Taurine – a potential natural biomarker of apoptosis in gliomas

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Introduction: Neoplastic cells accumulate by increased cellular proliferation and/or decreased cellular turnover, and dysregulation of apoptosis is common in many cancer cells. Hence, the apoptotic pathways are potential targets for new cancer therapies and if the outcome of such treatments is to increase apoptosis, new methods for *in vivo* monitoring of early treatment response, prior to tumour shrinkage, are needed. One such modality is magnetic resonance spectroscopy (MRS). High Resolution Magic Angle Spinning (HRMAS) enables ^{1}H spectra to be obtained from intact biopsy tissue and correlated with subsequent detailed histological analysis. Our study aim was to determine a biochemical correlate of apoptosis in gliomas that could provide a robust surrogate marker for apoptosis with *in vivo* MRS.

Methods: 41 astrocytoma biopsies of grades II, III and IV were obtained from consenting patients undergoing routine surgery. HRMAS measurements were performed on a 600 MHz Bruker Avance spectrometer (5 kHz spin rate, 4 °C) using 10-15 mg of liquid N_2 frozen tissue placed in a 50 µl insert. Presaturation and water spectra were acquired from each biopsy. Spectra were quantified using LCModel with the biopsy water signal as reference and a total of 23 metabolite and 18 lipid/macromolecule peaks [1]. Following HRMAS ^{1}H MRS, the biopsies were removed, embedded in OCT and re-frozen on cardice for cryostat sectioning. Biopsies were sectioned at 10 µm and arranged on microscope slides so that each slide had 5 sections to represent the length of the post-HRMAS biopsy. One slide from each biopsy underwent TUNEL staining for apoptosis detection and a consecutive slide underwent H&E staining for determination of cell density and percentage necrosis. TUNEL staining was manually counted and number of TUNEL positive nuclei per mm^2 determined. Individual metabolite profiles were compared with principal component analysis (PCA) of selected biochemicals (creatine, choline, glutamine, glutamate, glycerophosphocholine, glutathione, *myo*-inositol, N-acetyl aspartate, phosphocholine, taurine and ca. 0.9, 1.3, 2 and 2.8 ppm lipid/macromolecules) with the aim of finding a ^{1}H MRS metabolic signature of apoptosis. These biochemicals have previously been shown to remain stable over a period of ischemia during biopsy surgical excision [2].

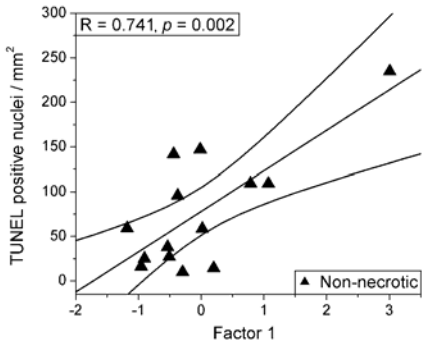

Figure 1

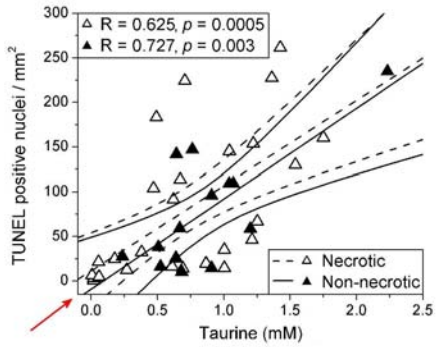

Figure 2

Results: Of the selected 10 metabolites and 4 lipid/macromolecules, one principal component (PC) was found to significantly correlate with TUNEL staining in non-necrotic biopsies (Figure 1). The PC loadings greater than 0.5 and in order of decreasing loading were ca. 0.9 ppm, ca. 1.3 ppm, phosphocholine, glutathione, taurine and ca. 2.8 ppm. No significant correlations could be found between the PC factors (with and without inclusion of the lipid/macromolecule peaks) and apoptosis in the necrotic biopsies. In contrast, a linear regression analysis between the individual metabolite concentrations and the number of TUNEL positive nuclei per mm^2 revealed a significant correlation for taurine in both non-necrotic and necrotic biopsy sample groups (Figure 2). The linear correlations have similar slopes and intercept very close to the origin (red arrow). Correlations were also found between TUNEL and the ca. 2.8, 1.3 and 0.9 ppm lipid/macromolecule peaks in the non-necrotic biopsies and phosphocholine in the necrotic biopsies.

Discussion: Our data suggest that for low-grade (i.e. non-necrotic) gliomas there is a pattern of metabolites and lipids that relates to apoptosis, and since whole biopsy tissue has been used, a similar pattern may likely be observed *in vivo*. However, as gliomas progress in malignancy they generally become necrotic and a positive correlation is found between the amount of necrosis and the ^{1}H MRS lipid signals [3]. The variability caused by the presence of necrosis and lipid signals, combined with decreasing cellular density with increasing necrosis, most likely explains the lack of any correlation between the PC factors and apoptosis in the necrotic biopsies. A PC analysis of just the metabolites (i.e. excluding the lipid/macromolecules that are strongly associated with necrosis) also did not result in any correlation with apoptosis. In contrast, our analysis of individual metabolites suggests that the quantified taurine signal in astrocytoma spectra is a biomarker for tumor apoptosis and is independent of tumor necrotic status. Taurine has been previously implicated in the mechanism of cell shrinkage during apoptosis, with a basal release of taurine shown in different cell types [4,5]. Other studies have shown an accumulation of the polyunsaturated fatty acid peak at ca. 2.8 ppm during gene therapy-induced apoptosis in experimental gliomas [6], and we find a similar correlation between apoptosis and ca. 2.8 ppm peak, but in the non-necrotic biopsies only.

In conclusion, the strong correlation between ^{1}H MRS taurine and TUNEL-stained apoptotic nuclei in both non-necrotic and necrotic human brain tumor biopsies suggests that taurine is a natural biomarker of apoptosis in glial tumors, and that non-invasive *in vivo* ^{1}H MRS could be a useful technique for monitoring tumor apoptosis in the clinic. Further studies are now required to confirm these results for taurine *in vivo*.

References

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