Topotecan-Triggered Degradation of Topoisomerase I Is p53-Dependent and Impacts Cell Survival

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Abstract

The anticancer drug topotecan belongs to the group of topoisomerase I (topo I) inhibitors. In the presence of topotecan, topo I cleaves the DNA but is unable to relegate the single-strand break. This leads to stabilization of topo I-DNA-bound complexes and the accumulation of DNA strand breaks that may interfere with DNA replication. The molecular mechanism of controlling the repair of topo I-DNA covalent complexes and its impact on sensitivity of cells to topotecan is largely unknown. Here, we used mouse embryonic fibroblasts expressing wild-type p53 and deficient in p53, in order to elucidate the role of p53 in topotecan-induced cell death. We show that p53-deficient mouse embryonic fibroblasts are significantly more sensitive to topotecan than wild-type cells, displaying a higher frequency of topotecan-induced apoptosis and DNA strand breaks. Treatment of p53 wild-type cells with pifithrin-α, an inhibitor of the trans-activating activity of p53, caused reversal of the phenotype, making wild-type cells more sensitive to topotecan. Upon topotecan treatment, topo I was degraded in wild-type but not in p53-deficient cells. Topo I degradation was attenuated by the proteosomal inhibitor MG132. Similar data were obtained with human glioblastoma cells. U138 cells (p53 mutated) were significantly more sensitive to topotecan than U87 cells (p53 wild-type). Furthermore, U87 cells showed significant degradation of topo I upon topotecan treatment, whereas in U138 cells, this response was abrogated. Topo I degradation was again attenuated by pifithrin-α. The data suggest that p53 causes resistance of cells to topo I inhibitors due to stimulation of topotecan-triggered topo I degradation which may impact topotecan-based cancer therapy. (Cancer Res 2005; 65(19): 8920-6)

Introduction

Topotecan is an anticancer drug widely used in the therapy of melanoma, ovarian, and colorectal carcinoma, and glioblastoma. It is a camptothecin derivative belonging to the class of topoisomerase I (topo I) inhibitors (1). The catalytic cycle of topo I starts with the formation of a DNA single-strand break after which it covalently binds to the 3’-end of the DNA phosphodiester backbone. Topo I covalently bound to the nicked DNA forms the so-called topo I-DNA cleavable complex that represents a unique type of DNA damage (2). Topo I-DNA complexes are reversible covalent intermediates catalyzing the cleavage-religation reaction of the enzyme (3). Camptothecin, the prototype of topo I poisons, and its derivatives such as the clinically relevant drug topotecan, stabilize the cleavable complex and thus prevent religation of topo I-mediated DNA single-strand breaks (4, 5). This block of religation of topo I-mediated DNA single-strand breaks gives rise to stabilization and accumulation of topo I-DNA covalent complexes (5). The cytotoxic mechanism of camptothecins is largely S phase-dependent, indicating that it is triggered by a collision between the replication fork and the camptothecin-stabilized cleavable complex. This may result in blockage of fork movement, and finally, the formation of DNA double-strand breaks (6–9). At higher concentrations, camptothecins can also kill non–S phase cells through apoptosis by an unknown mechanism (10).

The variables controlling cellular sensitivity to camptothecin and its derivatives are largely unknown. Strong differences in sensitivity were found in colorectal and breast cancer cell lines that were neither due to variation in the cellular accumulation of camptothecin nor the level of topo I-DNA complexes formed (11). Also, none of the other variables studied thus far, such as expression and activity of topo I, the cell’s doubling time, and expression of MDR-1, Bcl-2, and Bax was predictive for camptothecin sensitivity (12). A factor supposed to be involved in determining the sensitivity of cells to topo I poisons is p53. Here, however, only limited and contradictory data are available. Thus, for breast cancer cells, the p53 status was not found to be predictive for sensitivity to camptothecin (12). Comparing a panel of glioma cell lines, no influence of p53 on cell death induced by topotecan was observed (13). In contrast, in ovarian cancer cells that regained p53 function upon p53 transfection, a protective effect of p53 on topotecan treatment was found (14). The reason for the observed p53-mediated protection remained unclear. In order to elucidate the role of p53 in topotecan sensitivity, we conducted experiments using nontransformed mouse embryonic fibroblasts (MEF) proficient and deficient (knock-out) in p53. We show that p53-deficient (p53−/−) MEFs are significantly more sensitive to the cytotoxic activity of topotecan than the p53-proficient isogenic counterpart. This was related to increased topotecan-triggered topo I degradation. Similar data were obtained with glioblastoma cells. Pharmacologic inhibition of p53 imitated the p53-deficient phenotype, i.e., it blocked degradation of topo I and increased topotecan-induced apoptosis. Overall, the data strongly suggest that p53 mediates the repair of topo I-cleavable complex which causes resistance of cells to topotecan.

