Parallel Reaction Monitoring Study of Micro-heterogeneity of Haptoglobin from Human Blood Serum

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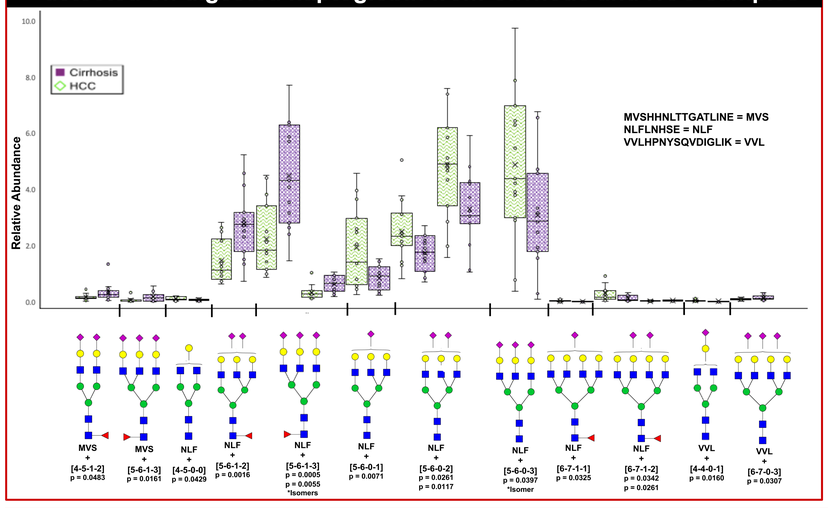
Introduction

Hepatocellular Carcinoma (HCC) is a common cancer type with high mortality rate and a large percentage of cases develop from liver Cirrhosis. Unfortunately, effective biomarkers for early detection are still lacking. Recent studies demonstrated that glycosylation patterns derived from human serum haptoglobin were different for Cirrhosis and HCC. Those studies also suggest that the distribution of isomeric glycans were different in abundance between two evaluated cohorts, in which LC-MS/MS analysis was utilized to determine specific isomeric glycan structures. This work attempts to demonstrate how Parallel Reaction Monitoring (PRM) can be applied for the quantitation of glycopeptide micro-moieties. Subsequently, the micro-heterogeneity differences among cirrhosis and HCC samples from human blood serum samples were evaluated using Principle Component Analysis (PCA).

