Sensitization of Cells to TRAIL-induced Apoptosis by Decoy Receptor 3*

Decoy receptor 3 (DcR3)/TR6/M68 is a soluble receptor that binds to the Fas ligand LIGHT and TL1A. Elevated levels of DcR3 expression have been found in many tumors. We report an unexpected effect of DcR3 by sensitizing Jurkat and U937 cells to apoptosis induced by tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). Cell death triggered by anti-Fas and tumor necrosis factor was unaffected by DcR3. DcR3 by itself did not stimulate apoptosis. The ability to augment TRAIL-initiated cell death was not observed with soluble lymphotxin β receptor or soluble death receptor 3, indicating that binding to LIGHT or TL1A alone is insufficient to trigger TRAIL sensitivity. Incubation with DcR3 did not increase the surface expression of TRAIL receptor, and the level of Fas-associated death domain protein and cellular FLICE-like inhibitory protein was not altered. Instead, in the presence of DcR3, TRAIL engagement resulted in an increased activation of caspase-8, an elevated cleavage of Bid, and enhanced release of Smac and cytochrome c from mitochondria to cytosol compared with TRAIL alone. This led to increased activation of caspase-9 and caspase-3. The unusual ability of DcR3 to promote TRAIL-triggered death may be used to potentiate TRAIL efficacy during treatment of tumors overexpressing DcR3.

DcR3 has also been shown to promote angiogenesis through neutralization of TL1A (9).

Recent studies have revealed the profound modulatory effects of DcR3 on many cells. DcR3 regulates CD14+ monocyte differentiation into dendritic cells, with down-regulation of HLA-DR, CD56/ICAM-1, and CD80, and up-regulation of CD86 in mature dendritic cells, leading to skewed Th2 differentiation in primed T cells (10). DcR3 attenuates T cell activation and reduces T cell response to mouse heart allograft (11). DcR3 also decreases T cell interaction with antigen-presenting cells (12) and inhibits T cell chemotaxis (13). In addition, DcR3 triggers actin re-organization, increases the adhesion of monocytes (14), and reduces phagocytic activity and proinflammatory cytokine production in macrophages (15).

TRAIL induces apoptosis by binding to TRAIL receptor (16, 17). There are five different TRAIL receptors, including TRAIL-R1/DR4, TRAIL-R2/DR5, TRAIL-R3/DcR1, TRAIL-R4/ DcR2, and osteoprotegerin. Binding of DR4 and DR5 to TRAIL leads to apoptosis, whereas DcR1, DcR2, and osteoprotegerin are likely decoy receptors. FADD and pro-caspase-8 are recruited to the engaged DR4 and DR5 to form a death-inducing signaling complex; the subsequent activation of caspase-8 initiates a death receptor apoptotic pathway (18–20). TRAIL-induced apoptosis, however, also requires Bax-dependent mitochondrial events (21, 22). Caspase-8 cleaves Bid to generate truncated Bid (tBid), which is translocated into mitochondria (23, 24). The interaction of tBid with Bax (and Bak) then promotes the release of cytochrome c and Smac/DIABLO from mitochondria. Cytochrome c directly activates caspase-9. Smac binds to XIAP and prevents XIAP from blockage of caspase activation (22). The essential role of Smac is further demonstrated by the facts that ectopic overexpression of Smac enhances TRAIL-induced apoptosis (25, 26), and that extracellular signal-regulated kinase 1/2 inhibition sensitizes melanoma cells to TRAIL-induced apoptosis through increased Smac release (27).

