Metabolite profiling of a gene knockout in a human tumor cell line

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Introduction

^{1}H MR spectroscopy has been widely used for metabolite profiling in tumours, both by *in vivo* MRS and high-resolution spectroscopy of tissue extracts [1]. Thus, it offers the potential to determine biomarkers of direct clinical relevance. However, equally interesting is the possibility to use this metabolomic approach to investigate which metabolites are affected in a highly-controlled system, and then use this to derive hypotheses about the possible biochemical mechanisms that relate to a specific aspect of oncogenesis. For example, Griffiths et al. used MRS profiling to conclude that hypoxia-inducible factor (HIF) deletion also caused an unexpected reduction in ATP levels, possibly by disrupting glycine synthesis [2]. In this study, we have used p300 knockout cells – p300 is a transcriptional coactivator with a wide range of cellular targets, including HIF. It has recently been shown to play a role in the regulation of apoptosis [3], and is of potential interest as a drug target.

Methods

A human carcinoma cell line (HCT116) was selected (wild type – WT), and three independent targeted p300 knockout cell lines (KO) were created by homologous recombination. Cells were grown as adherent cultures on 14 cm plates using both normal and serum-free medium, and metabolites were extracted in ice-cold 6% perchloric acid. Neutralized extracts were then profiled by ^{1}H NMR spectroscopy using a Varian Inova 400 MHz spectrometer. (The identity of some metabolites was also confirmed using ^{31}P NMR spectroscopy.) Spectra were phased, baseline-corrected, and then data-reduced by dividing into 0.01 ppm 'bins' between δ 9.5—5 and δ 4.5—0.5 ppm. This reduced each spectrum to a vector of length 850, which was then suitable for further data analysis. Principal components analysis (PCA) was performed after log transformation of the data using log (n + c), with a value of 'c' chosen to stabilize the variance across the entire data range.

Results and discussion

PCA analysis of the data gave complete separation between the WT and KO strains along PC 1. A number of different metabolites could be identified from the loadings as particularly associated with p300 status: e.g. nicotinamide mononucleotide (high in KO), and glutamate and glutamine (high in WT). In addition, visual inspection of the spectra indicated that glycerophosphocholine (GPC) concentrations were increased in p300 KO cells. Although metabolomics is extremely good at detecting phenotypic differences between samples with different genotypes, there is as yet no rational way of deciding whether these are direct consequences of the gene deletion or if they result from indirect effects, which may be trivial and uninteresting. The KO cells grow more slowly than the WT cells, and thus metabolic differences may well be an indirect consequence of growth rate. To test this, we also grew the cells in serum-free medium, in which the growth rates are reversed (KO grows faster than WT). A PCA plot of the data now shows that the choice of medium is – unsurprisingly – the major source of variance in the data, with samples separated along PC 1. However, the samples are still perfectly separated into WT and KO along PC2, and hence we can infer that there is a common set of metabolites that is affected by p300 status independently of the growth medium. It is also clear that the relative effects of p300 deletion are greater under serum-free conditions. The clearest biomarker of p300 deletion was GPC, which was increased in the p300 KO cell lines under both normal and serum-free conditions. Phosphocholine was the clearest differentiator between normal and serum-free samples.

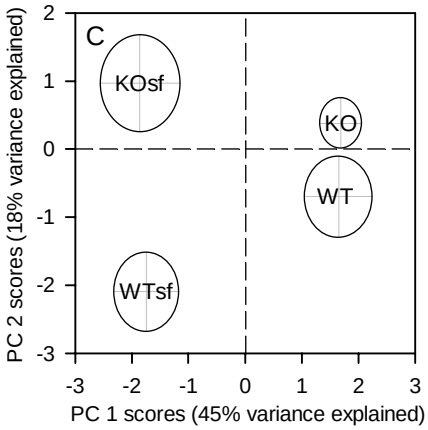

Figure 1. PCA plot of 400 MHz ^{1}H NMR data for p300 WT and KO cells, under both normal and serum-free (SF) conditions. Centre of each ellipse represents mean and the size represents +/- SE.

Thus, we have in this experiment effectively modulated the biological response of the different cell lines, which then gives us a rational basis for selecting a reduced set of metabolites for further study. (The principle would be identical for protein or gene transcript profiles.)

Conclusions

Samples were readily classified according to p300 status using metabolomics. A number of metabolites contributed to distinguishing the two groups, but experimental modulation of cell growth conditions showed that some of these metabolite changes were not observed under a different physiological context. GPC was the clearest biomarker of p300 deletion. Ongoing analysis of transcript profiles using DNA microarrays may help to explain the observed metabolite changes.

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