Longitudinal MR studies of human mammary carcinoma cell brain metastasis in nude mice at 7 Tesla

R. Simoes[1,2], A. Martinez[3], S. Cerdán[4], A. Sierra[3], C. Arús[1]

[1]Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, Barcelona, Spain, [2]Centro de Neurociências, Universidade de Coimbra, Coimbra, Portugal, [3]Centre d'Oncologia Molecular, Institut de Recerca Oncològica, Barcelona, Spain, [4]SIERMAC, Instituto de Investigaciones Biomédicas Alberto Sols UAM-CSIC, Madrid, Spain

INTRODUCTION: Animal models of brain metastasis using intracarotid injection of human cell lines in *nude mice* have been described for melanoma cells [1] and its characterization by MRI reported [2].

PURPOSE: To characterize the morphologic (MRI) and metabolic (MRS) development stages of brain metastasis in BALB/c *nude mice* induced by intracarotid injection of MDA-MB-435 human mammary carcinoma cells.

METHODS: 7 female 22-24g BALB/c nude mice were inoculated each with 10^6 MDA-MB-435/BRAIN human mammary carcinoma cells in the right internal carotid artery (i.c.) essentially as described [1]. MR was carried out at high-field (Bruker *PharmaScan*, 7.0 Tesla) using isoflurane anaesthesia at 1-2.5% in O2 and maintaining the respiratory frequency between 40-60 breaths/min. Tumour growth was characterized by T2, CE-T1 (Gd-DTPA, *i.v.* 0.2 mmol/Kg) and Diffusion weighted imaging (b=100, 400, 800 s/mm^2) and also by single voxel ^{1}H MRS (TE 35 and 136 ms). Volume rendering of brain metastasis was performed with *ImageJ*, *IDL* homemade software used to generate ADC maps (courtesy of Dr. ML García-Martín) and spectra processed with *MestreC*. Tentative pattern recognition analysis of selected spectra was carried out in a Decision Support System (DSS) developed for human brain tumour spectra classification: *INTERPRET SV v1.2d* [3]. Post-mortem histology studies were carried out by H&E and HSP27 staining.

RESULTS: Metastases were detected *in vivo* at different progression stages by T2 and CE-MRI (figures 1-A and 1-D) in 5 of 7 mice inoculated. Histology results agreed with these findings (figure 1-B). ADC maps showed higher values for metastasis than for non-afflicted tissue: 0.89±0.07 and 0.55±0.02 (x10^{-3} mm^2/s), respectively (figure 1-C). ^{1}H MRS allowed a preliminary metabolic characterization of the metastasis at each progression stage and the INTERPRET DSS was able to place the spectral patterns, at both short and long TE, in a clear progression towards malignancy, resembling human cases of healthy tissue being replaced by low grade glial and finally evolving towards an aggressive pattern (GBM/metastasis) (figures 1-E and 1-F).