Among the TNF family, TRAIL is unique for its potent anticancer effect and low toxicity toward normal tissues in vivo (28, 29). Many different molecules regulate TRAIL-mediated apoptosis, including interferon, cellular FLICE-like inhibitory protein (c-FLIP), cytotoxic drugs, or p53 (16, 17, 30, 31). In the present study, we found that DcR3, presumably overproduced by cancer cells to escape the cytotoxic effect of FasL and LIGHT, greatly enhanced TRAIL-induced apoptosis in selected lymphoma cell lines. DcR3 increased TRAIL stimulated caspase-8 activation and Bid cleavage, enhanced Smac release from mitochondria, and augmented caspase-3 and caspase-9 activation. Our results suggest that tumors overexpressing DcR3 could be treated with TRAIL.
**EXPERIMENTAL PROCEDURES**

Reagents—CH-11 was purchased from Upstate Biotechnology (Lake Placid, NY). Recombinant human TRAIL, human TNF-α, rabbit anti-human Smac/DIABLO antibodies were obtained from R&D Systems (Minneapolis, MN). Anti-cytochrome c (clone 7H5) was obtained from eBioscience. Rabbit polyclonal anti-FADD (H181), rabbit polyclonal anti-FLIP (H150), and rabbit polyclonal anti-Bcl-2 (N-19) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-β-tubulin, horseradish peroxidase-conjugated goat anti-rabbit Ig, and horseradish peroxidase-conjugated rabbit anti-mouse Ig were obtained from Amersham Biosciences.

Preparation of Mitochondrial and Cytosolic Fractions—Subcellular fractionation of different cancer cells were carried out as described previously with small modifications (27, 32, 33). In brief, cells were harvested by centrifugation at 1,000 × g for 5 min at 4 °C. Cell pellets were washed once with ice-cold phosphate-buffered saline and resuspended in 5 volumes of buffer A (20 mM HEPES-KOH, pH 7.5, 10 mM KCl, 1.5 mM MgCl2, 1 mM sodium EDTA, 1 mM sodium EGTA, 1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride, 20 μg/ml leupeptin, and 10 μg/ml aprotinin) containing 250 mM sucrose. After incubation on ice for 15 min, the cells were homogenized with a 26-gauge needle for 20 strokes, and the homogenates were centrifuged at 1,000 × g for 5 min at 4 °C. The supernatant was collected and centrifuged at 10,000 × g for 15 min at 4 °C, and the mitochondria-enriched pellets were resuspended in buffer A. The supernatants of the 10,000 × g spin were further centrifuged at 100,000 × g for 1 h at 4 °C, and the supernatants were designated as the S-100 cytosolic fraction. The S-100 fraction was collected, and the protein concentrations determined by the Bradford method (Bio-Rad, Hercules, CA).

Production of Recombinant DcR3.Fc, LTβR.Fc, DcR3.FLAG, and DcR3.FLAG Fusion Proteins—DcR3.Fc, LTβR.Fc, DcR3.FLAG, and DcR3.FLAG proteins were produced as described previously (10, 15). The supernatant from recombinant virus-infected SF21 cells was filtered, and the DcR3.Fc and LTβR.Fc proteins were loaded on protein A-Septasorose beads. The bound DcR3.Fc and LTβR.Fc were then eluted with 0.1 M glycine, pH 3.0, followed by dialysis against phosphate-buffered saline. For DcR3.FLAG and DcR3.FLAG, supernatant from recombinant virus-infected SF21 cells was filtered and loaded onto an anti-Flag M2 affinity gel. The bound DcR3.Flag protein was then eluted with 0.1 M glycine buffer, pH 3.0, followed by dialysis against phosphate-buffered saline.

Surface DR5 Staining—Jurkat and U937 cells were harvested and washed twice with fluorescence-activated cell sorting buffer (2% fetal calf serum in phosphate-buffered saline). The cells were incubated with phycoerythrin-conjugated rabbit anti-human DR5 polyclonal antibodies (eBioscience) on ice for 1 h in the dark. The stained cells were washed twice with fluorescence-activated cell sorting buffer, followed by analysis on a FACScan (BD Biosciences).

