A multi-targeted tyrosine kinase inhibitor lenvatinib for the treatment of mice with advanced glioblastoma

JIA LI[1*], CHANG-LIN ZOU[2*], ZHI-MING ZHANG[1], LIAN-JIE LV[1], HAI-BO QIAO[1] and XIU-JU CHEN[1]

Departments of [1]Neurosurgery and [2]General Surgery, Tianjin Nankai Hospital, Tianjin 300100, P.R. China

Received February 14, 2016; Accepted February 20, 2017

DOI: 10.3892/mmr.2017.7456

Abstract. Glioblastoma is the most aggressive primary brain tumor that originates from the glial cells in adults. Aberrant angiogenesis is essential for malignant glioblastoma tumorigenesis, development and metastasis. Lenvatinib is a multi-targeted anticancer agent that targets of receptor tyrosine kinases including vascular endothelial growth factor receptor 1 and 2, fibroblast growth factor receptor 1, platelet-derived growth factor receptor β and v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog. In the present study, the therapeutic effects of lenvatinib as a treatment for glioblastoma were investigated *in vivo* and *in vitro*. The maximum dose toxicity (MDT) and treatment-associated adverse events of lenvatinib were identified by cytotoxicity assay in experimental mice. Increasing levels of the pro-apoptosis genes caspase-3, -8, -9 and -10 following lenvatinib treatment were determined by reverse transcription-quantitative polymerase chain reaction, and apoptosis of the malignant gliomas cells was analyzed by FACS. *In vivo* treatment with lenvatinib for BV-2 bearing male BALC/c nude mice was assessed via tumor growth suppression and long-term observation of survival. Subsequent cytotoxic T lymphocyte responses were further analyzed to determine the *in vivo* efficacy of lenvatinib treatment in mice with glioblastoma. The MDT of lenvatinib was identified as 0.24 mg, with relatively few side effects and improved efficacy in mice. Lenvatinib (0.24 mg) significantly increased apoptosis in BV-2, C6, BC3H1 and G422 glioma cell lines. Tumor growth was significantly inhibited and tumor-bearing mice demonstrated an improved survival rate following treatment with lenvatinib. In conclusion, lenvatinib provided an effective treatment outcome, and the results of the present study may help to achieve a comprehensive therapeutic schedule for clinical application.

Correspondence to: Professor Chang-Lin Zou, Department of General Surgery, Tianjin Nankai Hospital, 6 Changjiang Road, Tianjin 300100, P.R. China
E-mail: changlinzou@yeah.net

*Contributed equally

Key words: glioblastoma, lenvatinib, targeted therapy, oncolytic therapy

Introduction

Glioblastoma is the most aggressive primary brain tumor that originates from the glial cells in adults or develops from existing malignant cells (1,2). Glioblastoma is characterized by the appearance of vascular proliferation, aggressive invasion and necrosis around human normal brain tissues (3). Patients with advanced glioblastoma commonly present with seizures and/or stroke, which increases the difficulty and risk of clinical treatment (4). Statistical analysis in previously published data revealed that glioblastoma accounts for ~75% of all malignant tumors associated with the brain (5). According to characteristics of pathologic evaluation and infiltrative growth, different malignant grades result in diverse glioblastoma shapes (6). Therefore, developing treatments for glioblastoma has been a focus of research.

The majority of conditional treatment schedules for human cancers remain ineffective and are often toxic for normal cells (7). Targeted therapy has demonstrated beneficial potential as it specifically targets cancer cells and elicits low toxicity to normal human tissues, demonstrating an increased capacity to eradicate human cancer (8-11). Targeted therapies often involve the application of a specific antibody or receptor for tumor molecules, proteins, peptides, or nucleic acids, as well as efforts that use adoptive transfer of effector cells that directly target antigens on tumor cells (12,13). Clinical research has demonstrated that these novel treatments are effective in treating glioblastoma (14,15). The present study evaluated the preclinical outcomes of lenvatinib targeting of receptor tyrosine kinases for glioblastoma therapy, which promoted cytotoxic T lymphocyte (CTL) responses and interferon-γ (IFN-γ) release through stimulating immunization and adoptive transfer of effector cells that directly target glioblastoma cells in xenograft mice. In addition, the present study discussed the advantages and efficacy of lenvatinib and how over the next decade investigators will attempt to broaden the reach, increase the efficacy and simplify the application of lenvatinib.

Lenvatinib is a multi-targeted tyrosine kinase inhibitor that targets fibroblast growth factor receptors 1-4, vascular endothelial growth factor (VEGF) receptors 1-3, ret proto-oncogene, v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene

homolog and platelet-derived growth factor receptor β (16). Previous studies have demonstrated that angiogenesis mediated by these receptors is involved in the tumorigenesis, development and metastasis of glioblastoma (17,18). Lenvatinib has demonstrated significant anticancer potential against the majority of human cancers in clinical trials, mediated by its inhibition of angiogenesis (19,20). In addition, several reports have revealed that inhibition of the VEGF pathway presented beneficial clinical outcomes in cancer therapy, indicating that targeted therapy using lenvatinib for the treatment of differentiated thyroid cancer and renal cell carcinoma has significant efficacy (16,19). Furthermore, as well as the clinical benefits presented by lenvatinib treatment alone for human cancer therapy, previous studies have also demonstrated that lenvatinib combined with everolimus extended overall survival significantly compared with everolimus alone in patients with metastatic renal cell cancer (20,21). However, few studies have reported the therapeutic effects of lenvatinib regarding the treatment of glioblastoma.

