Phosphorylation and Stabilization of Topoisomerase IIα Protein by p38γ Mitogen-activated Protein Kinase Sensitize Breast Cancer Cells to Its Poisons*

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Cancer drugs suppress tumor cell growth by inhibiting specific cellular targets. However, most drugs also activate several cellular nonspecific stress pathways, and the implications of these off-target effects are mostly unknown. Here, we report that p38γ, but not p38α, MAPK is specifically activated by treatment of breast cancer cells with topoisomerase II (Topo II) drugs, whereas paclitaxel (Taxol) does not have this effect. The activated p38γ in turn phosphorylates and stabilizes Topo IIα protein, and this enhances the growth inhibition by Topo II drugs. Moreover, p38γ activity was shown to be necessary and sufficient for Topo IIα expression, the drug-p38γ-Topo IIα axis is only detected in intrinsically sensitive but not resistant cells, and p38γ is co-overexpressed with Topo IIα protein in primary breast cancers. These results reveal a new paradigm in which p38γ actively regulates the drug-Topo IIα signal transduction, and this may be exploited to increase the therapeutic activity of Topo II drugs.

Current cancer chemotherapeutics are thought to inhibit tumor growth by acting on specific cellular targets. Most drugs, however, also have some off-target effects whose implications for drug effectiveness remain mostly unknown (1). DNA topoisomerase IIα (Topo IIα or Topo II) is a nuclear protein critical for DNA topology, and drugs that directly target Topo II represent an important class of anti-tumor agents (2, 3). Although p38 MAPKs (mitogen-activated protein kinases), including JNKs (C-terminal c-Jun kinases) and p38s, play a critical role in transduction and conversion of extracellular signals into various biological responses (6). Although these kinases are readily activated by various stimuli, evidence about their specific roles in stress response are just emerging (7). p38γ is a member of the p38 MAPK family and can be activated by both stress and mitogenic signals (8). In contrast to the tumor suppressor role of the p38α isoform, p38γ expression is induced by Ras oncogene, and p38γ in turn functions to promote the Ras transforming and invasive activity (9–12). Moreover, p38γ expression is increased in primary tumor tissues and is required for malignant growth (9, 13). Although p38γ and p38α are similarly activated by stress signals (11, 14), some studies showed that p38γ can specifically mediate certain stress responses such as γ-radiation (15, 16). Because Ras activates the p38 pathway (9, 10, 17) and induces Topo IIα gene expression (18, 19) and because Topo II drugs can lead to the activation of p38 MAPKs (20), we tested the hypothesis that p38 MAPKs may regulate signal transduction between Topo IIα and its poisons. Our results show that p38γ, but not p38α, is specifically activated by Topo II drugs. In turn, the activated p38γ phosphorylates Topo IIα at Ser-1524, and this increases its stability leading to an increased growth inhibition. Moreover, this drug-kinase-target axis only exists in intrinsically Topo II drug-sensitive cells, and there is a positive correlation between the levels of p38γ and Topo IIα proteins in primary tumors. These results reveal a new paradigm in which the intrinsic sensitivity to Topo II drugs does not depend on levels of Topo IIα protein, but on the activity of p38γ. Enhancing p38γ MAPK expression and/or activity may therefore prove useful for improving the anti-tumor effects of Topo II drugs.

EXPERIMENTAL PROCEDURES

cDNA Constructs and shRNAs—FLAG-tagged full-length Topo IIα (wild-type, WT) and its S1524A mutant (MT) in pcDNA3 were described previously (21). The HA-tagged MKK6-p38α (constitutively active (CA) p38α) and MKK6-p38γ (CA p38γ) fusion protein expression constructs were reported previously (11). The human Topo IIα luciferase promoter was previously described (19). For gene silencing, human p38γ target sequences (#1: CTCTGAAAACATGAGAACCTA; #2 GAAAGGATCATGGAAGGTGCAC) were cloned into a plenti6/Block-iT vector by including a sequence

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§ The abbreviations used are: Topo II or Topo IIα, topoisomerase IIα; AMSA, amasacrine; CA, constitutively active; ER, estrogen receptor; MT, mutant; VP16, etoposide.

