DNA Damage-induced G₂/M Checkpoint in SV40 Large T Antigen-immortalized Embryonic Fibroblast Cells Requires SHP-2 Tyrosine Phosphatase*

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DNA damage induced by radiation or DNA-damaging agents leads to apoptosis and cell cycle arrest. However, DNA damage-triggered signal transduction involved in these cellular responses is not well understood. We previously demonstrated an important role for SHP-2, a ubiquitously expressed SH2 domain-containing tyrosine phosphatase, in the DNA damage-induced apoptotic response. Here we report a potential role for SHP-2 in a DNA damage-activated cell cycle checkpoint. Cell cycle analysis and the mitotic index assay showed that following DNA damage induced by cisplatin or γ-irradiation, the G₂ (but not S) arrest response was diminished in SV40 large T antigen-immortalized embryonic fibroblast cells lacking functional SHP-2. Notably, re-introduction of wild-type SHP-2 into the mutant cells fully restored the DNA damage-induced G₂ arrest response, suggesting a direct role of SHP-2 in the G₂/M checkpoint. Further biochemical analysis revealed that SHP-2 constitutively associates with 14-3-3β, and that Cdc25C cytoplasmic translocation induced by DNA damage was essentially blocked in SHP-2 mutant cells. Additionally, we showed that following DNA damage, activation of p38 kinase was significantly elevated, while Erk kinase activation was decreased in mutant cells, and treatment of SHP-2 mutant cells with SB203580, a selective inhibitor for p38 kinase, partially restored the DNA damage-induced G₂ arrest response. These results together provide the first evidence that SHP-2 tyrosine phosphatase enhances the DNA damage G₂/M checkpoint in SV40 large T antigen immortalized murine embryonic fibroblast cells.

Genetic stability is maintained by cell cycle checkpoints (1, 2). In response to DNA damage, mammalian cells arrest at certain points in the cell cycle. This regulatory mechanism inhibits cell cycle progression until the cell has adequately repaired the DNA damage. For instance, arrest in G₁ and S phases prevents damaged DNA from replicating, and arrest in the G₂ phase prevents segregation of damaged chromosomes. Failures in cell cycle checkpoints can lead to the acquisition and accumulation of genetic alterations. These changes may result in the activation of oncogenes and/or the inactivation of tumor suppressor genes, both of which can ultimately lead to tumorigenesis. However, the precise mechanisms of cell cycle checkpoints and the signaling components involved are not fully understood.

In many cases, DNA damage-triggered signaling pathways induce cell cycle arrest by inhibiting the activities of the cyclin-dependent kinases that are required to drive cell cycle progression. The biochemical details of the G₁ checkpoint are relatively well understood. A delay in the G₁ phase results largely from the activation of p53 and consequent transcriptional induction of the cyclin-dependent kinase inhibitor p21WAF1 (3, 4). By comparison, the DNA damage-induced G₂/M checkpoint is more complex. The G₂/M transition is regulated by Cdc2 kinase and cyclin B1 as part of the mitosis promoting factor that determines entry into mitosis. It has been demonstrated that the G₂ arrest is largely dependent on inhibitory phosphorylation of Cdc2 at tyrosine 15 (Tyr¹⁵) and threonine 14 (Thr¹⁴) and is therefore likely to result from changes in the activities of the opposing kinases and phosphatases that act on Cdc2. Among these upstream regulators, Cdc25C has been identified as crucial for the activation of Cdc2 by dephosphorylating its inhibitory tyrosine sites (5, 6). Dephosphorylation of Cdc2 by Cdc25C and association with cyclin B1 results in rapid entry into mitosis whereas phosphorylation of negative regulatory sites on Cdc2 by Wee1/Myt1 kinases and cyclin B degradation or export to the cytoplasm block entry into mitosis. Following DNA damage, Cdc25C is phosphorylated by checkpoint kinases Chk1 and 2, which are activated by ATM and its related kinase ATR (7–12). Upon phosphorylation, Cdc25C binds to 14-3-3 adaptors and is thereby sequestered in the cytoplasm (7, 13). Separation of Cdc25C from the nucleus then results in elevated Cdc2 phosphorylation and a reduced Cdc2 kinase activity. As a result, cells arrest in the G₂ phase.

