Phosphocholine/Glycero-phosphocholine ratio is a potential marker for cellular senescence

B. Madhu[1], M. Narita[2], M. Narita[2], and J. R. Griffiths[1]

[1]Molecular Imaging, Cancer Research UK Cambridge Research Institute, Cambridge, England, United Kingdom, [2]Cellular Senescence and Tumour Suppressor Lab, Cancer Research UK Cambridge Research Institute, Cambridge, England, United Kingdom

INTRODUCTION: Senescence, which is a stable cell cycle arrest, is thought to act as a fail-safe mechanism to prevent the malignant transformation of pre-neoplastic cell populations. Oncogene-Induced Senescence (OIS) can be induced in human diploid fibroblasts (HDFs) by activating oncogenes such as Ras or MEK. Replicative senescence can be induced by repeated passage of cells, thus shortening the telomeres. Senescent cells are metabolically active, even though their cell growth is stably suppressed. Hence we have undertaken a ^{1}H NMR based metabolomics study in which oncogenic Ras and MEK–induced senescence, replicative senescence and malignant transformation induced by Ras and E1A, were compared with with quiescent and control HDFs. The aim was to find potential metabolic markers for cellular senescence.

METHODS: IMR90 HDFs (n=53) were cultured in DMEM, 10% FBS. OIS was induced using tamoxifen-regulatable ER-Ras (H-RasV12) (n=67) or MEK-ER (MEKQ56P) (n=36), while addition of viral E1A with ER-Ras caused malignant transformation (n=18). Cells of around passage number 37 became replicatively senescent (n=29). Quiescence was induced by 3 days serum starvation (n=33). Each cohort of HDFs was grown to a population of 10^6 cells before harvesting with perchloric acid. After neutralisation and lyophilisation these samples were re-dissolved in D_2O for ^{1}H NMR analysis. 500 µL of the sample was placed in a 5mm NMR tube. ^{1}H NMR spectroscopy 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, thus allowing for changes in cell size. Time domain spectra were Fourier transformed and pre-processed by zero and first order phase correction. Pattern recognition by principal component analysis (PCA)[2] was used for group classification.

RESULTS: PCA analysis (Figure 1) showed not only a clear separation of senescent samples from normally growing cells, but also separation depending on the mode of senescence induction. The NMR spectra of cell metabolites in cells made senescent, whether by OIS, or by replicative senescence, showed marked difference from normally growing, quiescent and E1A/RAS transformed HDFs. The relative concentrations of two of the choline metabolites, phosphocholine/glycerophosphocholine (PC/GPC) showed (in Figure 2) a significant reduction in OIS and replicatively-senescent cells compared to non-senescent cells. On the other hand quiescent and transformed cells showed significantly higher PC/GPC values.

DISCUSSION: Clear separation of the ER-Ras and MEK-ER OIS cell groups from the replicatively senescent cells 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. All these senescent groups were separated from the quiescent and normally growing cells. E1A/RAS transformed HDFs are also separated from all other cells groups, indicating that their metabolism is different from senescent, quiescent and normal growing HDFs. The PC/GPC ratio, typically elevated in aggressive cancer, is significantly decreased in senescent cells, suggesting that it could be used as a metabolic marker for cellular senescence. The higher PC/GPC ratio in E1A/RAS treated cells confirms that transformed cells exhibit an altered choline phospholipid metabolism[2].

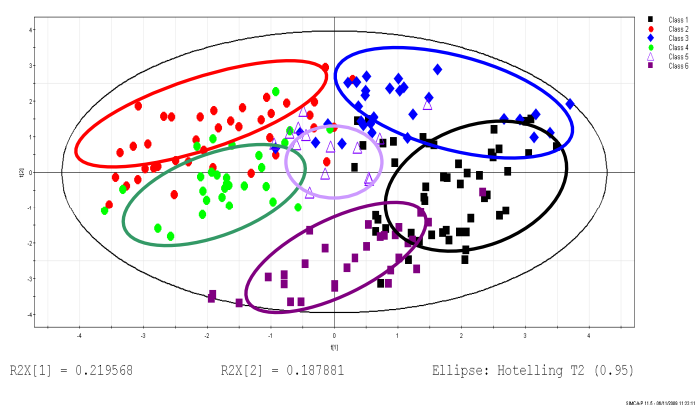**CONCLUSIONS:** PC/GPC is a potential metabolic marker for cellular senescence.

Figure 1. PCA analysis of 1H NMR data from normally growing (black squares), ER-Ras OIS (red circles) , MEK-ER OIS (green triangles) , replicative senescence cells (blue diamonds) , quiescent (purple squares) and E1A/RAS transformed (violet triangles)
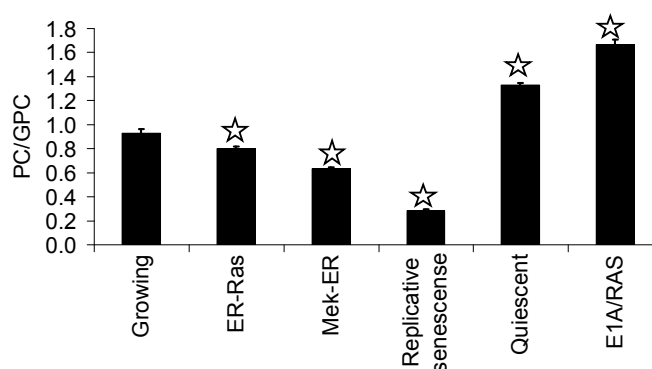
Figure 2. Plot of PC/GPC values from ^{1}H NMR spectroscopic data of senescence and non-senescent cells.

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