

# Pro-apoptotic effect of TRAIL-transfected endothelial progenitor cells on glioma cells

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**Abstract.** Glioma is one of the most common aggressive neuroepithelial malignant tumors in the central nervous system. It has a high recurrence rate and poor prognosis, primarily due to the fact that novel therapeutic agents cannot penetrate the blood-brain barrier (BBB). Endothelial progenitor cells (EPCs) have been reported to move across the BBB and access the tumor site. However, whether EPCs expressing the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) induce glioma cell apoptosis requires further investigation. In the present study, EPCs were transfected and stably expressed with TRAIL through lentiviral infection. The pro-apoptotic effect of these TRAIL-expressing EPCs on the SHG44 glioma cell line was investigated. The migration ability of TRAIL-expressing EPCs toward SHG44 cells through the Transwell culture system was investigated via a high-content screening assay. The apoptotic rate and the expression of cleaved caspase-8 and -3 in addition to the cleaved poly(ADP-ribose) polymerase in SHG44 cells significantly increased in the TRAIL-overexpressing EPC treatment group compared with the controls. The increased apoptotic rate was reversed using a caspase inhibitor. The findings suggested that the TRAIL-expressing EPCs induced apoptosis in the SHG44 cells by activating the death receptor pathway, indicating that the TRAIL-expressing EPCs may be a useful strategy for glioma treatment.

## Introduction

Glioma is the most common malignant tumor in the central nervous system (CNS). It occurs in any part of the CNS and exhibits highly aggressive and malignant behavior. The survival rates of glioma remain at a low level after the traditional

treatment, including surgical resection, radiotherapy, and chemotherapy (1,2). Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) has become one of the hotspots in the last several years (3). TRAIL has shown great anticancer activity by selectively binding to death receptor 4 (DR4) and death receptor 5 (DR5) and inducing tumor cell apoptosis, while healthy cells remain affected (3-6). However, blood-brain barrier (BBB), as a protective mechanism for CNS tumors, prevents TRAIL from reaching tumor sites (7,8). TRAIL cannot be applied in CNS cancer therapy owing to its short half-life (9,10). TRAIL was combined with stem cells to address these issues. Both neural stem cells (NSCs) and mesenchymal stem cells (MSCs) were engineered to express TRAIL and restrict glioma cells, showing bright prospects (11-15). However, SCs had some limitations, such as uncertain differentiation and difficulties in acquisition from adults.

Endothelial progenitor cells (EPCs) are the precursors of endothelial cells, with CD31 and CD34 as their specific surface markers. They were first isolated in 1997 from human peripheral blood (16,17). EPCs are recruited in the neovascularization of tumor and involved in tumor vascular network (18,19). Owing to these characteristics, EPCs have the potential to be involved in anti-tumor therapies. Most previous studies explored anti-angiogenic treatment by suppressing the mobilization and homing of EPCs to tumors (20). Increasing attention has been paid to the use of EPCs as a vector for treatment (21,22). Recent studies have demonstrated that EPCs can move across the BBB from peripheral blood and participate in the angiogenesis of gliomas (23,24). Compared with NSCs and MSCs, EPCs can be obtained from peripheral blood, which means that EPCs can be easily acquired from adults. This makes the autotransplantation of EPCs possible and avoids immunological rejection. Therefore, EPCs have been recognized as an ideal vector to deliver antineoplastic molecules to CNS tumors. However, whether the TRAIL-expressing EPCs home to glioma cells through the BBB and induce cell apoptosis needs further investigation.

In this study, EPCs engineered with a lentivirus encoding TRAIL were generated and used to induce apoptosis of a glioma cell line SHG44. First, the Transwell assay was used to verify that EPCs could home to glioma cells. Then, the co-culture system of TRAIL-overexpressing EPCs and SHG44 cells was established, and the apoptotic state of SHG44 cells

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was examined. Interestingly, the apoptosis percentage and change in protein levels of caspase-8 and -3, as well as poly ADP-ribose polymerase (PARP), in SHG44 cells were found to increase remarkably. Taken together, the study demonstrated that the TRAIL-modified EPCs could be a feasible approach for glioma therapy.

## Materials and methods

**Cell cultures.** Human glioma cell line, SHG44 cells, were purchased from Cell Bank of the Chinese Academy of Sciences and cultured in Dulbecco's modified Eagle's medium (DMEM)/high-glucose medium (HyClone; GE Healthcare, Logan, UT, USA) with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA), 0.5 mm glutamine, and 100 U/ml penicillin/streptomycin at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub> and 95% air.

EPCs were extracted from the blood of neonatal Sprague-Dawley rats by the density gradient method, as previously described (25), and cultured in endothelial cell growth medium-2 (EGM-2; Lonza, Group, Ltd., Basel, Switzerland) culture medium with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.), 0.5 mm glutamine, and 100 U/ml penicillin/streptomycin in a 5% CO<sub>2</sub> atmosphere at 37°C.

**Immunocytochemical analysis.** EPCs were washed twice with phosphate-buffered saline (PBS) and fixed with 4% paraformaldehyde at 4°C overnight. After removing paraformaldehyde and washing the cells with PBS, 3% H<sub>2</sub>O<sub>2</sub> was used to inactivate endogenous peroxidase for 10 min. The cells were incubated with 5% bovine serum albumin (BSA) for 1 h at room temperature to block the nonspecific binding. Then, the primary antibodies (CD31 and CD34, from Abcam, Cambridge, MA, USA) were added onto cells and incubated at 4°C for 12 h. Only PBS with 5% BSA was used as negative control. The cells were washed with PBS three times to remove unbound antibody and then incubated with secondary antibody conjugated to horseradish peroxidase (HRP; ZSGF-BIO, China) for 30 min at room temperature. After removing the secondary antibody by washing with PBS, the cells were incubated with 3,3'-diaminobenzidine developer, counterstained with hematoxylin, and observed under a microscope.

