Biochemical and Biophysical Research Communications 421 (2012) 585–590

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Valproic acid enhances anti-tumor effect of mesenchymal stem cell mediated HSV-TK gene therapy in intracranial glioma

Chung Heon Ryu [a], Kwang Ywel Park [a], Seong Muk Kim [a], Chang Hyun Jeong [a], Ji Sun Woo [a], Yun Hou [a], Sin-Soo Jeun [a,b,*]

[a] Department of Biomedical Science, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea
[b] Department of Neurosurgery, Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul, Republic of Korea

ARTICLE INFO

Article history:
Received 30 March 2012
Available online 14 April 2012

Keywords:
Mesenchymal stem cells
Herpes simplex thymidine kinase
Valproic acid

ABSTRACT

Suicide gene therapy of glioma based on herpes simplex virus type I thymidine kinase (HSV-TK) and prodrug ganciclovir (GCV) suffers from the lack of efficacy in clinical trials, which is mostly due to low transduction efficacy and absence of bystander effect in tumor cells. Recently, stem cells as cellular delivery vehicles of prodrug converting gene has emerged as a new treatment strategy for malignant glioma. In this study, we evaluated the anti-glioma effect of suicide gene therapy using human bone marrow mesenchymal stem cells expressing HSV-TK (MSCs-TK) combined with valproic acid (VPA), which can upregulate the gap junction proteins and may enhance the bystander effect of suicide gene therapy. Expression of HSV-TK in MSCs was confirmed by RT-PCR analysis and the sensitivity of MSCs-TK to GCV was assessed. A bystander effect was observed in co-cultures of MSCs-TK and U87 glioma cells by GCV in a dose-dependent manner. VPA induced the expression of the gap junction proteins connexin (Cx) 43 and 26 in glioma cell and thereby enhanced the bystander effect in co-culture experiment. The enhanced bystander effect was inhibited by the gap junction inhibitor 18-β-glycyrrhetinic acid (18-GA). Moreover, the combined treatment with VPA and MSCs-TK synergistically enhanced apoptosis in glioma cells by caspase activation. In vivo efficacy experiments showed that combination treatment of MSCs-TK and VPA significantly inhibited tumor growth and prolonged the survival of glioma-bearing mice compared with single-treatment groups. In addition, TUNEL staining also demonstrated a significant increase in the number of apoptotic cells in the combination treated group compared with single-treatment groups. Taken together, these results provide the rational for designing novel experimental protocols to increase bystander killing effect against intracranial gliomas using MSCs-TK and VPA.

1. Introduction

Malignant gliomas are the most common and aggressive primary intracranial tumors in adults. While conventional treatments including surgical resection, irradiation and chemotherapy may extend survival by weeks, the prognosis of malignant glioma patients is still poor [1].

A number of experimental treatments have been developed to improve the survival of malignant glioma patients [2–4]. Herpes simplex virus thymidine kinase gene (HSV-TK)/ganciclovir (GCV) gene therapy has been considered as one of the promising therapeutic strategies for malignant gliomas. The therapeutic potential is enhanced by the bystander effect that consists of the transfer of phosphorylated GCV as a cytotoxic metabolite from TK-expressing cells toward neighboring TK negative cells. Transfer of phosphorylated GCV requires cell contact and is mainly mediated through gap junctions. Connexin (Cx) 43 and 26, one member of Cx family, is considered as a gap junctions (GJ), which are formed of connexins [5].

Despite impressive results in experimental studies for malignant glioma, clinical trials using HSV-TK/GCV treatment have failed [6]. One of the main problems is the insufficient distribution of the gene therapy vehicles, mostly viruses, over the whole volume of the tumor. A second problem is that both the conversion of the prodrug into the cytotoxic compound and its delivery to neighboring tumor cells not expressing the suicide gene suffers from low efficiency. To overcome these problems, we used human bone marrow mesenchymal stem cells (BM-MSCs) as HSV-TK gene delivery vehicles with innate glioma targeting migration capabilities and valproic acid (VPA) to enhance gap junction communication in glioma cells.

VPA, an anti-epileptic drug and an anticancer drug, inhibits histone deacetylase (HDAC) and induces tumor cell differentiation,

* Corresponding author at: Department of Neurosurgery, Seoul St. Mary's Hospital, The Catholic University of Korea, 505 Banpo-daero, Seocho-gu, Seoul 137-701, Republic of Korea. Fax: +82 2 3482 1853.
E-mail address: ssjeun@catholic.ac.kr (S.-S. Jeun).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.04.050

apoptosis, and growth arrest [7,8]. VPA has been examined as an HDAC inhibitor (HDACI) in preclinical and clinical trials for solid tumors and leukemias [9,10]. Recently, HDACI compounds like phenyl butyrate have been reported to modulate the gap junction component Cx43 expression and enhance gap junction communications in glioma cells. Furthermore, VPA enhanced tumor cell kill in adenovirus-HSV/TK mediated suicide gene therapy in a HNSCC xenograft mouse model [11].

