Correlation and Mechanism Study of Multivoxel ^1H -MRS Parameters, Cho / Cr and CHKA

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Abstract

Background Glioma is a common malignant brain tumor with strong invasiveness and poor prognosis. In order to improve the survival rate of patients, there is an urgent need to find the targeted molecular mechanism affecting its invasion and development.

Methods In this study, the differential regions of Cho/Cr metabolism of tumor cells were identified by multi-voxel ^1H -MRS and the tumor tissues of Cho/Cr high metabolism group and Cho/Cr low metabolism group were accurately obtained by combining neuronavigation system. The expression of CHKA in different Cho/Cr metabolic groups was verified by Real-timePCR and Western-blot. Then we used CHKA inhibitor MN58b and short hairpin RNA to study the role of CHKA in the proliferation and invasion of U251 glioma cells, and the relationship between CHKA and EMT in nude mice.

Results We found that the expression of CHKA in Cho/Cr high metabolism group was significantly higher than that in Cho/Cr low metabolism group. Decreased CHKA activity can inhibit the proliferation and invasion of glioma cells and slow down the process of EMT.

Conclusion CHKA may be an oncogenic gene, which affects the invasion and development of glioma cells by regulating the process of tumor EMT, and is expected to become a molecular target for glioma therapy, and multi-voxel ^1H -MRS parameter Cho/Cr may become an important clinical index for detecting CHKA expression.

Introduction

Glioma is the most common primary central nervous system tumor, accounting for 40% - 50% of intracranial tumors. The average survival time after diagnosis is less than 15 months[1, 2]. Because of the strong invasive nature of glioma[3] and extensive infiltration during the growth process it cannot be completely removed by surgery, and the recurrence rate is high[4]. Therefore, on the premise of protecting normal brain tissue, striving to remove tumor tissue to the greatest extent is the fundamental purpose of glioma treatment. Although MRI is a common method for the diagnosis of brain tumors in the central nervous system, it cannot evaluate the changes of metabolites around the whole lesion tissue, and it also has some limitations in judging the pathological grade and infiltration range of glioma. However, ^1H -MRS can be used to monitor the changes of metabolites around tumor tissue in vivo[5]. Quantitative and qualitative analysis of some specific areas is carried out by using magnetic resonance imaging and chemical shift, and the disease is evaluated and diagnosed according to the quantitative changes, which improves MRI at the molecular level. The diagnostic value of MRI for brain tumors can be used as a powerful supplement to conventional MRI detection methods[6]. NAA, CR, Cho, LAC, and LIP were detected by multivoxel ^1H -MRS[7, 8], Among them, Cr peak is located at 3.02ppm, which is the second highest peak of normal brain wave spectrum. It is composed of γ - aminobutyric acid, creatine, phosphocreatine, lysine and glutathione. Its total amount is relatively stable among different tissues of the same individual, so it is often used as a reference value to measure the content of other metabolites.

Cho peak is located at 3.2ppm, which is one of the main components of phospholipid metabolism in brain[9]. Its main components include PCho, PC and GPC. The increase of Cho peak can infer that the phospholipid metabolism of cell membrane is accelerated and the cell division and proliferation are vigorous.

Choline kinase is a key enzyme in Kennedy's Choline metabolic pathway[10]. It is encoded by two genes, CHKA and CHKB, through homodimer or heterodimer. In vivo[11] CHKB can only play the role of ethanolamine kinase, while CHKA has the dual activity of Choline kinase and ethanolamine kinase. CHKA with Choline kinase activity can phosphorylate free Choline to Choline phosphate. PhosphatidylCholine is an important second messenger molecule, which plays a key role in DNA synthesis and promotes cell proliferation. Thus, CHKA can indirectly participate in cell mitosis through Choline phosphate and affect the physiological activities of cells. In recent years, some studies have also confirmed that[12-14] overexpression of CHKA can induce malignant transformation of some normal cells, and silencing or using inhibitors can inhibit these malignant biological behaviors.