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from luciferase gene (shLuc: GTGCGTTGCTAGTACCAAC) as a control. Human p38γ and p38α cDNA were gifts from Dr. Jiahui Han (22) and were cloned into a His-tagged bacterial expression pET plasmid to produce the recombinant p38γ and p38α proteins.

Cell Culture, Reagents, and Tet-on Inducible System—MEM and other reagents for cell culture were purchased from Invitrogen. A rabbit antibody against Ser-1524-phosphorylated Topo IIα was generated as described previously (21), p38γ and p38α antibodies were purchased from Santa Cruz Biotechnology or R&D Systems, whereas p-p38 and p-ERK antibodies were from Cell Signaling. A mouse monoclonal antibody against FLAG (M2) was purchased from Sigma. The Topo IIα antibody and Topo II assay kit were from TopoGen. Etoposide (VP16), amasacrine (AMSA), paclitaxel (Taxol), cycloheximide, and MG132 were all purchased from Sigma. Human breast cancer cell lines (estrogen receptor (ER)-positive, MCF-7 and T47D; ER-negative, MDA-MB-231 (231) and MDA-MB-468 (468)) were obtained from ATCC and maintained in MEM containing 10% FBS and antibiotics at 37 °C with 5% CO2. Early passages of embryonic fibroblasts from p38γ−/− mice were provided by Ana Cuenda (23) and were used to establish Ras-transformed sublines (12). The Tet-on expression system (T-Rex) was purchased from Invitrogen and used to express CA p38α and CA p38γ (10).

Transfection, Viral Infection, and Colony Formation—Transient transfection of 293T cells was performed by calcium phosphate. To deplete p38γ, lentiviral shLuc and shp38γ were transfected into packaging cells, and supernatants were then collected for infecting target cells followed by an antibiotic selection (13). To overexpress p38γ in T47D breast cancer cells, adenoviral-mediated gene delivery was used as described previously (10). For colony formation assays, cells were treated with various drugs as indicated for 24 h and plated in a 6-well plate, and colony formed were then stained and annually counted about 2 weeks later as we described previously (24).

Topo IIα Phosphorylation Assays in Vitro and in Vivo—FLAG-tagged WT and MT Topo IIα were expressed in 293T cells, and the expressed proteins were purified from cell lysates using a FLAG antibody. FLAG precipitates were then incubated with His-p38γ or BSA as a control. The in vitro kinase assay was performed as described previously (17). Phosphorylated Topo IIα proteins were separated on a SDS-PAGE and detected with an antibody specific for phosphorylated Ser-1524 Topo IIα antibody. For in vivo Topo IIα phosphorylation analysis, FLAG-Topo IIα cDNA was co-transfected with CA p38γ into 293T cells and Ser(P)-1524-Topo IIα was assessed by Western blotting. Additional methods are described in supplemental Experimental Procedures.

Statistical Analysis—Results of colony formation were analyzed by Student’s t test, and studies for a correlation of increased p38γ and Topo IIα protein expression in primary tumor tissues were assessed by the χ² test.

RESULTS

Increased Topo II Drug Sensitivity Correlates with Sustained Levels of Topo IIα protein—To search for a signaling pathway involved in the activity of Topo II drugs, a group of human breast cancer cell lines were treated with two clinically used Topo II drugs, VP16 (etoposide) and AMSA (amasacrine), and cell growth was assessed by colony formation compared with paclitaxel (Taxol), an important cancer drug that does not inhibit Topo IIα. Results in Fig. 1A show that ER-negative 231 and 468 breast cancer cells are more sensitive to both Topo II drugs than are the ER-positive counterparts MCF-7 and T47D. However, no such difference was observed with paclitaxel (Taxol). In contrast to other reports (18, 25), the increased sensitivity does not correlate with a higher level of endogenous nuclear Topo IIα expression (Fig. 1B). Of interest, Topo IIα protein was decreased in the ER-positive T47D and MCF-7 cells that were treated with both Topo II drugs (26, 27), but there was no decrease noted in the ER-negative 231 and 468 cells (Fig. 1C). Paclitaxel did not significantly affect the Topo IIα levels in any of the cell lines (Fig. 1C). Given that the cell lines 231 and 468 that are sensitive to Topo II drugs did not show a decrease in Topo IIα when treated...
p38γ is specifically phosphorylated in response to Topo II drugs in intrinsically sensitive cells. Cells were treated with the indicated drugs for 6 h as described in Fig. 1C and subjected to p-p38 immunoprecipitation (IP) and Western blot analyses. A portion of cell lysates was also analyzed by direct Western blotting (Input) (*, the numbers indicate the relative levels of p38γ protein detected in the p-p38 precipitates relative to the solvent control. The 0 values for T47D and MCF-7 cells indicate that p38γ was undetectable in these immunoprecipitates).

