Enhancement of Lexatumumab-Induced Apoptosis in Human Solid Cancer Cells by Cisplatin in Caspase-Dependent Manner

Xiu-Xian Wu1,2 and Yoshiyuki Kakehi1

Abstract

Purpose: This study was designed to evaluate the apoptotic effect of mapatumumab or lexatumumab, human agonistic antibodies that target the tumor necrosis factor–related apoptosis-inducing ligand receptor 1 (TRAIL-R1) and receptor 2 (TRAIL-R2), in combination with chemotherapeutic agents, against human solid cancer cells.

Experimental Design: Cytotoxicity was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Synergy was assessed by isobolographic analysis.

Results: Treatment of ACHN human renal cell carcinoma cells with cisplatin combined with mapatumumab did not overcome resistance to these agents. However, treatment with cisplatin in combination with lexatumumab had a synergistic cytotoxicity. Synergy was also achieved in six primary renal cell carcinoma cell cultures. Lexatumumab and cisplatin also synergistically enhanced apoptosis. Pretreatment with cisplatin followed by lexatumumab resulted in high cytotoxicity compared with the reverse sequence. Cisplatin significantly increased TRAIL-R2 expression at both the mRNA and the protein levels. Furthermore, the combination of lexatumumab and cisplatin significantly enhanced caspase-8 activity, Bid cleavage, up-regulation of Bax, cytochrome c release, and caspase-9, caspase-6, and caspase-3 activities. Importantly, the activation of caspase-8 was significantly abrogated by the specific inhibitors of caspase-9, caspase-6, and caspase-3. Furthermore, combination-induced cytotoxicity was significantly suppressed by the DR5:Fc chimeric protein and the specific inhibitors of caspase-8, caspase-9, caspase-6, and caspase-3. A similar effect was observed in prostate cancer, bladder cancer, lung cancer, and cervical cancer cells.

Conclusions: Cisplatin sensitizes solid cancer cells to lexatumumab-induced apoptosis by potentiation of the extrinsic and intrinsic apoptotic pathways that lead to amplification of caspase activation, particularly caspase-8, suggesting the combination treatment of solid cancers with cisplatin and lexatumumab might overcome their resistance.

The tumor necrosis factor–related apoptosis-inducing ligand (TRAIL), a member of the tumor necrosis factor family, has potential as an effective anticancer agent, because it selectively induces apoptosis in a variety of tumor cells, yet is relatively nontoxic to normal cells (1, 2). TRAIL triggers apoptosis by binding to two death receptors: TRAIL-R1 (DR4) and TRAIL-R2 (DR5; ref. 3). The activation of these death receptors results in a signal transduction cascade that initiates both intrinsic and extrinsic apoptotic pathways (4). In addition, TRAIL binds to two other receptors, TRAIL-R3 (DrC1) and TRAIL-R4 (DrC2), which lack a functional cytoplasmic death domain (3). These decoy receptors have been proposed to inhibit TRAIL-induced apoptosis. Therefore, developing agonistic monoclonal antibodies (mAb) to TRAIL-R1 and TRAIL-R2 may avoid competitive interaction with decoy receptors (5).

It was reported that mouse or rabbit mAbs to human TRAIL-R1 or TRAIL-R2 have antitumor activities in vitro and in vivo (6, 7). These agonistic antibodies work by activating TRAIL-mediated apoptotic pathways in a manner similar to TRAIL (8). It was also reported that mapatumumab (HGS-ETR1), a fully human agonistic mAb specific for TRAIL-R1, reduced the viability of multiple types of tumor cells in vitro and inhibited tumor growth in vivo (9). We recently reported that lexatumumab (HGS-ETR2), a human agonistic TRAIL-R2 mAb, induced apoptotic cell death in renal cell carcinoma cells (10).

The results of phase I and II clinical trials using mapatumumab orlexatumumab have shown good compatibility and only mild nonspecific toxicity in patients with advanced cancers (11–13). Although these clinical trials are promising, it is recognized that only 29% to 39% of patients had stable disease among the patients who received mapatumumab or lexatumumab therapy. Therefore, developing ways to optimize the effects of mapatumumab or lexatumumab, particularly through combinations with chemotherapy agents, is warranted.

