Tumor Necrosis Factor-related Apoptosis-inducing Ligand Retains Its Apoptosis-inducing Capacity on Bcl-2- or Bcl-xL-overexpressing Chemotherapy-resistant Tumor Cells

Henning Walczak, Axel Bouchon, Heiko Stahl, and Peter H. Krammer

Tumor Immunology Program, German Cancer Research Center (DKFZ), 69120 Heidelberg, Germany

ABSTRACT

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a member of the tumor necrosis factor family and has recently been shown to exert tumoricidal activity in vivo in the absence of any observable toxicity. The signaling pathways triggered by TRAIL stimulation and the mechanisms involved in resistance against TRAIL-mediated apoptosis are still poorly defined. We show here that TRAIL-induced apoptosis involves late dissipation of mitochondrial membrane potential (ΔΨm) and cytochrome c release. These events follow activation of caspase-8 and caspase-3 and induction of DNA fragmentation. In addition, caspase-8-deficient cells are resistant against TRAIL-induced apoptosis, and inhibition of caspase-8 but not caspase-9 prevents mitochondrial permeability transition and apoptosis. In contrast, various Bcl-2- or Bcl-xL-overexpressing tumor cell lines are sensitive to TRAIL-induced apoptosis; however, they show a delay in TRAIL-induced mitochondrial permeability transition compared with control transfectants. This indicates that TRAIL-induced apoptosis depends on caspase-8 activation rather than on the disruption of mitochondrial integrity. Because most chemotherapeutic drugs used in the treatment of malignancies lead to apoptosis primarily by engagement of the mitochondrial proapoptotic machinery, we tested whether drug-resistant tumor cells retain sensitivity for TRAIL-induced apoptosis. Tumor cells overexpressing Bcl-2 or Bcl-xL become resistant to apoptosis induced by the chemotherapeutic drug etoposide. However, these cells are not protected or are only marginally protected against TRAIL-induced apoptosis. Thus, TRAIL may still kill tumors that have acquired resistance to chemotherapeutic drugs by overexpression of Bcl-2 or Bcl-xL. These data will influence future treatment strategies involving TRAIL.

INTRODUCTION

In the immune system, apoptosis is an essential process during development and maintenance of homeostasis. So far, three members of the TNFα superfamily, TNF-α, CD95L (FasL/APO-1L), and TRAIL/APO-2L (1, 2), have been shown to directly induce apoptosis in sensitive target cells on binding to those cognate receptors that are capable of transmitting a caspase-activating signal due to the presence of a so-called cytoplasmic DD (3, 4).

TNF-α and CD95L are involved in different physiological and pathological apoptotic processes such as activation-induced cell death of T cells, immune privilege, tumor evasion from the immune system, and autoimmunity (4). In addition, TNF-α and CD95L kill various tumor cell lines in vitro. However, the use of these ligands for anticancer therapy is complicated by their severe toxic side effects on systemic administration such as shock and acute hepatic failure (5–7).

Like CD95L and TNF-α, TRAIL induces apoptosis in various tumor cell lines in vitro (8–10). Furthermore, we and others have recently demonstrated that administration of TRAIL suppresses the growth of TRAIL-sensitive human tumor cells in severe combined immunodeficient (SCID) mice or nonhuman primates without showing any toxicity to normal tissue (11–13). The tumoricidal activity of TRAIL is also highlighted by recent data showing that the TRAIL system may be involved in endogenous tumor and viral surveillance mediated by IFN-γ-activated monocytes and IFN-α/β-activated T cells (14, 15).