Materials and Methods

Reagents and antibodies. Topotecan (Hycaintin) was obtained from Smithkline Beecham, United Kindom. Mouse anti-Cdc25A monoclonal antibody, rabbit anti-topo I, and anti-ERK2 pAb were from Santa Cruz Biotechnology (Heidelberg, Germany). Mouse anti-p53 monoclonal antibody were from BD PharMingen (Heidelberg, Germany). Rabbit anti-p21/Waf1 was from Calbiochem (Merck, Darmstadt, Germany). Rabbit anti-phospho-Chk1 was purchased from Cell Signaling Technology (Beverly, MA). Horseradish peroxidase-coupled secondary anti-mouse and anti-rabbit IgG...
antibodies were from Amersham Pharmacia Biotech (Freiburg, Germany). Colorimetric caspase activity assays were from R&D (Wiesbaden, Germany).

**Cell lines.** Wild-type and p53-deficient MEFs were spontaneously immortalized and not additionally transformed. They were used in passages 15 to 25 upon immortalization. The wild-type cell line was grown in DMEM with high glucose and glutamine containing 15% inactivated fetal bovine serum, whereas p53 knock-out cells were grown in the same medium with 10% serum, at 37°C in an atmosphere containing 7% CO₂. Under the cultivation conditions used, p53 wild-type and knock-out cells had the same growth rate with a doubling time of 18 hours. U87 and U138 glioma cells were kindly provided by Dr. N. de Tribolet (Lausanne, Switzerland), Dr. M. Weller (Tübingen, Germany) and characterized as described before (15, 16). They were grown in DMEM containing 10% inactivated fetal bovine serum at 37°C in an atmosphere containing 7% CO₂.

**Drug treatment.** In all experiments, cells were continuously exposed to topotecan, in accordance to its administration during the anticancer therapy. For short exposure periods (up to 24 hours), higher drug concentrations (1–4 μg/mL) were applied, whereas for longer exposure times, lower drug concentrations (<1 μg/mL) were chosen. Pifithrin-α was continuously applied at a concentration of 30 μM/L.

**WST-1 viability and colony-forming assay.** Cells (up to 10⁶) were seeded in 96-well plates and 36 hours later, exponentially growing cells were treated with different concentrations of topotecan for 72 hours. WST-1 reagent was added for colorimetric reaction (measurement of metabolizing activity) up to a final concentration of 10% (v/v). Plates were incubated up to 2 hours at 37°C. The extinction was measured on an ELISA reader at 450 nm. The data are the mean of three independent experiments done in triplicates. For colony formation, 1 to 2.5 × 10⁵ cells were seeded per 6 cm dish and treated 6 hours later by the addition of topotecan to the medium. Colonies were fixed, stained, and counted 10 days after seeding.

**Determination of apoptosis and necrosis.** For monitoring drug-induced apoptosis and necrosis within the same cell population, annexin V-FITC/propidium iodide double-staining combined with flow cytometry was applied. Exponentially growing cells were chronically treated with different doses of topotecan and 48 to 96 hours thereafter subjected for analysis. The protocol was conducted as previously described (17).

**Cell cycle analysis.** Progression of cells through the cell cycle was analyzed by flow cytometry as previously described (18). In brief, cells were continuously treated with topotecan, and after different time points, fixed with ethanol, incubated with 0.1 mg/mL RNase in PBS for 1 hour, and stained with propidium iodide (25 μg/mL) prior to flow cytometry (CellQuestPro, BD, Heidelberg, Germany).

