG conjugated with fluorescein isothiocyanate and rhodamine-labeled goat anti-mouse IgG antibodies were used as secondary antibodies. Cells in the same field were visualized and photographed under a fluorescence microscope. Only the rabbit IgG control is displayed. Shown are representatives of two to three independent experiments.

Because SHP-2 is localized both in the cytoplasm and the nucleus, we next examined phosphatase activity of SHP-2 in cytosolic and nuclear extracts from cisplatin-treated WT cells to determine which fraction of SHP-2 participates in DNA damage signaling. In response to DNA damage, tyrosyl phosphorylation and phosphatase activity of the nuclear fraction of SHP-2 were enhanced, while catalytic activity of cytosolic SHP-2 remained unchanged (Fig. 7). This result suggests that nuclear but not cytosolic SHP-2 is involved in DNA damage signaling, further confirming the nuclear function of SHP-2.

Fig. 6. Physical associations between SHP-2 and c-Abl and ATM. A, WT and SHP-2<i>Δ</i>A mutant cells were treated with 25 μM cisplatin for 2 h. Whole cell lysates were prepared and immunoprecipitated with anti-c-Abl and ATM antibodies. Immunocomplexes were then resolved by SDS-PAGE followed by anti-SHP-2 immunoblotting. Loading controls were examined by stripping and re-probing the blots with anti-c-Abl and anti-ATM antibodies. B, whole cell lysates prepared from WT and mutant cells were incubated with the indicated GST fusion proteins. Proteins bound with glutathione-Sepharose 4B beads were then resolved by SDS-PAGE and immunoblotted with anti-SHP-2 antibody. Anti-SHP-2 immunoprecipitations were used as positive controls. C, cytosolic (C) and nuclear (N) extracts prepared from untreated and cisplatin-treated (25 μM, for 2 h) WT embryonic fibroblast cells were immunoblotted with anti-SHP-2, anti-c-Abl, and anti-tubulin Abs. Cytosolic and nuclear extracts (500 μg) were also subjected to anti-c-Abl immunoprecipitation followed by anti-SHP-2 immunoblotting. D, methanol-fixed WT cells were double-immuno-stained with anti-SHP-2 (rabbit IgG) and anti-tubulin (mouse IgG) antibodies according to standard procedures. Only the rabbit IgG control is displayed. Shown are representatives of two to three independent experiments.

SHP-2 and DNA Damage Response 15213
DNA damage-induced cellular response.

We next attempted to dissect the potential mechanism by which SHP-2 regulates DNA damage-induced c-Abl activation, and examined c-Abl upstream activators. To date, ATM kinase and possibly the ATM-related kinase (ATR) have been demonstrated to be responsible for the activation of c-Abl after DNA damage (19, 20, 51). Therefore, we wanted to determine whether SHP-2 might modulate c-Abl activation through its upstream ATM/ATR kinases. To examine ATM/ATR kinase activity, the phosphorylation status of p53 on serine 15, a putative in vivo substrate of ATM/ATR kinases (2, 52–54), was analyzed using a specific anti-phospho-p53Ser-15 antibody. Phosphorylation of p53 on serine 15 was dramatically induced upon cisplatin treatment (Fig. 8A). Interestingly, p53 phosphorylation response in WT and SHP-2/H9004/H9004 mutant cells was not significantly altered. Furthermore, as the ATM/ATR substrate proteins contain specific Ser/Thr-Gln motifs that are phosphorylating sites of these kinases (51, 52, 55), the activation status of ATM/ATR kinases was also determined by examining the phosphorylation status of ATM/ATR substrate proteins in whole cell lysates using a specific anti-phospho-Ser/Thr-Gln antibody. Phosphorylation of ATM/ATR substrates in both WT and SHP-2/H9004 cells treated with cisplatin was comparable (Fig. 8B). Taken together, these results suggest that ATM/ATR kinases were equally activated by cisplatin-induced DNA damage in WT and mutant cells. Therefore, the SHP-2 mutation appears to block c-Abl activation through a mechanism(s) other than ATM/ATR kinases.

DISCUSSION

In this report, we defined a novel nuclear function of SHP-2 tyrosine phosphatase distinct from its critical cytoplasmic role in the regulation of growth factor and cytokine signal transduction. We provided evidence that SHP-2 phosphatase is involved in the DNA damage-triggered signaling pathway whereby its tyrosyl phosphorylation and catalytic activity become significantly increased upon DNA damage treatment. Mutant cells lacking functional SHP-2 demonstrated markedly decreased sensitivities to DNA damage-induced apoptosis. Activation of c-Abl tyrosine kinase and induction of p73 and its effector p21\(^{Cip1}\) were essentially blocked by the SHP-2 mutation. We also show that a substantial amount of SHP-2 protein is localized in the nucleus, and that SHP-2 constitutively associates with nuclear c-Abl via its SH3 domain. These results represent the first demonstration of a connection between SHP-2 phosphatase and c-Abl kinase and consequently the c-Abl-mediated DNA damage response.

It appears that impaired activation of c-Abl kinase in SHP-2/H9004/H9004 mutant cells accounts for their decreased apoptotic response to DNA damage. p73 induction and hence the induction of its downstream effector p21\(^{Cip1}\) were essentially blocked in SHP-2/H9004 cells. Although p53 in WT and SHP-2 mutant fibroblast cells was not significantly induced following DNA damage...
presumably because of SV40 T antigen immortalization (47–49), in the embryonic stem cell system no significant change in p53 induction was observed between WT and SHP-2/−/− mutant embryonic stem cells exposed to DNA damage. Therefore, it is conceivable that p73 but not p53 induction is blocked by the loss-of-function mutation of SHP-2. Because p73 (but not p53) induction and tyrosyl phosphorylation are fully attributed to c-Abl kinase activity (8–10), the complete blockade of the induction of p73 and its downstream effector p21<sup>Cip1</sup> together with the abolished phosphorylation response of baseline p73 in SHP-2/−/− mutant cells suggests that DNA damage-induced c-Abl activation is impaired in the mutant cells. Consistent with this notion, c-Abl phosphorylation analyses and in vitro kinase assays (Fig. 4) showed that c-Abl kinase activation in mutant cells lacking functional SHP-2 phosphatase was significantly reduced. Moreover, this conclusion is supported by c-Abl transfection data, in which the effects of overexpressed WT and dominant negative c-Abl on DNA damage-induced cell death were abrogated in SHP-2/−/− mutant cells (Fig. 5).