However, in addition to the ATM/Chk1, 2/Cdc25C/Cdc2 pathway, other mechanisms contributing to the G₂/M checkpoint also exist. p21CIP1, a major downstream effector of p53 and p73 transcription factors, contributes mainly to the G₁ and S arrest (3, 4); however, its role in inducing the G₂ arrest has also been reported, i.e. cells deficient in p21CIP1 are unable to maintain stable G₂ arrest when exposed to DNA-damaging agents (14, 15). More recently, several signaling enzymes important for growth factor and cytokine-induced signal transduction, such as Erk, p38, and Akt kinases, have also been found to be involved in the regulation of the G₂/M transition of the cell cycle. For example, Erk kinases have been shown to be re-

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¶ The abbreviations used are: Erk, extracellular signal-regulated kinase; WT, wild type; Ab, antibody; PI, propidium iodide; FACS, fluorescence-activated cell sorting.

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required for normal G2/M progression (16, 17) and DNA damage-induced G2/M arrest (18). p38 kinase has also been shown to be involved in the G2/M DNA damage cell cycle checkpoint (19, 20). Therefore, it appears that multiple pathways contribute to the regulation of the G2/M checkpoint following genotoxic stress.

SHP-2, a 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 (21, 22). 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 (23–25), 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 mutation for SHP-2. As a result of this mutation, homozygous mutant (SHP-2<sup>−/−</sup>) embryos die at midgestation with multiple developmental defects (26, 27). 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 model and the mutant fibroblast cell lines derived from SHP-2<sup>−/−</sup> mutant embryos through SV40 large T antigen immortalization (27–31).

Using the SV40 large T antigen-immortalized SHP-2<sup>−/−</sup> embryonic fibroblast cell lines, we have recently demonstrated that SHP-2 plays an important role in DNA damage-induced cell death, and that it enhances the cellular apoptotic response to DNA damage by promoting activation of nuclear kinase c-Abl (32). During the course of that work, we inadvertently noticed that the DNA damage-induced G2 arrest response was diminished in SHP-2 mutant cells. We therefore investigated the potential role of SHP-2 phosphatase in DNA damage-induced cell cycle checkpoint. These results suggest that SHP-2 enhances the DNA damage G2/M checkpoint by modulating Cdc25C cytoplasmic translocation and the MAP kinase pathways.

**EXPERIMENTAL PROCEDURES**

**Cell Lines and Reagents**—Wild-type (WT) and SHP-2<sup>−/−</sup> mutant embryonic fibroblast cell lines were derived from day 9.5 embryonic fibroblast cell lines (rescued cells) completely corrected cell cycle profiles. As Erk kinase activity has been shown to be required for the G2/M transition of the cell cycle (16, 17) and SHP-2 plays a positive role in the growth factor and cytokine-induced Erk pathway (23–25), the prolonged G2/M phase is presumably attributed to the decreased Erk kinase activity in the mutant cells under normal culture conditions.

More interestingly, in response to the treatment of the DNA damaging chemotherapeutic drug cisplatin, the percentage of WT cells in the G2/M phase was significantly increased (G2/M arrest), suggesting activation of the cell cycle G2/M checkpoint. However, the DNA damage-induced G2/M arrest response in SHP-2<sup>−/−</sup> cells is diminished (Fig. 1B), indicating a defect in the G2/M checkpoint control in mutant cells. Notably, the G2/M arrest response to cisplatin treatment was fully restored in the rescued cell line (Fig. 1B), suggesting that the diminished cell cycle response to DNA damage in mutant cells is attributed directly to loss of SHP-2 function. It is noteworthy that the defect in DNA damage cell cycle control caused by the SHP-2 mutation appears to be specific for the G2/M but not S checkpoints, since the DNA damage-induced response of the arrest in the S phase is not altered in SHP-2 mutant cells (Fig. 1C).

