Role of p21 in Apoptosis and Senescence of Human Colon Cancer Cells Treated with Camptothecin*

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Treatment of cells with the anti-cancer drug camptothecin (CPT) induces topoisomerase I (Top1)-mediated DNA damage, which in turn affects cell proliferation and survival. In this report, we demonstrate that treatment of the wild-type HCT116 (wt HCT116) human colon cancer cell line and the isogenic p53<sup>−/−</sup> HCT116 and p21<sup>−/−</sup> HCT116 cell lines with a high concentration (250 nM) of CPT resulted in apoptosis, indicating that apoptosis occurred by a p53- and p21-independent mechanism. In contrast, treatment with a low concentration (20 nM) of CPT induced cell cycle arrest and senescence of the wt HCT116 cells, but apoptosis of the p53<sup>−/−</sup> HCT116 and p21<sup>−/−</sup> HCT116 cells. Further investigations indicated that p53-dependent expression of p21 blocked apoptosis of wt HCT116 cells treated with 20 nM, but not 250 nM CPT. Interestingly, blocking of the apoptotic pathway by Z-VAD-FMK, in p21<sup>−/−</sup> HCT116 cells following treatment with 20 nM CPT did not permit the cells to develop properties of senescence. These observations demonstrated that p21 was required for senescence development of HCT116 cells following treatment with low concentrations of CPT.

The “p53 → p21 pathway” is activated in cells after DNA damage. Activation of this pathway temporarily arrests cells at the G<sub>1</sub> and G<sub>2</sub> checkpoints of the cell cycle, and terminates DNA replication and cell division (1–3). These events provide the cells with enough time to repair damaged DNA and prevent accumulation of deleterious mutations in the genome that would otherwise be subsequently transferred to daughter cells (4, 5). DNA damage is sensed by the ataxia-telangiectasia mutated protein, which is a member of the phosphoinositol-3 lipid kinase family (6, 7). p53 is one of the key targets that are subjected to activation by ataxia-telangiectasia mutated catalyzed phosphorylation (7). Activated p53, in turn, induces the expression of many proteins including p21, which is a universal inhibitor of the cyclin-dependent kinases (Cdks)<sup>1</sup> (8), and is required to arrest cells at the G<sub>1</sub> and G<sub>2</sub> checkpoints of the cell cycle after DNA damage (9–11).

DNA damaging agents including γ-irradiation and inhibitors of the nuclear topoisomerases I and II (Top1 and Top2) are widely included in therapies for cancer patients. The development of many types of human cancers (>50%) is associated with the loss of p53 or mutations in p53 (5). Therefore, the relationship between p53 status and the sensitivity of cancer cells to a variety of drugs, especially DNA damaging agents, has been extensively investigated (12–14). Initially, a relationship was established between p53 and drug sensitivity in mouse embryonic fibroblasts (MEFs) transformed by ras and E1A oncogenes, showing that p53<sup>−/−</sup> MEFs were more sensitive than p53<sup>+/−</sup> MEFs to the apoptotic effect of γ-irradiation or DNA damaging drugs (15). In contrast, p21 was not required for apoptosis of the MEFs after DNA damage (9). This led to the hypothesis that apoptosis of cancer cells after DNA damage was p53-dependent, but p21-independent, and that cancer cells containing a mutated p53 gene should be resistant to chemotherapies that utilize DNA damaging agents (12). However, this view was re-investigated when, by utilizing clonogenicity assays rather than short-term apoptosis assays, researchers were led to the conclusion that the p53 status was unrelated to the long-term survival of transformed MEFs after DNA damage (14). Also, studies of the RKO human colon cancer cell line and its derivative cell lines with altered p53 status led to the conclusion that the long-term survival of the cells after DNA damage was independent of p53 (16). In contrast, the apoptotic effect of DNA damaging agents on the HCT116 human colon cancer cells was shown to be blocked by p21, which was expressed by a p53-dependent mechanism (17, 18). Once again, subsequent results obtained from a clonogenicity assay led to the conclusion that the long-term survival of HCT116 cells after DNA damage was independent of either p53 or p21 (14). Therefore, these studies collectively indicate that the requirement for p53 in the process of apoptosis depends on whether the cells are of rodent or human origin. In addition, contradictory conclusions can be drawn about the relationship between p53 and drug sensitivity because of variability in the experimental conditions applied to investigate the effect of DNA damaging agents (14).