**Immunofluorescence.** EPCs were washed with PBS and fixed with 4% paraformaldehyde after 3 days of lentiviral transfection. The cells were blocked with 5% BSA for 30 min and then incubated with primary antibodies (TRAIL, DR4, and DR5; Abcam) at 4°C for 12 h. After the cells were washed with PBS, secondary antibodies were used for staining (Alexa 594 conjugated; Thermo Fisher Scientific, Inc.; Alexa 405 conjugated; Wuhan Sanying Biotechnology, Wuhan, China). Cells were observed using laser scanning confocal microscopy (Nikon Corporation, Tokyo, Japan).

**Angiogenesis assay.** BD Matrigel basement membrane matrix (BD Biosciences, Franklin Lakes, NJ, USA) was melted at 0°C, implanted in a 24-pore culture plate, and then placed in a 37°C incubator for 1 h. EPCs were inoculated on the surface of solidified Matrigel (5x10<sup>4</sup> cells per well) and cultured in a 5% CO<sub>2</sub> atmosphere at 37°C. The cells were observed

under a microscope after 6 h, and images were taken under magnification, x40 and x100.

**Lentiviral infection.** The lentivirus with the TRAIL sequence was delivered to overexpress the TRAIL protein in EPCs. The negative control virus carrying green fluorescent protein (GFP) only was used as a control. Both viruses were constructed and packed by Shanghai GeneChem Co., Ltd., Shanghai, China. EPCs were infected using lentiviruses with a multiplicity of infection of 10 on the second day after the first passage of EPCs.

**RNA extraction, cDNA synthesis, and reverse transcription-quantitative polymerase chain reaction (RT-qPCR).** After a week of lentiviral infection, total RNA was extracted from EPCs in both the groups using TRIzol reagent (Solarbio, Beijing, China), chloroform, and isopropyl alcohol. Then, 1 µg of extracted RNA was reverse transcribed into strand cDNA after removing genomic DNA using a PrimeScript Reverse Transcription Reagent kit with gDNA Eraser (TaKaRa Bio Co., Ltd., Otsu, Japan). Real-time quantitative polymerase chain reaction (PCR) was performed on Roche LightCycler (Roche Applied Science, Rotkreuz, Switzerland) using the same reagent kit with cDNA synthesis for the desired gene. The primers were designed and synthesized by Sangon Biotech as follows:

TRAIL sense, 5'-AACTGGGACCAGAGGAAGAAGCAA-3' and antisense, 5'-ATGCCCACTCCTTGATGATTC CCA-3'; Glyceraldehyde-3-phosphate dehydrogenase sense, 5'-TCCTGCACCACCAACTGCTTAG-3' and antisense, 5'-TCCTGCACCACCAACTGCTTAG-3'.

**Co-culture.** SHG44 cells were cultured with TRAIL-expressing EPCs as the experimental group, or with EPCs expressing only GFP as the control group. Both groups were in the same culture environment: EGM-2 culture medium was used with 10% FBS, 0.5 mM glutamine, and 100 U/ml penicillin/streptomycin in a 5% CO<sub>2</sub> atmosphere at 37°C.

**Transwell migration assay.** *In vitro* cell migration assays were performed using 24-well Transwell chambers (12-µm pores; Corning Inc., Corning, NY, USA). Nontransfected EPCs and TRAIL-transfected EPCs (2x10<sup>4</sup> cells per well) were cultured in the top chamber, whereas SHG44 cells, as a positive group, were cultured in the lower chamber. DMEM medium with 10% FBS only in the lower chamber was used as the control group. After 24 h of cultivation, the cells on the upper side were removed and the migrated cells were fixed in 4% paraformaldehyde, stained with 4',6-diamidino-2-phenylindole (DAPI) solution, and examined using a high-content screening system (HCS; Thermo Fisher Scientific, Inc.).

**Propidium iodide and Annexin V assays.** The cells were gently washed once with PBS on the third day of co-culture and then stained with Annexin V-kFluor594 (Nanjing KeyGen Biotech Co., Ltd., Nanjing, China) for 15 min at room temperature away from light. The cells were observed under a fluorescence microscope after replacing the staining solution with PBS. The flow cytometry assay was performed using Annexin V-APC (Nanjing KeyGen Biotech Co., Ltd.) and propidium iodide (PI) (Nanjing KeyGen Biotech Co., Ltd.), whose fluorescence

