Myosin VI is an unconventional motor protein and functions in a variety of intracellular processes such as cell migration, vesicular trafficking, and homeostasis of the Golgi complex. Previously, we found that myosin VI is up-regulated in RKO, LS174T, and H1299 cells by DNA damage in a p53-dependent manner and mediates the pro-survival function of p53. Here, we showed that the levels of myosin VI protein were markedly inhibited in MCF7 and LNCaP cells by topoisomerase I-II inhibitors. However, the levels of myosin VI transcript were decreased only by topoisomerase I inhibitors. We also found that the levels of myosin VI protein were markedly increased in MCF7 cells by wild-type p53 but not tumor-derived mutant p53. Surprisingly, we found that the level of myosin VI transcript was slightly increased instead of decreased in MCF7 cells by p53, suggesting that a mechanism other than transcriptional repression is involved. Additionally, we found that on the myosin VI promoter, the level of acetylated histone H3 was markedly decreased, whereas that of p53 and acetylated histone H4 was slightly increased in MCF7 cells upon treatment with topoisomerase I-II inhibitors. Finally, we showed that overexpression of myosin VI enhances, whereas knockdown of myosin VI decreases, DNA damage-induced stabilization of p53, and consequently, knockdown of myosin VI de-sensitizes MCF7 cells to DNA damage-induced apoptosis. Taken together, as a mediator of the p53 pro-survival pathway and a marker of malignancy in some tumors, differential regulation of myosin VI in various tumor cells by topoisomerase inhibitors dictates whether knockdown of myosin VI inhibits, rather than enhances, the susceptibility of tumor cells to some therapeutic agents, which might be explored for designing a proper therapeutic strategy.

Myosins are motor proteins to move cargos along actin filaments using the energy derived from ATP hydrolysis by actin-activated Mg\(^{2+}\)-dependent ATPase activity (1–3). Myosins are categorized into 18 distinct classes according to variations of their amino acid sequence in the motor domain. The differences in motor and tail domains are linked to their specific biological functions in the cell (4–5). Myosin VI is an unconventional actin-based motor protein and carries out a diverse set of functions such as maintenance of the Golgi complex, cargo sorting and vesicle formation, protein secretion, endocytosis, cell migration, spindle orientation, spermatogenesis, and cell-cell contacts (1, 6–10). In addition, myosin VI was shown to enhance RNA polymerase II-dependent transcription through physical interaction with RNA polymerase II (11), suggesting that myosin VI has a critical function in the nucleus as well as in the cytoplasm and on the plasma membrane.

Myosin VI was found to be overexpressed in high-grade ovarian carcinoma cells and knockdown of myosin VI led to impediment of cell spreading, migration, and dissemination (12). Myosin VI was also found to be one of the highly expressed genes in clinical prostate specimens and knockdown of myosin VI in LNCaP prostate cancer cells led to inhibition of cell migration and colony formation (13). Because of its overexpression in the Golgi apparatus in prostate cancers, myosin VI might be used as a prostate cancer biomarker (14).

Multiple regulatory mechanisms have been shown to modulate the activity of myosin VI rather than to act as an on/off switch (15). For example, phosphorylation of potential sites in the motor domain and tail region of myosin VI is correlated with its translocation to the ruffling membrane at the leading edge upon epidermal growth factor treatment (16) and binding of calcium to the lever-arm region of myosin VI via calmodulin is correlated with its motor activity (17). Previously, we showed that myosin VI is induced by p53 and DNA damage in a p53-dependent manner and myosin VI mediates the p53 pro-survival pathway (18). A recently study showed that in response to water-immersion restrain stress, a form of traumatic/psychological stress, myosin VI was found to be increased transcriptionally in mouse hippocampus (19). Nevertheless, very little is known about how myosin VI, especially its expression, is regulated. Thus, we further explored how p53 and various chemotherapeutic DNA damage agents modulate myosin VI expression. We made several novel observations that myosin VI expression can be activated or repressed at the transcriptional and post-transcriptional levels dependent upon the cell type and DNA damage agent.

