Correlative 2D HPLC Maps of Glutathione for 13C Spectroscopy

M.P. Gamcsik (UNC, Biomedical Engineering), P.E. Thelwall, D. Clark (UF, AMRIS), N.E. Simpson (UF, Endocrinology), T. Schehl (UNC, Biomedical Engineering), J.J. Flint (UF, Neuroscience), J.M. Macdonald (UNC, Biomedical Engineering), S.J. Blackband (UF, Neuroscience)

Introduction

Over the past two years and reported to the NHMFL last year as part of an NIH-funded pilot project, we succeeded in developing a magnetic resonance method combined with stable isotope infusion to monitor glutathione metabolism in vivo in subcutaneous tumors in rats (1), reporting chemical shift images of the glutathione (GSH) distribution in the tissue and for the first time demonstrating a heterogeneous distribution of GSH. To support these studies and understand the origins of this heterogeneity, additional imaging (Gd-contrast enhanced) has been performed and illustrates heterogeneity. In this report, we present correlative studies to assess glutathione heterogeneity, level of apoptosis and determine the activity profiles of enzymes involved in glutathione metabolism in support of the MR data.

Experimental

A new multiblade slicing technique was developed so that an entire tumor cross section could be examined. Flash-frozen FSA tumors were then sliced into these uniform tissue sections and extracted and GSH and oxidized glutathione disulfide (GSSG) was determined by HPLC. Only complete square sections were used in order to eliminate the need to correct for tissue wet weight. This was desirable as weighing the samples is time consuming and can lead to oxidation of GSH to GSSG. The drawback is that partial tissue sections at the very edge of the tumor were not assayed. Future studies will attempt to correct for these partial sections.

Results and Discussion

The results of one of these studies from the analyses of 44 1.5 x 1.5 x 0.5 cm tumor sections are shown in Figure 1. The edge of sections, roughly corresponding to the edge of the tumor tissue is denoted by the dashed line. The variation of GSH and GSSG across the tissue sections was ‘mapped’ by converting the assayed levels into a 2D image using ImageJ software. Color images of a data set are shown in Figure 1 and show the GSH levels are greatest for regions on or near the periphery of the tumor. This is consistent with our in vivo studies and histological staining of ex vivo tumor sections. The GSSG levels are fairly uniform throughout the tumor so it does not appear that areas showing lower GSH show higher GSSG.

Conclusions

A new tumor sectioning technique allows us to generate pixelated ‘images’ of metabolite concentrations across the tumor obtained from HPLC data. Interestingly these images do not show enhanced GSSG levels in areas where GSH is low which is important since our MR methods currently cannot distinguish between GSH and GSSG. Further studies will directly correlate MR and HPLC data on the same tumors.

Acknowledgements

Support was provided by NIH grant RO1 CA1 14365, P41 RR 16105 and the National High Magnetic Field Laboratory.

References