A HRMAS 1H NMR spectroscopy and Quantitative-PCR analysis of apoptosis in glioma

J. L. Griffin[1], C. Blenkiron[2], P. Valonen[3], C. Caldas[2], R. A. Kauppinen[4]

[1]Biochemistry, University of Cambridge, Cambridge, Cambridgeshire, United Kingdom, [2]Oncology, University of Cambridge, Cambridge, Cambridgeshire, United Kingdom, [3]A.I. Virtanen Institute for Molecular Sciences, University of Kuopio, Kuopio, NA, Finland, [4]Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom

Introduction: Programmed cell death (PCD) can readily be induced in BT4C glioma using ganciclovir/thymidine kinase gene therapy. We have previously shown that there is a marked increase in lipids, and in particular polyunsaturated fatty acids (PUFAs), during PCD [1]. However, the cause of this lipid accumulation and how it interrelates with PCD are still unknown. In conjunction with pattern recognition techniques, high resolution Magic Angle Spinning (HRMAS) ^{1}H NMR spectroscopy is one of the analytical techniques currently being used to produce metabolic fingerprints of intact biological tissues [2,3]. In this study we have examined the metabolic profile of rat glioma undergoing apoptosis, and compared metabolite changes with the transcription of key apoptotic genes using Quantitative (Q)-PCR.

Methods: *Animal handling and tissue preparation:* BT4C gliomas with HSV-tk gene were induced as described previously [1]. When the tumours reached diameter of 4-5 mm, GCV treatment was introduced for the duration of the study. Tumours were removed, following funnel freezing, at days 0, 4 and 8 (n=3 for each). Tumours were dissected frozen on dry ice for sampling for either HRMAS ^{1}H NMR spectroscopy followed by Q-PCR or Q-PCR alone.

HRMAS spectroscopy: Tumour tissue samples (5-10 mg) were examined using a HRMAS ^{1}H NMR probe interfaced with a 9.4 Tesla superconducting magnet. Spectra were acquired at 4 °C using a conventional solvent suppressed pulse/acquire sequence (4 kHz spinning rate).

Q-PCR: Q-PCR was performed using the Applied Biosciences "Assays On Demand" gene expression system and used to analyse transcription levels of the pro-apoptotic rat transcripts BCL-2 (Rn00591516), BAK-1 (Rn00587491), Caspase-9 (Rn00581212) and FAS (Rn00594913).

Pattern recognition: NMR spectra and the combined data set consisting of NMR spectra and transcription results were analysed using partial least squares (PLS) within the SIMCA package (Umetrics, Umea, Sweden).

Results and Discussion: Figure 1 shows the metabolic changes that accompany GCV/thymidine kinase induced PCD in glioma across the time period investigated. Marked increases in a number of lipid resonances were detected, particularly for those associated with unsaturated lipids. Despite the relatively high spinning speeds used during HRMAS, RNA degradation was minor in terms of the reproducibility of the Q-PCR results. We observed that FAS varied significantly across the three time points according to an ANOVA test (p=0.013). Cross correlating metabolite profiles against the selected mRNA changes, time of treatment and cell density of the tumour demonstrated that the PUFA resonances at 5.3 ppm (from vinyl protons) and 2.75 ppm (from bis-allylic protons) were highly correlated with the progression of PCD as measured by time of treatment and cell density, as well as inversely correlated with the expression of FAS. These observations indicate that metabolic profiling through HRMAS can be conducted alongside transcriptional analysis based techniques, suggesting that NMR could be used as a screening tool to identify tumours with distinctive metabolic phenotypes for further analysis by approaches that are more costly on a per sample basis (e.g. using DNA microarrays).

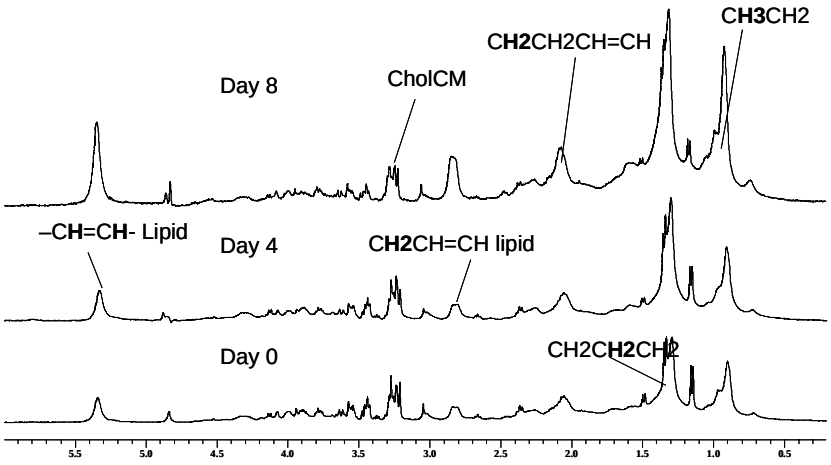

Figure 1: Three typical HRMAS ^{1}H NMR spectra obtained from tumours undergoing PCD. By day 8 large increases in a number of lipid resonances were clearly detected, and in particular those associated with unsaturated resonances. Bold type signifies proton positions associated with a given resonance.

References: 1. Griffin, J.L., Lehtimaki, K.K., Valonen, P.K., Grohn, O.H., Kettunen, M.I., Yla-Herttuala, S., Pitkanen, A., Nicholson, J.K. and Kauppinen, R.A. *Cancer Res.* **63(12)**, 3195-201 (2003). 2. Cheng, L.L., Lean, C.L., Bogdanova, A., Wright, S.C., Ackerman, J.L., Brady, T.J. and Garrido L. *Magn Reson Med.* **36**:653-8 (1996). 3. Millis, K., Weybright, P., Cambell, N., Fletcher, J.A., Fletcher, C.D. Cory, D.G. and Singer, S. *Magn Reson. Med.* **41**, 257-267 (1999).