Nevertheless, it remains to be elucidated why short-term topoisomerase; MEF, mouse embryonic fibroblasts; BrdUrd, 5-bromodeoxyuridine; CPT, camptothecin; PBS, phosphate-buffered saline; X-gal, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside; β-gal, β-galactosidase; DDS, DNA damage signal.
survival, unlike long-term survival, of some cells after DNA damage is affected by p53 and/or p21. Furthermore, it should be noted that the loss of clonogenicity of cells does not necessarily result from loss of cell viability. In this context, it was demonstrated that normal human fibroblasts entered a state of senescence after DNA damage (19). Therefore, it is possible that loss of clonogenicity of cancer cells after DNA damage can result from an irreversible arrest of the cell cycle rather than loss of viability. Pertinent to this is the demonstration that p53 and p21 play important roles in senescence development of normal human cells (20–24). Accordingly, it is plausible that p53 and p21 are required for senescence development of human cancer cells after DNA damage. To address this issue, we investigated both long- and short-term effects of CPT-induced apoptosis and Top1-mediated DNA damage on wild-type HCT116 (wt HCT116) and isogenic p53−/− p21−/− (p53−/−) and p53+/+ p21−/− (p21−/−) HCT116 cells (17, 18). Our results demonstrated that under treatment with a concentration (250 nM) of CPT, wt HCT116, p53−/− HCT116, and p21−/− HCT116 cells underwent apoptosis indicating that apoptosis was independent of the cellular status of p53 and p21 status under this treatment. In contrast, treatment of p53−/− HCT116 and p21−/− HCT116 cells with 20 nM CPT resulted in them becoming apoptotic whereas wt HCT116 cells did not lose their clonogenicity. These results indicated that p53 and p21 were required to block apoptosis of HCT116 cells treated with 20 nM CPT. The loss of clonogenicity of wt HCT116 cells treated with 20 nM CPT was not due to the loss of long-term viability, but was rather the result of senescence development. Our results imply that the expression of p21 by a p53-dependent mechanism is required to fully develop senescence properties after DNA damage.

**EXPERIMENTAL PROCEDURES**

**Materials**—Fetal bovine serum, X-gal, antibodies to BrdUrd (clone BU-33), Cy3- or fluorescein isothiocyanate-conjugated antibodies, and camptothecin (CPT) were obtained from Sigma. The pan-caspase inhibitor, Z-VAD-FMK, was obtained from BIOMOL Inc. (Plymouth Meeting, PA). Antibodies to p53 (FL-393; sc-6243), p21 (C19, sc-397), cyclin A (C19, sc-596), cyclin B1 (GNSI, sc-245), and Cdk2 (M2, sc-163) were obtained from Santa Cruz Biotechnologies, Inc. (Santa Cruz, CA). Antibodies to Top1 (685168), Rh (G3, 245), Top1 (556597), and Top2 (T90920) were obtained from BD Pharmingen (San Diego, CA); and the antibody (9284) to phosphoserine 15 of p53 was obtained from New England Biolabs, Inc. (Beverly, MA). The human autoantibody antibody to Top1 (614–451-5810) used for immunocytochemistry studies was obtained from Topogen, Inc. (Columbus, OH).

**Cells and Culture Conditions**—The HCT116 human colon cancer cell lines (wt, p53−/−, and p21−/−) were generously provided by Dr. Bert Vogelstein (Johns Hopkins University). The cells were grown in McCoy's 5A medium supplemented with 10% fetal bovine serum and antibiotics. All cell cultures were incubated at 37 °C in a humidified incubator containing 5% CO2.

**Detection of Apoptosis**—Apoptotic fractions in cultures of control and drug-treated cells were identified by flow cytometry analysis of cells stained with propidium iodide (35).

