Tumor Necrosis Factor-related Apoptosis-inducing Ligand’s Antitumor Activity in Vivo Is Enhanced by the Chemotherapeutic Agent CPT-11

Brian Gliniak1 and Tiep Le

Department of Molecular Immunology, Immunex Corp., Seattle, Washington 98101

ABSTRACT

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) can induce apoptosis in a wide variety of transformed human cells in vitro. In this study, the antitumor activity of recombinant TRAIL was analyzed in mice bearing human colon carcinoma tumors. We found that these tumors displayed a differential sensitivity to TRAIL in vivo that paralleled their susceptibility to TRAIL-induced apoptosis in vitro. Treatment of TRAIL-sensitive tumors 3 days after tumor challenge resulted in a dose-dependent inhibition of growth and the elimination of tumors in many mice. Colon carcinoma cell lines could be further sensitized to TRAIL-induced apoptosis in vitro by the addition of the chemotherapeutic agent camptothecin. Moreover, the combination of TRAIL and CPT-11, a water-soluble analogue of camptothecin, greatly enhanced the antitumor activity of TRAIL in vivo. TRAIL plus CPT-11 treatment of both 3- and 10-day established TRAIL-sensitive tumors resulted in both a significant inhibition of tumor growth and a high proportion of complete tumor regressions. Treatment of TRAIL-resistant tumors with TRAIL and CPT-11 dramatically slowed tumor growth and induced a transient tumor regression. These data demonstrate that TRAIL alone is a potent antitumor agent in vivo, and its activity can be significantly enhanced in combination with the chemotherapeutic agent CPT-11.

INTRODUCTION

The ability of TRAIL2 to induce apoptosis in a wide variety of human transformed cells in vitro has been well documented (1–3). Transformed cells killed by TRAIL in vitro include those derived from breast, colon, skin, prostate, and various hematopoietic malignancies. Whereas many of these transformed cells are resistant to cell killing by other members of the TNF ligand superfamily, a majority of these tumor cell lines can be killed by TRAIL without any prior sensitization. For example, a panel of human melanomas was uniformly resistant to killing by TNF-α, CD40 ligand, and FAS ligand, but TRAIL-induced apoptosis in a majority of the cell lines (4). Likewise, human colon carcinoma cell lines were found to be resistant to TNF-α-induced apoptosis, but several of the cell lines were very sensitive to TRAIL-induced apoptosis.3

The ability of TRAIL to induce apoptosis in many transformed cells in vitro suggests that it might be a potent antitumor agent in vivo. To study this experimentally, a soluble form of TRAIL that includes a leucine zipper incorporated at its NH2 terminus to promote the formation and stabilization of TRAIL trimers was generated (5). Analysis of the LZ-huTRAIL molecule in mice has demonstrated that it is not overtly toxic at therapeutic doses and maintains its antitumor activity in vivo (5). Specifically, multiple treatments with LZ-huTRAIL suppress the growth of the TRAIL-sensitive human mammary adenocarcinoma cell line MDA-231 in CB.17 (SCID) mice and lengthen their mean survival times. Histological analysis of the LZ-huTRAIL-treated tumors demonstrates an increase in apoptotic necrosis and confirms the ability of LZ-huTRAIL to induce apoptosis in vivo (5). Likewise, treatment of two human colon carcinoma xenografts with LZ-huTRAIL prevents tumor formation in a majority of treated animals and dramatically slows tumor growth in tumor-bearing animals (5).

Analysis of TRAIL-induced apoptosis in vivo has demonstrated that there are both TRAIL-sensitive and TRAIL-resistant human melanoma and colon carcinoma cell lines (4).3 The reason for the differential sensitivity remains unknown, but it is not regulated solely by the differential expression of the known TRAIL receptors (4).3 Instead, it appears that an intracellular inhibitor(s) acting downstream of the TRAIL receptors renders specific transformed cell lines insensitive to TRAIL (4). Treatment of TRAIL-resistant cell lines with metabolic inhibitors of protein synthesis can convert them to TRAIL-sensitive cell lines (4),3 suggesting that the antitumor activity of TRAIL may be enhanced in vivo by combining it with chemotherapeutic agents that are known disrupt a transformed cell’s metabolism or mitotic activity. In support of this, it was recently shown that the combination of doxorubicin or 5-FU with TRAIL could augment TRAIL-induced apoptosis in breast cancer cells in vitro (6).

