

Original article

Relationship between magnetic resonance imaging and molecular pathology in patients with glioblastoma multiforme

LI Wen-bin, TANG Kai, ZHANG Wei, YAN Wei, YOU Gang, LI Shao-wu, ZHANG Long, HUANG Yan-jie and JIANG Tao

Keywords: glioblastoma multiforme; magnetic resonance imaging; molecular pathology

Background Glioblastoma multiforme (GBM) is the most common and lethal primary brain tumor in adults. Magnetic resonance imaging (MRI) is routinely used in the diagnosis, characterization and clinical management of GBM. The diagnosis and treatment of GBM is largely guided by histopathology and immunohistochemistry. This study aimed to identify the relationship between magnetic resonance features and molecular pathology of GBM.

Methods MRI images of 43 glioblastoma patients were collected. Four imaging features, degree of edema, contrast tumor enhanced/T2 ratio, multiple lesions and tumor across the midline, were selected to identify their relationship with P53, Ki-67 and O⁶-methylguanine-DNA methyltransferase (MGMT) expression in patients with GBM. The relationship between imaging features and molecular pathology was studied by chi-square test using the software SPSS 13.0.

Results High expression of P53 was found correlated with low contrast tumor enhanced/T2 ratio, low expression of Ki-67 was correlated with multiple lesions and high expression of Ki-67 may be related with tumor across the midline, low expression of MGMT was correlated with edema.

Conclusion Some MRI features such as the degree of edema, contrast tumor enhanced/T2 ratio, multiple lesions and tumor acrossing the midline are correlated with P53, Ki-67 and MGMT of GBM.

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Glioblastoma multiforme (GBM) is the most common primary fatal brain tumor in adults. Magnetic resonance imaging (MRI) is the most commonly used means of non-invasive diagnostic imaging and it has been used to diagnose GBM and to guide clinical treatment of this disease. However, in clinical practice, we find that the same pathological grades of glioma may show different imaging features with MR, and sometimes even vary greatly. This suggests that MRI may contain more information, especially of genetic information. These image features could reflect the biological characteristics of the tumor, thereby influencing the clinical treatment and prognosis.

The development of functional genomics technology, such as DNA chip technology, has provided a platform for the diagnosis of cancer at a genetic level. However, due to various reasons, the genetic chip is difficult to be used in the clinical practice. If the imaging material could be combined with the functional genomics, the corresponding relationship could be established between gene expression patterns and MR images of GBM, which could be easily applied in clinical practice.

Studies¹ have shown that some GBM imaging such as the enhanced performance may predict gene expression that indicates hypoxia and proliferation. The over-expression of epidermal growth factor receptor may also be implicated by the imaging features of tumors and, at the same time, imaging can also explain the distribution of gene expression in the tumor.

P53, Ki-67 and O⁶-methylguanine-DNA methyltransferase (MGMT) are important changeable genetic indicators related to the occurrence and development of GBM. The expression of these genes may reflect biological characteristics of GBM and affect the clinical treatment and prognosis of the patients. This study focuses on the association between some MRI features of glioblastoma and these molecular pathology factors.

METHODS

Patients and image data

Patients and diagnosis

Totally 43 patients with GBM were selected who were treated in the Glioma Center, Beijing Tiantan Hospital, Capital Medical University, China during the period from January 2006 to July 2008. Pathological diagnoses of

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Department of Neurosurgery (Li WB, Tang K, Zhang W, Yan W, You G and Jiang T), Department of Radiology (Li SW), Beijing Tiantan Hospital, Capital Medical University, Beijing 100050, China

Cancer Center, Beijing Shijitan Hospital, Capital Medical University, Beijing 100038, China (Li WB)

College of Public Health, Peking University, Beijing 100083, China (Zhang L and Huang YJ)

Correspondence to: Dr. JIANG Tao, Department of Neurosurgery, Capital Medical University, Beijing 100050, China (Fax: 86-10-67118050. Email: jiangtao369@sohu.com)

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glioblastoma (glioma of WHO grade IV) were confirmed for all cases by two senior neural pathologists.