Glioblastoma therapy has attracted scientists and scholars to research more effective and comprehensive treatments (22,23). In the present study, the therapeutic effects of lenvatinib were investigated in a glioblastoma mouse model. The results indicated that targeted therapy of lenvatinib significantly suppressed tumor growth and prolonged the survival of tumor-bearing mice. These results supported a clinical application for lenvatinib in patients with glioblastoma.

Materials and methods

Ethics statement. All animal experiments were performed according to the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (Bethesda, MD, USA). The operation was approved by Chinese Association for Laboratory Animal Sciences, Animal Health Products and the Committee on the Ethics of Animal Experiments Defense Research. All surgery and euthanasia were performed under anesthesia with sodium pentobarbital (50 mg/kg; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany). All efforts were made to minimize the suffering of the experimental mice.

Cell culture and reagents. BV-2, C6, BC3H1 and G422 glioma cell lines were purchased from the American Type Culture Collection (Manassas, VA, USA). The BV-2 and C6 cells were cultured in RPMI-1640 medium (Sigma-Aldrich; Merck KGaA) supplemented with 10% heat-inactivated fetal bovine serum (FBS; BioWhittaker; Lonza Group, Basel, Switzerland), 50 μg/ml gentamicin (BioWhittaker; Lonza Group), 3 mM L-glutamine (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and 1% penicillin/streptomycin (Gibco; Thermo Fisher Scientific, Inc.). BC3H1 and G422 cells were cultured in Eagle's minimum essential medium (Sigma-Aldrich; Merck KGaA) supplemented with 10% FBS.

Apoptosis assays following lenvatinib treatment. BV-2, C6, BC3H1 and G422 ($1x10^3$) cells were incubated with lenvatinib in 96-well plates for 48 h in triplicate for each condition, and PBS was added instead of lenvatinib as a control. BV-2, C6, BC3H1 and G422cells were grown at 37˚C with 5% CO_2 until 80% confluence was reached. Following incubation for 48 h, apoptosis was assessed by incubation of these cells with lenvatinib. The BV-2, C6, BC3H1 and G422 cells were trypsinized and collected into EP tubes. The cells were then washed with cold PBS three times and adjusted to a concentration of $1x10^6$ cells/ml with PBS. The cells were subsequently labeled with Annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (Annexin V-FITC kit; BD Biosciences, San Jose, CA, USA) and analyzed with a FACScan flow cytometer using WinMDI software (version 2.9; BD Biosciences).

Flow cytometry analysis. Tumors from experimental mice were ground into monoplast suspensions and washed three times with PBS for flow cytometric analysis. Tumor cell suspensions were filtered through a 100 μm nylon strainer and centrifuged (1,000 x g for 5 min at 4˚C) to remove cell debris. The tumor cells were subsequently incubated with CD3 and CD45-labeled CD4 and CD8 to analyze the degree of CD4 and CD8 cell subsets in the total infiltrated immune cells. The stained cells were analyzed using a Becton Dickinson FACScan flow cytometer using WinMDI software (version 2.9; BD Biosciences).

IFN-γ release and CTL response assays. Spleens were obtained from the euthanized mice, which had been treated by lenvatinib or PBS. Splenocytes were subsequently isolated by passing the spleens through 100 μm nylon mesh filters. The cells were washed three times with PBS and incubated with mitomycin-inactivated BV-2 cells (mitomycin-C, cat. no. ab120797; Abcam, Cambridge, UK), and IFN-γ was measured in the supernatants on day 3 using a sandwich ELISA kit (cat. no. ab193969; Abcam). In addition, T cells ($1x10^6$) from the splenocytes were purified, as previously described (24) and co-cultured with fresh BV-2 cells for 4 h at the effector:target ratios of 5:1, 15:1 and 45:1. Specific CTL responses to the target cells (BV-2) were analyzed using MTT cytotoxicity assays.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total mRNA was isolated using an mRNeasy Extraction kit (Qiagen, Inc., Valencia, CA, USA). Extracted mRNA (1 μg) was transcribed into cDNA using a reverse transcription kit (Qiagen, Inc.). The cDNA (10 ng) was used for qPCR using the SYBR Green Master Mix system (Bio-Rad Laboratories, Inc., Hercules, CA, USA) for 30 cycles. All the forward and reverse primers were synthesized by Invitrogen; Thermo Fisher Scientific, Inc. (Table I). PCR amplification followed preliminary denaturation at 94˚C for 2 min, followed by 35 cycles of 94˚C for 30 sec, annealing at 64˚C for 30 sec, and 72˚C for 10 min. The reaction was performed at a volume of 20 μl, containing 50 ng genomic DNA, 200 μM dNTP, 2.5 units Taq DNA polymerase and 200 μM primers. Relative mRNA expression changes were calculated by $2^{-\Delta\Delta Cq}$ (25). The results were expressed as n-fold of the expression of β-actin.

Animal experiments. Male BALC/c nude mice (n=100; age, 6-8 weeks; 30-35 g) were purchased from the West China Experimental Animal Center of Sichuan University (Chengdu, China). All animals were housed in a temperature-controlled

Table I. Sequences of primers were used in the present study.