**RESULTS**

**DcR3 Enhances TRAIL-induced Apoptosis**—During the analysis of purified DcR3.Fc for its ability to block FasL-initiated apoptosis, we observed that DcR3.Fc (10 μg/ml) increased TRAIL-induced apoptosis in Jurkat T cells (Fig. 1A). This is contrary to the generally accepted view that DcR3 possesses antiapoptotic properties that may contribute to the survival of tumor cells (1, 2). We tested the effect of DcR3 on another TRAIL-sensitive tumor cell U937 and observed a similar enhancement of TRAIL-triggered apoptosis (Fig. 1A). DcR3 by itself did not induce apoptosis of Jurkat and U937 cells (Fig. 1, B and C); instead, the augmentative effect of DcR3 was most marked at suboptimal apoptotic doses of TRAIL. DcR3.Fc increased TRAIL-triggered Jurkat cell death by 40% at 40% TRAIL concentrations of 1–2 μg/ml (Fig. 1B), whereas TRAIL-mediated U937 apoptosis was enhanced by 50% at 2–4 μg/ml of TRAIL (Fig. 1C). Fig. 1D illustrates the dose-dependent effect of DcR3 on TRAIL-induced cell death.

DcR3.Fc binds to Fc receptor and may initiate signal transduction through its receptor (34). We thus used lymphotixin β receptor-Fc (LTβR.Fc) to examine whether binding to Fc receptor would increase TRAIL-triggered apoptosis. In addition, LIGHT is a common ligand for LTβR and DcR3 (2); binding to LIGHT has accounted for some of the biological activities of DcR3 (11, 13). Jurkat T and U937 cells were incubated with TRAIL in the presence or absence of 10 μg/ml LTβR.Fc. LTβR.Fc had no effect on TRAIL-induced cell death in either Jurkat T or U937 cells (Fig. 2A; data not shown for U937). It seems that the Fc-fusion protein-Fc receptor interaction did not promote TRAIL-initiated apoptosis, and LIGHT engagement did not contribute to TRAIL-triggered cell death. To further rule out the synergistic effects of Fc-Fc receptor binding on DcR3-augmented, TRAIL-induced apoptosis, a recombinant DcR3 without Fc portion, DcR3.FLAG, was tested. DcR3.FLAG was as effective as DcR3-Fc in enhancing TRAIL-triggered cell death in Jurkat and U937 cells (Fig. 2, C and D). Therefore, DcR3.Fc augments TRAIL-initiated apoptosis independent of Fc receptor. Because DcR3 binds to TL1A, we also examined the effect of DcR3.FLAG, the soluble receptor for TL1A, on sensitivity to TRAIL. DcR3.FLAG did not sensitize U937 and Jurkat cells to TRAIL-initiated apoptosis (Fig. 2B; data not shown for Jurkat cells). Binding to LIGHT or TL1A alone failed to reproduce the augmentative effect of DcR3. We screened other TRAIL-sensitive cells, and found that HeLa cells and SKW6.4 B lymphoma were tolerant to sensitization by DcR3 in TRAIL-mediated apoptosis (data not shown), indicating heterogeneity among tumor cells on DcR3 augmented TRAIL-induced apoptosis.

**DcR3 Does Not Affect Anti-Fas-induced and TNFα-induced Cell Death**—Fas, TRAIL receptor, and TNF receptor share the common death receptor apoptotic pathway (18–20). We examined whether cell death initiated by Fas and tumor necrosis factor receptor could be similarly increased by DcR3. The effect of DcR3 on Fas-induced apoptosis was studied using Fas agonistic antibody CH11 because FasL would be neutralized by DcR3. Treatment of Jurkat T cells with 10 μg/ml DcR3.Fc did not affect CH11-induced cell death (Fig. 3A). We also determined the regulatory effect of DcR3 on TNF-induced apoptosis. TNFα-sensitive L929 fibroblasts were tested, to avoid the use of protein synthesis inhibitors required for TNFα-induced apoptosis in many tumor cell lines. DcR3.Fc did not sensitize L929 cells to TNFα-induced cell death (Fig. 3B). Therefore, DcR3 increased apoptosis initiated by TRAIL but not those from anti-Fas and TNFα.

