# Synergistic Effects of Topoisomerase I Inhibitor, SN38, on Fas-mediated Apoptosis

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**Abstract.** *Inhibitors of topoisomerase I, such as camptothecin, have proven to be among the most promising new classes of anti-neoplastic agents introduced into the clinical setting in recent years. Irinotecan (CPT-11) is one of the most widely used camptothecin analogs and is converted to form the active metabolite SN-38. The present study was designed to explore apoptosis induced by SN38 and anti-Fas antibody (CH11) in WR/Fas-SMS1 cells and its possible mechanisms. The results demonstrate that combination of SN38 and CH11 synergistically enhanced cell apoptosis in WR/Fas-SMS1 cells. Western blotting analysis showed that combination of SN38 and CH11 activated the ATM-Chk1-p53 pathway, increased protein expression of phospho-p53 and cleavaged caspase-3, but down-regulated expression of phospho-p21. Our data suggest that combination of SN38 and CH11 enhanced apoptosis through down-regulation of p21 phosphorylation. In conclusion, inhibition of p21 could be a new adjuvant approach in cancer therapy.*

Irinotecan (CPT-11) is a semisynthetic analog of camptothecin, originally isolated from the Chinese/Tibetan ornamental tree *Camptotheca acuminata* (1, 2). It is a chemotherapy agent that causes S-phase-specific cell killing by poisoning topoisomerase I (Topo I) in the cell. Irinotecan has shown activity against colorectal, esophageal, gastric, non-small cell and small cell lung cancer, leukemia and lymphoma, as well as central nervous system malignant glioma (2). SN38 (10-hydroxy-7-ethyl-camptothecin), the active metabolite of CPT-11, exerts its cytotoxic activity through interaction with the nuclear enzyme Topo I and prevents it from resealing the DNA break, resulting in a double-strand DNA break and cell death (1, 3-5). SN38 is at least 1000-fold more cytotoxic than the parent compound and shows anticancer activity against a wide range of tumors (2, 6).

Apoptosis or programmed cell death is a process with typical morphological characteristics including plasma membrane blebbing, cell shrinkage, chromatin condensation and fragmentation. Fas (CD95/APO-1) belongs to the TNF receptor superfamily and mediates programmed cell death upon Fas ligand (FasL) or anti-Fas antibody (CH11) binding (7). Ligation of the death receptors leads to apoptosis through a common transcription/translation-independent pathway: ligand binding induces the formation of the death-inducing signaling complex (DISC), which in turn cleaves and activates the initiator caspases (caspase 8 or 10). The initiator caspases in turn activate a second group of caspases, known as effector caspases (caspase-3, -6, -7), leading to the dismantling of the cellular structure and to the cleavage of genomic DNA (8).

Several investigators have suggested that apoptosis is an important mechanism in SN38-induced tumor cell death. Usually, SN38 is used in combination with other cytotoxic agents. We have cloned the gene encoding sphingomyelin (SM) synthase, *SMS1*, and subsequently established SM synthase-restored cells (WR/Fas-SMS1) by transfection of *SMS1* gene into SM synthase-defective WR19L/Fas cells that were

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transfected with the human Fas gene (9). Using this cell line, we have reported the mechanisms of Fas-mediated apoptosis and cisplatin-mediated apoptosis (10, 11). The aim of the present study was to investigate whether SN38 enhances Fas-mediated apoptosis of WR/Fas-SMS1 cells and to elucidate mechanisms of such effect. We found that combination of SN38 and Fas stimulation functioned in a complementary manner, and synergistically induced apoptosis in WR/Fas-SMS1 cells.

## Materials and Methods

*Antibodies and reagents*. Anti-Fas antibody (CH11, mouse IgM) was purchased from Medical & Biological Laboratories Co., Ltd. (Nagoya, Japan). Antibodies against phospho-ATM (Ser1981), phospho-Chk1 (Ser345), phospho-p53 (Ser15), p53, α/β Tubulin and cleaved caspase-3 (Asp175) were purchased from Cell Signaling Technologies Japan (Tokyo, Japan). Mouse phospho-p21 antibody and ATM antibody were obtained from Santa Cruz Biotechnology, Inc (Santa Cruz, CA, USA). Antibody against β-actin was purchased from Rockland Immunochemicals, Inc (Gilbertsville, PA, USA). SN38 was kindly provided by Yakult pharmaceutical (Tokyo, Japan) and was reconstituted as 1M stock solution in DMSO. The ECL immunodetection system and horseradish peroxidase-conjugated goat anti-mouse or anti-rabbit IgG mAb were obtained from Amersham Bioscience Inc. (Piscateway, NJ, USA) and GE Healthcare Japan (Tokyo, Japan), respectively. The kit for extracting genomic DNA from cells was purchased from Sigma-Aldrich, Inc (St. Louis, MO, USA).