**Preparation of cell extracts and immunoblotting.** Whole-cell extracts were prepared by lysis in ice-cold sample buffer [25 mmol/L Tris-HCl (pH 6.8), 1 mmol/L EDTA, 5% glycerol, 2.5% mercaptoethanol; 1 mmol/L phenylmethylsulfonyl fluoride was added last], followed by sonification (Branson sonifier, 30 kHz, 3 x 10 seconds) on ice. Protein extracts (20 μg) were separated by 7% to 12% SDS-PAGE and electroblotted onto a nitrocellulose membrane (Schleicher & Schuell, Dassel, Germany). The antibodies used were mostly diluted (1:500) in 5% nonfat dry milk, 0.02% T-PBS. Protein-antibody complexes were detected by enhanced chemiluminescence (ECL, Amersham Pharmacia Biotech). For Western blot analysis with phospho-specific antibodies, cells were directly lysed in 1× SDS-PAGE sample buffer and subsequently sonified, as proposed by the manufacturer (Cell Signaling Technology). Phospho-specific antibodies were diluted in 5% bovine serum albumin, TBS, and washed with 0.1% Tween-TBS. For immunoblotting with anti-topo I antibody, untreated and topotecan-treated cells were directly lysed in 1× SDS-PAGE sample buffer (for monitoring trapped covalent topo I-DNA complexes). For reversal of topo I-cleavable complexes, topotecan-exposed cells were rinsed up to five times with culture medium to get rid of the drug and incubated in fresh topotecan-free medium for 30 minutes (for monitoring total cellular levels of topo I) prior to lysis and sonification. Proteins were transferred onto nitrocellulose membrane using semidyblotting for 30 minutes. Quantification of signal intensity was done using the MultiAnalyst Software and the Gel Doc 1000 system from Bio-Rad (Hercules, CA). Signal intensity of ERK2 served as a reference for calculating the ratio of protein expression.

**Preparation of nuclear extracts and electromobility shift assay.** Nuclear cell extracts of untreated and topotecan-treated cells were prepared and subjected to electromobility shift assay (EMSA) as described previously (19). The sequence of the oligonucleotides specific for the p53-binding site was: TGGCCATCTAGAACTACGACACATGCCAAACATGTTGACCTGCGCA.

**Single-cell gel electrophoresis.** After exposure to topotecan, subconfluent cultures were rinsed with ice-cold PBS and trypsinized. Cells were centrifuged and washed, and 10 μL of cell suspension (10⁶ cells per mL) was mixed with 120 μL low–melting point agarose at 37°C and poured onto agarose-overlayed slides. The alkaline cell lysis procedure after Singh et al. (20) was slightly modified as previously described (17).

**Results**

**Cells deficient in p53 are hypersensitive to topotecan.** The p53 phenotype of cell lines used in this study was analyzed by Western blot analysis and EMSA. For immunodetection of p53 and p21, nuclear extracts of topotecan-exposed MEFs were prepared. A transient induction of p53 protein was observed in wild-type cells 2 hours after exposure to topotecan (Fig. 1A). p21 was concomitantly induced and remained at higher level of expression. Neither p53 nor p21 protein was detectable in p53-deficient cells (Fig. 1A). Induction of p53 by topotecan was verified by EMSA, using nuclear extracts of untreated and topotecan-treated MEFs mixed with oligonucleotides harboring the p53-binding site of the p21 promoter. A clear increase in binding activity was detected after a 4-hour exposure period to topotecan in the wild-type but not in the p53-deficient cells (Fig. 1B).

To elucidate the role of p53 in the sensitivity of MEFs to topotecan, wild-type and p53−/− cells were exposed for 72 hours to topotecan. Cellular viability was determined by the WST-1 assay. As shown in Fig. 1C, p53-deficient cells were much more sensitive to topotecan than wild-type cells. Thus, whereas p53−/− cells were unable to survive treatment with a dose of 2.5 μg/mL only 20% of wild-type cells were killed. To analyze the mode of cell-killing in more detail, the frequency of apoptosis was determined. In accordance with data obtained with the WST-1 assay, p53−/− cells were more sensitive than wild-type cells. They showed a dose-dependent induction of apoptosis reaching levels up to 25% (Fig. 1D). p53 wild-type cells were highly resistant to topotecan, displaying only a low level of apoptosis (<10%) over the whole dose range tested. Similar results were obtained measuring apoptosis by flow cytometry at different times after treatment (sub-G1 method; Fig. 2A, left). Overall, the data revealed that cell death induced by topotecan is largely due to apoptosis against which p53 acts protectively.

