The effect of intravascular interventional embolization and craniotomy on MMP-2, MMP-9 and caspase3 in serum of intracranial aneurysm patients

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Abstract. The effect of intravascular interventional embolization and surgical operation on matrix metalloprotein (MMP)-2, MMP-9 and caspase3 in serum of intracranial aneurysms was analyzed to study the mechanisms. Seventy-nine patients of intracranial aneurysms from September 2011 to August 2014 were divided into the intervention group (n=41) and the craniotomy group (n=38) based on treatment methods, and 40 cases of normal volunteers with normal physical examination were selected as the control group. Patients in the intervention group were treated with intravascular interventional embolization, and the surgical group was treated with craniotomy and no treatment was performed in the control group. The survival rate and adverse reaction rate were calculated. ELISA kit was used to detect the level of reactive oxygen species (ROS), interleukin-6 (IL-6), tumor necrosis factor (TNF-α) and IL-10 in serum; the mRNA and protein expression levels of MMP-2, MMP-9 and caspase3 in serum were detected by qPCR and western blotting; the protein expression levels of p-adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK), Bcl-2 and Bax were also detected. The survival rate of patients in the intervention group was significantly higher than that in the craniotomy group (P<0.05). The ROS level in the intervention and craniotomy groups was significantly higher than that in the control group (P<0.01), while the levels of IL-6 and TNF-α in the serum of the intervention group were lower than those in the craniotomy group (P<0.01). The mRNA and protein expression of MMP-2, MMP-9 and caspase3 of the intervention group was significantly lower than that of the craniotomy group (P<0.01). The protein expression of p-AMPK and Bcl-2/Bax in the intervention group were lower than that in the craniotomy group (P<0.01). Intravascular interventional surgery can significantly increase the patient's survival time, and effectively reduce the expression of MMP-2, MMP-9 and caspase3 in the serum of intracranial aneurysm patients. The mechanism may be through the impact of ROS levels, thereby affecting the p-AMPK and Bcl2/Bax expression.

Introduction

Intracranial arterial wall abnormal bulge is called intracranial aneurysm, which often causes subarachnoid hemorrhage and leads to a series of neurological symptoms, with high mortality. Epidemiological investigations show that the incidence of intracranial aneurysms has an increasing trend year by year (1). Surgical intervention and the development of interventional materials have made endovascular and surgical procedures the preferred treatments for patients with intracranial aneurysms, and the use of imaging techniques has led to a significant increase in the safety of endovascular treatment (2). At present, there is scarce research on the difference of therapeutic effects between craniotomy and endovascular embolization for patients with intracranial aneurysms. Li *et al* (3) found that serum MMP-2 is closely related to the onset of intracranial aneurysm, and serum MMP-2 expression in rats with intracranial aneurysm was significantly increased.

MMP-2 is a member of the MMP family which can degrade the extracellular matrix and regulate the remodeling of the vascular wall (4). Brinjikji *et al* (5) found that in patients with abdominal aortic aneurysm and animal model group, MMP-2 mRNA and protein expression was significantly increased, suggesting that MMP-2 is closely correlated with the formation of aneurysm. Fujii *et al* (6) found that adenosine 5'-monophosphate (AMP)-dependent protein kinase (AMPK) can regulate the transcription of MMP-2 *in vivo* and affect the expression of MMP-2.

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In recent years, a number of studies have shown that the increased levels of proinflammatory cytokines (IL-6 and TNF-α) as well as the increased levels of reactive oxygen species (ROS) caused by inflammatory infiltration are important factors in the formation of multiple hemangiomas. At the same time, caspase3 as a protease present in the cytoplasm can mediate cell apoptosis by degrading the polymeric polymerase PARP, thereby affecting the occurrence and development of hemangiomas (7,8). At present, there is no study on the effect of endovascular embolization and craniotomy on the expression of MMP-2, MMP-9 and caspase3 in the serum of patients with intracranial aneurysms. This study investigated the difference of serum MMP-2, MMP-9 and caspase3 expression in patients with intracranial aneurysm treated by endovascular embolization and craniotomy, and explored the possible mechanism.