*Cell and culture*. WR19L cells, a mouse T cell lymphoma cell line, were transfected with the cDNA of the human *FAS* gene (WR19L/Fas cells) (12). SM-defective cells were obtained from the original WR19L/Fas cells by limiting dilution. *SMS1* gene was subcloned into the pLIB expression vector and transfected into the WR/Fas-SM(–) cells in VSV-G retroviral particles to establish SM synthase restored cells (WR/Fas-SMS1) (9). The WR/Fas-SMS1 cells were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin at 37°C with 5% $CO_2$ in a humidified incubator. For apoptosis assays, cells were seeded in 24-well plates ($5\times10^5$ cells/well). After a 2-hour rest, cells were treated with 25 ng/ml CH11 and 50 nM SN38 for the indicated times.

*Cell cycle analysis by flow cytometry*. Cells were seeded in 24-well plates ($5\times10^5$ cells/well). After a 2-hour rest, cells were treated with different concentrations of CH11 and SN38 overnight. After harvesting, cells were resuspended in permeabilization solution (0.5% paraformaldehyde and 0.5% saponin) and treated with 50 μg/ml RNase A for 30 min at room temperature, and propidium iodide (PI; Invitrogen Corporation, CA, USA) was added to a final concentration of 20 μg/ml. After 20 min, the fluorescence of the PI-stained DNA was quantitated on a per cell basis using FACSCalibur (BD Biosciences, NJ, USA), and cells with subdiploid content were considered to be apoptotic cells.

*Cell lysis and Western blotting*. Cells were solubilized with lysis buffer containing 50 mM Tris-HCl, pH 7.6, 1% Brij 97, 300 mM NaCl, 5 mM EDTA, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 1 mM PMSF, and 1 mM sodium orthovanadate with gentle rocking for 30 min at 4˚C. Cell lysates were eluted by boiling in SDS-containing sample buffer and fractionated by SDS-PAGE (6-12% polyacrylamide gels) (13). Proteins were electrophoretically transferred to polyvinylidene difluoride (Immobilon-P) membranes (Sigma-Aldrich). Membranes were blocked in a buffer solution containing 5% nonfat milk for 2 hours at room temperature. The membranes were incubated with primary antibodies overnight at 4°C and then washed three times in TBS containing 0.5% Tween 20. Peroxidase-conjugated secondary antibodies (GE Healthcare) were used at a 1:1,000 dilution, and immunoreactive bands were visualized using ECL (Amersham).

*Statistical analysis*. All data were expressed as mean±standard deviation (SD). Comparisons between two values were performed by unpaired *t*-tests. A *p*-value of less than 0.05 was considered to represent a statistically significant difference.

## Results

*Synergistic effects of SN38 and CH11 on apoptosis in WR/Fas-SMS1 cells*. We first examined whether SN38 enhanced Fas-mediated apoptosis in WR/Fas-SMS1 cells. Apoptosis in SN38- and CH11-treated cells was analyzed using PI staining. In our previous study using WR/Fas-SMS1 cells, apoptosis was only 13.2% after incubation with 25 ng/ml CH11 antibody (10). In this study, apoptotic cells comprised 24.86±3.08% and 12.29±5.37% after being incubated for 12 hours by SN38 (50 nM) or CH11 (25 ng/ml), respectively. But the cells underwent stronger apoptosis with the stimulation of SN38 and CH11 at the same time, exhibiting 47.73±10.41% apoptosis (Figure 1A). We also examined apoptosis using the TUNEL assay. Although the cells incubated with a combination of SN38 and CH11 showed more significant DNA fragmentation than those with CH11 alone, a DNA fragment was barely recognized in the lane of SN38 only (Figure 1B). Furthermore, we examined the morphological change of WR/Fas-SMS1 cells under culture with SN38 and/or CH11. Although neither SN38 nor CH11 treatment caused morphological changes in WR/Fas-SMS1 cells, the combination of these two reagents showed the strongest cytotoxicity in WR/Fas-SMS1 cells (Figure 1C).