TRAIL interacts with five distinct receptors: (a) TRAIL-R1 [DR4/apo-2L (16)]; (b) TRAIL-R2 [DR5/TRICK/Killer (9, 17–20)]; (c) TRAIL-R3 [Dr3/trid/lit (18, 21)]; (d) TRAIL-R4 [DcR2/tru/ndd (21, 22)]; and (e) osteoprotegerin (23). Both TRAIL-R1 and -R2 contain the intracellular DD that is essential for the induction of apoptosis on receptor ligation (9, 16–20). In contrast, neither TRAIL-R3 nor TRAIL-R4 can mediate apoptosis due to a complete or partial lack of the intracellular DD, respectively (17, 19, 21, 22, 24). Osteoprotegerin is a soluble receptor for TRAIL and thus blocks TRAIL-induced apoptosis (23).

Although caspase-8 and caspase-3 activation could be detected after triggering of TRAIL-R1 or TRAIL-R2 (10), thus far the TRAIL-induced signal transduction pathway and the influence of mitochondria-protecting antiapoptotic proteins such as Bcl-2 or Bcl-xL (25) on TRAIL-induced apoptosis remain poorly defined.

Many known chemotherapeutic drugs trigger a receptor-independent mitochondria-controlled apoptotic pathway that is induced by early loss of mitochondrial membrane potential (ΔΨm) (26–30) and the subsequent release of the proapoptotic proteins cytochrome c, procaspase-2, -3, and -9; and apoptosis-inducing factor from the mitochondria into the cytosol (31–35). Together with Apaf-1 and dATP as cofactors, cytosolic cytochrome c facilitates the autoproteolytic cleavage of caspase-9, leading to subsequent activation of downstream caspases (36–39). Therefore, we examined to what extent mitochondria contribute to the pathway leading to TRAIL-induced and chemotherapeutic drug-induced apoptosis. We were particularly interested in whether TRAIL can bypass mitochondria and thus also bypass the antiapoptotic effect of Bcl-2 or Bcl-xL, two known protooncogenes that have been shown to be overexpressed in several drug-resistant tumors observed in distinct clinical settings (40–44). We found that during TRAIL-induced apoptosis, mitochondrial PT and cytochrome c release occur after caspase-8 and caspase-3 activation and induction of DNA fragmentation. In addition, caspase-8-deficient Jurkat cells were resistant to TRAIL-induced apoptosis. In contrast, Bcl-2 overexpression protects only against early loss of ΔΨm but not against late disruption of mitochondrial integrity and apoptosis during TRAIL-induced apoptosis. These observations suggest that caspase-8 is necessary and, together with effector caspases, sufficient for TRAIL-induced apoptosis, whereas disruption of mitochondrial integrity is a secondary rather than an initial step after triggering of TRAIL.
TRAIL-R. By applying caspase-8-, caspase-9-, and caspase-3-specific inhibitors, this sequence of events was confirmed. In contrast, overexpression of Bcl-2- or Bcl-xL led to resistance against the chemotherapeutic drug etoposide, which induces apoptosis primarily through the mitochondria-dependent pathway (26–30). Thus, because overexpression of Bcl-2 or Bcl-xL at levels that protect cells against chemotherapeutic drugs does not inhibit TRAIL-induced apoptosis, Bcl-2 and Bcl-xL-overexpressing, chemoresistant tumors may still respond to treatment with TRAIL.

MATERIALS AND METHODS

Cell Lines. The human B-cell lines BL60 and BJAB and the human T-cell lines Jurkat (clone J16) and CEM were maintained in RPMI 1640 (Life Technologies, Inc.), 2 mg/ml Gentamycin (Life Technologies, Inc.) and 10% FCS (Life Technologies, Inc.). Jurkat cells deficient for caspase-8 were a kind gift of John Blenis and Peter Juo (Harvard Medical School, Boston, MA). Wild-type, transfected, or caspase-8-deficient Jurkat cells were cultured as described previously (45). CEM cells transfected with vector control or Bcl-xL were cultured in RPMI 1640 containing 10% FCS (Life Technologies, Inc.), and Bcl-2-transfected SKW6 and vector-transfected control cells were obtained from A. Strasser (Walter and Eliza Hall Institute, Melbourne, Australia) and maintained in DMEM containing 10% FCS (Life Technologies, Inc.) and 1 mg/ml G418 (Sigma, St. Louis, MO).

