

Original Article

Quantitative MRI assessment of glioma response to bevacizumab in a mouse model

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Abstract: We aimed to investigate the changes of MRI parameters in glioma after bevacizumab treatment and to explore possible pathological mechanism to these changes. Forty-two nude mice with intracranial gliomas were divided two groups: bevacizumab group and control group. Various MRI parameters (tumor volume, apparent diffusion coefficient [ADC], T1w + contrast, dynamic contrast-enhanced-MRI [DCE-MRI]) were acquired before and after treatment. The expression of Ki67, claudin-5, occludin and CD34 were detected and tight junction changes of blood brain barrier were observed. Neovascularization pattern (intussusceptive microvascular growth) in tumor was also observed after treatment. The results showed bevacizumab reduced tumor growth rate and suppressed tumor cell proliferation. Normalized ADC was negatively correlated with the number of Ki67 positive cells in the tumor tissue ($R^2 = 0.733$). Contrast enhancement on T1w + contrast and the K_{trans} value acquired from DCE-MRI showed a significant reduction in glioma. Transmission electron microscope showed tight junction was partially regained after bevacizumab treatment. Meanwhile, claudin-5 and occludin expression increased. In bevacizumab group, the number of intussusceptive microvascular growth (3.83 ± 1.17) in tumor was higher than that in control group (1.16 ± 0.75) ($P < 0.05$). K_{trans} value was negatively correlated with intussusceptive microvascular growth ($R^2 = 0.7396$) and normalized contrast enhancement value was negatively correlated with claudin-5 and occludin expression ($R^2 = 0.831, 0.924$) in tumor. In conclusion, bevacizumab effectively reduced the K_{trans} and T1w + contrast values. The mechanisms might involve neovascularization pattern change and reformation of tight junction. And ADC, K_{trans} and T1w + contrast might be noninvasive biomarkers to predict cell proliferation, neovascularization pattern and tight junction change in a U87 glioma model. These findings might provide some new guidance to glioma therapy.

Keywords: Bevacizumab, glioma, MRI, neovascularization, biomarker

Introduction

Malignant glioma has a high recurrence and mortality rate. Current strategy contains surgical debulking, radiation therapy and chemotherapy. Despite the increase in therapy methods, the median survival of patients with glioblastoma multiforme (GBM) remains at 12-14 months [1-3]. GBM is associated with high levels of angiogenesis and has increased expression of angiogenic factors and irregular extensive vascular proliferation. Vascular endothelial growth factor (VEGF) is the central factor in regulating GBM neovascularization [4]. High VEGF expression could cause proliferation and growth of endothelial cells, which is associated

with hypoxia and necrosis in glioma [5, 6]. And these factors can promote tumor angiogenesis and progression [7].

Antiangiogenic therapy is the focus of glioma therapeutic development in recent years. Strategies that inhibit VEGF have been explored the most [4]. Bevacizumab is a humanized monoclonal antibody and it could specifically bind to the VEGF-A isoform. Although bevacizumab was initially approved by the FDA for treatment of metastatic colorectal cancer, its potential for widespread use in other tumor types still need further investigation. Bevacizumab have been approved for use in recurrent and newly diagnosed GBM and promising

results have been obtained with the treatment of brain tumor patients [8-12]. However, bevacizumab could reduce vascular permeability and temporally reverse abnormal capillary leakage. The transient vascular normalization restricts further drugs penetration into brain parenchyma and reduces therapy effect on glioma [13, 14]. The mechanism to this phenomenon still needs further investigation.

Magnetic resonance imaging (MRI) plays a major role in management of brain tumors, providing a noninvasive strategy to characterize cellular and vascular properties of tumors in addition to response assessment following treatment [15-17]. Clinical studies have a limitation that they could not acquire repeat multiple biopsies before and after treatment in a same patient. Therefore, preclinical studies have an advantage of making direct comparison between tumor correlative pathology and MRI parameters before and after bevacizumab treatment. So, quantitative longitudinal MRI measures of tumor volume, apparent diffusion coefficient (ADC) and vascular responses would show a comprehensive evaluation of glioma response to bevacizumab therapy.

In this study, we applied bevacizumab to treat U87 intracranial xenografts. Then, we obtained several noninvasive MRI parameters for the assessment of glioma response to bevacizumab. Furthermore, we investigated the possible mechanism involved in the changes of MRI parameters after bevacizumab therapy.

Materials and methods

Cell culture

Human glioma U87MG cell line was obtained from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, PR China). Cells were grown in MEM with Earle Salts and L-glutamine (MEM 1*Mediatech, Inc.) supplemented with 10% fetal bovine serum (Hyclone, Logan, AR), 0.1 mmol/L MEM-nonessential amino acids, 2 mM/L sodium pyruvate and penicillin-streptomycin (Hyclone, Logan, AR). The cells were grown at 37°C in a humidified atmosphere containing 95% air and 5% CO₂.

Orthotopic GBM xenograft model

BALB/c nude mice (male, 5-6 weeks old) were supplied by the Department of Experimental

Animals (Daping Hospital, Third Military Medical University, PR China). All animal experiments were carried out according to institutional Animal Care Committee guidelines. Orthotopic GBM models were established by intracerebral implantation of U87MG cells. Mice were anesthetized and placed in a stereotaxic apparatus. Then, 1×10^6 U87MG cells suspended in 5 μ L of PBS were injected into the right striatum region (2.5 mm behind the bregma, 2.0 mm lateral and 3.75 mm deep) of mouse with a microinjector. The injection was given slowly over a period of 5 min. Then the needle was kept in for 5 min and slowly withdrawn [18]. Two weeks after U87 cell implantation, the mice were prepared for experiments. All of the procedures were performed in accordance with the approval of the Institutional Review Board of the Hospital. All animals received care in accordance with the Guide for the Care and Use of Laboratory Animals.

