UNRAVELING MOUSE GLIOMA HETEROGENEITY WITH DCE-T1 MRI AND 1H-CSI METABOLOME PATTERN PERTURBATION

R. V. Simões[1,2], M. L. García-Martín[3], T. Delgado-Goñi[1,4], S. Lope-Piedrafita[4,5], and C. Arús[1,4]

[1]Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Barcelona, Spain, [2]Bioquímica, Universidade de Coimbra, Coimbra, Coimbra, Portugal, [3]Resonancia Magnética, Clínica Nuestra Señora del Rosario, Madrid, Spain, [4]CIBER-BBN, Cerdanyola del Vallès, Spain, [5]Servei de Resonància Magnètica Nuclear, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain

INTRODUCTION: MRS-detected metabolome pattern perturbation has been described [1] and the effects of acute hyperglycemia in mouse gliomas reported by dynamic proton chemical shift imaging (^{1}H-CSI) [2]. The relevance of moderate brain hypothermia in this model has also been addressed [3].

PURPOSE: To investigate the heterogeneity of GL261 mouse glioma microenvironments *in vivo* by correlating the information obtained from dynamic contrast-enhanced T1 MRI (DCE-T1) with that from dynamic ^{1}H-CSI during acute an hyperglycemic challenge.

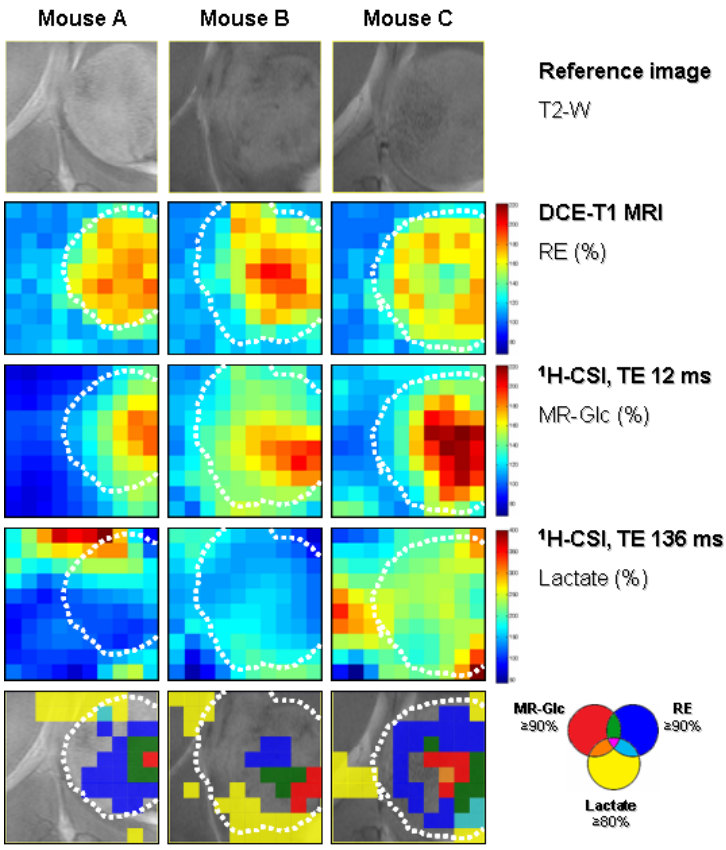

Figure 1 – *In vivo* monitoring of GL261 mouse gliomas in 3 mice (columns A, B and C), showing only the voxel windows used for each ^{1}H-CSI study (5.5x5.5mm). Rows (top to bottom): 1, T2-W reference image (tumor on the right side); 2, RE maps (%); 3, MR-Glc maps at 88min of acute hyperglycemia (%); Lactate maps at 66min of acute hyperglycemia (%); Fusion images – reference image overlaid with maximum intensity regions from each map (red, ≥90% of maximum MR-glc increase; blue, ≥90% of maximum RE; yellow, ≥80% maximum lactate increase; orange, red + yellow; green, red + blue; indigo, yellow + blue; pink, red + blue + yellow). Dotted white lines highlight the tumor borders on the maps. Scale: dark blue, minimum; *bordeaux*, maximum.