The above experiments were conducted with asynchronized cells. To better determine the role of SHP-2 in the cell cycle response to DNA damage, WT, SHP-2<sup>−/−</sup>, and rescued cells were synchronized at the G1/S boundary as described under...
Experimental Procedures, and cell cycle responses of the synchronized cells to DNA damage were then examined. Similar to the asynchronized cells, synchronized SHP-2\textsuperscript{+/+} cells did not show a significant increase in the percentage of G2/M following DNA damage (Fig. 1D), further confirming the potential role of SHP-2 in DNA damage-induced cell cycle regulation. Remarkably, the role of SHP-2 in G2/M checkpoint control is not specific to cisplatin-induced DNA damage; the γ-irradiation-induced G2/M arrest response is also diminished in SHP-2 mutant cells (Fig. 2). Collectively, these results demonstrate an important role of SHP-2 phosphatase in the DNA damage-induced G2/M checkpoint in SV40 large T antigen-immortalized murine embryonic fibroblast cells.

Since SHP-2\textsuperscript{+/+} cells showed a defect in the G2/M arrest response, we next wanted to determine whether this defect was specific to DNA damaging treatment. WT, SHP-2\textsuperscript{+/+}, and rescued cells were treated with nocodazole, a compound that disrupts nuclear microtubules and arrests the cell cycle in mitosis (35). As shown in Fig. 3A, all cell types were efficiently blocked in the G2/M phase of the cell cycle. To further validate the potential role of SHP-2 in the DNA damage-induced G2/M checkpoint, we treated cells with cisplatin in conjunction with caffeine, a specific inhibitor of ATM/ATR kinase, which has been demonstrated to play an important role in the DNA damage G2/M checkpoint (2). Cell cycle analyses showed that although caffeine treatment alone did not disturb cell cycle distribution, combined caffeine treatment abolished cisplatin-induced G2/M arrest in WT as well as rescued cells. However, this drug did not show any effect in SHP-2 mutant cells (Fig. 3B).

To precisely determine whether the increase of G2/M cells observed in our cell cycle analyses resulted from a delay in G2 or M phases, the mitotic index assay (which directly determines the fraction of cells in mitosis) was conducted. In response to DNA damage, mitotic activity of WT cells was quickly and progressively decreased, indicating a G2 delay in the cell cycle. Although mitosis of SHP-2 mutant cells was also decreased shortly following DNA damage, this G2 delay could not
To more rigorously examine the cell cycle response of SHP-2 mutant cells to DNA damage, we synchronized the cells in the G0/G1 phase or at the G1/S boundary by serum deprivation or thymidine treatment (see “Experimental Procedures”), respectively. Synchronized cells were released and treated with cisplatin for 4 and 8 h. Mitotic cells were counted and compared with the untreated cells that were released from synchronization for the same periods of time. Consistent with the data obtained from asynchronized cells (Fig. 4A), mitotic activity of synchronized WT cells was significantly decreased following DNA damage. By contrast, this response in synchronized SHP-2 mutant cells was diminished (Fig. 4, B and C), further confirming the role of SHP-2 in DNA damage cell cycle regulation.

As the G2/M transition of the cell cycle is mainly controlled by Cdc2 kinase, and phosphorylation of Tyr15 and Thr14 in Cdc2 negatively regulates its kinase activity, we next examined the phosphorylation status of Cdc2 in response to DNA damage by using a specific Ab. As shown in Fig. 5A, Cdc2 (Tyr15) phosphorylation was gradually induced in WT cells following DNA damage. By contrast, phosphorylation of this site in SHP-2Δ/Δ cells was slightly induced and then quickly decreased to the basal level. This observation is fully consistent with the mitotic index data (Fig. 4). Furthermore, we examined Cdc2 kinase activity by using histone H1 as the substrate. As demonstrated in Fig. 5B, after DNA damage (8 h), Cdc2 kinase activity was markedly decreased in WT as well as rescued cell lines. However, no appreciable change in Cdc2 kinase activity is detected in SHP-2 mutant cells. It is important to emphasize that re-introduction of WT SHP-2 in SHP-2Δ/Δ cells completely rescued the response of Cdc2 kinase activity to DNA damage, again suggesting a direct role for SHP-2 in DNA damage-induced cell cycle response, and that although the truncated SHP-2 with a low expression level exists in the mutant cells, the SHP-2 mutation appears to be a loss-of-function mutation rather than a gain-of-function mutation.