This study examined for the first time the potential of VPA to increase the therapeutic efficiency of the bystander effect by enhancing gap junction communication in glioma cells, using combined therapy with MSCs-TK and VPA. Although further studies are needed to determine the exact mechanism underlying the expression of gap junction by VPA, these results suggest that the clinical therapeutic efficacy of MSC-mediated HSV-TK gene therapy for malignant glioma can be enhanced by combination with VPA.

2. Materials and methods

2.1. Stem cell culture and reagents

Human MSCs derived from the bone marrow were purchased from Lonza (Walkersville, Maryland, USA). The MSCs were subcultured at a concentration of 5×10^4 cells/cm^2 in MSC growth medium (Lonza) and used for experiments during passages 5–8. MSC growth medium was supplemented with 100 units/ml penicillin, 100 μg/ml streptomycin (Invitrogen, Carlsbad, CA, USA), and supplement mix (Lonza). Cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2. GCV was acquired from InvivoGen (San Diego, CA, USA). Gap junction inhibitor 18 beta-glycyrrhetinic acid (18-GA) was purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Adenoviral vectors and infection

The recombinant adenoviral vector encoding the gene for green fluorescent protein (Ad-GFP) and HSV-TK (Ad-HSV-TK) was constructed and produced using the Ad-Easy vector system, following the manufacturer's instructions (Quantum Biotechnologies, Carlsbad, CA, USA). MSCs were infected with 50 multiplicity of infection of Ad-GFP or Ad-HSV-TK.

2.3. Reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was prepared from MSCs using TRIzol (Invitrogen) according to the manufacturer's instructions. cDNA was synthesized using 2 μg total RNA and oligo (dT) primer and Superscript II polymerase for reverse transcription PCR (Invitrogen). PCR amplifications consisted of a total of 20 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, extension at 72 °C for 1 min with a first denaturation at 94 °C for 7 min, and final extension at 72 °C for 7 min. Target primers sequences used were as follows: HSV-TK forward: 5′-CGCGAACATCTACACCACAC-3′, HSV-TK reverse: 5′-GTTATACAGGTCGCCGTTGG-3′ and GAPDH forward: 5′-TCCATGACAACT TT GGTATCG-3′, GAPDH reverse: 5′-TGTAGCCAAATT CGTTGTCA-3′.

2.4. Assessment of cell viability by MTT assay

To test the sensitivity to GCV, wild-type MSCs, MSCs-GFP or MSCs-TK were cultured in the MSC growth medium containing various concentrations of GCV (0.1–1000 μg/ml) and were incubated for 3 days. The viability of cells was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma–Aldrich) assay. Briefly, MTT tetrazolium salt added to the cells and incubated for 30 min at 37 °C. The formazan dye formed by viable cells was solubilized in isopropanol. Aliquots of the solutions were transferred to 96-well microplates. The absorbance at 570 nm was measured with a spectrophotometer (Molecular Devices, Sunnyvale, CA, USA). For co-culture experiments, MSCs-GFP and MSCs-TK (1×10^4 cells) were plated in the Transwell inserts (0.4 μm pores ; Costar, NY, USA) containing various concentrations of GCV (0.1–1000 μg/ml) and then U87 cells (1×10^4 cells) were grown in the lower well of the Transwell plates. VPA and 18-GA were added to the lower wells. After 3 days, the viability of U87 cells in the lower well was also analyzed by MTT assay.

2.5. Western blotting

Connexin (Cx) 43 antibody (Cell Signaling Technology, Danvers, MA, USA), β-actin antibody (Sigma–Aldrich), and Cx 26 antibody (Abcam, Cambridge, MA, USA) were used for the analyses. Cells were rinsed with phosphate-buffered saline (PBS) and lysed for 30 min on ice in RIPA-B buffer (0.5% Nonidet P-40, 20 mM Tris, pH 8.0, 50 mM NaCl, 50 mM NaF, 100 μM Na_3VO_4, 1 mM dithiothreitol, and 50 μg/ml phenylmethanesulfonylfluoride). Insoluble material was removed by centrifugation at 12,000 rpm for 5 min at 4 °C. The proteins in the supernatant were resolved by 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), and the resolved proteins were transferred to a nitrocellulose blot membrane. Each blot was blocked using TBS-0.05% Tween 20 containing 5% skim milk, incubated with the appropriate antibodies, and incubated with the secondary antibodies conjugated to horseradish peroxidase (HRP). The bands were detected using the enhanced chemiluminescence detection system (Amersham Pharmacia Biotech, Piscataway, NJ, USA).

2.6. Animals and intracranial glioma model

Nude mice (6–8 weeks old; Charles River Laboratories, Wilmington, MA, USA) were used in accordance with institutional guidelines under the approved protocols. For the intracranial implantation of human glioma cells in the brain of mice, animals were stereotactically inoculated with 1×10^5 U87 cells (in 3 μl phosphate buffered saline, PBS) into the right frontal lobe (2 mm lateral and 1 mm anterior to bregma, at 2.5 mm depth from the skull base) using a Hamilton syringe (Hamilton Company, Reno, NV, USA) and a microinfusion pump (Harvard Apparatus, Holliston, MA, USA).

