

# Assessment of the tumoral microenvironment in the development of a glioblastoma rat model by in vivo MRI and ex vivo HRMAS

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## Target Audience

The present work may be addressed to people interested in testing animal models of brain tumor with magnetic resonance (MR) approaches and with interest in characterizing the tumoral and inflammatory microenvironment by using MRI parameters.

## Purpose

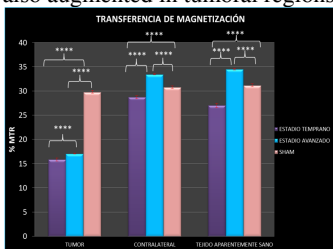
Alterations in inflammation and microvasculature underlying the most complicated brain pathologies, like cancer. A deeper characterization of the inflammatory and tumoral microenvironment would improve the diagnosis and prognosis of neoplastic process and the validation of new-targeted therapies. In this line, magnetic resonance imaging (MRI) is established as a powerful tool for obtaining structural, functional and molecular information<sup>1,2</sup>. Glioblastoma multiforme (GBM) is the most frequent, aggressive and lethal intracranial tumor. On the other hand, MR is a widely used and versatile technique in neurooncology<sup>3</sup> that provides information about blood flow/volume, edema and cellularity<sup>4</sup>. In this line, this research focuses on the identification of MR parameters that allow characterizing the evolution of the tumoral and inflammatory microenvironment in the glioma as it grows, in order to understand pathophysiological and biochemical basis of the coupling between inflammation and microvasculature. We used for that a glioblastoma multiforme animal model and structural, functional and molecular MR methodologies.

## Methods

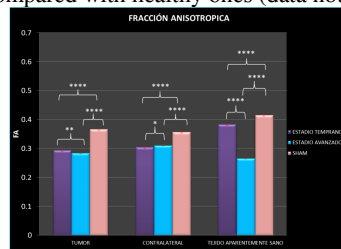
Experiments were carried out using sham adult male Wistar (n=5) and glioma bearing animals in two stages of the tumor growth (early, n=10, tumors  $\leq 50\mu\text{l}$ ; late, n=10 tumors  $\geq 75\mu\text{l}$ ). High-grade gliomas were induced in Wistar rats by stereotaxic injection of  $10^5$  C6 cells in the right caudate nucleus. MRI experiments were performed on a 7.0-T horizontal system equipped with a <sup>1</sup>H selective birdcage resonator. Imaging protocol was: magnetization transfer -MT- contrast imaging, (TR/TE = 2500/9.85ms, 50 pulses, 5.5  $\mu\text{T}$ , 1500 Hz); diffusion tensor imaging -DTI- (TR/TE= 3000/40ms,  $\Delta/\delta$ = 20/4ms, 7 directions, b values 0, 300 and 1400 s/mm<sup>2</sup>); and dynamic contrast enhanced -DCE- imaging (TR/TE= 100/6ms, 50 repetitions, and Gd-DTPA as extrinsic contrast agent). Images were computed on a pixel-by-pixel basis using My Map Analyzer (in-house software developed in Matlab) to obtain the parametric maps: magnetization transfer ratio (MTR), fractional anisotropy (FA) and mean diffusivity (MD). Color based images were analyzed in three brain regions: tumor, contralateral hemisphere and apparently healthy tissue in all experimental groups. Signal intensities of images from DCE studies were plotted versus time and the maximum enhancement of the curve was used as an indicator of the BBB permeability. At the end of the experiment, animals were euthanized with a 5 KW focalized microwave and the same ROIs assessed by MRI were excised (10 mg) and <sup>1</sup>H spectra were acquired in an HRMAS probe -CMPG sequences (TE = 44ms)- and processed with LcModel software.

## Results

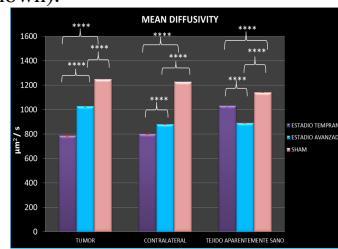
Figures 1-4 depict the main obtained results in the in vivo MRI studies performed. Values measured in all cases showed significant differences between the regions and groups of animals studied. Changes in MT and MD values are linked to the tumor progression, the concomitant inflammation and edema. Percentage of MTR (fig.1) decreased in tumor compared with non-tumoral regions, and increased in late stages indicating alteration in the macromolecular-bound proton pool associated with the pathology and its progression. Decreases in FA (fig.2) were correlated with the structural integrity loss in the brain because of the tumor's presence, while MD (fig.2) reflected the extension of the cellular swelling due to the inflammatory process taking place in the brain just as the tumor grows. A higher  $\Delta\text{SI}$  of larger tumors in DCE studies was in accordance with the expected increase in the disruption of the microvasculature in late stages. From ex vivo spectra, metabolites related to malignancy and necrosis, were also augmented in tumoral regions compared with healthy ones (data not shown).



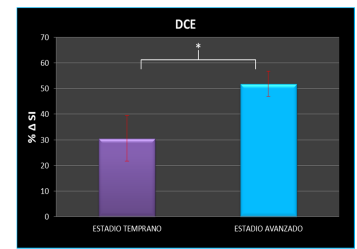
**Fig. 1.** MTR obtained results from MT studies by comparing early and advanced stages and sham animals



**Fig. 2.** FA obtained results from DTI studies by comparing early and advanced stages and sham animals



**Fig. 3.** MD obtained results from DTI studies by comparing early and advanced stages and sham animals



**Fig. 4.** Obtained results from MT studies by comparing early and advanced stages and sham animals

## Discussion

Multiparametric MRI studies in this glioma model have allowed establishing clear and significant differences between regions in the brain, associated with the development not only of the tumor but also of the associated inflammation. MTR and MD measurements have clearly indicated that apparently healthy regions in the brain are actually affected in the late stage of the tumor development. DCE analysis indicates either an increased in the vascular permeability in large tumors compared with smaller ones. Ex vivo obtained molecular data correlates with findings from in vivo studies.

## Conclusion

MR is able to assess the microenvironment during the tumoral growth allowing to identify parameters that can be used as surrogate markers not only in the characterization of the tumors but also in the validation of new therapies, specially those anti-inflammatory or anti-angiogenic targeted.

## References

<sup>1</sup>Lizarbe B, Benitez A. et.al, *Neuroimage*, 2013, 64:448-457; <sup>2</sup>Lopez-Larrubia P. et.al, Cap.12, 2012, ISBN 978-953-307-284-5; <sup>3</sup>Borges AR, Lopez-Larrubia P, et al. *AJNR. American Journal of neuroradiology*. 2012;33:24-36; <sup>4</sup>Calli C, et al. *European journal of radiology*. 2006;58:394-403