Anti-angiogenic therapy

Forty-two glioma-bearing mice were randomized into bevacizumab group and the control group. In bevacizumab group, bevacizumab (15 mg/kg) was injected in a single dose via the tail vein after U87 cell implantation for 14 days. And in control group, PBS was injected via the tail vein instead of bevacizumab.

Magnetic resonance imaging

The animals (n = 6 for each group) were performed MRI with a Bruker BioSpec 7T/20 cm system (Bruker, Ettlingen, Germany), using a head surface coil. During all MRI procedures, animals were anesthetized with a 1-2% isoflurane/air mixture. T2-weighted image (T2WI) was used to detect tumor volume changes at days 14, 17, 21 after intracranial tumor cell implantation. Other MRI parameters were used to detect real-time changes in physiological properties of tumor at days 14 and 17 after tumor cell implantation. The sequences used in this study were as follows: 1) T2WI: repetition time = 4000 ms, echo time = 45 ms, field of view = 25 mm $\times$ 25 mm, slice thickness = 0.8 mm, NEX = 4, flip angle = 90°; 2) T1 RARE: repetition time = 1000 ms, echo time = 8 ms, field of view = 25 mm $\times$ 25 mm, slice thickness = 0.8 mm, NEX = 4, flip angle = 90°; Diffusion weighted image (DWI): (TR = 4000 ms; TE = 30 ms; field

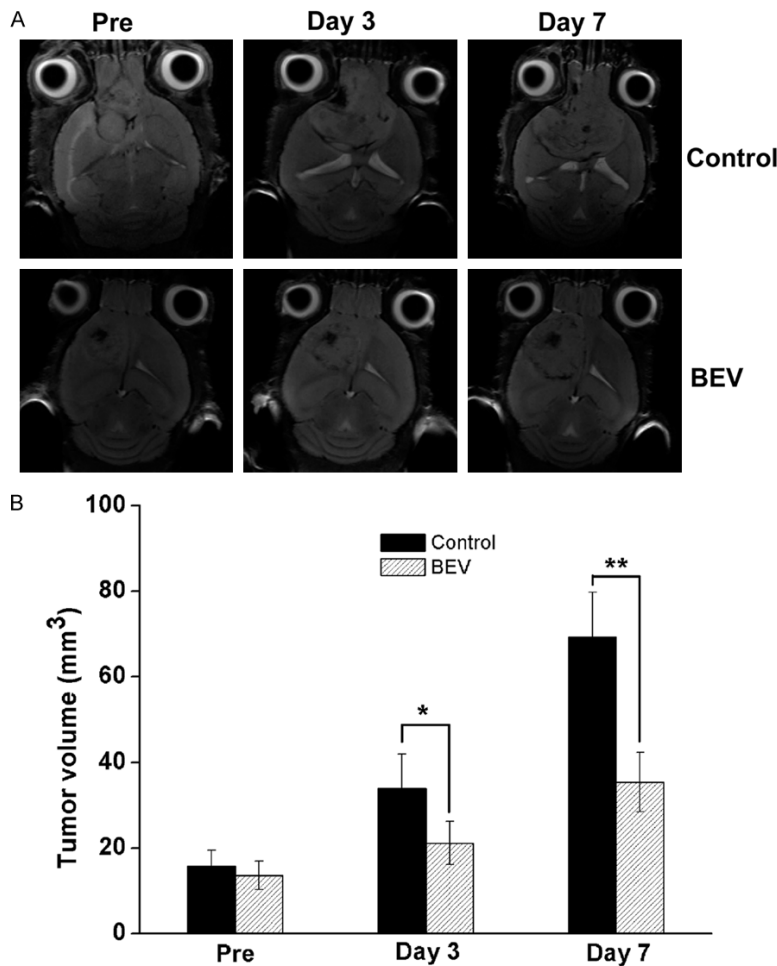


Figure 1. Tumor growth changes at the different time points in two groups. A. T2WI MRI images acquired at different time points: pre-treatment and at 3 and 7 days after treatment in two groups. B. Tumor volumes as measured by T2WI MRI at the different time points: pre-treatment and at 3 and 7 days after treatment in two groups ($n = 6$ for each group). *and** indicate a significant difference compare with the control group ($P < 0.05$).

of view = 25 mm $\times$ 25 mm; b-values = 29, 816 and 1199).

The mice were performed by dynamic contrast-enhanced-MRI (DCE-MRI) to evaluate tumor vessel permeability. To generate T1 maps from precontrast images, DCE FLASH images with multiple flip angles of 10°, 20°, 30°, 40° and 45° were acquired. The following parameters were used to acquire the DCE FLASH images: TR = 70 ms, TE = 1.8 ms using a 256 $\times$ 256 matrix, FOV = 25 mm $\times$ 25 mm, and NEX = 1. Acquisition of DCE FLASH images started before the administration of contrast to have baseline T1 signals. When the third dynamic loop finished, 0.5 mmol/mL Omniscan (GE Healthcare, Cork, Ireland) was administered at

a dosage of 0.1 mmol/kg body weight by hand push within 4 s.

Magnetic resonance image analysis

Tumor volume was calculated by multiplying the maximal anterior-posterior diameter with the maximal superior-inferior diameter and maximal left-right diameter and dividing the value in half in T2WI. DCE-MRI data were transferred to an independent workstation for quantitative analysis by OmniKinetics (GE Healthcare, Shanghai, PR China). After calculating the original T1 value with FA data, the maximal tumor section was selected and three regions of interest (3 mm $\times$ 3 mm) were outlined inside the tumor region. The reference region model was used to calculate the forward transfer constant K_{trans} . Apparent diffusion coefficient (ADC) was obtained from DWI images. For diffusion analysis, the maximal tumor section was selected and three regions of interest (3 mm $\times$ 3 mm) were outlined inside the tumor region. Percentage change in ADC

value of tumor, as compared with contralateral normal brain region, was calculated based on evidence of improved motion sensitivity. For contrast enhancement analysis, the maximal tumor section was selected and three regions of interest (3 mm $\times$ 3 mm) were outlined inside the tumor region. The percentage change in contrast enhancement of the tumor on T1-weighted (T1w) + contrast images was normalized to contralateral normal brain region. Contrast enhancement regions that were overlapping with ventricles would be excluded.