Gene name	Sequence
Caspase-3	F: 5'-AAAGTTTTCAATGACCAAGC-3'
	R: 5'-TCTGACGAATCTCCTCCAC-3'
Caspase-8	F: 5'-AGTCTATTTTATTATGGGCTCG-3'
	R: 5'-TGGATGTTTATGTCACCTTTTC-3'
Caspase-9	F: 5'-ATGGAGAACACTGAAAACTC-3'
	R: 5'-TGTGAGCATGGAAACAATAC-3'
Caspase-10	F: 5'-CTTATCTATGGGACAGACGGGC-3'
	R:5'-GCTGCTCCATTTCTTCACAGGTCCGA-3'
β-actin	F: 5'-AGCCTTCTCCATGGTCGTGA-3'
	R: 5'-CGGAGTCAACGGATTTGGTC-3'

Table II. Treatment-related adverse events of lenvatinib with an overall incidence ≥10%.

Adverse event	Total (n=50)	0.08-0.16 mg (n=20)	0.24 mg (n=10)	0.32-0.40 mg (n=20)
Hypertension	7	2	2	3
Nausea	6	1	2	3
Proteinuria	8	2	2	4
Vomiting	5	1	2	2
Lethargy	6	1	2	3
Fatigue	5	1	2	2
Constipation	5	1	2	2

facility at 23±1˚C and relative humidity 50±5%, with a 12-h light/dark cycle. BV-2cells ($5x10^6$) in 20 μl PBS were subcutaneously injected into the right forelimb of nude mice under aseptic conditions (n=100). The glioblastoma-bearing mice were divided into two groups, and each group contained 50 mice. Each mouse in the treated group received 0.24 mg lenvatinib by intravenous injection once daily, administered continuously in 14 day cycles. The mice in the control groups received normal saline, serving as an injection control. Tumor dimensions were measured every 2 days for a total of 14 times. The tumor volumes were calculated according to the following formula: length x $width^2$ x 0.52. Mice were sacrificed when tumor diameter reached 12 mm. On day 25 following inoculation, tumors from the mice were used for RT-qPCR assay.

Evaluation of toxicity. The median overall duration of treatment for dose-limiting toxicity (DLT) and maximum tolerated dose (MTD) was 14 days for the lenvatinib dosing cohorts: 0.08, 0.16, 0.24, 0.32 and 0.40 mg (8 mice/group). Toxicity was graded using the National Cancer Institute Common Toxicity Criteria (version 3.0) (26). DLT and MTD were defined as any of the drug-related toxicities described in a previous study (27).

Statistical analysis. All data are reported as the mean ± standard deviation. The statistical significance of differences between mean values was assessed by Student's t-test for unpaired data. Comparisons of data between multiple groups were performed using one-way analysis of variance followed by the Student-Newman-Keuls post hoc test. $P<0.05$ was considered to indicate a statistically significant difference. Analysis was performed using SPSS software version 20.0 (IBM Corp., Armonk, NY, USA).

Results

DLT and MTD. The median overall duration of treatment for DLT and MTD was 14 days for lenvatinib dosing cohorts: 0.08, 0.16, 0.24, 0.32 and 0.40 mg. Treatment with 0.24 mg and 0.40 mg lenvatinib once daily was identified as the MTD and DLT, respectively. The lowest dose lenvatinib had the least toxicity in term of experimental date. In addition, at least one dose of lenvatinib for all experimental mice for study therapy with post baseline safety evaluation was included in the safety population. Furthermore, the most common treatment-related adverse events were hypertension, vomiting, lethargy, proteinuria, nausea, constipation and fatigue following the last dose of lenvatinib (Table II).

Lenvatinib effectively induces apoptosis glioblastoma in vitro. To confirm the efficacy of lenvatinib on glioma cells, BV-2, C6, BC3H1 and G422 cells were used to detect the apoptosis rate *in vitro* induced by lenvatinib (0.24 mg/ml). The apoptosis rate of BV-2 (Fig. 1A), C6 (Fig. 1B), BC3H1 (Fig. 1C) and G422 cells (Fig. 1D) was significantly upregulated following treatment with lenvatinib compared with the control group. In addition, apoptosis-associated gene expression was analyzed in lenvatinib-treated BV-2, C6, BC3H1 and G422 cell lines *in vitro*. The mRNA expression levels of pro-apoptotic genes were analyzed using RT-qPCR, including caspase-3, -8, -9 and -10. Expression levels of caspase-3 (Fig. 2A), caspase-8 (Fig. 2B), caspase-9 (Fig. 2C) and caspase-10 (Fig. 2D) were significantly increased following 48 h treatment with lenvatinib compared with the control group. These results suggested that lenvatinib induced apoptosis and upregulated pro-apoptotic genes inBV-2, C6, BC3H1 and G422 glioma cell lines.