FIGURE 2. p38γ is specifically phosphorylated in response to Topo II drugs in intrinsically sensitive cells. Cells were cultured with the indicated drugs for 6 h as described in Fig. 1C and subjected to p-p38 immunoprecipitation (IP) and Western blot analyses. A portion of cell lysates was also analyzed by direct Western blotting (Input) (*, the numbers indicate the relative levels of p38γ protein detected in the p-p38 precipitates relative to the solvent control. The 0 values for T47D and MCF-7 cells indicate that p38γ was undetectable in these immunoprecipitates).

with VP16 or AMSA, there may be a signal transduction pathway in these cells that specifically regulates the drug-Topo II interaction thereby preventing Topo IIα depletion.

p38γ MAPK Is Specifically Activated by Topo II Drugs in Intrinsically Sensitive but Not Resistant Cells—Stress MAPKs are major signaling cascades that are activated by a number of cancer therapeutics (28). Western blot analysis showed a consistently stronger phosphorylation of p38, but not JNK, in sensitive cells treated with Topo II drugs (Fig. 1C). Of the four p38 MAPKs (α, β, γ, and δ), p38α and p38γ are predominant forms expressed in breast cancer cells with levels of p38γ expression higher in ER-negative breast cancer cells (10, 29) (supplemental Fig. S1A). To determine which of these two p38 proteins is responsive to Topo II drugs, total phosphorylated p38 (p-p38) proteins were isolated with a p-p38 specific antibody (reacting with all p-p38 proteins), and resulting precipitates were examined by Western blotting using p38α and p38γ isoform-specific antibodies. Of great interest, results in Fig. 2 showed that p38γ is specifically activated by AMSA and VP16 (but not paclitaxel) in the intrinsically sensitive 468 and 231 cell lines (Fig. 2). However, p38γ activation is not seen in the resistant T47D and MCF-7 cell lines in which p-p38α is predominantly elevated instead. Although a selective p38γ activation (with and without p38α) was previously observed in response to certain cancer therapeutics (30) and hypoxia (31), our results may be the first that demonstrate the coupling of a specific p38γ activation with an increased growth inhibition by Topo II poisons. The lack of p38γ activation in ER-positive cells may be due to a decreased p38γ expression (supplemental Fig. S1A) and/or the antagonistic activity of ER protein (10). Together with the sustained levels of Topo IIα and an enhanced growth inhibition by the Topo II drugs in 231 and 468 cells, these results indicate that p38γ may have a role in preventing loss of the Topo IIα protein. Sustained levels of Topo IIα in these cells could increase their sensitivity to the Topo II drugs.

p38γ MAPK Regulates Topo IIα Expression and Growth Inhibition of Topo II Drugs—To investigate whether p38γ plays a role in regulating Topo IIα expression in response to Topo II poisons, a CA p38γ (a MKK6-p38γ fusion protein) (11) was expressed using a tetracycline-inducible system (Tet-on) in MCF-7 cells, and its effect on Topo IIα expression and sensitivity of the cells to AMSA/VP16 was analyzed. Results in Fig. 3, A and B, show that the overexpression of p38γ prevents the loss of
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Topo IIα and increases the sensitivity of MCF-7 cells to VP16 and AMSA. This effect appears to be p38γ-specific because the overexpression of CA p38α using the Tet-on system does not cause these effects (supplemental Fig. S1, B and C). A complete prevention of the Topo IIα depletion and consequently a more substantial increase in the sensitivity to these drugs were observed in T47D cells in which p38γ was overexpressed by adenovirus-mediated infection (supplemental Fig. S2, A and B).