2.7. Animal tumor size and survival evaluation

Tumor size was determined as described previously [12]. Briefly, brains from mice given therapeutic treatment at day 35 after tumor inoculation were serially sectioned (18 μm-thick, obtained every 200 μm into the tumor) and then stained with hematoxylin and eosin (H&E). The section with the maximum tumor area was calculated via a computer using MetaMorph software (Molecular Devices, Sunnyvale, CA, USA). For survival experiments, intracranial glioma-bearing mice were randomly divided into four groups after tumor implantation and treated with intratumoral injections of saline (PBS), intraperitoneal injections of VPA (300 mg/kg, i.p), MSCs infected with Ad-HSV-TK (MSCs-TK) and combination therapy (MSCs-TK and VPA). GCV (50 mg/kg) was injected 1 day after MSCs transplantation and continued everyday for 7 days.

2.8. Histological evaluation of apoptosis by TUNEL assay

Mouse brains were perfused with PBS followed by 4% paraformaldehyde and postfixed overnight. Fixed brains were embedded, snap frozen in liquid nitrogen, and stored at −70 °C until use.

Apoptotic cells were visualized using a terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay kit (Roche, Basel, Switzerland) developed using Cy3-conjugated streptavidin (Jackson ImmunoResearch Laboratories, West Grove, PA, USA). Briefly, endogenous peroxidase activity was blocked by 3% H_2O_2 for 10 min at a room temperature. After washing with PBS, 50 μl of TUNEL reaction mixture was pipetted onto the sections, which were then incubated in a humidified chamber at 37 °C for 1 h. The reaction was stopped by adding wash buffer. In all sections, nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI; Sigma–Aldrich) for counterstaining. Apoptotic cells were also measured via a computer using MetaMorph software (Molecular Devices).

2.9. Statistical analysis

All data are expressed as mean ± SEM. Statistical differences between different test conditions were determined using Student's *t* test. Probability values <0.05 were considered significant. Statistical analysis of survival was performed by a log-rank test.

3. Results and discussion

3.1. In vitro sensitivity and bystander effect between MSCs-TK and U87 cells

In previous studies, we demonstrated that MSCs have a strong migratory capacity toward human glioma cells, and that genetically modified MSCs also have tropism for human gliomas and inhibit glioma growth after intratumoral injection [13–15]. Moreover, MSCs are easier to obtain and expand in vivo, and fewer ethical concerns are associated with their use. Recently, adipose tissue derived MSCs have been genetically modified to express HSV-TK with potent tropism for disseminating glioma cells and have shown strong anti-tumor effects both in vitro and in vivo [16,17]. In this study, we used human bone marrow-derived MSCs as vehicles to deliver HSV-TK gene therapy for intracranial gliomas. HSV-TK-transduced MSCs (MSCs-TK) was analyzed using semi quantitative RT-PCR, and adenovirus-induced cytotoxicity was determined by the MTT-based viability assay (data not shown). MSCs-TK were incubated with increasing concentrations of GCV (0.1–1000 μg/ml). The viability of MSCs-TK was drastically decreased at 10 μg/ml of GCV and almost no MSCs-TK survived at 50 μg/ml of GCV (Fig. 1A). The EC50 concentration of GCV for MSCs-TK was 1 μg/ml. In contrast, wild-type MSCs (WT-MSCs) and GFP-transduced MSCs (MSCs-GFP) were resistant to these concentrations of GCV. In addition, to investigate the bystander effect of GCV-treated MSCs-TK in U87 glioma cells, U87 cells were co-cultured with MSCs-TK, WT-MSCs or MSCs-GFP in a ratio 1:1. The bystander killing effect of MSCs-TK significantly reduced the number of viable glioma cells in a GCV concentration-dependent manner ($P < 0.05$). Proliferation inhibition of 50% was observed at 10 μg/ml of GCV, and almost no viable cells were observed at 100 μg/ml of GCV. No bystander killing effect was seen when the glioma cells were co-cultured with WT-MSCs and MSCs-GFP in the absence or presence of GCV (Fig. 1B). These results indicated that MSCs can be used as a promising delivery vehicle for HSV-TK gene, and besides MSCs-TK exhibits strong antitumor activity via bystander killing effect for glioma cells.

