IMPROVED QUALITATIVE AND QUANTITATIVE ANALYSIS OF THE HUMAN MITOCHONDRIAL PROTEOME BY HYBRID ACQUISITION

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INTRODUCTION

Mitochondria are essential organelles for the regulation of cell life and death. Literature suggests the involvement of mitochondrial dysfunction in many human diseases, some strictly linked to mutations in the mitochondrial genome, and others somehow connected to mitochondrial functionality by proteins sequence defects. At the same time, proteins possibly related to mitochondrial function await validation at the transcript and protein level. A novel hybrid acquisition mode, named Multi-Mode Acquisition, which is the product from combining of DDA and DIA in a single experiment, and sequential analysis tools, are expected to provide a comprehensive proteomic and mitochondrial complement. A dramatic fragmentation spectra quality increase was observed, which in turns improves confidence and coverage of identifications compared to conventional acquisition and processing modes.

METHODS

Sample preparation

Mitochondria were isolated from various human cell line sample as shown in Figure 1. Following mitochondria isolation, mitochondrial proteins were solubilized in a non-denaturing buffer containing EDTA and Triton X-100. The resulting mitochondrial protein extract was separated by a 40% sucrose gradient and fractionated into 12 mito fractions. DDA and DIA data were acquired in top 20 data dependent MS and IM runs for each fraction. LC-nano-ESI-MS/MS analysis was performed using a Waters SYNAPT G2 HDMS mass spectrometer (Waters Corporation, Wilmslow, United Kingdom) as illustrated by the right hand (green) component of the Multi-Mode Acquisition (MMA) and MMA processing with xiSPEC (University of Edinburgh, United Kingdom). The deconvoluted DDA and DIA spectra were visualized and compared with reference protein sequences. The MMA acquisition and processing details are illustrated in Figure 2. The small (red) box represent the MMA component in the flowchart and are the combination of DIA and DDA data and vice versa.

RESULTS

A detailed example of the de-multiplexing process for a single protein is shown in Figure 4. The spectra acquired with MMA workflow (red) are compared to individual DDA (green) and DIA (blue) data. The MMA DIA and MMA DDA data streams were further cross correlated with custom written tools (see Figure 5) with DIA* = MMA processed IM data and DDA* = MMA processed DDA. The MMA processed DDA and MMA processed DIA spectra were compared with reference protein sequences and the MMA processed DDA spectra were searched against the reference protein sequences with custom written tools. A significant increase in sequence coverage, better peptide ion detection/purity, charge state assignment, and ion alignment was observed with MMA processed DDA and MMA processed DIA spectra. The MMA processed DDA and MMA processed DIA spectra were also cross correlated with reference mitochondrial protein sequences. A detailed analysis of the mitochondrial functional proteome was performed on MMA processed DDA and MMA processed DIA spectra.

CONCLUSION

- Multi-mode acquisition methods allow the ability to assign multi-dimensional ion properties across data dependent and data independent data streams
- Enhanced multi-mode acquisition processing tools allow for improved charge state assignment, ion sequence identification, and the de-multiplexing of unknown data dependent and data independent data streams
- Qualitative sequence coverage and quantitative accuracy were both enhanced using the collective ion properties from all data streams and samples
- On average a 3.1 fold increase in number of peptides, a 1.7 fold increase in number of proteins, and a 50% reduction in VID quantitation was observed

References

5. Golick D, Ciavarini S, Gorenstein MV, Richardson K, Hoyes JB, Vissers JP, Langridge JI. Characterization of open reading frame 51 (CCD51_HUMAN) by contrasting MMA data streams and product ion spectra from multiple cell line samples. The top part illustrated an increase in sequence coverage. On average a 2.1 fold increase in number of peptides, a 1.7 fold increase in number of proteins, and a 50% reduction in VID quantitation was observed.