Defective G2/M Checkpoint in SHP-2 Mutant Cells—To elucidate why the G2 arrest response induced by DNA damage is attenuated in the mutant cells lacking functional SHP-2, we attempted to dissect the molecular mechanism by which SHP-2 modulates the G2/M checkpoint. Previous studies have demonstrated that several pathways contribute to the DNA damage-induced G2/M arrest, among which the ATM, ATR/Chk1, 2/Cdc25C/Cdc2 pathway is well characterized (2, 14). To determine whether this pathway is targeted by the SHP-2Δ/Δ mutation, we examined the phosphorylation status of Chk1 kinase (Ser345) that has been shown to be critical for its kinase activity (10) and thereby downstream signaling, by using a specific anti-phospho-Chk1 (Ser345) Ab. As shown in Fig. 6A, phosphorylation of Chk1 is comparably induced by DNA damage in WT synchronized WT cells was significantly decreased following DNA damage. By contrast, this response in synchronized SHP-2 mutant cells was diminished (Fig. 4, B and C), further confirming the role of SHP-2 in DNA damage cell cycle regulation.

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and SHP-2 mutant cells. This data, together with our previous observation that phosphorylation of p53^Ser^{15} by ATM/ATR kinase was not changed in SHP-2 mutant cells (32), suggest that Chk kinase activation is not affected by the SHP-2 mutation.

We next examined the downstream processes of the ATM, ATR/Chk1, 2/Cdc25C/Cdc2 pathway. Previous studies have shown that Cdc2 phosphorylation and thereby kinase activity are regulated by Cdc25C, and that both phosphorylation (activation) induced by Chk kinases and cytoplasmic translocation by association with 14-3-3/H9252 are essential for Cdc25C activity on Cdc2 kinase (7, 13). Since Chk1 activation is not affected in SHP-2 mutant cells, we focused on the Cdc25C translocation response. Nuclear extracts were prepared from G1/G0 synchronized cells and subjected to quality control as we previously reported (32). Anti-Cdc25C immunoblotting showed that in response to DNA damaging treatment, Cdc25C in the WT nucleus was decreased, suggesting its cytoplasmic translocation. By contrast, DNA damage-induced Cdc25C translocation was essentially blocked in SHP-2^−/− mutant cells. Remarkably, this response is efficiently restored in the rescued cells (Fig. 6B). It is important to mention that the Cdc25C level in the cytoplasm was not significantly changed in the three cell types following DNA damage (data not shown). This is because a large amount of the Cdc25C is localized in the cytoplasm of the cell lines; small amounts of translocated nuclear Cdc25C did not make a significant difference in the Cdc25C level in the cytoplasm.

To further elucidate the molecular mechanism by which SHP-2 modulates DNA damage-induced Cdc25C translocation, we examined potential interactions between SHP-2 and 14-3-3/Cdc25C. As shown in Fig. 6C, 14-3-3β was constantly detected in the anti-SHP-2 immunocomplex, and reciprocally, SHP-2 was always present in the anti-14-3-3β immunocomplex (data not shown), suggesting their physical association and that this association is independent of DNA-damaging treatment. Therefore, it is likely that SHP-2 promotes Cdc25C translocation by physically interacting with 14-3-3β.
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the effect of the SHP-2 mutation on the MAP kinase pathway
modulating DNA damage-induced Erk and p38/Jnk pathways.
SHP-2 mutation (32), SHP-2 appears to play opposing roles in
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increased in SHP-2 mutant cells (Fig. 7). As DNA damage-
conversely regulated by SHP-2 phosphatase; p38 activity was
increasingly, following DNA damage, activation of p38 kinase was
increased in SHP-2 mutant cells. Additionally, we showed that DNA damage-in-
dered Erk and p38 activation were respectively suppressed and
increased by the SHP-2 mutation. Treatment of mutant cells
with p38 inhibitor partially restored the DNA damage-induced
G2 arrest response in mutant cells. These results represent the
first demonstration of a connection between SHP-2 phosphatase
and DNA damage cell cycle regulation.