Immunohistochemistry

Mice ($n = 6$ for each group) were sacrificed by an overdose of chloral hydrate. The brains were

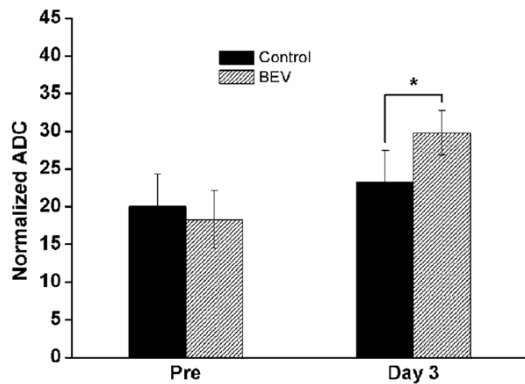


Figure 2. Normalized apparent diffusion coefficient (ADC) value changes in two groups. Normalized ADC value was measured at the different time points: pre-treatment and at 3 day after treatment in two groups (n = 6 for each group). Bar graphs represent percentage change in ADC value from contralateral normal brain region. *indicate a significant difference compare with the control group ($P < 0.05$).

extracted from skull after perfusion with 150 mL heparinized saline and then 150 mL 4% paraformaldehyde. Then put the brains into fixative for one week and immersed them in 30% sucrose solution with PBS for 4 days. Coronal sections were serially cut into pieces at a thickness of 6 μm on cryostat and at intervals of 0.8 mm on a cryostat. Before immuno-staining, they were rehydrated in PBS. Then they were incubated of a 0.3% H_2O_2 /methonal solution for 10 min at room temperature so that endogenous peroxidase activity was inactivated. The next step was to wash and block the sections by using normal goat serum. Then, the sections were incubated with primary antibody at 4°C overnight. Anti-human primary antibody included anti-CD34 (Millipore, CA, USA; 1:100 dilution), anti-Ki67 (Millipore, CA, USA; 1:50 dilution), anti-occludin (Abcam, Cambridge, UK; 1:50 dilution) and anti-claudin-5 (Abcam, Cambridge, UK; 1:50 dilution). Negative controls were conducted by PBS instead of primary antibody. Other procedures were performed according to the immunohistochemistry standard procedures. For semi-quantitative measurements of claudin-5 and occludin, the slides were photographed and the mean optical density values were analyzed by using a image analyzing system (Motic Images Advanced 3.2). CD34-positive folds across the lumens of newly formed blood vessels were the key feature of intussusceptive microvascular growth (IMG).

The numbers of folds in 20 cases of intussusceptive microvascular growth were counted under light microscopy. The average number of folds across the lumens in 20 cases was regarded as the number of intussusceptive microvascular growth in tumor. Ten fields were randomly selected under a 400 $\times$ light microscope and Ki67 positive cells were counted. The average number of Ki67 positive cells per 400 $\times$ field was determined for the entire area of each tumor.

Transmission electron microscopy (TEM) analysis

Mice (n = 6 for each group) were sacrificed by an overdose of chloral hydrate. Then, they were perfused with 150 ml heparinized saline and 150 ml fixative (4% paraformaldehyde). The tumor tissues were obtained and divided into some pieces of 1 mm^3 , which were fixed with 2.5% glutaraldehyde-4% paraformaldehyde for several days at 4°C. After that, according to the standard procedures, semi-thin and ultra-thin sections were made and stained with uranyl acetate and lead citrate, and the changes of tight junction were examined by TEM. All examination procedures by TEM were assigned to use a double-blind method to measure tight junction changes.

Statistical analysis

All data are expressed as mean $\pm$ standard deviation values. Student's t-test was performed to determine the significant difference between two groups. Spearman correlation analysis was used to compare the correlation between K_{trans} value and IMG, T1 + contrast and tight junction proteins expression, ADC value and Ki67 positive cells. A P value < 0.05 was deemed statistical difference. All data were analyzed using SPSS 18.0 software.

Results

Tumor growth in response to bevacizumab therapy

T2WI MRI showed that all tumors were still growing in size within 7 days after treatment in two groups. However, treatment with bevacizumab reduced the growth rate of GBM xenografts compared with the control group ($P < 0.05$) (Figure 1B). This suggested bevacizumab