*Effects of SN38 and CH11 on ATM activation in WR/Fas-SMS1 cells*. According to current understanding, DNA double-strand breaks and other changes in chromatin structure lead to autophosphorylation of the ataxia telangiectasia mutated (ATM) homodimer (14). ATM is one of the major molecular sensors of DNA damage (15, 16). Therefore, we next examined the expression of phospho-ATM and ATM in WR/Fas-SMS1 cells stimulated by SN38 and CH11 for the indicated time. Western blot analysis demonstrated a higher level of phospho-ATM expression at 0.5 and 1 hours (Figure 2). But the expression of total ATM did not show a significant time-dependent increase.

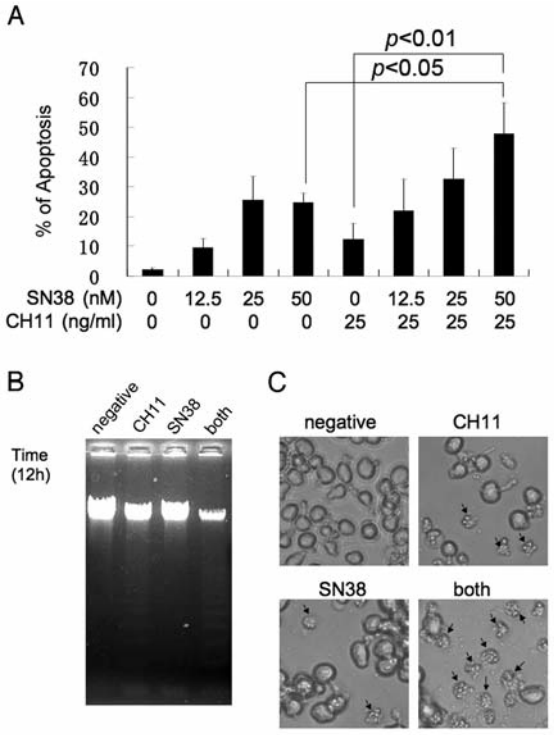


Figure 1. *Effects of SN38 and CH11 on apoptosis in WR/Fas-SMS1 cells. A: Cells (5×10$^5$ cells) were cultured for 12 h in the presence of SN38 and CH11 at the indicated concentrations. PI-positive cells, considered to be apoptotic cells, were counted and are shown as the percentage of apoptotic cells of total cells. Bars indicate mean±SD. Data shown are from three independent experiments and statistical analysis was performed with the t-test. B: Degradation of chromosomal DNA in WR/Fas-SMS1 cells after SN38 and/or CH11 stimulation. The cells (5×10$^5$ cells ) were cultured for 12 h in the presence of SN38 (50 nM) and/or CH11 (25 ng/ml). Chromosomal DNA was extracted from 2×10$^6$ cells each group and analyzed by 1.5% agarose gel and visualized under UV light after ethidium bromide staining. C: Morphological changes of WR/Fas-SMS1 cells after SN38 and/or CH11 stimulation. Cells (5×10$^5$ cells ) were cultured for 12 h in the presence of SN38 (50 nM) and/or CH11 (25 ng/ml). Cells were examined using an Olympus CKX41 inverted microscope and images were taken with an Olympus C7070 digital camera system. Magnifications are at ×400. Data are representative of more than three independent experiments. Arrows indicate apoptotic cells.*

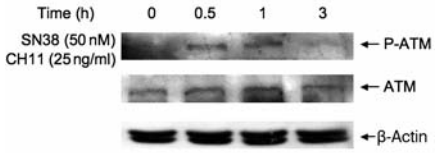


Figure 2. *ATM activation during SN38 and CH11 treatment of WR/Fas-SMS1 cells. WR/Fas-SMS1 cells (5×10$^5$ cells) were cultured for the indicated time in the presence of SN38 (50 nM) and CH11 (25 ng/ml). The ATM protein expression and phosphorylation were determined using Western blotting. β-Actin was used as the loading control. Data are representative of three independent experiments.*

