Effective gene-viral therapy of leukemia by a new fiber chimeric oncolytic adenovirus expressing TRAIL: *in vitro* and *in vivo* evaluation

Jie Jin,1 Hui Liu,1 Chunmei Yang,1 Gongchu Li,2 Xinyuan Liu,2,4 Qijun Qian,2,3 and Wenbin Qian1,2

1Institute of Hematology, The First Affiliated Hospital, College of Medicine, Zhejiang University, Key Lab of Combined Multi-Organ Transplantation, Ministry of Public Health; 2Xinyuan Institute of Medicine and Biotechnology, College of Life Sciences, Zhejiang Sci-Tech University, Hangzhou, People’s Republic of China and 3Laboratory of Viral and Gene Therapy, Eastern Hepatobiliary Surgery Hospital, Second Military Medical University; 4Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, People’s Republic of China

Abstract

Conditionally replicating adenoviruses (CRAd) have been under extensive investigations as anticancer agents. Previously, we found that ZD55, an adenovirus serotype 5-based CRAd, infected and killed the leukemia cells expressing coxsackie adenovirus receptor (CAR). However, majority of leukemic cells lack CAR expression on their cell surface, resulting in resistance to CRAd infection. In this study, we showed that SG235, a novel fiber chimeric CRAd that has Ad35 tropism, permitted CAR-independent cell entry, and this in turn produced selective cytopathic effects in a variety of human leukemic cells *in vitro* and *in vivo*. Moreover, SG235 expressing exogenous tumor necrosis factor-related apoptosis-inducing ligand (SG235-TRAIL) effectively induced apoptosis of leukemic cells via the activation of extrinsic and intrinsic apoptotic pathway and elicited a superior antileukemia activity compared with SG235. In addition, normal hematopoietic progenitors were resistant to the inhibitory activity of SG235 and SG235-TRAIL. Our data suggest that these novel oncolytic agents may serve as useful tools for the treatment of leukemia. [Mol Cancer Ther 2009;8(5):1387–97]

Received 10/6/08; revised 2/3/09; accepted 2/19/09; published OnlineFirst 5/5/09.

Grant support: National Natural Science Foundation of China grants 30470745 and 30600257 and Key Social Development Project of Zhejiang Province grant 2004c23005 (W. Qian), Chinese National Natural Science Foundation of China grants 30470745 and 30600257 and Key Social Development Project of Zhejiang Province grant 2004c23005 (W. Qian), Chinese National “973” Project Foundation grants 2003AA216031 and 2002AA216021, and Natural Science Foundation of China grant 3012506823 (X. Liu).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Wenbin Qian, Institute of Hematology, The First Affiliated Hospital, College of Medicine, Zhejiang University, 79 Qingchun Road, Hangzhou 310003, People’s Republic of China.

E-mail: qianwenb@yahoo.com.cn

Copyright © 2009 American Association for Cancer Research.

doi:10.1158/1535-7163.MCT-08-0962

Introduction

Novel therapeutic approaches for cancer treatment are urgently needed because conventional treatment regimens are frequently acutely toxic, nonselective, or ineffective. Oncolytic virotherapy provides a new platform to treat cancer as biotherapeutic agents that lack cross-resistance with currently available treatments (1, 2) and represents a novel approach for gene therapy (3, 4). Results from preclinical studies with several viral types are promising and some oncolytic viruses have entered or even completed clinical trials (5, 6); however, little of the previously used therapeutic viruses is able to infect hematopoietic malignant cells. Recently, oncolytic measles viruses have been shown to be active in preclinical *in vitro* and animal models of multiple myeloma (7–9) and lymphoma (10–12). These data have raised the possibility of clinical application of virotherapy for leukemia.