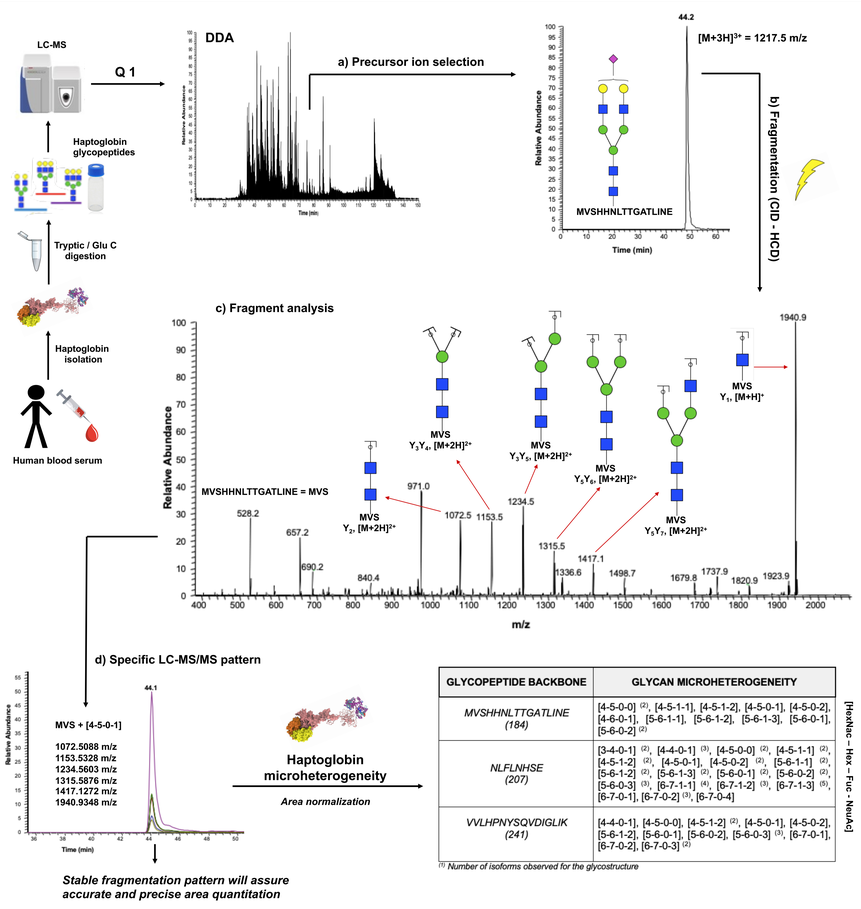
Method

Serum samples from Cirrhosis and HCC patients were provided by The University of Michigan Hospital, Ann Arbor, Michigan. Haptoglobin was purified from a 20-μL aliquot of serum for each patient by using an HPLC-based antibody-immobilized column. Haptoglobin samples were enzymatically digested by Trypsin and Glu C enzymes at 37 °C for 16 h respectively with an enzyme-to-protein ratio of 1:20 by mass. Then, the Tryptic-Glu C peptides were desalted using a solid phase extraction and dried. Samples for analysis were reconstituted in water containing 0.1% formic acid and analyzed by a C18(50cm)-LC-MS/MS chromatography system using an Ultimate 3000 nano-UHPLC (Dionex) coupled to an Q Exactive (Thermo Scientific). Analysis of glycopeptides in haptoglobin was performed using PRM in CID and HCD modes.

Box plot; relative abundance for significant glycopeptide structures detected on digested haptoglobin from Cirrhotic and HCC samples



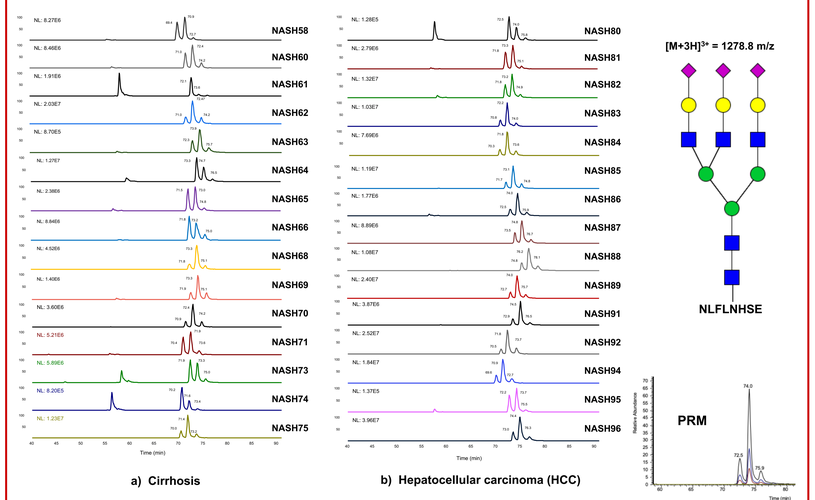
Parallel reaction monitoring (PRM); workflow applied for the glycopeptide quantitation of purified haptoglobin samples from Cirrhotic and HCC patients



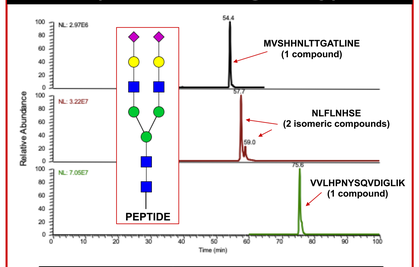
GLYCOPEPTIDE BACKBONE	GLYCAN MICROHETEROGENEITY
MVSHHNLTTGATLINE (184)	[4-5-0-0] (2), [4-5-1-1], [4-5-1-2], [4-5-0-1], [4-5-0-2], [4-6-0-1], [5-6-1-1], [5-6-1-2], [5-6-1-3], [5-6-0-1], [5-6-0-2] (2)
NLFLNHSE (207)	[3-4-0-1] (2), [4-4-0-1] (3), [4-5-0-0] (2), [4-5-1-1] (2), [4-5-1-2] (2), [4-5-0-1], [4-5-0-2] (2), [5-6-1-1] (2), [5-6-1-2] (2), [5-6-1-3] (2), [5-6-0-1] (2), [5-6-0-2] (2), [5-6-0-3] (3), [6-7-1-1] (4), [6-7-1-2] (3), [6-7-1-3] (5), [6-7-0-1], [6-7-0-2] (3), [6-7-0-4]
VVLHPNYSQVDIGLIK (241)	[4-4-0-1], [4-5-0-0], [4-5-1-2] (2), [4-5-0-1], [4-5-0-2], [5-6-1-2], [5-6-0-1], [5-6-0-2], [5-6-0-3] (3), [6-7-0-1], [6-7-0-2], [6-7-0-3] (2)

(1) *Number of isoforms observed for the glycostructure*

EICs of glycopeptide NLFLNHSE + [5-6-0-3] derived from (a) Cirrhosis and (b) HCC samples

