A biological model of the ageing metabolome reveals potential clinically relevant biomarkers

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Overview

- HFF-1 cells and media from an early passage versus a later passage representative of a senescent state have been studied as a biological model of the ageing metabolome.
- A workflow for untargeted metabolomics analysis is presented based on an untargeted HILIC-LC-DIA-MS/MS method for data acquisition, MS-DIAL and MetaboAnalyst for data analysis and LabSolutions Insight for metabolite identification.
- A number of metabolites have been found to be statistically significantly different between passage 3 and passage 20 cells and media which could be potential clinically relevant biomarkers of ageing.

1. Introduction

Ageing can have a profound effect on the metabolome through mechanisms including mitochondrial dysfunction, genomic instability, altered intercellular communication and cellular senescence. In this research, serially passaged human foreskin fibroblasts (HFF-1 cells) and their media have been considered as a biological model to increase our understanding of the ageing metabolome. Untargeted metabolomic analysis of cell and media extracts has revealed potential clinically relevant biomarkers of ageing.

2. Materials and Methods

A biological model of cell senescence considered the comparison of HFF-1 cells and media from an early passage versus a later passage representative of a senescent state. Extracts from HFF-1 cells and culture media at passage 3 and passage 20 were analysed with high resolution LC-MS/MS (LCMS-9030 Q-TOF system, Shimadzu Corporation, Japan).

- **HILIC LC Separation**.
  - Shim-pack Velox HILIC (2.1x100mm 2.7µm); 40ºC, flow rate 0.3 mL/min.
  - Binary gradient; water + 10mM ammonium formate 0.1% formic acid, and acetonitrile:water + 10mM ammonium formate 0.1% formic acid [92:8].
- **Mass Spectrometry Detection**. QTOF LCMS-9030 using external mass calibration
  - MS mass scan m/z 60-1000; 100 msecs; positive and negative ion modes.
  - DIA-MS/MS mass scans m/z 60-1000; precursor isolation window 35 Da collision energy spread 5-55V.
  - DDA-MS/MS; 20 MS/MS mass scans.
  - Cycle time less than 1 second for MS and MS/MS mass scans.
- **Data processing**.
  - **MS-DIAL |** raw data file processing, feature detection, alignment, filters applied (to retain components detected in at least 80% of samples in the passage 3 or the passage 20 group). Positive and negative ion data arrays were combined for statistical analysis of cells or media.
  - **MetaboAnalyst 5.0 |** used to find features that were found to be statistically significantly different by volcano plot analysis (FC>1.5; p<0.05) between passage 3 and passage 20 in cells or in media.
  - **LabSolutions Insight |** Compound Verification/Identification using an in-house metabolomics library and the MS-DIAL MSP spectral kit converted .msp files (http://prime.psc.riken.jp/compms/msdial/main.html).

2.1 Applying a generic HILIC LC-MS/MS method

The method has been optimised for highly polar metabolite analysis

Polar metabolites and lipids
Amino-acids, nucleotides, nucleosides, vitamins, carnitines, lyso-phospholipids

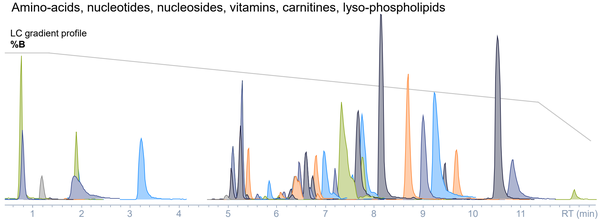

Figure 1. Mass chromatograms for a panel of highly polar metabolites (precursor ion ± 5 ppm) separated using a HILIC LC phase.

2.2 Identifying features of statistical significance

Transfer the statistically significant feature list into LabSolutions Insight and search against MS/MS libraries;

- In-house MS/MS library of authentic standards with defined HILIC retention times (Metabolomics Standards Initiative (MSI) level 1 identifications)
- MS-DIAL metabolomics MSP spectral kit includes repositories from MassBank, GNPS, RIKEN and CASMI. Elution order was considered for metabolites or lipids within the same chemical class to increase the reporting confidence of MSI level 2 annotations.