EMT refers to the biological process in which epithelial cells lose their epithelial phenotypic characteristics and transform into fibroblast-like stromal phenotypes[15]. EMT plays an important role in the invasion and migration of malignant tumor cells. The expression of epithelial cell adhesion protein E-cadherin is decreased, while the expression of mesenchymal cell characteristic proteins Vimentin and N-cadherin is up-regulated, which leads to the weakening of intercellular polarity and adhesion ability and the enhancement of tumor cell invasion and migration ability. Although in recent years, more and more studies have found that EMT is closely related to the occurrence and development of tumors of various epithelial sources, such as lung cancer, breast cancer, liver cancer and so on, the role and specific molecular mechanism of EMT in brain gliomas are still unclear.

In multivoxel ^1H -MRS studies, Cho / Cr is commonly used to reflect the level of Cho metabolism. The increase of Cho / Cr is correlated with the grade of glioma.[16, 17] Cho / Cr guiding the resection range of glioma can definitely improve the therapeutic effect and prognosis[18]. Whether CHKA, as a key enzyme in Choline metabolism is involved in the occurrence and development of glioma, and whether it is related to multivoxel ^1H -MRS parameters, to verify these conjectures, this study first verified the correlation between the multivoxel ^1H -MRS parameter Cho / Cr and the expression of CHKA from clinical samples with different Cho / Cr metabolism. Then, we used lentiviral transfection to down regulate the expression of CHKA in U251 glioma cells, and used MN58b, a CHKA inhibitor, to inhibit the biological activity.

Materials And Methods

2.1 Clinical samples

Nineteen patients with high-grade gliomas were selected from the Department of Neurosurgery of Ningxia Medical University General Hospital (March 2015 to November 2018, all confirmed by postoperative pathology). The patients included, 9 males and 10 females, aged 7-68 years, with an average age of 45.6

years. Before multivoxel ^1H -MRS examination, all patients did not receive radiotherapy and chemotherapy, and had no history of brain injury and/or brain surgery. This study has been reviewed by the research ethics committee of Ningxia Medical University, and informed consent forms were signed with patients or their families before operation.

2.2 Image processing

Using signa HDX 3.0T superconducting MRI system made by GE company in the United States, multi voxel ^1H -MRS was performed after conventional MRI plain scans and enhanced scans. MRI was used to determine the location of the lesion. The multi voxel ^1H -MRS image and structure image data were transmitted to the post-processing workstation through LAN. The multi voxel analysis is carried out for each layer by using the self-contained software, and a 3D chemical displacement imaging is generated and converted into gray value image. The image contains standardized Cho / Cr information, and the Cho / Cr level of each voxel is represented by pseudo color image. Select the highest and lowest points of Cho / Cr as the region of interest and save them in DICOM format. The saved MRS images were imported into 3D interface, reconstructed and saved in axial direction. After being imported into navigation workstation, MRS sequences and structural images were fused and reconstructed, and the reconstructed sequences were exported to cranial software. In the fusion interface, the conventional anatomical sequences and MRS sequences were combined and multivoxel ^1H -MRS was added. The images were accurately fused into the intraoperative neuro-navigation system. With the aid of neuro-navigation, the highest and lowest Cho / Cr levels were accurately selected, and the tumor tissues in these two regions were taken as tissue samples, which were Cho / Cr high metabolism group and Cho / Cr low metabolism group, respectively.

2.3 Quantitative real time PCR

Total RNA was extracted from tumor tissue samples of Cho / Cr high metabolism group and Cho / Cr low metabolism group by Omega Kit (Omega , USA), and SYBR PremixExTaqTM. Gamma kit (Takara , Japan) was used to reverse the total RNA into cDNA, and then real-time PCR was performed. The target gene CHKA primer sequence: upstream 5'-CGGAAAAGTGCTCCTGCGGCT-3', downstream 5'-AACCAAAGCTGTGCAGCCAA-3'. β -actin was selected as the internal reference gene. Samples were added according to the 20 μl PCR system in the instruction manual and tested on the computer.

