Effective Combination Therapy for Malignant Glioma with TRAIL-Secreting Mesenchymal Stem Cells and Lipoxygenase Inhibitor MK886

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Abstract

The apoptotic ligand TRAIL is believed to have promise as a cancer gene therapy, yet many types of cancer, including gliomas, have exhibited resistance to TRAIL-induced apoptosis. Here, we show that therapeutic combination of the lipoxygenase inhibitor MK886 and TRAIL-secreting human mesenchymal stem cells (MSC-TRAIL) provide targeted and prolonged delivery of TRAIL both in vitro and in orthotopic mouse models of glioma. Treatment of either TRAIL-sensitive or TRAIL-resistant human glioma cells with MK886 and MSC-TRAIL resulted in significantly enhanced apoptosis compared with each agent alone. MK886 effectively increased the sensitivity to TRAIL-induced apoptosis via upregulation of the death receptor 5 and downregulation of the antiapoptotic protein survivin in human glioma cell lines and in primary glioma cells. This regulation was accompanied by a substantial increase in caspase activation after combined treatment. Furthermore, in vivo survival experiments and imaging analysis in orthotopic xenografted mice showed that MSC-based TRAIL gene delivery combined with MK886 into the tumors had greater therapeutic efficacy than single-agent treatment. Together, our findings indicate that MK886 combined with MSC-based TRAIL gene delivery may represent a novel strategy for improving the treatment of malignant gliomas. Cancer Res; 72(18); 4807–17. ©2012 AACR.

Introduction

Glioblastoma multiforme (GBM), the most common type of primary brain tumor, is highly aggressive and invasive. The median survival of GBM patients undergoing conventional treatment (i.e., surgery, radiotherapy, and chemotherapy) is 14.6 months for radiotherapy plus temozolomide and 12.1 months for radiotherapy alone (1). The intrinsic high capacity of these tumors to invade and infiltrate the adjacent functional normal brain parenchyma, which hampers the complete surgical removal of tumors and results in tumor recurrence, and their resistance to most chemotherapy agents available currently are the main obstacles to GBM treatment (2).

Mesenchymal stem cells (MSC) have tumor-targeting properties, can be isolated easily, and can be engineered with viral vectors, suggesting a potential clinical use in cancer gene therapy (3, 4). The use of MSC-based gene therapy as an effective therapeutic gene delivery vehicle represents a promising strategy for improving the efficacy of the treatment of glioma. We and others have shown that MSCs can selectively deliver therapeutic agents, such as immune-modulating, apoptosis-inducing, and suicide or prodrug converting genes, to tumors and yield a significant antitumor efficacy in glioma models (5–10).

The TRAIL induces apoptosis selectively in transformed cells, but not in most normal cells (11, 12), which renders it a promising candidate for brain tumor therapy. TRAIL-based cancer therapies with recombinant TRAIL (rTRAIL) or an adenovirus bearing the TRAIL gene have been reported (13, 14); however, potential issues of toxicity and short protein half-life of rTRAIL remain to be addressed. In addition, the limitations of adenoviral gene therapy include the infiltration of the brain parenchyma by outgrowing glioma cells. To extend the release time of TRAIL and deliver it to infiltrated tumor cells, we evaluated MSCs as delivery vehicles for TRAIL (7). However, despite the expression of death-inducing TRAIL receptors (15), many types of cancer, including most glioma cells, are resistant to TRAIL-induced apoptosis, suggesting that TRAIL alone may be ineffective. Thus, the identification of novel drugs that sensitize glioma cells to TRAIL-induced apoptosis or of new strategies to overcome TRAIL resistance is needed. Recent reports showed that chemotherapeutic agents or radiotherapy can enhance sensitivity to TRAIL by increasing the expression of TRAIL receptors in a range of tumors (16, 17), suggesting that a synergistic antitumor effect may be achieved by using combination therapies.