To assess the role of endogenous p38γ, 468 cells were infected with control (shLuc) and shp38γ-containing lentivirus (13) and analyzed for protein expression and sensitivity to Topo II drugs. Results in Fig. 3C showed that depletion of p38γ by two separate shRNAs decreases Topo IIα protein expression and confers a resistance to AMSA and VP16. Similar results were also obtained in 231 cells (supplemental Fig. S2C). Together, these results indicate a critical role of p38γ in maintaining Topo IIα protein expression and sensitivity to Topo II drugs.

p38γ MAPK Phosphorylates Topo IIα at Ser-1524, and This Is Required for the Topo IIα Stability and Sensitivity to Topo II Drugs—Topo IIα protein is phosphorylated at several residues including Ser-1524 (32) and others (33, 34). Because the Ser-1524 phosphorylation (21) and Topo II inhibitors (35) both act at the decatenation checkpoint, we examined whether p38γ phosphorylates Topo IIα at this residue and thereby regulates the drug-target interaction. Topo IIα phosphorylation was first assessed in vitro by incubating FLAG antibody-isolated WT and Ser-1524 MT (S1524/A) Topo IIα proteins (expressed in 293T cells) with bacterially expressed His-tagged p38γ, followed by analysis with a specific antibody against Ser-1524-phosphorylated Topo IIα (21). Results in Fig. 4A showed that p38γ, but not p38α, increases the p-Topo IIα signal when the WT protein was used as a substrate (left). The co-expression of FLAG-Topo IIα and CA p38γ in 293T cells also resulted in enhanced phosphorylation of Topo IIα (Fig. 4A, right). Of interest, the increased p-Topo IIα signal in 293T cells couples with an elevated Topo IIα protein expression (Fig. 4A, right), suggesting that the phosphorylation of Topo IIα leads to increased expression and/or decreased degradation.

To demonstrate whether the Ser-1524 is required for p38γ binding Topo IIα, the FLAG-tagged WT and MT enzymes were co-expressed with the CA-p38γ, and FLAG precipitates were analyzed. Results in Fig. 4B (left) showed that although there is a Ser-1524-independent complex formation of Topo IIα with p38γ, the overexpression of p38γ with the WT Topo IIα enhances the Topo II levels, whereas the p38γ overexpression decreases the levels of MT (S1524A) Topo IIα. This suggests that Ser-1524 in Topo IIα is important for increased Topo IIα expression by p38γ. Moreover, p38γ overexpression and depletion lead to an increased and decreased Topo IIα protein stability in breast cancer cells, respectively (Fig. 4B, right). Furthermore, WT Topo IIα is much more stable than the Ser-1524 MT protein in 293T cells (Fig. 4C). Additional experiments showed that p38γ increases Topo IIα protein stability, leading to its decreased degradation by the proteasome pathway (supplemental Fig. S2D, upper). Together, these results indicate that the p38γ phosphorylation of Topo IIα at Ser-1524 enhances Topo IIα protein stability.

To examine whether the p38γ activation affects Topo IIα catalytic activity, nuclear proteins were prepared from Tet-on CA p38γ MCF-7 cells and assessed for the DNA decatenation activity using a Topogen kit (upper left). To measure Topo IIα-DNA covalent complexes, cells were cultured overnight with and without Tet then treated with 100 μM VP16 or AMSA or solvent for 30 min and assessed for Topo IIα-mediated DNA cleavage using a Topogen ICE bioassay kit as described (48, 49). Results of fractions 6 and 7 are shown (lower left). For the effects on growth, cells transfected with pDNA3 or MT Topo IIα were incubated with and without VP16/AMSA and assessed for colony formation (right), mean ± S.D. (error bars), n = 3, *p < 0.05 versus the MT transfected cells, with the right blot showing the expressed FLAG-MT Topo IIα protein.