3.2. VPA treatment enhanced bystander-mediated glioma cell killing of MSCs-TK

Cx43 is a major constituent of gap junctions that allow the transfer of phosphorylated GCV from cell to cell. It was observed that Cx43 could be downregulated in malignant gliomas [18]. Here, Western blot was carried out to check expression of genes involved in gap junctions including Cx43 and 26 on U87 cell in the presence of various concentrations of HDACI VPA (0–4 mM). The expression of Cx43 and 26 in U87 cells was increased by VPA in a dose-dependent manner (Fig. 2A). The expression of the gap junction connexins Cx43 and 26 play an important role in mediating bystander effect [19]. Increased gap junction expression from cancer cells has been demonstrated upon treatment with inhibitors of HDACs [20,21]. The HDACI phenylbutyrate has been reported to modulate Cx43 expression and enhance gap junction communications in glioma cells. Phenylbutyrate increases bystander killing of HSV-TK transfected glioma cells in vitro by an increase in gap junctions [22]. Here, we evaluated the bystander killing effect under the concentrations of VPA (0–4 mM) and GCV (10 μg/ml). The bystander killing effect was increased by VPA in a dose-dependent manner. Moreover, the bystander effect induced by VPA could be significantly alleviated by gap junction inhibitor 18-GA (20 μM) ($P = 0.032$, combination treatment vs inhibitor treatment). Treatment with VPA in vitro was able to enhance the MSCs-TK-mediated cell killing effect significantly when compared to MSCs-TK treatment alone ($P = 0.041$). This could be attributed to increase in gap junctions resulting in increased bystander effect and passage of toxic metabolites to neighboring cells. Mechanisms underlying the bystander effect using HSV-TK that have been reported include transfer of toxic nucleoside analogs through gap junctions or, alternatively, by phagocytosis of apoptotic vesicles [23]. To identify effects of apoptotic cell death by these effects, we performed Western blot analyzes using antibody against caspase-3 in U87 cells treated with 1 mM VPA and/or MSCs-TK. Cleaved caspase-3 (CL), which is a terminal effector of apoptosis, was also increased by MSCs-TK and also to a much greater extent by the VPA and MSCs-TK combination. The enhanced apoptosis was also inhibited by gap junction inhibitor 18-GA. Taken together, these results suggest that VPA upregulates gap junction proteins and enhanced apoptosis by bystander killing effect. Therefore, VPA could be used in combination with HSV-TK based suicide gene therapy.

3.3. Combined therapeutic effects of MSCs-TK and VPA on tumor growth and survival of glioma-bearing mice

To determine whether the combination with MSCs-TK and VPA showed antitumor effects on gliomas, tumor sizes in MSCs-TK and VPA-treated mice were measured by histological staining from glioma-bearing mice. The average tumor areas in MSCs-TK and VPA-treated mouse were decreased compared with PBS-, VPA- or MSCs TK-treated mice. This decrease in tumor size associated with MSCs-TK and VPA treatment was significant at day 35 ($P = 0.007$, MSCs-TK/VPA vs VPA, $P = 0.042$, MSCs-TK/VPA vs MSCs-TK) (Fig. 3A). There was no detectable difference in tumor size between animals treated with VPA and MSCs-TK alone. These results showed that MSCs-TK/VPA reduced the rate of tumor growth in glioma-bearing mice. Next, the survival of MSCs-TK/VPA-treated mice was significantly prolonged compared with control mice treated with PBS, VPA or MSCs-TK alone ($P < 0.01$) (Fig. 3B). It is worth noting that 18% of MSCs-TK-treated mice survived over day 60 after tumor inoculation, whereas in our previous report all TRAIL-secreting MSCs-treated mice died within 30 days [24], suggesting that TK-mediated suicide gene therapy of glioma is more potent than TRAIL-mediated direct tumor killing strategy.

3.4. Apoptosis induction of MSCs-TK and VPA treatment in intracranial glioma

HSV-TK inhibits growth in experimental tumors in vivo and induces apoptosis by gap junction after GCV treatment [23]. To

