The analysis of lipid and macromolecule signals in HR-MAS data reveals information on the nature of cytoplasmic lipid droplets

M. Wilson[1,2], G. M. Reynolds[3], R. A. Kauppinen[4], T. N. Arvanitis[2,3], and A. C. Peet[1,2]

[1]Cancer Sciences, University of Birmingham, Birmingham, West Midlands, United Kingdom, [2]Oncology, Birmingham Childrens Hospital Foundation Trust, Birmingham, West Midlands, United Kingdom, [3]School of Electronic, Electrical & Computer Engineering, University of Birmingham, Birmingham, West Midlands, United Kingdom, [4]Dartmouth Medial School, Dartmouth College, Hanover, New Hampshire, United States

Introduction

Cytoplasmic lipid droplets (CLD) are associated with tumour drug resistance and cell death[1,2]. These signals can be observed in-vivo using short echo MRS providing a promising clinical tool for predicting and monitoring tumour response to treatment. One of the key factors determining the success of this technique is the accurate measurement of CLD signals. Whilst large differences in CLD signals can be easily detected, smaller changes are error prone as CLD signals are broad and overlap heavily with signals from other molecules, such as proteins.

1H High-Resolution Magic Angle Spinning (HR-MAS) is an in-vitro technique, which greatly improves the spectral resolution of the CLD and macromolecule signals. The improved resolution of these signals offers two advantages: firstly, knowledge gained can be incorporated into fitting algorithms to improve the measurement of CLD signals using in-vivo MRS and secondly, analysis may offer insight into the composition of the CLDs, improving the understanding of the mechanisms behind the formation and function of CLDs in tumour cells.

The aim of this study is to investigate the lipid and macromolecule signals present in the HR-MAS spectra in a panel of tumour cell lines. Firstly a lineshape model is developed for lipid and macromolecule signals which are then incorporated into an automated fitting algorithm to examine correlations between broad signals aiding the assignment of CLDs.

Method

HR-MAS was performed on a panel of 18 cell lines, derived from a range of childhood nervous system tumours (13-neuroblastoma, 2-medulloblastoma, 2-retinoblastoma and 1-ST-PNET). Three or more repeats were available for each cell line. The broad signals at 5.3 and 3.0ppm were chosen to be used as measures of CLDs and protein signals respectively, since the peaks can be clearly resolved from other signals. The intensity of these signals was estimated for each cell line using a modified version of the TARQUIN algorithm[3]. A simple Gaussian lineshape model was assumed at this stage in the analysis.

Cell lines with the highest and lowest ratio of CLD/macromolecule were identified and used in the next stage of the analysis. An in-house implementation of the Total Lineshape (TLS) fitting algorithm was used to build a lineshape model of the CLD and macromolecule peaks between 0.7 and 1.9ppm for the cell lines with high and low levels of CLDs. These models were then imported into the TARQUIN algorithm and used to re-analyse all the cell line data. Fitted signals were divided by the intensity of the macromolecular peak at 3.0ppm and we investigated correlations between the fitted broad components.

Results and Discussion

The cell lines Be2M17 (neuroblastoma) and WERI (retinoblastoma) were found to have the highest and lowest CLD/macromolecule ratios respectively and the high levels of CLD in Be2M17 were confirmed using nile red staining. Lineshape models were constructed using these lines for the following peaks: Lip 0.9, MM 0.9, Lip 1.3, MM 1.3, Lip 1.6 and MM 1.7. Figure 1 shows an example fit using the lineshape models while the good match between signal and fit validates the approach taken.

Figure 2 shows that all of the fitted lipid components are highly correlated to each other implying that there is little difference between the composition of CLDs across the cell lines investigated. Of particular interest is the observation that all lipid peaks are well correlated with the -CH= peak at 5.3ppm implying that a significant proportion of the CLDs are unsaturated.

Figure 3 shows that the macromolecular components are also correlated but have greater spread when compared to figure 2. This may imply that the ratio between macromolecular peaks is cell line dependant.

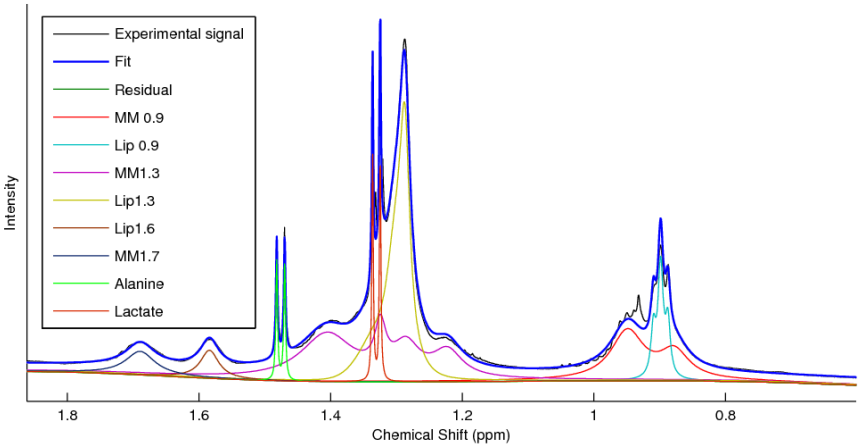

Figure 1 An example fit to a cell line with equal levels of CLD and macromolecules.

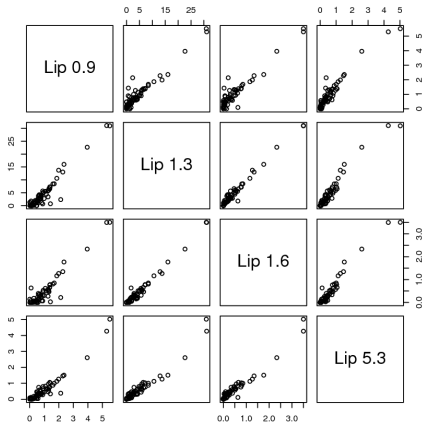

Figure 2 A scatter plot of fitted CLD signals.

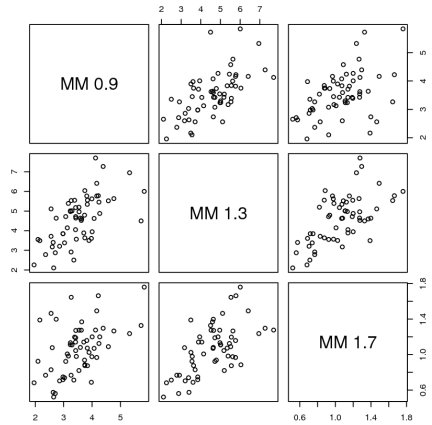

Figure 3 A scatter plot of fitted MM signals.

Conclusion

The proposed method of fitting yields stable results for HR-MAS data despite the strong overlap of signals at 0.9 and 1.3ppm. The high correlation between the CLD components analysed implies that the ratio between the various signals is fixed which may indicate the CLD signals originate from a homogenous group of species. This prior knowledge can potentially be used to improve the accuracy of CLD measurements both in-vitro and in-vivo.

Acknowledgements

This work was funded by the Medial Research Council and the EU Framework 6 project eTUMOUR.

References

1. Morjani H et al. Int J Cancer. 2001 Oct;94(2):157-65.

2. Griffin JL, et al. Cancer Res. 2003 Jun;63(12):3195-201.

3. Reynolds G et al. MRM 2006 Dec;56(6):1211-9.