FIGURE 4. p38γ phosphorylates Topo IIα/Ser-1542 which is important for Topo IIα stability, activity, and growth inhibition by Topo II poisons. A, p38γ phosphorylates Topo IIα at Ser-1542 in vitro and in vivo. The FLAG-WT and FLAG-MT Topo IIα proteins were incubated with His-p38γ or His-p38α in vitro, and the phosphorylated signal was examined by an antibody specific for p-Topo IIα/Ser-1524 (left); the middle panel shows an input control. Results of p38γ expression on the phosphorylation of Topo IIα (in vivo) are shown in the right panel. B, co-transfection with p38γ increases the WT but decreases the MT Topo IIα protein levels in a manner that is independent of their binding activity (left), and p38γ expression enhances, whereas its depletion reduces, the endogenous Topo IIα stability in breast cancer cells (right). C, the WT Topo IIα is more stable than its S1524A mutant in 293 cells. D, CA p38γ increases Topo IIα catalytic activity and Topo IIα-DNA cleavage complexes induced by VP16 and AMSA, and the Ser-1524 residue of Topo IIα plays a role in the drug sensitivity. Nuclear proteins from Tet-on CA p38γ MCF-7 cells were prepared and assessed for the DNA decatenation activity using a Topogen kit (upper left). To measure Topo IIα-DNA covalent complexes, cells were cultured overnight with and without Tet then treated with 100 μM VP16 or AMSA or solvent for 30 min and assessed for Topo IIα-mediated DNA cleavage using a Topogen ICE bioassay kit as described (48, 49). Results of fractions 6 and 7 are shown (lower left). For the effects on growth, cells transfected with pDNA3 or MT Topo IIα were incubated with and without VP16/AMSA and assessed for colony formation (right), mean ± S.D. (error bars), n = 3, *p < 0.05 versus the MT transfected cells, with the right blot showing the expressed FLAG-MT Topo IIα protein.
Ser-1524 residue of Topo IIα contributes to the cellular sensitivity to Topo II drugs, the MT S1524A Topo IIα (21) was stably transfected in MCF-7 cells, and G418-resistant cells were pooled and analyzed for VP16/AMSA-induced growth inhibition. The WT Topo IIα transfection failed to yield a stable clone, likely as a result of cell death as reported previously (37). Results in Fig. 4D (right) show that stable expression of the S1524A mutant significantly decreases the sensitivity to both drugs, which may be mediated through enhanced degradation of Topo IIα protein (Fig. 4B) and/or an inhibition of the endogenous enzyme. Together, these results indicate that Topo IIα may be a natural substrate for p38γ MAPK, and the resultant phosphorylation of Ser-1524 plays an important role in maintaining Topo IIα protein stability and activity, thereby enhancing Topo II drug sensitivity.