Antibodies and Reagents. mAbs against cytochrome c oxidase/subunit II (12C4-F12), cytochrome c (TH8.2C12), Bcl-xL, and Bcl-2 were purchased from Molecular Probes, Inc. (Eugene, OR), PharMingen (San Diego, CA), Transduction Laboratories (San Diego, CA), and Calbiochem (La Jolla, CA), respectively. The mouse mAb against PARP (C-11) was a kind gift of Dr. A. Bürkle (German Cancer Research Center, Heidelberg, Germany). The C15 mAb recognizes the p18 subunit of caspase-8 (33), whereas the anti-caspase-3 mAb recognizes the p17 subunit of caspase-3, but not the p12 subunit (Transduction Laboratories, Lexington, Kentucky). LZ-TRAIL and LZ-CD40L are stable trimers of the respective cytokines, and LZ-TRAIL induces apoptosis upon binding to TRAIL-sensitive cells (9). Both reagents were kindly provided by Immunex Corp. (Seattle, WA). The horseradish peroxidase-conjugated goat antimouse IgG1, IgG2a, and IgG2b polyclonal antibodies were obtained from Southern Biotechnology Associates (Birmingham, AL). The caspase inhibitors zVAD-fmk and zDEVD-fmk were purchased from Bachem (Basel, Switzerland), and zIETD-fmk and zLEHD-fmk were obtained from Calbiochem. All other chemicals used were of analytical grade and were purchased from Merck (Darmstadt, Germany) or Sigma. The chemotherapeutic agent etoposide was kindly provided by Ganti Chemo Supply (Heidelberg, Germany).

Fluorescence-activated Cell-sorting Analysis. Wild-type or caspase-8-deficient Jurkat cells were incubated with mAbs M271 (anti-TRAIL-R1), M413 (anti-TRAIL-R2), and M430 (anti-TRAIL-R3). M444 (anti-TRAIL-R4; Immunex Corp.) or isotype-matched control IgG followed by phycoerythrin-conjugated secondary goat antimouse antibodies (Southern Biotechnology Associates). Surface staining was determined on a FACScan cytometer (Becton Dickinson, San Jose, CA).

Determination of Mitochondrial Membrane Potential and ROS Production. Measurement of ROS generation and mitochondrial transmembrane potential was performed as described previously (46). Briefly, 5 × 10^4 cells were incubated in PBS containing 40 nm DIOC3(3) (Molecular Probes Inc.) and 2 μM dihydroethidin (Molecular Probes, Inc.) for 15 min at 37°C in the dark, followed by analysis on a FACScan cytometer (Becton Dickinson).

Measurement of Cell Viability. To determine the integrity of the cell membrane, cells were incubated for 5 min in 2.5 μg/ml PI in PBS (Sigma) at 4°C in the dark, followed by analysis on a FACScan cytometer (Becton Dickinson).

Determination of DNA Fragmentation. As a direct measurement of apoptotic cell death, DNA fragmentation was quantitated essentially as described previously (47). Briefly, 2.5 × 10^5 cells were incubated in 24-well plates (Costar, Cambridge, MA) with or without apoptotic stimuli in 0.5 ml of medium at 37°C. Cells were collected by centrifugation at 600 × g for 10 min at 4°C, washed twice with PBS, and then resuspended in 100 μl of lysis buffer containing 0.1% (w/v) Triton X-100, 0.1% (w/v) sodium citrate, and 50 μg/ml EDTA. Apoptosis was quantitatively determined by flow cytometry after incubation at 4°C in the dark for at least 24 h as cells containing nuclei with subdiploid DNA content.