Conditionally replicating adenoviruses (CRAd) have attracted considerable interest owing to their several attributes including lytic replication, high stability, efficient genome transfer, and low pathogenicity (13). The most studied CRAd in cancer therapy is adenovirus serotype 5 (Ad5), which requires expression of coxsackie adenovirus receptor (CAR) on target cells for successful transduction (14–16). Unfortunately, hematopoietic and leukemia cells express few CARs, which essentially renders the tumor cell resistant to Ad5 infection (14, 15, 17). To enhance tumor cell infectivity, concerted efforts have been made to modify fiber knob domain of Ad5. In this regard, recent reports showed that a nonreplicating adenovirus independent of CAR by substitution of a chimeric Ad5/35 fiber improved infectious efficiency and antitumor gene therapy effects of the Ad5 tropism in leukemic cells (18, 19). In this study, we constructed a novel fiber chimeric Ad5 oncolytic vector (SG235) that has Ad35 tropism generated through the substitution of the Ad5 fiber protein by the Ad35 knob domain. Then, SG235 was armed to express exogenous apoptosis-inducing gene tumor necrosis factor-related apoptosis-inducing ligand (SG235-TRAIL). The infectivity and cytotoxic effects of SG235 and SG235-TRAIL on leukemic cell lines and primary blasts obtained from leukemia patients were determined, and the potent antitumor activity in human leukemia xenograft mouse model was evaluated.

Materials and Methods

**Primary Leukemia Samples, Cell Lines, and Reagents**

Bone marrow aspirates from the patients with acute myeloid leukemia (AML; *n* = 70), acute promyelocytic leukemia (*n* = 8), B-cell acute lymphocytic leukemia (*n* = 37), and T-cell acute lymphocytic leukemia (*n* = 7) were obtained.
after institutional review board approval and informed patient consent. The percentage of infiltrating blasts in the bone marrow was >85%. Mononuclear cells were prepared as described (3). To examine the apoptosis of primary leukemic cells induced by CRAds, we established a short time culture of patient’s blasts as reported previously (20). Briefly, mononuclear cells from another 6 patients with AML were allowed to settle in MEM (Life Technologies) for 17 min at 37°C to eliminate cell sticking. The non-sticking cells were cultured in MEM with 15% heat-inactivated FCS (Hyclone Laboratories). Viability was better than 96% after 72 h as determined by trypan blue dye exclusion.

Human T-cell leukemia lines Molt-4 and Jurkat, chronic myelogenous leukemia line K562, AML line HL-60, myeloma line RPMI 8226, and lymphoma line L428 were purchased from the American Type Culture Collection. B-cell leukemia line Mutz-1 was provided by Dr. Z Hu, and myelogenous leukemia line K562, AML line HL-60, myeloid leukemia line Kasumi was kindly provided by Prof. S Chen. Cells were cultured in RPMI 1640 (Hyclone) supplemented with 10% FCS (Hyclone) and 1% L-glutamine (Life Technologies). Human embryonic kidney cell line (Microbix Biosystems) and cervical cancer cell line HeLa (Shanghai Cell Collection) were maintained in MEM supplemented with FCS. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was purchased from Sigma, and caspase inhibitors z-DEVD-FMK and z-IETD-FMK were from Bio Vision.

Construction of Recombinant Adenovirus Vectors

Vectors ZD55, pZD55-TRAIL, and pZD55-enhanced green fluorescence protein (GFP) were constructed as described previously (21, 22). Plasmid DC311-TRAIL was constructed as follows: the TRAIL expression cassette of pZD55-TRAIL was cloned into shuttle plasmid (pDC311) by cutting with Bgl II (New England Biolabs) and religating to generate pDC311-TRAIL. The resulting shuttle vector (pDC311-TRAIL) or pZD55-TRAIL was cotransfected with a serotype 35 adenovirus vector (pPE35) into 293 cells to generate the Ad-TRAIL and SG325-TRAIL CRAd, respectively, through homologous recombination.

Cell Viability Assay

Leukemic cells were plated on 96-well plates at 1 × 10^4 per well 1 day before virus infection. Cells were then infected with viruses at the indicated multiplicity of infection (MOI) for 48 h. PBS was used as a control. The cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay and trypan blue dye exclusion as described previously (23).

Western Blotting

Western blot analysis was done as described previously (23), with 40 mg protein loaded on 12% SDS-PAGE. The primary antibodies used were as follows: caspase-9, caspase-8, caspase-3, poly(ADP-ribose) polymerase, Bcl-1, Bid, Bcl-xL, and Bax antibodies were purchased from Cell Signaling Technology. Bin and Mcl-1 were obtained from Epitomics. GAPDH and actin were used as housekeeping protein control and provided by Kangchen. After incubation with secondary antibodies (KPL), blots were revealed by enhanced chemiluminescence procedures according to the manufacturer's recommendation.