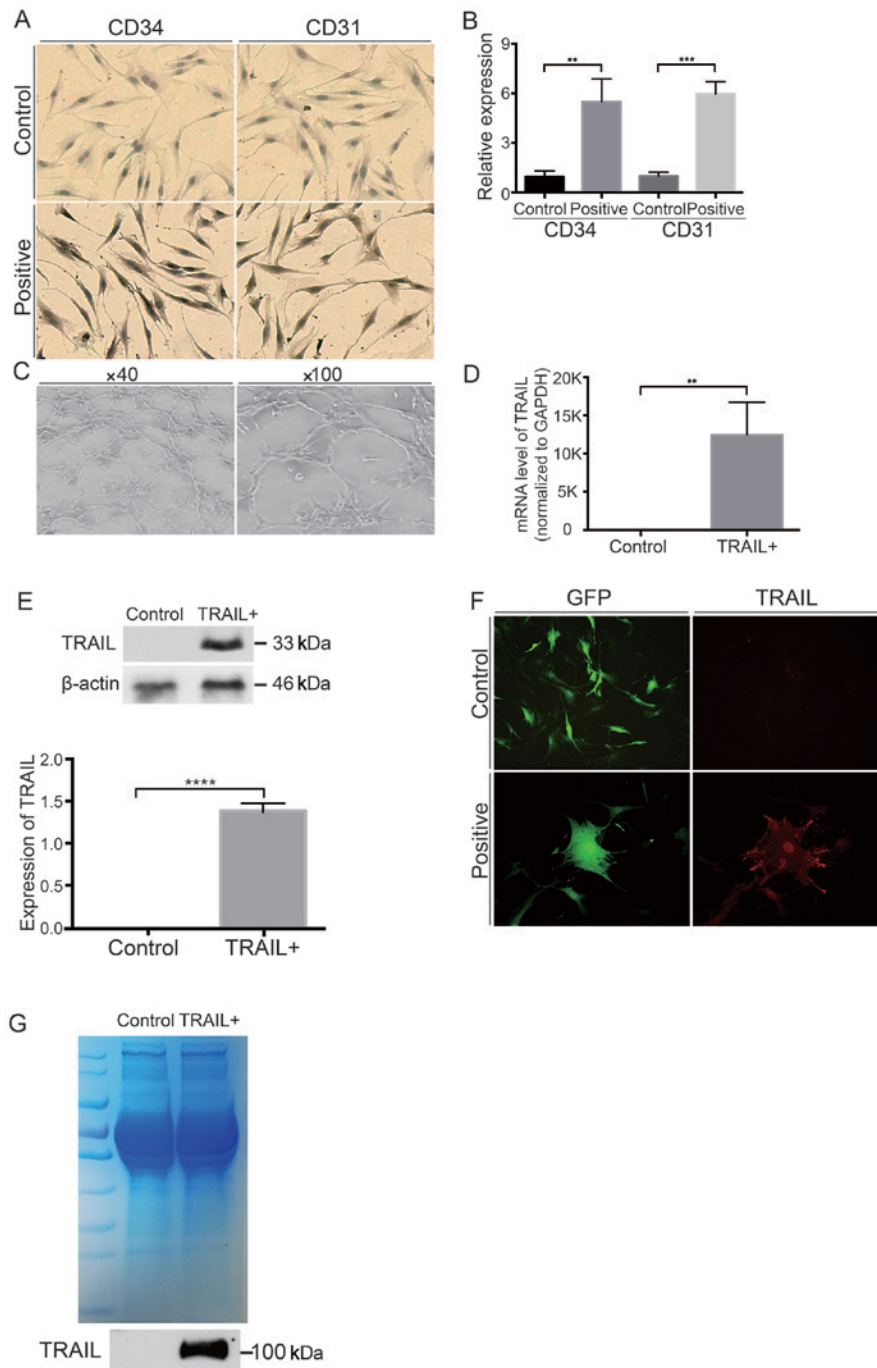


Figure 1. Construction of TRAIL-expressing EPCs. (A and B) Immunocytochemical staining showed that the cells were CD31 and CD34 positive (magnification, x100) ( $P<0.05$ , compared with the control group). (C) EPCs formed capillary-like networks after being seeded onto the Matrigel surface for 6 h. (D and E) EPCs were infected with lentiviruses encoding GFP or TRAIL. The mRNA and protein levels of TRAIL in EPCs were determined using qPCR (D) ( $P<0.05$ , compared with the control group) and western blot analysis.  $\beta$ -Actin was used as a loading control (E,  $P<0.05$ , compared with the control group). (F) EPCs harboring GFP showed green fluorescence, and EPCs harboring TRAIL showed red fluorescence (magnification, x200). (G) The sTRAIL was detected using western blot and Coomassie blue staining. Data are presented as means  $\pm$  SD. Three individual experiments were performed for each group. \*\* $P<0.01$ , \*\*\* $P<0.0001$  and \*\*\*\* $P<0.001$ , compared with the control group. TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; EPCs, endothelial progenitor cells; GFP, green fluorescent protein qPCR, quantitative polymerase chain reaction; sTRAIL, soluble extracellular TRAIL; SD, standard deviation.

signals were excited at 633 and 488 nm and collected at 660 and 610 nm, respectively, to determine the degree of apoptosis of SHG44 cells further. More than 10,000 cells per sample group were collected and divided into EPCs and SHG44 cells on the basis of GFP fluorescence intensity. The percentage of Annexin V-positive SHG44 cells was calculated as an indicator of apoptosis.