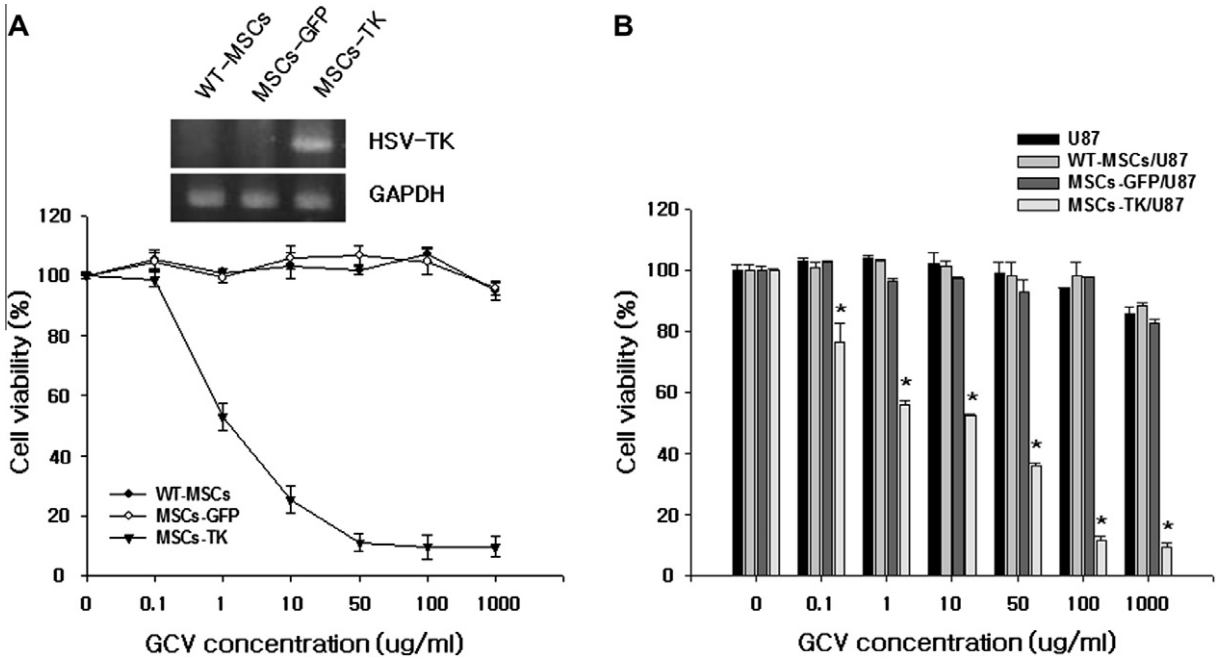

Fig. 1. In vitro sensitivity and bystander effect between MSCs-TK and U87 cells. (A) Semi quantitative RT-PCR analysis for HSV-TK gene in MSCs after Ad-HSV-TK infection. WT-MSCs and MSCs-GFP were used as a control. The sensitivity of WT-MSCs, MSCs-GFP and MSCs-TK to GCV (0–1000 μg/ml) evaluated by the MTT assay. (B) The bystander killing effect of U87 cells co-cultured with MSCs-TK was significantly increased at the GCV concentration as low as 1 μg/ml. The proliferation of U87 cells was not inhibited by GCV when they were co-cultured with WT-MSCs or MSCs-GFP. Columns, mean; bars, SE. $^{*}P < 0.05$, Student's t test. The results are representative of three independent experiments.

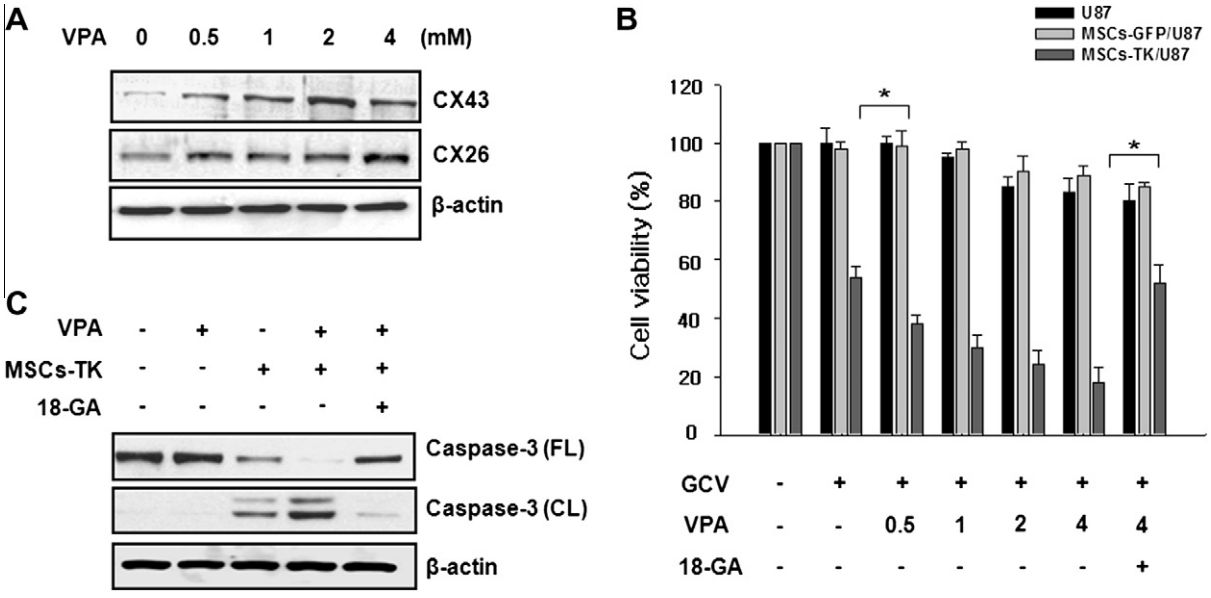

Fig. 2. VPA treatment enhanced bystander-mediated glioma cell killing of MSCs-TK. (A) U87 cells were incubated with VPA for various concentrations (0–4 mM), and the expression of gap junction proteins was determined using Western blot analysis with Cx43, Cx26, or β-actin antibody. (B) U87 cell and co-cultured MSCs-GFP or MSCs-TK with U87 cells cultured in increasing concentrations of VPA (0–4 mM) for 3 days with or without GCV (10 μg/ml). Columns, mean; bars, SE. $^{*}P < 0.05$, Student's t test. (C) U87 cells were treated with VPA (1 mM), MSCs-TK, or their combination with or without 18-GA (20 μM). After 3 days, cell extracts were analyzed by Western blot with antibodies against full-length (FL) caspase-3, cleaved (CL) caspase-3. β-actin was used as a loading control. The results are representative of three independent experiments.

determine whether the apoptotic effects were involved in the antitumor activity of transplanted MSCs-TK with VPA, tissue sections from glioma-bearing mice were analyzed by immunohistochemistry at 14 days after treatment. TUNEL staining demonstrated a significant increase in the number of apoptotic cells in the group treated with MSCs-TK/VPA compared with controls treated with VPA or MSCs-TK ($P < 0.01$) (Fig. 4). These results demonstrate that the antitumor activity of combination of MSCs-TK and VPA is mediated by apoptosis and enhances apoptotic cell death in intracranial gliomas. Further studies are needed to identify the detailed mechanisms of therapeutic effect by combination of MSCs-TK and VPA in vivo.