**Western Blot Analysis**—Aliquots of whole cell extracts containing 50 μg of protein were used for Western blot analysis as described (26, 27).

**SA-β-Galactosidase Activity**—To detect senescence associated β-galactosidase (SA-β-galactosidase) activity, cells were washed in PBS, fixed in 0.5% glutaraldehyde in PBS at room temperature for 15 min, washed three times in PBS (pH 6.0), and then incubated for 2 h in X-gal/PBS solution (pH 6.0) as described (28).

**BrdUrd Incorporation and Immunofluorescence Assays**—DNA synthesis was determined by measuring BrdUrd incorporation into DNA as described (29). Briefly, cells were incubated with BrdUrd (24 h for normal cells, and 72 h for CPT-treated cells), fixed for 20 min in 2% glutaraldehyde in PBS at room temperature, and the cell membranes were permeabilized with acetone and methanol (1:1). The cells were then incubated for 30 min in 2 × HCl followed by a 30-min incubation in 0.1 M sodium borate (pH 8.5), washed three times in PBS, incubated for 60 min in 1% bovine serum albumin/PBS, and followed by a 45-min incubation in PBS containing 2 μg/ml mouse monoclonal antibody to BrdUrd. The cells were then washed three times in PBS (10 min per wash), incubated with a Cy3-conjugated secondary antibody (1:200) against mouse IgG for 30 min, and washed three times in PBS (20 min per wash). BrdUrd-labeled nuclei were observed and photographed under a fluorescent microscope. The protocols for immunofluorescent detection of various cellular proteins have been described (30).

**Clonogenicity Assays**—Approximately 300 cells were seeded into each well of a 6-well cell culture plate, and then incubated in 5 ml of medium for 2 weeks or longer. Subsequently, the medium was removed, and the cells were fixed for 5 min in 5 ml of methanol. The methanol was removed, the wells were rinsed with water, the cell colonies stained for 10 min in 2 ml of 4% (w/v) methylene blue solution in PBS, washed once again with water, and then counted.

**RESULTS**

**Effects of Camptothecin on HCT116 Cell Lines**—To induce Top1-mediated DNA damage, wt HCT116, p21−/− HCT116 and p53−/− HCT116 cell lines were treated with CPT (31). Cells were exposed either to low (i.e. 20 nM) or high (i.e. 250 nM) concentrations of CPT, and the effects on cell proliferation and survival were monitored. Treatment of all three HCT116 cell lines with either concentration of CPT for 24 h was sufficient to induce cell cycle arrest (data not shown). Treatment with 250 nM CPT resulted in apoptosis of all three cell lines (Fig. 1A), whereas treatment with 20 nM CPT resulted in significant apoptosis only of p53−/− HCT116 and p21−/− HCT116 cell lines (Fig. 1A). Thus, the response of HCT116 cells to the apoptotic effects of CPT on HCT116 cell lines. A, HCT116 cell lines (wt, p53−/−, and p21−/−) were treated with 20 or 250 nM CPT for the indicated time, and apoptosis was determined by flow cytometry. B, whole cell extracts were prepared from wt, p53−/−, and p21−/− HCT116 cells treated with 20 nM CPT for 0, 24, 48, 72, and 96 h, and then subjected to Western blot analysis for the presence of p53, phosphoserine 15 in p53, and p21.
The effect of low and high concentrations of CPT was differentially affected by the p53 and p21 status.

Western blot analysis of proteins from the wt HCT116 cells, treated with 20 nM CPT, demonstrated accumulation and phosphorylation of Ser15 in p53, i.e. activation of p53, and expression of p21 (Fig. 1B). In contrast, a very delayed expression of B of p21 was required to block the apoptotic effect of 20 nM CPT in subsequent experiments. Since the effect of long-term treatment of cancer cells with low doses of CPT appears to be beneficial to their growth, the apoptotic effect of 20 nM CPT in subsequent experiments was not observed (26). The results indicated that p21 is involved in the mechanism of CPT-induced inhibition of DNA synthesis, although the exact role of p21 in this process is not yet clear.