Flow Cytometric Analysis

FITC-conjugated anti-human CD46 (BD Pharmingen) and phycoerythrin-conjugated anti-human DR4, DR5, DcR1, and DcR2 (eBioscience) antibodies were used. Stained cells were analyzed on a FACSCalibur flow cytometer (Becton Dickinson).

Apoptosis was assessed by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining (Roche) and phycoerythrin-conjugated Annexin V apoptosis detection kit (Becton Dickinson) following the manufacturer’s instructions. For mitochondrial membrane potential evaluation, cells were stained with 5 μg/mL rhodamine 123 (Invitrogen) at 37°C for 30 min and then measured at 530 nm using flow cytometry.

ELISA for TRAIL

An ELISA for levels of TRAIL in leukemic cell supernatant was done using a Quantikine kit as per the manufacturer’s instructions (R&D Systems).

Colony-Forming Cell Assay

Leukemic colony formation assay was done as described elsewhere (24, 25). Briefly, blasts were collected from the peripheral blood or bone marrow of untreated AML patients. Cells were cultured in MEM for 20 min at 37°C in plastic flasks, and nonsticking cells were collected and resuspended in serum-free MEM with or without viruses at MOI of 50 for 4 h and plated at 2 × 10^5/mL in methylcellulose, growth medium, and 10% phytohemagglutinin-leukocyte conditioned medium for incubation at 37°C for 6 days. Colonies containing in excess of 20 cells were counted under an inverted microscope (Olympus). For normal hematopoietic progenitor assays, bone marrow cells (2 × 10^7/mL) were exposed to viruses (50 MOI) in serum-free medium for 4 h and cultured in 0.8% methylcellulose and 20% FCS/Iscove’s modified Dulbecco’s medium supplemented with granulocyte-macrophage colony-stimulating factor (Sigma). The cultures in triplicates were maintained for 6 days with humidity and 5% CO₂, after which colonies were enumerated.

Animal Studies

The local animal care and use committee approved this study. Cell implantation and virus treatment were done as described (3). Briefly, 3- to 4-week-old female severe combined immunodeficient mice (Shanghai Experimental Animal Center of the Chinese Academy of Sciences) were injected with 1 × 10^7 Kasumi cells in 100 μL s.c. into the hind flanks. Tumor volume was measured and calculated as π/6 length × width². When the tumors reached a volume of ~120 mm³, animals were randomly assigned to treatment groups and received three intratumoral injections of 1.7 × 10^8 plaque-forming units viruses diluted in a volume of 100 μL PBS. The untreated control received PBS. On the day 5 after treatments, one mouse of each group was humanely killed, and tumors were harvested and then processed for TUNEL assay using In situ Cell Death Detection Kit (Roche).
Statistical Analysis
Differences among the treatment groups were assessed by ANOVA using GraphPad Prism 4. *P < 0.05* was considered significant.

Results
Infection and Replication of Chimeric Oncolytic Adenoviruses in Leukemia Cells

First, we analyzed transduction efficiency of SG235 in a panel of leukemia cell lines. Infection of Kasumi cells, which were shown to be CAR negative (3), with SG235-GFP at MOIs of 5, 25, and 50 resulted in a dose- and time-dependent shift in fluorescence (Fig. 1A). Flow cytometric analysis showed that SG235 infected all tested hematopoietic malignant cell lines (Fig. 1B). To assess the replication ability of SG235, we compared the viral production of SG235 with that of ZD55, a 5 type oncolytic adenovirus. The total viral particles in the culture medium and cell fraction were determined by performing plaque assay on 293 cells. After 48 h of virus infection, the viral yield increased significantly for SG235. In contrast, ZD55 replicated in CAR-positive Mutz-1 cells but not in CAR-negative Kasumi cells (Fig. 1C). These results confirm that SG235 infects and replicates effectively in a variety of leukemic cells through a CAR-independent mechanism.