**DcR3 Does Not Enhance Surface DR5 Expression**—Augmentation of TRAIL-induced cell death through increased TRAIL receptor expression has been previously reported for ionization radiation, etoposide, Ara-C, doxorubicin, 5-fluorouracil, paclitaxel, and interferon-α (32, 35–40). To investigate whether DcR3 treatment up-regulates surface TRAIL receptors, the expression of surface TRAIL receptors were monitored. DR5 (TRAIL receptor 2) is the only TRAIL receptor expressed in both Jurkat and U937 cells (37, 41). Surface DR5 expression was unaffected by DcR3.Fc treatment for 2 h (Fig. 4, A and B) or for 20 h (data not shown) in Jurkat and U937 leukemia cells. Treatment of Jurkat and U937 cells with TRAIL resulted in a modest decrease in cell surface DR5 expression (Fig. 4, A and B), probably as a result of internalization of DR5 upon TRAIL engagement. Combined treatment of cells with TRAIL and DcR3.Fc did not alter surface DR5 expression relative to cells treated with TRAIL alone (Fig. 4, A and B). Therefore, DcR3 does not increase DR5 expression, suggesting an apoptotic enhancing mechanism distinct from that exhibited by chemotherapeutical agents on TRAIL-induced apoptosis.

**DcR3 Does Not Alter FADD and c-FLIP Expression**—We also examined the effect of DcR3 on the expression of other components along the death receptor apoptotic pathway. FADD is immediately downstream of TRAIL receptor. DcR3 treatment alone did not change FADD levels in Jurkat and U937 cells (Fig. 4C, right lane in each figure). FADD protein levels re-
TRAIL remained constant upon ligation of DR5 by TRAIL in Jurkat and U937 cells and were not further altered by co-incubating cells with DcR3 (Fig. 4). c-FLIP, another modulator of DR5-initiated apoptotic signals, binds to FADD and pro-caspase-8 and inhibits TRAIL receptor-mediated apoptosis (42). Down-regulation of c-FLIP has been reported to sensitize cell to TRAIL-induced cell death. A, Jurkat T cells and U937 cells were incubated with recombinant human TRAIL (1 ng/ml for Jurkat and 2 ng/ml for U937) in the presence or absence of 10 μg/ml DcR3.Fc for 18 h. Cell death assays were performed by propidium iodide staining, followed by flow cytometry for sub-G₁ fraction quantitation. B and C, effect of DcR3 on Jurkat and U937 cells from three independent experiments expressed in means ± S.D. D, dose-dependent enhancing effect of DcR3. U937 cells were treated with TRAIL (4 ng/ml) in the presence of the indicated amount of DcR3. Cell death was quantitated 18 h later.
induced cell death, while c-FLIP overexpression inhibits TRAIL-initiated apoptosis (43–45). DcR3 treatment alone did not affect c-FLIP levels, and co-incubation of DcR3 and TRAIL did not alter c-FLIP levels in Jurkat and U937 cells (Fig. 4). Therefore, the ability of DcR3 to promote TRAIL-induced apoptosis was not mediated by modulation of FADD/c-FLIP expression.

DcR3 Co-treatment Enhances Caspase-8 Activation and Bid Truncation—We then monitored different stages along the TRAIL-induced apoptotic pathway to examine whether any of the biochemical steps was enhanced by DcR3. Jurkat and U937 cells were treated with TRAIL in the presence or absence of DcR3. Caspase-8 is the apical caspase activated during TRAIL-induced apoptosis in a FADD-dependent manner (18, 19). Pro-caspase-8 remained unprocessed 1.5 h after TRAIL stimulation, and processing of pro-caspase-8 was evident 2.5 h after TRAIL treatment in Jurkat cells (Fig. 5A). Co-incubation of DcR3 greatly accelerated TRAIL-induced cleavage of pro-caspase-8, which was prominent 1.5 h after TRAIL treatment. It is noteworthy that DcR3 alone was unable to activate caspase-8 (Fig. 5A, right lane). A similar finding was observed in U937 cells (data not shown). Therefore, co-ligation of leukemia cells with TRAIL and DcR3 enhanced the activation of caspase-8.