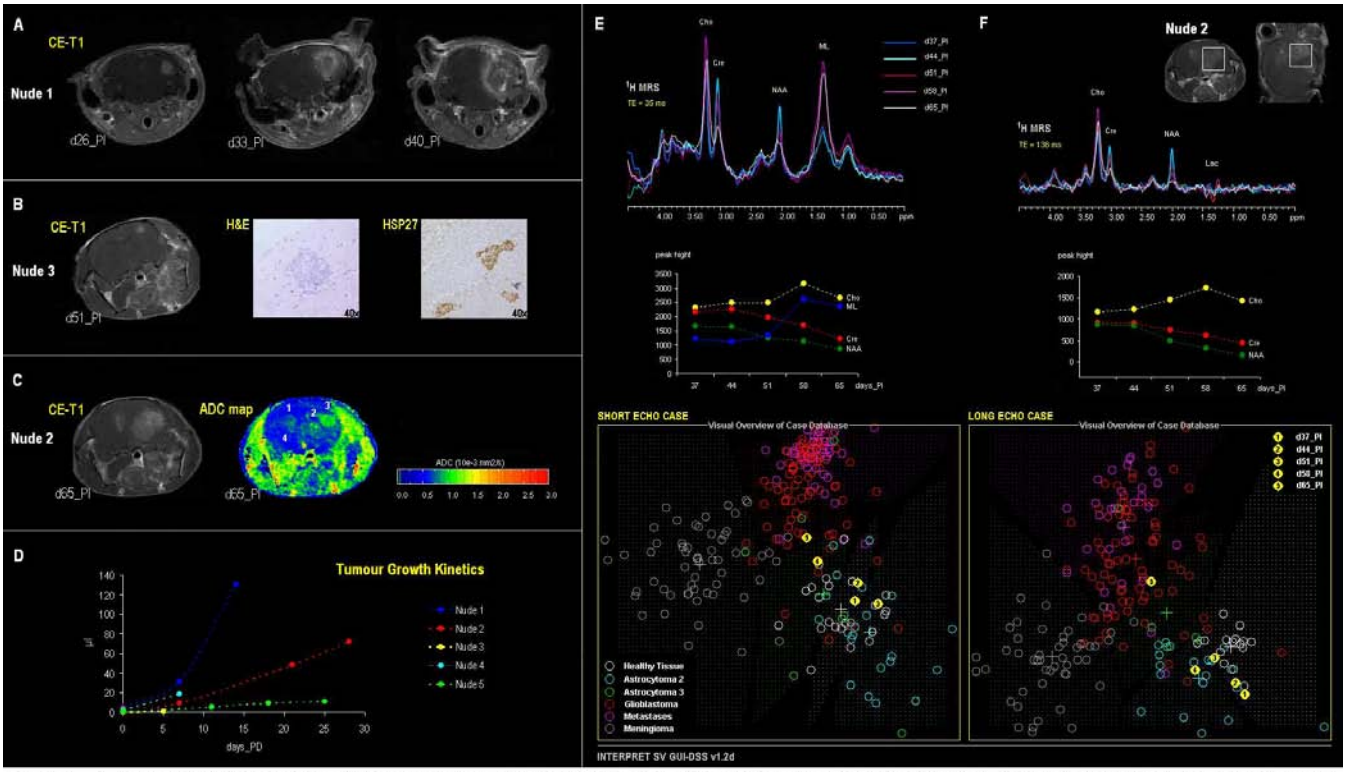

Figure 1 – A, Mouse 1: CE-T1 detection of brain metastasis (brighter regions) at different days post-injection (d_PI). B, Mouse 3: CE-T1 showing a small metastasis (0.6 µl) and post-mortem H&E and HSP27 staining of the fixed brain (metastasis seen as darker regions). C, Mouse 2: CE-T1 showing different metastasis and the ADC map of the same slice showing these lesions in green (1, 2, 3). ADC values (x10^{-3} mm^2/s): 1, 0.77; 2, 0.83; 3, 1.02; 4, 0.55. D, Tumour growth kinetics after first detection (post-detection, PD) for five mice (1-5). E and F, Longitudinal MRS studies of Mouse 2 at both short and long echo time, respectively. Top, spectra acquired at different days PI with voxel localization details in the right corner. Center, MRS peak intensity pattern changes for major metabolites as metastasis grow. Bottom, tentative pattern recognition analysis of the metastasis spectra (yellow dots) using the INTERPRET SV GUI-DSS v1.2d.

CONCLUSIONS: Brain Metastasis developed with heterogeneous growth kinetics after first detection (days 26-62 *post*-injection) in 71% of the animals studied. ADC values suggest low cellularity in these masses and histology studies support this assumption. MRS pattern changes indicate replacement of normal brain parenchyma by aggressive tumour cells (high *Cho*, low *NAA*) and automated pattern recognition analysis, using a non-optimized classifier, shows the potential of this approach as a non-invasive tool for tumour staging and grading in animal models.

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