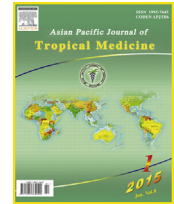


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The relationship between fractional anisotropy value and tumor microarchitecture in late-stage rat glioma

Xiang-Ying Li^{1,2}, Jian-Qiang Chen², Yi-Kai Xu^{1✉}, Xiang-Jun Han³¹Department of Medical Imaging Center, Nanfang Hospital, Southern Medical University, Guangzhou, Guangdong, PR China²Department of Radiology, Central South University Xiangya School of Medicine Affiliated Haikou Hospital/Haikou People's Hospital, Haikou, Hainan, PR China³Department of Radiology, Kangya Hospital, Yiyang, Hunan, PR China

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ABSTRACT

Objective: To explore the magnetic resonance diffusion tensor imaging (MR-DTI) features of in the late stage of Wistar rat C6 brain glioma, and the relationship between fractional anisotropy value and tumor microarchitecture.

Methods: The concentration of more than $1.0 \times 10^6/10 \mu\text{L}$ glioma cells and complete medium were injected stereotactically into the right caudate nucleus of the experimental group ($n = 35$) and control group ($n = 10$), respectively. Conventional MRI, DTI, and enhanced T1WI scans were Performed using the GE Signa HD $\times 3.0\text{T}$ MRI scanner about 3–4 weeks after implantation for the rats. Postprocessing was done using the DTI specific software Function Tool to gain FA image. Many ROIs were drawn avoiding hemorrhage, necrosis areas in tumor parenchyma, the value of FA was recorded. Each surviving rat brain was examined histologically using HE and immunohistochemical staining for VEGF and CD34. Pearson correlation analysis was used to determine the relationships between FA values and VEGF, MVD, cell density, respectively.

Results: A total of 35 tumor-bearing rats were confirmed the tumor formation by the subsequent MRI and pathological examination. The mean FA values of the tumor and the contralateral brain tissue were 0.17 ± 0.03 and 0.31 ± 0.05 respectively, and the difference was statistically significant ($t = 12.80$, $P < 0.05$). The mean FA value of grade III glioma ($n = 12$) was 0.16 ± 0.03 , and the average FA value of grade IV glioma ($n = 23$) was about 0.18 ± 0.04 . There was no significant difference between the two groups ($t = 1.92$, $P > 0.05$). FA value in the late stage of Wistar rat C6 brain glioma has significant positive correlation to VEGF, MVD, cell density. The correlation coefficients between FA and VEGF, MVD, and cell density were 0.67, 0.65 and 0.71 ($P < 0.05$), respectively.

Conclusions: The FA value of rat glioma tumor in the late stage can preoperatively provide an accurate, reliable and noninvasive imaging monitoring method to evaluate the microstructure of glioma (cell density, the extent of angiogenesis, fiber bundle integrity and tumor cell infiltration and so on), predict the biological behavior of the tumor and make out surgical plan.

First author: Xiang-Ying Li, Department of Radiology, Central South University Xiangya School of Medicine Affiliated Haikou Hospital/Haikou People's Hospital, Haikou, Hainan, PR China.

Tel: +86 13876959224

Email: lxdoc@126.com

✉Corresponding author: Yi-Kai Xu, Department of Medical Imaging Center, Nanfang Hospital, Southern Medical University, Guangzhou 510515, China.

Tel: +86 13729846668

E-mail: Yikai.xu@163.com

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1. Introduction

The recurrence of glioma is a long-standing clinical problem, especially in high-grade glioma, and preoperative assessment of glioma microstructure (cell density, the extent of angiogenesis, fiber bundle integrity and tumor cell infiltration and so on), to predict the biological behavior of the tumor, the development of surgical programs is of great significance. Magnetic resonance diffusion tensor imaging (MR-DTI) is a noninvasive functional magnetic resonance imaging technique that can reflect the changes of microstructure *in vivo*, such as cell density, degree of vascular proliferation, fiber bundle preservation and destruction and degree of tumor infiltration invasion. It is widely used in the evaluation of microstructure [1–5]. In view of the biological characteristics of rat C6 glioma model similar to human glioma, we established the late stage rat glioma model to study the value of diffusion tensor imaging (DTI) in assessing the microstructure characteristics of the high grade glioma to ensure that the experimental results are more reliable and reproducible.

2. Materials and methods

2.1. Glioma cell inoculation

The animal study was conducted in accordance with the guidelines and approval of the institutional animal care and use committee. Female Wistar 45 rats (220–315 g) were purchased from Dong Chuang Laboratory Animal Technology Services, Kaifu District, Changsha city. Rats were divided into the experimental group ($n = 35$), and the sham group ($n = 10$) according to random number tables. C6 glioma cells were bought from the Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. The cells were cultured in RPMI-1640 medium, supplemented with 10% fetal cattle serum and 1% penicillin streptomycin mixed liquid. During the logarithmic growth phase, the cells were tackled with pancreatic enzymes, and a small sample of cells were removed for calculation using a microscope. The Trypan Blue Exclusion Test was conducted to ensure that cell viability was over 95% prior to inoculation.