CPT-treated wt HCT116 Cells Are Senescent—Treatment of wt HCT116 cells with CPT for 24, 48, 72, and 96 h inhibited cell growth in a time-dependent fashion, expression of cyclin A, cyclin B1, Cdk1, E2F1, and activated, as shown by dephosphorylation, by the Rb protein (Fig. 2A). Of interest, an initial decrease in Top1 expression was observed at 24 h of CPT treatment, but no further decrease was detected thereafter (Fig. 2A). In contrast, Top2 expression was dramatically down-regulated after 48 h of CPT treatment, and virtually no expression was detected at 72 and 96 h of treatment (Fig. 2A). The CPT-induced suppression of the expression of cyclin A, cyclin B1, Cdk1, E2F1, and Top2 was irreversible, because it remained unaltered for a prolonged period of time after CPT was removed from the culture medium as assessed by immunofluorescence studies of the cells (Fig. 2B). Although the expression level of Cdk2 was not significantly altered during CPT treatment (Fig. 2A), it was reduced to an undetectable level in the senescent cells after the treatment was terminated (Fig. 2B). Thus, it appeared that down-regulation of Cdk2 was a very late event.

After a 96-h treatment of wt HCT116 cells with CPT, the size of the cells had dramatically increased, the nuclei were enlarged and prominent, and the nucleolar content was highly heterochromatic (Fig. 3A). Also, the CPT-treated cells stained positive, at pH 6.0 for SA-β-gal activity in the cytoplasm (Fig. 3A). SA-β-gal has been identified as a specific marker for senescence. Therefore, the results suggested that the CPT-treated wt HCT116 cells developed properties of senescence. After a 96-h treatment with 20 nM CPT, the wt HCT116 cells became senescent and concurrently lost their clonogenicity (Fig. 3C). Thus, it appeared that the CPT-treated wt HCT116 cells became senescent and concurrently lost their clonogenicity. Selective Defect in the Apoptotic Pathway Activated by DNA Damage in the CPT-treated wt HCT116 Cells—The results described above demonstrated that the presence of p21 in CPT-treated wt HCT116 cells enabled them to escape apoptosis and enter senescence, thus maintaining their viability. Therefore, the question raised by this observation was whether p21 selectively blocks the apoptotic pathway activated by DNA damage or whether it impaired the process of apoptosis in general. To investigate this, the CPT-treated wt HCT116 cells were also treated with 1 μM staurosporine to induce apoptosis by a pathway that is not associated with DNA damage (26, 42–44). The cells were sensitive to the apoptotic effect of staurosporine at
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Development of Senescence Requires p21—The findings described above raised the issue of how p21 affects senescence of CPT-treated wt HCT116 cells. Is p21 required to simply block apoptosis and thus provide the cells time to develop into senescence? Or, is p21 required for blocking both apoptosis and inducing senescence? To investigate whether the CPT-treated p21−/− HCT116 cells were able to enter senescence while the apoptotic pathway was blocked, the cells were treated for 48 h, and 96 h with CPT in presence of 100 μM Z-VAD-FMK, a pan-caspase inhibitor that blocks apoptosis of cells subjected to various apoptotic conditions. The presence of Z-VAD-FMK blocked apoptosis (Fig. 5A). At the end of the treatment, the cells stained positive for the presence of SA-β-gal activity (Fig. 5B), suggesting that they had become senescent. However, the expression and nuclear localization of cyclin A, cyclin B1, Cdk1, Cdk2, E2F1, and Top2 remained essentially unaltered (Figs. 5C and 6). Furthermore, these cells rapidly underwent apoptosis upon withdrawal of CPT and Z-VAD-FMK from the medium (Fig. 5D). Collectively, these results indicated that blocking apoptosis alone in the CPT-treated p21−/− HCT116 cells was insufficient to allow them to develop full senescent properties. Therefore, p21 appeared to be required not only to block apoptosis but also induce senescence of HCT116 cells after CPT treatment.