In this report, we have further characterized the in vivo antitumor activity of TRAIL, both alone and in combination with the chemotherapeutic agent CPT-11. We demonstrate that the sensitivity to TRAIL seen in vivo for the colon carcinoma tumors parallels the differential sensitivity to TRAIL-induced apoptosis seen in vitro for these cell lines. Moreover, by combining TRAIL with CPT-11, the antitumor activity of TRAIL is greatly enhanced and results in the complete elimination of tumors in many animals.

MATERIALS AND METHODS

Cell Culture. Cells were obtained from the American Type Culture Collection (Manassas, VA) and cultured as suggested by the manufacturer. Briefly, HT-29 cells were grown in DMEM supplemented with 10% FBS, SW620 cells were grown in DMEM:Ham’s F-12 plus 10% FBS, HCT-15 cells were grown in RPMI 1640 plus 20% FBS, and COLO 205 cells were grown in RPMI 1640 plus 10% FBS. All media were supplemented with 100 μg/ml streptomycin and penicillin.

Purification of LZ-huTRAIL. Expression and purification of LZ-huTRAIL was performed as described previously (5, 7). Purified fractions containing recombinant proteins were pooled and dialyzed against TBS, and aliquots were stored at −70°C. Protein concentrations were determined by amino acid analysis, and endotoxin content was determined by Limulus Ameboocyte Lysate analysis. The endotoxin content of LZ-huTRAIL used in the studies was less than 9 pg/mg recombinant protein.

Cell Viability Assays. Cells were plated at 40,000 cells/well in 96-well plates and allowed to attach overnight. Members were added at the indicated concentration, and the cells were cultured at 37°C for 24 h. Camptothecin (Sigma Chemical Co., St. Louis, MO) was diluted in DMSO, and all cultures not receiving camptothecin received an equivalent amount of DMSO. Cell viability was measured by crystal violet staining and quantified by reading the A490 nm as described previously (1). The percentage viability was calculated by multiplying the ratio staining of experimental versus control cultures by 100.

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1 To whom requests for reprints should be addressed, at Department of Molecular Immunology, Immunex Corp., 51 University Street, Seattle, WA 98101. Phone: (206) 587-0430, ext. 4661; Fax: (206) 233-9733; E-mail: gliniak@immunex.com.

2 The abbreviations used are: TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; TNF, tumor necrosis factor; SCID, severe combined immunodeficient; LZ-huTRAIL, leucine zipper human TRAIL; FBS, fetal bovine serum; TBS, Tris-buffered saline; 5-FU, 5-fluorouracil.

3 B. Gliniak, T. Le, and T. Griffith. TRAIL receptor expression by human colon carcinoma cells is not predictive of sensitivity to TRAIL-induced apoptosis, submitted for publication.
Treatment of Tumor-bearing Mice with LZ-huTRAIL. Female CB.17 (SCID) mice (Taconic Farms, Germantown, NY) were pretreated 24 h before tumor challenge with a single injection (100 µl, i.p.) of purified asialo GM-1 antibody (Wako Chemicals, Richmond, VA). Mice were injected s.c. with 3 x 10^6 human colon carcinoma cells, and treatment began 3, 10, or 17 days after tumor implantation, as noted. Treatments with TBS or LZ-huTRAIL were administered by i.p. injection, and CPT-11 (Pharmacia and Upjohn Co., Kalamazoo, MI) was administered i.v. as described in the text. All dilutions of LZ-huTRAIL and CPT-11 were made with TBS. For the 10- and 17-day established tumor study, changes in tumor size were calculated as follows: [(Tumor size posttreatment) - (Tumor size at day 10 or 17)]/[(Tumor size at day 10 or 17)] x 100%.

Statistical Analysis of Data. Tumor growth analyses were performed by ANOVA, with P values obtained by t test. Only tumor-bearing mice were included in the analysis. Analysis of tumor growth rates was performed using a generalized linear model, with P values obtained via likelihood ratio x^2 tests. All statistical analyses were performed using SAS software (SAS Institute Inc., Cary, NC).