**Co-immunoprecipitation.** The cells from co-culture were homogenized in cell lysis buffer (Solarbio), supplemented with 1 mM phenylmethanesulfonyl fluoride (PMSF) and complete protease inhibitor mixture (Solarbio). The homogenate was centrifuged at 11,000 rpm for 15 min at 4°C. The supernatant was incubated with anti-TRAIL (Abcam) crosslinked beads at 4°C overnight with rotation. The Pierce Crosslink Magnetic

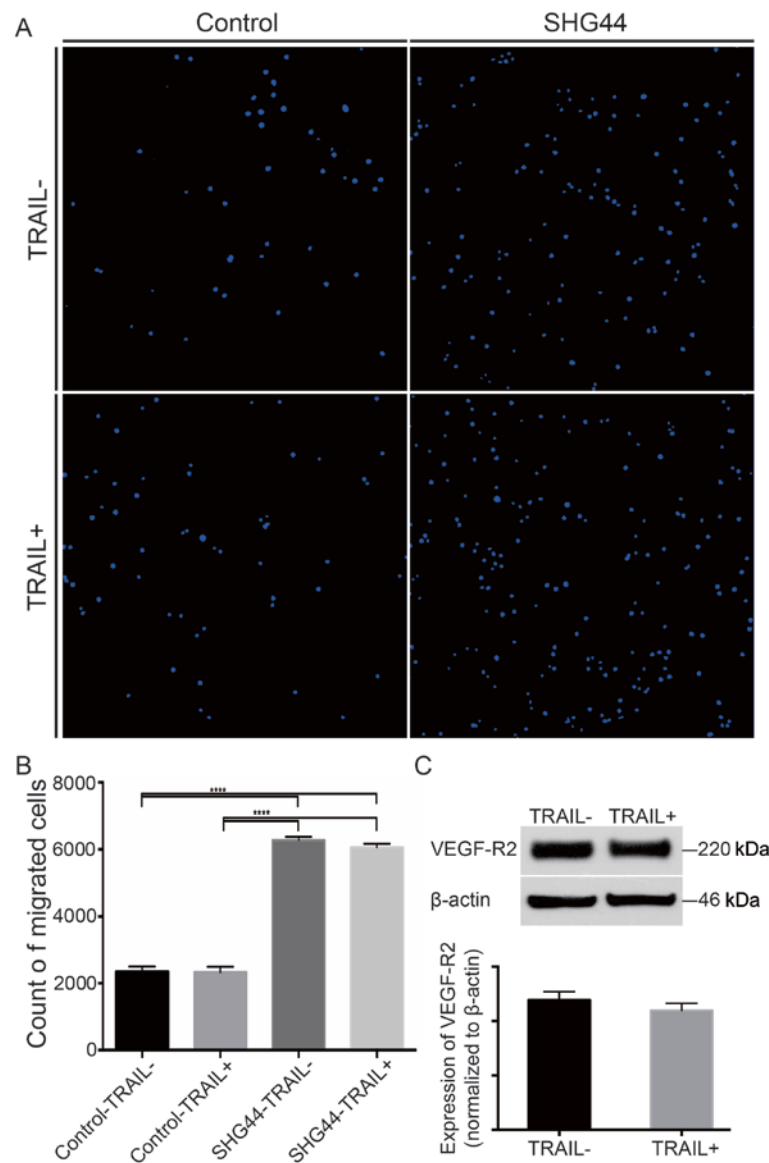


Figure 2. Transwell migration assay of EPCs. (A) Migrated EPCs were stained using DAPI, and images were obtained using HCS of 25 continuous visual fields. (B) Numbers of migrated cells were counted using HCS. The migrated EPCs increased obviously when SHG44 cells were cultured in the lower chamber (magnification,  $\times 100$ ) ( $P < 0.05$ , compared with the control group). No significant difference was found between EPCs with or without the overexpression of TRAIL ( $P > 0.05$ ). (C) Expression of VEGF-R2 on control and TRAIL + EPCs.  $\beta$ -Actin was used as a loading control. Data are presented as means  $\pm$  SD. Three individual experiments were performed for each group. \*\*\*\* $P < 0.0001$ , compared with the control group. EPCs, endothelial progenitor cells; DAPI, 4',6-diamidino-2-phenylindole; HCS, high-content screening; SD, standard deviation.

IP kit instruction was used for the pretreatment of beads and immunoprecipitation. The associated proteins were detected using western blot analysis. Homogenates from co-culture cells were used as positive controls.

**Quantitative immunoblot analysis.** The cells in the two groups were collected on third and fifth days of co-culture, homogenized in 1X cell lysis buffer (Solarbio) supplemented with 1 mM PMSF and complete protease inhibitor mixture (Solarbio) for 30 min on ice, and then centrifuged at 11,000 rpm for 10 min at 4°C. The supernatant was measured using a Bicinchoninic Acid Protein Assay kit (Solarbio). The measurement samples were combined with 5X SDS loading buffer and boiled at 100°C for 10 min. For each protein, equal amounts of samples (20-100  $\mu$ g) from each group were analyzed using sodium dodecyl sulfate-polyacrylamide gel

electrophoresis as previously described (26). After proteins were transferred onto a polyvinylidene difluoride membrane, the membrane was incubated with 5% BSA at room temperature for 2 h to block the nonspecific protein site and then corresponded with primary antibodies [TRAIL from Abcam; DR4 and DR5 from Abcam; vascular endothelial growth factor receptor 2 (VEGF-R2), caspase-8 and -3, and PARP from Cell Signaling Technology, Inc. (Danvers, MA, USA);  $\beta$ -actin from ZSGF-BIO] at 4°C overnight. This step was followed by incubation with HRP-conjugated secondary antibodies (ZSGF-BIO). Visualization was achieved using a SuperSignal West Pico Trial kit (Thermo Fisher Scientific, Inc.).