**Cell cycle progression, Chk1 activation and Cdc25A degradation.** To analyze whether increased sensitivity of p53-deficient cells to topotecan is accompanied by blockage of progression of cells within a specific cell cycle phase, cell cycle distribution upon exposure of cells to 2 μg/mL topotecan was determined. As shown in Fig. 2A (right), p53−/− cells were arrested in S phase 48 hours after topotecan treatment at higher level than p53 wild-type cells. Conversely, their proportion in G1 phase was clearly lower 48 hours after treatment compared with the corresponding wild-type cells. This indicates that p53−/− cells are largely unable to leave S phase upon topotecan treatment, whereas p53 wild-type cells are only transiently blocked in S phase; they obviously recover and progress at later times into G1.

S phase arrest induced by topo I poisons is dependent on ATR kinase that provokes Chk1 phosphorylation (21). Chk1 mediates S phase arrest through Cdc25A degradation (22). To elucidate...
whether these checkpoints are activated upon topotecan exposure in our cell system, we measured Chk1 phosphorylation and Cdc25A degradation by Western blot analysis. As shown in Fig. 2B, Chk1 was phosphorylated at higher levels in p53-deficient cells after topotecan treatment. Thus, quantification of the Chk1 signal revealed a 6- to 8-fold increase in Chk1 phosphorylation in p53−/− cells, whereas in the p53 wild-type cells, only a 3- to 4-fold increase was observed (measured 1 and 4 hours after topotecan exposure.
and set in relation to ERK2). Topotecan-triggered activation of Chk1 gives rise to degradation of Cdc25A, which again occurred more pronounced in p53−/− cells (Fig. 2C). An inhibitor of ubiquitin/26S pathway (MG132) blocked this process in wild-type and in p53−/− cells (Fig. 2C), indicating that down-modulation of Cdc25A expression upon topotecan treatment is mediated by proteosomal degradation. The finding that phosphorylation of Chk1 and degradation of Cdc25A upon topotecan treatment is dependent on the p53 status indicates that the Chk1-Cdc25A pathway is involved in the accumulation of p53−/− cells in S phase, which accompanies their increased topotecan sensitivity.

**Induction of DNA strand breaks.** Upstream of Chk1 phosphorylation is DNA strand break–dependent ATR signaling. Therefore, it was reasonable to speculate that the increased sensitivity of p53−/− cells to topotecan is based on an altered processing of topotecan-stabilized topo I-DNA complexes that cause DNA breaks. To analyze the formation of DNA single-strand breaks, wild-type and p53−/− cells were incubated with topotecan for up to 24 hours and subjected to alkaline single-cell gel electrophoresis (comet assay). One to six hours after topotecan exposure, only a very low induction of single-strand breaks was detected in wild-type cells, whereas in p53−/− cells, a dramatic increase in the level of single-strand breaks was observed. In wild-type cells, single-strand breaks were completely repaired 24 hours after topotecan exposure, whereas in p53−/− cells, they kept on accumulating reaching very high levels (Fig. 3A). Treatment of wild-type cells with the p53 inhibitor pifithrin-α caused them to behave like the mutant; they showed a high level of topotecan-induced DNA strand breaks (Fig. 3B). The data suggest that p53 is involved in protection against topo I-mediated cell killing by prevention of DNA strand break formation upon topotecan treatment.

**Topo I degradation depends on p53 activity.** An increased formation of DNA single-strand breaks upon topotecan treatment could indicate that the cleavable complex is more stable in p53−/− cells. To analyze whether topotecan affects the stability of topo I bound on DNA in a p53-dependent manner, we examined the expression of topo I upon topotecan treatment in wild-type and p53−/− cells. As shown in Fig. 4A, wild-type and p53−/− cells displayed a similar basal level of topo I. However, there was a clear decline in topo I level in topotecan-treated wild-type cells, which was not observed in p53−/− cells (Fig. 4A). The decline of topo I in wild-type cells started 2 hours after the beginning of topotecan treatment and was most pronounced 4 to 12 hours later. Decline of topo I in wild-type cells was abrogated by coinubcation of cells with a 26 S proteosomal inhibitor MG132 (Fig. 4B), indicating that it is due to ubiquitin-mediated degradation.

