Metabolomic analysis of *ex-vivo* ^{1}H MRS profiles from experimental tumours

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INTRODUCTION:

As the complete genome of many species is now available in the public domain, the focus is now shifted to functional genomics in which genomic studies are often followed by proteomics and metabolomic analysis. Metabolomics is the study of global variation of all metabolites in cells or tissues. The huge volume of data, generated by tissue metabolite profiles, needs a robust and unbiased method of analysis for determining changes in the metabolites. Pattern recognition methods have been used in previous studies to recognise the type, nature and grades of brain tumours. Here, we present the use of pattern recognition methods to differentiate experimental tumours by analysis of ^{1}H NMR spectra of perchloric acid tumour extracts.

METHODS:

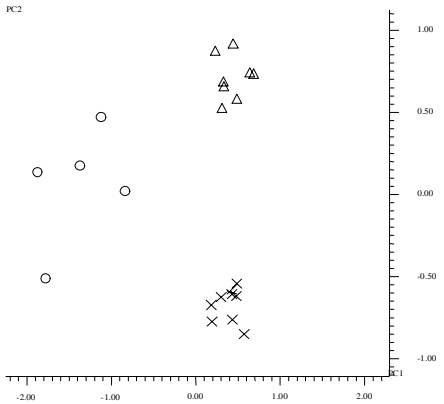
Tumour models: HT-29 xenografts were grown subcutaneously to an average volume of 500mm^3 in female MFI nude mice (n=9). RIF-1 tumours were grown subcutaneously in female C3H mice (n=8) to an average volume of 500mm^3. Mammary tumours developed spontaneously in female C-neu oncomice[3] between 18-20weeks of age; the average volume was 580mm^3 (n=5).

Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H NMR): Snap-frozen tumours were extracted in 6% PCA. Neutralised extracts were freeze-dried and reconstituted in D_2O and 0.5ml of the extracts was placed in 5mm NMR tubes. ^{1}H NMR spectra were obtained using a Bruker 600MHz spectrometer. The water resonance was suppressed by using gated irradiation centred on the water frequency. Sodium 3-trimethylsilyl-2, 2, 3, 3-tetradeuteropropionate (TSP) was added to the samples for chemical shift calibration. Immediately before the NMR analysis, the pH was readjusted to 7 with PCA or KOH.

Pattern Recognition Analysis: Proton metabolic profiles from 0 to 4 ppm with positive intensities scaled to total intensity in the spectrum were sampled by generating rectangular buckets with a width of 0.005 ppm. Principal Component Analysis (PCA) included Pareto scaling with a minimum variability of 5% and principal components (PCs) were selected by minimum explained variance of 99.9%.

RESULTS:

Figure 1 shows the PC1 and PC2 scores plot of the ^{1}H MRS data obtained from oncomouse mammary carcinomas, RIF-1 and HT-29 tumours. The plot shows a clear separation of the PC scores for all the three tumour models. The loadsplot has shown that these separations are caused by the chemical shifts around the regions of 1.33-1.34 and 3.22-3.25.



O – Oncomouse mammary carcinomas,
Δ – RIF-1, X – HT29

Figure 1. Plot of PC1 and PC2 scores

DISCUSSION:

The huge volume of data generated by metabolomics needs unbiased and robust methods for quantitative assessment of changes in metabolites. PCA analysis is a popular method for pattern recognition of metabolite profiles. In this study the application of PCA analysis clearly separated the three different tumours from each other in the scatter plot of PC1 and PC2 scores. This information about the separations are given by the loadings plot which revealed that these separations are caused by variations of metabolites appearing at 1.33 (lactate) and 3.23 (choline containing compounds) ppm. Tumours have more lactate than normal tissues and the enhanced lactate metabolism in tumours is still a matter of discussion[4]. The enhanced signal of choline-containing compounds which appears in the ^{1}H NMR spectrum around 3.20 to 3.23 ppm mainly consists of choline, phosphocholine (PC) and glycero-phosphocholine (GPC). The tumours used in this study have different modes of tumourigenesis, different growth rates and patterns. This study used volume-matched tumours and the average tumour volume for this study was around 500mm^3. RIF-1 reached the required volume in 2 weeks, HT29 in 3-4 weeks and the oncomouse mammary carcinoma in 3-6 weeks. The utilisations of the glycolytic pathway of each tumour type, as indicated by their lactate content, and their tendency towards proliferation and malignancy, which are associated with choline-containing compounds, seem to be markedly varied for these tumours even though the tumour volumes were similar.

REFERENCES:

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