Table I. PCR primers.

Genes	Sequence
MMP-2	Sense: 5'-atgacagctgaccactgag-3' Antisense: 5'-atttgttgcccaggaaagtg-3'
MMP-9	Sense: 5'-ttggtttctgccctagtgagaga-3' Antisense: 5'-aaagatgaacgggaacacacagg-3'
Caspase3	Sense: 5'-tgtcgatgcagcaaacctca-3' Antisense: 5'-gacttctacaacgatcccctc-3'
β-actin	Sense: 5'-ctggaacggtgaaggtgaca-3' Antisense: 5'-gggacttcctgtaacaatgca-3'

Patients and methods

Research object. A total of 79 patients with intracranial aneurysms treated in the Second Affiliated Hospital of Soochow University (Suzhou, China) from September 2011 to August 2014 were enrolled in this study. All the patients selected were diagnosed as intracranial aneurysms by experts and imaging examination, and confirmed that there was no local or distant metastasis. All patients were eligible for endovascular embolization and craniotomy for treatment. Patients were divided into the intervention group (n=41, vascular intervention embolization) and craniotomy group (n=38, craniotomy) based on treatment methods. The intervention group consisted of 23 males and 18 females, aged 65.2±19.7 years. The craniotomy group consisted of 21 males and 17 females, with an average age of 64.6±21.9 years. Inclusion criteria: diagnosed as intracranial tumors by the whole brain digital angiography and head CT examination and diagnosed by experts as intracranial tumors. Exclusion criteria: severe wasting disease, not eligible for the conditions of surgery, long-term chronic inflammation, severe liver and kidney dysfunction, traumatic brain injury and cerebral infarction. All patients underwent the same treatment and nursing program after surgery. All patients signed informed consent and all clinical and pathological data during the hospital stay were retained. Forty cases of normal volunteers with normal physical examination were selected as the control group, and all of them signed informed consent. This study was approved by the Ethics Committee of The Second Affiliated Hospital of Soochow University (Suzhou, China) and The Second Clinical Medical School of Inner Mongolia University for Nationalities (Hulunbuir, China).

Surgical methods. All the patients were administered midazolam 0.1 mg/kg (Sichuan Baili Pharmaceutical Co., Ltd., Sichuan, China) 30 min before the anesthesia and then performed with intravenous injection of fentanyl 3 μg/kg. Patients in the intervention group were treated with endovascular embolization: The patient was punctured on the right femoral artery after anesthesia and inserted with the microcatheter. One end of the catheter was placed in the aneurysm of the cerebral artery for embolization (according to the size of the tumor, a corresponding spring circle can be added), and the operation condition was determined through angiography. When the aneurysm cavity disappeared, and no significant blood could be detected, the microcatheter was extubated, and the puncture point was wrapped. After the craniotomy, low molecular weight heparin 5000 units were injected twice a day for a total of 5 days.

The patients in the craniotomy group were treated with craniotomy: After anesthesia, the position of intracranial aneurysms was determined according to brain DSA and cranial CT. The meninges were incised after the surgical route was determined, and the aneurysm was closed under the microscope (Olympus, Tokyo, Japan). The arteries near the intracranial aneurysm were blocked. When the aneurysm was closed by a blocking clip, the blocking clip should be released and the blood supply was restored. After craniotomy, the success rate of operation was evaluated in both groups. Venous blood (5 ml) of the selected patients and volunteers in the control group were drawn, and the serum was separated and stored at -80˚C for future use.

Observation of survival and adverse reactions. Postoperative complications were observed in both groups of patients. The incidence of cerebral hemorrhage, aneurysm rupture, thrombus shedding, infection and other adverse reactions were closely monitored.Then the two groups of patients were followed up for 3 years. The patient survival rate was recorded, and the two treatment methods for the treatment of intracranial tumors were evaluated.