Topo IIα/Ser-1524 Is Phosphorylated in Intrinsically Sensitive but Not Resistant Cells, and p38γ Activity Is Required for Ser-1524 Phosphorylation—Results in Fig. 1 showed that 231 and 468 cells are more sensitive to Topo II poisons than their ER-positive counterparts. If Ser-1524 phosphorylation plays a role in the drug sensitivity, there should be an increased p-Topo IIα expression in sensitive cells. To this end, normal MCF-7 cells were incubated with different drugs as described in Fig. 2, and endogenous Topo IIα was isolated with a specific antibody and examined by Western blotting. Results in Fig. 5A show that precipitated Topo IIα is decreased after treatment of the cells with VP16 and AMSA but not with paclitaxel. Phosphorylated Topo IIα/Ser-1524 was not detected in these cells, however, regardless of pre-treatment (Fig. 5A). Moreover, there was no detectable p38γ protein in these precipitates (Fig. 5A). Similar results were observed in T47D cells (data not shown). There is, however, a complex formation between Tet-inducible CA p38γ and endogenous Topo IIα, which couples with increased p-Topo IIα levels in the MCF-7 cells overexpressing p38γ (supplemental Fig. S2D, lower). Therefore, the lack of Topo IIα phosphorylation and p38γ binding in normal MCF-7 cells (Fig. 5A) is likely due to very low endogenous p38γ protein expression. In sensitive 231 and 468 cells, on the other hand, precipitated Topo IIα proteins were phosphorylated at Ser-1524 whether or not the cells were pretreated with VP16 in which endogenous p38γ proteins were also present (Fig. 5B). These results, together with the increased p38γ levels in the sensitive over the resistant cells (supplemental Fig. S1A), indicate that the intrinsic p38γ activity may play a critical role in the Topo IIα phosphorylation. To demonstrate whether p38γ activity is required for p-Topo IIα/Ser-1524 expression, a nonphosphor-able p38γ mutant (p38γ/AGF, dominant negative) was overexpressed in 231 and 468 cells by infection with adenoviral constructs. These cells were then treated with or without VP16, and the Topo IIα precipitates were analyzed by Western blotting. The results in Fig. 5B show that expression of DN p38γ suppresses the p-Topo IIα levels in both lines relative to the β-gal control, indicating that the endogenous p38γ is required for the phosphorylation of Topo IIα expression. These results, together with the stimulation of p-Topo IIα levels by CA p38γ shown in supplemental Fig. S2D, lower, reveal that p38γ is necessary and sufficient to support the phosphorylation of Topo IIα/Ser-1524.

The ability of Topo II drugs to induce p38γ (but not p38α) activity and the resulting phosphorylation of Topo IIα at Ser-1524 suggest that p38γ may actively be involved in signal transduction between Topo IIα and its poisons. To demonstrate whether Topo II drugs directly regulate its levels through Ser-1524 phosphorylation, WT and MT Topo IIα proteins were transiently expressed in 293T cells. The effects of Topo II drugs versus paclitaxel treatment on the endogenous Topo IIα protein expression were examined. Treatment with Topo II drugs increased the levels of ectopically expressed WT but not MT Topo IIα protein (Fig. 5C). In contrast, paclitaxel increased levels of both WT and MT Topo IIα (Fig. 5C). Overall, these results indicate that p38γ is specifically activated by Topo IIα drugs, and activated p38γ phosphorylates Ser-1524 of Topo IIα which prevents its degradation and thereby enhances the growth-inhibitory effects of these drugs.
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p38γ Stimulates Topo IIα Gene Expression, and Immune Histology Staining and Proteomics Analysis Verify Existence of the p38γ-Topo IIα Axis—We showed previously that Ras stimulates Topo IIα gene expression (19). Because p38γ signals downstream of Ras (9, 10, 13), we next explored whether p38γ may also stimulate Topo IIα gene expression thereby serving as a second mechanism to sustain Topo IIα levels. Indeed, transient CA p38γ expression stimulates the luciferase activity driven by the human Topo IIα promoter (19) in 293T cells. A similar stimulation was observed with Tet-induced CA p38γ expression in MCF-7 cells (supplemental Fig. S3, A, B, and C). Moreover, levels of Topo IIα RNA were increased by adenovirus-mediated expression of p38γ in 468 cells and by Tet-on-induced expression of CA p38γ in MCF-7 cells (supplemental Fig. S3, C and D). These results are consistent with their elevation of endogenous Topo IIα protein expression under similar conditions (supplemental Figs. S2D and S3C). Overall, these data indicate that p38γ also stimulates Topo IIα gene expression.

To demonstrate further the regulatory role of p38γ in Topo IIα expression, a group of primary breast cancer tissues was analyzed by immune histochemistry (12, 13). Results in Fig. 6A (top) show that the most of Topo IIα staining is in the nucleus (38), whereas the p38γ signals are predominantly cytoplasmic (13). Although we were not be able to examine p-p38γ expression in these tissues due to the lack of the antibody, our previous studies showed that the phosphorylated p38γ is localized inside the nucleus (11) where it may interact with Topo IIα and thereby regulate its activity in response to the chemotherapy stress. Most importantly, there is a significant correlation between increased p38γ and elevated Topo IIα protein levels in this group of tumor tissues versus their matched normal controls (Fig. 6A, bottom). These results indicate that p38γ may also positively regulate Topo IIα expression in primary cancer tissues.