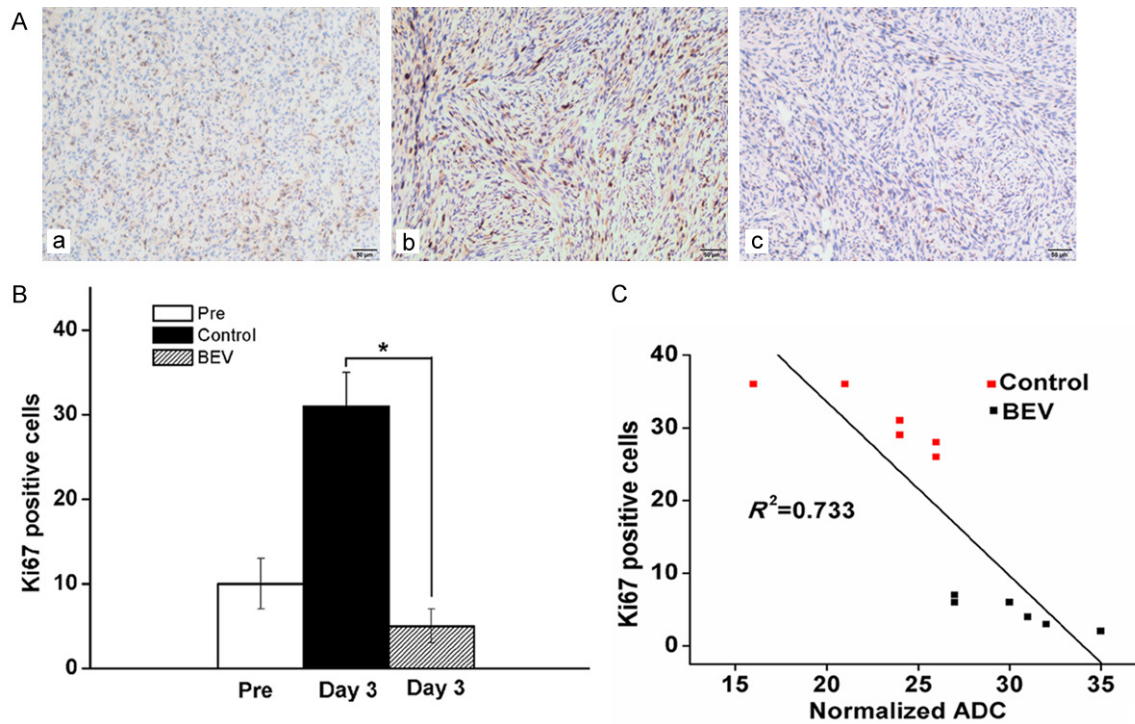


Figure 3. Ki67 positive tumor cells changes in two groups. A. Distribution and expression of Ki67 by immunohistochemistry (a: pre-treatment, b: 3 day after treatment in control group, c: 3 day after treatment in bevacizumab group). B. Bar graphs represent the number of Ki67 positive tumor cells at the different time points: pre-treatment and at 3 day after treatment ($n = 6$ for each group). C. Scatter plots and fitted linear correlation lines of the number of Ki67 positive cells versus the normalized ADC at 3 day after treatment in the tumor tissues. *indicate a significant difference compare with the control group ($P < 0.05$).

had a potent anti-tumor activity. Furthermore, in bevacizumab group, the MRI feature of tumors in T2WI showed distinct differences compared with control group in which tumors became more heterogeneous on the T2WI and this might be associated with necrosis (Figure 1A).

ADC and Ki67 expression changes in glioma

At 14 days post-implantation, normalized baseline ADC values were similar for all groups higher than contralateral brain parenchyma. ADC values were still rising within 3 days after treatment in two groups. However, the magnitude of ADC rise was greater in bevacizumab group (29.8 ± 3.9) compared with the control group (23.3 ± 4.2) ($P < 0.05$) (Figure 2).

Ki67 was used as a marker for cell proliferation. In control group, the number of Ki67 positive cells (31 ± 4) increased significantly after treatment compared with the pre-treatment (10 ± 3). In bevacizumab group, the number of

Ki67 positive cells (5 ± 2) decreased after treatment compared with the control group ($P < 0.05$) (Figure 3A, 3B). These results demonstrated that bevacizumab suppressed cell proliferation in glioma.

Furthermore, we found the number of Ki67 positive cells correlated negatively with normalized ADC value in the tumor tissue ($R^2 = 0.733$, $P < 0.05$) (Figure 3C). This demonstrated that ADC value could be a noninvasive biomarker to evaluate cell proliferation in glioma.

T1w + contrast and DCE-MRI changes in glioma

At 14 days post-implantation, U87 tumors showed almost uniform signal enhancement and the range of contrast enhancement was rising after treatment in control group (Figure 4A). In bevacizumab group, contrast enhancement on T1w + contrast decreased after treatment (37.5 ± 7.4) compared with the control group (74.5 ± 9.2) ($P < 0.05$) (Figure 4A, 4B).

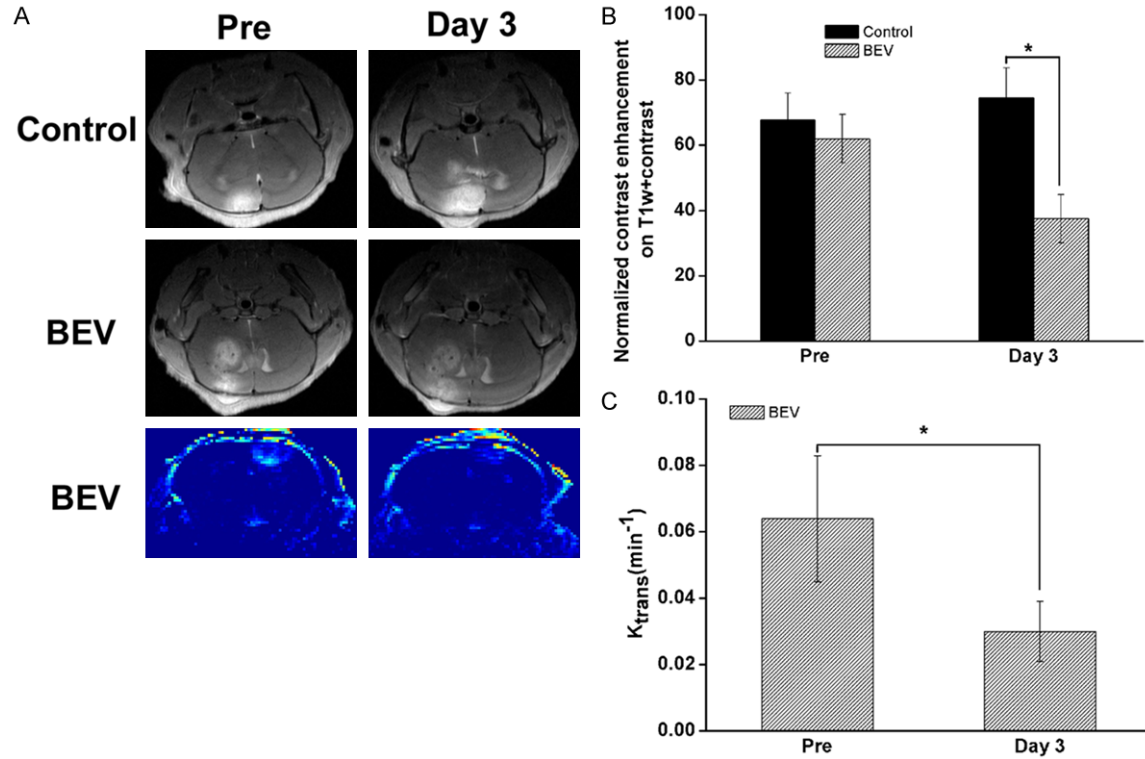


Figure 4. T1w + contrast enhancement and K_{trans} changes in two groups. A. T1w + contrast enhancement and K_{trans} images acquired at different time points: pre-treatment and at 3 day after treatment. B. Bar graphs represent percentage change in T1w + contrast from contralateral normal brain region (n = 6 for each group). C. Bar graphs represent the K_{trans} value at the different time points: pre-treatment and at 3 day after bevacizumab treatment (n = 6 for each group). *indicate a significant difference compare with the control group ($P < 0.05$).