Reactive oxygen species (ROS) and inflammatory cytokine content detection. The serum levels of related factors in each group were measured by ELISA kit (Wuhan Boster Biological Technology Co., Ltd., Wuhan, China) using ROS, IL-6, TNF-α and IL-10 ELISA kit, respectively. The operation was performed strictly according to the instructions of the ELISA kit. The standards of ROS, IL-6, TNF-α and IL-10 were diluted to the standard concentration for the production of standard curve, and working fluid was prepared in advance. The samples, antibodies, enzyme, color solution and termination solution were added in strict accordance with the steps in the instructions, and each sample was repeated three times. After terminating the reaction, the sample was placed on a microplate reader (Bio Rad, Hercules, CA, USA), and the optical density (OD) value of each sample was measured at 450 nm. CurveExpert1.4 software was used to draw a standard

Table II. The general information of patients (mean ± SD).

Groups	Sex (M/F)	Age (year)	BMI (kg/m^2)	Tumor site		
				Anterior communicating artery aneurysm (case)	Posterior communicating artery aneurysm (case)	Middle cerebral artery aneurysm (case)
Intervention	(23/18)	65.2±19.7	24.8±1.5	28	5	8
Operation	(21/17)	64.6±21.9	24.6±1.6	25	6	7
P-value	0.396	0.528	0.652	0.089	0.352	0.287
t value	0.613	0.762	0.876	0.896	0.962	0.931

M, male; F, female; BMI, body mass index.

curve. The concentration of the sample in each well was calculated, and then ROS, IL-6, TNF-α and IL-10 concentration of the original sample was measured in pg/ml as a unit.

qPCR detection of gene expression levels. The serum stored at -80˚C was thawed. TRIzol (Millipore) (1 ml) was added per 100 mg and allowed to stand for 5 min in an ice bath to completely lyse the cells. After adding 200 µl of chloroform, the mixture was mixed completely, then allowed to stand for 5 min in an ice bath and centrifuged at 3,000 x g at 4˚C for 10 min. The supernatant was discarded, and 1 ml of freshly prepared 75% ethanol was added. After centrifugation, 60 µl of DEPC water was added to dissolve the precipitate. The RNA was obtained, and the OD value of the corresponding RNA and $A_{260/280}$ were measured to verify the concentration and purity of the RNA.

According to the reverse transcription kit (Millipore), 500 ng of RNA template was added, and 20 µl of the reaction system was used to obtain the cDNA by reverse transcription. Reverse transcription conditions were: 37˚C for 15 min, and 85˚C for 5 sec. The reaction system was amplified by qPCR using β-actin as internal reference. The reaction conditions were as follows: 95˚C for 5 min, 95˚C for 30 sec, 63˚C for 50 sec, 72˚C for 60 sec, 30 cycles, and 72˚C for 5 min. The primers were synthesized by Inventec Bio-Technology Co., Ltd., Taipei City, Taiwan. The sequences are shown in Table I. The relative expression levels of the target genes were calculated by $2^{-\Delta\Delta Cq}$ (9), and expressed as MMP-2/actin, MMP-9/actin and caspase3/actin.

Western blot detection of protein expression. After the serum of each group was obtained, the RIPA lysate (Biotime Biotechnology Co., Ltd., Shanghai, China) was added in the proportion of 0.5 ml:1 ml and then homogenized with an ultrasonic homogenizer (containing 1% phosphatase inhibitor and 1% protease inhibitor). After incubating for 5 min in an ice bath and centrifuging at 10,800 x g for 15 min at 4˚C, the supernatant was transferred to a new EP tube, and protein quantification was carried out by using a BCA Protein assay kit (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Protein samples were placed in the same concentration of the loading system for boiling denaturation 15 min, configured 12% SDS polyacrylamide gel and 5% concentrated gel for electrophoresis. The wet transfer method was performed to transfer the protein to a PVDF membrane, and closed for 1 h. The purpose band was cut and incubated with the corresponding primary rabbit anti-human polyclonal antibodies: MMP-2 (dilution, 1:1,000; cat. no. ab37150; Abcam, Cambridge, UK), MMP-9 (dilution, 1:1,000; cat. no. ab73734; Abcam), caspase3 (dilution, 1:1,000; cat. no. ab13847; Abcam), p-AMPK (dilution, 1:1,000; cat. no. ab3760; Abcam), Bcl-2 (dilution, 1:1,000; cat. no. ab196495; Abcam), Bax (dilution, 1:1,000; cat. no. ab53154; Abcam) and β-actin (dilution, 1:1,000; cat. no. ab8227; Abcam) overnight. The membrane was washed three times with TBST, and incubated with goat anti-rabbit horseradish peroxides enzyme conjugate secondary polyclonal antibody (dilution, 1:5,000; cat. no. ab6721; Nanjing Jiancheng Biotechnology Institute, Nanjing, China) at room temperature for 1 h, then washed three times with TBST, 5 min each time, adding appropriate ECL light Liquid (liquid A and liquid B were mixed 1:1) in the dark environment, then developed and fixed, and then the gray value analysis was performed by ImageJ software after scanning the band to calculate the relative expression level of the protein.