COX-2 and 5-lipoxygenase (5-LOX) are key enzymes involved in the metabolism of arachidonic acid to eicosanoids (18).
These arachidonic acid metabolites (i.e., prostaglandins and leukotrienes) are related to tumor development and progression (19, 20), thus pinpointing these enzymes as potential therapeutic targets against cancer; moreover, their selective inhibitors have been considered as antitumor agents (21, 22). MK886, a specific inhibitor of the 5-LOX-activating protein, induced antiproliferative effects and apoptosis in human myeloid leukemia, prostate cancer, and breast cancer (23–25). Previously, we also investigated the overexpression of 5-LOX and the effect of MK886 on cell proliferation and apoptosis in human malignant glioma cells (26). Importantly, celecoxib, a COX-2 selective nonsteroidal antiinflammatory drug, exhibited therapeutic activity in cancer and restored or enhanced sensitivity to TRAIL in a range of tumors (27, 28). Because these two arachidonic acid-metabolizing enzymes (COX-2 and 5-LOX) are so closely related regarding their mechanisms of action and substrate, we hypothesized that a 5-LOX inhibitor, such as MK886, could be a potent sensitizer to overcome the resistance to TRAIL-induced apoptosis in malignant gliomas.

In this study, first we showed that malignant glioma cells were sensitized to TRAIL-induced apoptosis after administration of MK886, via modulation of the apoptotic machinery. In addition, we found that a combined treatment using MK886 and MSC-delivered TRAIL (MSC-TRAIL) enhanced apoptosis in glioma cells in vitro and tumor regression in mouse xenograft models in vivo.

Materials and Methods

Human MSCs and glioma cells

Primary human MSCs were obtained from PromoCell. These cells are harvested from normal human bone marrow and their ability to differentiate in vitro into adipocytes, chondrocytes, and osteoblasts was tested according to the manufacturer’s instructions (29). These cells have also been tested for the presence of surface antigens (CD44/CD105, >95% positive; CD31/CD45, >95% negative). MSCs were cultured in Mesenchymal Stem Cell Growth Medium containing SupplementMix (PromoCell) and were used for experiments during passages 5 to 8. Human glioma cell lines (U-87MG, U-138MG, U-251MG, and U-373MG) were obtained from the American Type Culture Collection, and primary glioma cells (GBM) were obtained from fresh specimens of glioma-bearing patients who underwent surgery, after ethical approval and written informed consent. Glioma cell lines and GBM cells were maintained in Dulbecco’s Modified Eagle’s Media (DMEM; Invitrogen). Normal human astrocytes were obtained from the Applied Cell Biology Research Institute and cultured in DMEM. Firefly luciferase (Luc)-expressing U-87MG cells (U87-Luc) were stably transduced using a lentivirus expressing Luc (30). All cells were supplemented with antibiotics and 10% FBS and were incubated at 37°C in a humidified atmosphere containing 5% CO2.

Production of gene-modified MSCs

The adenovirus carrying the secretable trimeric form of the TRAIL gene (Ad-TRAIL) was engineered as described previously (31). The recombinant replication-deficient adenoviral vector encoding the gene for enhanced green fluorescent protein (Ad-EGFP) was constructed using the AdEasy vector system (Quantum Biotechnologies). Cell permeable peptides-mediated adenoviral transduction was carried out as described previously (7, 32).

Transfection with siRNAs

TRAIL death receptor 5 (DR5), cyclophosphamide–Adriamycin–vincristine–prednisone (Oncovin; CHOP), and survivin siRNAs were obtained from Santa Cruz Biotechnology. To transfet tumor cells with siRNA, cells (2 × 105 cells per well) were seeded into 6-well plates and transfection was carried out using Lipofectamine 2000 (Invitrogen). Cells were exposed to a scrambled siRNA (Santa Cruz Biotechnology) and mock transfection with Lipofectamine 2000 only was used as controls.