The underlying mechanism(s) by which SHP-2 promotes c-Abl activation and c-Abl-mediated DNA damage signaling pathways remains to be determined. The tyrosine phosphatase activity of SHP-2 plays a positive role in a number of signaling pathways induced by growth factors and cytokines. However, a detailed biochemical basis for its positive function is ill-defined. Likewise, it is unclear how SHP-2 promotes c-Abl activation in response to DNA damage. It is unlikely that SHP-2 regulates c-Abl kinase through its upstream activators, as ATM/ATR kinases appeared to be equally activated by DNA damage in both WT and SHP-2/−/− mutant cells (Fig. 8). In addition to ATM/ATR kinases, c-Abl activity can be regulated by several other mechanisms. It is especially important to emphasize that the SH3 domain of c-Abl negatively regulates its kinase activity through an intramolecular interaction between SH3 and the linker region between its SH2 and kinase domains, either deletion or point mutations within the SH3 domain result in constitutive tyrosine kinase activity and oncogenic activation (56–61). c-Abl kinase can also be regulated by its inhibitors (62–64). In our studies, because both in vitro immunoprecipitation and in vitro binding assays showed that SHP-2 phosphatase constitutively associated with c-Abl kinase via its SH3 domain (Fig. 6), and a potential motif for c-Abl SH3 binding (a proline-rich consensus sequence XPXXXPHXPS (43, 65, 66)) has been identified in SHP-2 (228WPDHGPSEP436), it is possible that SHP-2 phosphatase facilitates c-Abl activation by directly acting on c-Abl or c-Abl-associated inhibitors. It is interesting to note that SHP-2 phosphatase also binds to the SH3 domain of c-Src kinase and activates Src by a non-enzymatic mechanism (67). Because c-Abl shares high homology with c-Src kinase, it is likely that SHP-2 promotes c-Abl activation through a similar mechanism, i.e. SHP-2 may displace the inhibitory SH3 domain away from the linker region, thereby co-stabilizing the active state of c-Abl kinase. As the association between c-Abl and the mutant form of SHP-2 was diminished because of either its N-terminal truncation or its decreased protein expression, activation of c-Abl in response to DNA damage in SHP-2 mutant cells was compromised. Nevertheless, because phosphatase activity of WT SHP-2 is significantly increased upon DNA damage, it remains possible that SHP-2 might promote c-Abl activation through inactivating c-Abl inhibitor(s). If this is the case, it would be very interesting to determine the direct downstream substrate(s) of SHP-2 phosphatase and the biochemical significance of its enzymatic activity in this context.

The mutant cell lines used in this study harbor a deletion and loss-of-function mutation of SHP-2. Although the truncated form of SHP-2 lacking N-terminal amino acids 46–110 (including the N-SH2 domain) is expressed, the protein level is only about 25% of WT SHP-2. Previous studies using these cell lines have suggested that it is biologically inert (32–39). Consistent with those studies, the mutant form of SHP-2 also appears to be non-functional in the DNA damage-triggered signaling pathway, because its tyrosyl phosphorylation and phosphatase activity remained unchanged following DNA damage (Fig. 3, A and B). More importantly, reintroduction of functional SHP-2 into the mutant cells partially corrected their biological and biochemical responses to DNA damage. Although the rescue effects were not complete because of the low expression level of reintroduced WT SHP-2 in the rescued cell line, the partial correction of the defective signaling and cellular responses by WT SHP-2 clearly suggests that the targeted mutation of SHP-2 is a hypomorphic mutation and that the phenotype displayed by the mutant cells resulted from loss-of-function rather than gain-of-function of the truncated form of SHP-2 expressed in mutant cells. Regardless of whether the defective cellular response to DNA damage in SHP-2 mutant cells results from the N-terminal truncation of SHP-2 or the decreased expression level of the mutant form of SHP-2, our results obtained using this mutant cell model still support an important role for SHP-2 in DNA damage-induced cell death responses. Additionally, it is noteworthy that because of very early (embryonic day 8.5–10.5) embryonic lethality of SHP-2/−/− mice, the number of primary embryonic fibroblast cells that can be derived from mutant embryos at this stage is extremely limited. For this reason, the fibroblast cell lines we used were immortalized with SV40 T antigen. Even though p53 function in these cell lines is blocked, p73 is fully functional, as SV40 T antigen selectively inactivates p53 but not p73 (47–49). Indeed, p21<sup>Cip1</sup>, a downstream effector of p73, was significantly induced in the immortalized WT cells following DNA damage (Fig. 2A).

In summary, the biological and biochemical evidence presented in this report suggest a previously uncharacterized nuclear function of SHP-2 tyrosine phosphatase, i.e. it plays an important role in the DNA damage-induced apoptosis. SHP-2 is constitutively associated with c-Abl kinase and promotes c-Abl activation in response to genotoxic insult. These findings represent the first demonstration of a link between SHP-2 phosphatase and c-Abl-mediated DNA damage responses, and suggest a potential application of SHP-2 phosphatase in cancer treatment.

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