Chimeric Oncolytic Adenovirus SG235 Induces Apoptosis of Leukemia Cell Lines

Next, a panel of leukemic cell lines was examined for apoptosis by Annexin V staining and fluorescence-activated...
cell sorting analysis. Infection with SG235 at a MOI 50 resulted in apoptosis of all tested leukemic cells (data not shown). The apoptosis induced by SG235 was verified by TUNEL assay in Kasumi cells. The number of cells with TUNEL positivity increased in a dose-dependent manner after treatment (Fig. 2A). To determine the underlying mechanism by which SG235 induces apoptosis of leukemia cell, the activation of caspases was examined by Western blot analysis. After infection with SG235 at the time interval indicated, pro-caspase-9 levels decreased and the cleavage of caspase-9, caspase-3, and poly(ADP-ribose) polymerase was observed (Fig. 2B). These data showed that infection with SG235 triggers activation of caspase cascade. Similar results were obtained from human HeLa cell line (Supplementary Fig. S1), suggesting the broad activity of SG235 against tumor cells.

---

SG235-TRAIL Exhibits Enhanced Cytotoxicity and Induction of Apoptosis via Activation of Caspase

To determine whether TRAIL expression could improve the antitumor activity of fiber chimeric CRAd, cell proliferation was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Both SG235 and SG235-TRAIL significantly inhibited growth of leukemic cells in a dose-dependent manner compared with no viral treatment (P < 0.001). Moreover, SG235-TRAIL achieved stronger cytotoxicity than did SG235 (Fig. 3A). Similarly, when cells were exposed to SG235-TRAIL, a significant increase in apoptosis was observed in Kasumi cells (Fig. 3B) and Mutz-1 cells (data not shown). The activation of caspase-8 and the caspase-8 substrate Bid was observed in the cells treated with SG235-TRAIL for 48 h but not in the control cells. Dysfunction of mitochondria, up-regulation of Bax, and activation of caspase-9 and caspase-3 were detected as well. In contrast, SG235 only showed a slight activation of caspase-9 and caspase-3 but not caspase-8 (Fig. 3C and D). Specific inhibitors of caspases were used to further confirm involvement of the extrinsic apoptotic pathway. Either z-DEVD-FMK (caspase-3 inhibitor) or z-IETD-FMK (caspase-8 inhibitor) provided a partial protection against cytotoxicity of SG235-TRAIL. Moreover, the inhibitory effect of caspase-3 inhibitor appeared to be stronger than that of caspase-8 inhibitor (Fig. 3E). We further analyzed activation of caspase-9 and caspase-3 in a panel of cell lines including HL-60 (AML), K562 (chronic myelogenous leukemia), RPMI 8226 (myeloma), and Jurkat (T-cell leukemia). The activation of caspases was detected in all tested cell lines (Supplementary Fig. S1). Together, these data are consistent with the previous observation that both membrane and mitochondrial pathways were activated on TRAIL exposure in K562 cells (25), indicating that increased cell killing by SG235-TRAIL is mainly dependent on TRAIL-mediated apoptosis.

---

**Effects of SG235-TRAIL on the Expression of TRAIL Receptors and Bcl-2 Family Proteins**

TRAIL induces apoptosis in a wide variety of tumor cells by interacting with death signaling receptors (DR4 and DR5). The decoy receptors (DcR1 and DcR2) compete with death receptors for TRAIL engagement (26). We thus examined the expression of TRAIL receptors on leukemia cell lines. The expression of DR4 and DR5 was undetectable on Kasumi, Mutz-1, and K562 cells, which is consistent with a previous report showing similar phenomena in leukemic cell lines and primary blasts (27). A weak expression of DcR1 and DcR2 at the protein level was observed (Fig. 4A). DR4 and DR5 expression was not up-regulated by SG235-TRAIL (data not shown). We further determined whether SG235-TRAIL virus secreted TRAIL into the medium of infected cells using an ELISA. Figure 4B showed that TRAIL could be detected in the culture supernatants of Kasumi and K562 cells treated with SG235-TRAIL. In contrast, the medium of cells infected with Ad-TRAIL or SG235 showed that TRAIL levels were below the detectable range of the assay (<31.2 pg/mL).

