

# Tumor growth analysis by magnetic resonance imaging of the C6 glioblastoma model with prospects for the assessment of magnetohyperthermia therapy

Monitoramento por imagem de ressonância magnética do crescimento tumoral no modelo C6 de glioblastoma com perspectivas de avaliação da terapia de magnetohipertermia

André César da Silva<sup>1</sup>, Francisco Romero Cabral<sup>1</sup>, Javier Bustamante Mamani<sup>1</sup>, Jackeline Moraes Malheiros<sup>2</sup>, Roberson Saraiva Polli<sup>2</sup>, Alberto Tannus<sup>2</sup>, Edson Vidoto<sup>2</sup>, Mateus José Martins<sup>2</sup>, Tatiana Tais Sibov<sup>1</sup>, Lorena Favaro Pavon<sup>1</sup>, Liza Aya Mabuchi Miyaki<sup>1</sup>, Walter Humberto Zavala Cárdenas<sup>1</sup>, Suzana Maria Fleury Malheiros<sup>3</sup>, Reynaldo André Brandt<sup>4</sup>, Edson Amaro Junior<sup>1</sup>, Lionel Fernel Gamarra<sup>1</sup>

## ABSTRACT

**Objective:** The objective was to establish a pattern of tumor growth of the C6 model of glioblastoma multiforme in Wistar rats via magnetic resonance imaging (MRI) for the subsequent verification of tumor volume reduction due to magnetic hyperthermia therapy. **Methods:** Young male Wistar rats weighing between 250 and 300 g were used for the C6 model. After the rats were anesthetized (55 mg/kg ketamine and 11 mg/kg xylazine), C6 lineage tumorigenic cells suspended in culture medium ( $10^5$  cells in  $10 \mu\text{l}$ ) were stereotaxically injected into the right frontal cortex (bregma coordinates: 2.0 mm anteroposterior, 3.0 mm laterolateral, and 2.5 mm depth) of the rats using a Hamilton syringe. For the control group, the rats were injected with culture medium without cells. MRI scans were performed at 14, 21, and 28 d after the injection using a 2.0 T MRI scanner (Bruker BioSpec, Germany). The animals were anesthetized with 55 mg/kg ketamine and 11 mg/kg xylazine before being examined. Coronal multilayers were acquired using a standard spin echo sequence with the following parameters: repetition/echo time = 4.000 ms/67.1 ms, field of view = 3.50, matrix = 192, slice thickness = 0.4 mm, and slice separation = 0 mm. **Results:** The MRI analysis enabled a clear visualization of the tumor mass, and it was possible to establish the tumor volume parameters on the various days that were examined. The volume at 14 d after induction was  $13.7 \pm 2.5 \text{ mm}^3$ . On days 21 and 28, the tumor volumes were  $31.7 \pm 6.5 \text{ mm}^3$  and  $122.1 \pm 11.8 \text{ mm}^3$ , respectively. **Conclusion:** These results demonstrated

that it is possible to evaluate the C6 model tumor volume in rats, which will allow for the future implementation and verification of magnetic hyperthermia therapy.

**Keywords:** Magnetic resonance imaging; Glioblastoma/therapy; Brain neoplasms; Rats, Wistar

## RESUMO

**Objetivo:** Estabelecer um padrão de crescimento tumoral (volume) em ratos Wistar submetidos ao modelo C6 de glioblastoma multiforme por meio de imagens de ressonância magnética para posterior verificação de redução de volume tumoral com a terapia de magnetohipertermia. **Métodos:** Para o modelo C6, utilizamos ratos Wistar, machos, jovens, pesando entre 250 e 300 g. Após anestesiados (cetamina 55 mg/kg e xilazina 11 mg/kg) foram injetadas estereotaxicamente células tumorigênicas linhagem C6 suspensas em meio de cultura ( $10^5$  células em  $10 \mu\text{L}$ ) no córtex frontal direito (coordenadas a partir do bregma: anteroposterior = 2,0 mm; látero-lateral = 3,0 mm; profundidade = 2,5 mm) com uma seringa Hamilton. No Grupo Controle, houve a injeção do meio de cultura sem as células. Posteriormente, foram feitas imagens mediante a técnica de imagem por ressonância magnética em 14, 21 e 28 dias após a injeção em um escâner de imagem por ressonância magnética 2.0 T (Bruker BioSpec, Germany). Para o exame, os animais foram

Study carried out at the Brain Institute, Hospital Israelita Albert Einstein - HIAE, São Paulo (SP), Brazil.