And the signal enhancement was heterogeneous. Furthermore, we can see contrast enhancement on T1w + contrast decreased after bevacizumab treatment compared with pre-treatment (Figure 4A, 4B). These results demonstrated that bevacizumab reduced contrast enhancement on T1w + contrast in glioma.

K_{trans} is considered to be an indicator of tumor vessel permeability. In bevacizumab group, K_{trans} value ($0.03 \pm 0.009 \text{ min}^{-1}$) exhibited a significant decrease after treatment compared with pre-treatment ($0.064 \pm 0.019 \text{ min}^{-1}$) ($P < 0.05$) (Figure 4C). K_{trans} maps images shows similar results. In bevacizumab group, the tumor color of K_{trans} maps images exhibited darker compared with pre-treatment (Figure 4A). This result demonstrated that bevacizumab reduced tumor vessel permeability in glioma.

Tight junction changes in glioma

Although tumor microvessels were disrupted locally in glioma at 14 days post-implantation,

we still observed that tight junction appeared as a series of discrete electron-dense zone by using TEM (Figure 5A). It lay in the adjacent plasma membranes of the endothelial cells and sealed the intercellular cleft. The cleft index of tight junction in tumor was different between control group and bevacizumab group. In bevacizumab group, the cumulated length of clefts in tight junction was attenuated after treatment (Figure 5D-F) compared with the control group (Figure 5B, 5C). These results showed that discrete tight junction in glioma tissue was partially regained after bevacizumab treatment.

Immunohistochemistry showed that claudin-5 and occludin proteins expressed mainly in capillary in glioma (Figures 6A, 7A). In bevacizumab group, the expression of claudin-5 (0.38 ± 0.06) and occluding (0.4 ± 0.06) increased significantly after treatment compared with the control group (0.25 ± 0.04 , 0.19 ± 0.05) ($P < 0.05$) (Figures 6B, 7B). These results also demonstrated that bevacizumab partially regained tight junction in glioma tissue, which was consistent to TEM results.

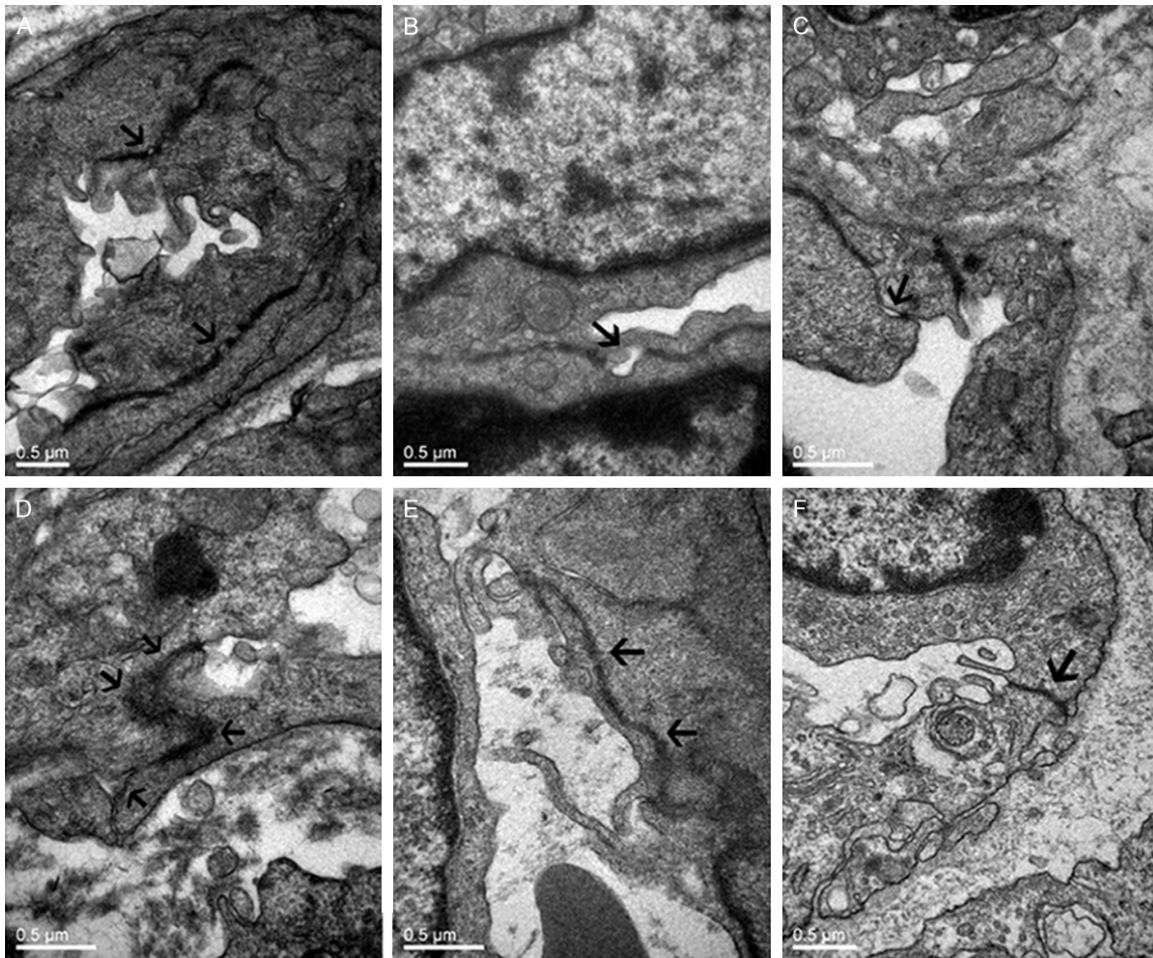


Figure 5. Tight junction changes in tumor. Tight junction (black arrowhead) changes of tumor at the different time points in two groups (A: Pre-treatment; B, C: Discrete tight junction at 3 day after treatment in control group; D-F: Partially regained tight junction 3 day after treatment in bevacizumab group).