Several studies have shown that combination treatment with agonistic TRAIL-R1/TRAIL-R2 mAbs and chemotherapeutic...
agents had a synergistic apoptotic effect in some tumor cell lines, such as lymphoma, breast cancer, colorectal cancer, and malignant mesothelioma (14–17). Recently, we also reported that low concentrations of doxorubicin sensitized human renal cell carcinoma cells to mapatumumab- and lexatumumab-induced apoptosis through the induction of TRAIL-R1 or TRAIL-R2 (18, 19). However, the molecular mechanisms of the synergistic apoptosis are not fully understood.

In this study, we investigated whether the cytotoxic effect of lexatumumab or mapatumumab would be enhanced in combination with chemotherapeutic agents and immunotherapeutic agents clinically used in cancer therapy, such as cis-diamminodichloroplatinum (II) (cisplatin, CDDP), 5-fluorouracil (FU), vinblastine, IFN-α, or IFN-γ. Furthermore, we explored the molecular mechanisms that may be involved in the reversal of drug resistance.

**Materials and Methods**

**Reagents.** Lexatumumab and mapatumumab were kindly provided by Human Genome Sciences (9). ELISA and/or Biacore analyses were used to determine that lexatumumab and mapatumumab are highly specific for binding to TRAIL-R2 and TRAIL-R1, respectively. CDDP was purchased from Japan Chemical Pharmaceutical. Z-LETD-FMK, Z-LEHD-FMK, Z-VEID-FMK, and Z-DQMD-FMK were purchased from Sigma, and Z-VA/D-FMK was from ICN Pharmaceuticals.

**Cell lines and primary renal cell carcinoma cells.** The human renal cell carcinoma (ACHN), prostate cancer (DU145 and PC3), bladder cancer (T24), lung cancer (MAC10), and cervical cancer (Hela) cell lines from the American Type Culture Collection were used.

Primary renal cell carcinoma cells were separated from surgical specimens of six untreated renal cell carcinoma patients, as described previously (20). All patients were diagnosed with clear cell renal cell carcinoma by histologic examination. Pathologic stage and grade were consistent with the 2000 WHO criteria, as follows: T2N0M0 grade 2 in patient 1; T1N0M0 grade 2 in patient 2; T2N0M0 grade 1 in patient 3; T2N0M0 grade 2 in patient 4; T1N0M1 grade 2 in patient 5; and T2N0M0 grade 2 in patient 6.

**Cytotoxicity assays.** A 100-μL suspension of 1 × 10⁴ cells was seeded into a 96-well flat-bottom microtiter plate. Cells were grown for 24 h, after which 100 μL of drug solution or medium (control) were added to the plates in triplicate. Each plate was incubated for 12 to 48 h. In sequential experiments cells were preincubated for 8 h with medium only, lexatumumab, or CDDP, washed three times with medium, and exposed to lexatumumab and/or CDDP for 16 h. Cytotoxicity was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, as described previously (20).

Cell viability was determined using the trypan blue dye-exclusion test. Cells were seeded in a 6-well plate at 3 × 10⁵ cells per well and cultured for 24 h. Cells were then treated in duplicate with lexatumumab and/or CDDP for 24 h. Following treatment, the cells were harvested, and viable cells were counted with 0.5% trypan blue dye (Sigma). Cell death was also documented, and photographs of adherent cells were taken under a phase-contrast microscope after removal of the medium containing floating cells.

**Apoptosis assays.** Apoptosis was determined in two ways. Following incubation with lexatumumab and/or CDDP for 24 h, both floating and adherent cells were harvested. Disruption of mitochondrial transmembrane potential in apoptotic cells was analyzed by flow cytometry using MitoCapture Mitochondrial Detection Kit (BioVision). Quantification of DNA fragmentation was assessed with Cell Death Detection ELISA Kit (Roche) according to the manufacturer’s instructions.