To show whether endogenous p38γ is required for Topo IIα expression, tumor samples from Ras-transformed wild-type (p38γ+/+) and p38γ knock-out (p38γ−/−) mouse embryonic fibroblasts (12) were analyzed for protein expression. Results in supplemental Fig. S4A showed a decreased Topo IIα levels in p38γ−/− tumors, consistent with a role of p38γ in maintaining Topo IIα expression. To demonstrate whether Topo IIα is a natural substrate of p38γ, the same amount of proteins from the p38γ+/+ and p38γ−/− samples was precipitated with an antibody against protein tyrosine phosphatase H1 (PTPH1), a p38γ-specific phosphatase (13); the immunoprecipitates were separated on SDS-PAGE (supplemental Fig. S4B) followed by digestions and proteomics analysis. We recently showed that PTPH1 binds and dephosphorylates p38γ via PSD-95/Dlg/ZO-1 homology (PDZ)-mediated complex formation (13). Because PTPH1 protein is expressed similarly in p38γ+/+ and p38γ−/− tumors (supplemental Fig. S4A) and a substrate may form a complex both with its kinase and the associated phosphatase (39), comparative analyses of PTPH1 precipitates from the p38γ+/+ and p38γ−/− samples may identify p38γ-dependent PTPH1-binding proteins. Indeed, results from the proteomics analysis (supplemental Fig. S4C and data not shown) show that PTPH1 binds Topo IIα only in p38γ+/+ but not in p38γ−/− tumors, indicating a natural complex of Topo IIα with PTPH1 and p38γ. Thus, a correlation of increased p38γ with up-regulated Topo IIα protein levels in primary breast cancer. Representative p38γ- (open arrow) and Topo IIα- (filled arrow) positive (top four panels) and negative (bottom two panels) staining pictures are shown at the top, and the results are summarized at bottom of A, B, an experimental model illustrates how p38γ regulates the signal transduction between Topo II drugs and their target leading to increased Topo II drug therapeutic activity through phosphorylation and stabilization of Topo IIα (short term effect) and stimulation of Topo IIα gene expression (long term effect).

FIGURE 6. Increased p38γ protein expression in primary breast cancer correlates with up-regulated Topo IIα. A, there is a correlation of increased p38γ with up-regulated Topo IIα protein levels in primary breast cancer. Representative p38γ- (open arrow) and Topo IIα- (filled arrow) positive (top four panels) and negative (bottom two panels) staining pictures are shown at the top, and the results are summarized at bottom of A, B, an experimental model illustrates how p38γ regulates the signal transduction between Topo II drugs and their target leading to increased Topo II drug therapeutic activity through phosphorylation and stabilization of Topo IIα (short term effect) and stimulation of Topo IIα gene expression (long term effect).

DISCUSSION

Cancer chemotherapeutic drugs remain a major component of anticancer therapy. Although multiple pathways are involved in drug-induced growth inhibition and/or cell death, there is a pronounced lack of information about the mechanisms that
regulate drug-target signal transduction (1, 5). Topo IIα is perhaps the most well-established therapeutic target in human cancer, and its direct interaction with Topo II poisons is believed to be the foundation for the anti-tumor activity (40). It is not known, however, whether any cellular signaling pathways are involved in regulating the interaction of Topo IIα with its poisons. Here, we provide several pieces of evidence that together indicate that p38γ may act as a specific kinase to regulate the signal transduction between Topo IIα and its poisons. First, p38γ but not p38α MAPK is selectively activated by Topo II-targeting drugs (but not by paclitaxel) in intrinsically sensitive cells leading to sustained Topo IIα expression. Second, p38γ phosphorylates Topo IIα at Ser-1524 in vitro and in vivo, which is important for Topo IIα protein stability, for the regulation of Topo IIα expression, and for the growth inhibition by Topo IIα poisons. Third, the manipulation of p38γ activity by its forced expression and/or inhibition/depletion shows that it positively regulates Topo IIα expression, the phosphorylation of Topo IIα at 1524, and the sensitivity of breast cancer cells to Topo II drugs. Moreover, the intrinsic Topo II drug sensitivity correlates with the endogenous levels of p38γ but not with those of Topo IIα. Also, p38γ stimulates Topo IIα transcription, and there is a positive correlation of increased p38γ levels with Topo IIα protein expression in primary tumor tissues. Finally, Topo IIα forms a complex with the phosphatase PTPH1 in a p38γ-dependent manner. Overall, these results indicate that p38γ specifically regulates the signal transduction between Topo IIα and its poisons and thereby promotes increased chemotherapeutic activity of these drugs by maintaining their target Topo IIα (Fig. 6B).