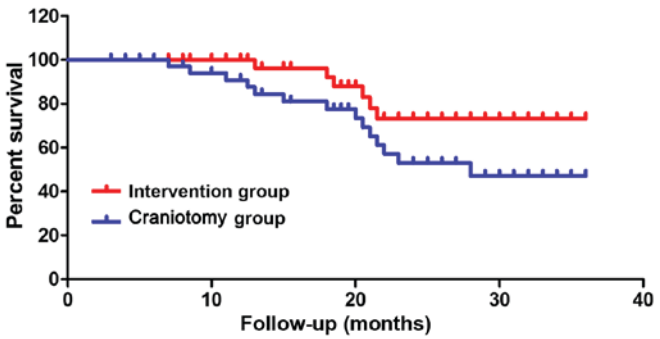

Figure 1. Survival curves by Kaplan-Meier analysis. Three-year survival rate in the intervention group was higher than that in the craniotomy group.

Statistical analysis. Data of this study are presented as mean ± standard deviation. Data were analyzed by SPSS 19.0 software (IBM Corp., Armonk, NY, USA). The t-test was used to compare the data between two groups. Chi-square test was used to compare the enumeration data. Analysis of variance was used for multiple comparisons and Dunnett's test was the post hoc test. $P<0.05$ was considered to indicate a statistically significant difference.

Table III. Adverse reaction rate.

Groups	Cerebral hemorrhage (case)	Aneurysm rupture (case)	Thrombosis (case)	Infection (case)	Other (case)	Total
Intervention	1	1	1	2	2	7
Operation	1	2	4	8	5	20
P-value	>0.05	>0.05	<0.05	<0.01	<0.05	<0.01
t value	0.613	0.762	0.436	0.486	0.563	0.396