One of the immediate downstream targets of caspase-8 is Bid, which is cleaved by caspase-8 and translocated into mitochondria (23, 24), providing a link between the DR apoptotic and mitochondrial apoptotic pathways. Consistent with an increased caspase-8 activation, there was a significant decrease in Bid and a concomitant increase in tBid when U937 cells were treated with a combination of TRAIL and DcR3 relative to cells treated with TRAIL alone (Fig. 5B). A similar enhanced processing of Bid was found in TRAIL-treated Jurkat cells in the presence of DcR3 (data not shown). In contrast, co-treatment of Jurkat T cells with DcR3 did not accelerate the Fas-mediated Bid cleavage (Fig. 5C). In summary, DcR3 enhanced TRAIL-induced, but not CH11-induced, caspase-8 activation and Bid processing.

DcR3 Enhances TRAIL-mediated Smac/DIABLO Release from Mitochondria—Recent studies reveal a requirement for the mitochondrial pathway, including Smac release, in TRAIL-induced apoptosis (21, 22). We examined whether DcR3 also promotes TRAIL-induced Smac release. U937 and Jurkat T cells were treated with TRAIL in the presence or absence of DcR3. Caspase-8 is the apical caspase activated during TRAIL-induced apoptosis in a FADD-dependent manner (18, 19). Pro-caspase-8 remained unprocessed 1.5 h after TRAIL stimulation, and processing of pro-caspase-8 was evident 2.5 h after TRAIL treatment in Jurkat cells (Fig. 5A). Co-incubation of DcR3 greatly accelerated TRAIL-induced cleavage of pro-caspase-8, which was prominent 1.5 h after TRAIL treatment. It is noteworthy that DcR3 alone was unable to activate caspase-8 (Fig. 5A, right lane). A similar finding was observed in U937 cells (data not shown). Therefore, co-ligation of leukemia cells with TRAIL and DcR3 enhanced the activation of caspase-8.
cells were treated with TRAIL in the presence or absence of DcR3, followed by fractionation into cytosolic (S-100) and heavy membrane fractions (mitochondria-enriched). The purity of S-100 extracts and mitochondrial-enriched fractions was monitored by the expression of cytoplasmic marker β-tubulin and mitochondrial marker Bcl-2, respectively (Fig. 6). TRAIL treatment resulted in the release of Smac from mitochondria into the cytoplasm, as shown by the appearance of Smac in cytosol (Fig. 6A), and a concomitant reduction in mitochondrial Smac (Fig. 6B) in both U937 and Jurkat cells. There was a significant increase in cytoplasmic Smac and a clear decrease in mitochondrial Smac in cells co-treated with DcR3 and TRAIL compared with cells treated with TRAIL alone. In contrast, DcR3 alone had no effect on Smac release (Fig. 6). These results illustrate that DcR3 co-treatment enhances TRAIL-mediated Smac release in Jurkat and U937 cells.

**DcR3 Co-treatment Promotes TRAIL-induced Cytochrome c Release and Caspase-9 Maturation**—We further examined whether cytochrome c release from mitochondria was affected by DcR3. Under the same conditions we used for studying Smac, a moderate amount of cytochrome c was detected in the S-100 fraction of U937 cells treated with TRAIL (Fig. 6A). Co-treatment of U937 cells with DcR3 and TRAIL greatly enhanced the release of cytochrome c into the cytoplasm. Likewise, TRAIL triggered a small release of cytochrome c in Jurkat cells (Fig. 6A), which was also strongly augmented by the addition of DcR3. The increased release of cytochrome c mediated by DcR3, with a predictable accelerated formation of apoptosome, was accompanied by enhanced caspase-9 activation in Jurkat cells (Fig. 7A). DcR3 alone had no effect on the level of pro-caspase-9. DcR3 did not modulate CH11-induced caspase-9 activation in Jurkat cells (Fig. 7A). Augmentation of TRAIL-initiated caspase-9 activation by DcR3 was also found in U937 cells (data not shown). TRAIL-induced cytochrome c release and caspase-9 activation was clearly enhanced by DcR3 co-stimulation.