Determination of Phosphatidylserine Translocation to the Outer Leaflet of the Plasma Membrane by Annexin-V-FITC Surface Staining. Cells (1 × 10^5) were incubated in 96-well plates (Costar), with or without apoptotic stimuli, in 200 μl of medium at 37°C. Cells were collected by centrifugation at 600 × g for 10 min at 25°C, washed twice in binding buffer (2.5 mM CaCl_2, 140 mM NaCl, and 10 mM HEPES (pH 7.4)), and then resuspended in 50 μl of staining solution [0.2 μg/ml annexin-V-FITC (R&D Systems, Minneapolis, MN), 4 μg/ml 7-AAD Molecular Probes], 2.5 mM CaCl_2, 140 mM NaCl, and 10 mM HEPES (pH 7.4). After incubation for 15 min in the dark at 37°C, 150 μl of cold binding buffer was added, and the degree of single positive (FITC^-/7-AAD^+ early apoptotic cells) and double-positive cells (FITC^-/7-AAD^++ late apoptotic and necrotic cells) was determined by flow cytometry.

Preparation of Cell Lysates. Cells (1 × 10^7 per sample) were harvested by centrifugation at 600 × g for 10 min at 4°C and washed twice with PBS, and lysates were prepared by resuspending the resulting cell pellets in 160 μl of lysis buffer (PBS and 1% Triton X-100) supplemented with protease inhibitors (2 mg/ml aprotinin, 20 mg/ml aminophenylmethanesulfonyl fluoride, 100 μg/ml leupeptin, and 40 μg/ml bestatin). After a 30-min incubation on ice, the lysates were centrifuged once at 15,000 × g at 4°C to remove nuclei. The supernatants were stored at −20°C or directly subjected to SDS-PAGE.

Subcellular Fractionation. BL60 cells (1 × 10^7 per sample) were harvested by centrifugation at 600 × g for 10 min at 4°C. The cell pellets were washed twice in PBS, resuspended in 160 μl of isocratic buffer (250 mM sucrose, 20 mM HEPES, 20 mM KCl, 1.5 mM MgCl_2, 1 mM EDTA, 1 mM EGTA, and 1 mM DTT) supplemented with protease inhibitors (17 μg/ml phenylmethylsulfonyl fluoride, 8 μg/ml aprotinin, and 2 μg/ml leupeptin), and allowed to swell during a 20-min incubation on ice. The cells were gently broken up using a Dounce homogenizer. Small aliquots of the lysate were taken and stained with trypan blue to determine the progression of cell lysis. Homogenization was continued until ≤80% of the cells were broken. The homogenate was centrifuged once at 750 × g at 4°C to remove unbroken cells and nuclei. The supernatant was centrifuged at 10,000 × g for 25 min at 4°C to collect the mitochondria-enriched heavy membrane pellet (marked as Mito in the figures). The resulting supernatant was centrifuged at 100,000 × g for 60 min at 4°C to yield the light membrane pellet and the soluble cytosolic fraction (marked as Cytos in figures).

Western Blot Analysis. For Western blot analysis, the resulting postnuclear supernatants, mitochondrial or cytosolic fractions, were supplemented with 40 μl of 5-fold concentrated standard reducing sample buffer (5 × reducing sample buffer), and proteins equivalent to 10^6 cells or 20 μg of protein as determined by the BCA method (Pierce) were separated on 12% SDS-polyacrylamide gels (Novex, San Diego, CA) by standard electrophoresis. After protein transfer onto nitrocellulose membranes by electroblotting, membranes were blocked with 3% BSA and 3% FCS in PBS/Tween overnight, washed with PBS/Tween, and incubated with primary antibodies against caspases-8 (1:20-diluted C15 hybridoma supernatant), caspase-3 (1:1,000; Transduction Laboratories), PARP (mAb C-11; 1:20), cytochrome c (TH8.2C12; 1:500; PharMingen), cytochrome c oxidase/subunit II (12C4-F12; 1:1,000; Molecular Probes, Inc.), Bcl-2 (1:500; Calbiochem), or Bcl-xL (1:500; Transduction Laboratories) for 3 h. After six washes for 5 min each in PBS/Tween, the blots were incubated with horseradish peroxidase-conjugated isotype-specific secondary antibody diluted 1:20,000 in PBS/Tween. After washing six times for 10 min with PBS/Tween, the blots were developed by enhanced chemiluminescence following the manufacturer’s protocol (Amersham). For stripping, blots were incubated for 30 min in a buffer containing 62.5 mM Tris/HCl (pH 6.8), 2% SDS, and 100 mM β-mercaptoethanol at 60°C. Then blots were washed six times for 10 min in PBS/Tween and blocked again. To confirm equal loading and purity of the cytosolic fraction, Western blots were also developed with an antibody directed against either extracellular–signal-regulated kinase (Santa Cruz Biotechnology, Santa Cruz, CA; data not shown) or against mitochondrial cytochrome c oxidase (Molecular Probes, Inc.).

