

Applying a High Throughput IMS-QTOF MS Platform to Complex Samples for Increased Molecular Coverage

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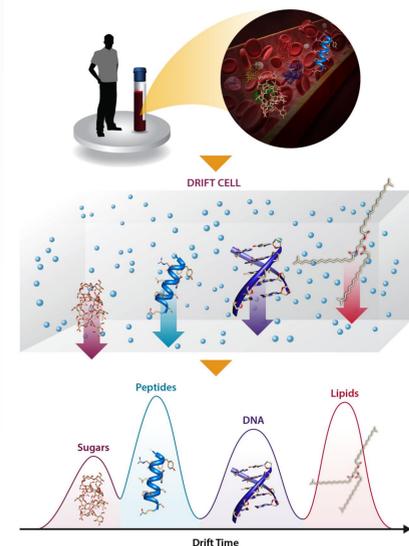
Overview

- A well characterized cohort of patients was utilized to identify protein markers in serum that discriminate among patients with liver fibrosis
- Discovery and verification phases were performed with multiple mass spectrometry and immunoassay platforms
- Overall platform performance and utility for initial large scale verification studies are summarized

Introduction

Rapid diagnosis of disease states using less invasive, safer, and more clinically acceptable approaches is an imperative goal in the field of medicine. While mass spectrometry (MS)-based proteomics approaches have attempted to meet these objectives, challenges such as the enormous dynamic range of protein concentrations in clinically relevant biofluid samples coupled with the need to address human biodiversity have slowed their employment.

To address these shortcomings, we have coupled technical advances in rapid gas phase multiplexed ion mobility spectrometry (IMS) separations with liquid chromatography (LC) and MS for dramatically increased measurement sensitivity and throughput that further enable exciting MS-based clinical applications.



IMS separation of different molecular classes from serum.

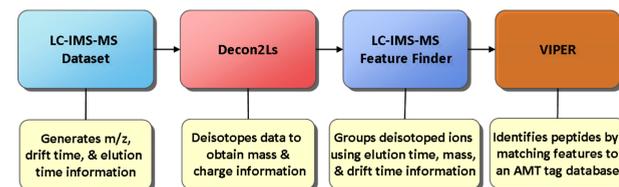
Methods

Experimental

- Blood serum samples from 60 post-liver transplant and 60 non-transplant were analyzed by LC-IMS-MS
- Patients were classified as non-, slow and fast progressors (shown below)
- Potential liver disease progression diagnostic/therapeutic biomarkers were determined



Informatics



- THRASH [1] algorithm implemented in Decon2LS [2] used to deisotope data
- Deisotoped ions are grouped into features utilizing drift time, monoisotopic mass and elution time information
- VIPER used to match features to sample information for identifications
- Heat maps for differentially expressed proteins were created in DaNTe using Rrollup [3] for significant peptides with p and q < 0.05

LC-IMS-QTOF MS Platform

High pressure automated LC system
Four 30-cm long, 150 μm i.d. reversed-phase capillary columns packed in-house with porous 3 μm C18 bonded particles

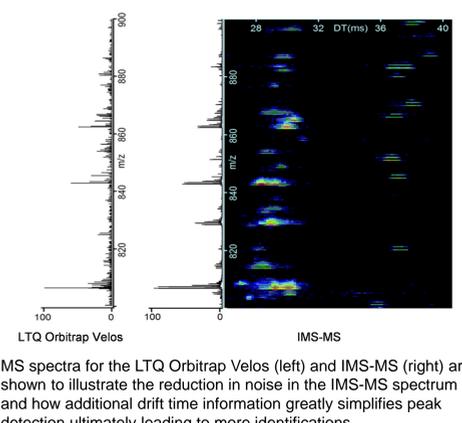
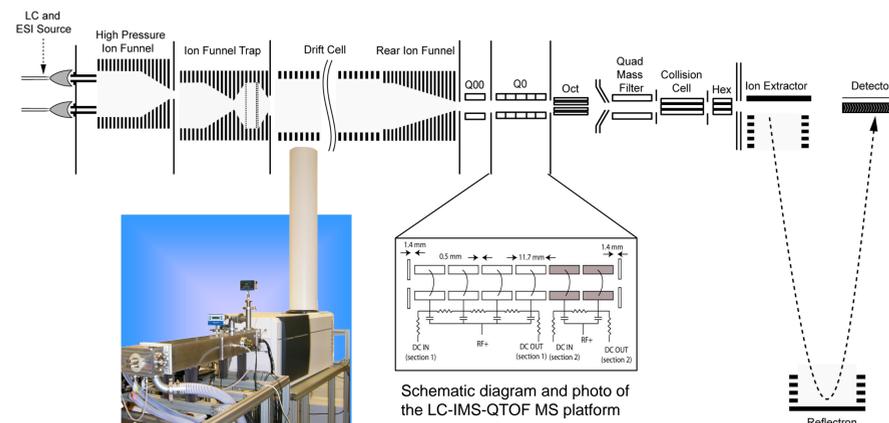
Future ion source advances

- Near perfect efficiency nanoESI-MS SPIN interface [4,5]
- High sensitivity droplet-based microfluidics [6]

IMS-QTOF MS [7, 8, 9, 10]