**Flow cytometric analysis of TRAIL-R2.** Cell surface expression of TRAIL-R2 in renal cell carcinoma cells was determined using flow cytometry with EPICS XL (10). Briefly, renal cell carcinoma cells were seeded in 60-mm dishes at 5 × 10⁶ cells per dish, and cultured for 24 h. Cells then were treated with 1 to 10 μg/mL CDDP for 6 to 24 h. Following treatment, cells were harvested from the substrate using 0.05% trypsin and 0.02% EDTA, and washed twice in PBS containing 0.2% fetal bovine serum and 0.01% NaN₃. The number of cells was counted, and 2 × 10⁴ cells were incubated with phycoerythrin-conjugated anti-TRAIL-R2 mAb (Genzyme Technne) at 4°C for 30 min, washed, and analyzed.

**Real-time reverse transcription-PCR.** RNA was extracted from renal cell carcinoma cells using TRIzol Reagent (Life Technologies) and cDNA was synthesized with the First-Strand cDNA Synthesis kit (Amersham Pharmacia Biotech). The primer set for TRAIL-R2 was as follows: forward 5′-GGGAGGCGCTCCTGAGGAATGGC-3′ and reverse 5′-GGCAATCTCCTCTCCAGGCTCC-3′ (21). The real-time reverse transcription-PCR was done using LightCycler Fast-Start DNA Master SYBR Green 1 (Roche Diagnostics). The protocol applied for TRAIL-R2 was 40 cycles at 95°C for 10 s, 63°C for 1 s, and 72°C for 4 s. Quantitative analysis of the data was done using LightCycler software version 3.5 (Roche Diagnostics). Standard curves for templates of TRAIL-R2 and glyceraldehyde-3-phosphate dehydrogenase were generated by serial dilution of the PCR products.

**Western blot analysis.** Western blot analysis was carried out as described previously (13). Forty micrograms of protein were loaded in each lane. Goat anti-Bid polyclonal antibody, and rabbit anti-Flip-associated polypeptide with death domain (FADD)-like interleukin-1–converting enzyme-inhibitor protein (FLIP) antibody, mouse anti-caspase-8 mAb (MBL), Bcl-2, Bcl-xL, Bax, and cytochrome c mAbs (Santa Cruz Biotechnology), and mouse anti-β-actin mAb (Abcam) were used as primary antibodies. Signals were detected using ECL kit (Amersham Pharmacia Biotech).

**Caspase activity and caspase-inhibition assays.** The activities of caspase-8, caspase-9, caspase-6, and caspase-3 were measured by a quantitative colorimetric assay with Caspase-8, Caspase-9, Caspase-6, and Caspase-3 Colorimetric Protease Assay kits (MBL), as described previously (22).

The caspase-inhibition assay was done with specific inhibitors of caspase-8, caspase-9, caspase-6, and caspase-3, and a general caspase
inhibitor. Cells were pretreated with these caspase inhibitors (50 μmol/L) for 1 h, and were then exposed to 10 μg/mL CDDP and 100 ng/mL lexatumumab for 23 h. Cell viability was assessed using the MTT assay.

**Statistical analysis.** All determinations were done at least three times and results are expressed as mean ± SD of three experiments. Significance was analyzed by Student’s t test with P < 0.05 considered significant. Synergy was assessed by isobolographic analysis, as described previously (23).

**Results**

**Synergistic cytotoxicity of lexatumumab and CDDP against renal cell carcinoma cells.** We examined whether the apoptotic effect of lexatumumab or mapatumumab would be enhanced in combination with CDDP, FU, vinblastine, IFN-α, or IFN-γ in renal cell carcinoma cells. When ACHN cells were treated with a combination of lexatumumab (1-100 ng/mL) and CDDP (1-10 μg/mL) for 24 hours, a significant potentiation of cytotoxicity and synergy was achieved (Fig. 1A and B), although there was no synergistic effect of lexatumumab in combination with FU (0.1-10 μg/mL), vinblastine (0.1-10 μg/mL), IFN-α (10-1,000 ng/mL), or IFN-γ (10-1,000 ng/mL; data not shown). In contrast, there was no synergistic effect of mapatumumab in combination with these chemotherapeutic or immunotherapeutic agents. Therefore, we selected lexatumumab for further analysis. The synergistic effect was also achieved when the treatment with lexatumumab and CDDP was shortened from 24 hours to 18 hours, although it was not when the treatment

![Graph A](image1.png)