Lenvatinib significantly inhibited tumor growth in a glioblastoma mouse model. Following the *in vitro* apoptosis assays, the present study investigated whether lenvatinib-treated mice demonstrated tumor growth inhibition. BV-2 cells were subcutaneously injected into BALB/c nude mice. Lenvatinib treatment via intravenous injection was initiated when the tumor diameter reached 5-6 mm on day 7 following the first tumor inoculation. The treatment was continued for 14 day cycles at frequency of once daily at the MDT dose (0.24 mg/day). The tumor diameter was recorded and tumor volume was calculated. Tumor growth was significantly inhibited in lenvatinib-treated mice (Fig. 3A). In addition, lenvatinib prolonged the survival of tumor-bearing mice across 120 days of observation compared with control group (Fig. 3B). These results suggested that the therapeutic effects of lenvatinib on the BV-2-bearing mice were strong enough to suppress the glioma tumor growth, which translated into long-term survival.

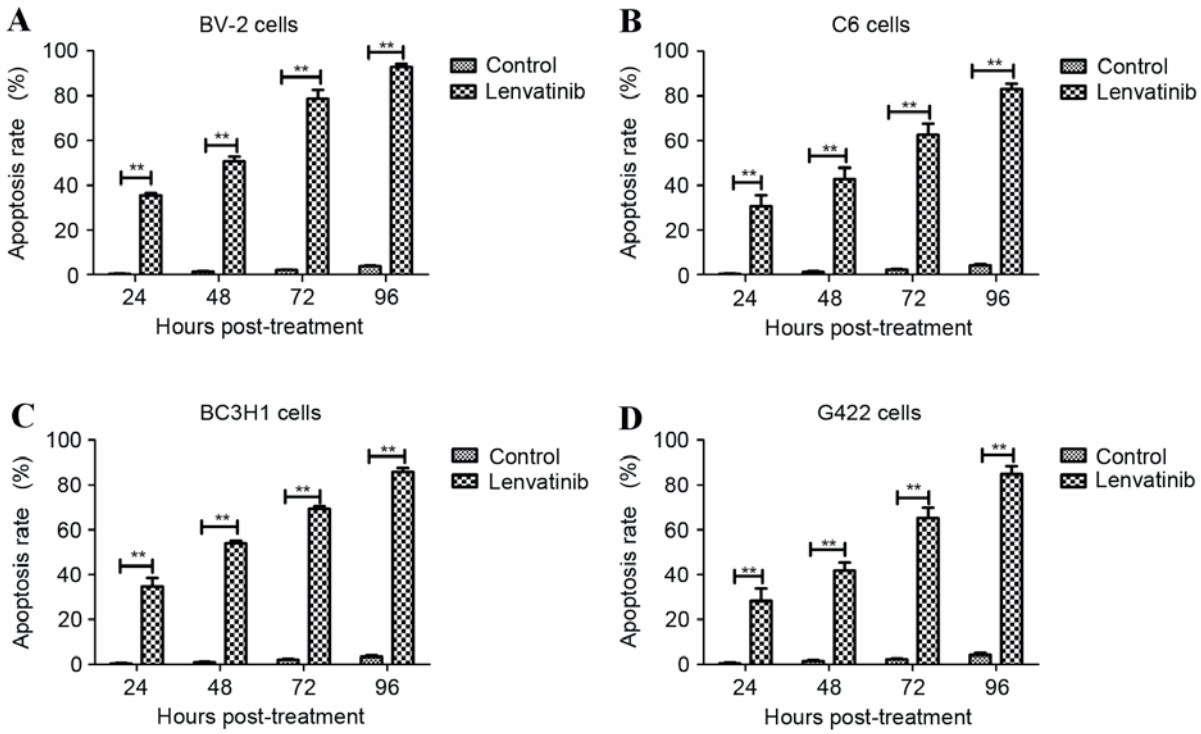

Figure 1. Effect of 0.24 mg/ml lenvatinib treatment (48 h) on apoptosis rate in (A) BV-2, (B) C6, (C) BC3H1 and (D) G422 glioma cell lines *in vitro*. **P<0.01 vs. control.