<sup>1</sup> Brain Institute, Hospital Israelita Albert Einstein - HIAE, São Paulo (SP), Brazil.

<sup>2</sup> Institute of Physics, Universidade de São Paulo - USP São Carlos (SP), Brazil.

<sup>3</sup> Universidade Federal de São Paulo - UNIFESP, São Paulo (SP), Brazil; Department of Oncology, Hospital Israelita Albert Einstein, HIAE, São Paulo (SP), Brazil.

<sup>4</sup> Neuro-Oncology Center, Sociedade Beneficente Israelita Brasileira Albert Einstein - SBIBAE, São Paulo (SP), Brazil.

Corresponding author: Lionel Fernel Gamarra, Instituto Israelita de Pesquisa, Avenida Albert Einstein 627/701, Morumbi, Zip code: 05651-901, São Paulo (SP), Brazil; E-mail: lgamarra@einstein.br

Sources of research support: Brazilian Federal Agency for the Support and Evaluation of Graduate Education (Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior, CAPES), Funder of Studies and Projects (Financiadora de Estudos e Projetos, FINEP), National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq).

Received on: Jan 1, 2011; Accepted on: Feb 24, 2012

Conflict of interest: None

anestesiados com cetamina 55 mg/kg e xilazina 11 mg/kg. Multifatias coronais foram adquiridas utilizando uma sequência *spin-echo* padrão com os seguintes parâmetros: TR/TE = 4,000 ms/67,1 ms, FOV = 3,50, Matrix 192, *slice thickness* = 0,4 mm e *slice separation* = 0 mm. **Resultados:** A análise das imagens de ressonância magnética do tumor possibilitou a clara visualização da massa tumoral, sendo possível ainda estabelecer parâmetros de volume tumoral nos diferentes dias analisados. O volume de 14 dias após a indução do foi de  $13,7 \pm 2,5 \text{ mm}^3$ . Aos 21 dias, o volume alcançado foi de  $31,7 \pm 6,5 \text{ mm}^3$  e, aos 28 dias, a massa tumoral atingiu  $122,1 \pm 11,8 \text{ mm}^3$ . **Conclusão:** Estes resultados mostraram a possibilidade de avaliação do volume tumoral no modelo C6 em ratos, o que possibilitará, no futuro, a aplicação da terapia de magnetohipertermia bem como verificação de seus resultados.

**Descritores:** Imagem por ressonância magnética; Glioblastoma/terapia; Neoplasias encefálicas; Ratos Wistar

## INTRODUCTION

Gliomas are neuroepithelial tumors that originate in glial cells and correspond to 31% of primary tumors and 80% of malignant tumors of the central nervous system (CNS). Astrocytomas correspond to 76% of all gliomas, and glioblastomas represent 53.7% of these cases<sup>(1)</sup>. Glioblastoma multiform (GBM) is the most common and malignant astrocytoma, and despite numerous advances in the diagnosis and treatment of these tumors, the prognosis for GBM remains limited with a life expectancy of less than 1 year<sup>(2,3)</sup>. Thus, it is extremely important to search for new therapeutic approaches that provide a better quality of life for brain cancer patients.

Accordingly, several therapies for gliomas have been proposed, such as chemotherapy and radiotherapy strategies<sup>(4)</sup>. In recent decades, thermotherapy has been used in combination with these therapies. Thermotherapy involves increasing with tumor temperature (43 to 47°C) with the intent of destroying the tumor cells<sup>(5-9)</sup>. Recent studies have shown that hyperthermia can be induced in a localized manner by applying magnetic nanoparticles that heat the tumor region under an alternating magnetic field. This therapy, called magnetic hyperthermia (MHT), has shown good results in preclinical<sup>(10)</sup> and clinical<sup>(11)</sup> studies.