**Fig. 1.** The synergistic cytotoxicity and apoptosis of lexatumumab and CDDP on renal cell carcinoma cells. ACHN cells were treated for 24 h with lexatumumab (1-100 ng/mL) alone, CDDP (1-10 μg/mL) alone, or a combination of the two. A, cytotoxicity was measured using the MTT assay. B, synergy was assessed by isobolographic analysis. ACHN cells were treated for 24 h with 100 ng/mL lexatumumab and/or 10 μg/mL CDDP. C, cytotoxicity of lexatumumab and CDDP used in combination for primary renal cell carcinoma cells from six patients was measured using the MTT assay. Synergy was assessed by isobolographic analysis. D, MitoCapture monomers in apoptotic cells were determined by flow cytometry using MitoCapture Mitochondrial Detection Kit. LXA, lexatumumab.
was shortened to 12 hours (data not shown). Furthermore, synergy was confirmed by the trypan blue dye-exclusion test and morphologic examination using phase-contrast microscope (data not shown).

We further examined the apoptotic effect of lexatumumab and CDDP in primary renal cell carcinoma cells derived from six patients. In all primary renal cell carcinoma cells a significant synergy was achieved, regardless of the sensitivity of renal cell carcinoma cells to CDDP or lexatumumab when each was used alone (Fig. 1C).

Taken together, these findings clearly showed that treatment of human renal cell carcinoma cells with a combination of lexatumumab and CDDP resulted in the potentiation of cytotoxicity.

**Induction of apoptosis.**

To assess whether the synergistic cytotoxicity of lexatumumab and CDDP was mediated by apoptosis, ACHN cells were analyzed. When lexatumumab and CDDP were used in combination, obvious disruption of mitochondrial transmembrane potential was observed, although the treatment of lexatumumab or CDDP alone disrupted slightly the mitochondrial transmembrane potential (Fig. 1D). The synergistic apoptosis of cells treated with lexatumumab and CDDP was confirmed using a quantitative apoptosis-specific ELISA kit (data not shown). In contrast, lexatumumab-induced apoptosis was only slightly enhanced by cotreatment with either FU or vinblastine. These results indicate that the synergistic cytotoxicity of lexatumumab and CDDP was related to their ability to trigger apoptotic cell death.

**Sensitization of renal cell carcinoma cells to lexatumumab-induced cytotoxicity by CDDP.**

The findings above showed that simultaneous treatment with lexatumumab and CDDP resulted in synergy. The effect of sequential treatment with these two agents was examined next. Pretreatment of ACHN and primary renal cell carcinoma cells with 5 μg/mL CDDP for 8 hours, followed by treatment with 100 ng/mL lexatumumab for 16 hours, induced more cytotoxicity than either reverse treatment or simultaneous treatment using these two agents (P < 0.05; Fig. 2). This sequential effect was also observed with different concentrations of CDDP (data not shown). These findings indicate that CDDP sensitized renal cell carcinoma cells to lexatumumab-induced cytotoxicity.

**Synergistic cytotoxicity of lexatumumab and CDDP is TRAIL-R2–dependent in renal cell carcinoma cells.**

Cytotoxic drugs have been reported to increase the expression of TRAIL-R2 in cancer cells (19, 24). We used flow cytometry to determine whether this mechanism could account for the sensitization of renal cell carcinoma cells to lexatumumab-induced apoptosis by CDDP. Treatment of ACHN cells with 1 μg/mL CDDP for 24 hours increased the expression of TRAIL-R2 (Fig. 3A). In contrast, CDDP had no effect on the expression of TRAIL-R1 (data not shown). Quantitative real-time reverse transcription-PCR showed that CDDP significantly increased the mRNA levels of TRAIL-R2 in ACHN cells (Fig. 3B).

To further assess the molecular mechanism of synergistic cytotoxicity of lexatumumab and CDDP, the effect of a human recombinant DR5:Fc chimeric protein that has dominant negative function against TRAIL-R2 on the synergistic cytotoxicity was examined. As shown in Fig. 3C, CDDP-enhanced lexatumumab-induced cytotoxicity was significantly inhibited by the DR5:Fc chimeric protein in ACHN cells. The inhibitory effect of the DR5:Fc chimeric protein was also observed when its concentration was decreased from 10 μg/mL to 1 μg/mL and increased to 20 μg/mL, and in primary renal cell carcinoma cells (data not shown). In contrast, the synergistic cytotoxicity was not inhibited by a human recombinant DR4:Fc chimeric protein that has dominant negative function against TRAIL-R1. Furthermore, the induction of apoptosis by the combination of lexatumumab and CDDP in ACHN cells was also blocked by the DR5:Fc chimeric protein (Fig. 3D).