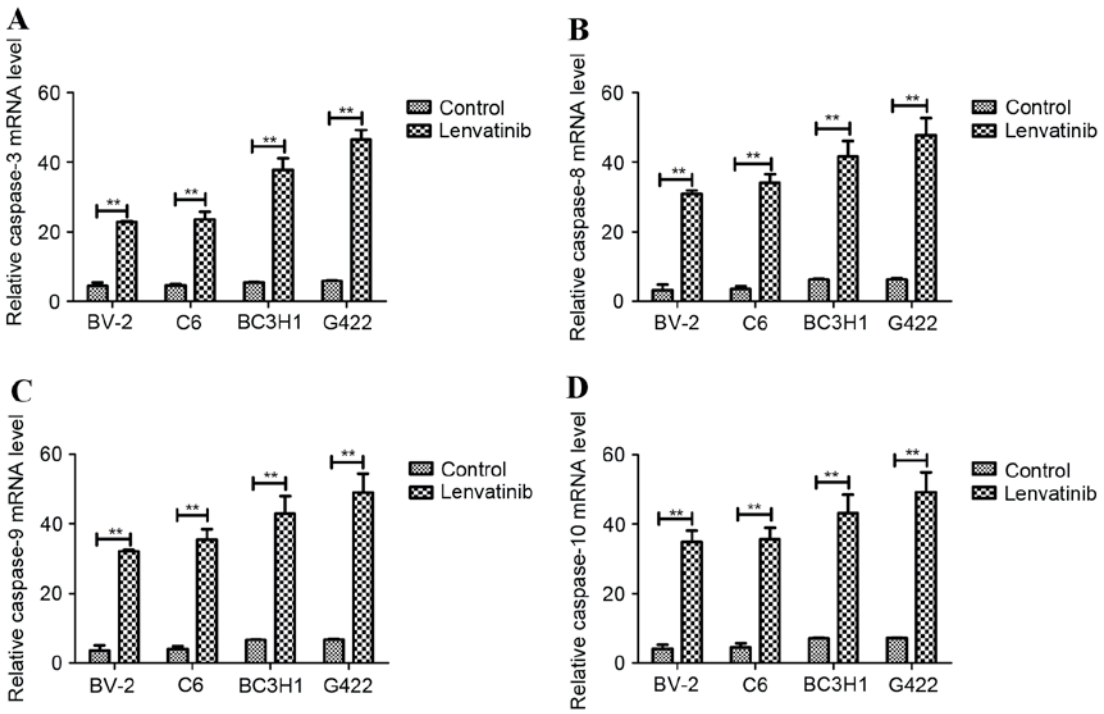

Figure 2. Lenvatinib treatment (0.24 mg/ml, 48 h) induced upregulated mRNA expression levels of the apoptosis-associated genes (A) caspase-3, (B) caspase-8, (C) caspase-9 and (D) caspase-10. **P<0.01 vs. control.

Treatment with lenvatinib resulted in immunological cytotoxicity and a CTL response in glioblastoma tumors. Tumor-bearing mice from the lenvatinib and PBS-treated groups (n=8 mice/group) were sacrificed on day 30 to further analysis of beneficial outcomes. Tumors were collected on day 30 in each group. The tumors were subsequently ground, filtered and stained for $CD4^+$ and $CD8^+$ expression on tumors. Tumors from the mice treated with lenvatinib exhibited a significantly increased degree of $CD4^+$ (Fig. 4A) and $CD8^+$ (Fig. 4B) cell infiltration in the glioblastoma tumor model, as determined by

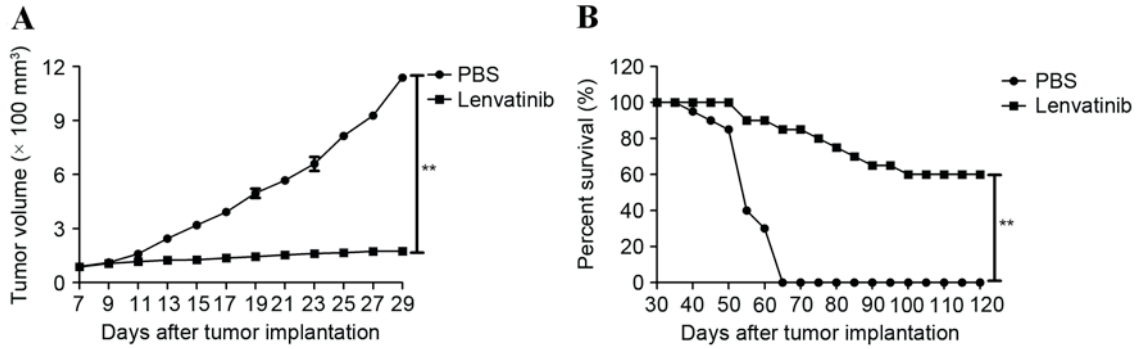

Figure 3. (A) Inhibition of tumor growth and (B) increased long-term survival of the BV-2-bearing BALB/c nude mice following intravenous injection of lenvatinib. **P<0.01 vs. control.

