"Bottom-Up against FIRE": Comparing bottom-up proteomics and a modified Edman degradation methodology for automated, untargeted protein adductomics analysis within the Galaxy platform.

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I. INTRODUCTION

Humans are exposed to large numbers of endogenous and exogenous electrophilic compounds which form covalent adducts at nucleophilic sites in biomolecules. Interpreting these adducts gives a record of the exposures of an individual and can be indicative of underlying health problems. While DNA adducts are detectable and directly impact replication and translation, these adducts are relatively shortlived and of variable abundance in tissues. Protein adducts in the blood form an attractive source of adducts with information of the exposures.

The Törnvik lab has developed the FIRE methodology to interrogate biomolecular adducts in hemoglobin. This research compares the ability of FIRE methodology and bottom-up proteomics to detect hemoglobin adducts in an untargeted fashion and begins the development of adductomics inside in Galaxy.

II. SAMPLE PREPARATION

Donor blood was aliquoted and stored with industrial blood transfusion bags (AA, AD, and AC) and control donor (CARD, POST, and DONOR). Hb samples were treated with a cocktail of reductant and buffer (100 mM succinate, 100 mM L-cysteine, and 0.25% Tween) to reduce intermolecular disulfides in hemoglobin. A mixture of electrophilic stock solutions for variable concentrations (100 nM or 500 nM) and incubations times (1, 2, 3, or 4 hours).

Samples of individual incubations were mixed to create combinations of electrophilic with known-mass concentrations. Post of electrophilic were mixed with individual blood aliquots to examine the effects. Samples were analyzed within 4 hours from extraction.

III. BOTTOM-UP PROTEOMICS

Nonproteolytic protein adducts are isolated via a modified Edman degradation procedure. Hemoglobin was incubated overnight at 37°C with benzoyl isothiocyanate (FITC) and potassium persulfate, releasing the fluorescein isothiocyanate (FITC) labeled peptide and the acrylamide-guanine adduct (AG). The purified peptides were digested and analyzed via LC-MS on a Q Exactive Hybrid Orbitrap Mass Spectrometer (ThermoFisher).

IV. BOTTOM-UP PROTEOMICS DETECTS MORE ADDUCTS THAN FIRE

100 µg of hemoglobin from each sample was alkylated in aliquots buffer followed by overnight trypsin digestion and desalting 1 µg of samples for mass analysis onto a Q Exactive Hybrid Orbitrap Mass Spectrometer for LC-MS analysis. Raw data was searched using Protein Discoverer with add variable peptide modifications (acrylamide; AG, Cys93-2-MGN, Cys93-2-MGN, and S35-H77). The resulting peptide spectra were cropped and searched using the Orbitrap using the Alpha and Beta hemoglobin chains.

Initial normal MS data for hemoglobin and selected adducts was used to populate an exclusion list for targeted validation.

Target mass and parent mass analyses demonstrate greater adduction at lower concentration-time value. These adducts show greater levels of adduction than the most reactive adducts, DC95.

V. AUTOMATED DETECTION, (SEM)-QUANTITATION OF HEMOGLOBIN ADDUCTS IN BOTTOM-UP PROTEOMICS

FIRE analysis of blood samples shows variable responses among clinical patients. Detection of adducted peptides at any concentration-time value

VI. SUMMARY

The FIRE methodology and bottom-up proteomics were compared for the untargeted detection of hemoglobin adducts. FIRE had a more consistent dose response, though proteomics was able to detect more adducts. Adductomics has been able to be adapted to detect adducts in human and rat hemoglobin, and is currently being wrapped into Galaxy MSI.

VII. FUTURE DIRECTIONS

Complete the assembly and test automated bioinformatics workflows for the detection and quantitation of novel hemoglobin adducts with Ligand FIRE and/or TABA. Further analysis for high throughput analyses of large sample cohorts.

The FIRE methodology for more applications.

Analyze blood samples from human patients to characterize the impact of chronic cigarette smoking.

Validate novel hemoglobin adducts in smoking exposure through targeted mass spectrometry experiments

References relevant to this work can be found here:

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