An investigation of human brain tumor lipids by HRMAS ^{1}H MRS and histological analysis

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Introduction

NMR-visible mobile lipids have been associated with tumor aggression [1], but there is still debate as to the exact origin of the lipid signals found in ^{1}H MR spectra. The current consensus is that ^{1}H MRS lipid signals arise from cytoplasmic lipid droplets found in tumor cells. Tumor model [2] and tumor cell [3] studies have suggested a correlation between lipid droplets and ^{1}H MRS lipid signals. However, one study concluded that there was no correlation between Nile Red staining of the lipid droplets and ^{1}H MRS lipid signals [4]. In this study we have performed HRMAS ^{1}H MRS and Nile Red staining on human brain tumor biopsies to further investigate the origin of NMR-visible mobile lipids.

Methods

18 astrocytoma biopsies of grades II, III and IV were obtained from consenting patients undergoing routine surgery for their tumors. ^{1}H MRS measurements were performed on a 600 MHz Bruker Avance spectrometer, using an HRMAS probe spun at 5000 Hz and 4 °C. 10 - 15 mg of liquid N_2 frozen tissue were placed in a 50 µl insert and the remaining space filled with D_2O. Presaturation and water spectra were acquired from each biopsy, using a repetition time of ~8 s. All biopsy presat spectra were quantified using LCModel with the biopsy water signal as reference and a total of 23 metabolite and 18 lipid/macromolecule peaks [5]. Following ^{1}H MRS, the biopsy samples 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 entire length of the biopsy. A drop of Nile Red, prepared in glycerol, was added to each section which was incubated in the dark for 5 mins prior to observation by fluorescence microscopy. Composite images of each section acquired at x10 magnification were analysed using Olympus analySIS software for particle detection. The lipid droplet size distribution and the average total lipid droplets per mm^2 were determined for each biopsy.

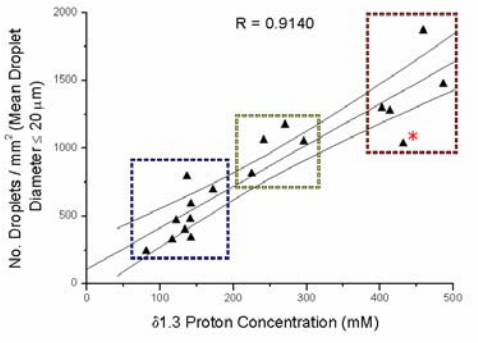

Figure 1

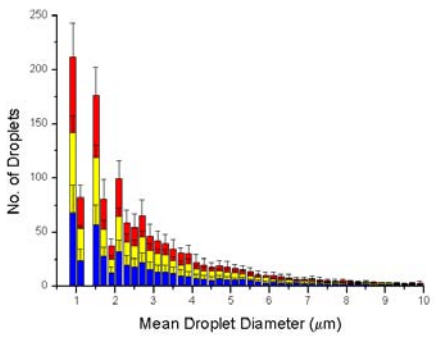

Figure 2

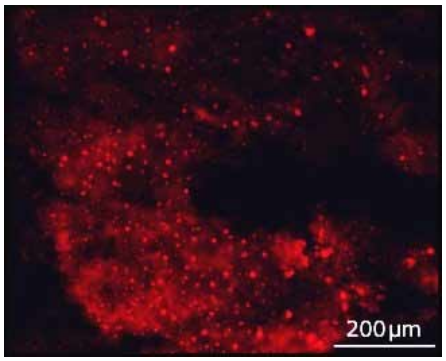

Figure 3

Results

Figure 1 shows a plot and linear regression analysis (± 95% confidence interval) of the estimated total number of lipid droplets per mm^2 (with diameter ≤ 20 µm) versus the δ1.3 lipid/macromolecule proton concentration determined by HRMAS. The coloured boxes highlight three groups of biopsies for which the average lipid droplet distribution (displayed over the range 0 to 10 µm in bins of 0.2 µm) is given in Figure 2. Figure 3 shows Nile Red staining of lipid droplets taken from the biopsy indicated by the red asterisk in Figure 1.

Discussion

The strong correlation between number of Nile Red stained droplets and δ1.3 lipid/macromolecule proton concentration supports the view that ^{1}H MRS visible lipid signals in human brain tumors arise from cytoplasmic lipid droplets, the same as observed in cell and tumor model studies [2,3]. Comparing the mean data in Figures 1 and 2, relative to the blue group of biopsies, we observe an increase over all droplet sizes of 2.0 ± 0.2 (yellow group, mean ± SD) and 2.7 ± 0.3 (red group), for average increases in the δ1.3 lipid/macromolecule proton concentrations of 2.0 (yellow) and 3.3 (red) respectively. The relative proportion of number of droplets in the three groups is constant up to droplet sizes of 50 µm, a size beyond which there are negligible number of droplets. Thus, the increase in NMR observed lipid concentrations is simply an increase in the number of droplets and not due to changes in the distribution of the droplet size.

In conclusion, we have shown a strong correlation between ^{1}H MRS visible lipid signals and Nile Red histological staining of human brain tumor biopsies, from the same tissue samples, giving good evidence that the origin of NMR-visible mobile lipids is cytoplasmic lipid droplets.

References

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