**DcR3 Co-treatments Enhance TRAIL-induced Caspase-3 Maturation**—The above studies demonstrate that DcR3 increased TRAIL-mediated caspase-8 activation, tBid truncation (Fig. 7B and C) and caspase-9 activation. We went on to determine whether activation of the effector caspases was indeed enhanced by DcR3. The cleavage of poly-(ADP-ribose) polymerase, a caspase-3 specific substrate, was monitored in TRAIL-treated Jurkat cells with or without DcR3. The cleavage of poly-(ADP-ribose) polymerase cleavage was significantly increased in the presence of DcR3 in Jurkat cells (Fig. 7B) and in U937 cells (data not shown). In comparison, CH11-activated caspase-3 activity was not changed by DcR3 co-treatment of Jurkat cells. Our results indicate that the enhanced upstream apoptotic events observed on DcR3-co-stimulated cells did lead to increased effector caspase activation stimulated by TRAIL but not by CH11.
In the present study, we found that unexpectedly DcR3 could enhance TRAIL-induced apoptosis. The augmentative effect of DcR3 was most prominent at suboptimum apoptotic doses of TRAIL (Fig. 1). We examined various apoptotic processes downstream of TRAIL receptor and found that DcR3 enhanced TRAIL-mediated caspase-8 activation, Bid truncation, release of Smac and cytochrome c, and caspase-3 activation. In addition, DcR3 by itself triggered none of the apoptotic events (Figs. 5–7).

TRAIL receptor genes are linked to sensitivity to TRAIL in different cancer patients (31). The surface expression of DR5, the sole functional TRAIL receptor in Jurkat and U937 cells (37, 41), was not affected by incubation with DcR3 (Fig. 4A). This is in contrast to the enhanced TRAIL-induced apoptosis mediated by increased DR4/DR5 expression reported for /H9253-irradiation, chemotherapeutical agents, and interferon-/H9251 (32, 35–40). It is noteworthy that /H9253-irradiation, chemotherapeutical agents, or interferon-/H9251 alone is sufficient to up-regulate TRAIL receptor expression, mediated by either activation of transcription factors specific for DR4/DR5 gene promoters or enhanced synthesis of DR4/DR5 protein (35, 37, 38). On the contrary, neither DR5 expression nor any of the apoptotic processes downstream of DR were regulated by DcR3 alone (Figs. 4–7). The inability of DcR3 to increase DR4/DR5 expression also suggests cells ligated by DcR3 apparently transmit signaling distinct from those by cytotoxic drugs and interferon-a.

c-FLIP is another molecule linked to the sensitivity to TRAIL-induced apoptosis (31, 43–45). c-FLIP overexpression in cancer cells confers resistance to TRAIL, whereas c-FLIP down-regulation promotes TRAIL-triggered cell death. However, DcR3 treatment did not alter the levels of c-FLIP (Fig. 4B). c-FLIP expression therefore does not contribute to the augmentative apoptotic effect of DcR3.

All the apoptotic processes downstream of DR5 were enhanced by DcR3 in the presence of TRAIL stimulation. The observations that DcR3 increased TRAIL-mediated caspase-8 activation and Bid truncation (Fig. 5) suggest that DcR3 promotes apoptotic events proximal to DR5 engagement. Because DcR3 did not affect DR5 expression and c-FLIP expression, a stage likely to be regulated by DcR3 is the formation of death-inducing signaling complex, a process that is probably enhanced by cytoskeleton mobilization. In this context, DcR3 has been shown to exhibit a profound effect on cell mobility. DcR3 suppresses phagocytosis of macrophages (15) and increases adhesion of monocytes (14). DcR3 induces angiogenesis of endothelial cells by neutralization of TL1A (9). DcR3 also inhibits T cell pseudopodium formation and represses T cell chemotaxis (12, 13). DcR3 interaction seems to induce cytoskeleton reorganization of the targeted cells. It is therefore plausible to postulate that death-inducing signaling complex formation is enhanced by DcR3-induced actin mobilization in the presence of TRAIL engagement.