As DNA damage results in cellular stress, it is possible that the
effect of the SHP-2 mutation on the MAP kinase pathway
results from a stress response unrelated to the DNA damage-
induced G2/M checkpoint. To further determine whether in-
creased p38 kinase activation in SHP-2−/− cells might be respon-
sible for the decreased G2 arrest response in SHP-2 mutant
cells, we treated the cells with cisplatin in combination with
SB203580, a selective inhibitor of p38 kinase. This inhibi-
tor alone did not affect cell cycle distribution in WT and SHP-
2−/− cells. However, when the p38 inhibitor was combined with
DNA-damaging treatment, the DNA damage-induced G2/M ar-
est response was partially restored in SHP-2 mutant cells
(Fig. 8A). The percentage of G2/M cells was increased from 37%
(cisplatin-treated) to 47% (combined treatment with cisplatin
and SB203580). And the rescue effect of SB203580 is dose-de-
pendent (data not shown). As SB203580 treatment indeed blocked
DNA damage-induced p38 activation (Fig. 8B), the effective restoration of the G2 arrest in SHP-2 mutant cells by
inhibition of p38 kinase suggests that the excessive p38 activity
is at least in part associated with the attenuated G2 arrest
response of the mutant cells.

**Differential Roles of SHP-2 in DNA Damage-induced MAP**
**Kinase Activation**—In addition to the ATM/Chk1, 2/Cdc25C
pathway, other mechanisms involved in the regulation of Cdc2
kinase and thereby the G2/M transition following DNA damage
have emerged. Recent studies have suggested that DNA dam-
age-induced MAP kinase pathways play important roles in the
DNA damage-induced G2/M checkpoint (18–20). To determine
whether the role of SHP-2 in cell cycle regulation is mediated
by the MAP kinase pathways, we examined DNA damage-
duced Erk and p38 kinase activation in SHP-2 mutant cells.
As shown in Fig. 7, in response to cisplatin treatment, Erk
kinases are activated, but the DNA damage-induced Erk activa-
tion in SHP-2−/− cells was significantly decreased, demon-
strating a positive role of SHP-2 in the DNA damage-induced
Erk pathway, consistent with its promoting role in the growth
factor and cytokine-induced Erk pathways (23–25). Interest-
ingly, following DNA damage, activation of p38 kinase was
conversely regulated by SHP-2 phosphatase; p38 activity was
increased in SHP-2 mutant cells (Fig. 7). As DNA damage-
duced Jnk activation was also significantly increased by the
SHP-2 mutation (32), SHP-2 appears to play opposing roles in
modulating DNA damage-induced Erk and p38/Jnk pathways.

In this report, we have presented several novel results im-
portant for understanding the function of tyrosine phosphatase
SHP-2 in cell signaling. SHP-2 appears to be involved in the
DNA damage-induced G2/M checkpoint control. Mutant embry-
onic fibroblast cells lacking functional SHP-2 showed a diminished
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biochemical analyses revealed that SHP-2 constitutively asso-
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increased by the SHP-2 mutation. Treatment of mutant cells
with p38 inhibitor partially restored the DNA damage-induced
G2 arrest response in mutant cells. These results represent the
first demonstration of a connection between SHP-2 phospha-
tase and DNA damage cell cycle regulation.

The mutant cell lines used in this study harbor a deletion
and loss-of-function mutation of SHP-2. Although the trunc-
cated 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 (26–31). In
agreement with those studies, our recent work showed that the
mutant form of SHP-2 also appeared to be non-functional in the
DNA damage-triggered signaling pathway, as its tyrosyl phos-
phorylation and catalytic activity remained unchanged follow-
ing DNA damage (32). Moreover, in the present study, we
demonstrated that reintroduction of functional WT SHP-2 into
the mutant cells fully corrected their cell cycle responses to
DNA damage, suggesting that the cell cycle phenotype dis-
played 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 cell cycle
response to DNA damage in SHP-2 mutant cells results from
the N-terminal truncation of SHP-2 or the decreased expres-
sion level of the mutant form of SHP-2, the results obtained
using this mutant cell model support a role for SHP-2 in DNA
damage cell cycle response. Additionally, it is noteworthy that
due to 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

**DISCUSSION**
extremely limited, precluding large scale biological and biochemical analyses. For this reason, the fibroblast cell lines we used were immortalized with SV40 large T antigen. The immortalized cell lines have been widely used to define the molecular mechanisms of SHP-2 function in growth factor and cytokine signal transduction (29, 30, 33, 34).