2.4 Western-blot Testing

The protein from Cho/Cr high metabolic group and Cho/Cr low metabolic group was extracted by full protein extraction kit and quantified by BCA. SDS-PAGE gel electrophoresis was carried out on the 40 micron g/ pore size. After electrophoresis, the protein on the gel was transferred to PVDF membrane at 300mA and 45 minutes. PVDF membrane was placed in 5% skimmed milk powder and sealed at room temperature for 2 hours then, it was added into CHKA primary antibody (Abcam , USA) for overnight on a 4 °C shaking table. The next day, the PVDF membrane was washed 5 times with TBST for 5 minutes

each time. The second antibody of the corresponding species was incubated for 2 hours. After cleaning 5 times with TBST, the ECL emitted light.

2.5 Cell culture and lentiviral transfection

U251 cells (Shanghai cell bank of Chinese Academy of Sciences) were taken out from liquid nitrogen and placed in a 37°C constant temperature water bath for 3-5min for resuscitation. The cell suspension in the cryopreserved tube was inhaled into a centrifuge tube containing 3ml culture medium (GIBCO ,USA) in an ultra-clean bench. Centrifugation was performed at 1000 RMP for 5 min. the supernatant was discarded. The cells at the bottom of the tube were used with 10% fetal bovine serum (GIBCO, USA) The DMEM with 1% double antibody was resuspended and seeded into a culture bottle, and then cultured in a cell incubator containing 5% CO₂ at 37°C. U251 cells in logarithmic growth phase were seeded in a 6-well plate at the density of 1×10^5 . After 24 hours, CHKA silencing lentivirus diluted with DMEM and its control lentivirus were added. After 12 hours of incubation, the DMEM medium containing 10% fetal bovine serum was replaced. After 48 hours of culture, the fluorescence of transfected cells was observed by fluorescence microscope. The cells were screened with puromycin (2 μ g / ml), and then identified by real-time PCR and Western blot.

2.6 Clonal activity was detected by clonal assay.

The cells in logarithmic growth phase of CHKA group and vector group were digested and inoculated into a 6-well plate containing 2 ml medium at a density of 200 cells / well. The cells were evenly inoculated by shaking gently and then cultured in a cell incubator with 5% CO₂ at 37°C for 2-3 weeks. The cells were observed every 3 days. When cell clones were visible in the 6-well plate, the culture was terminated and the supernatant was discarded. The bacteria were washed twice with PBS, fixed for 15 min with 4% paraformaldehyde, stained with 0.1% crystal violet solution for 30 min, and stained with 0.2% crystal violet solution for 30 min. finally, the cell clone formation rate was calculated.

2.7 CCK8 was used to detect cell proliferation

The cells were digested into single cells by trypsin and diluted to cell suspension with density of 3×10^4 cells / ml in culture medium. 100 μ L cell suspension was inoculated into 96 well plate and placed in 37°C constant temperature cell incubator containing 5% CO₂ for 4H to make the cells adhere to the wall. DMSO were added to each treatment hole of 96 hole plate and MN58b with different dosages of DMSO at 10 μ m, 20 μ m and 40 μ m, were cultured in the cell incubator for 24 h, 48 h, 72 h and 96 h. Then 10 μ CCK8 solution was added into each treatment hole of 96 well plate, and then cultured in the incubator for 2 h. The absorbance values of each well at 450 nm at different time points were measured by enzyme labeled instrument.

2.8 Transwell invasion assay was used to detect the invasion ability of the cells

The diluted Matrigel matrix glue was spread on the upper chamber of the small chamber, and the treated cells were diluted to 2×10^5 cells / ml with serum-free DMEM medium. 200 μ L cell suspension was added into the upper chamber and the lower chamber was added with DMEM medium containing 20% fetal bovine serum. The cells in the upper chamber were wiped off with cotton swab and fixed with 4% polymethanol for 30 min, and stained with 0.1% crystal violet solution for 30 min. The cells were then observed under a microscope and photographed.