Taken together, these results indicate that the synergistic cytotoxicity of lexatumumab and CDDP is TRAIL-R2–dependent.

**Effect of the combination of lexatumumab and CDDP on the expression of compounds of TRAIL-R2–mediated apoptosis.**

By Western blot analysis, we analyzed whether the combination of
lexatumumab and CDDP regulates the expression of compounds of the TRAIL-R2–mediated apoptosis. FADD, FLIP, Bcl-2, or AIF expression was not affected when renal cell carcinoma cells were treated with CDDP and/or lexatumumab for 24 hours. Strong activation of caspase-8 and caspase-3 with cleavage of Bid, up-regulation of Bax, and mitochondria release of cytochrome c was noted in combination-treated cells but not in cells treated with either drug alone (Fig. 4A).

Functional activation of caspase-8, caspase-9, caspase-6, and caspase-3 following cotreatment with lexatumumab and CDDP. There was no detectable activation of caspase-9 following lexatumumab treatment in ACHN cells, whereas it slightly activated caspase-8, caspase-6, and caspase-3. Exposure to CDDP alone activated caspase-8, caspase-6, caspase-9, and caspase-3, but they were significantly lower than those obtained with the combination of lexatumumab and CDDP. Remarkably, the combination of lexatumumab and CDDP induced activation of caspase-8, caspase-9, caspase-6, and caspase-3 (Fig. 4B). In contrast, there was no activation of caspase-8, caspase-9, or caspase-6 following treatment with lexatumumab in combination with either FU or vinblastine (data not shown). Although the combination of lexatumumab and FU activated caspase-3, it was much lower than that obtained with the combination of lexatumumab and CDDP. The functional assay of caspase activity mirror-imaged the Western blot analysis, indicating very high levels of mature activated caspase-8 and caspase-3 (Fig. 4A).

To confirm that the synergistic cytotoxicity of lexatumumab and CDDP is mediated through the activation of caspases, we further examined the effects of caspase-8 inhibitor Z-LETD-FMK, caspase-9 inhibitor Z-LEHD-FMK, caspase-6 inhibitor Z-VEID-FMK, caspase-3 inhibitor Z-DQMD-FMK, and a general caspase inhibitor Z-VAD-FMK on the cell death of lexatumumab and CDDP. The synergistic cytotoxicity was significantly inhibited by the specific inhibitors of caspase-8, caspase-9, caspase-6, and caspase-3, and the general caspase inhibitor (data not shown). The inhibition effect of these caspase inhibitors was also obtained by morphologic studies.
Activation of caspase-8 was suppressed by the specific inhibitor of caspase-9, caspase-6, and caspase-3. To assess the position of the activated caspase-8 in the hierarchy of the caspase cascade in combination-treated cells, we further analyzed the activity of caspase-8, caspase-9, caspase-6, and caspase-3 in cells treated with lexatumumab in combination with CDDP in the absence or presence of the specific inhibitors of caspase-8, caspase-9, caspase-6, caspase-3, or the general caspase inhibitor. The activation of caspase-8, caspase-9, caspase-6, and caspase-3 in ACHN cells treated with drug combination, as expected, was totally suppressed by the specific caspase inhibitor. It is interesting to observe, however, that the specific inhibitors of caspase-9, caspase-6, and caspase-3 completely abrogated the elevated caspase-8 activity in the cells exposed to the drug combination as well as the general caspase inhibitor (Fig. 5A).

We further analyzed the activity of caspase-8, caspase-9, caspase-6, and caspase-3 in cells treated with lexatumumab in combination with CDDP in the absence or presence of the DR5:Fc chimeric protein. The elevated caspase-8, caspase-9, caspase-6, and caspase-3 activities in the cells exposed to the drug combination was also partly suppressed by the DR5:Fc chimeric protein (Fig. 5B).