Cell viability assay

Cell viability was measured using a Cell Counting Kit-8 (CCK-8; Dojindo Laboratories) allowing sensitive colorimetric assay by using highly water-soluble tetrazolium salt that is reduced by dehydrogenases in living cells to give a colored product (formazan). Glioma cells were seeded in 96- or 24-well plates to measure TRAIL-induced cytotoxicity and MK886-mediated cell death. Cells were treated with MK886 (Merck), rTRAIL (R&D Systems), or both reagents at adequate concentrations and were analyzed 48 hours later. For coculture experiments, MSC-TRAIL were plated in Transwell inserts with 0.4 μm pores (Corning Inc.) at various cell densities and tumor cells (4 × 103) were grown in the lower well of the 24-well plates. For inhibition studies, D5/Fc chimera protein (100 ng/mL; R&D Systems) was added or anti-TRAIL (R&D systems) and caspase inhibitor, z-VAD-fmk (10 μmol/L; R&D Systems) were preincubated for 1 hour at each experiment. The cytotoxicity induced by another lipoxygenase inhibitor, AA861 (Enzo Life Sciences), was also confirmed using the method described above. For the visualization of induced apoptosis, U87-Luc cells were seeded in 96-well plates and analyzed 48 hours after treatment using the Maestro In Vivo Imaging System (CRI Inc.).

Apoptosis analysis

Apoptosis was determined using Annexin V and propidium iodide (PI) staining-based fluorescein isothiocyanate Annexin V Apoptosis Detection Kit (BD Biosciences). U-87MG cells (2 × 105 cells/well) were seeded in a 6-well plate, incubated with MK886 (20 μmol/L) in the absence or presence of TRAIL (10 ng/mL) for 24 hours, and analyzed by flow cytometry (MoFlo; Beckman Coulter Inc.).

Flow cytometry for death receptors

Cells were analyzed for the surface expression of TRAIL death receptors using phycoerythrin (PE)-conjugated antihuman DR4 and DR5 antibodies (R&D Systems), as described previously (17). PE-conjugated mouse IgG1 (R&D Systems) was used as an isotype control.

Western blotting

Preparation of cell lysates and Western blot analysis were carried out as described previously (7). Proteins transferred
onto a nitrocellulose membrane (Invitrogen) using the iBlot Dry Blotting System (Invitrogen) were incubated with primary antibodies against DR4, DR5 (R&D Systems), p53, CHOP, Bid, caspase-3, caspase-8, caspase-9, Bcl-2, Bcl-XL, Bax, survivin, X-linked inhibitor of apoptosis protein (XIAP; Cell Signaling Technology), cellular FLICE-inhibitory protein (c-FLIP; Alexis), and \( \beta \)-actin (Sigma). For each Western blot, one representative of at least 2 independent experiments was shown.

**Immunohistochemistry and in vivo apoptosis assay**

To evaluate tumor growth inhibition using histologic analysis, mice were sacrificed at day 14 after tumor inoculation, during the imaging experiment. Mouse brains were perfused with 4% paraformaldehyde under deep anesthesia. Fixed tissues were cryosectioned (14 \( \mu \)m-thick sections) and then stained with hematoxylin and eosin (H&E). Apoptotic activity was detected via staining using a terminal deoxyribonucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) assay kit (Roche) and developed with Cy3-conjugated streptavidin (Jackson ImmunoResearch Laboratories). Nuclei were counterstained with 4', 6-diamidino-2-phenylindole (DAPI; Sigma).

**Statistical analysis**

All data are expressed as means ± SEM from at least 3 independent experiments. Statistical differences between different test conditions were determined using Student \( t \) test. Probability values less than 0.05 were considered significant.

**Figure 1.** Cytotoxicity of combined treatment using MK886 and TRAIL on glioma cells. A and B, cytotoxic effect on different glioma cell lines (U-87MG, U-138MG, U-251MG, and U-373MG) and GBM treated with increasing doses of rTRAIL (0–300 ng/mL) and MK886 (0–60 \( \mu \)mol/L) at 48 hours by CCK-8 cytotoxicity assay. C, cytotoxic effect on glioma cells cotreated with MK886 (20 \( \mu \)mol/L) and rTRAIL (100 ng/mL) at 48 hours by CCK-8 cytotoxicity assay. \( * \), \( P < 0.05 \) in the comparison of each treatment with untreated control; \#, \( P < 0.05 \) as compared with treatment with TRAIL alone. Student \( t \) test. D, sensitization effect of MK886 on TRAIL-induced apoptosis in U-87MG cells was evaluated by flow cytometry analysis. Apoptosis was determined using Annexin V and PI staining at 24 hours after treatment with MK886 (20 \( \mu \)mol/L) in the absence or presence of TRAIL (10 ng/mL).

