Role of Antiapoptotic Proteins in Tumor Necrosis Factor-related Apoptosis-inducing Ligand and Cisplatin-augmented Apoptosis

Jin H. Kim, Mubasshir Ajaz, Anna Lokshin, and Yong J. Lee

Departments of Surgery [J. H. K., M. A., Y. J. L.] and Pathology [A. L.], University of Pittsburgh, Pittsburgh, Pennsylvania, 15213

ABSTRACT

Purpose and Experimental Design: The purpose of this study was to examine the effect of combined treatment with tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and cisplatin in human head and neck squamous cell carcinoma. HNSCC-6 cells were treated with 0.1–1 μg/ml TRAIL and/or 1–10 μg/ml cisplatin for 24 h.

Results: TRAIL alone or cisplatin alone caused minimal cytotoxicity. The combination of TRAIL and cisplatin synergistically enhanced apoptotic death, caspase-8 and caspase-3 activation, as well as poly(ADP-ribose) polymerase cleavage. However, the total cellular levels and the surface expression of TRAIL receptor proteins, such as death receptors 4 and 5 and decoy receptors 2 and 1, were not significantly changed by treatment with TRAIL and cisplatin. Interestingly, the level of the short form of Fas-associated death domain-like interleukin-1β-converting enzyme-inhibitory protein (FLIPS) but not the long form of Fas-associated death domain-like interleukin-1β-converting enzyme-inhibitory protein was reduced through cleavage. Benzylxoyacarbonyl-Asp-Glu-Val-Asp-fluoromethylketone a caspase-3 inhibitor, blocked the cleavage of FLIPS and caspase-3 activation. Overexpression of FLIPS protected cells from apoptotic death and FLIPS cleavage during treatment with TRAIL in combination with cisplatin.

Conclusions: These results suggest that caspase-3 is responsible for FLIPS cleavage, and the cleavage of FLIPS is one of facilitating factors for TRAIL-induced apoptotic death.

INTRODUCTION

HNSCC is the sixth most common solid tumor worldwide, accounting for about 5% of all new cancers diagnosed annually in the United States (1). Although recent advances in management with a multidisciplinary approach including chemotherapy in combination with radiotherapy or surgery result in improved local and regional disease control, the overall survival rate has not improved much during the last decade. Obviously, greater intervention will be required to significantly enhance HNSCC cancer therapy.

Cisplatin is indicated in various combinations of chemotherapeutic regimens for ovarian, head, neck, bladder, cervical, and other neoplasms. In addition to its toxic side effects, a major limitation of cisplatin chemotherapy is drug resistance. The dose scale necessary to overcome even a small increase in cellular resistance can cause severe cytotoxicity. Understanding the molecular basis of cisplatin-mediated apoptosis could significantly improve clinical protocols.

TRAIL/APO-2 ligand is a type II integral membrane protein belonging to the TNF family. TRAIL is a 281-amino acid protein, related most closely to Fas/APO-1 ligand. Like Fas ligand and TNF, the COOH-terminal extracellular region of TRAIL (amino acids 114–281) exhibits a homotrimeric subunit structure (2). It induces apoptosis in a variety of tumor cell lines more efficiently than normal cells (3). The apoptotic signal induced by TRAIL is transduced by its binding to the DRs, TRAIL-R1 (DR4) and TRAIL-R2 (DR5), which are members of the TNF receptor superfamily. Both DR4 and DR5 contain a cytoplasmic death domain that is required for TRAIL receptor-induced apoptosis. TRAIL also binds to TRAIL-R3 (DcR1) and TRAIL-R4 (DcR2), which act as DcRs by inhibiting TRAIL signaling (4–10). Unlike DR4 and DR5, DcR1 does not have a cytoplasmic domain, and DcR2 retains a cytoplasmic fragment containing a truncated form of the consensus death domain motif (4). The relative resistance of normal cells to TRAIL has been explained by the presence of large numbers of the DcRs on normal cells (11, 12). Recently, this hypothesis has been challenged, based on results showing poor correlations between DR4, DR5, and DcR1 expression and sensitivity to TRAIL-induced apoptosis in normal and cancerous breast cell lines (13). This discrepancy indicates that other factors such as death

3 The abbreviations used are: HNSCC, head and neck squamous cell carcinoma; DcR, decoy receptor; DED, death effector domain; DR, death receptor; EGF, enhanced green fluorescent protein; FLIP, Fas-associated death domain-like interleukin-1β-converting enzyme-inhibitory protein; PARP, poly(ADP-ribose) polymerase; TNF, tumor necrosis factor; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; FLIPs, short form of FLIP; DcR2, long form of FLIP; DISC, death-inducing signaling complex; Z-DEVD-FMK, benzylxoyacarbonyl-Asp-Glu-Val-Asp-fluoromethylketone.
inhibitors (FLIP, Fas-associated protein-1, or inhibitor of apoptosis) are also involved in the differential sensitivity to TRAIL.