Apoptosis Is the Fate of wt HCT116 Cells Treated with 250 nm CPT—Treatment of wt HCT116 cells with 250 nm CPT induced the stable accumulation of p53, transient phosphorylation of Ser15 on p53, and the up-regulation of p21 expression (Fig. 7). This treatment did not significantly alter the expression level of Cdk1 and Cdk2, but induced down-regulation of the expression of cyclin A, cyclin B1, Rb, and Top2, and degradation of Top1 (Fig. 7). However, the E2F1 expression level was increased in the cells following drug treatment (Fig. 7). Nevertheless, the elevated presence of p21 suggested that it might be possible for wt HCT116 cells to develop along the senescent pathway following treatment with 250 nm CPT if the apoptotic pathway could be blocked. However, although the apoptotic effect of 250 nm CPT on the wt HCT116 cells was blocked by the presence of 100 μM Z-VAD-FMK (Fig. 8A), the cells did not develop any senescent properties, including the expression of SA-β-gal activity (Fig. 8A). Furthermore, the cells rapidly underwent apoptosis after withdrawal of both Z-VAD-FMK and CPT from the medium (Fig. 8B). Thus, even in the presence of elevated p21 levels, wt HCT116 cells were unable to escape apoptosis induced by a high concentration of CPT and enter senescence.

**DISCUSSION**

**The Relationship between p21 and Transduction of Apoptotic Signals Induced by DNA Damage**—In this study, we demonstrated that Top1-mediated DNA damage induced by 20 nm CPT resulted in the apoptotic death of p53−/− and the p21−/− HCT116 cells, both of which were deficient in p21 expression (Fig. 1B). In contrast, wt HCT116 cells that expressed robust levels of p21 became senescent, and remained viable for a prolonged period of time (Figs. 1, 3, and 4). Furthermore, we demonstrated that the CPT-treated wt HCT116 cells that remained viable were still sensitive to the apoptotic effect of staurosporine (Fig. 4). Staurosporine is an inhibitor of many
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protein kinases and triggers apoptotic signals in the absence of DNA damage (26, 42, 44). Therefore, our results indicated that the ability of p21 to block apoptosis was selectively associated with the apoptotic pathway activated by a nuclear DNA damage signal (DDS) in CPT-treated cells (Fig. 9).

Although p21 expression was also induced in wt HCT116 cells treated with 250 nM CPT (Fig. 7), it failed to prevent the cells from undergoing apoptosis (Figs. 1A, 3, and 8). Since it has been shown that the extent of CPT-induced DNA damage in cells is proportional to the concentration of CPT used (45), it is very likely that the amount of DNA damage in HCT116 cells caused by 250 nM CPT was more extensive than that caused by 20 nM CPT. Thus, when HCT116 cells (wt, p53<sup>−/−</sup>, and p21<sup>−/−</sup>) were exposed to only 20 nM CPT a limited amount of DNA damage should have been produced, resulting in the production of a low strength DDS which was nonetheless sufficient to induce apoptosis. In wt HCT116 cells, however, this low dose of CPT resulted in the p53-dependent induction of p21 (Fig. 1). As a consequence of p21 induction, the commitment to apoptosis was blocked and senescence was instead induced (Fig. 9). When wt HCT116 cells were treated with 250 nM CPT, extensive DNA damage presumably occurred in the cells, which, in turn, resulted in the production of a high strength DDS. Although the high strength DDS induced expression of p21 in wt HCT116 cells by a p53-dependent mechanism, the presence of elevated p21 failed to override the apoptotic effect of the signal (Fig. 9). This observation also suggests that the mechanism mediating the apoptotic effect of 250 nM CPT (or high strength DDS) on wt HCT116 cells was in this instance not regulated by p21 but by another, unidentified, factor(s) that was defective in the cells. The fact that blocking apoptosis with the use of Z-VAD-FMK of the wt HCT116 cells treated with 250 nM CPT failed to enable the cells to decrease the level of proteins, such as Cdk1 and E2F, and enter senescence (Fig. 8), suggests that the molecular mechanism for senescence was impaired in the wt HCT116 cells treated with 250 nM CPT. Moreover, these data imply that senescence and apoptosis are not competing pathways per se.