These results indicate that the activation of caspase-8 in the setting is mediated not only by TRAIL-R2 activity, but also by the intrinsic apoptotic pathway-derived caspase-9 and the extrinsic apoptotic pathway-derived caspase-6 and caspase-3 via the amplification feedback loop.

Sensitization of other human solid cancer cells to lexatumumab-induced cytotoxicity by CDDP. We next investigated whether CDDP also sensitizes other human solid cancer cells to lexatumumab-induced cytotoxicity, using DU145, PC3, T24, MCA10, and Hela cells. In all of the cases, significant synergy was observed with lexatumumab in combination with CDDP as well as in renal cell carcinoma cells, irrespective of the sensitivity of these cells to either lexatumumab or CDDP when each was used alone (Fig. 6A). Furthermore, the synergistic effects of drug combination were also observed in DU145, PC3, T24, MCA10, and Hela cells.

**Fig. 4.** Activation of caspases by lexatumumab and CDDP in renal cell carcinoma cells. ACHN cells were treated for 12 to 24 h with 100 ng/mL lexatumumab alone, 10 μg/mL CDDP alone, or a combination of the two. A, the protein levels of FADD, FLIP, caspase-8, Bid, Bax, Bcl-2, Bcl-xL, cytochrome c, and AIF were determined using Western blot analysis. β-Actin was used as loading control. B, time course of caspase activation. Activities of caspase-8, caspase-9, caspase-6, and caspase-3 were measured by a quantitative colorimetric assay.
cytotoxicity was blocked by the DR5:Fc chimeric protein (Fig. 6B), and specific inhibitors of caspase-8, caspase-9, caspase-6, and caspase-3 (Fig. 6C).

**Discussion**

Studies reported here show that: (a) lexatumumab and CDDP had a synergistic effect on human solid cancer cells, including renal cell carcinoma, bladder cancer, prostate cancer, lung cancer, and cervical cancer cells, which were resistant to each agent used alone, and in all cases synergy was achieved with subtoxic concentrations of CDDP; (b) the synergistic cytotoxicity of lexatumumab and CDDP was realized by induction of apoptosis and activation of caspase cascades in a TRAIL-R2–dependent manner; and (c) the activation of caspase-8 in the caspase cascade in combination-treated cells is mediated not only by TRAIL-R2 activity, but also by the intrinsic apoptotic pathway-derived caspase-9 and the extrinsic apoptotic pathway-derived caspase-6 and caspase-3 via the amplification feedback loop.

A synergistic effect is achieved by the reciprocal interaction of two agents. The present study shows that pretreatment of renal cell carcinoma cells with CDDP followed by lexatumumab could induce more significant cytotoxicity than either the reverse treatment or the simultaneous treatment using these two agents. These results indicate that CDDP sensitizes renal cell carcinoma cells to lexatumumab-mediated apoptosis and cytotoxicity. Similar results also reported in malignant mesothelioma cells cotreated with CDDP and lexatumumab (16).
These sequential effects might provide a foundation to optimize administration of these drugs for application in the clinical setting.

The cell surface expression of TRAIL-R1 or TRAIL-R2 is essential for TRAIL-induced apoptosis, although tumor cells expressing these death receptors are not always sensitive to TRAIL due to intracellular mechanisms (25). It was reported that the efficacy of TRAIL correlates with cell surface expression of TRAIL-R1 and/or TRAIL-R2 in leukemia cells (26). In a previous study, we also reported that the surface levels of TRAIL-R1 and TRAIL-R2 mainly qualify the susceptibility of human renal cell carcinoma cells to mapatumumab and lexatumumab, as well as TRAIL (10). CDDP also augmented TRAIL-mediated apoptosis in squamous cell carcinoma by the up-regulation of TRAIL-R1 and TRAIL-R2 (27). Some studies showed, however, that TRAIL receptor expression does not correspond to the synergy of TRAIL and chemotherapeutic agents in certain cell lines (28–31).

Furthermore, the synergistic cytotoxicity and apoptosis of lexatumumab and CDDP was significantly inhibited by the DR5:Fc chimeric protein that has dominant negative function against TRAIL-R2. These findings indicate that lexatumumab and CDDP synergistically induce apoptosis and cytotoxicity in renal cell carcinoma cells in a TRAIL-R2-dependent manner.