Combined Therapy with MSC-TRAIL and MK886
The statistical analysis of survival was carried out using a log-rank test.

Results

MK886 sensitizes glioma cells to TRAIL-mediated apoptosis

First, we determined the cytotoxicity of rTRAIL or MK886 alone in human glioma cells. Most of the human glioma cell lines and GBM were resistant to TRAIL (Fig. 1A), and MK886 treatment exhibited a cytotoxic effect in a dose-dependent manner (Fig. 1B). Another 5-LOX inhibitor, AA861, also induced cell death in a pattern that was similar to that observed for the MK886 treatment (Supplementary Fig. S1). Next, we examined whether TRAIL treatment combined with MK886 enhanced the cytotoxic effect. A subtoxic dose of MK886 (20 μmol/L) induced cell death significantly in the presence of 100 ng/mL TRAIL in U-87MG, U-138MG, and GBM cells (Fig. 1C), indicating that MK886 strongly sensitizes cells to TRAIL-induced cell death. We also examined the effect of MK886 on the TRAIL-induced apoptosis by Annexin V/PI-based flow cytometric analysis. The respective percentages of apoptotic cells were 14% or 17% when treated with MK886 or TRAIL (Fig. 1D). However, we found that apoptosis was dramatically increased to 62% by the combination of the two. Although we showed enhanced TRAIL-induced apoptosis after treatment with MK886 in several glioma cells, there was only a slight or no effect after treating U-251MG and U-373MG cells, which have high resistance at this concentration of TRAIL. Therefore, the identification of a more powerful modulation that induces cell death by treatment with a high concentration of TRAIL is of great importance.

Effect of MSC-TRAIL together with MK886 in TRAIL-resistant glioma cells

We previously found that MSC-based gene therapy had the advantages of offering continuous and concentrated local delivery of secretable TRAIL (7). Thus, we used MSC-TRAIL in the present study to overcome the resistance to rTRAIL. First, we examined the cytotoxic effects of MK886 and TRAIL on MSCs (Fig. 2A) or normal human astrocytes (data not shown), because it is important that MK886 and TRAIL do not induce cell death to the delivery vehicle (MSCs) or normal cells in the therapeutic setting. MSCs are resistant to both the combination and single-agent treatments. In addition, DR5 expression levels did not change in MK886-treated MSCs (Fig. 2B). We also showed that MSC-TRAIL had greater...
therapeutic efficacy than rTRAIL by coculture experiments with U-87MG (Fig. 2C). On the basis of these findings, we examined whether treatment with MSC-TRAIL combined with MK886 had the effect on cell death in U-87MG cells in response to 24-hour treatment with increasing doses of MK886 in comparison with untreated control. Mouse isotype IgG antibody served as a negative control. B, Western blot analysis of DR5 on U-87MG cells exposed to MK886 dose dependently (0–40 μmol/L) for 24 hours. C. Bioluminescence images for viable cells of U87-Luc treated with 5-LOX inhibitors (MK886, 20 μmol/L; AA861, 100 μmol/L), rTRAIL (10 ng/mL), or a combination with or without DR5/Fc chimeric protein (100 ng/mL) by In Vivo Imaging System at 48 hours. Standard image shows dose-dependent bioluminescence intensity. D, viability of U-87MG cells in response to the combined treatment with 5-LOX inhibitors (MK886 or AA861) and TRAIL, with or without DR5/Fc chimeric protein. * P < 0.05, Student t test. E, Western blot analysis of DR5 knockdown on glioma cells transfected with either control or DR5 siRNA. F, effect of DR5 knockdown on the viability of glioma cells in response to 48-hour treatment of MK886 and TRAIL. Mock-treated (lipofectamine only) cells or scrambled siRNA-treated cells were used as controls. * P < 0.05, Student t test. Ctrl, control.