RESULTS

Human Colon Carcinoma Tumors Show a Differential Sensitivity to LZ-huTRAIL in Vivo. Previous work has shown that human colon carcinoma cell lines are differentially sensitive to LZ-huTRAIL-induced apoptosis in vitro. To determine the antitumor activity of LZ-huTRAIL in vivo, tumors derived from human colon carcinoma xenografts were analyzed for their sensitivity to LZ-huTRAIL (Fig. 1). Beginning 3 days after tumor challenge, mice were treated with LZ-huTRAIL (500 or 1000 µg) or a control solution (TBS) for 14 days. Consistent with the in vitro findings, tumors derived from COLO 205 and HCT-15 cells were very sensitive to LZ-huTRAIL treatment (Fig. 1A). The growth rate for both tumors was significantly reduced in the LZ-huTRAIL-treated groups compared with that of control mice. Treatment of COLO 205 tumors with 500 or 1000 µg of LZ-huTRAIL resulted in a 74% and 93% reduction in tumor size, respectively (P = 0.004 and 0.0012, respectively, by ANOVA). Likewise, treatment of HCT-15 tumors with 500 µg of LZ-huTRAIL resulted in a >50% reduction in tumor size (P = 0.03). Moreover, a dose-dependent inhibition of COLO 205 tumor formation was also observed. Treatment of COLO 205 tumor-bearing mice with 500 µg/day LZ-huTRAIL resulted in a 90% incidence of tumor formation 6 weeks after tumor challenge, whereas treatment with 1000 µg/day LZ-huTRAIL resulted in a 30% incidence of tumor formation (P = 0.0002, using a generalized linear model). For HCT-15 tumor-bearing mice, the number of tumor-positive animals at 6 weeks was also significantly less (60%; P = 0.03) after treatment with 500 µg/day of LZ-huTRAIL. In contrast, tumors derived from the HT-29 and SW620 cell lines showed less sensitivity to LZ-huTRAIL (Fig. 1B). HT-29 tumor growth was reduced slightly (~25%) after LZ-huTRAIL treatment in comparison with TBS-treated animals (P = 0.007), and SW620 tumor growth was not significantly affected (P = 0.23). In addition, no difference in the frequency of HT-29 or SW620 tumor formation was observed between the treated and untreated groups. Thus, the sensitivity of these four human colon carcinomas to LZ-huTRAIL in vivo appears to closely parallel their susceptibility to LZ-huTRAIL-induced apoptosis in vitro.

Camptothecin Enhances LZ-huTRAIL Cytotoxic Activity in Vitro. Treatment of colon carcinoma lines with the transcription inhibitor actinomycin D enhances the cytotoxic activity of LZ-huTRAIL in vitro and converts the LZ-huTRAIL-resistant cell lines to LZ-huTRAIL-sensitive cell lines. Similar results have been shown using metabolic inhibitors on human melanoma lines (4). These findings suggest that the antitumor activity of LZ-huTRAIL might be enhanced in vivo by combining it with chemotherapeutic agents that are capable of disrupting a transformed cell’s metabolism or mitotic activity. To first test this on the colon carcinoma lines in vitro, a variety of chemotherapeutic agents were assayed for their ability to synergize with LZ-huTRAIL. Combining LZ-huTRAIL with cisplatin, 5-FU, mitomycin, etoposide, or Adriamycin did not result in any enhancement of cytotoxic activity in vitro (data not shown). In contrast, camptothecin was found to be a potent cytotoxic agent both alone and in combination with LZ-huTRAIL.

Camptothecin is a topoisomerase I inhibitor that has antitumor activity in vitro and in vivo (8–11). The addition of camptothecin (1 µg/ml) to colon carcinoma cell lines in vitro reduced the cell viability of all four cell lines by 40–60% within 24 h (Fig. 2). Incubation for 48 h resulted in a complete loss of cell viability (data not shown). Combining camptothecin with LZ-huTRAIL converted the LZ-huTRAIL-resistant cell lines, HT-29 and SW620, into LZ-huTRAIL-sensitive cell lines. Likewise, the LZ-huTRAIL-sensitive lines, COLO 205 and HCT-15, became more sensitive to LZ-huTRAIL-induced apoptosis in combination with camptothecin. These results are similar to those seen with the metabolic inhibitors actinomycin D and cyclohexamide and confirm that a chemotherapeutic agent has the potential to enhance the cytotoxic activity of LZ-huTRAIL.