Because proapoptotic Bcl-2 family members are involved in tumor cell resistance to TRAIL (28, 29), we investigated

---

5 Supplementary material for this article is available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).
the behavior of Bcl-2 family member proteins in leukemic cells infected with the viruses. Cells treated with SG235 and SG235-TRAIL showed a decreased expression of Bcl-2 and Mcl-1, suggesting an inhibitory effect of the CRAds on Bcl-2 and Mcl-1 proteins. The expression levels of Bim, Bak, and Bcl-xL were not affected by SG235 or SG235-TRAIL infection (Fig. 4C). These observations were further confirmed by quantitative real-time PCR analysis (data not shown).

Figure 3. SG235-TRAIL induces apoptosis via activation of both membrane and mitochondrial pathways and results in enhanced cytotoxicity. A, leukemic cells were treated with SG235 and SG235-TRAIL at the indicated viral particles for 48 h. Ad-TRAIL was used as the vector control. Cell viability was determined by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide assay. Mean ± SD of three independent assays. B, Kasumi cells were infected with the indicated vectors at MOI of 50. On day 2 after the treatment, apoptosis was assessed by flow cytometric analysis. C, mitochondrial membrane potential was evaluated by rhodamine 123 staining in Kasumi cells treated with SG235-TRAIL at 50 MOI for 48 h. D, cell extracts prepared from Kasumi cells untreated or exposed to the viruses for 48 h were examined with Western blotting using antibodies that recognize the indicated polypeptide. E, Kasumi cells were treated with SG235-TRAIL in the presence or absence of z-DEVD-FMK (50 μmol/L) or z-IETD-FMK (50 μmol/L) for 3 d and then examined for cell apoptosis by flow cytometric analysis. *, P < 0.001 versus SG235-TRAIL alone; #, P < 0.01 versus SG235-TRAIL alone.
Expression of CD46 and Selective Cytotoxic Effect of Chimeric CRAds in Primary Leukemic Cells

Because Ad35 uses CD46 as a high-affinity primary attachment receptor (15, 30), the expression level of CD46 in primary leukemic cells from 122 patients was determined by flow cytometry. Most of the tested primary blasts expressed CD46 (data not shown). As determined, 60 of 70 cases with AML were found to be CD46+ (≥30% of reactive cells), and 7 of 8 acute promyelocytic leukemia patient samples were CD46+. In B-cell acute lymphocytic leukemia, 24 of 37 patient samples were CD46+, and in T-cell acute lymphocytic leukemia, 5 of 7 patients were CD46+.

To examine infectivity and cytotoxicity effects of SG235 on primary leukemic cells, the blasts from 6 AML patients were infected with SG235-GFP at MOI 50 for 48 h, and GFP-positive cells were checked by a fluorescence microscope or counted by fluorescence-activated cell sorting. Results showed that SG235 effectively infected primary AML cells and achieved 25.64% of GFP-positive cells on average (Fig. 5A). Furthermore, leukemic cells treated with Ad-TRAIL, SG235, and SG235-TRAIL vector showed average of 9.2%, 14.9%, and 30.5% Annexin V-positive/propidium iodide-negative cells, respectively (Fig. 5B). The increased apoptosis induced by SG235-TRAIL was also confirmed through analyzing the cleaved forms of caspase-3 and poly(ADP-ribose) polymerase (Fig. 5C). Next, we examined the ability of infectious and cytopathic effect of the CRAds on primary leukemic cells by performing the colony formation assay. AML cells from 2 patients and incubated for 5 days in methylcellulose culture with SG235-GFP showed bright green fluorescence dots (Fig. 6A). As expected, SG235 significantly inhibited leukemic colony formation in all tested 6 AML specimens (SG235 versus untreated control; P < 0.01). On infection by SG235-TRAIL, almost no colonies were recovered in any cases, suggesting enhanced cytopathic effects induced by SG235-TRAIL. In contrast, Ad-TRAIL slightly inhibited clonogenic survival of primary AML samples.

To assess the cytotoxicity of CRAds to normal hematopoietic progenitor cells, CFU-GM assays were done by plating appropriate numbers of mononuclear cells obtained from normal bone marrow samples in methylcellulose medium supplemented with recombinant human granulocyte-macrophage colony-stimulating factor. The data presented in Fig. 6C revealed that the number of normal myeloid progenitor cells (CFU-GM) was not significantly affected by Ad-TRAIL and SG235. Treatment with SG235-TRAIL resulted in 17% reduction of the colony number. These results indicate that the cytopathic effect of SG235 and SG235-TRAIL on normal hematopoietic progenitors is minimal.