MK886 upregulates the expression of DR5 in glioma cells
Several chemotherapeutic agents enhance TRAIL-induced apoptosis through upregulation of the TRAIL-mediated death receptors in many types of cancer. Thus, we examined whether treatment with MK886 had an effect on death receptor expression in glioma cells. Flow cytometry and Western blot analysis showed that MK886 induced DR5 expression in a dose-dependent manner in U-87MG (Fig. 3A and B) and other glioma cell lines (Supplementary Fig. S2). The expression of another death receptor, DR4, was not affected by MK886 treatment (data not shown). To confirm that the increase of apoptosis is mediated by DR5 upregulation, we used the DR5/Fc chimeric protein to block the interaction between DR5 and TRAIL. Treatment with 5-LOX inhibitors (MK886 and AA861) and TRAIL alone slightly induced cell death, whereas the combined treatment markedly induced apoptosis (Fig. 3C); however, DR5/Fc efficiently blocked the apoptosis induced by cotreatment (Fig. 3C and D). We also showed a dose-dependent increase in DR5 levels and enhanced TRAIL-induced apoptosis after treatment with AA861 (Supplementary Fig. S3A and S3B). Moreover, to determine the role of DR5 in TRAIL-induced apoptosis, we used an siRNA specific to DR5, which blocked the upregulation of DR5 after MK886 treatment (Fig. 3E). The effect of MK886 on TRAIL-induced apoptosis was effectively abolished in cells transfected with DR5 siRNA (Fig. 3F).

Figure 3. DR5-mediated cytotoxic effect of combined treatment with MK886 and TRAIL. A, flow cytometry analysis of TRAIL receptors (DR4 and DR5) on U-87MG cells in response to 24-hour treatment with increasing doses of MK886 in comparison with untreated control. Mouse isotype IgG antibody served as a negative control. B, Western blot analysis of DR5 on U-87MG cells exposed to MK886 dose dependently (0–40 μmol/L) for 24 hours. C. Bioluminescence images for viable cells of U87-Luc treated with 5-LOX inhibitors (MK886, 20 μmol/L; AA861, 100 μmol/L), rTRAIL (10 ng/mL), or a combination with or without DR5/Fc chimeric protein (100 ng/mL) by In Vivo Imaging System at 48 hours. Standard image shows dose-dependent bioluminescence intensity. D, viability of U-87MG cells in response to the combined treatment with 5-LOX inhibitors (MK886 or AA861) and TRAIL, with or without DR5/Fc chimeric protein. * P < 0.05, Student t test. E, Western blot analysis of DR5 knockdown on glioma cells transfected with either control or DR5 siRNA. F, effect of DR5 knockdown on the viability of glioma cells in response to 48-hour treatment of MK886 and TRAIL. Mock-treated (lipofectamine only) cells or scrambled siRNA-treated cells were used as controls. * P < 0.05, Student t test. Ctrl, control.
These results indicate that induction of DR5 could be a mechanism of which MK886 enhances the TRAIL-induced apoptosis in glioma cells.

**MK886-induced DR5 upregulation is mediated through induction of CHOP**

Several studies have shown that the expression of death receptors can be induced by p53 and CHOP (33, 34). Thus, we determined to see whether these transcription factors are involved in MK886-induced DR5 upregulation. Western blot analysis showed that DR5 expression was markedly increased after treatment and that MK886 induced CHOP expression but had no effect on the expression of p53 (Fig. 4A). To elucidate the role of CHOP in MK886-induced upregulation of DR5, we used an siRNA specific to CHOP, which blocked the upregulation of CHOP after MK886 treatment. Whereas MK886 upregulated the expression of DR5 in nontransfected and control-transfected U-87MG cells, transfection with CHOP siRNA suppressed MK886-induced DR5 upregulation (Fig. 4B). Moreover, the suppression of CHOP by siRNA significantly reduced the effects of MK886 on TRAIL-induced apoptosis (Fig. 4C). These results indicate that CHOP plays an important role in DR5 upregulation and contributes to the sensitizing effect of MK886 on TRAIL-induced apoptosis.