However, the search for new therapies requires the use of animal models that can mimic disease progression in humans. Currently, several animal models have been proposed, most of which utilize rats and mice<sup>(12,13)</sup>.

A rat model of the Wistar strain, called C6, has been widely used in recent decades and has shown good applicability<sup>(14-19)</sup>. Benda et al.<sup>(20)</sup> developed this

model and found that they were able to develop brain tumors in Wistar rats after multiple injections of N-methylnitrosourea over approximately 8 months. The tumorigenic cells were cultured, and the lineage was developed from the sixth clone (C6). These tumors had a glial origin and characteristics similar to GBM development, thus displaying similar growth and invasion to that found in human tumors<sup>(21)</sup>. Although the C6 model is a widely used for studies of tumor progression, the published patterns of growth show a large variability. To implement MHT, as well as other therapies, it is of paramount importance that we obtain these tumor progression parameters.

## OBJECTIVES

To establish the tumor growth pattern (volume) of C6 GBM model Wistar rats using MRI and to verify the reduction of tumor volume due to MHT.

## METHODS

### Animals

This work was developed in accordance with the standards of the *Hospital Israelita Albert Einstein* (HIAE) Ethics in Animal Research Committee (*Comitê de Ética em Pesquisa Animal do Hospital Israelita Albert Einstein*). For all procedures, care was taken to minimize the number of animals used as well as their amount of suffering and stress.

For the C6 model, 2-month-old male Wistar rats weighing between 250 and 300g were used. The animals were housed in the vivarium of the Experimentation and Surgery Training Center (*Centro de Experimentação e Treinamento em Cirurgia*, CETEC) of the HIAE Brain Institute (*Instituto do Cérebro*, InCe) in individual polypropylene cages that were lined with autoclaved sawdust. They were provided with feed and water *ad libitum* via stainless steel cover grids with divisions for balanced feed and water. This vivarium is accredited by the International Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). The environment followed a 12 hour light/dark cycle (7 am to 7 pm) and was maintained at a constant temperature ( $21 \pm 2^\circ\text{C}$ ) according to international specifications.

For the MRI, the animals were transported to the Magnetic Resonance Imaging and *in vivo* Magnetic Resonance Spectroscopy Center for the Study of Animal Models (*Centro de Imagens e Espectroscopia in vivo por Ressonância Magnética para Estudo de Modelos Animais*, CIERMag) of the *Universidade de São Paulo*

Institute of Physics, São Carlos, where they were housed in the vivarium under the same aforementioned conditions until the final phase of the experiments.

### **C6 glioma cell culture**

The C6 glioma cells of the Wistar rats were grown at 37°C (5% CO<sub>2</sub>) in Dulbecco's Eagle medium (Gibco, Gaithersburg, MD) that contained 20% fetal bovine serum (Invitrogen). At 90% cell confluence, the medium was removed, and the cells were released for incubation using trypsin (0.04% of trypsin/EDTA). The cells were centrifuged at 800 rpm for 5 min, resuspended in Dulbecco's medium at a final concentration of 10<sup>5</sup> cells/10 μL, and kept cool until implanted.

### **Stereotaxic implantation of C6 cells**

The animals were anesthetized with ketamine (55 mg/kg) and xylazine (11 mg/kg) to implant the C6 cells. The hair was then removed from the top of the head. The animal was fixed to the stereotaxic apparatus (Stoelting®, model 51700) using in-ear and upper teeth bars. After making a skin incision on the dorsal region of the skull and removing the periosteum, a trepanation of the bone cap was made using a dental drill. The implantation position was determined and marked on the bone according to Swanson's Stereotaxic Atlas guidelines (1992) at the following coordinates: 2.0 mm anteroposterior, 2.0 mm laterolateral, and a depth of 2.5 mm. A Hamilton syringe was used to implant 10<sup>5</sup> tumorigenic glioma cells in 10 μL of culture medium cells into the right frontal cortex. The cells were slowly injected over a 10 min period. For the control group, culture medium was injected without the cells. The syringe was kept in position for an additional 2 min before being withdrawn. To avoid drawing the injected solution back into the needle, the syringe was slowly raised until it was completely removed from the brain. The bone was then reassembled using bone wax, and the skin was sutured using cotton thread.