DcR3 binds to FasL, LIGHT, and TL1A. FasL expression has not been undetectable on the cell surface of untreated Jurkat cells (46) and U937 cells (data not shown) and is thus unlikely...
to participate in the modulatory effect of DcR3 observed here. LTβR.Fc did not regulate TRAIL-induced apoptosis in Jurkat and U937 cells (Fig. 2), indicating that LIGHT is not involved in the enhanced TRAIL-induced apoptosis seen in this study. DcR3.FLAG also failed to mimic the stimulatory activity of DcR3 (Fig. 2), suggesting that TL1A does not participate in this enhanced TRAIL-mediated cell death. Therefore, any interaction of DcR3 with FasL, LIGHT, or TL1A alone cannot account for the enhancement of TRAIL-initiated apoptosis. However, we cannot exclude the possibility that the observed effect of DcR3 was caused by a simultaneous interaction of DcR3 with two or more ligands on the cell surface of leukemia cells. On the other hand, augmented TRAIL-mediated cell death could be caused by cross-linking of an as-yet-unidentified DcR3 ligand (14, 15). These possibilities are currently being examined.

IAP proteins inactivate caspase-3 through the binding of the linker segment N terminus to the BIR2 domain of IAP proteins to the catalytic site of caspase-3 (47). IAP proteins may also prevent the complete processing of caspase-3 mediated by caspase-8 and caspase-9 (48). The binding of Smac to the BIR2 domain of IAP proteins interferes with the interaction of IAP to caspase-3 and relieves the inhibitory effect of IAP on caspase-3. Previous studies suggest that release of Smac into cytosol, but not release of cytochrome c and activation of caspase-9, plays a major role in TRAIL-induced caspase-3 activation and cell death (22). In the present study, we found DcR3 co-treatment promoted an increased release of Smac from mitochondria to cytoplasm (Fig. 6). The elevated level of Smac could be a consequence of enhanced caspase-8 activation and accelerated Bid processing (Fig. 5) by DcR3 co-treatment (22). However, the possibility that DcR3 cell binding triggers Bid-independent mitochondrial events that lead to Smac release cannot be completely excluded in the present study.

Fas apoptotic signals are known to be distinct between type I and type II cells (49). In type I cells, Fas ligation triggers caspase-8 generation sufficient for downstream effector caspase activation and cell-killing independent of mitochondria. In type II cells, Fas-induced apoptotic signals requires amplification through a mitochondrial pathway mediated by caspase-8 truncation of Bid. The presence of type I and type II cells in response to TRAIL has also been documented (50). In the present study, we found that DcR3 increased TRAIL-induced apoptosis in Jurkat and U937 cells but not in HeLa and SKW6.4 cells. Whether different DcR3 sensitivity between these two groups of cells is correlated with classification of type I and II cells remains unknown. Jurkat is a type II cell for Fas-induced apoptosis, yet is not a type I cell for TRAIL-triggered apoptosis (51). The linkage between the ability of DcR3 to promote TRAIL-mediated apoptotic processes and the dependence of target cells on mitochondrial pathways is still being determined.

DcR3 levels are elevated in many types of tumors. A likely function of DcR3 overexpression in tumor cells could be to reduce the cytotoxic attack from two TNF family members, FasL and LIGHT, leading to increased cell survival. The observation that DcR3 augmented cell death initiated by TRAIL, another TNF family member, is intriguing. TRAIL is a promising anticancer reagent, because it effectively triggers apoptosis in many tumor cells but leaves normal cells untouched. The ability of DcR3 to increase TRAIL-triggered cell death in selected T leukemia cell lines suggest the possibility that antitumor TRAIL could be further enhanced in cancer cells overexpressing DcR3. Such feasibility deserves further exploration.

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