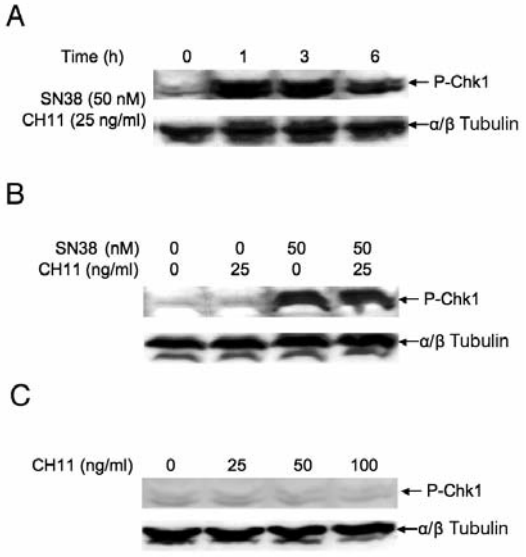


Figure 3. *Chk1 activation during SN38 and CH11 treatment of WR/Fas-SMS1 cells. A: WR/Fas-SMS1 cells (5×10$^5$ cells) were treated with SN38 (50 nM) and CH11 (25 ng/ml) for the indicated time. Whole cell lysate was collected for Western blotting analysis of phosphorylated Chk1 (serine 345) and α/β tubulin. B: Cells (5×10$^5$ cells) were treated with SN38 and/or CH11 for 1 h. Cells were harvested and analyzed by Western blotting for expression of phosphorylated Chk1 (serine 345) and α/β tubulin. C: The cells (5×10$^5$ cells) were treated with CH11 alone for 1 h to harvest and analyze the expression of phosphorylated Chk1 (serine 345) and α/β tubulin using Western blotting. Data are representative of more than three independent experiments.*

*Effects of SN38 and CH11 on Chk1 activation in WR/Fas-SMS1 cells*. Previous studies have demonstrated that Chk1 was activated after treatment with the topoisomerase I inhibitor, camptothecin (17). Aime *et al*. reported that S-phase arrest induced by the topoisomerase I inhibitor resulted from adtivation of Chk1 (18). Chk1 was one of the downstream mediators of ATM. Therefore, we examined Chk1 phosphorylation after the cells were stimulated with SN38 and/or CH11 for the indicated time and at the indicated concentration. First, the expression of phospho-Chk1 was examined after cells were stimulated for 0, 1, 3 and 6 hours by SN38 and CH11. The results of the kinetic studies demonstrated that phospho-Chk1 protein expression increased significantly at 1, 3 and 6 hours compared with that of no stimulation. The maximum level of phospho-Chk1 protein was observed at 1 hour and then decreased with time (Figure 3A). To elucidate whether the activation of Chk1 is induced by SN38 or by CH11, we cultured the WR/Fas-SMS1 cells with a combination of SN38 and CH11 or each one alone at the indicated concentration for 1 hour. The

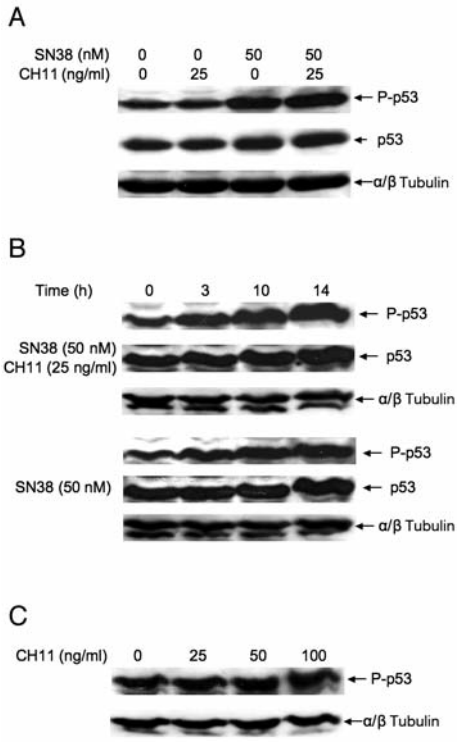


Figure 4. *Analysis of the p53 response following incubation of WR/Fas-SMS1 cells with SN38 and CH11. A: WR/Fas-SMS1 cells ($5\times10^5$ cells) were cultured with SN38 and/or CH11 at the indicated concentrations for 12 h. p53 protein expression and Ser15 phosphorylation in the cells was determined using Western blotting. α/β-Tubulin was used as the loading control. Data are representative of three independent experiments. B: Cells ($5\times10^5$ cells) were cultured with SN38 (50 nM) and CH11 (25 ng/ml) or SN38 (50 nM) only for the indicated time. Cells were harvested and analyzed by Western blotting using phospho-p53, p53 and α/β tubulin antibody. C: The cells ($5\times10^5$ cells) were cultured with CH11 at different concentrations for 1 h. Total cellular proteins were subjected to Western blotting analysis with anti-phospho-p53 and α/β tubulin antibodies. Data are representative of more than three independent experiments.*