RESULTS

DNA Fragmentation and Caspase-3 Activation Occur before Mitochondrial PT and Production of ROS during TRAIL-induced Apoptosis. To assess the contribution of mitochondria to TRAIL-induced apoptosis, we monitored the sequential appearance of different apoptotic features after stimulation of BL60 (Fig. 1A) and Jurkat cells (Fig. 1B) with TRAIL. In accordance with previous reports (48), phos-
phosphatidylserine translocation to the outer leaflet of the plasma membrane could be detected as early as 30 min after TRAIL receptor engagement by LZ-TRAIL. In addition, DNA fragmentation clearly preceded loss of $\Delta \Psi_m$, suggesting a mitochondria-independent pathway that leads directly to DNA fragmentation. Because the degree of DNA fragmentation is identical to the observed amount of total cell death determined by PI uptake (Fig. 1), we conclude that TRAIL-induced cell death is exclusively apoptotic and does not include any additional necrosis in these cell lines. Although it was previously reported that ROS might initiate mito-
chondrial PT in some cellular systems (49), we could not detect an initial or early increase in ROS production during TRAIL-induced apoptosis. Thus, increased production of ROS seems to be a secondary effect following mitochondrial PT and does not play a role at the initiation of TRAIL-induced apoptosis. Stimulation of BL60 or Jurkat cells with LZ-CD40L, as a control, did not induce any of the hallmarks of apoptosis induced by LZ-TRAIL (data not shown).

To study the sequence of apoptotic events after TRAIL treatment at the biochemical level, we determined the cleavage kinetics of caspase-8, caspase-3, and the caspase-3-substrate PARP and investigated the distribution of cytochrome $c$ between cytosol and mitochondria at different time points. The active caspase-8 subunit p18 could be detected as early as 15 min after stimulation with LZ-TRAIL (Fig. 2A). This strongly suggests that caspase-8 might be the caspase initiating TRAIL-induced apoptosis. In accordance with the observed onset of DNA fragmentation after 1–2 h (Fig. 1), the active p17 subunit of caspase-3 (Fig. 2B) and processing of its substrate PARP (Fig. 2C) could already be observed 1 h after stimulation. In contrast, release of cytochrome $c$ from the mitochondria to the cytosol was detected after the onset of caspase-3 cleavage and DNA fragmentation (Fig. 2, D and E) yet before loss of $\Delta \Psi_m$ (Fig. 1). Thus, TRAIL-induced apoptosis involves early activation of caspase-8, which then leads to cleavage of caspase-3, subsequent caspase-3 substrate cleavage, and DNA fragmentation. Although the TRAIL-generated apoptotic signal is also transmitted to the mitochondria and leads to mito-
chondrial PT and cytochrome $c$ release, these mitochondrial changes occur late during TRAIL-induced apoptosis, clearly following caspase-8 activation and the onset of DNA fragmentation.