In vivo Antitumor Activity of the Chimeric Oncolytic Adenoviruses

We subsequently investigated the relative antileukemia efficacy of SG235 in vivo. Treatment of CAR-negative Kasumi xenograft-bearing severe combined immunodeficient mice with direct intratumor injection of 5 × 10⁸ plaque-forming units SG235 achieved significant tumor growth inhibition compared with the PBS control (P < 0.001). In contrast, treatment with ZD55 almost had no effect on the tumor growth (Fig. 7A). We next assessed the therapeutic efficacy of SG235 versus SG235-TRAIL against leukemic cells in vivo (Fig. 7B). SG235-TRAIL was more effective than SG235 in tumor growth inhibition (SG235-TRAIL versus SG235; P < 0.001 at the conclusion of the treatment). The antitumor therapeutic effect of nonreplicative Ad-TRAIL was not observed. Furthermore, one animal from each treatment group was euthanized 5 days after treatment, and tumors were harvested for histochemical examination. Results of in situ TUNEL assay showed a marked
increase of apoptotic cells within the tumor treated with SG235-TRAIL compared with the treatment of SG235 (Fig. 7C).

Discussion

This study found that SG235, a new fiber chimeric CRAd, could effectively infect a variety of malignant hematopoietic cell lines regardless of their CAR expression status. Leukemia cells treated with SG235 showed a significant growth inhibition and apoptosis evidenced by Annexin V staining and TUNEL assay. Importantly, in addition to an effect of SG235 on commercially available permanent tumor cell lines, a strong cytotoxicity effect on primary leukemic cells was also observed, whereas almost no effect on normal hematopoietic progenitors was detected. Because chimeric Ad5 vectors possessing Ad35 fibers use CD46, often abundant on tumor cells compared with normal cells (31), as a primary attachment receptor, we analyzed CD46 expression on bone marrow samples from the patients with AML and acute lymphocytic leukemia by flow cytometry. CD46 was expressed in a majority of tested primary blasts. These findings, along with our data that leukemic cells are sensitive to SG235, suggest that this new fiber chimeric CRAd is an attractive vector for treating hematopoietic tumors including leukemia.

TRAIL, a member of the tumor necrosis factor superfamily, has garnered considerable attention as a novel anticancer agent, as it appears to selectively induce apoptosis of cancer cells but not of normal cells in various tissue types (32). Human recombinant TRAIL is reported to induce apoptosis of multiple myeloma (33, 34) and myeloid leukemia
cells (35, 36) and elicit potent antitumor activity and synergistic effect with chemotherapy (37, 38). However, some leukemic cell lines and fresh blasts are unresponsive to TRAIL treatment (39, 40). In the present study, we have shown for the first time that SG235-TRAIL, a fiber chimeric CRAd expressing TRAIL, exhibits enhanced oncolytic potency in a variety of leukemic cells compared with its parent vector SG235. Moreover, SG235-TRAIL is much stronger than SG235 in killing established leukemia xenografts, which is related to apoptosis induction. The data reported herein have confirmed the results of others and our previous observation in different cancer cell models (21, 41) and suggested that integrating TRAIL gene therapy into an oncolytic adenovirus overcomes the weaknesses of the TRAIL gene therapy and virotherapy used individually.

TRAIL activates the extrinsic apoptotic pathway in type I cells. In type II cells, the intrinsic mitochondrial pathway is recruited to amplify the apoptotic signal through cleavage of Bid by caspase-8 (39). Our study revealed that the intrinsic mitochondrial pathway is also involved in SG235-TRAIL-induced apoptosis of leukemic cells evidenced by the activation of caspase-8 and caspase-9, decreased Bid, and dysfunction of mitochondria. Moreover, Bcl-2 family proteins are critical for the determination of sensitivity to TRAIL (28, 29). Recent studies have shown that degradation of Mcl-1, a Bcl-2 family member, initiates mitochondrial apoptotic cascade (42). In this study, we observed down-regulated expression of Bcl-2 and Mcl-1 in SG235-treated or SG235-TRAIL-treated cells. This loss of Mcl-1 might contribute to the activated intrinsic pathway.