### **MRI tumor analysis**

Prior to each imaging session, the animals were anesthetized using a mixture of ketamine (95 mg/kg) and xylazine (12 mg/kg). The animals were then placed in a ventral decubitus position on a soft, absorbent surface. After positioning the head inside the coil, the head was fixed using the ear and nasal bars, and the set was shielded. Finally, this entire set assembly was introduced to the magnet, which had a 150 mm internal diameter.

The magnetic resonance images were acquired using a horizontal superconducting magnet from Oxford Instruments (2 T field; model 65310HR) that operated in conjunction with a Bruker® spectrometer. The rapid acquisition with relaxation enhancement (RARE) sequence was used to acquire T<sub>2</sub> weighted images with the following parameters: repetition time = 4.000 ms, echo time = 67.1, RARE factor = 6, means = 18, and bandwidth = 12.5 KHz. The acquisition time was approximately 50 min per animal. The field of view was 35x35 mm<sup>3</sup> with an array of 192x192 points to produce a spatial resolution of 182x182 μm. A total of 26 slices were used at a 0.5 mm thickness without spacing between the slices.

For the 26 total slices taken during each imaging session, the slices for which the tumor was visible were selected, and regions of interest were drawn using Paravision 5® software. The tumor area was identified for each slice and then summed and multiplied by the slice thickness to obtain the tumor volume.

### **Histopathological analysis of tumor tissues**

After image acquisition, the animals were anesthetized and transcardially perfused with a buffered saline solution and 4% paraformaldehyde (PFA). The brains were removed and stored in PFA for 24 hours; they were then cryoprotected in a 40% sucrose solution for 48 hours. Subsequently, 40 μm thickness coronal sections were cut using a cryostat (Leica) and stained via the standard hematoxylin-eosin technique.

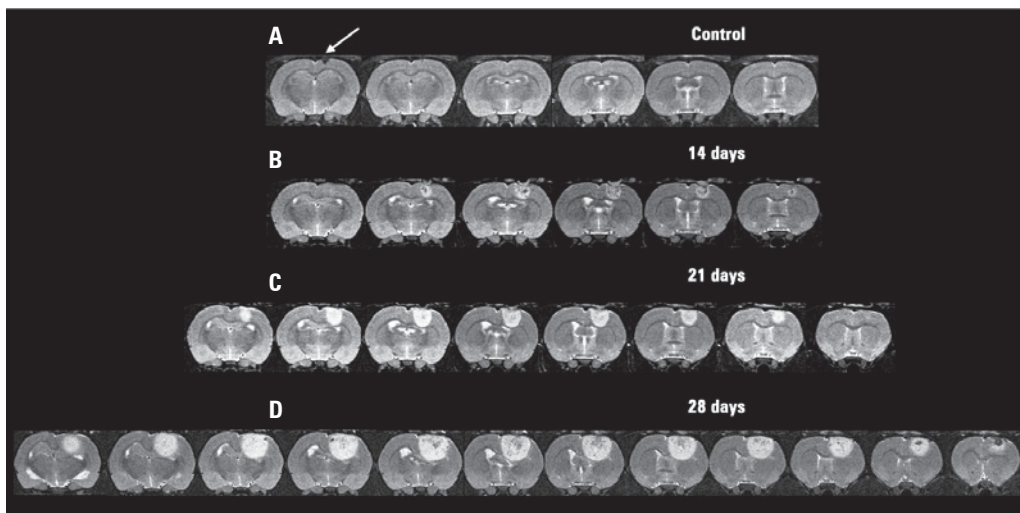
## **RESULTS**

### **MRI analysis of tumor volumes**

The progression of tumor growth of the C6 model was monitored using MRI (Figure 1). In the controls that were injected with culture medium only, the presence of tumor tissues was not detected over the 28-day period (Figure 1A).