2.9 Animal model and tumor cell implantation

Six-week-old female nude mice (Beijing Weitong Lihua Experimental Animal Technology Co., Ltd., 18-22g) were raised in a SPF environment for one week. U251 cells suspended in serum-free medium (1×10^7) were injected into the axilla of the right forelimb of each mouse. When the volume of the tumor on the right side of the mouse reached about 100 mm^3 , the mice were divided into two groups, 6 in each group. The experimental group was intraperitoneally injected with MN58b (2mg/kg/ days), and the control group was injected with normal saline. After 14 days of continuous treatment, all nude mice were killed and samples were taken as required.

2.10 Immunohistochemistry

The paraffin-embedded tumor specimens were cut into $4 \mu\text{m}$ thick slices, which were routinely dewaxed and placed in sodium citrate buffer for antigen repair, cooled naturally for 2 hours, incubated with 3% hydrogen peroxide solution at room temperature for 10 minutes, and sealed with goat serum. Removing the sealing solution, the primary antibody E-cadherin (Servicebio, 1:300), N-cadherin (Servicebio, 1:300) and Vimentin (Servicebio, 1:200) were incubated overnight at 4°C , and the second antibody (Servicebio, 1:500) was added the next day. After DAB staining, the expression of E-cadherin, N-cadherin and Vimentin in glioma was observed under microscope.

2.11 Statistical analysis

SPSS 24.0 software was used for statistical analysis. The data results were expressed as mean $\pm$ standard deviation ($\bar{x} \pm s$). t-Test was used for comparison of two samples, one-way ANOVA was used for diversity comparison, and SNK-q test was used for comparison between two samples. The difference was statistically significant ($P < 0.05$).

Results

3.1 Image findings

Conventional MRI results showed that the left frontal lobe of glioma patients showed low signal on T1, high signal on T2, and high signal on T2 Flair. No obvious enhancement was found after conducting an enhancement. There were different degrees of peritumoral edema (Fig. 1A).

The results of multivoxel ^1H -MRS showed that compared with normal brain tissue, the peak of NAA was significantly decreased, the peak of Cho was significantly increased, and the peak of Cr was slightly decreased. The ratio of Cho / Cr in different regions of glioma was significantly different (Fig. 1B). The processed spectral images were accurately fused into the neuro-navigation workstation, and the tumor tissues at the highest and lowest points of Cho / Cr were accurately obtained intraoperatively with combined neuro-navigation (Fig. 1C).

3.2 The expression of Cho / Cr in multivoxel ^1H -MRS was correlated with the expression of CHKA

Through real-time PCR and Western blot, we found that the expression of CHKA in the tumor tissues of Cho / Cr high metabolism group was significantly higher than that of Cho / Cr low metabolism group. PCR results showed that there was significant difference between Cho / Cr high metabolism group (40.77 ± 24.18) and Cho / Cr low metabolism group (0.58 ± 0.52) ($P < 0.001$, $t = 6.44$, Fig.2A). Western blot results showed that Cho / Cr high metabolism group (1.26 ± 0.14) and Cho / Cr low metabolism group (0.49 ± 0.13) had significant difference ($P < 0.001$, $t = 8.92$, Fig. 2B, C). It can be seen that the expression of Cho / Cr in multivoxel ^1H -MRS is correlated with the expression of CHKA. The higher the Cho / Cr metabolic value, the higher the expression of CHKA.

3.3 Down regulation of CHKA expression can inhibit the clonal ability of glioma cells

In order to verify the relationship between the expression of CHKA and the clonal activity of glioma cells, we performed the cloning experiment of U251 glioma cell line transfected with CHKA silencing lentivirus and its control cell lines (shCHKA group and vector group). The experimental results showed that there was significant difference between shCHKA group (25 ± 5.48) and vector group (134.40 ± 14.79) ($t = 15.51$, $P < 0.001$, Fig. 3). The results showed that down regulating the expression of CHKA could significantly inhibit the clonal ability of glioma cells.