Several researchers have also shown that genotoxic agents such as chemotherapeutic agents (13–15) and ionizing radiation (16, 17) can sensitize cells to killing by TRAIL. The synergistic effect is probably due to up-regulation of DR5 expression (15, 16) or down-regulation of FLIP expression (14). In this study, we examined whether the synergistic induction of apoptosis by treatment with TRAIL and cisplatin in HNSCC is associated with modulation of TRAIL receptors or FLIP. Our results show that TRAIL in combination with cisplatin did not alter the level of DR4, DR5, DcR2, and a FLIPL. However, it resulted in cleavage of FLIPs. Our results also suggest that the cleavage of FLIPs is mediated through activation of caspase-3.

MATERIALS AND METHODS

Cell Culture and Survival Determination. Human HNSCC cells (HNSC-6 and HNSC-30) were cultured in DMEM/Ham’s F-12 supplemented with 10% fetal bovine serum (HyClone, Logan, UT), 0.1 mg/ml human epidermal growth factor, 5 μg/ml insulin, 0.5 μg/ml hydrocortisone, 2 mM l-glutamine, and 26 mM sodium bicarbonate for monolayer cell culture. The dishes containing cells were kept in a 37°C humidified incubator with a mixture of 95% air and 5% CO2. Two humid culture. The dishes containing cells were kept in a 37°C humidified incubator with a mixture of 95% air and 5% CO2. Two days before the experiment, cells were plated into 60-mm dishes. The trypan blue exclusion assay was used for survival determination. Cells were trypsinized, pelleted, and resuspended in 0.2 ml of medium, 0.5 ml of 0.4% trypan blue solution, and 0.3 ml of PBS solution. The samples were mixed thoroughly, incubated at room temperature for 15 min and examined.

Production of Recombinant TRAIL. A human TRAIL cDNA fragment (amino acids 114–281) obtained by reverse transcription-PCR was cloned into a pET-23d (Novagen, Madison, WI) plasmid by Dr. D. W. Seol (18), and expressed protein was purified using Ni-NTA His-Bind Resin Superflow according to the manufacturer’s instructions (Qiagen, Santa Clarita, CA).

Treatment with TRAIL and/or Cisplatin. Cells were replaced with fresh medium containing TRAIL and/or cisplatin (Bristol-Myers, Evansville, IN).

Morphological Evaluation. Approximately 5 × 10^5 cells were plated into 60-mm dishes overnight. Cells were treated with TRAIL and/or cisplatin and then analyzed by phase-contrast microscopy for signs of apoptosis (19).

Shuttle Vector Construction. A FLAG-tagged FLIPs gene was isolated from pcDNA3-FLAG-FLIPs (kindly provided by Dr. P. M. Kramer; German Cancer Research Center, Heidelberg, Germany) by digestion with HindIII and SpHl and cloned into the HindIII and SpHl site of pAdlox shuttle vector (20). The complete shuttle vector was cotransfected into CRE8 cells with ψ5 viral genomic DNA for homologous recombination as described below.

Adenoviral Vector Construction. The adenovirus containing FLAG-tagged FLIPs was constructed by using the Crelox recombination system (20). The selective cell line CRE8 has a β-actin-based expression cassette driving a Cre recombinase gene with a NH2-terminal nuclear localization signal stably integrated into 293 cells. Transfections were done by using Lipofectin Reagent (Invitrogen, Carlsbad, CA). Cells (5 × 10^5) were split into a 6-well plate 1 day before transfection. For the production of recombinant adenovirus, 2 μg of SfiI/ScaI-digested Adlox/FLAG-FLIPs and 2 μg of ψ5 viral genomic DNA were cotransfected into CRE8 cells. The recombinant virus was generated by intermolecular homologous recombination between the shuttle vector and ψ5 viral DNA. A new virus has an intact packaging site and carries a recombinant gene. Plaques were harvested, analyzed, and purified. The insertion of FLAG-FLIPs to adenovirus was confirmed by Western blotting after infection of corresponding recombinant adenovirus into HNSC-6 cells.