On day 14, a tumor mass was observed (tumor + edema + necrotic area) that had a well-defined circular shape compared to the adjacent brain tissue, and thus, it was possible to outline the mass due to the intense signal in the affected region. The calculated volume on day 14 was 13.7 ± 2.5 mm<sup>3</sup> (Figure 1B). On day 21, the tumor was clearly demarcated, with a volume of 31.7 ± 6.5 mm<sup>3</sup>, and it began to cause compression of the right ventricular region (Figure 1C). On day 28, the tumor growth reached 122.1 ± 11.8 mm<sup>3</sup> (Figure 1D). After 28 days, the animals were sacrificed, and their brains were removed for later analysis.





**Figure 1.** MRI follow-up of tumor progression in the C6 model. The images show sequences of brain slices for which it was possible to observe the tumor. (A) No tumor growth was observed for the brains in which only culture medium was injected. The arrow superficial to the cortex indicates the region where the syringe was inserted. (B) After 14 d, it was possible to observe an intense signal in the region inoculated with tumorigenic cells, and the tumor volume reached  $13.7 \pm 2.5 \text{ mm}^3$ . (C) On day 21, a well-defined circular tumor that measured  $31.7 \pm 6.5 \text{ mm}^3$  was evident. (D) On day 28 after inoculation, a large tumor with a calculated mass of  $122.1 \pm 11.8 \text{ mm}^3$  was observed; this tumor began to compress the ventricular region

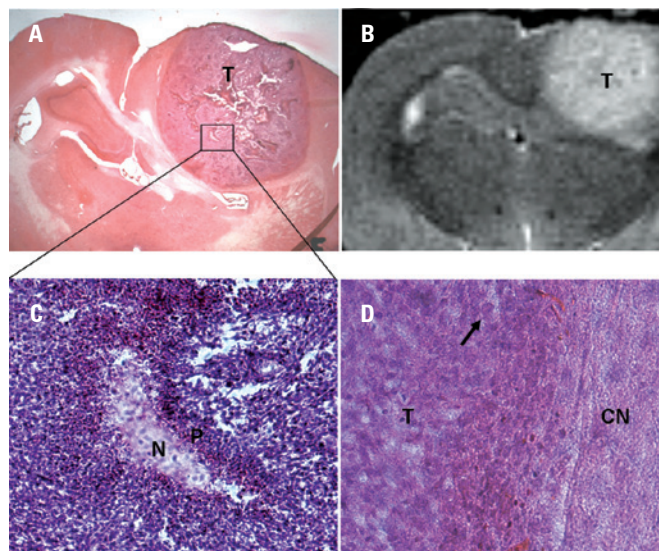
### Histopathological analysis

The histopathological evaluation of the gliomas implanted in the Wistar rats is presented in figure 2. The tumors presented GBM characteristics that were consistent with those previously demonstrated by Morrone et al.<sup>(22)</sup>. Figure 2 shows a histological section (Figure 2A) and an MRI (Figure 2B) of a glioblastoma tumor. In accordance with the GBM characteristics, necrotic lesions, pseudopalisade-type atypical cells (Figure 2C), giant cells (Figure 2D), and hemorrhagic areas are highlighted.

### DISCUSSION

GBM is a highly aggressive primary tumor of the CNS, and the average survival time of patients with GBM does not exceed 15 months of diagnosis, regardless of all available therapies<sup>(4,23)</sup>. Thus, attempts to develop new therapies are of extreme importance. MHT has been used in combination with existing therapies and has provided favorable results<sup>(11)</sup>. Studies conducted since the 1990s have shown that the use of magnetic nanoparticles is extremely efficient in generating heat after the application of a magnetic field, and this heat is capable of destroying cancer cells of the nervous system<sup>(24)</sup>. In addition, the recent work of Van Landeghem et al.<sup>(25)</sup> in humans showed the ability of MHT to increase the life expectancy of GBM patients.

The C6 model features histopathological characteristics, such as the presence of pseudopalisade cells, necrotic areas, and microvascular proliferation, that are comparable to those found in GBM patients<sup>(26)</sup>.