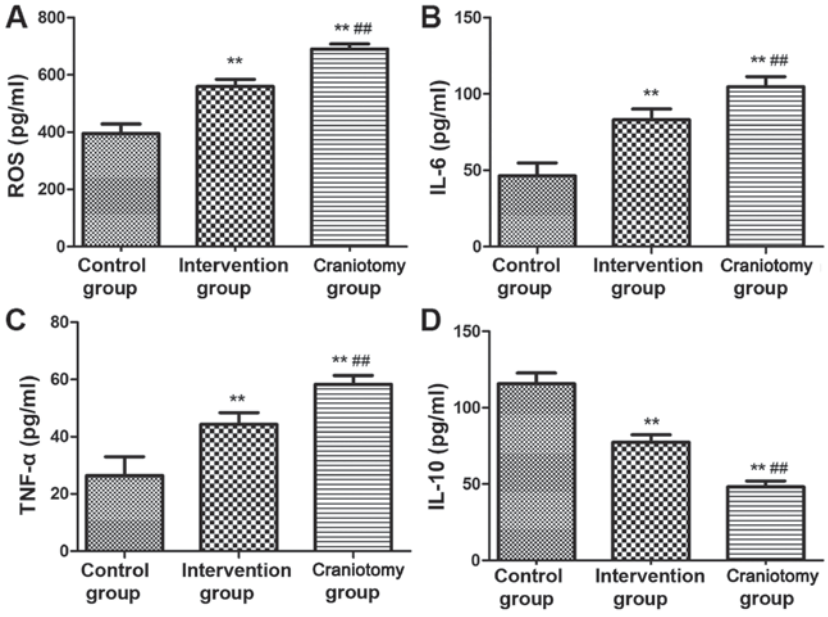

Figure 2. Levels of ROS and inflammatory cytokines. (A) The level of ROS in serum; (B) the concentration of IL-6 in serum; (C) the level of TNF-α in serum, and (D) the level of IL-10 in serum. Serum levels of ROS, IL-6 and TNF-α in intervention group and craniotomy group were significantly higher than those in control group, and IL-10 content was significantly lower than that in control group, **P<0.01. Serum ROS, IL-6 and TNF-α in intervention group were significantly lower than those in the craniotomy group, and the content of IL-10 was significantly higher than that in the craniotomy group, $^{\#\#}$P<0.01.

Results

General information. A total of 78 patients with intracranial aneurysm were selected, and the patients treated with vascular interventional embolization were selected as the intervention group, a total of 41 cases. The patients treated by craniotomy were selected as the craniotomy group, a total of 38 cases. The patients were examined within 24 h of admission. In addition, sex, age, body mass index (BMI) and tumor site were recorded. The general information of both groups of patients are shown in Table II, and the differences in sex, age, BMI and tumor site of each group of patients had no statistical significance (P>0.05).

Survival and adverse reaction rate. The survival rate of both groups of patients and the incidence of adverse reactions were recorded within 3 years after operation. The results are shown in Fig. 1 and Table III, and the 3-year survival rate of the intervention group was higher than that of the craniotomy group. Adverse reactions occurred in 7 patients in the intervention group, and there were 20 cases of adverse reactions in the craniotomy group, suggesting the incidence of adverse reactions in the intervention group was significantly lower than that in the craniotomy group (P<0.01).

ROS and inflammatory cytokine content. The serum levels of ROS and inflammatory cytokines in each group were detected by ELISA kit after surgery. The results are shown in Fig. 2: the contents of ROS, IL-6 and TNF-α in the serum of the intervention and craniotomy groups were significantly higher than that of the control group, and the level of IL-10 was significantly lower than that of the control group (P<0.01). The serum levels of ROS, IL-6 and TNF-α in the intervention group were significantly lower than those in the craniotomy group, and the content of IL-10 in the serum was significantly higher than that in the craniotomy group (P<0.01).

mRNA expression levels. The qPCR was used to detect the mRNA expression of related genes in the serum of each group. The results are shown in Fig. 3. The relative expression levels of MMP-2, MMP-9 and caspase3 mRNA in the serum of intervention and craniotomy groups were significantly higher than those of the control group (P<0.01). The relative