Protein Extracts and PAGE. Cells were lysed with 1× Laemmli lysis buffer [2.4 M glycerol, 0.14 M Tris (pH 6.8), 0.21 M SDS, and 0.3 mM bромphenol blue] and boiled for 10 min. Protein content was measured with BCA Protein Assay Reagent (Pierce, Rockford, IL). The samples were diluted with 1× lysis buffer containing 1.28 M β-mercaptoethanol, and an equal amount of protein was loaded on 8–12% SDS-polyacrylamide gels. SDS-PAGE analysis was performed according to Laemmli (21) using a Hoefer gel apparatus.

Immunoblot Analysis. Proteins were separated by SDS-PAGE and electrophoretically transferred to nitrocellulose membrane. The nitrocellulose membrane was blocked with 7.5% nonfat dry milk in PBS-Tween 20 (0.1%, v/v) at 4°C overnight. The membrane was incubated with anti-PARP (Biomol Research Laboratory, Plymouth Meeting, PA), anti-caspase-8 (Upstate Biotechnology, Lake Placid, NY), anti-caspase-3 (Santa Cruz Biotechnology, Santa Cruz, CA), anti-DR5 (Stressgen, Victoria, British Columbia, Canada), anti-DR4 (Upstate Biotechnology), anti-DcR2 (Stressgen), or anti-FLIP antibody (Calbiochem, Darmstadt, Germany) for 1 h. Horseradish peroxidase-conjugated antirabbit or antimouse IgG was used as the secondary antibody. Immuno-reactive protein was visualized by the chemiluminescence protocol (ECL; Amersham, Arlington Heights, IL).

Flow Cytometry. Cells were pelleted and washed with fluorescein-activated cell-sorting buffer (PBS, 1% BSA, and 0.1% sodium azide). Cells were incubated with optimal concentrations of four recently developed phycoerythrin-conjugated monoclonal antibodies against extracellular domains of functional (DR4 and DR5) and decoy (DcR1 and DcR2) Apo2 ligand/TRAIL receptors (all from eBioscience, San Diego, CA) for 30 min at 4°C. Matched isotype control IgG antibodies were included. Analysis was performed using the FACSscan flow cytometer, and results were analyzed with CellQuest software (both from Becton Dickinson Immunocytometry Systems, San Jose, CA).

RESULTS

Synergistic Induction of Cytotoxicity by the Combination of TRAIL and Cisplatin. The effect of TRAIL on cell death in conjunction with cisplatin was assessed in human HNSCC-6 cell line. Fig. 1A shows that treatment with 0.1–1.0 μg/ml TRAIL alone or 1–10 μg/ml cisplatin alone for 24 h caused minimal cytotoxicity: approximately 10–30% of cells were killed. However, the cytotoxicity was significantly enhanced by the combination treatment (Fig. 1A, TRAIL + Cisplatin). For example, more than 70% of cells were killed when cells were exposed to 0.5 μg/ml TRAIL in combination
with 5 μg/ml cisplatin. These observations were consistent with morphological features (Fig. 1B). Most cells underwent apoptosis during TRAIL treatment in combination with cisplatin (Fig. 1B, bottom right panel) as shown by cell surface blebbing and formation of apoptotic bodies. Additional studies were designed to determine whether the combination of TRAIL and cisplatin enhances PARP cleavage, the significant event of apoptosis, in HNSCC-6 cells.

Fig. 1 Synergistic cytotoxicity by combination treatment with TRAIL and cisplatin in human head and neck squamous carcinoma HNSCC-6 cells. A, cells were treated with 0.1–1.0 μg/ml TRAIL alone, 1–10 μg/ml cisplatin alone, or a combination of 0.1–1.0 μg/ml TRAIL and 1–10 μg/ml cisplatin for 24 h, and then survival was analyzed by trypan blue exclusion assay. Error bars represent ±SE from three separate experiments. B, cells were treated with 0.5 μg/ml TRAIL alone, 5 μg/ml cisplatin alone, or a combination of 0.5 μg/ml TRAIL and 5 μg/ml cisplatin for 24 h, and then morphology was evaluated with a phase-contrast microscope. Con, untreated control cells.