p21 Involvement in Development of Senescence after DNA Damage—p21 has been identified as a senescent cell-derived inhibitor of DNA synthesis (20), and other reports on the development of senescence in human fibroblasts also demonstrated an association with increased expression of p53 and p21 (46–48). Finally, the requirement of p21 for human cell senescence was established by the demonstration that normal human fibroblasts, with a targeted knock-out of the p21 gene, bypassed senescence (22), and that ectopic expression of p21 in human fibroblasts forced their entry into a state of premature senescence (49). In this context, our studies demonstrated that subsequent to p53-dependent expression of p21 in the wt HCT116 cell treated with 20 nM CPT, inhibition of expression of cyclin A, cyclin B1, and Cdk1 (Fig. 2), activation of Rb (Fig. 2), lack of BrdUrd incorporation into DNA, loss of clonogenicity, and expression of SA-β-gal activity occurred (Fig. 3). These events collectively characterize cell senescence (21, 38, 48, 50–53). Therefore, the loss of clonogenicity of the CPT-treated wt HCT116 cells was likely a consequence of the development of senescence.

We have demonstrated that blocking the apoptotic pathway by Z-VAD-FMK in the CPT-treated p21<sup>−/−</sup> HCT116 cell did not allow the cells to reduce the expression of cyclin A, cyclin B1, Cdk1, and Top2 (Fig. 5C) but did result in elevated SA-β-gal activity (Fig. 5B). These results indicated that the cells were unable to develop full senescent properties in the absence of p21. Given the fact that CPT-treated p21<sup>−/−</sup> HCT116 cells expressed active p53 (Fig. 1C), our results strongly suggest that p21 is the salient downstream target of p53, which is required not only for blocking apoptosis but also inducing senescence development of colon cancer cells after exposure to DNA damage.

Recently, it was demonstrated that SA-β-gal activity can be expressed by a mechanism independent of p53 and p21 in several cancer cell lines including the HCT116 cell line after DNA damage (54). Thus, it was suggested that senescence development of the cells was independent of p53 and p21 (54). It should be noted, however, that although SA-β-gal expression has been primarily associated with development of cell senescence under various conditions (23, 29, 34–36, 55, 56), SA-β-gal activity has also been detected in cultured aging human fibroblasts before they enter senescence (28) as well as in other non-senescent cells (57). We have demonstrated that CPT-treated p21<sup>−/−</sup> HCT116 cells, in which apoptosis was suppressed by Z-VAD-FMK, expressed SA-β-gal activity (Fig. 5B), indicating that expression of SA-β-gal is independent of p21. However, these cells were not senescent (Fig. 5). Therefore, the presence of SA-β-gal activity alone in cells lacking p53 or p21 is not necessarily indicative of senescence development.

How does p21 induce senescence? Recently, it was shown that ectopic overexpression of p21, in a p21-deficient human...
with 250 nM CPT (Fig. 7). Therefore, E2F1 regulation was upregulated in wt HCT116 cells following treatment with 20 nM (Fig. 2).

E2F1 expression occurred in wt HCT116 cells treated with 20 nM CPT in the absence or presence of 100 μM Z-VAD-FMK and then examined for induction of apoptosis and expression of SA-β-gal. Alternatively, the cells were incubated in Z-VAD-FMK/CPT-free medium and subsequently apoptosis was determined.

It is of particular interest to note that the down-regulation of E2F1 expression occurred in wt HCT116 cells treated with 20 nM CPT, A, wt HCT116 cells were left untreated or treated for 72 h with 250 nM CPT in the absence or presence of 100 μM Z-VAD-FMK and then examined for induction of apoptosis and expression of SA-β-gal. B, alternatively, the cells were incubated in Z-VAD-FMK/CPT-free medium and subsequently apoptosis was determined.

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Fig. 8. The apoptotic fate of wt HCT116 cells treated with 250 nM CPT. A, wt HCT116 cells were left untreated or treated for 72 h with 250 nM CPT in the absence or presence of 100 μM Z-VAD-FMK and then examined for induction of apoptosis and expression of SA-β-gal. B, alternatively, the cells were incubated in Z-VAD-FMK/CPT-free medium and subsequently apoptosis was determined.