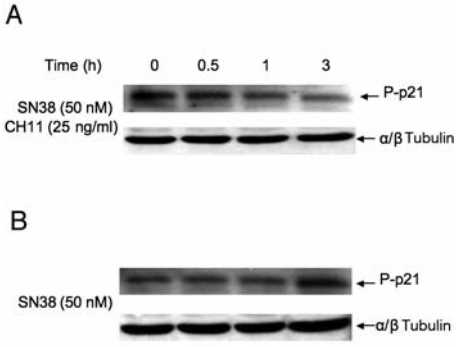


Figure 5. *Effect of SN38 and CH11 on P-p21 protein expression level in WR/Fas-SMS1 cells. The WR/Fas-SMS1 cells ($5\times10^5$ cells) were treated with SN38 (50 nM) and CH11 (25 ng/ml), or SN38 (50 nM) only for different time intervals. Total cellular proteins were extracted and applied to Western blot of phospho-p21 and α/β tubulin. The data are representative of three independent experiments.*

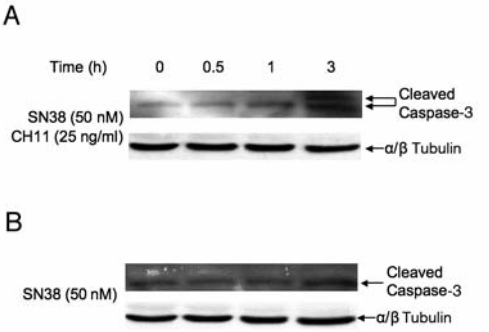


Figure 6. *SN38 and CH11 mediated caspase-3 activation in WR/Fas-SMS1 cells. WR/Fas-SMS1 cells ($5\times10^5$ cells) were treated with (A) a combination of SN38 (50 nM) and CH11 (25 ng/ml) or (B) SN38 (50 nM) only for the indicated time. The expression of cleaved caspase-3 was determined using Western blotting. α/β Tubulin was used as the loading control. The data are representative of three independent experiments.*

expression of phospho-Chk1 was found to be increased only with the stimulation of SN38 (either alone or with CH11), but not CH11 (Figure 3B). To analyze whether phospho-Chk1 protein expression is induced by high concentrations of CH11, cells were stimulated with CH11 at the indicated concentrations. There was no phosphorylation of Chk1 expression after the cells were cultured with CH11 alone up to 100 ng/ml (Figure 3C).

*Effects of SN38 and CH11 on p53 activation in WR/Fas-SMS1 cells.* Wang and El-Deiry reported that irinotecan increases p53 transcriptional activity *in vivo* (19). Takeba *et al.* reported that p53 expression in SN38 treated Huh7 cells was significantly greater than that in controls at 24 h (20). As we observed that ATM and Chk1 in WR/Fas-SMS1 cells were activated during SN38 treatment, we hypothesized that DNA damage was induced by p53 activation during culture of WR/Fas-SMS1 cells with SN38. Therefore, we examined phospho-p53 expression by western blotting after SN38 and/or CH11 treatment. The expression of phospho-p53 increased only with the stimulation of SN38, either alone or in combination with CH11 (Figure 4A). To further assess whether the activation of p53 was induced by SN38, we performed the following experiments. Cells were cultured with both CH11 and SN38 or SN38 alone for 0, 3, 10, 14 h. The results showed that p53 was activated in a time-dependent manner starting from 3 h following stimulation with both CH11 and SN38. Similar results were also

observed after the cells were treated with SN38 alone (Figure 4B). These results indicated that SN38 alone or with CH11 was able to activate p53 expression in WR/Fas-SMS1 cells. To analyze whether the activation of p53 is induced by high concentrations of CH11 antibody, cells were stimulated with CH11 at the indicated concentrations. As shown in Figure 4C, the expression of phospho-p53 was not dose-dependent on CH11 up to 100 ng/ml after 1 h of treatment. These results suggest that the activation of p53 is mainly caused by the stimulation of SN38.