METHODS: Fifteen female C57BL6 mice (20-23g) were used in this work. Animals were anesthetized during the studies with isoflurane and kept at moderate hypothermia using a water blanket (28.5-29.5 ºC body temperature). Acute hyperglycemia was induced in mice as described in [3]. Six mice were used as controls for time-course blood glucose measurements, with a glucometer, during euglycemia (n=3, 3.5h) and hyperglycemia (n=3, 1.5h euglycemia + 3.5h hyperglycemia) – samples periodically collected from the tail vein. The remaining animals harbored a GL261 brain glioma, induced as described in [1], and were divided into 2 groups (I and II). Group I mice (n=6) were kept at either euglycemia (n=3, 1.5h) or hyperglycemia (n=3, 1.5h euglycemia + 1.3h hyperglycemia), and sacrificed immediately after by focused microwave irradiation (5kW). Their brains were removed and samples collected from normal/peritumoral parenchyma and tumor regions for *ex vivo* HR-MAS analysis at 9.4T. Micro-rotors (12 µl) were used and the studies carried out at room temperature, with 3000 Hz spinning rate, using both unsuppressed and water-suppressed pulse-and-acquire sequences. Group II mice (n=3: mice A, B and C), after being cannulated i.p. (26G catheter connected to a syringe loaded with 1.4M Glucose) and i.v. (30G 2-way catheter connected to 2 independent syringes loaded with saline and Gd-DTPA 50 mM) were studied *in vivo* at 7T as follows: 1, acquisition of reference T2-W images of the mouse brain; 2, DCE-T1 study, carried out as described in [4]; 3, 4 h washout period (animal left untouched inside the magnet), with visual confirmation of contrast agent clearance by periodic T1-W scans; 4, dynamic ^{1}H-CSI during acute hyperglycemic challenge. ^{1}H-CSI was performed as described in [3], over the same FOV geometry-position used in the previous DCE-T1 study. 2 reference CSI scans were acquired (12ms and 136 ms TE) followed by 4 additional scans during acute hyperglycemia (time post-injection of glucose): 22 min, 12 ms TE; 44 min, 12 ms TE; 66 min, 136 ms TE; 88 min, 12 ms TE. Glucose (α, 5.22 ppm; β, 4.63 ppm) and taurine (3.42 ppm) were quantified from HR-MAS data, using the unsuppressed water peak as reference (*MestRec* software). DCE-T1 resolution was reduced (from 128x128 to 32x32 matrix size) to match that of CSI studies and 2 types of maps were generated: time to maximum (min) and relative enhancement (RE, %), as in [4] (*IDL* home-written scripts). CSI data were processed as described [3] to generate relative intensity-change maps for MR-detectable glucose (MR-Glc: 3.43 ppm, 12 ms TE scans) and for lactate (1.32 ppm, 136 ms TE scans) (*3DiCSI* software and *MatLab* home-written scripts). For each study, maps were correlated pixel-by-pixel (Pearson's P-MC coefficient) and fusion images were generated to show simultaneously the regions of maximum intensity from each map overlaid on the reference image.

RESULTS: Control studies showed a maximum 5.0-fold blood glucose increase at 60 min post-induction of hyperglycemia (mM: euglycemia, 5.3±1.4; hyperglycemia, 26.9±1.8), which matches well with the average 5.8-fold glucose changes observed *ex vivo* by HR-MAS from independent GL261 tumors (µmol glucose/g water: euglycemia, 2.4±0.6; hyperglycemia, 14.0±2.1). Glucose changes monitored *in vivo* (MR-glc, Figure 1) during hyperglycemia (maximum 2.2-fold increase, in agreement with previous work [2,3]) were underestimated due to the large taurine contribution (12.8±2.4 µmol/g water, *ex vivo*) to the basal MR-Glc signal. Still, MR-Glc and RE maps showed significant linear correlations in the tumor regions of Mouse A and Mouse B (R= 0.56 and 0.61, respectively, $p<0.05$) but not in Mouse C. MR-Glc and lactate maps however, showed no significant correlations in these regions. Fusion images also showed that regions of higher RE overlapped partially (and sometimes poorly: Mouse C) with regions of higher MR-Glc increase while there was a spatial mismatch between MR-Glc and lactate maximum accumulations, as described by others between lactate and extracellular pH [5].

CONCLUSIONS: The distinct enhancement patterns given by DCE-T1 and ^{1}H-CSI maps indicate different vascularization/permeability and metabolism, respectively, in the different regions of each tumor. Other metabolite challenges should be tested to better understand *in vivo* sampled GL261 glioma regional heterogeneity.

ACKNOWLEDGMENTS: Mr. Oscar Tibaduisa, for help with *MatLab* scripts. Work funded by: *MEC* (Spain), SAF 2005-03650; *ISCIII* (Spain), PI051845; *FCT* (Portugal), SFRH/BD/17643/2004.

REFERENCES: [1] Simões *et al.* NMR Biomed. 2008; 21: 251-264; [2] Simões *et al.* Proc. ISMRM 2008 (Toronto,Canada) [3] Simões *et al.* Proc. ESMRMB 2008 (Valencia, Spain); [4] Rodríguez et al. Magn Reson Mater Phy. 2007; 20: 27-37. [5] Provent et al. Cancer Res. 2007; 67: 7638-7645