A Combination of TRAIL and Cisplatin Treatment Promotes the Cleavage of PARP and the Activation of Caspases. It is well known that apoptosis induced by TRAIL or cisplatin is characterized by the cleavage of PARP and activation of caspases (22). Thus, we examined the effect of the combination of TRAIL and cisplatin on the cleavage of PARP and the activation of caspases, in particular, caspase-8 (Fas-associated death domain-like interleukin-1β-converting enzyme) and caspase-3 (CPP32) activation. HNSCC-6 cells were treated with TRAIL (0.1–1 μg/ml), cisplatin (1–10 μg/ml), or a combination of the two for 24 h and then analyzed. Fig. 2A shows that TRAIL (0.1–1 μg/ml) alone failed to induce PARP cleavage. In contrast, PARP (116 kDa) was cleaved, yielding a characteristic 85-kDa fragment in the presence of a high concentration of cisplatin (10 μg/ml) alone. The amount of cleavage was markedly enhanced when TRAIL and cisplatin were combined (Fig. 2A). These results consistently show that the combination of TRAIL and cisplatin synergistically induces apoptotic death in HNSCC-6 cells. Similar results were also observed in the activation of caspases. Fig. 2B shows that...
TRAIL (0.1–1 μg/ml) alone failed to activate caspase-8. In contrast, procaspase-8 (55 kDa), the precursor form of caspase-8, was cleaved to the intermediates (43 and 41 kDa) and active form (18 kDa) by treatment with a high concentration of cisplatin (10 μg/ml) alone. The combined treatment with TRAIL and cisplatin resulted in an increase in caspase-8 activation compared with treatment with cisplatin alone (Fig. 2B). Similar results were observed in the activation of caspase-3. Cisplatin (10 μg/ml) induced proteolytic processing of procaspase-3 into its signature forms (17 and 12 kDa). The activation of caspase-3 was promoted by the combined treatment with TRAIL and cisplatin (Fig. 2B). These results indicate that synergistic induction of apoptosis by the combination of TRAIL and cisplatin is due to promotion of caspase activation. Previous studies have shown that HNSCC-6 cells contain mutated p53. Missense point mutation is present in the p53 cDNA sequence derived from HNSCC-6 cells at codon 179 (CAT→CTT; Ref. 23). In contrast, HNSCC-30 cells contain wild-type p53. To examine whether p53 status is associated with the synergistic effect during treatment with TRAIL in combination with cisplatin, cells were treated with 0.5 μg/ml TRAIL alone, 10 μg/ml cisplatin alone, or the combination for 24 h. Fig. 3 shows that the combination of TRAIL and cisplatin synergistically induces cell death (Fig. 3A), PARP cleavage (Fig. 3B), and activation of caspases (Fig. 3B) in HNSCC-30 cells. Our results were similar to those in the HNSCC-6 cell line. Thus, these results suggest that the synergistic effect can be generalized regardless of p53 status.

**Total Cellular Levels of TRAIL Receptors, Surface Expression of Functional TRAIL Receptors, and the Level of FLIP<sub>S</sub> during Combined Treatment with TRAIL and Cisplatin.** Previous studies have shown that genotoxic agents such as chemotherapeutic agents (13–15) and ionizing radiation (16) can sensitize TRAIL-induced cytotoxicity by decreasing intracellular levels of FLIP (14) or increasing DR5 gene expression in response to genotoxic stress (15, 16, 24). To investigate whether these proteins are associated with the enhancement of apoptosis by TRAIL combination with cisplatin, we examined the intracellular levels of DR4, DR5, DcR2, and FLIP by Western blot (Fig. 4, A and C). The surface expression of TRAIL receptors was examined by flow cytometry (Fig. 4B). The total cellular levels of DR4, DR5, DcR2, and FLIP, and the surface expression of DcR1, DcR2, and DR4 were not significantly changed in HNSCC-6 cells treated with TRAIL and/or cisplatin relative to untreated control cells (Fig. 4). Interestingly, Fig. 4B shows that cisplatin induced surface expression of DR5. However, combination of cisplatin and TRAIL did not enhance the DR5 expression. Unlike DR5, cisplatin alone (10 μg/ml) or in combination with TRAIL resulted in a decrease in the level of 28-kDa FLIP<sub>S</sub>, whereas there was an increase in the level of the fragment form of FLIP<sub>S</sub> (23 kDa; Fig. 4C).