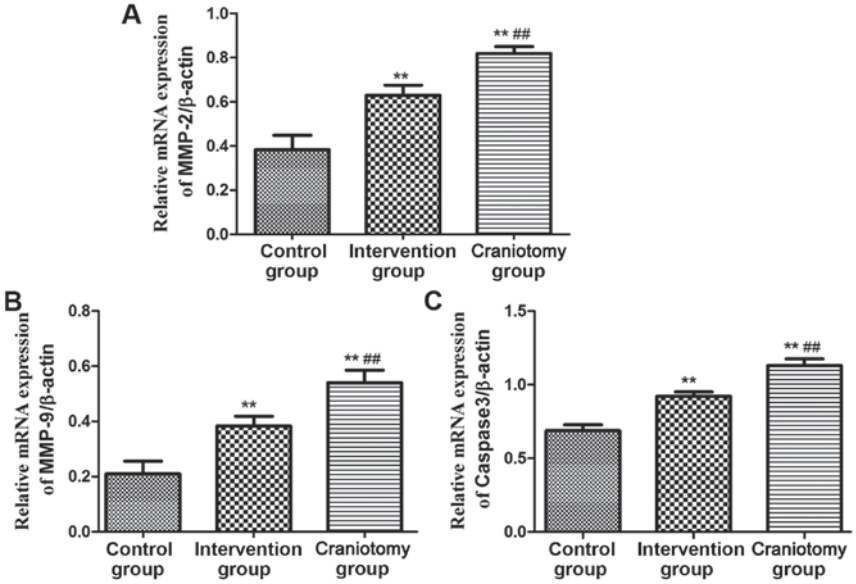

Figure 3. mRNA expression level. (A) The relative expression level of MMP-2 mRNA; (B) the relative expression of MMP-9 mRNA; (C) the relative expression of caspase3 mRNA, and in the intervention group and craniotomy group, the level of MMP-2, MMP-9 and caspase3 mRNA were significantly higher than those in the control group, $^{**}P<0.01$. The relative expression levels of MMP-2, MMP-9 and caspase3 mRNA in the intervention group were significantly lower than those in the craniotomy group, $^{\#\#}P<0.01$.

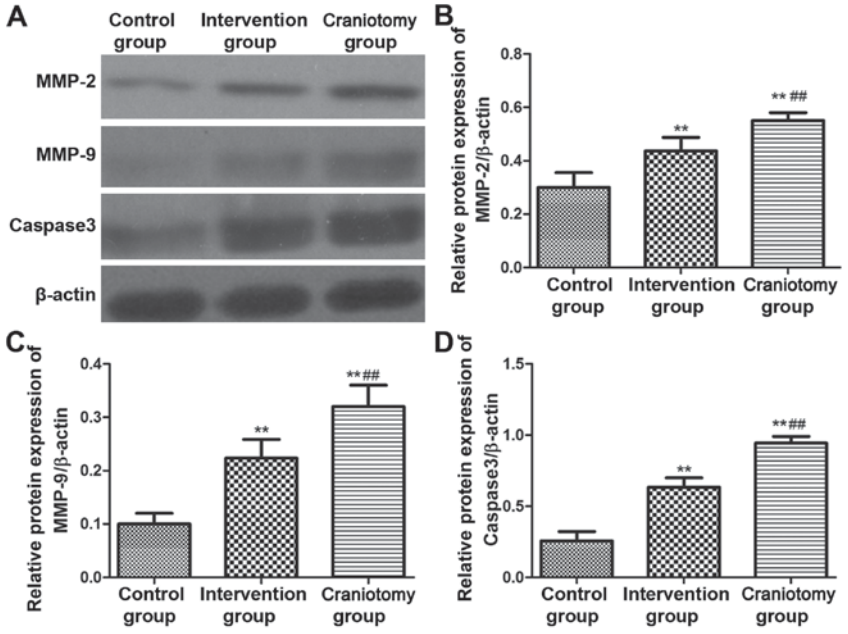

Figure 4. Protein expression level. (A) Protein band; (B) the expression level of MMP-2; (C) the expression level of MMP-9, and (D) the expression level of caspase3; serum MMP-2, MMP-9 and caspase3 protein expression levels in intervention group and craniotomy group were significantly higher than those in control group, $^{**}P<0.01$; serum levels of MMP-2, MMP-9 and caspase3 in the intervention group was significantly lower than those in craniotomy group, $^{\#\#}P<0.01$.

expression levels of MP-2, MMP-9 and caspase3 mRNA in the intervention group were significantly lower than those in the craniotomy group (P<0.01).

Protein expression level. Western blotting was used to detect the expression levels of corresponding protein, and the results are shown in Fig. 4. The results of protein expression were consistent with the mRNA expression results: the levels of the serum MMP-2, MMP-9 and caspase3 protein expression in the intervention and craniotomy groups were significantly increased (P<0.01). The serum levels of MMP-2, MMP-9 and caspase3 in the intervention group were significantly lower than those in the craniotomy group (P<0.01). Further study was made on the expression level of its upstream protein, and the results (Fig. 5) showed that the expression levels of p-AMPK and Bcl-2/Bax in the intervention group and surgery groups were significantly higher than those in the control group (P<0.01). The serum p-AMPK, Bcl-2/Bax in the intervention group was significantly lower than those in the craniotomy group (P<0.01).