**Figure 2.** Histopathological characteristics of the C6 model. (A) A histological section and (B) an MRI image showing the circular tumor "T" clearly outlined and compressing ventricular structures. (C) *N*, necrosis area, and *P*, pseudopalisade, cells adjacent to the necrosis area. (D) The edge of the tumor and the presence of giant cells (arrow) and *CN*, normal brain tissue. Magnification: (A) x10, (B) x10, (C) x100, and (D) x400

The present study confirmed these characteristics, which supports this model as a useful tool for the development of new therapies, such as MHT. However, for the assessment of MHT, monitoring tumor development is essential because the objective of MHT is to reduce tumor volume. MRI allows for the follow-up of tumor volume as well as modifications and deformations of surrounding regions<sup>(27)</sup>. In the present study, the rapid growth of a tumor mass using MRI was evaluated. The

tumors progressed and compressed the ventricular region along the rostro-caudal axis. Tumor volumes in rats, which were measured 20 d after the implantation of cancer cells and analyzed *in vivo* by MRI, were similar to the volumes found in histological studies performed by Morrone et al.<sup>(22)</sup>. This finding demonstrates that tumor measurement using imaging techniques can generate reliable data on the magnitude of tumors *in vivo* and can provide information regarding other brain structures that may be compressed are compromised. MRI data also permit the correlation analyses of behavioral and histological data that are acquired from the same animal.

## CONCLUSION

The MRI analysis enabled the clear visualization of tumor mass and growth, which allowed tumor volume parameters of the C6 model to be analyzed on various days. The results obtained here are important for future applications of the MHT technique because tumor growth progression is directly related to the power of the magnetic field and the duration of the therapy that is applied.