Low dose CPT
Limited DNA damage
Low strength DDS
p53
Other genes
Senescence
Apoptosis

High dose CPT
Extensive DNA damage
High strength DDS
p21

DDS: DNA Damage Signal

Fig. 9. A hypothesis of how p21 regulates apoptosis and senescence after CPT-induced DNA damage. Treatment of cells with low and high concentrations of CPT induces limited and extensive DNA damage, respectively, and consequently p53 activation, which in turn induces expression of p21 and other genes. p21 is capable of blocking the apoptotic effect of low but not high strength DDS. Also, senescence development is induced by p21 in cells with limited DNA damage. In contrast, senescence development is impaired in cells with p21 and extensive DNA damage.

It is of particular interest to note that the down-regulation of E2F1 expression occurred in wt HCT116 cells treated with 20 nM CPT (Fig. 2A), but not 250 nM, CPT (Fig. 7). In fact, the E2F1 level was increased in wt HCT116 cells following treatment with 250 nM CPT (Fig. 7). Therefore, E2F1 regulation was associated with the fate of HCT116 cells treated with CPT. Down-regulation of E2F1 has been shown to be intimately associated with senescence development of human fibroblasts (39, 59), whereas ectopic expression of E2F1 in presence of MDM2 in senescent human fibroblasts stimulated DNA synthesis (60), suggesting that down-regulation of E2F1 is critical for the maintenance of senescence. Thus, the down-regulation of E2F1 expression in wt HCT116 cells treated with 20 nM CPT is probably an important event for the p21-dependent senescence program. However, it should also be noted that severe DNA damage in cells can result in stabilization and thus accumulation of E2F1 (61) (Fig. 7), and E2F1 is required for DNA damage-induced apoptosis (61–64). Therefore, as was observed, the elevated levels of E2F1 in wt HCT116 treated with 250 nM CPT and in p21−/− HCT116 cells treated with 20 nM CPT facilitated apoptosis, whereas the down-regulation of E2F1 in wt HCT116 cells treated with 20 nM CPT reduced the apoptotic potential of the cells.

The Apoptotic Activity and Anti-cancer Efficacy of CPT—In the past decade, studies on mechanisms mediating the anti-cancer effect of a broad spectrum of drugs have led to the suggestion that the ability of a drug to cause apoptosis of cancer cells is an important determinant for the drugs’ efficacy. In addition, cancer cells exhibiting no apoptotic response to a drug are assumed to have developed resistance to this drug (12, 65–69). Consequently, studies of cellular factors that regulate apoptosis have become very important for either the development of new anti-cancer drugs or the improvement of the efficacy of existing anti-cancer agents (66, 70–75). However, caution should be taken in the initial phase of drug discovery if the apoptotic action of a drug against cultured cancer cells is the sole criteria determining whether further studies of the drug should be conducted. We have demonstrated that a low concentration (20 nM) of CPT treatment induced apoptosis of the p53−/−/HCT116 and p21−/−/HCT116 cell lines, whereas the same drug concentration induced senescence of the wt HCT116 cell line. In contrast, a high concentration (250 nM) of CPT treatment induced apoptosis of all three cell lines. Thus, there is no simple answer to the question of how CPT induces apoptosis of colon cancer cells in the presence or absence of p53 and p21. Nevertheless, it is difficult to achieve high CPT concentrations in patient’s plasma without causing severe toxicity (32, 33). In addition, it has been suggested that administration of low doses of CPT or its derivatives for extensive periods of time would be more beneficial for the patient than administration of high doses for short periods of time (32, 33, 76–78). Therefore, the observed apoptotic effect of 250 nM CPT on both p53-containing and p53-deficient colon cancer cells is unlikely of great clinical importance, whereas the effect of 20 nM CPT may be more relevant to clinical applications. However, it still remains to be investigated how patients with p53+/−/p21+/+, p53−/−, and p21−/− colon cancers respond to treatment with CPT analogues.

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