SV40 large T antigen is the major component for the transformation function of SV40 virus that has been connected to several types of human tumors such as mesotheliomas, ependymomas, and osteosarcoma (36, 37). It binds and inactivates many cellular target proteins including p53, retinoblastoma protein (pRb), and the transcriptional co-activators p300 and CBP, thereby permitting cell immortalization and transformation. SV40 large T antigen-immortalized cells display a markedly increased S phase of the cell cycle due to the release of E2F from pRb-mediated control. Consequently, the DNA damage-induced G1 checkpoint was inactivated. However, the DNA damage-induced G2/M checkpoint in SV40 immortalized cells appears to still be functional, because SV40 immortalized WT embryonic fibroblast cells significantly arrest in G2 phase fol-

*Fig. 6. DNA damage-induced Cdc25C translocation to the cytoplasm is blocked in SHP-2ΔΔ cells. WT and SHP-2ΔΔ cells were treated with cisplatin (25 μM) for the indicated time periods. A, whole cell lysates were prepared and examined by immunoblotting with anti-phospho-Chk1 (Ser345) Ab. The blot was stripped and re-probed with anti-Chk1 Ab to monitor protein loading. B, G0/G1 synchronized cells were treated with cisplatin for 6 h. Nuclear extracts were prepared and examined for Cdc25C by immunoblotting. Blots were stripped and re-probed with the Ab against histone H1, a nuclear protein, to monitor protein loading. Results shown are the mean ± S.D. of three independent experiments. C, whole cell lysates were prepared as above and then immunoprecipitated with anti-SHP-2 Ab followed by anti-14-3-3β immunoblotting. Results shown are a representative of two to three independent experiments.*
following DNA damage (Figs. 1B and 2). Since both WT and SHP-2\(^{+/−}\) cells were equally transduced with SV40 large T antigen (Fig. 1A), it should not cause biased influence on our analysis of the SHP-2 function in DNA damage-induced cell cycle regulation.

SHP-2 might contribute to the DNA damage-activated G2/M checkpoint by multiple mechanisms. We previously showed that DNA damage induction of p21\(^{CIP1}\) was abolished in SHP-2 mutant cells, which may in part explain the diminished G2/M arrest response in mutant cells, since p21\(^{CIP1}\) has been demonstrated to be also important for the DNA damage G2/M checkpoint (14, 15), in addition to the G1/S checkpoint. The results in this report suggest that SHP-2 also controls G2/M transition after DNA damage by modulating Cdc25C translocation. In response to DNA damage, Cdc25C detected in the nuclear extract of WT and rescued cells was significantly reduced. By contrast, the Cdc25C level in nuclear extracts from SHP-2\(^{−/−}\) cells remained unchanged (Fig. 6B), suggesting that DNA damage-induced Cdc25C translocation is blocked by the loss-of-function mutation of SHP-2. It is unclear at the present time how SHP-2 exactly functions in this context. Since SHP-2 is associated with 14-3-3\(\beta\), an important regulator of Cdc25C translocation, it is likely that SHP-2 enhances Cdc25C translocation from the nucleus to the cytoplasm by promoting 14-3-3\(\beta\) binding to Cdc25C or by stabilizing the association between these two interacting proteins. In SHP-2\(^{−/−}\) mutant cells, although the truncated SHP-2 (SHP-2\(^{−}\)) can also bind with 14-3-3\(\beta\), the association between 14-3-3\(\beta\) and Cdc25C induced by DNA damage might be decreased due to the lack of full-length functional SHP-2. Therefore, more Cdc25C is retained in the nucleus. As a result, DNA damage-induced changes in Cdc2 phosphorylation and its kinase activity are decreased and thus the G2/M arrest response is attenuated in mutant cells. Further investigations on the biological significance of the SHP-2/14-3-3\(\beta\) interaction in the DNA damage cell cycle response are ongoing in the laboratory.