**Downregulation of antiapoptotic proteins after MK886 treatment**

Antiapoptotic proteins are expressed at high levels in many tumors, including glioma, and have been associated with refractory disease and poor prognosis (35, 36). To identify intracellular signaling events responsible for the MK886-mediated sensitization to TRAIL-induced apoptosis, the expression of the antiapoptotic proteins FLIP, survivin, and XIAP by the treatment of MK886 was examined. Glioma cells showed a dose-dependent decrease in FLIP-L protein levels, with the exception of 1 cell line (U-138MG), and 2 glioma cell lines (U-138MG and U-251MG) showed decrease in XIAP levels (Fig. 5A). More importantly, all glioma cells exhibited downregulation of survivin. In contrast, the levels of other apoptosis-related proteins, Bcl-XL and Bax (data not shown), were not modulated in any of the cell lines tested. Next, we verified whether the TRAIL-mediated apoptosis was increased by downregulation of survivin. To verify whether apoptosis factors are affected by survivin knockdown, we measured the levels of a major effector of caspase (caspase-3) and DR5 (Fig. 5B). The results revealed no differences in the expression levels of procaspase-3; however, DR5 expression was increased by survivin knockdown. More importantly, knockdown of survivin in U-87MG cells induced more apoptosis than that observed in control-transfected cells after TRAIL treatment (Fig. 5C). These results showed that survivin is an important protein in elucidating the resistance to TRAIL-induced apoptosis.

**MK886 augments TRAIL-induced activation of caspases**

Next, we investigated whether the enhanced TRAIL-induced apoptosis observed after MK886 treatment is mediated by caspase activation (Fig. 5D). First, we investigated the levels of receptor-related proteins in the extrinsic apoptosis pathway.
inhibitor, z-VAD-fmk, which significantly inhibited the MK886-mediated decrease of U-87MG cell viability (Fig. 5E).

Therapeutic potential of combined therapy with MK886 and MSC-TRAIL in vivo

Because the combined treatment enhanced TRAIL-induced apoptosis in vitro, we evaluated the therapeutic efficacy of this treatment in an intracranial xenograft mouse model. To monitor tumor growth using in vivo bioluminescent imaging analysis, we established tumor-bearing mice with U87-Luc cells. A significant decrease in bioluminescence expression was observed in mice treated with MK886 and MSC-TRAIL compared with mice treated with the control or a single agent alone (Fig. 6A). In addition, treatment with MSC-TRAIL after MK886 injection significantly \( (P = 0.034) \) prolonged the survival of tumor-bearing mice compared with mice that were given MSC-TRAIL alone (Fig. 6C). Furthermore, H&E staining revealed that tumor growth in the mice treated with MK886
and MSC-TRAIL was more restricted than that induced by the control or single-agent treatments (Fig. 6D), and the combined treatment also induced greater apoptosis compared with the single-agent treatment, as shown by TUNEL staining (Fig. 6E). Interestingly, apoptotic cells were detected only in the tumor mass and were not seen in normal brain parenchyma, suggesting that the treatment induced tumor cell death specifically, and thus had no adverse toxic side effects. Taken together, these results show that the combination of MSC-TRAIL with MK886 is a potent therapeutic approach against malignant glioma.