## REFERENCES

- Central Brain Tumor Registry of the United States. CBTRUS statistical report. Primary brain and central nervous system tumors diagnosed in the United States in 2004-2006 [Internet]. Hinsdale (IL): CBTRUS; 2010 Feb [cited 2012 Feb 23]. Available from: <http://www.cbtrus.org/2010-NPCR-SEER/CBTRUS-WEBREPORT-Final-3-2-10.pdf>
- Brandes AA, Tosoni A, Franceschi E, Reni M, Gatta G, Vecht C. Glioblastoma in adults. *Crit Rev Oncol Hematol*. 2008;67(2):139-52.
- Wen PY, Kesari S. Malignant gliomas in adults. *N Engl J Med*. 2008;359(5):492-507.
- Shirai K, Chakravarti A. Towards personalized therapy for patients with glioblastoma. *Expert Rev Anticancer Ther*. 2011;11(12):1935-44.
- Overgaard J. The current and potential role of hyperthermia in radiotherapy. *Int J Radiation Oncology Biol Phys*. 1989;16(3):535-49.
- Herman TS, Teicher BA, Jochelson M, Clark J, Svensson G, Coleman CN. Rationale for use of local hyperthermia with radiation therapy and selected anticancer drugs in locally advanced human malignancies. *Int J Hyperthermia*. 1988;4(2):143-58.
- Anderson RL, Kapp DS. Hyperthermia in cancer therapy: current status. *Med J Aust*. 1990;152(6):310-5.
- Sneed PK, Stauffer PR, McDermott MW, Diederich CJ, Lamborn KR, Prados MD, et al. Survival benefit of hyperthermia in a prospective randomized trial of brachytherapy boost +/- hyperthermia for glioblastoma multiforme. *Int J Radiat Oncol Biol Phys*. 1998;40(2):287-95.
- Wust P, Hildebrandt B, Sreenivasa G, Rau B, Gellermann J, Riess H, et al. Hyperthermia in combined treatment of cancer. *Lancet Oncol*. 2002;3(8):487-97.
- Jordan A, Scholz R, Maier-Hauff K, van Landeghem FK, Waldoefner N, Teichgraber U, et al. The effect of thermotherapy using magnetic nanoparticles on rat malignant glioma. *J Neurooncol*. 2006;78(1):7-14.
- Maier-Hauff K, Ulrich F, Nestler D, Niehoff H, Wust P, Thiesen B, et al. Efficacy and safety of intratumoral thermotherapy using magnetic iron-oxide nanoparticles combined with external beam radiotherapy on patients with recurrent glioblastoma multiforme. *J Neurooncol*. 2011;103(2):317-24.
- Barth RF, Kaur B. Rat brain tumor models in experimental neuro-oncology: the C6, 9L, T9, RG2, F98, BT4C, RT-2 and CNS-1 gliomas. *J Neurooncol*. 2009;94(3):299-312.
- Fomchenko EI, Holland EC. Mouse models of brain tumors and their applications in preclinical trials. *Clin Cancer Res*. 2006;12(18):5288-97.
- Day SE, Kettunen MI, Cherukuri MK, Mitchell JB, Lizak MJ, Morris HD, et al. Detecting response of rat C6 glioma tumors to radiotherapy using hyperpolarized [1-13C]pyruvate and 13C magnetic resonance spectroscopic imaging. *Magn Reson Med*. 2011;65(2):557-63.
- Ulmer S, Reeh M, Krause J, Herdegen T, Heldt-Feindt J, Jansen O, et al. Dynamic contrast-enhanced susceptibility-weighted perfusion MRI (DSC-MRI) in a glioma model of the rat brain using a conventional receive-only surface coil with an inner diameter of 47 mm at a clinical 1.5 T scanner. *J Neurosci Methods*. 2008;172(2):168-72.
- Doblas S, He T, Saunders D, Pearson J, Hoyle J, Smith N, et al. Glioma morphology and tumor-induced vascular alterations revealed in seven rodent glioma models by *in vivo* magnetic resonance imaging and angiography. *J Magn Reson Imaging*. 2010;32(2):267-75.
- Doblas S, He T, Saunders D, Hoyle J, Smith N, Pye Q, et al. *In vivo* characterization of several rodent glioma models by (1)H MRS. *NMR Biomed* [DOI: 10.1002/nbm.1785]. 2011 [cited 2011 Sep 23]. Available from: <http://onlinelibrary.wiley.com/doi/10.1002/nbm.1785/full>
- Valable S, Barbier EL, Bernaudin M, Rousset S, Segebarth C, Petit E, et al. *In vivo* MRI tracking of exogenous monocytes/macrophages targeting brain tumors in a rat model of glioma. *Neuroimage*. 2008;40(2):973-83.
- Pannetier N, Lemasson B, Christen T, Tachrount M, Troprès I, Farion R, et al. Vessel size index measurements in a rat model of glioma: comparison of the dynamic (Gd) and steady-state (iron-oxide) susceptibility contrast MRI approaches. *NMR Biomed*. 2012;25(2):218-26.
- Benda P, Lightbody J, Sato G, Levine L, Sweet W. Differentiated rat glial cell strain in tissue culture. *Science*. 1968;161(3839):370-1.
- Grobben B, De Deyn PP, Slegers H. Rat C6 glioma as experimental model system for the study of glioblastoma growth and invasion. *Cell Tissue Res*. 2002;310(3):257-70.
- Morrone FB, Oliveira DL, Gamermann P, Stella J, Wofchuk S, Wink MR, et al. *In vivo* glioblastoma growth is reduced by apyrase activity in a rat glioma model. *BMC Cancer*. 2006; 23;6:226.
- Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med*. 2005;352(10):987-96.
- Silva AC, Oliveira TR, Mamani JB, Malheiros SM, Malavolta L, Pavon LF, et al. Application of hyperthermia induced by superparamagnetic iron oxide nanoparticles in glioma treatment. *Int J Nanomedicine*. 2011;6:591-603.
- van Landeghem FK, Maier-Hauff K, Jordan A, Hoffmann KT, Gneveckow U, Scholz R, et al. Post-mortem studies in glioblastoma patients treated with thermotherapy using magnetic nanoparticles. *Biomaterials*. 2009;30(1):52-7.
- Braganhol E, Huppel D, Bernardi A, Wink MR, Lenz G, Battastini AM. A comparative study of ectonucleotidase and P2 receptor mRNA profiles in C6 cell line cultures and C6 *ex vivo* glioma model. *Cell Tissue Res*. 2009;335(2):331-40.
- Puaux AL, Ong LC, Jin Y, Teh I, Hong M, Chow PK, et al. A comparison of imaging techniques to monitor tumor growth and cancer progression in living animals. *Int J Mol Imaging*. 2011;2011:321538.