EXPERIMENTAL PROCEDURES

Cell Culture and Generation of Cell Lines—MCF7 cell lines that inducibly express wild-type p53, or p53(R249S) were cultured as previously reported (20). MCF7-p53-KD in which endogenous p53 was stably knocked down by siRNA was cultured as previously reported (21). RKO, MCF7, and LNCaP cells were purchased from American Type Culture Collection and cultured as instructed.

To generate inducible myosin VI-knockdown cell lines, H1 promoter-based tetracycline-inducible expression vector car-
Differential Regulation of Myosin VI by DNA Damage

To generate inducibly myosin VI expression cell lines, a cDNA clone (MCG14D) carrying the 5' ‐ coding sequence of myosin VI was digested by EcoRI and HpaI and then cloned into the tetracycline ‐ inducible expression vector pcDNA4/TO (Invitrogen) via EcoRI and EcoRV sites. The resulting construct was designated as pcDNA4/TO‐MYO VI‐N. The C ‐ terminal region of myosin VI derived from an EST clone (MCG14A) was digested with BsrGI and XbaI and then cloned into the BsrGI and XbaI ‐ digested pcDNA4/TO‐MYO VI‐N. The resulting vector that expresses the full ‐ length myosin VI was designated as pcDNA4/TO‐MYO VI. An HA tag was added to the N ‐ terminal region of myosin VI in pcDNA4/TO‐MYO VI and the resulting construct designated as pcDNA4/TO‐HA‐MYO VI. The myosin VI expression vector was then transfected into MCF7 cells in which a tetracycline repressor is expressed by pcDNA6. Myosin VI ‐ KD MCF7 cell lines were selected with 1 μg/ml of puromycin and confirmed by Western blot analysis.

To monitor myosin VI acetylation, myosin VI ‐ KD MCF7 cell lines were treated with 1 μg/ml of doxorubicin for 48 h, collected and then fixed in 75% ethanol for at least 1 h. Cells were then washed with PBS and resuspended in a staining buffer containing 100 μg/ml of RNase A (Sigma) and 50 μg/ml of propidium iodide (Invitrogen). The percentage of cells in each phase of the cell cycle (sub ‐ G1/G0, G1/G0, S, and G2/M) was analyzed by a FACS ‐ Caliber cell sorter along with Cell Quest software (BD Biosciences).

RESULTS

Myosin VI Expression Is Inhibited in MCF7 and LNCaP Cells upon DNA Damage—Previously, we showed that myosin VI can be up ‐ regulated by p53 and DNA damage in a p53 ‐ dependent manner in several tumor cell lines, including H1299, RKO, and LS174T (18). In these cells, myosin VI acts as a mediator of the p53 ‐ dependent cell survival pathway upon DNA damage. To further investigate the function of myosin VI in the DNA damage pathway, myosin VI expression was measured by Western blot analysis in MCF7 and LNCaP cells upon DNA damage. We found that p53 was stabilized and then p21 was induced (Fig. 1, A and B, compare lanes 1, 4, 7, and 10 with lanes 2–3, 5–6, 8–9, and 11–12, respectively; Fig. 1C, compare lane 1 with lanes 2–5). Surprisingly, we found that the level of myosin VI protein was decreased in MCF7 and LNCaP cells upon treatment with doxorubicin and camptothecin in a manner dependent on the time (Fig. 1, A and B, compare lanes 1, 4, 7, and 10 with lanes 2–3, 5–6, 8–9, and 11–12, respectively) and dose (Fig. 1C, compare lane 1 with lanes 2–5). Considering that myosin VI is a protein with a long half ‐ life (more than 12 h), the effect of topoisomerase inhibitors on myosin VI expression is significant. We also found that activation of p53 led to increased expression of p21 and decreased expression of myosin VI in MCF7 cells upon treatment with Nutlin ‐ 3, which inhibits the ability of Mdm2 to inactivate p53 but does not directly induce DNA damage (Fig. 1C, compare lane 1 with lanes 6–7). To determine whether the decreased expression of myosin VI protein is due to decreased myosin VI transcription, Northern blot analysis and quantitative RT ‐ PCR were used to measure the levels of myosin VI transcript in MCF7 cells upon treatment with camptothecin and doxorubicin along with RKO cells as a control. Consistent with our previous observation (18), the levels of myosin VI transcript were increased by doxorubicin and camptothecin in RKO cells (Fig. 1D, compare lanes 1 and 3 with 2 and 4, respectively). In contrast, in MCF ‐ 7 cells, the level of myosin VI transcripts were markedly decreased upon treatment with camptothecin whereas doxorubicin had no effect (Fig. 1D, compare lanes 5–7). Similarly, qRT ‐ PCR showed that the level of myosin VI transcript was decreased in MCF7 cells treated with camptothecin but not doxorubicin (Fig. 1E, left panel) whereas the level of p21 transcript was decreased in MCF7 cells treated with doxorubicin (Fig. 1E, right panel).
Ectopic Expression of p53 Decreases the Level of Myosin VI Protein but Not Transcript in MCF7 Cells