**Statistical analysis.** Data were expressed as mean  $\pm$  standard deviation (SD). One-way analysis of variance was used for comparisons among multiple groups, followed by the Student



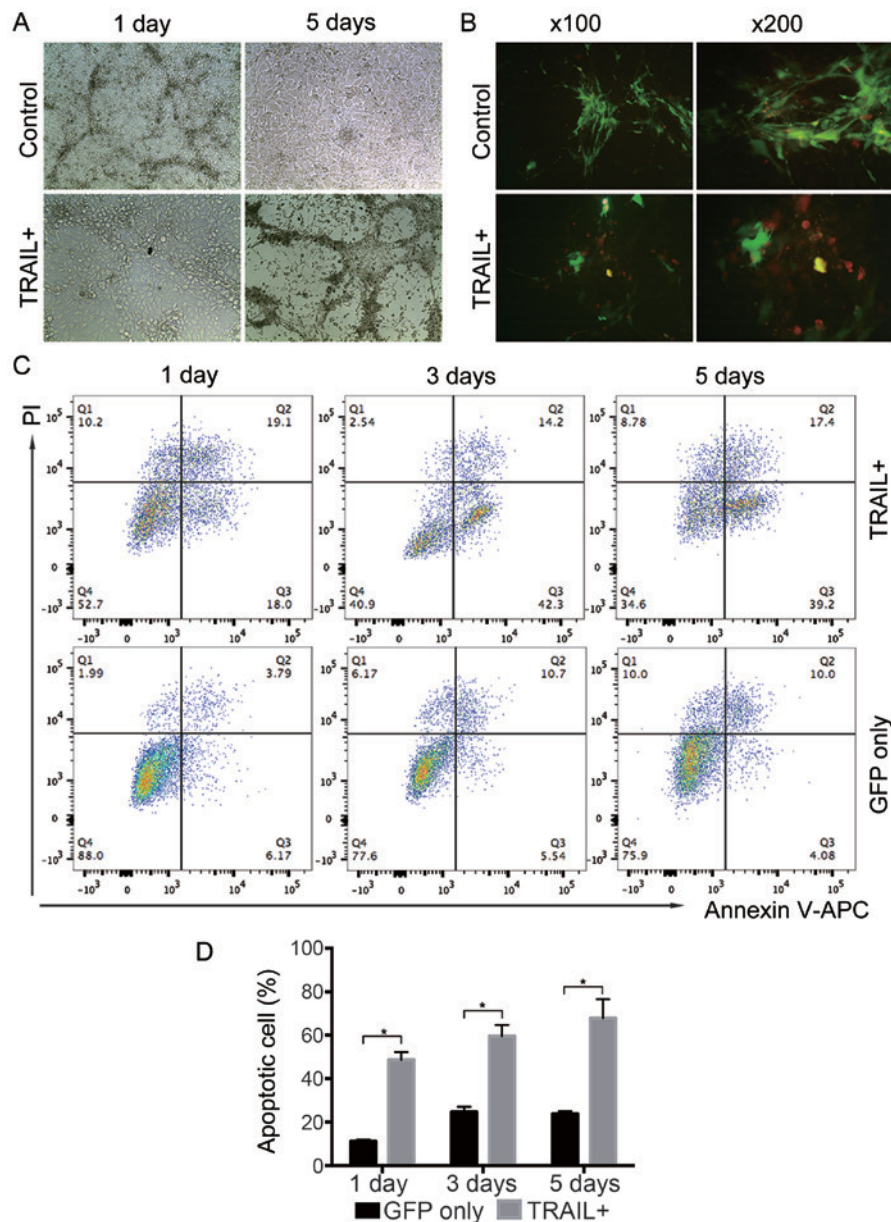


Figure 3. Apoptosis of SHG44 cells co-cultured with EPCs. (A) Cellular growth in the two groups on the first and fifth days after co-culture (magnification, x100). (B) Co-cultured cells were stained with Annexin V-kFluor594 on the fifth day. Apoptotic cells showed red fluorescence, and GFP-harboring EPCs showed green fluorescence. (C and D) The apoptotic rate of SHG44 cells was higher in the TRAIL-positive group than in the control group ( $P < 0.05$ ). Data are presented as means  $\pm$  SD. Three individual experiments were performed for each group. \* $P < 0.05$ , compared with the control group. EPCs, endothelial progenitor cells; GFP, green fluorescent protein; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; PI, propidium iodide; SD, standard deviation.

post hoc two-tailed test. The unpaired Student t-test was performed for comparisons between the means of two groups. GraphPad 6.0 (GraphPad Software, Inc., La Jolla, CA, USA) was used for all the statistical analyses.  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**Generation of TRAIL-expressing EPCs.** Blood cells were extracted from neonatal Sprague-Dawley rats, and the specific surface markers CD31 and CD34 were detected by immunohistochemical analysis (27). Most cells revealed positive reactions to CD31 and CD34 (Fig. 1A and B). The angiogenic ability of EPCs was also identified. The cells inoculated on the Matrigel surface formed tube-like structures within 6 h, as shown in representative

images (Fig. 1C). Thus, EPCs were isolated successfully from the blood of neonatal rats.