In addition to dysregulated Cdc25C translocation, DNA damage-induced Erk activation was decreased while activation of p38 kinase was enhanced in SHP-2\(^{−/−}\) cells (Fig. 8A). These changes in the MAP kinase pathways might result from a cellular stress response following DNA damage. However, since combined treatment with p38 inhibitor partially restored the DNA damage G2 arrest response in mutant cells (Fig. 8B), it is likely that elevated activation of p38 also contributes in part to the diminished G2 arrest in SHP-2 mutant cells. The role of the MAP kinase family in genotoxic stress-induced cellular responses has been controversial. Contradictory results exist in the published literature. For example, Erk kinases have been reported to promote the DNA damage apoptosis and G2/M arrest (18, 38). However, other studies suggest that Erk inhibits DNA damage-induced apoptosis (39–41). Likewise, it has been uncertain whether p38 and Jnk kinases protect (40, 42, 43) cells from apoptosis or enhance (44, 45) cell death in response to genotoxic insult. The possibility remains that the contradictory observations may be due to different DNA damage stimuli or different tumor cell lines used. It is important to note that various tumor cell lines may harbor different mutations in the cell cycle regulatory proteins such as p53 and pRb. Some tumor cell lines contain highly activated oncogenic enzymes such as RAS GTPase and Src tyrosine kinase. Other tumor cell lines were immortalized with virus proteins like E1A antigen or SV40 T antigens. p38 kinase has been demonstrated to be essential for sustained G2 arrest induced by γ-irradiation (20). However, a more recent report showed that p38 was only required for the initi-

**Fig. 7. Opposing roles of SHP-2 phosphatase in the DNA damage-induced Erk and p38 kinase activation.** WT and SHP-2\(^{−/−}\) cells were treated with cisplatin (50 \(\mu\)M) for the indicated time periods. Whole cell lysates were prepared and examined by immunoblotting with anti-phospho-Erk and anti-phospho-p38 Abs. Blots were stripped and re-probed with anti-Erk Ab to monitor protein loading. Results shown are representatives of three independent experiments.

**Fig. 8. Inhibition of p38 kinase activity in SHP-2\(^{−/−}\) cells partially restored the DNA damage-induced G2 arrest response.** Exponentially growing WT and SHP-2\(^{−/−}\) cells were treated with cisplatin, SB203580 (5 \(\mu\)M), or both. A, treated and untreated cells were examined for percentages of G2/M 24 h later. Results shown are the mean ± S.D. from three independent experiments. B, whole cell lysates were prepared and examined by immunoblotting with anti-phospho-p38 Ab. The blot was stripped and re-probed with anti-p38 Ab to monitor protein loading. Results shown are representatives of three independent experiments.
ation of UV but not γ-irradiation-induced G2/M checkpoint (19). In our system, inhibition of p38 activity did not affect the cisplatin-induced G2/M arrest in WT cells. However, it appears that excessive p38 activity inhibits the DNA damage-induced G2/M checkpoint. For this, the cell model used in this study is SV40 large T antigen immortalized murine embryonic fibroblast cells; as a result, some cell cycle regulatory proteins such as p53 and pRB are inactivated, so the defects in the cell cycle response in the mutant cells we observed is likely to be a combined effect of loss-of-function of SHP-2 and SV40 large T immortalization. Second, the DNA damage model used in our studies is cisplatin treatment and γ-irradiation. The intracellular signaling pathways triggered by these DNA damaging treatments might be different from those induced by other DNA damage stimuli. Lastly, p38 might indeed kinetically modulate the DNA damage-induced G2/M checkpoint. In the early phase, p38 is required. After this, p38 may then turn to attenuate the DNA damage-induced G2/M arrest. Certainly, further studies are required to determine which possibility is true and how excessive p38 activation contributes to diminished G2 arrest response.

In summary, the biological and biochemical evidence presented in this report suggest a previously uncharacterized function of SHP-2 phosphatase, i.e. its role in the G2/M cell cycle checkpoint. SHP-2 enhances the DNA damage-induced G2 arrest by promoting Cdc25C cytoplasmic translocation and by differentially regulating the MAP kinase pathways.

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