Furthermore, we found the expression of claudin-5 and occludin correlated negatively with normalized contrast enhancement on T1w + contrast in the tumor tissue ($R^2 = 0.831, 0.924, P < 0.05$) (Figures 6C, 7C). This demonstrated that T1w + contrast could be a noninvasive biomarker to evaluate tight junction changes in glioma tissues.

IMG change in glioma after bevacizumab treatment

IMG is a special neovascularization pattern in glioma. In control group, immunohistochemistry showed that most of neovascularization pattern was sprout angiogenesis and there was little IMG in glioma tissue after treatment (Figure 8A). In bevacizumab group, IMG (3.83 ± 1.17) exhibited a significant increase in tumor

tissue after treatment compared with the control group (1.16 ± 0.75) ($P < 0.05$) (Figure 8A, 8B). These results showed that bevacizumab changed neovascularization patterns and increased the number of IMG in tumor.

Furthermore, we found K_{trans} value correlated negatively with IMG in tumor tissue ($R^2 = 0.7396, P < 0.05$) (Figure 8C). This demonstrated that K_{trans} could be a noninvasive biomarker to evaluate neovascularization patterns change in glioma after bevacizumab treatment.

Discussion

The blood-brain barrier (BBB) restricts the delivery of most therapeutic agents with molecular weight greater than 400 Da in the brain [19]. Bevacizumab, with molecular weight

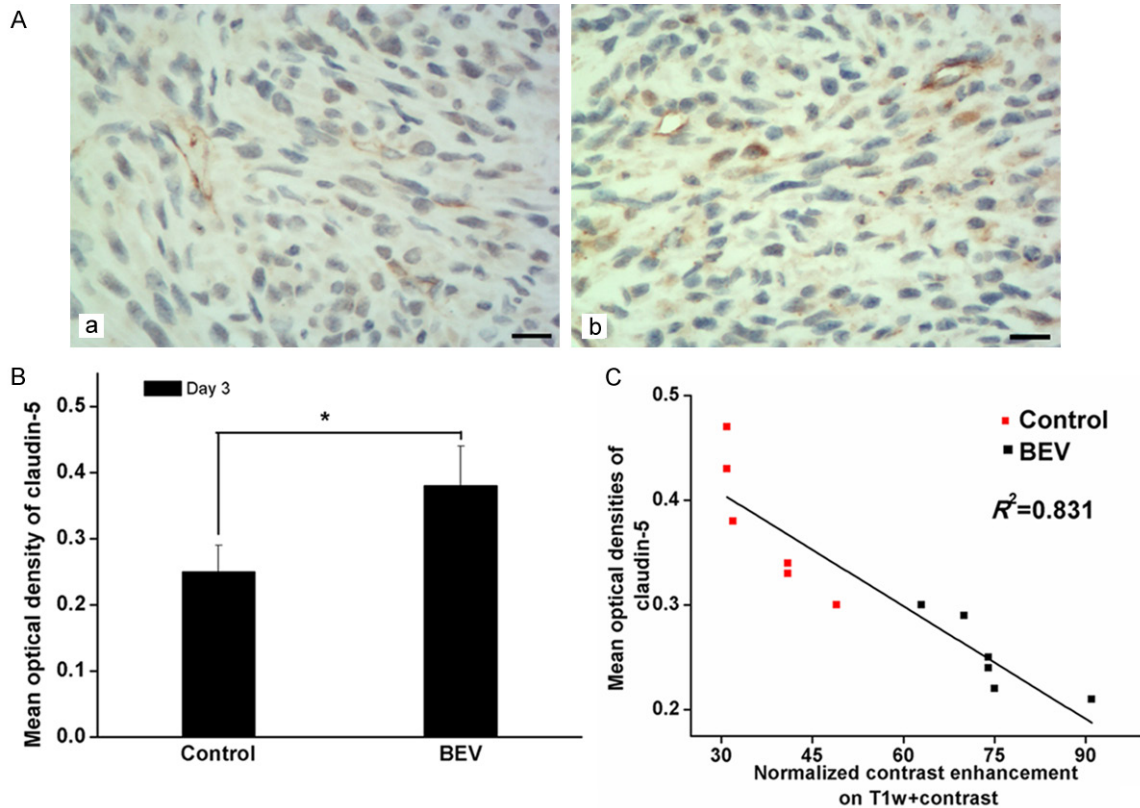


Figure 6. The expression changes of claudin-5 in tumor in two groups (scale bar = 20 μ m). A. The expression of claudin-5 by immunohistochemistry (a: 3 day after treatment in control group; b: 3 day after treatment in bevacizumab group). B. Mean optical densities of claudin-5 in two groups (n = 6 for each group). C. Scatter plots and fitted linear correlation lines of claudin-5 expression versus the normalized contrast enhancement on T1w + contrast at 3 day after treatment in the tumor tissues. *indicate a significant difference compare with the control group ($P < 0.05$).

about 149 KD, can not pass through fully intact BBB. However, VEGF is mainly produced in areas of florid microvascular proliferation in glioma, where disruption of the BBB is evident. And bevacizumab can go through disrupted BBB to penetrate into tumor parenchyma [20].