CPT-11 Enhances the Antitumor Activity of LZ-huTRAIL in Vivo. The synergism observed with LZ-huTRAIL plus camptothecin in vitro suggested that a similar combination in vivo might enhance the...
antitumor activity of LZ-huTRAIL. To study this, LZ-huTRAIL was combined with CPT-11, a water-soluble analogue of camptothecin. CPT-11 has a broad range of activity against a variety of human tumors in vivo, including several human colon carcinomas (12–17). Analysis of CPT-11 pharmacokinetics has shown that it is converted into its active form, SN38, in mouse serum and then cleared within a few hours (18, 19). Consistent with this observation, multiple low doses of CPT-11 are more effective than a single high dose (12–15). To test the combination therapy of LZ-huTRAIL plus CPT-11 in vivo, LZ-huTRAIL was administered as described previously (Fig. 1), and CPT-11 was administered by i.v. injection six times during the LZ-huTRAIL treatment period. In these experiments, we focused on the LZ-huTRAIL-resistant HT-29 and LZ-huTRAIL-sensitive COLO 205 tumors.

Consistent with the previous analysis (Fig. 1B), treatment of HT-29 tumors with LZ-huTRAIL alone slowed their growth slightly but did not result in any tumor regressions (Fig. 3A). Administration of CPT-11 alone resulted in a dose-dependent inhibition of HT-29 tumor growth, with six doses of CPT-11 at 25 mg/kg/dose resulting in ~50% reduction in tumor size, and six doses of CPT-11 at 50 mg/kg/dose resulting in ~75% reduction. For both treatments, the incidence of tumor formation was 100%. The combination of CPT-11 plus LZ-huTRAIL resulted in an additional inhibition of tumor growth, but the difference was not significantly greater than that observed with 25 or 50 mg/kg CPT-11 alone (P = 0.204 and 0.262, respectively). Thus, it appears that the treatment of HT-29 tumors with a combination of CPT-11 and LZ-huTRAIL results in an additive enhancement of antitumor activity.

Whereas the combination treatment of LZ-huTRAIL plus CPT-11 did not ultimately inhibit tumor formation at 6 weeks after tumor challenge, LZ-huTRAIL plus 50 mg/kg CPT-11 did dramatically inhibit tumor formation during and shortly after the treatment period (Fig. 3B). Control animals and those treated with LZ-huTRAIL alone were 100% positive for measurable tumors within 7 days of tumor challenge. Likewise, 90–100% of the animals treated with 50 mg/kg CPT-11 alone were tumor positive within the first 4 weeks. In contrast, LZ-huTRAIL plus CPT-11-treated animals were 70% tumor positive by the first week but had no measurable tumors at 3 weeks (4 days after treatment). However, these animals were not completely tumor free, and by 5 weeks, all of them had measurable tumors, albeit significantly smaller than those in untreated animals (Fig. 3A). Thus, treatment with LZ-huTRAIL and high-dose CPT-11 could induce a transient regression of LZ-huTRAIL-resistant HT-29 tumors, but tumor growth resumed with the cessation of treatment.

Results of the LZ-huTRAIL-sensitive COLO 205 tumor treated with LZ-huTRAIL and CPT-11 are shown in Fig. 4. The administration of either LZ-huTRAIL (500 μg) or CPT-11 (50 mg/kg) alone significantly inhibited the growth of COLO 205 tumors (P = 0.003 and 0.001, respectively) and induced tumor regression in 5 of 10 mice and 6 of 9 mice, respectively (Fig. 4A). The combination of LZ-huTRAIL and CPT-11 was even more effective, with all of the treated animals being tumor free 6 weeks after tumor challenge. However, in the CPT-11 treatment groups, with or without LZ-huTRAIL, animal mortality was observed during the administration of the drug. Thus, CPT-11 and LZ-huTRAIL synergized to eliminate COLO 205 tumors in all of the surviving animals, but the 50 mg/kg dose of CPT-11 proved toxic for some of the treated animals.

Multiple dosing of CPT-11 induces many toxicities (14, 15). In our...
The treatment of COLO 205 tumor-bearing mice with LZ-huTRAIL and/or CPT-11 resulted in many tumor-free animals after 6 weeks. To determine whether the tumors were completely eliminated, the animals were examined 9 and 12 weeks after tumor challenge (Table 1). The tumor-free animals treated with only CPT-11, 250 µg/day LZ-huTRAIL, or 250 µg/day LZ-huTRAIL plus low-dose CPT-11 (25 mg/kg) all developed measurable tumors within 12 weeks. In contrast, the majority of animals treated with 500 µg/day LZ-huTRAIL, alone or in combination with CPT-11, remained tumor free at 12 weeks. These results suggest that high-dose LZ-huTRAIL (500 µg/day) treatment, alone and in combination with CPT-11, can induce the complete elimination of COLO 205 tumors.