Degradation of topo I in wild-type cells was clearly abrogated by pifithrin-α (Fig. 4C). This indicates that either p53 itself or one of the p53-regulated proteins is involved in signaling DNA breaks (caused by the topotecan-topo I complex) upstream of the ubiquitin/26S proteosomal complex that degrades topo I. Repair of the cleavable complex would allow cells to proceed through the cell cycle and thus would increase the viability of the cells. If this is true, pifithrin-α should have an impact on cell survival after topotecan treatment. Indeed, wt cells exposed to pifithrin-α and topotecan were significantly more sensitive to the drug, as shown by overall survival (WST-1 assay; Fig. 4D, left) and an increase in the frequency of apoptosis (Fig. 4D, right).

To elucidate whether p53-dependent degradation of topo I also occurs in human cells, similar experiments with a pair of human glioblastoma cells wt and mutant for p53 were done. As shown in

![Figure 3. DNA single-strand breaks induced by topotecan. A, wild-type and p53-deficient cells were exposed to 2 μg/mL topotecan for the indicated times and thereafter alkaline single-cell gel electrophoresis (comet assay) was done. Columns, mean; bars, ± SD. B, wild-type cells were cultivated in the absence or presence of 30 μmol/L pifithrin-α and coexposed to 2 μg/mL topotecan for the indicated times. Thereafter, single-cell gel electrophoresis was done. Columns, mean; bars, ± SD.](image-url)

**Discussion**

Using an isogenic pair of MEFs, we analyzed the role of p53 in cell death induced by the topoisomerase I-inhibitor topotecan. We show that cells deficient in p53 are significantly more sensitive to topotecan than cells expressing wt p53. This pertained to the end points overall-cytotoxicity (as measured by WST assay) and apoptosis. Inhibition of the trans-activating activity of p53 by
p53-dependent mechanisms are more important in apoptosis induced by topotecan. We should note that p53−/− MEFs are more sensitive than p53 wt cells to UV-C light and the alkylating agent methyl methanesulfonate (23, 24). This was explained by the involvement of p53 in the processing or repair of UV-C and alkylating agent–induced DNA lesions (25–27). The mechanism by which p53 protects against topotecan-induced cell death seems to be related to the repair of the topo I cleavable complex. This is supported by the observation that p53−/− cells display a much higher level of DNA single-strand breaks than p53 wt cells upon topotecan treatment. Single-strand breaks induced by topotecan are the result of inhibition of the religation function of topo I (28). If this function is blocked, topo I remains stuck on DNA, forming a poisoned topo I-DNA complex. This complex, together with single-strand breaks that remained nonligated, interact with the DNA replication machinery, resulting in arrest of the replication fork. As reported for camptothecin, blocked replication forks may be converted into topo I-linked DNA double-strand breaks (6–9) that finally trigger apoptosis.

The basal expression level of topo I was similar in wt and p53-deficient cells. However, upon topotecan treatment, the amount of topo I decreased dramatically in wt but not in p53−/− cells. This suggests that p53 supports the degradation of topo I upon treatment with topotecan. For camptothecin, a similar topo I degradation was reported (29, 30). However, the role of p53 in this process has not been addressed. Here, we show for the first time that p53 stimulates topo I degradation. We also show that topo I degradation upon topotecan treatment occurs by proteosomal degradation because the 26S proteosomal inhibitor MG132 abrogated the topotecan-induced decline in topo I level. This is in line with a previous report using camptothecin (29, 30).

Degradation of topo I upon camptothecin or topotecan treatment is believed to occur with the topo I protein trapped to the DNA. Topo I degradation may therefore be considered as a "repair" mechanism that helps cells to recover from a potentially lethal lesion. In this process, p53 obviously acts as an essential factor. p53 may interact physically with topo I, facilitating topo I degradation upon topotecan treatment. This, however, is unlikely to occur because pifithrin-α that blocks the trans-activating activity of p53 (31) abrogated topotecan-induced topo I degradation in p53 wt cells. It should be noted that pifithrin at a very high dose level (100 μmol/L) blocks nuclear import of p53 (32), which does not occur at a lower dose level (30 μmol/L) that specifically blocks its transcriptional activity (33). In our experiments, a low dose of pifithrin-α (30 μmol/L) was used that inhibits p53 transactivation activity. Therefore, we conclude that p53 does not participate physically in the degradation of the topo I cleavable complex, but rather regulates the synthesis of a factor that mediates topo I degradation. This factor could be involved either in the ubiquitination step of topo I (29, 30) or in transcription-coupled repair, which was shown to facilitate topo I degradation.