For each patient, accurate assessment of tumor response to treatment is very important. It determines whether to continue current therapy or switch to an alternative therapy [21, 22]. However, tissue sampling is not always obtained for all tumor areas. Even surgery is possible and advised, choosing the area to target for tissue sampling is also difficult, because gliomas are heterogeneous. So, reliable MRI parameters that can predict treatment effects are urgently needed [23]. Many reports have suggested that treatment effects are associated with increasing ADC value in tumor [24]. In this study, we demonstrated an increase in ADC value for the group treated with bevacizumab at

3 day after treatment compared with the control group. Ki67 was used as a marker for cell proliferation. Our results showed the number of Ki67 positive cells decreased significantly at 3 day after bevacizumab treatment compared with the control group. So, the probable reason for ADC change was bevacizumab reduced tumor cell density and promoted water diffusion. Also, we found the number of Ki67 positive cells correlated negatively with normalized ADC values in the tumor tissue. This demonstrated that the normalized ADC value could be used as a noninvasive biomarker to evaluate cell proliferation in tumor. Treatment with bevacizumab also suppressed tumor growth rate compared with the control group and this suggested bevacizumab had a potent anti-tumor activity, which was consistent to above results.

DCE-MRI has been used to evaluate microvascular permeability and drug delivery efficiency after blood-brain barrier (BBB) disruption in

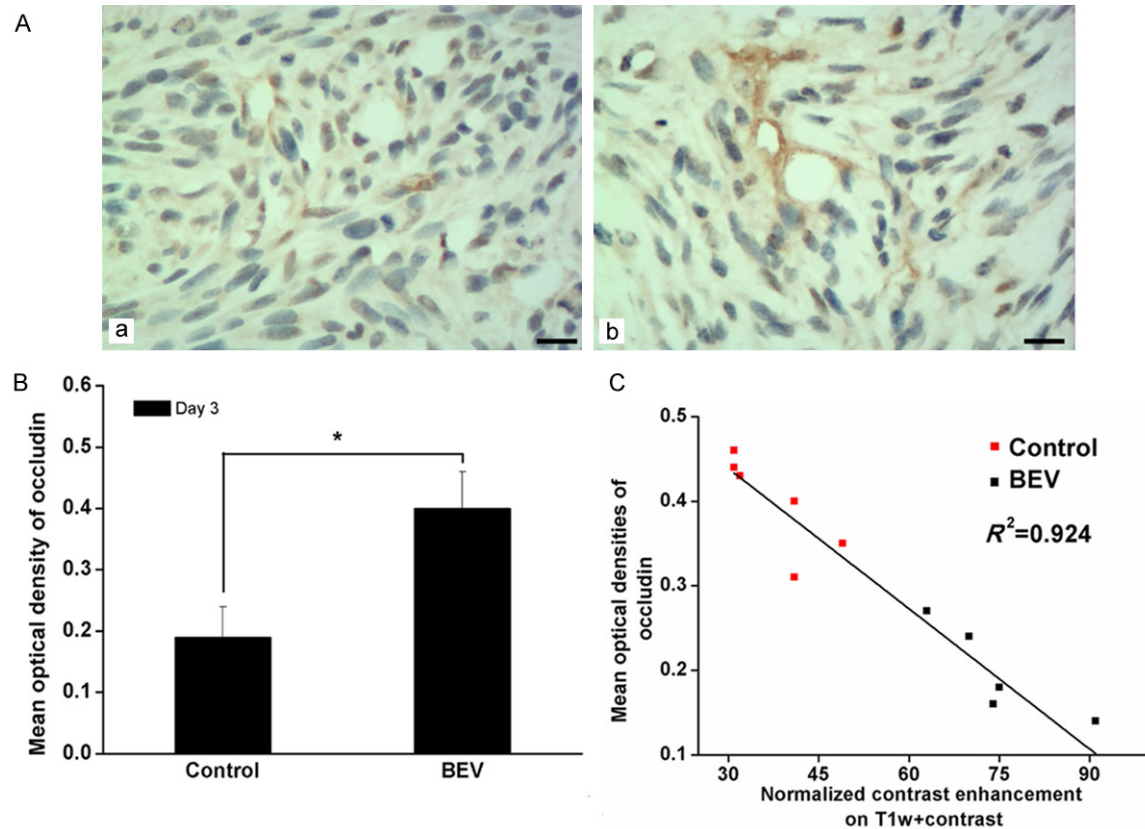


Figure 7. The expression changes of occludin in tumor in two groups (scale bar = 20 μ m). A. The expression of occludin by immunohistochemistry (a: 3 day after treatment in control group; b: 3 day after treatment in bevacizumab group). B. Mean optical densities of occludin in two groups ($n = 6$ for each group). C. Scatter plots and fitted linear correlation lines of occludin expression versus the normalized contrast enhancement on T1w + contrast at 3 day after treatment in the tumor tissues. *indicate a significant difference compare with the control group ($P < 0.05$).

brain [25, 26]. In this study, we adopted K_{trans} of DCE-MRI and T1w + contrast to measure tumor vessel permeability alteration. The results showed that both contrast enhancement on T1w + contrast and K_{trans} value decreased significantly at 3 day after bevacizumab treatment compared with the control group. This was consistent to previous report [27, 28]. But we did not acquire complete loss of enhancement on T1w + contrast or DCE images, which might be attributed to different bevacizumab dosage or animal model. BBB plays an important role in adjustment of microvascular permeability in tumor [29]. Transmembrane proteins regulating tight junction organization include claudins and occludin. Claudins act as the main determinants of tight junction properties [30].

In this study, the expression of claudin-5 and occludin in tumor increased significantly after bevacizumab treatment. And the expression of

claudin-5 and occludin correlated negatively with normalized contrast enhancement on T1w + contrast in the tumor tissue. Previous studies demonstrated VEGF could promote BBB breakdown by reducing claudin-5 protein expression [31]. So, we can conclude partially regained tight junction might be involved in reduction of T1w + contrast after bevacizumab treatment. TEM results confirmed above conclusion. In TEM, the cumulated length of clefts in tight junction was attenuated after bevacizumab treatment compared with the control group, showing that discrete tight junction was partially regained in tumor tissue.