- High-pressure converging hourglass ion funnel focuses and traps ions prior to ion injection
- 1-meter IMS drift cell

- Second high-pressure ion funnel focuses the diffuse ion beam post-IMS separation
- High efficiency CID fragmentation performed in segmented quadrupole (Q0)
- Encoding was dynamically varied between signal averaging, 4-bit, 5-bit and 6-bit pseudo-random sequences; each 60-ms long IMS frame was acquired over 1 s
- Orthogonal Agilent QTOF MS provides high mass measurement accuracy after IMS separation

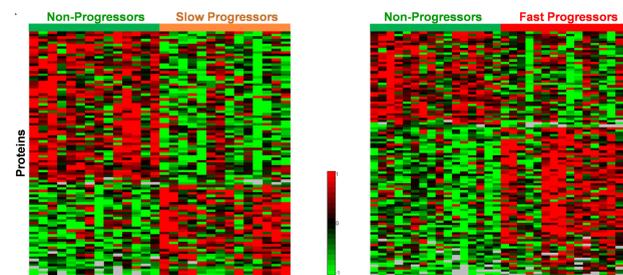


MS spectra for the LTQ Orbitrap Velos (left) and IMS-MS (right) are shown to illustrate the reduction in noise in the IMS-MS spectrum and how additional drift time information greatly simplifies peak detection ultimately leading to more identifications.

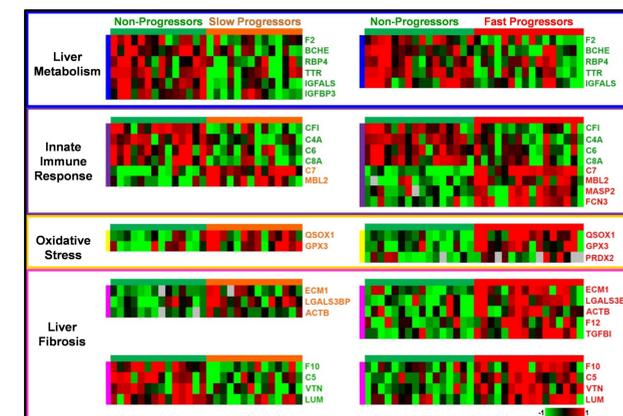
Results

Discovery Phase

- 60 individual post-liver transplant patients were analyzed with LC-IMS-MS
- Informatics/statistical analysis identified 77 proteins that discriminate fibrosis progression



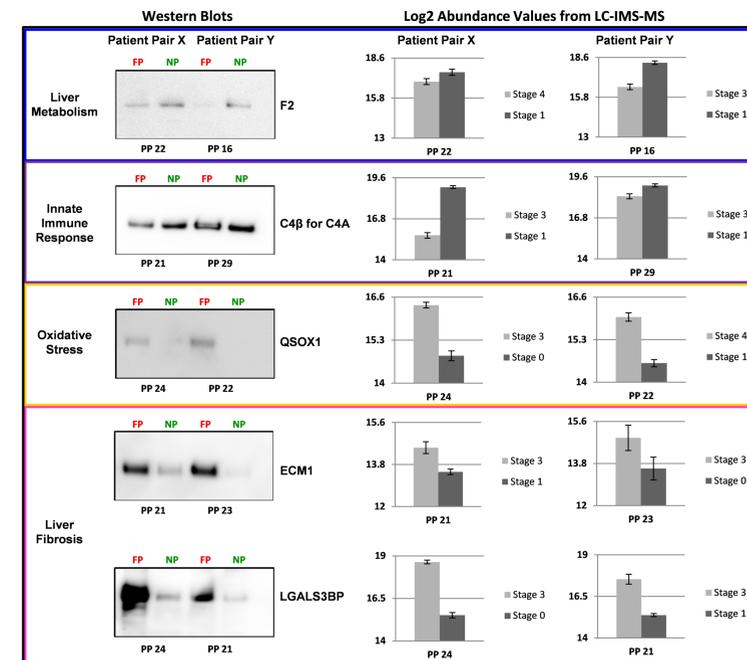
Heat maps representing the relative log₂ intensity change for proteins with significant differential abundance when non-progressors are compared to either slow progressors (left) or fast progressors (right).



Twenty-six selected proteins further characterized into 4 groups based on whether they could be correlated with liver metabolism, innate immune response, oxidative stress or liver fibrosis to illustrate the trend of each category.

Verification Phase

- 60 individual non-transplant patient serum samples were analyzed with the LC-IMS-MS platform
- 63 significant proteins were found to overlap with the post-liver transplant patients
- Western Blot immunoassays were performed on 5 proteins to verify their significance



Western blot (left) and LC-IMS-MS abundance (right) analyses of certain liver fibrosis patients where fast progressors (FP) were analyzed against their matched controls (NP) and patient fibrosis stage is noted on the bar graphs for the LC-IMS-MS abundance data.

Conclusions

- Application of an LC-IMS-QTOF MS platform allowed high throughput analysis of 120 liver fibrosis patients
- 63 statistically significant proteins that discriminate fibrosis progression were identified
- Analysis by Western Blots confirmed significant variation in 5 of the 63 statistically significant proteins
- Current results are also being correlated with complementary proteomic liver biopsy data and literature for mechanistic understanding of protein serum expression profiles

Acknowledgements

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