3.4 Down regulation of CHKA expression can significantly inhibit the invasion of glioma cells

In order to verify the relationship between the expression of CHKA and the invasive activity of glioma cells, Transwell assay was performed on U251 glioma cell transfected with CHKA silencing lentivirus and its control cell (shCHKA group and vector group). The number of invasive cells in CHKA group was (36.20 ± 11.82) and that of vector group was (104.40 ± 19.92), the difference was statistically significant ($t = 6.58$, $P < 0.001$, Fig. 4). The results showed that down regulating the expression of CHKA could significantly inhibit the invasion of glioma cells.

3.5 MN58b, an inhibitor of CHKA, can inhibit the proliferation of glioma cells

We treated U251 glioma cells with different concentrations of MN58b ($10 \mu\text{m}$, $20 \mu\text{m}$, $40 \mu\text{m}$) dissolved in DMSO solution, and then treated with CCK8 at different time points (24h, 48h, 72h, 96h). The absorbance of each group at 450 nm was detected (Fig. 5). The results showed that the proliferation ability of glioma cells was significantly inhibited with the increase of drug concentration and the

prolongation of action time, which indicated that the proliferation of glioma cells could be inhibited by using MN58b, which is a CHKA inhibitor.

3.6 MN58b, an inhibitor of CHKA can effectively inhibit the invasion of glioma cells

In order to verify the effect of MN58b, an inhibitor of CHKA, on the invasive activity of glioma cells, U251 cells were treated with 10 μ m MN58b and DMSO for 48 hours, then Transwell test was performed. Transwell invasion assay showed that the number of invasive cells in shCHKA group was (56.20 $\pm$ 9.42) and that of vector group was (188.60 $\pm$ 10.24), the difference was statistically significant (t = 21.28, P < 0.001, Fig. 6). The results showed that MN58b, an inhibitor of CHKA, could effectively inhibit the invasion of glioma cells.

3.7 MN58b, an inhibitor of CHKA can inhibit the EMT progression of glioma cells.

Through the tumor formation experiment in nude mice, we found that there were significant differences in the immunohistochemical results of EMT specific molecules E-cadherin, N-cadherin and Vimentin between the experimental group treated with MN58b and the control group injected with normal saline. The results showed that the positive area of epithelial cell adhesion protein E-cadherin in the experimental group was 29.74 ± 1.42 and that in the control group was 22.30 ± 1.49 , p < 0.05. The difference was statistically significant (Fig. 7). The positive area of mesenchymal cell characteristic protein N-cadherin and Vimentin in the experimental group was 24.39 ± 1.23 and 37.28 ± 0.51 and that in the control group was 33.79 ± 0.89 , 47.41 ± 0.39 p < 0.01. There was significant difference between the two groups (Fig. 7). The results showed that the expression of epithelial marker E-cadherin increased, the expression of mesenchymal markers N-cadherin and Vimentin decreased, the cell adhesion was strong, the invasive ability was weakened, and the process of EMT was inhibited in glioma cells.

Discussion

Multivoxel ^1H -MRS can detect the chemical components of brain cell metabolism noninvasively, and can evaluate and diagnose diseases according to their quantitative changes. It has become an important technical means for glioma research, clinical diagnosis and treatment[19, 20]. In this study, we first used multivoxel ^1H -MRS to identify the regions of Cho / Cr metabolism differences in the tumor cells and fuse the collected data with the neuro-navigation system. Neurosurgeons with many years of clinical experience accurately selected the tumor tissues with the highest and lowest Cho / Cr levels as clinical samples for real-time PCR and Western blot studies. The results showed that CHKA was found in Cho / Cr in glioma tissues with high metabolism was significantly higher than that in glioma tissues with low Cho / Cr metabolism. In recent years, a number of studies have found that Cho / Cr is related to the invasion and proliferation of glioma cells, and the increase of Cho / Cr is more common in high-grade gliomas[21-23]. Therefore, we infer that CHKA may be related to the multivoxel ^1H -MRS parameter Cho / Cr, and affect the occurrence and development of glioma through the role of Cho metabolism. On the one hand, we used lentiviral transfection to down regulate the expression of CHKA in U251 glioma cells, and on the