**Caspase-8 Is Essential for the Induction of TRAIL-induced Apoptosis.** To test whether caspase-8 activation is necessary for the initiation of TRAIL-induced apoptosis, we treated Jurkat cells defi-
cient for caspase-8 (Fig. 3B) with TRAIL. Whereas wild-type Jurkat cells were sensitive to TRAIL-induced apoptosis, caspase-8-deficient cells were resistant to TRAIL-induced apoptosis (Fig. 3A). This resistance was due to a deficiency in caspase-8 expression rather than to reduced expression of caspase-3 (Fig. 3B) or, conversely, increased surface levels of TRAIL-R3 or TRAIL-R2 (Fig. 3C) or TRAIL-R4 (Fig. 3D) yet before loss of $\Delta \Psi_m$ (Fig. 1). Thus, caspase-8 activation is a necessary event during TRAIL-mediated apoptosis in Jurkat cells.

**Bcl-2 Delays Mitochondrial PT but not Cell Death during TRAIL-induced Apoptosis.** Overexpression of Bcl-2 or Bcl-xL protects cells against various apoptotic stimuli (25–30, 45). However, the effect of Bcl-2-mediated protection of mitochondria on TRAIL-induced apoptosis is unknown. We therefore monitored cell death induction and changes in $\Delta \Psi_m$ after TRAIL treatment of Jurkat cells overexpressing Bcl-2 (Fig. 4A) compared with control transfectants. Whereas cell death progressed with similar kinetics in both cell lines tested, the loss of $\Delta \Psi_m$ was clearly delayed by about 2–4 h in the Bcl-2-transfected Jurkat cells (Fig. 4, C and D). Thus, Bcl-2 protects the mitochondria during the early stages of TRAIL-induced apoptosis, but not during the late phase. This might be due to caspase-8- or caspase-3-induced mechanisms that target the mitochondria later during apoptosis or finally occurring necrotic processes that cannot be inhibited by Bcl-2 (50). These data provide evidence that Bcl-2 overexpression can indeed inhibit mitochondrial PT during the early stages of TRAIL-induced apoptosis. However, independ-
ent of the occurrence of mitochondrial PT, TRAIL-induced apoptosis is initiated and executed with almost equal efficiency. These data clearly
involved in the induction of TRAIL-induced loss of mitochondria such as cytochrome c. These data provide further proof that TRAIL can lead to apoptosis via a mitochondria-independent pathway, whereas etoposide-induced apoptosis is at least substantially inhibited by overexpression of Bcl-2 or Bcl-xL. Thus, although conventional antitumor treatment failed to kill Bcl-2- and Bcl-xL-overexpressing tumor cell lines efficiently, these cells remain highly sensitive to TRAIL-induced apoptosis.

DISCUSSION

Apoptosis can be induced at both the mitochondrial and the death receptor levels (3, 50). In addition, mitochondria contribute to and, at least in some cellular systems, seem to be necessary for execution of death receptor-induced apoptosis (45). For the TRAIL system, however, the degree of mitochondrial contribution to the apoptotic process has not
been determined thus far. In this report, we provide biochemical and functional evidence that mitochondrial dysfunction occurs during TRAIL-induced apoptosis but is not a necessary requirement for TRAIL-induced apoptosis because it can be bypassed by direct caspase-8-mediated activation of downstream caspases. Whereas protection of mitochondrial function by overexpression of Bcl-2 or Bcl-xL in various cell lines led to resistance against typical agents mediating apoptosis primarily via the mitochondrial pathway, such as etoposide (25–30), these cells retained 60–100% of their sensitivity against TRAIL.