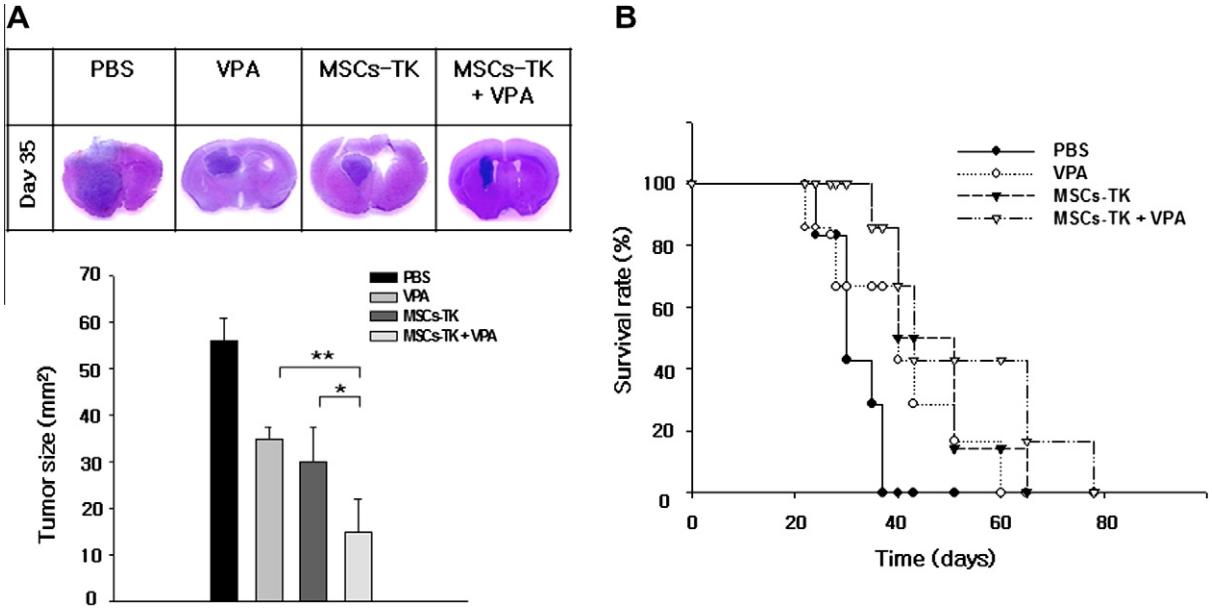

Fig. 3. In vivo effects of MSCs-TK and VPA on tumor growth and survival of glioma-bearing mice. MSCs-TK (1×10^5 cells) were given intratumorally to glioma-bearing mice at day 7 after U87 (1×10^5 cells) inoculation. GCV (50 mg/kg) and VPA (300 mg/kg) were administered intraperitoneally twice daily for 14 days after MSCs-TK transplantation. The phosphate-buffered saline (PBS) group was used as a control. (A) Representative photographs of hematoxylin and eosin (H&E) staining from each group show tumor growth. Magnification, ×1. Tumor size was also determined by histological analysis at 35 days after tumor inoculation ($n = 3$/treatment group). Columns, mean; bars, SE. $^{*}P < 0.05$; $^{**}P < 0.01$, Student's t test. (B) Survival curve of intracranial glioma-bearing mice. At day 7 after U87 inoculation, tumors were injected intratumorally with a single dose (1×10^5 cells) of MSCs-TK ($n = 7$) or/and intraperitoneally with VPA ($n = 7$). Analysis of survival was conducted by a log-rank test based on the Kaplan–Meier method. The results are representative of two independent experiments.