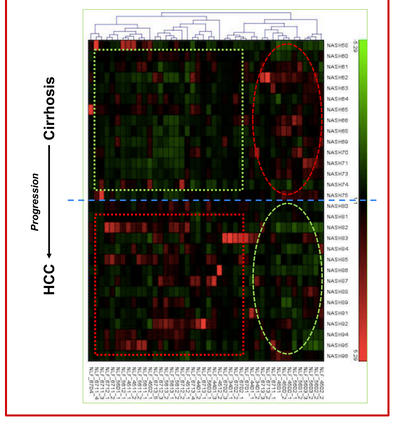


Isomer distribution for same glycan structure in different glycosylation sites (micro-heterogeneity)

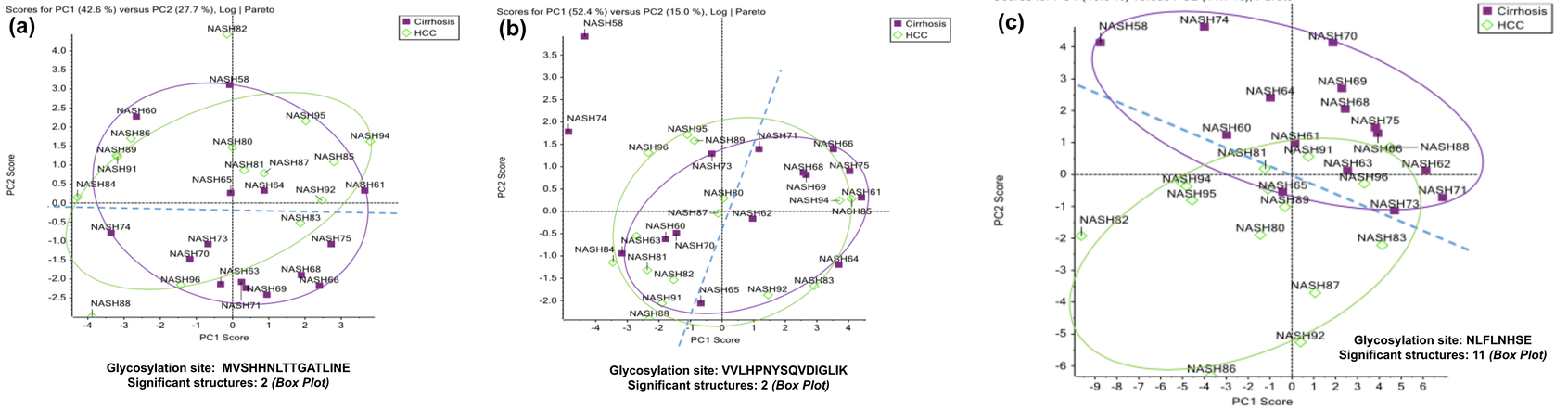


Glycan structure	Peptide backbone derivation
[4-5-1-1]	MVSHHNLTTGATLINE → *1 compound* NLFLNHSE → *2 isomeric compounds* VVLHPNYSQVDIGLIK → *NA*
[4-5-1-2]	MVSHHNLTTGATLINE → *1 compound* NLFLNHSE → *2 isomeric compounds* VVLHPNYSQVDIGLIK → *2 isomeric compounds*
[5-6-0-1]	MVSHHNLTTGATLINE → *1 compound* NLFLNHSE → *2 isomeric compounds* VVLHPNYSQVDIGLIK → *2 isomeric compounds*

Heat Map for normalized abundances of glycopeptide structures in glycosylation site NLFLNHSE (207)



PCA evaluation of the micro-heterogeneity of haptoglobin glycosylation sites; (a) MVSHHNLTTGATLINE (184), (b) VVLHPNYSQVDIGLIK (241), and (c) NLFLNHSE (207). The PCA analyzes were based on the normalized signal obtained from PRM analysis aforementioned



Glycosylation site: MVSHHNLTTGATLINE
Significant structures: 2 *(Box Plot)*

Glycosylation site: VVLHPNYSQVDIGLIK
Significant structures: 2 *(Box Plot)*

Glycosylation site: NLFLNHSE
Significant structures: 11 *(Box Plot)*

Conclusions

- A PRM analysis coupled with isomeric separation on 50cm C18 column were successfully performed on haptoglobin samples derived from Cirrhosis and HCC patients. Three out of four glycosylation sites were characterized and quantified. Totally 73 isoforms were detected, with 13, 44, and 16 structures on sites N184, N207, and N241, respectively.
- A grouping pattern was observed in PCA results using the normalized quantitation on site N207, however, the similar results were not detected on sites N184 and N241.
- The results suggests that the microheterogeneity of the glycosylation profiling is related to different disease states.
- PRM is a sensitive and high-throughput method to provide quantitative information in glycopeptide analysis.

Acknowledgements

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