A ^{1}H NMR-based metabolomics study of Cellular Senescence, Quiescence and Transformation

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INTRODUCTION: Metabolomics is the study of the global variation of metabolites in a cell or organism. Senescence, which is a permanent cell cycle arrest, is thought to act as a fail-safe mechanism to prevent the malignant transformation of pre-neoplastic cell populations. Senescence can be induced in human diploid fibroblasts (HDFs) by activating oncogenes such as Ras or MEK, or by treating with DNA damaging agents (e.g. etoposide). Replicative senescence can be induced by repeated passage of cells, thus shortening the telomeres. Oncogene induced senescence (OIS) is a tumour suppressing mechanism that shares conceptual and therapeutic similarities with the apoptosis machinery[1]. SA-β-gal activity, elevated p53 and p16 protein levels, coupled with morphological changes and gene expression, are used as senescence markers, though reliable metabolic markers for senescence are still required. Senescent cells are metabolically active, even though their cell growth is stably suppressed. Quiescence, a readily reversible cell cycle arrest, can be induced by either serum starvation or by cell confluence. In contrast, E1a in conjunction with RAS can by-pass senescence and induce malignant transformation of the HDFs. There is little information available about the metabolism of quiescent and transformed cells. Hence we have undertaken a ^{1}H NMR based metabolomics study of oncogenic Ras and DNA damage (etoposide)–induced senescence, along with replicative senescence, quiescence and malignant transformation (by E1a/Ras) in HDFs. The aim was to see whether senescence induced by a variety of methods led to a similar metabolic state.

METHODS: IMR90 HDFs were cultured in DMEM supplemented with 10% FBS and antibiotics. Ectopic genes were introduced to cells by retrovirally mediated gene transfer. OIS was induced by oncogenic H-RasV12 (Ras) expression, while transformation was induced by E1A and Ras co-expression. To generate DNA damage-induced senescence, cells were treated with 100μM etoposide for 2 days, and maintained for an additional 5 days. Passage numbers of around 35 were used to induce replicative senescence. Quiescence was induced by serum starvation for 3 days. Each cohort of HDFs was grown to a population of 10^6 cells before harvesting with perchloric acid, which extracted their water-soluble metabolites. After neutralisation and lyophilisation these samples were re-suspended in D_2O for ^{1}H NMR analysis. 500 μL of the extracts was placed in 5mm NMR tubes. ^{1}H NMR spectroscopy of perchloric acid extracts of cells was performed with a solvent-suppression sequence on a 600 MHz Bruker AVANCE NMR spectrometer. Time domain data were Fourier transformed and pre-processing of NMR spectra included zero and first order phase correction. Proton metabolic profiles from 0.5 to 10ppm (water signal region from 4.00 to 5.20ppm was excluded) with positive intensities scaled to total intensity in the spectrum were sampled by generating rectangular buckets with a width of 0.01ppm. A generalized logarithmic (glog) transformation[2] was applied on the binned data to minimize the technical variance in sample preparation and data acquisition. Unsupervised pattern recognition was performed by Principal Component Analysis (PCA) of the binned data using the SIMCA software package.

RESULTS: Application of generalized logarithmic transformation to the binned data improved the cell group classifications in PCA analysis. The PCA scores plot (Figure 1) showed a clear separation of the normally growing cell groups from the OIS cells, DNA damage-induced senescent cells and replicative senescent cells, transformed (E1a/RAS expressing) HDFs, indicating the perturbed metabolism of these cells. Quiescent cells did not show any separation from normally growing HDFs on PCA analysis, indicating their overall metabolism was not perturbed.

DISCUSSION: This study shows that ^{1}H NMR based metabolomics is a valuable tool to analyse the phenotypic effects of gene perturbations in cells, such as OIS, DNA damage or malignant transformations. Clear separation of cell groups showed on PCA analysis indicates that the metabolism of senescent cells varies, depending on the method of induction and on the gene(s) perturbed, even though all these gene aberrations lead to senescence. Malignant transformation by E1a also led to a clearly distinguishable phenotype. In contrast, the reversible cell cycle arrest characterised as cell quiescence induced a metabolic phenotype that could not be distinguished from that of normally growing cells.
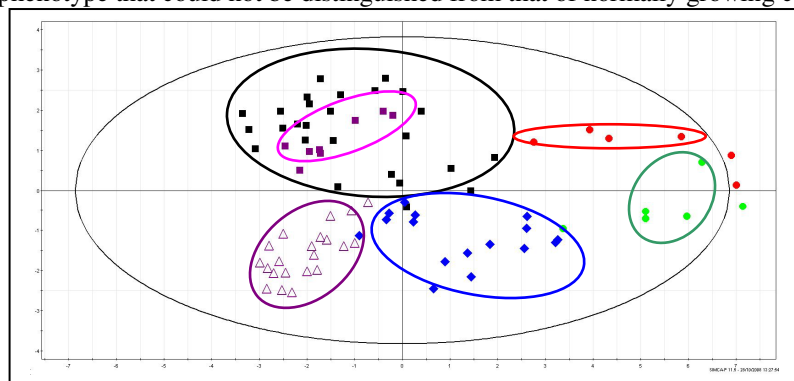

Figure 1: A) PCA scores plot (PC1 and PC2) of normal growing (black), RAS-induced (red) senescent, Replicative (purple) senescent, etoposide treated (green) senescent and E1a treated/transformed (blue), and quiescent (pink ellipse) HDFs.

REFERENCES:
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