other hand, we treated U251 glioma cells with MN58b, a specific inhibitor of CHKA, to reduce the biological activity of CHKA. Furthermore, cloning experiments, CCK8 and Transwell experiments proved that the ability of U251 cells to clone, proliferate and invade after the down-regulation of CHKA gene and the inhibition of biological activity of CHKA were confirmed to be inhibited.

As an important enzyme in Cho metabolism, CHKA has been widely concerned about its role in human tumorigenesis and development[24]. Recent studies by some scholars have shown that the expression level of CHKA in a variety of tumor tissues is significantly increased by [25, 26], and our experimental results are consistent with it. It has also been shown that CHKA interacts with a variety of tumor related signaling pathways, which can be activated by a variety of proto oncogenes. In breast cancer[27], CHKA promotes the proliferation of breast cancer cells through c-Src dependent protein interaction with EGFR. In cervical cancer [28], as a catalytic enzyme, indirectly affects Akt, MEK / ERK signaling pathways by regulating the production of phosphatidylcholine, Cytidine Diphosphate Choline in Choline metabolic pathway, and ultimately promotes the occurrence and development of cervical cancer. In T-cell lymphoma[29], CHKA is involved in the regulation of ras-akt / ERK signaling pathway, thus affecting the apoptosis and necrosis of T-lymphoma cells. In order to explore its role and mechanism in glioma, we searched the literature related to glioma and found that Katharina Koch et al reported in 2016 that CHKA is an important regulator of EMT in glioblastoma[30], but how it regulates and its specific mechanism has not been systematically studied.

In recent years, a large number of literature reported that there are a small number of stem cell characteristic cells in a variety of tumor cells[31, 32]. These cells have the potential of self-renewal and multi-directional differentiation, which are defined as cancer stem cells[33]. The origin of these tumor stem cell subpopulations is still unclear. It may originate from mutated normal stem cells, dedifferentiated somatic cells or cancer cells undergoing epithelial mesenchymal transition. Studies have shown that there are stem cell like tumor initiation cells in glioblastoma, called glioma stem cell[34]. Glioblastoma stem cells can self-renew and proliferate stably. They can form neurospheres in specific growth environment and have strong tumorigenicity[35]. Studies have found that compared with normal glioma cells, glioma stem cells are more resistant to radiotherapy and chemotherapy. Glioma stem cells may be the source of the occurrence and development of glioma, and are closely related to the poor prognosis of glioma[36]. Therefore, the research of glioma stem cells has become the current trend and focus of malignant glioma treatment.

In our previous studies, we found that the regions of Cho / Cr metabolism difference of multivoxel ^1H -MRS parameters were closely related to the enrichment of tumor stem cells in glioma[37]. The preliminary study confirmed that there were a small amount of glioma stem cells in the brain glioma tissue of Cho / Cr low metabolism area, while the content of glioma stem cells in Cho / Cr high metabolism area was significantly higher than that in Cho / Cr low metabolism area. The distribution of glioma stem cells was positively correlated with Cho content. This is consistent with the expression of CHKA in the region of Cho / Cr metabolism difference of multivoxel ^1H -MRS parameters. Therefore, we speculate that there may be some relationship between CHKA and glioma stem cells, Katharina Koch. Our results suggest that CHKA

is an important regulator of EMT in glioblastocytes, and EMT, as a key transformation pathway for malignant tumor cells to acquire invasiveness, may be an important reason for therapeutic resistance and tumor recurrence of glioma stem cells[38]. Our experimental results also show that CHKA is related to glioma stem cells and its inhibitor MN58b can slow down the progress of EMT, these are consistent with our conjecture.