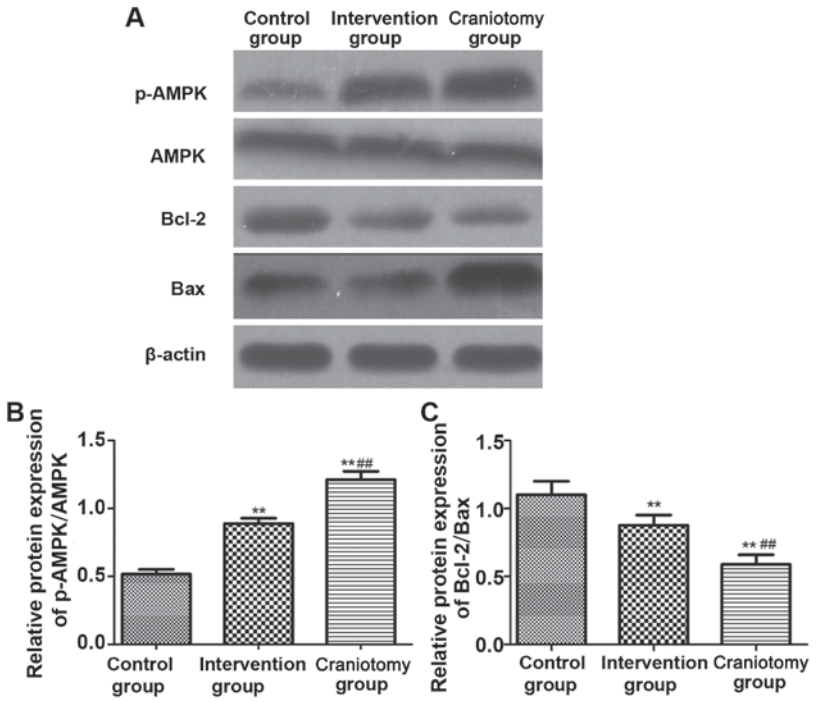

Figure 5. The expression of p-AMPK, Bcl-2 and Bax protein. (A) The protein band; (B) the expression level of p-AMPK; (C) the expression level of Bcl-2/Bax. The serum levels of p-AMPK and Bcl-2/Bax in the intervention group and the craniotomy group were significantly higher than those in control group, **P<0.01. The serum levels of p-AMPK and Bcl-2/Bax in intervention group were significantly lower than those in craniotomy group, $^{\#\#}$P<0.01.

Discussion

Intracranial tumor is caused by many factors, and intracranial atherosclerosis-induced degradation of arterial wall and the occurrence of inflammatory reaction is the main reason. The treatment of intracranial hemangiomas by intracranial vascular embolization increased in the 1970s, and intracranial vascular embolization and craniotomy are the most common means of treatment of intracranial aneurysms (10,11). In this study, by comparing the impact of vascular interventional embolization and craniotomy on patients with intracranial aneurysms, the results showed that i) vascular interventional embolization can effectively increase the 3-year survival rate of patients with intracranial tumors, and significantly reduce postoperative adverse reactions; ii) vascular embolization can effectively reduce the levels of intravascular ROS and proinflammatory cytokines, and increase the level of inhibitors, which can effectively control the intracranial inflammatory response; iii) vascular embolization can effectively reduce the serum levels of MMP-2, MMP-9 and caspase3 mRNA and protein expression, and effectively inhibit the expression of induced genes and proteins in intracranial tumors; and iv) the inhibitory effect of vascular interventional embolization on the expression of MMP-2, MMP-9 and caspase3 may be associated with inhibition in patients with the AMPK expression, and reduce the expression of apoptotic protein Bcl-2/Bax.

The most common adverse reactions after intracranial tumor treatment are aneurysm intraoperative and postoperative rupture and thrombosis, with a high mortality rate if the rescue is not timely (12). Kanematsu *et al* (13) found that among 152 cases of aneurysms with rupture after the treatment, mortality rate was 8%, and disability rate was 12%, and incidence of serious central nervous system injury was 21%. Improper operation during the blocking of intracranial surgery for intracranial tumors is very easy to cause rupture of aneurysm, and blocked intracranial aneurysm artery recanalization will occur after craniotomy (14). Wada *et al* (15) analyzed 398 patients who underwent intracranial aneurysm artery surgery with Mate analysis, and the postoperative recanalization rate was 28.9%. Incidence of trauma caused by intracranial surgery in patients was significantly higher than that caused by endovascular embolization, leading to significantly increased incidence of adverse reactions, seriously affecting the patient's postoperative survival rate and quality of life.