To determine whether the repression by DNA damage is mediated by p53, we examined myosin VI expression in MCF7 cells that inducibly express wild-type p53 and a mutated form. We found that the levels of myosin VI protein were decreased in MCF7 cells upon expression of wild-type p53 for 48 h (Fig. 2A, compare lanes 1–2). However, mutant p53 R249S had no effect on myosin VI expression (Fig. 2A, compare lanes 3–4). As a transcription factor, p53 is capable of activating as well as repressing gene expression (24). Thus, we examined whether the decreased expression of myosin VI protein by p53 is due to decreased transcription of myosin VI. To test this, Northern blot analysis was performed. Surprisingly, we found that the levels of myosin VI transcript were slightly increased instead of decreased in MCF7 cells upon induction of wild-type p53 (Fig. 2B, compare lanes 1–2). Mutant p53 had no effect (Fig. 2B, compare lanes 3–4). As a control, p21 was found to be induced by wild-type p53 but not mutant R249S (Fig. 2B, p21 panel, compare lanes 1 and 3 with 2 and 4, respectively). Similar patterns of myosin VI and p21 expression were detected by qRT-PCR in MCF7 cells that were uninduced or induced to express wild-type p53 or p53(R249S) (Fig. 2C).

Myosin VI Expression Can Be Inhibited by DNA Damage Independent of p53—Although myosin VI expression can be inhibited in MCF7 cells upon DNA damage and ectopic expression of wild-type p53, it is not clear whether p53 is required for inhibition of myosin VI expression by DNA damage. To test this, we examined the expression level of myosin VI in p53-knockdown MCF7 cells. We showed that upon DNA damage, the levels of p53 and its target genes were markedly increased in p53-competent, but not p53-knockdown, MCF7 cells (Fig. 3A, compare lanes 1–3 with 4–6). However, upon treatment with doxorubicin and camptothecin, myosin VI expression was inhibited in both p53-competent and p53-knockdown MCF7 cells (Fig. 3A, compare lanes 1–3 with 4–6, respectively). In addition, we found that myosin VI transcription was still inhibited by camptothecin in p53-knockdown cells as well as in p53-competent cells (Fig. 3B). As a control, we showed that DNA damage induction of p21 and Mdm2 was abrogated by p53 knockdown in MCF7 cells (Fig. 3A, compare lanes 1–3 with 4–6; Fig. 3B, right panel). Thus, we conclude that p53 participates in, but is not required for, inhibition of myosin VI expression by DNA damage.