LZ-huTRAIL plus CPT-11 Induces Regression of Established LZ-huTRAIL-sensitive Tumors. The treatment protocol used in the previous experiments started LZ-huTRAIL and/or CPT-11 treatment 3 days after tumor challenge. To analyze whether LZ-huTRAIL and CPT-11 could inhibit the growth of more established tumors, treatments were started at either 10 or 17 days after implantation of COLO 205 cells (Fig. 5). At 10 days after tumor challenge, LZ-huTRAIL (500 µg/day) was administered from day 10–23 (14 days), and six injections of CPT-11 were given at 25 or 40 mg/kg during the LZ-huTRAIL treatment period (Fig. 5A). The combination of LZ-huTRAIL and CPT-11, either...

eperiments, CB.17 (SCID) mice did not tolerate more than six injections of CPT-11 at 50 mg/kg over a 2-week period. As seen with the above experiments at this dosing level, this results in a mortality rate of ~10%. To determine whether a lower dose of CPT-11 could still synergize with LZ-huTRAIL, CPT-11 at 25 mg/kg was combined with 250 or 500 µg of LZ-huTRAIL. (Fig. 4B). The administration of LZ-huTRAIL (250 or 500 µg/day) or 25 mg/kg CPT-11 alone resulted in tumor growth inhibition, but with all treatments, ≥90% of mice were tumor positive 6 weeks after tumor challenge. As seen with the 50 mg/kg CPT-11 dose, the combination of LZ-huTRAIL (250 or 500 µg/day) plus 25 mg/kg CPT-11 resulted in a much greater tumor growth inhibition than that seen with either agent alone. Moreover, 5 of 10 animals treated with 250 µg/day LZ-huTRAIL and CPT-11 (P = 0.0041) were tumor free, and 18 of 19 animals treated with 500 µg/day LZ-huTRAIL plus CPT-11 (P = 0.0001) were tumor free 6 weeks after tumor challenge. These results demonstrate that multiple dosing of CPT-11 at 25 mg/kg is well tolerated (only 2% mortality) and can still synergize with LZ-huTRAIL at two different concentrations and induce tumor regression in the majority of treated animals.
Table 1  Treatment of COLO 205 tumor-bearing mice with TRAIL (500 µg) or TRAIL (500 µg) plus CPT-11 results in long-term tumor regression

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tumor-free micea</th>
<th>Treated miceb surviving mice</th>
<th>6 wks</th>
<th>9 wks</th>
<th>12 wks</th>
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<tr>
<td>25 mg/kg CPT-11</td>
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<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>50 mg/kg CPT-11</td>
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<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>TRAIL (250 µg)</td>
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<td>5</td>
<td>3</td>
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<tr>
<td>TRAIL (500 µg)</td>
<td>20/20</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>TRAIL (500 µg) + 25 mg/kg CPT-11</td>
<td>20/19</td>
<td>18</td>
<td>18</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>TRAIL (500 µg) + 50 mg/kg CPT-11</td>
<td>10/7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>0</td>
</tr>
</tbody>
</table>

a Number of mice beginning treatment.

b Surviving mice at 6 weeks after tumor implantation.

c Tumor-free animals resulting from treatment with TRAIL and/or CPT-11 at 6 weeks after tumor implantation (Fig. 4) were examined at 9 and 12 weeks for tumor development.

DISCUSSION

In this report, we have analyzed four human colon carcinoma cell lines for their sensitivity to the cytotoxic activity of TRAIL in vivo. Previous work using LZ-huTRAIL verified that this recombinant form of the molecule retains its activity in vivo (5). Using this form of TRAIL, we demonstrate that the colon carcinoma-derived tumors display a differential sensitivity to TRAIL in vivo. The sensitivity of these tumors in vivo parallels their susceptibility to TRAIL-induced apoptosis in vitro.

For the TRAIL-sensitive tumors, a dose-dependent antitumor activity was observed for both tumor growth inhibition and complete tumor regressions. In contrast, treatment of TRAIL-resistant tumors did not significantly slow tumor growth or result in the elimination of any tumors. Thus, consistent with in vitro observations, tumors derived from the same tissue type display a differential sensitivity to TRAIL in vivo.