**Figure 4.** Degradation of topoisoermerase I upon topotecan treatment. A, cells were exposed to 1 μg/mL topotecan for the indicated times. After exposure, the drug-treated cells were carefully rinsed with culture medium and incubated for another 30 minutes to reverse the cleavable complexes (see Materials and Methods). The total amount of topo I was detected by Western blot analysis using anti-topo I pAb followed by ECL detection. B, cells were exposed to 1 μg/mL topotecan for the indicated time points in the absence or presence of 5 μmol/L MG132. Then cells were thoroughly rinsed with culture medium and cultivated for another 30 minutes in the topotecan-free medium containing or not containing MG132. The total amount of topo I was detected by Western blot analysis using anti-topo I pAb followed by ECL detection. C, cells were cultivated in the absence or presence of 30 μmol/L pifithrin-α and coexposed for 7 hours to 1 μg/mL topotecan. Thereafter, they were carefully rinsed with culture medium and cultivated for another 30 minutes in topotecan-free medium containing or not containing pifithrin-α. The total amount of topo I was detected by Western blot analysis using anti-topo I pAb followed by ECL detection. Topotecan-induced topo I degradation was between 80% and 100%, as observed in several independent experiments. ERK2 served as a loading control. D, cells were coincubated with pifithrin-α and topotecan for 72 hours. Cell viability was measured by WST-assay (left) and the frequency of apoptosis was determined by annexin V/propidium iodide staining and flow cytometry (right); columns, mean; bars, ± SD.
Clinical studies have shown that during high-dose therapy with a drug that was not toxic for wild-type (wt) cells but was toxic for p53 mutant cells, degradation of topoisomerase I was observed. Degradation of topo I upon topotecan treatment in glioblastoma cells. A, human glioblastoma cells U87 (p53 wild-type) and U138 (p53 mutant) were exposed to 1 μg/mL topotecan for indicated times in the absence or presence of 30 μmol/L pifithrin-α. Thereafter, the cells were rinsed with culture medium and cultivated for another 30 minutes in topotecan-free medium containing or not containing pifithrin-α. The total amount of topo I was determined by Western blot analysis using anti-topo I pAb followed by ECL detection. B, determination of cell death by colony formation. Human glioblastoma cells were seeded in 6 cm dishes and treated with different concentrations of topotecan. Colonies were stained and counted 10 days after treatment. Points, mean; bars, ± SD; C, U87 and U138 cells were exposed for 6 hours to indicated concentrations of topotecan. Cells were rinsed with culture medium and cultivated for another 30 minutes in topotecan-free medium. The total amount of topo I was determined by Western blot analysis using anti-topo I pAb followed by ECL detection. D, U87 cells were cultivated in the absence or presence of 5 μmol/L MG132 for 30 minutes and thereafter coexposed for 6 hours to indicated doses of topotecan. Cells were rinsed with culture medium and cultivated for another 30 minutes in topotecan-free medium containing or not containing MG132. The total amount of topo I was determined by Western blot analysis using anti-topo I pAb followed by ECL detection. ERK2 served as a loading control.

In many cell systems, p53 has been identified as a proapoptotic player, stimulating either the mitochondrial or the death receptor pathway (37, 38). However, in some cell types, including MEFs, p53 acts rather antiapoptotic than proapoptotic (23–25). This newly defined role of p53 is presumably of significance for cancer therapy because we observed the effect not only in MEFs but also in glioblastoma cells treated with topotecan. Topo I degradation in glioblastoma cells correlated with their topotecan resistance. Degradation of topo I was observed in p53 wt cells at a concentration of 0.1 μg/mL topotecan, which was not toxic for wt cells but was toxic for p53 mutant cells. Clinical studies have shown that during high-dose therapy with topotecan, a peak plasma level of about 13 μg/mL 1 hour after administration is achieved (39). This is above the concentration that was effective in our in vitro setting, although because of clearance, the pharmacologically relevant dose in vivo might be lower. Under these conditions, during tumor therapy with topotecan, the p53 status might be of utmost importance. About one-third of primary glioblastomas are p53-mutated (40). This tumor group is supposed to respond to topotecan and other topo I inhibitors better than tumors expressing p53 wt cells. In view of the prognostic value of p53, it would be desirable to assess the p53 status of a given tumor, providing predictive information as to the success of topotecan-based therapy. Another conclusion derived from this work is that pharmacologic inhibition of p53 might be an efficient strategy for sensitizing p53 wt expressing tumors to topotecan-based therapy.

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