Myosin VI Expression Is Differentially Regulated by Various Topoisomerase Inhibitors—Based on the enzymatic activity, topoisomerases are categorized into class I and II (TOP1 and TOP2). As a result, there are two group of topoisomerase inhib-
itors, both of which are potent chemotherapeutic and DNA-damaging agents and capable of inducing p53 and its target genes (25–28). As shown above (Figs. 1 and 3), camptothecin, a TOP1 inhibitor, is more potent than doxorubicin, a TOP2 inhibitor, in repressing myosin VI expression. In addition, camptothecin, but not doxorubicin, is capable of inhibiting myosin VI transcription. Thus, we wanted to confirm whether myosin VI can be inhibited similarly by apigenin, a TOP1 inhibitor, and etoposide, a TOP2 inhibitor. We showed that like camptothecin, apigenin was a potent inhibitor of myosin VI expression (Fig. 4A, compare lane 5 with lanes 1 and 4, respectively). In addition, apigenin was a potent inducer of p53 and to a less extent of p21 (Fig. 4A, lane 5). Surprising, although p53 stability was increased by apigenin and activated p53 was able to induce p21, Mdm2 expression was markedly inhibited (Fig. 4A). This suggests that Mdm2 was repressed by a potent repression pathway activated by apigenin, which obliterates the ability of p53 to induce Mdm2. Like doxorubicin, TOP2 inhibitor etoposide was capable of stabilizing p53 and subsequently, decreasing myosin VI, but increasing p21 and Mdm2, expression (Fig. 4A, compare lane 3 with lanes 1–2). In addition, both apigenin and etoposide were capable of repressing myosin VI expression in p53-knockdown cells (Fig. 4B, compare lanes 1–5 with 6–10). Furthermore, myosin VI transcription was found to be inhibited by apigenin but little if any by etoposide (Fig. 4C, left panel), suggesting that only TOP1 inhibitors are capable of repressing myosin VI transcription. For a control, p21 transcription was measured and found to be increased by both TOP1 and TOP2 inhibitors (Fig. 4C, right panel). We would like to note that although p53 was highly accumulated in MCF7 cells upon treatment with apigenin (Fig. 4A, lane 5), the level of p21 transcript was only slightly increased (Fig. 4C, right panel), consistent with the extent of p53 induction of p21 protein (Fig. 4A, lane 5 with lanes 2–4).

To determine the mechanism by which TOP1 inhibitors decrease myosin VI expression, ChIP assay was performed to examine the level of acetylated histones H3 and H4 on the proximal myosin VI promoter and the level of p53 bound to the p53-responsive element (p53-RE) on the myosin VI promoter (Fig. 4D). As a control, the p21 promoter was similarly examined (Fig. 4D). We showed that on the p21 promoter, the binding of p53 to the p53-RE and the extent of acetylated histone H4 were markedly increased in MCF7 cells treated with camptothecin and apigenin (Fig. 4E, p53 and acetylated H4 panels). These results are consistent with previous observations (23, 29). Interestingly, the extent of acetylated histone H3 on the p21 promoter was slightly increased by camptothecin but markedly decreased by apigenin (Fig. 4E, acetylated H3 panel), suggesting that the lack of acetylated histone H3 on

**FIGURE 2.** Ectopic expression of wild-type p53 decreases the level of the myosin VI protein, but not the transcript. A, MCF7 cells were uninduced (−) or induced (+) to express wild-type p53, or mutant p53(R249S) for 48 h. The levels of myosin VI, p53, p21, and GAPDH proteins were determined by Western blot analysis with their respective antibodies. B, levels of myosin VI, p21, and GAPDH transcripts were measured in MCF7 cells uninduced (−) or induced (+) to express wild-type p53 or mutant p53(R249S) for 24 h by Northern blot analysis. The relative levels of myosin VI and p21 transcripts were normalized by the levels of GAPDH transcript.

**FIGURE 3.** The levels of myosin VI protein are decreased upon DNA damage in both p53-dependent and -independent manners. A, MCF7 and p53-KD MCF7 cells were mock-treated or treated with DOX (100 ng/ml) or CPT (100 nM) for 48 h. The levels of myosin VI, p53, Mdm2, and p21 proteins were determined by Western blot analysis with their respective antibodies. The relative levels of myosin VI protein were quantified by densitometry and normalized by GAPDH levels. B, levels of myosin VI and p21 transcripts in MCF7 and p53-KD MCF7 cells, which were untreated or treated with DOX (100 ng/ml) or CPT (100 nM) for 24 h, were measured by qRT-PCR. The relative levels of myosin VI and p21 transcripts were normalized by the levels of GAPDH transcript.