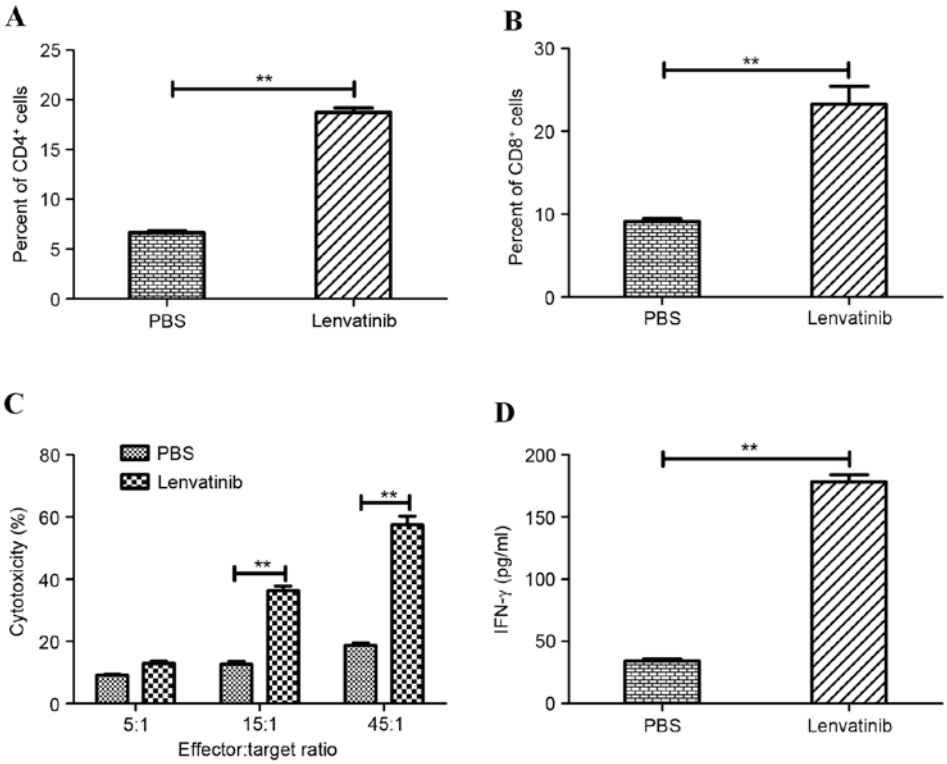

Figure 4. Lenvatinib treatment increased immune responses and protected mice against tumor cells *in vivo*. Percentage of the (A) $CD4^+$ and (B) $CD8^+$ was analyzed by flow cytometry. (C) Lenvatinib enhanced CTL responses against glioblastoma cells. (D) Tumor-specific IFN-γ release was increased in mice with glioblastoma following treatment with lenvatinib. **P<0.01 vs. control. IFN-γ, interferon-γ; CTL, cytotoxic T lymphocyte.

Student's paired t-tests. In addition, CTL responses against the BV-2 glioblastoma cells were assessed on day 30. BV-2-specific CTL activity was assessed following the purification of T cells co-cultured with tumor cells. Treatment with lenvatinib resulted in a significant promotion of CTL activity compared with the PBS-treated group at the effector:target ratios of 15:1 and 45:1 (Fig. 4C). Furthermore, IFN-γ release assay was analyzed to identify and explain the long-term survival in lenvatinib-treated mice. Lenvatinib treatment resulted in significantly increased IFN-γ release compared with PBS-treated mice (Fig. 4D). These results suggested that treatment of tumor-bearing mice with lenvatinib resulted in the generation of tumor-specific CTL responses and partial protection of the animals against the tumor cells, which may contribute to the long-lasting antitumor effects observed in the BV-2-bearing mice.

Discussion

Targeted therapy has demonstrated marked antitumor activities in the clinical treatment of several types of human cancer (16,28,29). Antineoplastic agents with targeted therapy agents can effectively target tumor cell-specific recognition domains, either antigens or receptors (30-33). Different targeted therapy drugs for cancer treatment are being studied clinically. The targeted therapy agents frequently use tumor cell-specific recognition to inhibit tumor angiogenesis, mediated by suppression of tumor-derived vascular endothelial cells, and have demonstrated encouraging results in the treatment of certain advanced tumors (34,35). However, more targeted therapy agents require development to cater to the needs of an ever-increasing number of cancer patients.

Therapeutic protocols that target VEGF-mediated pathways have been clinically applied to treat human cancers (36). However, single therapies that target VEGF remained challenges in clinical outcomes, and improvements are required to overcome ineffective drug treatments (37). Lenvatinib is a multi-targeted tyrosine kinase inhibitor with anticancer potential, which improved upon and overcame deficiencies of single anticancer agents, as well as demonstrated improved therapeutic benefits for patients with cancer (38,39). However, few studies among these previous reports studied the efficacy of lenvatinib as a therapy for glioblastoma. In the present study, an optimal treatment scheme of lenvatinib for glioblastoma in a murine model has been defined, and preclinical efficacy has demonstrated low toxicity and positive outcomes. The data support the use of the effective, multi-targeted lenvatinib agent for glioblastoma therapy, as it is associated with improved efficacy of treatment and manageable, lower toxicity compared with other anticancer agents (40).

Lenvatinib has previously demonstrated favorable antitumor potential for human cancer therapy (37). Lenvatinib is also well tolerated without serious treatment-associated adverse events. Despite targeted therapy providing the advantage of tumor specificity, it is conceivable that effectively invoked toxicity of immune cells for tumor cells may contribute to cancer therapy (40). The clinical application of lenvatinib would be more effective by intravenous injection to inhibit tumor cell growth and produce tumor-specific killer cells with improved immunogenicity to stimulate adaptive T cell mediated anti-tumor immunity (41).

Although previous studies have demonstrated the MTD of lenvatinib in phase II trial in advanced medullary thyroid cancer (36). In the present study, targeted therapy was introduced to treat glioblastoma in BV-2-bearing BALB/c nude mice. In addition, lenvatinib demonstrated manageable toxicity at the MTD, and lower-dose cohort and MTD dose presented an effective treatment for glioblastoma. Furthermore, the treatment-associated adverse events of lenvatinib treatment were representative and consisted with previous clinical research (41).

The responses observed with the present lenvatinib therapy conferred an advantage relative to other single-target therapy (42). In the present primary analysis of glioblastoma therapy, treatment with 0.24 mg lenvatinib once daily resulted in an increased CTL response, increased IFN-γ release and improved long-term survival in glioblastoma-bearing mice.

In conclusion, a novel therapeutic schedule for the treatment of glioblastoma using the multi-therapy anticancer agent lenvatinib was introduced as an MTD dose of 0.24 mg at a frequency of once daily. The treatment-associated adverse events were consistent with those of multi-targeted tyrosine kinase inhibitors and were managed effectively by administering continuously in 14 day cycles. However, the beneficial effects of targeted lenvatinib therapy require further elucidation to improve the clinical value of this regimen.