The isolated EPCs were infected and screened using lentivirus encoding TRAIL. Then, the expression level of TRAIL on EPCs was detected. Total mRNA was also extracted from both TRAIL-transfected EPCs and negative controls. Quantitative PCR was performed to quantify the mRNA levels. The TRAIL mRNA level in infected EPCs significantly increased compared with the control group ( $P < 0.05$ ) (Fig. 1D). Furthermore, the TRAIL protein level was also enhanced, as determined by western blot analysis ( $P < 0.05$ ) (Fig. 1E). The location of TRAIL was determined by immunofluorescence staining. TRAIL was found to be mainly expressed on the cell surface and partly in the cytoplasm and nucleus (Fig. 1F). The soluble extracellular TRAIL (sTRAIL) was also detected by

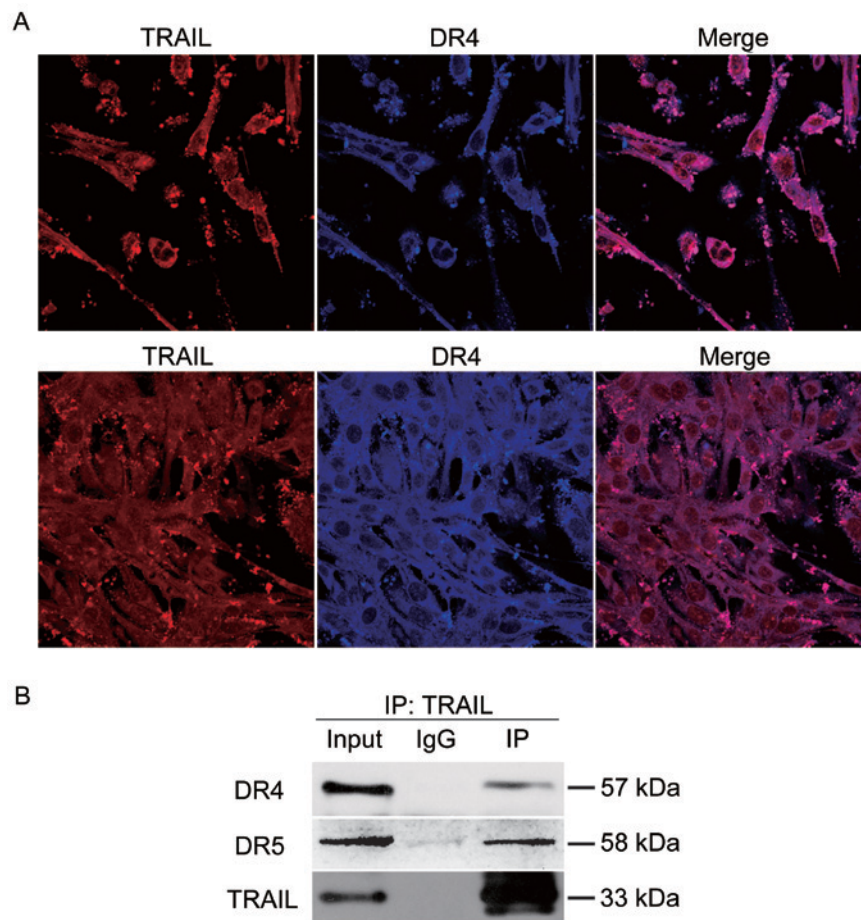


Figure 4. TRAIL combined with DR4 and DR5 on glioma cells. (A) Immunofluorescent co-localization of TRAIL and DR4/5. TRAIL was stained using Alexa 594-conjugated antibody, whereas DR4/5 was stained using Alexa 405-conjugated antibody (magnification, x400). (B) Western blots demonstrated that TRAIL was co-immunoprecipitated with DR4/5. DR4/5, death receptor 4/5; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand.

western blot analysis. It was found that the sTRAIL existed in the trimeric form in the culture medium of the TRAIL-positive group (Fig. 1G). These results showed that TRAIL was overexpressed in the EPCs after lentiviral transfection, and the overexpressed TRAIL was further distributed both on the cell membrane and in the culture medium.

*Overexpression of TRAIL did not affect the migration of EPCs toward SHG44 cells.* A Transwell migration assay was performed to assess the directional migration of EPCs. The number of migrated cells increased significantly when SHG44 cells were cultured in the lower chamber, as evident from statistics and HCS of the images ( $P < 0.05$ ) (Fig. 2A and B). However, similar numbers of the migrated cells were found in the TRAIL-transfected EPCs and controls, indicating that the overexpression of TRAIL did not affect the homing of EPCs to SHG44 cells ( $P > 0.05$ ) (Fig. 2A and B). Furthermore, the overexpression of TRAIL did not alter the protein levels of VEGF-R2 in the TRAIL-transfected EPCs compared with the controls ( $P > 0.05$ ) (Fig. 2C). These findings indicated that EPCs with the overexpression of TRAIL had the similar ability for directional migration and angiogenesis, which could be used in the following experiments.

*Apoptosis of SHG44 in co-culture system with EPCs.* A co-culture system in which SHG44 cells were co-cultured

with EPCs harboring TRAIL or GFP was established to determine the effect of TRAIL-overexpressing EPCs on SHG44 cells. After 5 days of continuous observation, it was found that the number of SHG44 cells in the TRAIL-expressing EPC-treated group obviously reduced compared with that in the GFP-harboring EPCs (Fig. 3A). Annexin V-kFluor594 was used on the fifth day of co-culture to detect cell apoptosis. More Annexin V-kFluor594-positive cells (red fluorescence) were found around the TRAIL-expressing EPCs (green fluorescence) (Fig. 3B) in the TRAIL-expressing EPC-treated group compared with the GFP group. It indicated that the TRAIL-expressing EPCs could induce SHG44 cell apoptosis. Then, the flow cytometry assay was used to measure the apoptotic rate of SHG44 cells. The cells were collected and stained with Annexin V-APC/PI on the first, third, and fifth days after co-culturing. The apoptotic rate of SHG44 cells significantly increased by co-culturing with the TRAIL-expressing EPCs, compared with that of the cells harboring GFP, after separating EPCs and SHG44 cells based on GFP fluorescence intensity, (Fig. 3C and D). These findings indicated that TRAIL-positive EPCs could increase SHG44 cell apoptosis.