Discussion

In this study, we showed that the 5-LOX inhibitor MK886 sensitized glioma cells to TRAIL-induced apoptosis and provided evidence that the combined therapy for MK886 and TRAIL is a more powerful modality compared with treatment using a single agent against glioma, both in vitro and in vivo. Moreover, we showed that MSC-based TRAIL delivery efficiently enhanced the therapeutic potential of the combined treatment by providing a continuous and substantial amount of TRAIL to glioma cells that were sensitized to TRAIL-induced apoptosis by treatment with MK886. Although several mechanisms have been implicated in TRAIL resistance, in the present study we verified that treatment with MK886 had the potential to overcome TRAIL resistance by decreasing in the expression levels of anti-apoptotic proteins related to the apoptotic signaling pathway at different stages of the caspase cascade, and increasing in death receptor-mediated apoptosis directly.
In particular, several groups, including ours, have reported the use ofMSCs expressing secretable TRAIL, which has been shown to be a potent anticancer agent in experimental glioma models (7, 8, 37, 38) and found an effective therapeutic potential by overcoming some of the hurdles of conventional TRAIL-based treatments that use soluble rTRAIL protein or agonistic antibodies to DR5 (39, 40). However, an additional issue with TRAIL-based therapy, that is, the resistance of tumor cells to TRAIL, remains unaddressed. In a previous report, we showed that the combination of MSC-TRAIL and irradiation synergistically enhances the antitumor effect by increasing the expression ofTRAIL receptors in the treatment of glioma (17). In this study, MK886 also effectively potentiated TRAIL-induced apoptosis by augmenting cell death in TRAIL-responsive glioma cells and by sensitizing TRAIL-resistant cells through a DR5-mediated pathway, which was also verified by experiments using DR5-specific knockdown. However, there was only one exception in the present study, flow cytometry analysis showed that MK886 did not induce DR5 expression in U-251MG cells (Supplementary Fig. S2). Although upregulation of DR5 expression was not detected, coculture experiments showed that MSC-TRAIL combined with MK886 had therapeutic efficacy (Fig. 2D), indicating that it is cell-type specific and other mechanisms of TRAIL-sensitizing effect including downregulation of antiapoptotic proteins, FLIP, survivin, and XIAP (Fig. 5A), could be related. In addition, we showed that treatment with MSC-based TRAIL gene therapy is more effective and useful than treatment using rTRAIL, especially human glioma cells that are highly TRAIL-resistant, indicating the advantageous effect of the cell-mediated delivery of TRAIL.

Resistance to TRAIL can be overcome by combining TRAIL-based agents with chemotherapeutic drugs, irradiation, or other novel therapeutic drugs in a variety of tumor types, however, the resistance to TRAIL may have various causes. Given the enhanced effects of MK886 and TRAIL in the present study, the identification of intracellular signaling events that are responsible for the MK886-mediated sensitization to TRAIL-induced apoptosis is of particular importance. Several molecular mechanisms have been revealed in TRAIL resistance, including intracellular blockage of the apoptotic-signaling pathway by down-regulation of proapoptotic proteins or upregulation of antiapoptotic proteins. The best-characterized inhibitor of TRAIL-mediated apoptosis is FLIP, which blocks the death signal at the level of the receptor by occupying the caspase-8 binding site on the Fas-associated protein with death domain, thus inhibiting the activation and cleavage of caspase-8 (41). Another important point related to the resistance to TRAIL, downstream of the receptors, is the high expression of conserved proteins of the family of inhibitors of apoptosis proteins (IAP), including XIAP, c-IAP1, c-IAP2, and survivin, which can block the activation and/or activity of caspase-9, -3, and -7 (42). Although the expression of antiapoptotic proteins did not decrease equally, MK886 treatment caused a marked decrease in the expression levels of survivin in all glioma cells tested in this study. Interestingly, the suppression of survivin by siRNA enhanced TRAIL-mediated apoptosis and Western blot analysis after survivin knockdown showed no activation of procaspase-3, indicating a caspase-independent cell death mechanism. This observation is in agreement with findings from previous report that described a caspase-independent and DR5-mediated sensitizing effect on TRAIL-induced cell death by silencing of survivin (43). These results suggest that the MK886-induced downregulation of antiapoptotic proteins could be one mechanism by which TRAIL-induced apoptosis is potentiated. Thus, the use of drugs targeting survivin could be beneficial to the therapy against glioma.

Although many agents that possess antitumor effects have been reported, the efficacy of single agents is insufficient. Therefore, identifying clinically safe drugs that, when administered concomitantly, sensitize tumor cells to TRAIL by generating new effects, rather than just adding the effects of the 2 agents, is of great importance. Moreover, understanding the intracellular mechanisms underlying the overcoming of the resistance to TRAIL is important for the development of novel and efficient modalities of combined treatment. In the present study, we showed for the first time that the combined therapy markedly induced apoptosis at suboptimal concentrations of MK886 and long-acting TRAIL delivered by MSCs in glioma cells, both in vitro and in vivo, with the findings suggesting that MK886 is a sensitizing or augmenting agent in cells that are sensitive or resistant to TRAIL-induced cell death.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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