Although Topo IIα is phosphorylated at several sites (32–34), phosphorylation of Ser-1524 is required both for the decatenation checkpoint (21) and cell cycle progression (34), indicating its critical role in maintaining the malignant progression. Although the Ser-1524 can be also phosphorylated by casein kinase II (32) and polo-like kinase 1 (34), functional roles of these kinases in regulating cancer cell response to Topo II drugs have not been reported. p38γ MAPK, on the other hand, is overexpressed in primary breast cancer (9), and its RNA/protein levels are increased in ER-negative breast cancer cells (10). Therefore, the regulation and stabilization of Topo IIα by p38γ have important clinical implications. Although it has long been recognized that Topo II-targeting drugs are more effective in ER-negative breast cancer patients, the mechanisms have been mostly unknown (41, 42). While the cytotoxicity of Topo II drugs is directly correlated with Topo IIα levels (40), clinical studies have failed to show any connection between Topo IIα and ER protein expression in primary breast cancer (43), indicating that other factor(s) must regulate Topo IIα levels and the activity of Topo II drugs. Here, we show that the intrinsic sensitivity of breast cancer cells to Topo II drugs correlates with the levels of p38γ protein expression in cultured breast cancer cells and that regulation of p38γ expression/activity positively impacts Topo IIα/Ser-1524 phosphorylation, Topo IIα protein stability, and the activity of Topo II drugs. Moreover, increased p38γ protein expression in primary breast tumors correlates with elevated Topo IIα protein, indicating a determinant role of p38γ in the clinical response to a Topo II drug-based therapy. Future experiments are warranted to investigate whether p38γ activity positively correlates with p-Topo IIα in primary breast cancers and whether their combined up-regulation predict an improved clinical response to a Topo II drug-containing chemotherapy.

Drug-target interaction is a dynamic process that may involve alterations of thousands of cellular proteins in space and time (1). Consequently, how the totality of these changes regulates a given drug-target interaction for a coordinated therapeutic response is poorly understood. For example, previous studies have shown that the NF-kB pathway plays a protective role in the cellular response to stress stimuli such as TNFα, ionizing radiation, and daunorubicin, but whether these effects are executed through their cellular targets has not been demonstrated (44). p38 MAPKs, on the other hand, can facilitate an apoptotic response together with the JNK pathways in stress response (45, 46), but whether any of these stress MAPKs can specifically regulate a given drug-target interaction and thereby modify their pharmacological outcomes still remains unknown. Here, we show that specific activation of p38γ MAPK by Topo II drugs in intrinsically sensitive cells leads to phosphorylation and stabilization of Topo IIα, a process that is required for their growth-inhibitory activity. Although the stimulation of Topo IIα gene expression by p38γ may also contribute to the increased Topo IIα levels and enhanced anti-tumor activity, this effect may be counteracted by an overall inhibitory effect of Topo II drugs on DNA synthesis and gene expression during the therapeutic stress. However, p38γ-mediated stimulation of Topo IIα gene expression may be important for a long-term therapeutic effect through regulating Topo II drug-induced DNA repair/checkpoint activation programs (47) (Fig. 6B). Further studies on the regulation of drug-target interaction by stress kinases could open a new avenue for novel cancer therapeutic development.

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