*TRAIL bound with DR4/5 on glioma cells.* The study demonstrated that TRAIL-positive EPCs could induce SHG44 cell apoptosis obviously. Next, immunofluorescent

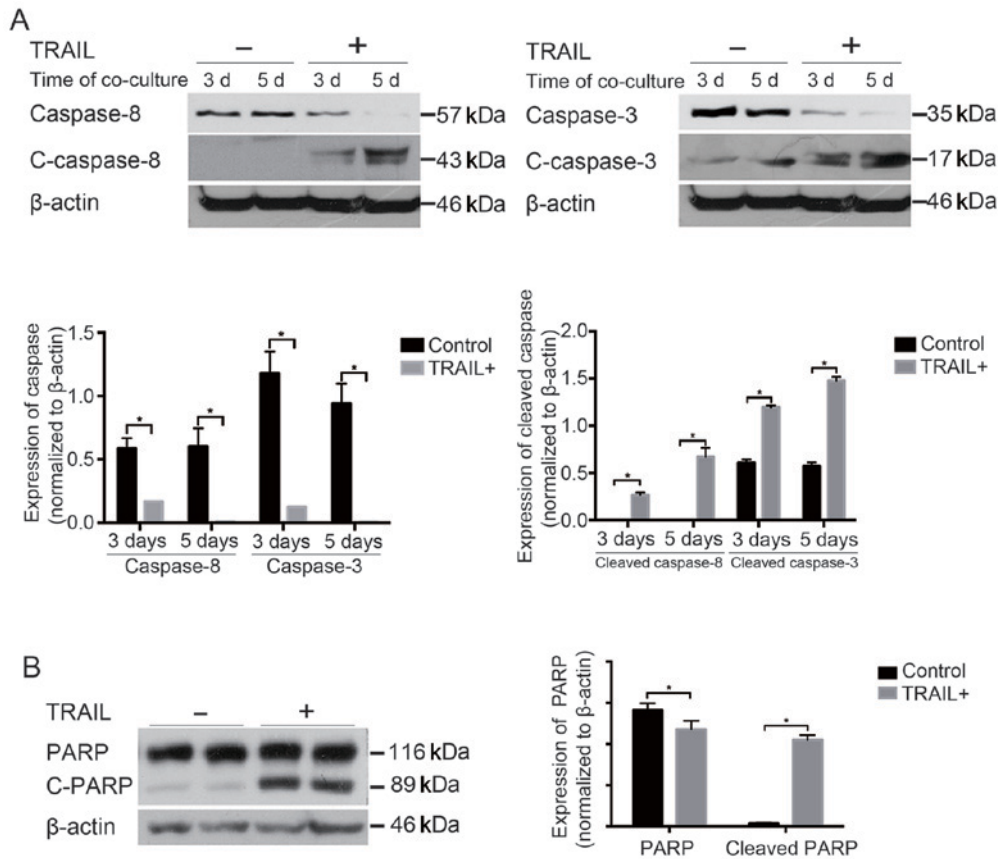


Figure 5. TRAIL enhanced the activation of caspases and PARP. (A) TRAIL expression induced the activation of caspase-8 and -3 significantly.  $\beta$ -Actin was used as a loading control ( $P < 0.05$ ). (B) The cleaved PARP increased obviously in the TRAIL-positive group, whereas the original PARP showed a mild decrease.  $\beta$ -Actin was used as a loading control ( $P < 0.05$ ). Data are presented as means  $\pm$  SD. Three individual experiments were performed for each group. \* $P < 0.05$ , compared with the control group. PARP, poly ADP-ribose polymerase; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; SD, standard deviation.

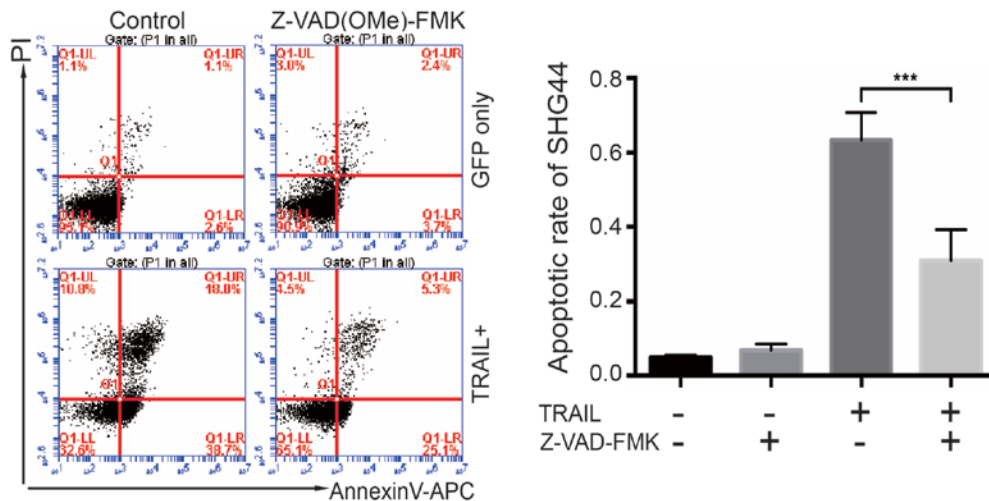


Figure 6. Apoptosis in the co-culture system could be reversed using a caspase inhibitor. The apoptotic rate significantly reduced with Z-VAD(OMe)-FMK ( $P < 0.05$ ). Data are presented as means  $\pm$  SD. Three individual experiments were performed for each group. \*\*\* $P < 0.001$ , compared with the control group. PI, propidium iodide; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; SD, standard deviation.