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**Differential Regulation of Myosin VI by DNA Damage**

| MCF7-p53 | WT | R249S |
|----------|----|-------|
| Induction | MYO VI | p53 | p21 | GAPDH |
| 1 | 2 | 3 | 4 |

| MCF7-p53 | WT | R249S |
|----------|----|-------|
| Induction | MYO VI | p21 | GAPDH |
| 1 | 2 | 3 | 4 |

**FIGURE 4.** A, MCF7 cells were treated with camptothecin (CPT) and apigenin (API) for 48 h. The levels of myosin VI, p53, p21, and GAPDH proteins were determined by Western blot analysis with their respective antibodies. B, levels of myosin VI and p21 transcripts were measured in MCF7 cells treated with camptothecin and apigenin for 24 h by qRT-PCR. The relative levels of myosin VI and p21 transcripts were normalized by the levels of GAPDH transcript.
the p21 promoter may be responsible for weak induction of p21 in MCF7 cells treated with apigenin (Fig. 4A, lane 5). However, on myosin VI promoter, the binding of p53 to the p53-RE was only slightly increased in MCF7 cells upon treatment with camptothecin and apigenin (Fig. 4E, compare lane 1 with lanes 2–3). In addition, the extent of acetylated histone H3 on the myosin VI promoter was markedly decreased, whereas that of histone H4 was slightly increased, in MCF7 cells treated with camptothecin and apigenin (Fig. 4E, compare lane 1 with lanes 2–3). Therefore, the decreased level of acetylated histone H3 on the myosin VI promoter in MCF7 cells treated with camptothecin and apigenin may be responsible for repressed expression of myosin VI transcript.

**Ectopic Expression of Myosin VI Increases p53 Stabilization and Subsequently Induction of p21 and Mdm2—**To determine whether myosin VI is a *bona fide* modulator of p53 expression, we generated multiple MCF7 cell lines in which HA-tagged myosin VI can be inducibly expressed under the control of the tetracycline-regulated promoter. We found that upon induction, myosin VI was found to be expressed as detected by anti-HA (Fig. 5, HA-MYO VI panel, compare lanes 1–2). When anti-myosin VI antibody was used, which detects both endogenous and HA-tagged myosin VI, we found that the level of myosin VI was also increased upon induction (Fig. 5, MYO VI panel, compare lanes 1–2). We would like to note that the level of exogenous myosin VI proteins was only 1–2-fold higher than that of endogenous myosin VI (Fig. 5, MYO VI and HA-MYO VI panels, compare lanes 1–2), suggesting that myosin VI was not highly overexpressed. Nevertheless, we found that upon treatment with doxorubicin for various times, DNA damage-induced stabilization of p53 was further increased by ectopic expression of myosin VI (Fig. 5, compare lanes 5, 9, and 13 with 6, 10, and 14, respectively). However, ectopic expression of myosin VI had no effect on the basal level of p53 under a non-stress condition (Fig. 5, lanes 1–2) or camptothecin-induced stabilization of p53 (Fig. 5, compare lanes 3, 7, and 11 with 4, 8, and 12, respectively). The latter observation is interesting but not surprising since the overall level of myosin VI was not significantly increased upon ectopic expression of HA-myosin VI in MCF7 cells treated with camptothecin (Fig. 5, MYO VI panel, compare lanes 3, 7, and 11 with 4, 8, and 12, respectively). This effect is likely due to strong repression of
Differential Regulation of Myosin VI by DNA Damage

