Metabolite-Metabolite correlation maps: A novel method to understand metabolic pathways

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INTRODUCTION: Metabolomics studies the global metabolites in a cell, tissue or organism, and plays vital role in understanding the phenotypic functionalities of cells. Novel bioinformatic methods such as metabolite-metabolite correlation analysis (1,2) are being developed to address problems in metabolomics. NMR-based metabolomic profiles are useful methods for metabolomic studies of cell function. We have recently developed a novel heat map method for analysing metabolite-metabolite correlations derived from ^{1}H NMR-based metabolomics data. Each metabolite concentration from the intracellular ^{1}H NMR data is plotted against all the other metabolite concentrations using optimized statistical methods for normalisation, multiple testing and false discovery rates. These correlations maps are helpful in understanding perturbed metabolic pathways in the cells due to gene modifications, enzymatic modulations (inhibition/over-expression), toxic and/or drug effects and nutrient supply. In this study we show the application of metabolite-metabolite correlation maps in Oncogene Induced Senescence (OIS).

METHODS: Cells and ^{1}H NMR data acquisition: IMR90 HDFs were cultured in DMEM, 10% FBS. OIS was induced using tamoxifen-regulatable ER-Ras (H-RasV12). Each cohort of HDFs (n=47) was grown to a population of 10^6 cells before harvesting with perchloric acid. After neutralisation and lyophilisation these samples were re-suspended in D_2O for ^{1}H NMR analysis. 500 µL of the sample was placed in 5mm NMR tubes. ^{1}H NMR spectroscopy of the samples was performed with a solvent-suppression sequence on a 600 MHz Bruker AVANCE NMR spectrometer. TSP was used for quantitation and protein content for normalisation of the metabolite data. Metabolite concentrations estimated from ^{1}H NMR data were used for constructing the correlation heat maps.

Statistical methods: Metabolite concentrations of the replicates within each treatment group were normalised so as to show the same median-absolute value (MAV), with the median also corrected to be equal in each sample within the same group. Correlations of metabolite levels within each group were calculated with the non-parametric Spearman test. To correct for multiple testing, the false discovery rate (the expected proportion of false discoveries among the rejected hypotheses) was controlled using the Benjamini-Hochberg method. All statistical analysis was performed using the open source statistical software R, and the LIMMA array analysis package (available through the Bioconductor repository).

RESULTS: Metabolite-metabolite correlation maps from ^{1}H NMR data of normally-growing and RAS-induced senescent HDFs are shown in Figure 1 A and B respectively, plotted according to the modularity of the main metabolomic pathways in which they participate. Normally growing cells showed mostly positive correlations even with off-diagonal metabolites, with the sole exception of choline metabolites which showed a negative correlation with glutamate, leucine and tyrosine. In contrast, Oncogenic RAS-induced senescent cells showed mainly negative correlations with other metabolites.

DISCUSSION AND CONCLUSIONS: The mainly positive correlations in the heatmap for normally-growing cells suggest that most of their major metabolic pathways are functioning without negative feedback controls. In contrast, the RAS-induced senescent cells showed many negative correlations, implying the control of pathways by negative feedback. Even lactate, which showed a negative correlation with choline and GPC in normally growing cells, showed more negative correlation with choline and PC in RAS-induced senescent cells. Positive correlations in the amino acid module in normally growing cells changed to negative correlations with the choline metabolite module and energy metabolite modules, suggesting that control of the amino acid metabolic pathways drastically changed in RAS-induced senescence.

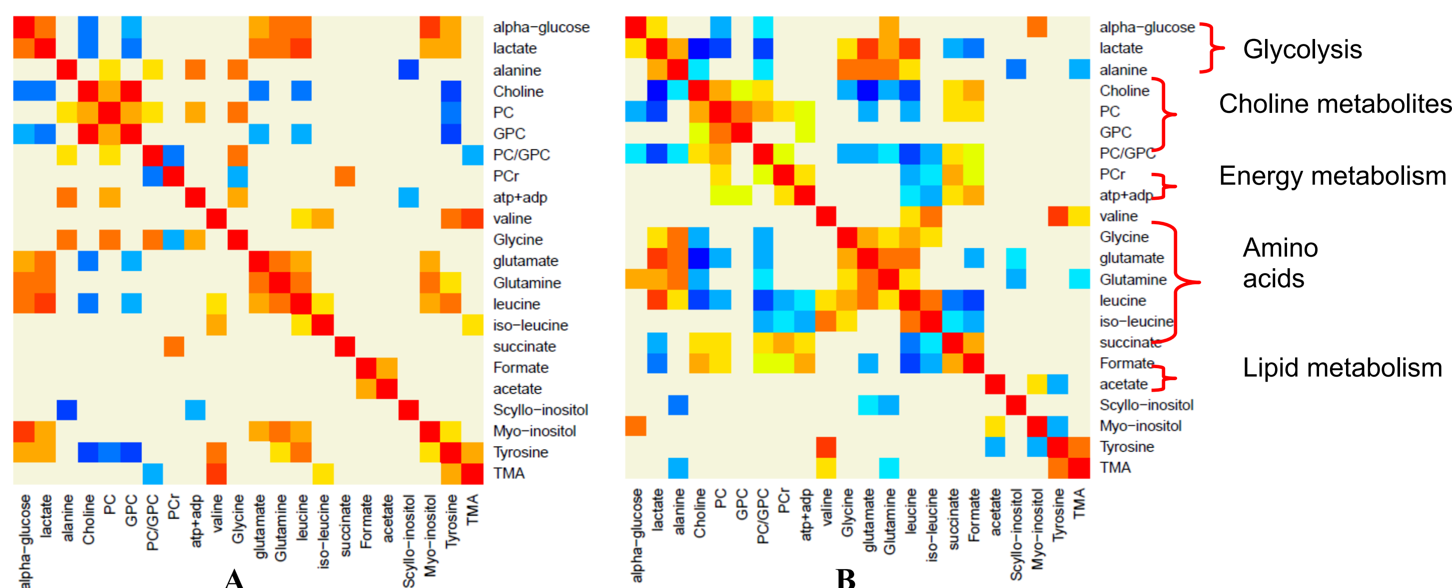

Figure 1. Metabolite-metabolite correlation map from normal growing (A) and ER-RAS induced senescence (B) cells.

References: 1. Steur R et al., Biochemical Soc. Trans. (2003) 31, 1476. 2. Fiehn O et al., Phytochemistry (2003) 62, 875

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