Conclusion

In , as a tumor promoting gene, CHKA plays an important role in the proliferation, cloning, invasion and other processes of glioma. We speculate that CHKA may be a pleiotropic negative regulatory factor related to glioma growth, which can induce EMT of glioma cells, thus endowing the transformed glioma cells with stem cell like characteristics. Gliomas with similar characteristics can lead to the occurrence and progression of tumors. The expression of these glioma stem cells and CHKA in glioma tissues can be detected non-invasively by multivoxel ^1H -MRS parameter Cho / Cr, which introduces a new theory for the treatment, is expected to guide the surgical resection range, improve the therapeutic effect and thereby, help improve the overall prognosis of Gliomas.

Abbreviations

^1H -MRS Proton magnetic resonance spectroscopy

CHKA Choline kinase α

CHKB Choline kinase β

CR Creatine

Cho Choline

DMEM Dulbecco's Modified Eagle Medium

EMT Epithelial mesenchymal transformation

NAA N-acetylaspartic acid

LAC Lactic acid

LIP Lipid

PCho PhosphatidylCholine

PC PhosphatidylCholine

GPC Glycerophosphate

Declarations

Availability of data and materials

The author can provide the data and materials used in this experiment.

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Contributions

The conception and design of this study were mainly completed by *Hui Ma*, *Fang Qian Yue* and *You Rui Zou*. *Sheng Yu Sun*, *Zhe Wang* and *Xin Yi Gao* were mainly responsible for the collection of samples during the operation, and the cell and animal experiments were performed by *Fang Qian Yue* and *Ling Huang*. data analysis and article writing were completed by *Fang Qian Yue* and *You Rui Zou*. Everyone reviewed and approved the final manuscript.

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Ethics declarations

Ethics approval and consent to participate

This study has been approved by Ethics Committee of General Hospital of Ningxia Medical University(2017-010) and obtained the informed consent of all patients.

The Experimental Animal Welfare Ethics Committee of the Experimental Animal Center of Ningxia Medical University examined that the study was in line with animal protection, animal welfare and ethical principles.(IACUC-NYLAC-2020-052)

Consent for publication

Our study has obtained the informed consent of individual patients.

Competing interests

The authors report no conflicts of interest.

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Figures

Figure 1

A shows left frontal gliomas (low signal on T1, high signal on T2, high signal on t2flair, no obvious enhancement after enhancement.) B shows the location of left frontal lobe lesion by multi voxel MRS, which indicates that Cho / Cr ratio is significantly increased. C shows the accurate acquisition of tumor tissue in Cho / Cr metabolism difference area during spectroscopy combined with neuro-navigation

Figure 2

A. shows the expression of CHKA in different Cho / Cr metabolic tissues detected by real-time PCR ($P < 0.001$), 2B represents the gray value statistics of chka protein in different Cho / Cr metabolic tissues ($P < 0.001$), and 2C is the protein electrophoretic map of CHKA in different Cho / Cr metabolic tissues.

Figure 3

A shows the colony formation of U251 glioma cells in vector group, B represents the colony formation of U251 glioma cells in shCHKA group, and C represents the numerical statistical results of clone formation in vector group and shCHKA group.

Figure 4

shows the cell invasion map and cell invasion result statistics of vector group and shCHKA group.

Figure 5

shows the OD values of U251 cells treated with different concentrations of MN58b at different time points.

Figure 6

shows the invasion map of U251 cells treated with DMSO and U251 cells treated with MN58b.

Figure 7

A shows the immunohistochemical staining of EMT specific molecules E-cadherin, N-cadherin and Vimentin in the MN58b experimental group and the control group. B is the statistics of the immunohistochemical staining positive cells in the MN58b experimental group and the control group.