Knockdown of Myosin VI Inhibits p53 Stabilization and Subsequently Induction of p21 and Mdm2, Leading to Enhanced Resistance of MCF7 Cells to DNA Damage-induced Apoptosis—Previously, we showed that myosin VI in RKO cells is induced by p53, which then modulates p53 stabilization induced by DNA damage (18). However, in MCF7 cells, myosin VI was found to be repressed by p53 and DNA damage. Thus, does myosin VI still play a role in DNA damage-induced p53 activation in MCF7 cells? To test this, we generated multiple MCF7 cell lines in which myosin VI can be inducibly knocked down by siRNA under the control of the tetracycline-regulated promoter. Three representative cell lines (37, 42, 79) were chosen for further studies. We showed that upon induction, the level of myosin VI protein was significantly decreased, especially considering that myosin VI is a structural protein with a long half-life (Fig. 6, A and B, compare lanes 1, 3, and 5 with 2, 4, and 6, respectively). We found that upon knockdown of myosin VI, the level of p53 protein was slightly decreased under a non-stress condition (Fig. 6, A and B, compare lanes 1–2). We also found that p53 was accumulated upon treatment with camptothecin and doxorubicin, but the extent of p53 accumulation was decreased by myosin VI knockdown (Fig. 6, A and B, compare lanes 3 and 5 with lanes 4 and 6, respectively). Consistent with decreased accumulation of p53, the extent of p53 induction of p21 and Mdm2 was also decreased (Fig. 6A, p21 and Mdm2 panels, compare lanes 1, 3, and 5 with 2, 4, and 6, respectively).

Our previous and other published studies showed that myosin VI is a pro-survival factor and serves as a mediator of the p53 pro-survival pathway (12–13, 18, 30). Thus, we examined whether myosin VI in MCF7 cells still possesses such an activity. To test this, DNA histogram analysis was performed to measure the extent of apoptosis in myosin V1-KD MCF7 cells (clone 42), which were uninduced or induced to knock down myosin VI followed by mock-treatment or treatment with doxorubicin for 48 h. We found that the cell cycle profile was not significantly effected by myosin VI knockdown alone (Fig. 6C, compare the relevant DNA contents between control and MYO VI-KD). However, upon treatment with doxorubicin, the sub-G1 DNA content induced by DNA damage was markedly decreased from 11.49 to 2.8% by myosin VI knockdown (Fig. 6C, compare the relevant DNA contents between DOX2 and DOX+MYO VI-KD panels). Sub-G1 DNA content is known to conservatively represent the percentage of cells undergoing apoptosis. We also showed that knockdown of myosin VI in another MCF7 cells (clone 37) led to a decreased level of apoptosis induced by treatment with doxorubicin (from 17.74 to 5.07%) (Fig. 6D). This suggests that lack of myosin VI inhibits, rather than increases, the susceptibility of MCF7 cells to doxorubicin-induced apoptosis.

DISCUSSION

Previously, we showed that myosin VI is activated by DNA damage in a p53-dependent manner and myosin VI mediates p53 pro-survival function (18). Here, we confirmed and further extended our previous observations. We showed that myosin VI expression can be increased in RKO cells upon treatment with other TOP1 and TOP2 inhibitors (apigenin and etoposide) in addition to camptothecin and doxorubicin. We also showed that myosin VI is necessary for proper activation of p53 in MCF7 cells upon DNA damage although myosin VI expression is found to be repressed by p53 and DNA damage. This suggests that the ability of myosin VI to modulate DNA damage-induced p53 activation is independent of the cell type. Most interestingly, we made several novel and surprising findings. First, we found that in MCF7 and LNCaP cells, both p53 and DNA damage decrease myosin VI expression. Second, we found that while p53 participates in DNA damage inhibition of myosin VI, p53 is not required. Third, we found that post-transcriptional regulation is likely to be responsible for the inhibition of myosin VI expression by p53 and TOP2 inhibitors since the levels of myosin VI transcripts were slightly increased by p53 or insignificantly altered by TOP2 inhibitors. Fourth, we found that the levels of myosin VI transcripts and the extent of acetylated histone H3 on the myosin VI promoter were markedly decreased in MCF7 cells by TOP1 inhibitors, suggesting that myosin VI expression can be increased in RKO cells upon treatment with other TOP1 and TOP2 inhibitors (apigenin and etoposide) in addition to camptothecin and doxorubicin. We also showed that knockdown of myosin VI in another MCF7 cells (clone 37) led to a decreased level of apoptosis induced by treatment with doxorubicin (from 17.74 to 5.07%) (Fig. 6D). This suggests that lack of myosin VI inhibits, rather than increases, the susceptibility of MCF7 cells to doxorubicin-induced apoptosis.