MRI features

MR studies were performed with a 1.5 T Magnetic resonance scanner (GE, USA). T1- and T2-weighted unenhanced and the contrast-enhanced images were obtained, with three dimensions (axial, sagittal, and coronal). Each case had a comparison of preoperative and postoperative images. All of MRI was read by two experienced neuroradiologists, who were blinded to the pathological result. The imagings were shot by SONY acybershot™ digital camera. The size of the pictures was 2272×1704, stored on a CD-ROM and hard disk storage. Four imaging features were selected, including the degree of edema (DE), contrast tumor enhanced/T2 (CTE/T2), multiple lesions (ML), and tumor crossing the midline (TCM). Each feature is divided into two categories for statistical analysis. The definitions of these imaging features are shown as followed. DE: "0" for mild edema; "1" for moderate to severe edema. CTE/T2: "0" on behalf of the ratio of <1; "1" on behalf of the ratio >1. ML: "0" for single lesion; "1" for multiple lesions. TCM: "0" for the tumor not crossing the midline; "1" for the tumor crossing the midline.

Molecular pathology of tumor specimens

Expression of Ki-67, mutant p53 and MGMT in the pathological specimens of patients by immunohistochemical staining

The 4–5 μm paraffin sections were pasted on slides and then baked at 60°C for 2 hours. Paraffin sections were dewaxed in xylene, rinsed in graded ethanol and finally rehydrated in double-distilled water. The sections were pretreated for 15 minutes at 100°C in sodium citrate buffer (10 mmol/L sodium citrate, pH 6.0) for antigen retrieval and added 1 drop or 50-μl 3% hydrogen peroxide to each section for 10-minute incubation at room temperature to negate the endogenous poly-peroxydaseactivity. After washing the sections with PBS three times for 3 minutes at a time, sections were incubated for 60 minutes at room temperature with a anti-human MGMT mouse monoclonal antibody (clone MT3.1, MAB-0361, MAIXIN, Fuzhou, China; dilution 1:100), or with a anti-human mutant p53 mouse monoclonal antibody (clone DO-7, MAB-0142, MAIXIN; dilution 1:100), or with a anti-human Ki-67 mouse monoclonal antibody (clone MIB-1, MAB-0129, MAIXIN; dilution 1:100). After three rinses with PBS three times for 3–5 minutes at a time, the sections were added 1 drop or 50 μl of MaxVision™ secondary reagent (MaxDS™ M&R Polymer Double Staining IHC Detection Kit, USA) reagent to each section following the kit instructions and incubated for 10–15 minutes at room temperature. Then wash with PBS flush three times for 3 minutes at a time, add 100 μl of fresh prepared DAB to each section, and observe 3–5 minutes by the microscope. At last, wash under tap water, counter stain with hematoxylin, and wash with PBS or tap water. And process slides through gradient alcohol dehydration and

xylene treatment and neutral gum cementing.

Molecular pathology

Select the area where the positive cells were most numerous, and count 1000 tumor cells. Labeling index (LI) was defined as the percentage of immunoreactive positive cells in total tumor cells. Assess the level of expression of each marker according to its average: "1" (LI ≥30%), as high expression, "0" (LI <30%) as low or no expression.

Statistical analysis

The chi-square test was used for analyzing the correlation between imaging features and molecular pathology indicators using SPSS 13.0 statistical software (SPSS Inc., USA). *P* values <0.05 were considered statistically significant.

RESULTS

Molecular pathology and imaging features on 43 patients with glioblastoma were shown in Table 1.

Relationship between expression of P53 and CTE/T2 ratio

Expression of P53 was detected in 42 patients. Ten cases (24%) showed low expression and 32 cases (76%) with high expression of P53. CTE/T2 ratio was measured in 43 cases, 31 patients (72%) with CTE/T2 ratio <1, and 12 cases (28%) with CTE/T2 ratio ≥1. The difference between the low/high expression of P53 and low/high CTE/T2 ratio is significant ($\chi^2=3.849$, $P=0.050$), which suggests a low CTE/T2 ratio may be related with high expression of P53 (Table 2).

Relationship between expression of Ki-67 and multiple lesions

Expression of Ki-67 was detected in 43 cases; 15 cases (35%) with low expression, and 28 cases (65%) with high expression. The number of lesions was measured in 41 cases; 38 patients (93%) have only one lesion, and 3 cases (7%) with multiple lesions (Table 3). Chi-square test results indicate that the high/low expression of Ki-67 and the number of lesions has significant differences ($\chi^2=5.611$, $P=0.018$), which suggests that low expression of Ki-67 may associate with multiple lesions (Table 3).