co-localization and co-immunoprecipitation were used to verify the interaction of TRAIL and death receptor so as to explore the apoptotic mechanism further. Double-channel confocal imaging revealed that TRAIL showed red fluorescence, TRAIL co-localized with DR4 and DR5 (DR4

and DR5) showed blue fluorescence (Fig. 4A). The total proteins from the co-culture system were also extracted to test the interaction between TRAIL and DR4/5 by immunoblotting. It was found that TRAIL co-immunoprecipitated with DR4/5 in SHG44 cells (Fig. 4B).



*TRAIL enhanced the activation of caspases and PARP.* Next, the expression levels of pro/cleaved caspase-8, pro/caspase-3, and PARP were examined. The expression levels of cleaved caspase-8 and -3 significantly increased and were further enhanced with time in the co-culture system, whereas the expression levels of pro-caspase-8 and -3 decreased significantly, compared with the GFP group ( $P < 0.05$ ) (Fig. 5A). Similar changes were observed in the cleaved PARP. In contrast, the amount of full-length PARP was much less in the TRAIL-positive group than in the GFP group ( $P < 0.05$ ) (Fig. 5B), indicating that PARP was largely activated under the effect of TRAIL on EPCs.

*Apoptosis in the co-culture system could be reversed by a caspase inhibitor.* The caspase inhibitor Z-VAD(OMe)-FMK was used for 5 days of co-culture, and the apoptosis of SHG44 cells was detected to verify the involvement of TRAIL-expressing EPCs further. Fig. 6 shows that the apoptotic rate was significantly reversed when Z-VAD(OMe)-FMK was added at the concentration of 20  $\mu\text{mol/l}$  (Fig. 6).

## Discussion

Gliomas, the most common malignant tumors in CNS, exhibit highly aggressive behavior. The therapeutic effect on glioma is still not improved drastically despite various new therapies reported for this tumor. In this study, the extracted EPCs from the blood of neonatal Sprague-Dawley rats were identified by detecting specific surface markers CD31 and CD34 as well as the capability of vasculogenesis. TRAIL was found to increase the apoptosis of SHG44 cells significantly. This trend could be reversed by caspase-8 inhibitor. The mechanism underlying this phenomenon might be related to the increased pro-apoptotic protein levels of cleaved caspase-3, caspase-8, and PARP.

EPCs can self-proliferate and differentiate into the endothelial lineage and express CD31, CD34, CD133, and VEGFR-2 on their surface (28,29). They have the capability of vasculogenesis and are involved in tumor vessel neogenesis. Many studies investigated the relationship between EPCs and tumors (22). Most previous studies focused on anti-angiogenic treatment by suppressing the mobilization and homing of EPCs to tumors (20). Only a few studies used EPCs as a vector for treatment (21). EPCs harboring specific matrix metallo-peptidase-12 (MMP-12) could inhibit melanoma growth (30). On the contrary, EPCs releasing CD40 ligand could induce the increase in cleaved caspase-3 and -7 in metastatic breast cancer (31). These findings proved that EPCs could be used for tumor treatment. Growing evidence indicated that EPCs could move across the BBB and home to brain tumors (23). EPCs can serve as a medium for anti-glioma therapy. The Transwell assay indicated that EPCs had the capability of homing to glioma cells, as previously reported. The present study also proved that lentiviral infection had little influence on the migration ability of EPCs. Therefore, it was feasible to use the TRAIL-expressing EPCs as a therapeutic vector after lentiviral infection.

TRAIL induces apoptosis through binding to DR4 or DR5 on the surface of cells, recruiting and activating caspase-8 via the death domains of DRs and adaptor protein Fas-associated death domain (FADD) (9,32). Activated caspase-8 cleaves

pro-caspase-3, inducing cell apoptosis (33,34). The low bioavailability of TRAIL limits its application despite its immense potential for anti-tumor therapy. Attempts were made to address this limitation through enhancing the stability of TRAIL (35-38) and concentrating TRAIL in the tumor site (39-42). In this study, EPCs were used as a vector for TRAIL, continuously producing TRAIL besides tumor cells. The findings indicated that TRAIL delivered by EPCs could bind to DR4 or DR5 on the surface of glioma cells. Correspondingly, the activated caspase-8 and -3, and cleaved PARP increased obviously. In other words, TRAIL-expressing EPCs could promote the apoptosis of SHG44 cells through activating the death receptor pathway.

In conclusion, the present study demonstrated that TRAIL-overexpressing EPCs could migrate toward SHG44 cells and induce apoptosis by activating the death receptor pathway. The findings suggested that TRAIL and EPCs could be combined for glioma therapy.

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