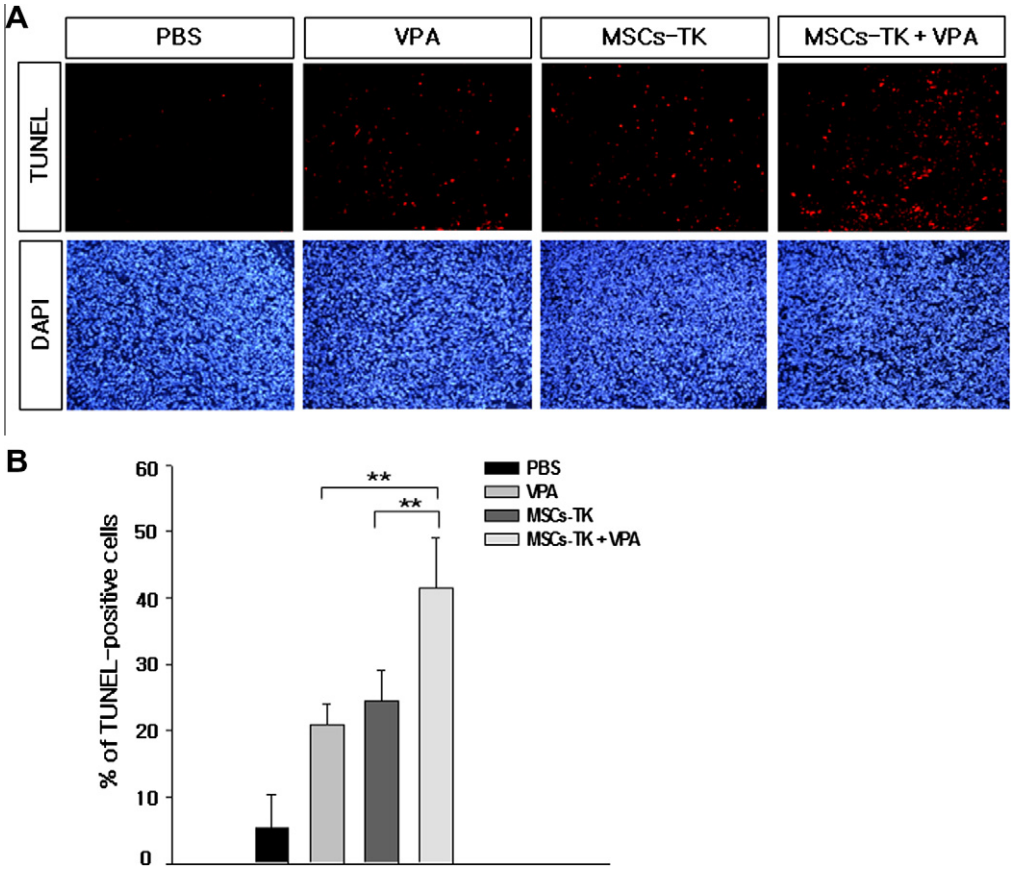

Fig. 4. Anti-tumor effect of MSCs-TK and VPA in xenograft mouse model. Brain tissues were removed from glioma-bearing mice at 14 days after VPA treatment (300 mg/kg) or/and transplantation of MSCs-TK (1×10^5 cells). The PBS group was used as a control. (A) For the detection of apoptotic cells in brain tissues, cryosections from each group were stained by TUNEL methods. TUNEL-positive nuclei (red) were stained and counterstaining was conducted with DAPI (blue). Magnification, ×100. (B) The TUNEL-positive cells were also quantified by computerized image analysis. Columns, mean; bars, SE. $^{**}P < 0.01$, Student's t test. The results are representative of three independent experiments.

Acknowledgments

This study was supported by grants of the Korea Health technology R&D Project (A110330, A110298, A092258), and by a grant from the National R&D Program for Cancer Control (0820040), Ministry of Health & Welfare, Republic of Korea.