2 The abbreviations used are: DOX, doxorubicin; CPT, camptothecin.
Differential Regulation of Myosin VI by DNA Damage

We provided compelling evidence that myosin VI expression is inhibited by p53 and DNA damage in MCF7 and LNCaP cells (this study) but increased in RKO, LS174, and H1299 cells (18), indicating that myosin VI expression is regulated in a cell type-dependent manner. Then, how does the cell type control the response of myosin VI expression to DNA damage? One possibility lies in the way these tumor cells were initially transformed. Because of different genetic aberrations, it is well-known that tumor cells would respond differently to p53 activation and DNA damage. For example, dependent on the presence or absence of adenoviral E1A oncogene or c-MYC in mouse embryo fibroblasts, p53 activation and/or DNA damage elicit different sets of gene activation and as a result, different sets of cellular responses, such as apoptosis versus cell cycle arrest (31). The other possibility is that p53 is a transcription factor as well as an adaptor protein involved in multiple cell signaling events (32). For example, when a co-activator is recruited to the myosin VI promoter in RKO and H1299 cells, p53 would activate myosin VI expression. However, as a signaling protein, p53 may interact with a protein that directly controls myosin VI expression via a non-transcriptional mechanism, and as a result, p53 would indirectly control myosin VI expression. Nevertheless, these possibilities warrant further investigation, which is beyond the scope of the current study.

We found that although both TOP1 and TOP2 inhibitors are capable of inhibiting myosin VI expression, camptothecin and apigenin are particularly active. In addition, the levels of myosin VI transcript can be markedly decreased by TOP1, but not TOP2, inhibitors. Thus, what is responsible for such an effect? First of all, the effect might be traced to different functions of TOP1 and TOP2 in normal cells and the consequences when their functions are inhibited by their respective inhibitors. The primary biological function for TOP1 is repair of DNA supercoiling. DNA tends to be overwound (positively supercoiled) upstream of transcription forks and underwound (negatively supercoiled) downstream of these forks (25). In addition, the positive supercoiling in the flank of the nucleosome is generated during nucleosome formation by wrapping DNA around the histone octamer. Thus, in order for transcription to proceed, TOP1 is needed to relax DNA supercoiling. The primary biological function for TOP2 is to disentangle DNA strands and thus resolve topological problems (33). DNA entanglement occurs frequently during DNA recombination and to less extent during transcription. Thus, inhibition of TOP1 may have more profound effect on transcription than that of TOP2, which may explain a greater effect on myosin VI transcription by TOP1 inhibitors than by TOP2 inhibitors. Alternatively, the differential effect by TOP1 and TOP2 inhibitors may be attributed to the ways by which DNA break is generated and their corresponding DNA damage response pathways. Inhibition of TOP1 initially leads to single-strand DNA breaks and eventually double-strand DNA breaks when DNA replication forks are encountered (34) whereas inhibition of TOP2 creates double-strand DNA breaks (33). Thus, different DNA damage response pathways are activated to sense the damaged DNAs induced by TOP1 and TOP2 inhibitors and as a result, different cellular responses, including transcriptional regulation, are induced. For example, p53 activation and subsequently induction of p53 target genes have been found to be differentially regulated by TOP1 and TOP2 inhibitors (35–36). However, because p53 is not required for transcriptional repression of myosin VI by TOP1 inhibitors, future study is warranted to identify such a transcription factor. Currently, many types of TOP1 and TOP2 inhibitors are used as anticancer agents (25, 27–28). Thus, the differential regulations of myosin VI by various TOP1 and TOP2 inhibitors along with the expression patterns of myosin VI in various cell types might be explored for designing a proper therapeutic strategy.

Myosin VI is known to function as an actin motor protein for membrane trafficking, cell migration, and endocytosis (2–3, 8–9, 12). In addition to aberrant cell cycle control, cell migration is one of the major factors responsible for malignancy. Not surprisingly, as a tumor suppressor, p53 inhibits cell spreading, polarization, filopodia formation, and consequently tumor cell invasion and migration (37–41). Moreover, dominant-negative mutant p53 R273H promotes tumor cell invasion and migra-
Differential Regulation of Myosin VI by DNA Damage

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