Atherosclerosis and other cardiomyopathy cause a large number of apoptosis, and apoptosis of cells leads to the reduction of vascular endothelial cells, causing the remodeling of the vessel wall, leading to the formation of aneurysm (16). It has been found that the signal transduction pathway can activate caspase family members, and the expression level of caspase3, which is apoptosis effector, and is significantly increased, leading to a significant increase in the expression of TNF-α (16). This study found that the formation of aneurysms leads to elevated p-AMPk in patients. Increased p-AMPk level affects the release of mitochondria and regulates cytochrome *c* by decreasing the expression level of Bcl-2, thereby regulating cell apoptosis, promoting the expression of MMP-2, MMP-9, and caspase3 and resulting in blood vessel wall reconstruction. Compared with craniotomy, interventional embolization can effectively reduce the expression level of p-AMPk, promote the expression of Bcl-2/Bax, and reduce the release of pro-inflammatory and apoptotic proteins, thus improving aneurysm. Oh *et al* (17) found a significant increase in the level of apoptosis in tumor tissue of patients with aortic aneurysm, which in turn led to increased release of inflammatory cytokines. The formation of aneurysms activates the Bcl-2 protein,

which regulates apoptosis through mitochondrial regulation of cytochrome *c* release, leading to remodeling of the vessel wall.

Interleukin-10 (IL-10) is an important inhibitory cytokine with immune-regulatory function and various biological activity. It could be produced by many kinds of cells, including $CD4^+$ Th cells, $CD8^+$ Th cells, dendritic cells, macrophages and regulatory T cells. IL-10 is an effective anti-inflammatory substance, which can inhibit the production of interleukin-2, leukotriene and other inflammatory factors. Therefore, IL-10 has a strong anti-inflammatory effect. The data showed that the contents of ROS, IL-6 and TNF-α in the intervention and craniotomy groups were significantly higher than that in the control group, and the level of IL-10 was significantly lower than that in the control group ($P<0.01$). The serum levels of ROS, IL-6 and TNF-α in the intervention group were significantly lower than those in the craniotomy group, and the content of IL-10 in serum was significantly higher than that in the craniotomy group ($P<0.01$). The results also demonstrated that IL-10 is an important inhibitory cytokine, with strong anti-inflammatory effect. Ma *et al* (18) found that ADR-treated rat cardiomyocytes significantly increased apoptotic cells, and the pro-apoptotic protein caspase3 activity and expression were significantly increased, and the anti-apoptotic protein Bcl-2 expression was significantly reduced.

AMPK can mediate the energy metabolism of cells, and activate the corresponding ATPase production, thereby increasing the level of ATP, maintaining energy supply (19). The phosphorylation level is increased when AMPK is activated, and then involved in the regulation of a variety of physiological signals. Jiang *et al* (20) found that pravastatin can increase AMPK phosphorylation in patients with abdominal aortic aneurysm and increase *in vivo* MMP-2 expression. ROS regulates the balance of oxidation and antioxidation *in vivo*. Liaw *et al* (21) found that the level of ROS in cardiomyocytes of myocardial infarction rats was significantly increased and the cells were in an oxidative stress state, leading to cell injury.

In summary, compared with craniotomy, endovascular embolization can effectively increase the patient's survival rate, reduce adverse reactions, reduce the level of intravascular MMP-2, MMP-9 and caspase3, and the mechanism may be associated with the impact on ROS levels, which in turn affect the expression of AMPK and Bcl2/Bax.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

LZ, YH and YD conceived and designed the study. LZ, YH and BY were responsible for the collection and analysis of the patient data. YH and SL interpreted the data and drafted the manuscript. LZ and YH revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of The Second Affiliated Hospital of Soochow University (Suzhou, China) and The Second Clinical Medical School of Inner Mongolia University for Nationalities (Hulunbuir, China). Signed informed consents were obtained from the patients or the guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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