Neovascularization has long been implicated as a salient feature of glioma progression. Several mechanisms by which glioma achieve neovascularization have been described: sprout angiogenesis, vascular co-option, vasculogenesis, mosaic vessel formation, vascular

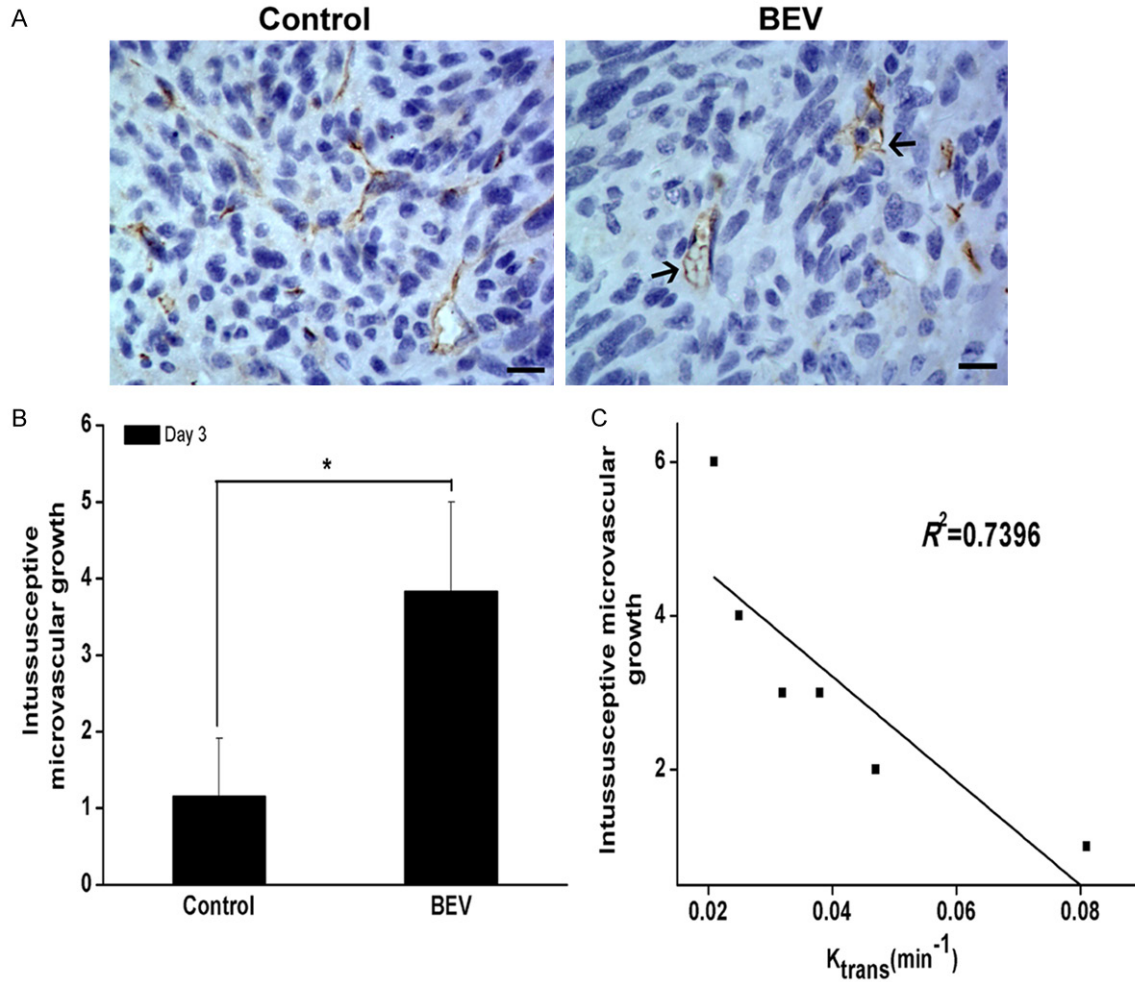


Figure 8. Intussusceptive microvascular growth (IMG) changes in tumor in two groups. A. IMG changes (black arrow) at 3 day after treatment in two groups. B. Bar graphs represent the number of IMG after treatment in two groups ($n = 6$ for each group). C. Scatter plots and fitted linear correlation lines of IMG versus K_{trans} value in tumor. * indicate a significant difference compare with the control group ($P < 0.05$).

mimicry and glioblastoma-endothelial cell transdifferentiation [32-34]. Different patterns are mediated via corresponding signaling pathway with distinct sensitivity to targeted drugs. IMG is an alternative to the sprouting mode of angiogenesis. The advantage of this mechanism of vascular growth is that blood vessels are generated more rapidly and the capillaries thereby formed are less leaky [35]. In this study, IMG exhibited a significant increase after bevacizumab treatment in tumor tissue compared with the control group. Furthermore, we found K_{trans} value correlated negatively with IMG in tumor tissue ($R^2 = 0.7396$). However, explicative molecular mechanism to tumor vessel intussusception is still unknown. From these results we can conclude neovascularization

patterns change in glioma might be involved in reduction of K_{trans} value after bevacizumab treatment. And K_{trans} could be a noninvasive biomarker to evaluate neovascularization pattern change in glioma after bevacizumab treatment.

Despite the findings, this study has many limitations. For example, we only adopted one time point to evaluate the effect of bevacizumab on a U87 glioma model. Also, we did not evaluate other neovascularization patterns change in glioma after bevacizumab treatment.

In conclusion, bevacizumab effectively reduced the K_{trans} and T1w + contrast values. The mechanisms might involve neovascularization pat-

tern change and reformation of tight junction. And ADC, K_{trans} and T1w + contrast might be noninvasive biomarkers to predict cell proliferation, neovascularization pattern and tight junction change in a U87 glioma model. These findings might provide some new guidance to glioma therapy.

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Disclosure of conflict of interest

None.

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