Relationship between Ki-67 and TCM

The MRI showed 11 of 43 patients (26%) with tumor infiltration crossing the midline and 32 cases (74%) without TCM. The chi-square test showed that the correlation between Ki-67 expression and the difference of TCM was significant ($\chi^2=4.329$, $P=0.037$), which suggests that high expression of Ki-67 may be related with TCM (Table 3).

Relationship between MGMT and degree of edema

MGMT was detected in 42 patients; low expression of MGMT in 16 cases (38%) and high expression in 26 cases (62%). Edema was measured in 43 cases in which edema

Table 1. Molecular pathology and image features

No.	P53	Ki-67	MGMT	DE	CTE/T2	ML	TCM
1	0	1	1	1	1	0	0
2	0	0	0	1	1	1	0
3	1	1	1	0	1	0	1
4	1	1	0	0	1	0	0
5	1	1	1	1	0		0
6	1	1	1	0	0	0	0
7	0	0	1	0	0	0	0
8	0	1	0	1	1	0	1
9	1	1	1	1	1		0
10	1	0	0	0	0	0	0
11	1	0	1	0	0	0	0
12	1	1	1	1	0	0	0
13	1	1	1	0	0	0	0
14	1	0	0	1	0	0	1
15	1	0	0	1	0	0	0
16	1	1	0	1	0	0	0
17	1	1	0	1	0	0	0
18	1	1	1	0	0	0	0
19	0	1	1	0	0	0	0
20	0	1	1	1	0	0	0
21	0	0	1	1	0	1	0
22	0	1	1	0	1	0	1
23	1	0	0	1	0	0	0
24	1	0	0	1	0	0	0
25	1	1	0	1	0	0	1
26	1	0	1	0	1	0	0
27	1	1	1	1	0	0	0
28	1	0	1	0	0	0	0
29	1	1	1	0	0	0	1
30	0	1	1	0	1	0	1
31	1	1	1	0	0	0	0
32	1	0	0	1	0	1	0
33	1	1	0	0	0	0	0
34	1	1	0	1	0	0	1
35	1	1	1	0	0	0	1
36	1	1	1	0	1	0	1
37	1	1	1	0	0	0	0
38		1	0	0	1	0	1
39	1	1	1	0	1	0	0
40	1	1	1	0	0	0	0
41	1	0	1	1	0	0	0
42	0	0	0	1	0	0	0
43	1	0	1	0	0	0	0

1: LI ≥30%, as high expression. 0: LI <30%, as low or no expression.

Table 2. Relationship between expression of P53 and CTE/T2 (n (%))

Image features	Low P53	High P53
CTE/T2 ratio <1 (n=31)	5 (16)	26 (84)
CTE/T2 ratio ≥1 (n=12)	5 (42)	6 (50)

Table 3. Relationship between Ki-67 expression, ML and TCM (n (%))

Image features	Low Ki-67	High Ki-67
No multiple lesions (n=38)	12 (31)	26 (68)
Multiple lesions (n=3)	3 (100)	0 (0)
No TCM (n=32)	14 (44)	18 (56)
TCM (n=11)	1 (9)	10 (91)

Table 4. Relationship between MGMT and edema (n (%))

Edema	Low MGMT	High MGMT
None to mild (n=22)	4 (17)	18 (78)
Moderate to severe (n=20)	12 (60)	8 (40)

of none/mild was seen in 23 patients (53%), and edema of moderate/severe in 20 (47%). As shown in Table 4, chi-square test results indicate *MGMT* expression associated with a high or low degree of edema had a significant difference ($\chi^2=7.769$, $P=0.005$), which

suggests that the low expression of *MGMT* is associated with edema (Table 4).

DISCUSSION

In the past 20 years, MRI has been used in clinical practice to diagnose brain glioblastoma and to guide clinical treatment. MRI is the most commonly used means of non-invasive diagnostic imaging to diagnose brain glioblastoma. However, the same pathological types of glioma may show different magnetic resonance imaging features. This suggests that MRI may contain more information, particularly include genetic information.

If the relationship between the phenotypic image and the expression of molecular pathology could be found, some image features and biological markers would be used to guide clinical diagnosis and treatment.