The regulatory mechanism that governs sensitivity to TRAIL-induced apoptosis remains unknown. However, it has been observed that the resistance to TRAIL-induced apoptosis can be overcome in vitro by treating the cells with metabolic inhibitors (4). This led us to examine a variety of chemotherapeutic agents for their ability to enhance TRAIL-induced tumor apoptosis. Although cisplatin, mitomycin, and 5-FU did show a dose-dependent cytotoxicity alone, none showed any synergy with TRAIL in vitro (data not shown). One agent, Adriamycin, did demonstrate a weak synergy with TRAIL in vitro, but no enhancement of TRAIL’s cytotoxicity was seen in vivo (data not shown). Of all of the chemotherapeutic agents we tested in the colon carcinomas, only the topoisomerase I inhibitor camptothecin was found to be a potent cytotoxic agent both alone and in combination with TRAIL.

Treating tumor-bearing mice with TRAIL plus CPT-11, a water-soluble derivative of camptothecin, resulted in a dramatic enhancement of the antitumor activity of TRAIL. Treatment of 3- or 10-day established COLO 205 tumors with TRAIL and CPT-11 resulted in both a dose-dependent reduction in tumor growth rate and the elimination of tumors in many of the treated animals. Analysis of these animals for 12 weeks confirmed that this treatment resulted in tumor-free animals. In contrast, none of the tumors allowed to establish for 17 days before treatment were eliminated, but a transient shrinkage (>50%) of tumor mass was observed. Likewise, combination treatment of TRAIL-resistant HT-29 tumors resulted in a greater tumor inhibition than observed with either agent alone. TRAIL plus high-dose CPT-11 reduced the size of HT-29 tumors >85% compared with the untreated controls and induced a transient tumor regression after treatment. However, all animals were eventually tumor positive 6 weeks after tumor challenge.
How camptothecin/CPT-11 synergizes with TRAIL at the cellular level remains unknown. Camptothecin has been shown to be an inhibitor of the nuclear enzyme topoisomerase I and is believed to block DNA transcription and replication through the inhibition of this enzyme (10, 11). Presumably, camptothecin/CPT-11 synergizes with TRAIL in a manner similar to that of actinomycin D and cycloheximide by ultimately inhibiting the synthesis of an apoptosis-regulatory protein. Recently, other chemotherapeutic agents have been shown to sensitize tumor cells to TRAIL-induced apoptosis in vitro. Keane et al. (6) demonstrated that both doxorubicin (Adriamycin) and 5-FU could augment TRAIL-induced apoptosis of breast cancer cells in vitro, and this was mediated through the selective activation of caspases by these drugs. Alternatively, Wu et al. (20) demonstrated that TRAIL receptor 2 (KILLER/DR5) expression is up-regulated after doxorubicin-induced DNA damage of transformed human cells. However, it remains to be proven whether the higher level of receptor expression makes these cells more sensitive to TRAIL. Treatment of the colon carcinoma cell lines in vitro with camptothecin did not result in the up-regulation of TRAIL receptor 2 expression (data not shown). Taken together, these results demonstrate that a variety of chemotherapeutic drugs can modulate the TRAIL-induced apoptosis signaling pathway and suggest that other agents may synergize with TRAIL in vivo.

Although TRAIL is a potent inducer of apoptosis in vitro, the administration of multiple doses of LZ-huTRAIL to mice was very well tolerated, both alone or in combination with CPT-11. This is not unexpected because transcripts for TRAIL and TRAIL receptors are abundantly expressed in many tissues (1, 2, 7, 21–23), suggesting that TRAIL-induced apoptosis is tightly regulated in normal cells and/or that TRAIL may have additional activities other than cell killing in vivo. These findings are consistent with previous in vivo studies that demonstrate no systemic toxicities in mice after repeated doses of either murine or human LZ-TRAIL (5). This apparent lack of toxicity associated with the administration of TRAIL is in direct contrast to other members of the TNF ligand superfamily whose utilization in vivo is limited by their toxicities (24–26).

Combining TRAIL with CPT-11 may have great clinical potential. CPT-11 is currently being tested clinically and has been shown to be consistently effective in metastatic colorectal cancers (27–29). However, myelosuppression and gastrointestinal damage are the two primary dose-limiting toxicities associated with CPT-11 (28, 29). An exciting outcome from our study is the observation that suboptimal doses of both TRAIL and CPT-11 can synergize to induce a strong antitumor activity. This suggests that a potent antitumor response may still be achieved by combining TRAIL with a better-tolerated dose of a chemotherapeutic agent. Thus, TRAIL may prove to be a potent antitumor agent alone and may enhance the antitumor potential of traditional chemotherapeutic drugs.

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Brian Gliniak and Tiep Le


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