Several intracellular events blocking TRAIL-mediated apoptosis have been identified by researchers, such as lack of expression of death receptor on the cell surface and competitive binding of TRAIL by the decoy receptors (39).
this regard, we found that the DR4/DR5 expression was absent in three leukemic lines, whereas dimly positive DcR1/DcR2 expression was detected, which is in line with the recent data that DR4 expressed in ~50% of AML cases and DR5 expressed only in a minority (~10%) of the cases analyzed (40). Moreover, there was no any modification of DR4/DR5 and DcR1/DcR2 expression in SG235-TRAIL-treated cells. Thus, the reason why SG235-TRAIL induced a massive apoptosis in leukemic cells with defect of the DR4/DR5 expressions is still unclear; however, it was reported that the status of signaling or decoy TRAIL receptors, assessed through either immunoblotting analysis or flow cytometry, cannot serve to reliably predict the TRAIL sensitivity (or resistance) of multiple myeloma cells (33). Recently, Wu et al. reported that a nonreplicative Ad5/35-TRAIL is more efficient than exogenous TRAIL in triggering apoptosis of HL-60 leukemia cells (43). The exact reason for this discrepancy is unclear. It is important to note that the authors reported TRAIL-induced apoptosis of HL-60 cells only at very high doses of TRAIL (10-100 ng/mL) and did not examine secreted TRAIL in the medium of Ad5/35-TRAIL-infected cells. Data from other studies support the observation that high doses of recombinant TRAIL are needed for apoptosis of leukemic cells (35, 40). Our results showed that low concentrations of TRAIL were detected in the medium of SG235-TRAIL-treated cells, indicating that the mechanisms by which SG235-TRAIL induces apoptosis may differ from that of exogenous TRAIL. Because constitutive endocytosis and predominant intracellular location of some of the TRAIL receptors were reported previously (44, 45), further studies are needed to determine whether TRAIL produced by SG235-TRAIL ligates the death

Figure 7. Antitumor activity of chimeric oncolytic adenoviruses in s.c. Kasumi xenografts in animal model. A, tumor-bearing mice (n = 7) were treated with either PBS or 5 × 10^8 plaque-forming units of the indicated viruses by intratumor injection. *, P > 0.05 from PBS control; #, P < 0.001 from control. B, animals with Kasumi tumors were intratumorally injected with 5 × 10^8 plaque-forming units of different vectors. The size of tumor was monitored and tumor volume was calculated. Points, mean of tumor volume (n = 7); bars, SD. *, P < 0.001 versus SG235-treated group. C, apoptotic cell death is induced by treatment with SG235 and SG235-TRAIL in vivo; apoptotic nuclei are stained in blue. TUNEL pictures were taken at ×50 original magnification.
receptors within intracellular organelles and thus triggers apoptosis. Additionally, adenovirus EIA gene expression in tumor cells was reported to enhance killing by TRAIL (46), which may contribute to enhanced apoptosis induced by SG235-TRAIL.

In conclusion, we showed that the fiber-modified oncolytic adenovirus SG235 permits CAR-independent cell entry and induces selective cytopathic effects in human leukemic cells in vitro and in vivo. Furthermore, SG235-TRAIL treatment could elicit much stronger cytotoxic effects through induction of apoptosis than did SG235. These findings suggest that the current gene-virotherapeutic strategy could be a promising approach for treating leukemia.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References


Molecular Cancer Therapeutics

Effective gene-viral therapy of leukemia by a new fiber chimeric oncolytic adenovirus expressing TRAIL: \textit{in vitro} and \textit{in vivo} evaluation

Jie Jin, Hui Liu, Chunmei Yang, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/1535-7163.MCT-08-0962

Supplementary Material
Access the most recent supplemental material at:
http://mct.aacrjournals.org/content/suppl/2009/04/29/1535-7163.MCT-08-0962.DC1

Cited articles
This article cites 46 articles, 23 of which you can access for free at:
http://mct.aacrjournals.org/content/8/5/1387.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.