Some studies have explored the relationship between MRI features and gene expression. For example, imaging features of the “infiltration” that affects patient survival as an independent indicator, especially the tumor imaging features of infiltration and edema can both reflect that tumors require different therapy. This imaging feature is associated with the gene expression of *BCAN*, *TRIO*, *PTP4A3*, and *ERF*.²⁻⁴ Imaging findings of enhancement and may reflect hypoxia and gene expression associated with proliferation. Over-expression of epidermal growth factor receptor can be inferred from the tumor image, and imaging can also explain the distribution characteristics of gene expression within the tumor.¹

MRI image of GBMs, enhanced and non-enhanced, may reflect different expression of protein. Some scholars⁵ have compared the areas of incompletely enhanced (IE) gene expression to complete enhancement areas (CE), and found eight gene set of 71 gene sets in IE and CE-rich areas. Matrix metalloproteinase (*MMP*)-7 is highly expressed in the CE. In contrast, the expression of tight junction protein-2 increased in the IE. The balance between *MMPs* and tight junction proteins may be responsible for maintaining the blood-brain barrier. Change in this balance may promote a more aggressive phenotype that is associated with edema, invasion and contrast enhancement. *ASCL1* is mainly expressed in the secondary glioblastoma tumors; and is highly expressed in the IE. On the contrary, genes such as caveolin 1, caveolin 2, interleukin-8, transgelin, and thrombospondin-1 are mainly expressed in primary glioblastoma CE tumor.⁶ IE tumor is rich in *OLIG2* and *ASCL1*, in contrast CE is rich in the tumor gene expression of caveolin 1.⁷ *OLIG2* genes are generally expressed in the GBM “shaozhi” components.⁸ The higher eight gene in IE tumors are not expressed in primary GBMs, and CE 71 has high tumor gene expression but not in secondary GBMs. So from the radiological point of view the enhanced appearance of tumors may identify primary and secondary GBMs. CE-related genes associate with excess of hypoxia-

edema-vascularity. For example, vascular endothelial growth factor (VEGF) is a key component of the pathway of angiogenesis, as well as a main factor that leads to increased edema.^{9,10} Our study showed a low CTE/T2 ratio may be related with the expression of *P53*.

P53, *Ki-67* are commonly used in clinical molecular pathology for GBM. They are important factors in the development of glioblastoma associated with genetic changes, and their variations or their expression levels reflect biological characteristics of glioblastoma are correlated with diagnosis and recurrent of GBM, that could affect the clinical treatment and prognosis.¹¹⁻¹⁴ In our study, we found that high expression of *P53* is correlated with low CTE/T2 ratio. This study focuses on the relationship between *Ki-67* and multiple lesions, and it is found that low expression of *Ki-67* is correlated with multiple lesions. In addition, high expression of *Ki-67* may be related with tumor across the midline.

MGMT is an important mechanism involved in glioblastoma resistance to alkylating chemotherapy drug. Hegi et al¹⁵ reported that during temozolomide (TMZ) treatment of 206 patients with glioblastoma, regardless of whether patients also received the TMZ chemotherapy, the patients with *MGMT* gene promoter methylation has significantly longer survival time than patients with non-methylated of the gene. They suggest that the effect of *MGMT* gene silencing is an improved response to chemotherapy. This study looked at the relationship between *MGMT* expression and peritumoral edema. The results showed that low expression of *MGMT* is correlated with edema.

There are some questions during correlative research between molecular diagnostics and imaging.¹⁶ Such as the image dimension and the number of whole genome-level analysis does not match; the methods of DNA chips commonly used to identify associated a particular image is inadequate, for example that DNA chips using several probes to measure expression of a specific gene may produce different results; several groups of studies are retrospective studies, and further prospective research remains to be conducted. For non-invasive medical imaging is widely used in clinical practice, if the image features can be bound to different molecular phenotypes it will help to promote development of personalized medicine for treatment of glioblastoma.

In conclusion, imaging features of brain glioblastoma, such as degree of edema, CTE/T2, multiple lesions, tumor across the midline have a close relationship with tumor molecular pathology indicators such as the *P53*, *Ki-67* and *MGMT*. MRI data could be applied to infer the molecular pathology of tumor target, and to guide clinical diagnosis and treatment.

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