*Effects of SN38 and CH11 on p21 activation in WR/Fas-SMS1 cells*. The cyclin-dependent kinase (cdk) inhibitor, p21 is a key downstream targent of p53 (21). Therefore, we wondered if SN38 and CH11 could synergistically induce p21 activation. To address this issue, WR/Fas-SMS1 cells were treated with both CH11 and SN38 or SN38 alone at the indicated times. The results demonstrated that the expression of phospho-p21 decreased with time following CH11 and SN38 treatment. On the contrary, there were no significant changes of phospho-p21 expression by the treatment of SN38 alone (Figure 5).

*Effects of SN38 and CH11 on caspase-3 activation in WR/Fas-SMS1 cells*. Two major apoptotic pathways, namely the extrinsic (through membrane death receptor) and intrinsic (through mitochondria), are governed by various caspases which have been divided into initiators or executioners of apoptosis. Caspase-3 is generally considered to be the primary executioner of apoptosis (22). Therefore, we next examined the expression of active caspase-3 in WR/Fas-SMS1 cells with the treatment of CH11 and SN38 for the indicated time. Western blot analysis using a cleaved caspase-3-specific antibody revealed that the active fragments (p17 and p19) of caspase-3 could be seen at 3 h after the stimulation by CH11 and SN38, whereas SN38 alone was ineffective at inducing these responses at any time point (Figure 6).

## Discussion

The cells studied here undergo stronger apoptosis after being stimulated by both SN38 and CH11 than by SN38 or CH11 alone, respectively. Importantly, DNA damage induced by SN38 and CH11 leads to activation of ATM-Chk1 and p53, and inactivation of p21. The subsequent apoptosis is induced by caspase-3 activation. Our results have shown the first evidence for the synergistic effects of SN38 and CH11 on apoptosis in WR/Fas-SMS1 cells.

SN38 is a topoisomerase I inhibitor that induces DNA damage and transient S-phase arrest. Topoisomerase I enzyme is an enzyme involved in regulating critical cellular functions including DNA replication, transcription and recombination. SN38 has the ability to stabilize the covalent binding of topoisomerase I to DNA forming a ternary complex (5, 23). The single-strand DNA breaks of the cleavable complex are reversible, but these lesions may be converted into irreversible double-strand breaks during DNA synthesis following collision with the replication complexes. Double-strand breaks, which are recognized as lethal lesions, activate DNA damage-response signaling pathways. Thus, the DNA damage response is a highly orchestrated and complex signaling event (24). One of the master sensor proteins that recognize damaged DNA is ATM (25, 26). In response to DNA damage or genotoxic stress, ATM is recruited to the site of DNA damage, forming nuclear 'foci' (16, 24, 25). This is followed by recruitment and activation of other signaling molecules, including Chk1 and Chk2, which arrests cell cycle progression so as to allow for repair and prevention of the transmission of damaged or incompletely replicated chromosomes (24, 27).

In the current study, ATM was phosphorylated in a time-dependent manner during SN38 and CH11 treatment (Figure 2). These results are consistent with the previous theory that SN38 induces ATM phosphorylation at Ser-1981 in proliferating cells (28). These results may indicate that lesions of DNA damage resulting from SN38 and CH11 are sensed by ATM and cause ATM phosphorylation. We also demonstrated that the phosphorylation of Chk1 on Ser-345 during SN38 and CH11 treatment of WR/Fas-SMS1 cells (Figure 3). These results are also consistent with the previous reports (28, 29). Chk1 is a serine/threonine kinase associated with DNA damage-linked S and $G_2$-M checkpoint control, which is activated in mammalian cells in response to DNA damage. It monitors the progression of cells through the S and $G_2$-M phases and may slow the rate of ongoing DNA synthesis in a transient manner (24). Cell cycle arrest allows time for the repair of DNA lesions. Cells re-enter the cell cycle if the damage can be adequately repaired, or they die by apoptosis if the damage is too severe (30). Activation of the Chk1 pathway might not only delay mitotic entry, but also increase the capacity of these cells to survive. In our study, Chk1 was phosphorylated on Ser-345 as early as 1 h following the combination treatment. These changes were then followed by decreased Chk1 phosphorylation at 3 and 6 h (Figure 3). Furthermore, the phosphorylation of Chk1 is induced only by SN38, not by CH11. These results show that treatment of cells with combination of SN38 and CH11 or SN38 only results in rapid cell cycle arrest, giving the cells enough time to repair.