References

1. Lu M, Zhang X, Zhang M, Chen H, Dou W, Li S and Dai J: Non-model segmentation of brain glioma tissues with the combination of DWI and fMRI signals. Biomed Mater Eng 26 (Suppl 1): S1315-S1324, 2015.
2. Chow KK, Naik S, Kakarla S, Brawley VS, Shaffer DR, Yi Z, Rainusso N, Wu MF, Liu H, Kew Y, *et al*: T cells redirected to EphA2 for the immunotherapy of glioblastoma. Mol Ther 21: 629-637, 2013.
3. Wang K, Kievit FM, Jeon M, Silber JR, Ellenbogen RG and Zhang M: Nanoparticle-mediated target delivery of TRAIL as gene therapy for glioblastoma. Adv Healthc Mater 4: 2719-2726, 2015.
4. Goudar RK, Shi Q, Hjelmeland MD, Keir ST, McLendon RE, Wikstrand CJ, Reese ED, Conrad CA, Traxler P, Lane HA, *et al*: Combination therapy of inhibitors of epidermal growth factor receptor/vascular endothelial growth factor receptor 2 (AEE788) and the mammalian target of rapamycin (RAD001) offers improved glioblastoma tumor growth inhibition. Mol Cancer Ther 4: 101-112, 2005.
5. Delfino KR, Serão NV, Southey BR and Rodriguez-Zas SL: Therapy-, gender- and race-specific microRNA markers, target genes and networks related to glioblastoma recurrence and survival. Cancer Genomics Proteomics 8: 173-183, 2011.
6. Koekkoek JA, Postma TJ, Heimans JJ, Reijneveld JC and Taphoorn MJ: Antiepileptic drug treatment in the end-of-life phase of glioma patients: a feasibility study. Support Care Cancer 24: 1633-1638, 2016.
7. Powell IJ, Banerjee M, Bianco FJ, Wood DP Jr, Dey J, Lai Z, Heath M and Pontes EJ: The effect of race/ethnicity on prostate cancer treatment outcome is conditional: A review of Wayne State University data. J Urol 171: 1508-1512, 2004.
8. Cafarotti S, Lococo F, Froesh P, Zappa F and Andre D: Target therapy in lung cancer. Adv Exp Med Biol 893: 127-136, 2016.
9. Sareddy GR and Vadlamudi RK: Cancer therapy using natural ligands that target estrogen receptor beta. Chin J Nat Med 13: 801-807, 2015.
10. Hamamoto R and Nakamura Y: Dysregulation of protein methyltransferases in human cancer: An emerging target class for anticancer therapy. Cancer Sci 107: 377-384, 2016.
11. Beck JT: Potential role for mammalian target of rapamycin inhibitors as first-line therapy in hormone receptor-positive advanced breast cancer. Onco Targets Ther 8: 3629-3638, 2015.
12. Jackson CM, Lim M and Drake CG: Immunotherapy for brain cancer: Recent progress and future promise. Clin Cancer Res 20: 3651-3659, 2014.
13. Choi SY, Xue H, Wu R, Fazli L, Lin D, Collins CC, Gleave ME, Gout PW and Wang Y: The MCT4 gene: A novel, potential target for therapy of advanced prostate cancer. Clin Cancer Res 22: 2721-2733, 2016.
14. Lapa C, Linsenmann T, Lückerath K, Samnick S, Herrmann K, Stoffer C, Ernestus RI, Buck AK, Löhr M and Monoranu CM: Tumor-associated macrophages in glioblastoma multiforme-a suitable target for somatostatin receptor-based imaging and therapy? PloS One 10: e0122269, 2015.
15. Vehlow A and Cordes N: Invasion as target for therapy of glioblastoma multiforme. Biochim Biophys Acta 1836: 236-244, 2013.
16. Cabanillas ME, Schlumberger M, Jarzab B, Martins RG, Pacini F, Robinson B, McCaffrey JC, Shah MH, Bodenner DL, Topliss D, *et al*: A phase 2 trial of lenvatinib (E7080) in advanced, progressive, radioiodine-refractory, differentiated thyroid cancer: A clinical outcomes and biomarker assessment. Cancer 121: 2749-2756, 2015.
17. Nijaguna MB, Patil V, Urbach S, Shwetha SD, Sravani K, Hegde AS, Chandramouli BA, Arivazhagan A, Marin P, Santosh V and Somasundaram K: Glioblastoma-derived macrophage colony-stimulating factor (MCSF) induces microglial release of insulin-like growth factor-binding protein 1 (IGFBP1) to promote angiogenesis. J Biol Chem 290: 23401-23415, 2015.
18. Krishnan S, Szabo E, Burghardt I, Frei K, Tabatabai G and Weller M: Modulation of cerebral endothelial cell function by TGF-β in glioblastoma: VEGF-dependent angiogenesis versus endothelial mesenchymal transition. Oncotarget 6: 22480-22495, 2015.
19. Kuznar W: Lenvatinib extends survival in metastatic renal-cell carcinoma. Am Health Drug Benefits 8: 18, 2015.
20. Molina AM, Hutson TE, Larkin J, Gold AM, Wood K, Carter D, Motzer R and Michaelson MD: A phase 1b clinical trial of the multi-targeted tyrosine kinase inhibitor lenvatinib (E7080) in combination with everolimus for treatment of metastatic renal cell carcinoma (RCC). Cancer Chemother Pharmacol 73: 181-189, 2014.