The bcl-2 gene was first shown to be dysregulated in the majority of follicular lymphomas in which a t(14:18) chromosomal translocation is present (40). However, Bcl-2 has now been found to be overexpressed also in the absence of gene rearrangements in most cases of B-cell chronic lymphocytic leukemia (41) and acute lymphoblastic leukemia (42). Interestingly, strongly increased levels of Bcl-2 in B-cell chronic lymphocytic leukemia or acute lymphoblastic leukemia were particularly pronounced in patients who had undergone chemotherapy or γ-irradiation and were subsequently found to be clinically unresponsive to these treatments (41, 43, 44). Because most clinically administered chemotherapeutic drugs mediate apoptosis mainly by inducing mitochondrial dysfunction, tumor cell resistance is likely to include overexpression of Bcl-2 or Bcl-xL. This leads to protection of mitochondria and subsequent cross-resistance to many mitochondria-targeting chemotherapeutic drugs (25–30). Our data show that TRAIL is still capable of killing tumor cells that have already acquired resistance to chemotherapeutic drugs by Bcl-2- or Bcl-xL overexpression-induced protection of mitochondria may still remain sensitive to TRAIL-induced apoptosis.

![Fig. 4. Jurkat cells overexpressing Bcl-2 show a delay in TRAIL-induced disruption of mitochondrial integrity but display similar kinetics of cell death progression. A and B, expression levels of Bcl-2 and Bcl-xL in wild-type, control-, Bcl-2-, and Bcl-xL-transfected Jurkat, CEM, and SKW6 cells, respectively. Cells (5 x 10⁶) were lysed and subjected to SDS-PAGE followed by Western blot analysis with anti-Bcl-2, anti-Bcl-xL, or caspase-8 antibodies. Migration positions of the detected proteins are indicated. C and D, Jurkat cells transfected with control vector (C) or Bcl-2 (D) were stimulated with 100 ng/ml LZ-TRAIL for the indicated time periods, followed by determination of cell death (increased fluorescence at 575 nm by PI uptake; ■) and loss of ΔΨm [reduced fluorescence in the presence of DOCC(3) at 525 nm; □]. All data points are the mean of two independent experiments.](download)

![Fig. 5. Low concentrations of caspase-8 inhibitor zIETD-fmk but not caspase-9 inhibitor zLEHD-fmk blocks loss of ΔΨm during TRAIL-induced apoptosis. BL60 cells were incubated with the indicated concentrations of zVAD-fmk (●), zIETD-fmk (■), zLEHD-fmk (▲), or zDEVDD-fmk (●) 30 min before stimulation with 100 ng/ml LZ-TRAIL. Loss of ΔΨm [reduced fluorescence with DOCC(3) at 525 nm] was determined 6 h after stimulation. All data points are the mean of three independent experiments. The values obtained at inhibitor concentrations that provide specific caspase inhibition are boxed.](download)
It has previously been shown that coadministration of TRAIL and 5-fluorouracil may have a beneficial effect on tumor eradication in vivo (12). However, the functional basis for this observation has not been investigated thus far. We provide evidence that TRAIL and chemotherapeutic drugs kill tumors by primarily targeting non-mitochondria-dependent and mitochondria-dependent apoptotic pathways, respectively. Thus, our findings suggest an explanation for the observed enhanced treatment effect of TRAIL together with mitochondria-targeting drugs in vivo (12). In addition, we show that TRAIL is still capable of inducing apoptosis in Bcl-2- and Bcl-xL-overexpressing tumor cells while these cells are protected against mitochondria-targeting cytotoxic agents.

TRAIL does not exhibit any observable toxicity on systemic treatment of mice or nonhuman primates, and it does not induce apoptosis in many different normal human cells in vitro (11–13). Thus, our data suggest that this cytokine may be a promising candidate for the treatment of patients carrying drug-resistant tumors. In addition, because chemotherapy and radiation therapy on the one hand and TRAIL on the other hand trigger tumor cell apoptosis primarily via two distinct pathways, cotreatment of malignancies that still react to chemotherapy or radiation therapy potentially diminishes the pool of tumor cells that may give rise to an outgrowing resistant clonal variant of the parental tumor. Therefore, concomitant treatment of tumor patients with TRAIL and chemotherapeutic drugs or radiation therapy may reduce the relapse rate in malignancies.

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TRAIL-INDUCED APOPTOSIS

REFERENCES


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