Each rat was anesthetized with 1% pentobarbital sodium (40 mg/kg) and placed in a stereotactic apparatus (RWD Life Science and Technology Co., Ltd.). The skin of the head was cleaned and an incision was made to expose the skull to identify the bregma and lateral positions, and a 1 mm hole was drilled using a dentist drill through the skull 1 mm anterior and 3 mm lateral to the bregma on the right side. C6 glioma cells ($1 \times 10^6/10 \mu\text{L}$) were injected at a depth of 6 mm from the dural surface, and then retracted 1 mm back. The injection was fully completed within 10 min ($1 \mu\text{L}/\text{min}$), a waiting time of 10 min was implemented following injection and then the needle was slowly withdrawn. The bone was sealed off with wax to prevent any cell suspension reflux. As control, five rats were inoculated with complete medium with no C6 cells in the same location.

2.2. MRI examination

MRI experiments were performed using a GE 3.0T Signa HD × medical magnetic resonance scanner (United States General Electrical Medical Group, GE Healthcare), with a gradient field of 50 mT/m, a switch rate of 150 mT/m/ms, and a rat-dedicated RF

coil (China Shanghai Chen Guang Company). Each survival rat was anesthetized by intraperitoneal injection of 1% pentobarbital sodium (40 mg/kg). In order to reduce effect of intravenous gadolinium-DTPA on diffusion tensor MR imaging for the evaluation of brain tumors [6,7]. Conventional MRI and DTI were done before contrast medium injection about 3–4 weeks after cells implantation, centered on optic chiasma. These times were based on the theory that it grew rapidly and became more infiltrative after 3–4 weeks after cell implantation [8–10].

Imaging parameters for T1-weighted images were as follows: repetition time (TR) = 350 ms, echo time = 19 ms, a slice thickness = 3.0 mm, interlayer spacing = 0 mm, field of view (FOV) = $(8 \times 8) \text{ cm}^2$, (192×192) matrix. The scanning parameters for T2WI were: TR = 3000 ms and TE = 120 ms, slice thickness, interlayer spacing, matrix, and FOV were the same as those of T1WI. The T2-Flair propeller parameters were imaged using the following parameters: TR = 9000 ms, TE = 155 ms, echo chain length = 36, bandwidth = 31.25 MHz, layer thickness = 3.0 mm, and FOV = $(15 \times 15) \text{ cm}^2$.

Diffusion tensor data were acquired with a spin echo multi-shot echo planar imaging (EPI) pulse sequence (TR = 2500 ms, TE = 91 ms, slice thickness = 3 mm, motivate times = 16). The diffusion-weighting gradient schemes with 15 non-collinear directions with a b value of 800 s/mm^2 were used; thereafter, we obtained an additional measurement without diffusion gradient encoding (b value, 0 s/mm^2). This based on the study theory that by applying the diffusion imaging gradients in a minimum of six directions, a diffusion tensor image can be measured [11].

T1WI in the transverse plane and T1-3D-Bravo (IR FSPGR, inversion recovery fast spoiled gradient echo) sequences were performed after the administration of 2.5–3.0 mL of gadolinium diethylenetriamine-pentaacid (Magnevist; Schering AG, Germany) per kilogram of body weight with T1-3D-Bravo parameters: a slice thickness = 0.8 mm, FOV = $(8 \times 8) \text{ cm}^2$, (256×256) matrix, flip angle = 15° , bandwidth = 31.25 MHz, prep time = 380 ms.

2.3. MRI image processing

Post-processing of the original images via Function Tool after DTI canning was required to avoid non-brain regions like CSF and the ventricle [12]. Combining T2WI, T2-FLAIR and T1WI with T1-3D-Bravo enhanced, we were able to locate the tumor. Under the guidance of experienced neurological surgeons and neuro-imaging experts, the fractional anisotropy (FA) values of the corresponding parts were obtained by selecting multiple isometrical ROIs of the tumor, the contralateral brain tissue and the control group.

2.4. Histological examination

2.4.1. HE staining

After scanning, the live rats were anesthetized with an overdose of 1% pentobarbital sodium, and then perfused with 4% paraformaldehyde liquid via the left ventricular to fix the whole brain, and then we proceeded to check the organs for metastasis. Following this, we put the specimens into 4% paraformaldehyde liquid for 24 h.

Based on the location of optic chiasma, the brain tissue was continuously sectioned. We randomly selected one section from

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