In addition to the Chk1-dependent manner, $G_2$/M transition is also regulated by other checkpoint-signaling pathways. The tumor suppressor p53 (and its downstream effectors p21) has been shown to play a key role in the maintenance of $G_2$/M checkpoint (21). We found that combination of SN38 and CH11 or SN38 alone increased p53 phosphorylation at Ser-15 (Figure 4A). Consequently, the activation of p53 is in a time-dependent manner with the treatment of combination or SN38 alone (Figure 4B). These results are also consistent

with the previous reports (31). The p53 pathway plays a significant role in the determination of cell death and survival by up-regulating the expression of genes which facilitate cell cycle arrest, apoptosis, DNA repair, or genomic stability at a transcriptional level. As shown in Figure 4, we clearly detected the p53 activation by SN38, but not by CH11. The opposite functions of p53 are well known in that p53 can protect cells from death and also induce cells to undergo cell death. It is not clear how p53 "decides" which of these two activities to implement. It is likely that p53 favors arrest and repair at low levels of DNA damage, and hence can be protective to cells. On the contrary, p53 stimulates apoptosis at high levels of DNA damage. In this study, the combination of SN38 and CH11 activated p53 and induced the cells to undergo stronger apoptosis.

The cdk inhibitor p21, a key downstream targent of p53, mediates both $G_1$ arrest and $G_2$/M phase arrest. The p53-p21 pathway is activated in cells after DNA damage. Activation of this pathway temporarily arrests cells at the $G_1$ and $G_2$ checkpoints of the cell cycle, and terminates DNA replication and cell division (32-35). P21 is transcriptionally activated by p53 and is responsible for the p53-dependent senescence pathway (30, 36). Since we observed that the combination of SN38 and CH11 affected p53 expression (Figure 4), we examined whether they could subsequently induce p21 expression. Our data shows that the activation of p21 decreased in a time-dependent manner with the combination of SN38 and CH11, whereas SN38 alone did not (Figure 5). It is likely that the stronger apoptosis induced by the combination of SN38 and CH11 may be caused by a decrease in p21 phosphorylation. These results have been explained by previous studies. Recently it has been reported that activation of p21 keeps tumor cells alive after DNA damage, favoring a survival advantage (32, 37). Waldman *et al*. has reported that cancer cells with a defective p21 response undergo cell death that occurs during or after mitosis (38) and Lazzarini *et al*. has reported that inhibition of drug-induced p21 expression strongly enhanced the apoptotic potential of these stimuli in malignant mesothelioma (39). Our results indicate that p21 might play an important role in chemosensitivity to anticancer agents, and that the suppression of its expression might be a potential therapeutic target for tumors.

Fas-mediated apoptosis can be further separated in several steps as follows (a) ligand-induced formation of CD95 microaggregates at the cell surface; (b) recruitment of FADD to form a DISC; (c) formation of large CD95 surface clusters, which is positively regulated by DISC-generated caspase-8; and (d) internalization of activated CD95 through an endosomal pathway, including the activation of caspase-8 and downstream cascade of caspase-3 activation (40). Caspase-3 is a cysteine protease with aspartic specificity and a well-characterized effector of apoptosis or programmed cell death signaling. Caspase-3 is synthesized in normal cells as inactive proenzymes; it can be rapidly activated by autoproteolytic cleavage or cleavage by other caspases at specific aspartic acid residues (22). Cleavage caspase-3 allows CAD, also known as DNA fragmentation factor, to translocate to the nucleus where it is responsible for internucleosomal DNA cleavage, generating oligonucleosomal DNA fragments. Therefore, cleavage caspase-3 is the executor in apoptosis signaling. In the present study, cleaved caspase-3 was present after the cells were treated with SN38 and CH11, whereas SN38 alone was ineffective at inducing these responses at any time point (Figure 6). This finding indicates that the combination of SN38 and CH11 makes the cells undergo greater apoptosis by finally activating caspase-3, whereas SN38 alone was ineffective at inducing these responses.

In conclusion, we show that the combination of SN38 and CH11 makes the WR/Fas-SMS1 cells undergo greater apoptosis, and that this action may be mediated in part by activation of ATM-Chk1-p53 pathway, by inhibition of p21 and activation of caspase-3. More experiments are necessary to better understand the mechanisms underlying this involvement. However, disruption of p21 function might provide a means of enhancing chemotherapeutic effect.

## Aknowledgements

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