SILAC and label-free approaches for quantitatively identifying caveolae proteome

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OVERVIEW

- Compare SILAC and label-free approaches using statistical analysis software to identify differential protein expression associated with membrane caveolae
- SILAC and label-free quantitation produced differing results, because samples are combined at different stages during experiments. Thus, protein normalization is a variable to be considered in organelle proteomics.

INTRODUCTION

Organelle proteomics involve fractionation/purification steps prior to mass spectrometry. While quantitation by chemical derivatization and label-free methods normalize protein loading after fractionation, Stable Isotope Labeling by Amino acids in Cell culture (SILAC) workflows routinely normalizes total cell lysate prior to fractionation. The differences in when this normalization step is applied can lead to apparent differences in the data, which has thus far been unexplored. To address this question, we apply some of these methods to the analysis of a detergent-resistant fraction from caveolin-1 (Cav1) null and wild type fibroblasts using software specifically designed for the label-free approach.

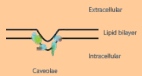

Figure.1 Cartoon representation of caveolae

METHODOLOGY

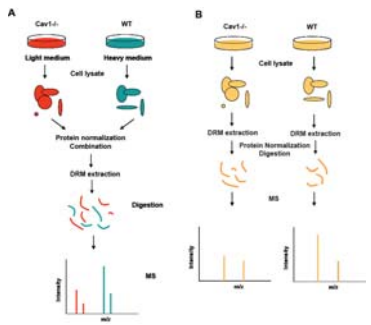

Figure.2 Methodology of the study A) SILAC B) label-free

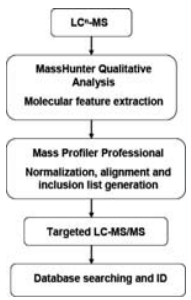

Figure.3 Agilent's ion intensity based label-free workflow

RESULTS AND DISCUSSION

- **Run reproducibility of Chip-Cube Q-TOF**

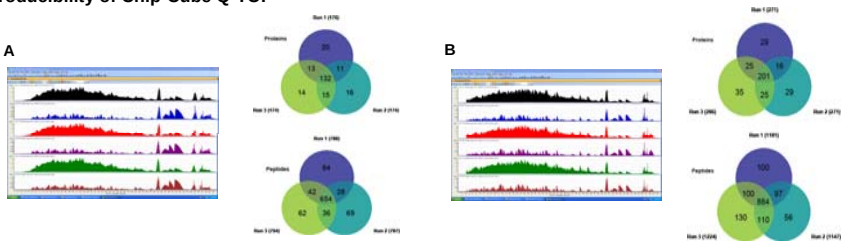

Figure.4 Testing the run reproducibility of Chip-Cube Q-TOF and the performance of different chips. A) 1ug protein 40nl trap chip B) 4ug protein 160nl trap chip

RESULTS AND DISCUSSION

- **SILAC analysis of MEF WT and Cav1 -/- DRMs**

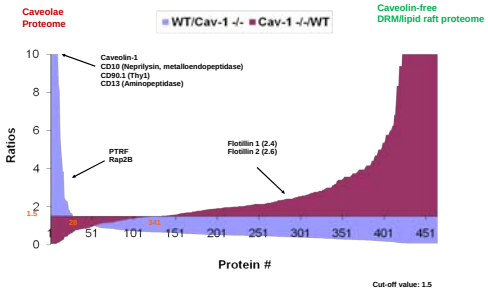

Figure.5 WT MEF cells were labeled with 4/6 and Cav1 -/- cells with 0/0. Protein samples were combined prior to DRM isolation. Protein ratios correspond to the enrichment in DRMs in either of the two cell types were plotted in decreasing order.

- **Label-free quantitative proteomics of DRMs from WT and Cav1-/- MEF**

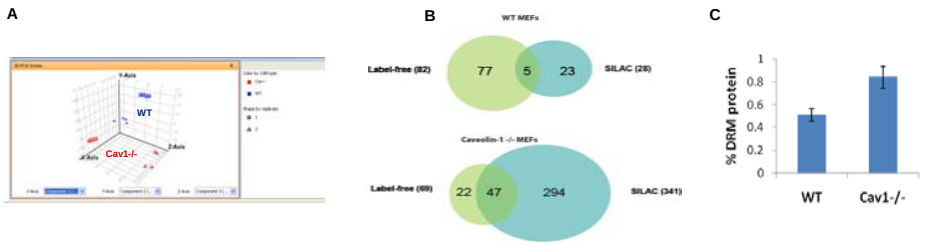

Figure.6 A) PCA analysis of repeated LC-MS analysis of WT and Caveolin-1 -/- MEF DRMs showing the high reproducibility of the runs. B) Overlap of DRM proteins that are identified to be enriched in the two cell types by using the two quantitative proteomics approaches: SILAC and label-free by Mass Profiler Professional. C) % DRM protein as DRM Protein/total protein from WT and Cav1-/- MEFs, and have significant increase by 1.51 ± 0.19 fold in cav1-/- MEF.

CONCLUSIONS

- Both SILAC and label-free quantitation are useful in subcellular proteomics
- For quantitative organellar proteomics the choice of procedure can lead to unintended bias in the data if the proteomes being compared are normalized too late in the procedure
- Therefore, potential differences in the relative abundance of the organelle/fraction between samples need to be considered during experimental design and data interpretation

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