References

[1] T.S. Surawicz, F. Davis, S. Freels, E.R. Laws Jr., H.R. Menck, Brain tumor survival: results from the National Cancer Data Base, J. Neurooncol. 40 (1998) 151–160.
[2] S.E. Lawler, P.P. Peruzzi, E.A. Chiocca, Genetic strategies for brain tumor therapy, Cancer Gene Ther. 13 (2006) 225–233.
[3] S. Yip, R. Sabetrasekh, R.L. Sidman, E.Y. Snyder, Neural stem cells as novel cancer therapeutic vehicles, Eur. J. Cancer 42 (2006) 1298–1308.
[4] H. Hamada, M. Kobune, K. Nakamura, Y. Kawano, K. Kato, O. Honmou, K. Houkin, T. Matsunaga, Y. Niitsu, Mesenchymal stem cells (MSC) as therapeutic cytoreagents for gene therapy, Cancer Sci. 96 (2005) 149–156.
[5] H. Namba, M. Tagawa, Y. Iwadate, M. Kimura, K. Sueyoshi, S. Sakiyama, Bystander effect-mediated therapy of experimental brain tumor by genetically engineered tumor cells, Hum. Gene Ther. 9 (1998) 5–11.
[6] N.G. Rainov, A phase III clinical evaluation of herpes simplex virus type 1 thymidine kinase and ganciclovir gene therapy as an adjuvant to surgical resection and radiation in adults with previously untreated glioblastoma multiforme, Hum. Gene Ther. 11 (2000) 2389–2401.
[7] M.M. Knupfer, F. Pulzer, I. Schindler, P. Hernaiz Driever, H. Knupfer, E. Keller, Different effects of valproic acid on proliferation and migration of malignant glioma cells in vitro, Anticancer Res. 21 (2001) 347–351.
[8] Y. Shao, Z. Gao, P.A. Marks, X. Jiang, Apoptotic and autophagic cell death induced by histone deacetylase inhibitors, Proc. Natl. Acad. Sci. USA 101 (2004) 18030–18035.
[9] A. Kuendgen, N. Gattermann, Valproic acid for the treatment of myeloid malignancies, Cancer 110 (2007) 943–954.
[10] A. Duenas-Gonzalez, M. Candelaria, C. Perez-Plascencia, E. Perez-Cardenas, E. de la Cruz-Hernandez, L.A. Herrera, Valproic acid as epigenetic cancer drug: preclinical, clinical and transcriptional effects on solid tumors, Cancer Treat Rev. 34 (2008) 206–222.
[11] V. Kothari, G. Joshi, S. Nama, K. Somasundaram, R. Mulherkar, HDAC inhibitor valproic acid enhances tumor cell kill in adenovirus-HSVtk mediated suicide gene therapy in HNSCC xenograft mouse model, Int. J. Cancer 126 (2010) 733–742.
[12] C.H. Ryu, S.H. Park, S.A. Park, S.M. Kim, J.Y. Lim, C.H. Jeong, W.S. Yoon, W.I. Oh, Y.C. Sung, S.S. Jeun, Gene therapy of intracranial glioma using interleukin 12-secreting human umbilical cord blood-derived mesenchymal stem cells, Hum. Gene Ther. 22 (2011) 733–743.
[13] C.H. Ryu, S.A. Park, S.M. Kim, J.Y. Lim, C.H. Jeong, J.A. Jun, J.H. Oh, S.H. Park, W.I. Oh, S.S. Jeun, Migration of human umbilical cord blood mesenchymal stem cells mediated by stromal cell-derived factor-1/CXCR4 axis via Akt, ERK, and p38 signal transduction pathways, Biochem. Biophys. Res. Commun. 398 (2010) 105–110.
[14] S.A. Park, C.H. Ryu, S.M. Kim, J.Y. Lim, S.I. Park, C.H. Jeong, J.A. Jun, J.H. Oh, S.H. Park, W. Oh, S.S. Jeun, CXCR4-transfected human umbilical cord blood-derived mesenchymal stem cells exhibit enhanced migratory capacity toward gliomas, Int. J. Oncol. 38 (2011) 97–103.
[15] S.M. Kim, J.H. Oh, S.A. Park, C.H. Ryu, J.Y. Lim, D.S. Kim, J.W. Chang, W. Oh, S.S. Jeun, Irradiation enhances the tumor tropism and therapeutic potential of tumor necrosis factor-related apoptosis-inducing ligand-secreting human umbilical cord blood-derived mesenchymal stem cells in glioma therapy, Stem cells 28 (2010) 2217–2228.
[16] Q. Huang, X.Z. Liu, C.S. Kang, G.X. Wang, Y. Zhong, P.Y. Pu, The anti-glioma effect of suicide gene therapy using BMSC expressing HSV/TK combined with overexpression of Cx43 in glioma cells, Cancer Gene Ther. 17 (2010) 192–202.
[17] M. Matuskova, K. Hlubinova, A. Pastorakova, L. Hunakova, V. Altanerova, C. Altaner, L. Kucerova, HSV-tk expressing mesenchymal stem cells exert bystander effect on human glioblastoma cells, Cancer Lett. 290 (2010) 58–67.
[18] S. Crespin, J. Bechberger, M. Mesnil, C.C. Naus, W.C. Sin, The carboxy-terminal tail of connexin43 gap junction protein is sufficient to mediate cytoskeleton changes in human glioma cells, J. Cell. Biochem. 110 (2010) 589–597.
[19] S. Cottin, K. Ghani, M. Caruso, Bystander effect in glioblastoma cells with a predominant cytoplasmic localization of connexin43, Cancer Gene Ther. 15 (2008) 823–831.
[20] O. Ammerpohl, D. Thormeyer, Z. Khan, I.B. Appelskog, Z. Gojkovic, P.M. Almqvist, T.J. Ekstrom, HDACi phenylbutyrate increases bystander killing of HSV-tk transfected glioma cells, Biochem. Biophys. Res. Commun. 324 (2004) 8–14.
[21] T. Asklund, I.B. Appelskog, O. Ammerpohl, I.A. Langmoen, M.S. Dilber, A. Aints, T.J. Ekstrom, P.M. Almqvist, Gap junction-mediated bystander effect in primary cultures of human malignant gliomas with recombinant expression of the HSVtk gene, Exp. Cell Res. 284 (2003) 185–195.
[22] T. Asklund, I.B. Appelskog, O. Ammerpohl, T.J. Ekstrom, P.M. Almqvist, Histone deacetylase inhibitor 4-phenylbutyrate modulates glial fibrillary acidic protein and connexin 43 expression, and enhances gap-junction communication, in human glioblastoma cells, Eur. J Cancer 40 (2004) 1073–1081.
[23] A.F. Andrade-Rozental, R. Rozental, M.G. Hopperstad, J.K. Wu, F.D. Vrionis, D.C. Spray, Gap junctions: the ‘‘kiss of death’’ and the ‘‘kiss of life’’, Brain Res. Brain Res. Rev. 32 (2000) 308–315.
[24] S.M. Kim, J.Y. Lim, S.I. Park, C.H. Jeong, J.H. Oh, M. Jeong, W. Oh, S.H. Park, Y.C. Sung, S.S. Jeun, Gene therapy using TRAIL-secreting human umbilical cord blood-derived mesenchymal stem cells against intracranial glioma, Cancer Res. 68 (2008) 9614–9623.