21. Hegazi M, Azadi A, Jain D, Redman R and Perez CA: Pharmacological and clinical profile of lenvatinib (E-7080) in the treatment of advanced, radioiodine-refractory, differentiated thyroid cancer. Drugs Today (Barc) 51: 689-694, 2015.
22. Iacovelli R, Alesini D, Palazzo A, Trenta P, Santoni M, De Marchis L, Cascinu S, Naso G and Cortesi E: Targeted therapies and complete responses in first line treatment of metastatic renal cell carcinoma. A meta-analysis of published trials. Cancer Treat Rev 40: 271-275, 2014.
23. Brugarolas J: Renal-cell carcinoma-molecular pathways and therapies. Brugarolas J 356: 185-187, 2007.
24. Greaves MF and Brown G: Purification of human T and B lymphocytes. J Immunol 112: 420-423, 1974.
25. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
26. Huynh-Le MP, Zhang Z, Tran PT, DeWeese TL and Song DY: Low interrater reliability in grading of rectal bleeding using national cancer institute common toxicity criteria and radiation therapy oncology group toxicity scales: A survey of radiation oncologists. Int J Radiat Oncol Biol Phys 90: 1076-1082, 2014.
27. Mehravi B, Alizadeh AM, Khodayari S, Khodayari H, Ashtari K, Mohseni M, Anaraki NI, Dana EA, Safari S and Amanlou M: Acute toxicity evaluation of glycosylated $Gd3^{+}$-based silica nanoprobe. Mol Imaging Biol 19: 522-530, 2017.
28. Tsuruoka A, Matsui J, Suzuki T, Koyama N, Watanabe T and Funahashi Y: Preclinical and clinical researches of lenvatinib mesylate (Lenvima capsule), a novel antitumor agent approved for thyroid cancer treatment. Nihon Yakurigaku Zasshi 146: 283-290, 2015.
29. Midorikawa Y, Sugiyama Y and Aburatani H: Molecular targets for liver cancer therapy: From screening of target genes to clinical trials. Hepatol Res 40: 49-60, 2010.
30. Husain SR, Behari N, Kreitman RJ, Pastan I and Puri RK: Complete regression of established human glioblastoma tumor xenograft by interleukin-4 toxin therapy. Cancer Res 58: 3649-3653, 1998.
31. Debinski W, Gibo DM, Obiri NI, Kealiher A and Puri RK: Novel anti-brain tumor cytotoxins specific for cancer cells. Nat Biotechnol 16: 449-453, 1998.
32. Bera TK, Viner J, Brinkmann E and Pastan I: Pharmacokinetics and antitumor activity of a bivalent disulfide-stabilized Fv immunotoxin with improved antigen binding to erbB2. Cancer Res 59: 4018-4022, 1999.
33. Ghetie MA, Richardson J, Tucker T, Jones D, Uhr JW and Vitetta ES: Antitumor activity of Fab' and IgG-anti-CD22 immunotoxins in disseminated human B lymphoma grown in mice with severe combined immunodeficiency disease: Effect on tumor cells in extranodal sites. Cancer Res 51: 5876-5880, 1991.
34. Sivashankari PR and Prabaharan M: Peptides to target tumor vasculature and lymphatics for improved anti-angiogenesis therapy. Curr Cancer Drug Targets 16: 522-535, 2016.
35. Collet G, Szade K, Nowak W, Klimkiewicz K, El Hafny-Rahbi B, Szczepanek K, Sugiyama D, Weglarczyk K, Foucault-Collet A, Guichard A, *et al*: Endothelial precursor cell-based therapy to target the pathologic angiogenesis and compensate tumor hypoxia. Cancer Lett 370: 345-357, 2016.
36. Grun D, Adhikary G and Eckert RL: VEGF-A acts via neuropilin-1 to enhance epidermal cancer stem cell survival and formation of aggressive and highly vascularized tumors. Oncogene 35: 4379-4387, 2016.
37. Mayor S: Lenvatinib improves survival in refractory thyroid cancer. Lancet Oncol 16: e110, 2015.
38. Schlumberger M, Jarzab B, Cabanillas ME, Robinson B, Pacini F, Ball DW, McCaffrey J, Newbold K, Allison R, Martins RG, *et al*: A Phase II trial of the multitargeted tyrosine kinase inhibitor lenvatinib (E7080) in advanced medullary thyroid cancer. Clin Cancer Res 22: 44-53, 2016.
39. Zhu C, Ma X, Hu Y, Guo L, Chen B, Shen K and Xiao Y: Safety and efficacy profile of lenvatinib in cancer therapy: A systematic review and meta-analysis. Oncotarget 7: 44545-44557, 2016.
40. Lorusso L and Newbold K: Lenvatinib: A new option for the treatment of advanced iodine refractory differentiated thyroid cancer? Future Oncol 11: 1719-1727, 2015.
41. Nair A, Lemery SJ, Yang J, Marathe A, Zhao L, Zhao H, Jiang X, He K, Ladouceur G, Mitra AK, *et al*: FDA approval summary: Lenvatinib for progressive, radio-iodine-refractory differentiated thyroid cancer. Clin Cancer Res 21: 5205-5208, 2015.
42. Whiteside TL: Inhibiting the inhibitors: Evaluating agents targeting cancer immunosuppression. Expert Opin Biol Ther 10: 1019-1035, 2010.