**Caspase-3 Is Involved in the Cleavage of FLIP<sub>S</sub>**. To investigate whether caspase-3 is involved in the cleavage of the precursor form of FLIP<sub>S</sub>, HNSCC-6 cells were pretreated with Z-DEVD-FMK, a caspase-3 inhibitor, before treatment with TRAIL and cisplatin. Fig. 5 shows that TRAIL in combination with cisplatin-induced caspase-3 activation as well as the cleavage of FLIP<sub>S</sub> were inhibited by pretreatment with Z-DEVD-FMK. These results suggest that caspase-3 is involved in the reduction of FLIP<sub>S</sub> level. Inhibition of caspase-3 activation also blocked the cytotoxicity of combined TRAIL and cisplatin (data not shown). Taken together, cell death by combination of TRAIL and cisplatin is due to caspase-3-mediated apoptosis, and activation of caspase-3 is involved in the cleavage of FLIP<sub>S</sub>.

**Overexpression of FLIP<sub>S</sub> Protects Cells from Cell Death and Cleavage of FLIP<sub>S</sub> during Treatment with TRAIL and Cisplatin.** Recent studies show that FLIP<sub>P</sub> and FLIP<sub>S</sub> prevent caspase-8 activation at different levels of procaspase-8 processing at the DISC. FLIP<sub>S</sub> completely inhibits cleavage of procaspase-8, whereas FLIP<sub>P</sub> inhibits the second cleavage step of procaspase-8 (25). We investigated whether overexpression of FLIP<sub>P</sub> inhibits FLIP<sub>S</sub> cleavage during treatment with TRAIL and cisplatin. HNSCC-6 cells were infected with Ad/EGFP or Ad/FLAG-FLIP<sub>P</sub> and then treated with TRAIL in combination with cisplatin (Fig. 6). Fig. 6A shows that FLIP<sub>P</sub> (28 kDa) was cleaved to the fragment form of FLIP<sub>S</sub> (23 kDa) during treatment with TRAIL (0.5–1 μg/ml) and cisplatin (5–10 μg/ml) in Ad/EGFP-infected HNSCC-6 cells. However, overexpression of FLIP<sub>P</sub> prevents the cleavage of FLIP<sub>S</sub> and PARP and activation of caspase-8 and caspase-3.
as well as cell death (Fig. 6C). We further examined the Ad/FLAG-FLIPS-infected HNSCC-6 cells with high dose of cisplatin (20–100 μg/ml). The combined treatment with 1 μg/ml TRAIL and 50–100 μg/ml cisplatin results in the cleavage of endogenous FLIPS as well as exogenous FLAG-FLIPS in HNSCC-6 cells (Fig. 6B). Fig. 7 shows a schematic diagram of a model based on the current data. This model illustrates that an activated caspase-3 cleaves FLIPS. Previous studies have shown that during the execution phase of apoptosis, caspase-3 is responsible for the proteolysis of various substrates, each of which contains a common Asp-Xaa-Xaa-Asp (DXXD) motif (26). Fig. 7 shows a putative cleavage site at Asp^{52}-Ile^{53}.

**DISCUSSION**

In the present study, we observed that the combination of cisplatin and TRAIL resulted in synergistic induction of cell death in HNSCC-6 and HNSCC-30 cells (Figs. 1 and 3). The synergistic cytotoxicity is probably due to the augmentation of caspase-mediated apoptosis (Fig. 2). The enhancement of TRAIL-mediated apoptosis by chemotherapeutic agents has been explained by up-regulation of DR4 and DR5 receptors or down-regulation of FLIP (14–16, 24, 27). However, our data show that intracellular levels of DR4, DR5, DcR2, and FLIP were not changed during treatment with TRAIL in combination...
with cisplatin in HNSCC-6 cells (Fig. 4, A and C). There were no conclusive results when the surface expression of TRAIL receptors was examined during combination treatment with TRAIL and cisplatin (Fig. 4B). This discrepancy may be due to the fact that regulation of DR5 expression by genotoxic drugs is dependent on the presence of wild-type p53 (15, 21, 27, 28), and HNSCC-6 cells contain mutant p53. However, recent reports demonstrate that expression of the DR4 receptor is regulated in a p53-independent manner (29). These results are consistent with previous reports in breast and ovarian cancer cells in which there was no correlation between enhancement in TRAIL-mediated apoptosis by chemotherapeutic agents and DR levels (13, 30).