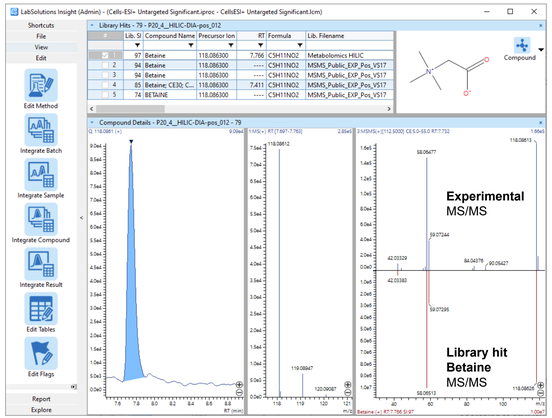

Figure 2. The criteria for reporting compounds included accurate mass error (nominally within ± 5 ppm), isotopic pattern matching score, retention time (if available) and product ion spectral library verification using in house developed MS/MS libraries or external MS/MS libraries.

3. Results

3.1 Intracellular metabolite profiling (cell extracts)

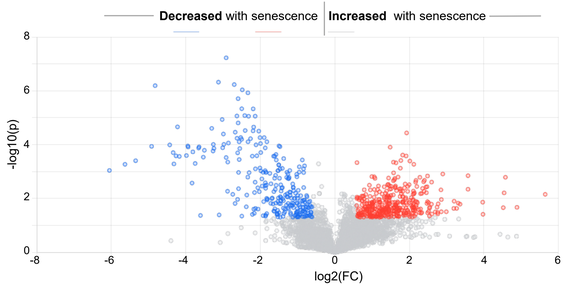

Figure 3. Volcano plot analysis was used to show the most significant differences (FC>1.5, p<0.05) between early passage cells (passage 3) and later passage cells representative of replicative senescence (passage 20).

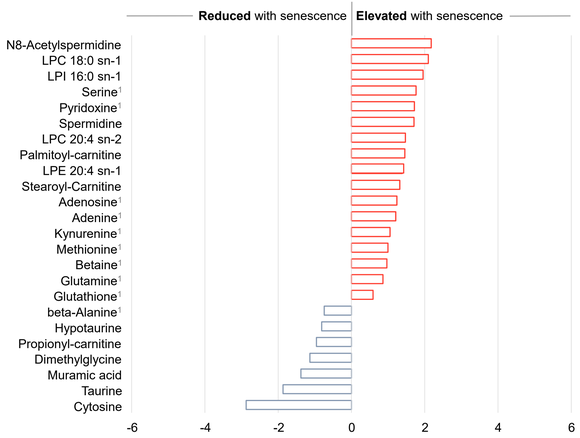

Figure 4. Metabolite features with a log2 fold change identified by MS/MS spectral library searching to MSI level 1 or 2. Metabolites that were elevated with senescence correspond to an increase metabolite level in passage 20 cell extracts compared to passage 3 cell extracts. Conversely, the level of several metabolites was reduced with senescence, comparing passage 20 cell extracts to passage 3 cell extracts.

[1]Indicates match against authentic standard (MSI level1).

3.2 Extracellular metabolite profiling (media extracts)

Comparing passage 20 media extracts to passage 3 media extracts

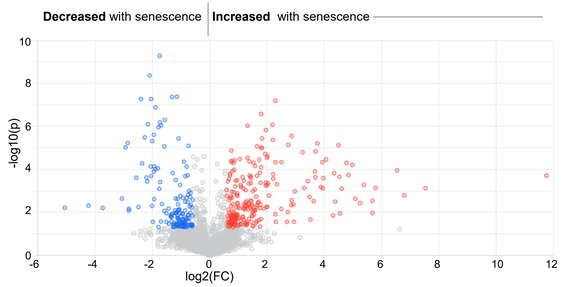

Figure 5. Volcano plot analysis was used to show the most significant differences (FC>1.5, p<0.05) between early passage media (passage 3) and later passage media representative of replicative senescence (passage 20).

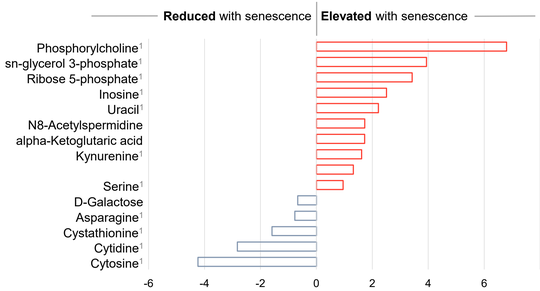

Figure 6. Metabolite features with a log2 fold change identified by MS/MS spectral library searching to MSI level 1 or 2. Metabolites that were elevated with senescence correspond to an increase metabolite level in passage 20 media compared to passage 3 media.

[1]Indicates match against authentic standard (MSI level1).

4. Conclusions

- Untargeted metabolomics has been applied to a cell culture model of replicative senescence to reveal potential clinically relevant biomarkers of ageing.
- The results from pairwise comparison of early and later passage cells and media representative of senescence.

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