Caspases are critical protease mediators of apoptosis triggered by different stimuli including TRAIL (4, 32, 33). In the present study, we found that the combination treatment with lexatumumab and CDDP significantly activated initiator caspases, such as caspase-8 and caspase-9, and effector caspases including caspase-6 and caspase-3. The activation of caspase-8, caspase-6, and caspase-3 in combination-treated renal cell carcinoma cells is the same pattern which reached a peak level after 18 hours of treatment, whereas caspase-9 activity reached a peak level after 24 hours of treatment. Furthermore, the synergistic cytotoxicity of lexatumumab and CDDP was significantly inhibited by the specific inhibitors of caspase-8, caspase-9, caspase-6, and caspase-3, and the general caspase inhibitor Z-VAD-FMK. These findings suggest that the activation of extrinsic and intrinsic apoptotic pathways plays a critical role in the synergistic cytotoxicity of lexatumumab and CDDP in renal cell carcinoma cells.

The processing of caspase-3 can be mediated by caspase-8 (extrinsic pathway) and/or by caspase-9 (intrinsic pathway). The strong activation of caspase-8 in combination-treated renal cell carcinoma cells may be the result of increased death-inducing signaling complex activity or secondary to activation by downstream caspases. A recent study showed that activation of caspase-8 was attributable to the amplification feedback loop mediated by mitochondria-derived caspase-9 and caspase-3 in esophageal cancer cells cotreated with TRAIL and CDDP (30). In the present study, we found that the activation of caspase-8 was significantly abrogated not only by a caspase-9–specific inhibitor but also by the specific inhibitors of caspase-6 and caspase-3. Furthermore, the elevated caspase-8, caspase-9, caspase-6, and caspase-3 activities in the cells exposed to the drug combination was partly suppressed by the DR5:Fc chimeric protein. These results indicate that the activation of caspase-8 in the setting is mediated not only by TRAIL-R2 activity, but also by both the intrinsic pathway–derived caspase-9 and the extrinsic pathway–derived caspase-6 and caspase-3 via the amplification feedback loop.

Altogether, these results suggest that CDDP sensitizes renal cell carcinoma cells to lexatumumab-induced apoptosis and cytotoxicity by potentiation of the extrinsic and intrinsic pathways that lead to amplification of caspase activation, particularly caspase-8, by the feedback loop to efficiently induce apoptosis.

In addition, the synergistic effect of lexatumumab and CDDP was achieved in other human solid cancer cells, including bladder cancer, prostate cancer, lung cancer, and cervical cancer cells. These results support those of previous studies showing that CDDP and antihuman TRAIL-R2 antibody induced synergistic apoptosis and cytotoxicity in some solid cancer cells (8, 14). Furthermore, the synergistic cytotoxicity of lexatumumab and CDDP was also blocked by the caspase inhibitors. These findings suggest that the activation of both the intrinsic and the extrinsic caspase cascades via the amplification feedback loop is not specific for renal cell carcinoma cells and might be a general mechanism in the human solid cancer cells.

The chemotherapeutic drug resistance of cancer cells remains a major obstacle to successful treatment, and a more effective therapy is needed. The present results indicate that lexatumumab and CDDP have a synergistic cytotoxicity in human renal cell carcinoma and other human solid cancer cells. The synergistic cytotoxicity of lexatumumab and CDDP occurs via induction of apoptosis and activation of extrinsic and intrinsic apoptotic pathways. CDDP is a commonly used chemotherapeutic drug for many solid cancers and lexatumumab is currently undergoing clinical testing (11–13). This combination might be promising in the treatment of solid cancers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

10. Zeng Y, Wu XX, Fiscella M, Shimada O, Humphreys R, Albert V. Monoclonal antibody to tumor necrosis...


Enhancement of Lexatumumab-Induced Apoptosis in Human Solid Cancer Cells by Cisplatin in Caspase-Dependent Manner

Xiu-Xian Wu and Yoshiyuki Kakehi


Updated version  Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/15/6/2039

Cited articles  This article cites 33 articles, 16 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/15/6/2039.full.html#ref-list-1

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.