Previous studies have shown that several FLIP splice variants exist on the mRNA level, but two endogenous forms, FLIPL and FLIPS, are detected on the protein level (31, 32). The role of FLIP in apoptosis signaling has been controversially discussed. Some reports have described it as an antiapoptotic molecule (33–35), and others have described it as a proapoptotic molecule (34, 36, 37). Our studies reveal that FLIPS has an antiapoptotic function (Fig. 6). Recent studies show that FLIPL and FLIPS prevent caspase-8 activation at different levels of procaspase-8 processing at the DISC (25). FLIP contains DEDs that are similar to the corresponding segment in Fas-associated death domain and caspase-8 (38). FLIPL possesses two DEDs and one caspase-like domain in which tyrosine is substituted for the active cysteine residue necessary for enzymatic activity. FLIPS possesses two DEDs but no caspase-like domain that is similar to viral FLIP (vFLIPS; Ref. 31). FLIP is recruited to the DISC and inhibits caspase-8 activation. Expressed at high levels in stable transfectants, FLIP completely blocks receptor-ligand apoptotic death through inhibition of caspase-8 processing at the DISC. Interestingly, recent studies revealed that FLIPS completely inhibits cleavage of procaspase-8, whereas FLIPL inhibits the second cleavage step of procaspase-8 (25). In this study,

**Fig. 5** Effect of caspase-3 inhibitor on TRAIL/cisplatin-induced caspase-3 activation and FLIPS cleavage. HNSCC-6 cells were pre-treated with (+) or without (−) 20 μM Z-DEVD-FMK for 30 min and then treated with TRAIL (0.1–0.5 μg/ml) in combination with cisplatin (1–5 μg/ml). After 24 h of treatment, cells were harvested. Cell lysates containing equal amounts of protein (20 μg) were separated by SDS-PAGE and immunoblotted using antibodies against caspase-3 or FLIPS.

**Fig. 6** Inhibition of combined TRAIL and cisplatin-induced FLIPS cleavage and cell death by FLIPS overexpression. HNSCC-6 cells were infected with adenoviral vectors containing EGFP (Ad/EGFP) or FLAG-tagged FLIPS (Ad/FLAG-FLIPS) at a multiplicity of infection of 100. A, after 48 h of incubation, cells were treated with TRAIL (0.1–0.5 μg/ml) in combination with cisplatin (1–5 μg/ml) and then cell lysates were immunoblotted using antibodies against caspase-8, PARP, caspase-3, and FLIPS. Anti-actin antibody was used to confirm the equal amount of proteins loaded in each lane. B, Ad/FLAG-FLIPS-infected cells were treated with 0.5 μg/ml TRAIL in combination with various concentrations of cisplatin (20–100 μg/ml) for 24 h, and then cell lysates were immunoblotted using antibodies against FLIPS, PARP, caspase-8, and caspase-3. Anti-actin antibody was used to confirm the equal amount of proteins loaded in each lane. C, Ad/EGFP- or Ad/FLAG-FLIPS-infected cells were treated with combined TRAIL (0.5–1 μg/ml) and cisplatin (5–10 μg/ml) for 24 h, and then the survival of the cells was measured. The results represent the mean ± SE for three independent experiments.
we observed that FLIP\textsubscript{S}, but not FLIP\textsubscript{L}, was cleaved during treatment with 10 \(\mu\)g/ml cisplatin. The cleavage of FLIP\textsubscript{S} was promoted by combined treatment with TRAIL and cisplatin (Fig. 4C). Our data also suggest that caspase-3 is involved in the cleavage of FLIP\textsubscript{S} (Fig. 5). At the present time, we can only speculate how the combination of TRAIL and cisplatin promotes the cleavage of FLIP\textsubscript{S}. Previous studies have shown that chemotherapeutic drugs can activate the initiator caspase-9 by stimulating cytochrome \(c\) release from the mitochondria (39, 40). Our previous studies have shown that an increase in cytochrome \(c\) release promotes TRAIL-induced apoptosis (18). Overexpression of Bcl-2 blocks the synergistic effect by inhibiting cytochrome \(c\) release (18). Thus, one possibility is that combined treatment with TRAIL and cisplatin enhances apoptosis by promoting cytochrome \(c\) release. This possibility needs to be examined.

REFERENCES


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