KOREA BASIC SCIENCE INSTITUTE

Poster #28d

Direct Monitoring of Fucosylated Glycopeptides of Alpha-Fetoprotein in Hepatocellular Carcinoma Serum by LC-MS/MS with Immunoprecipitation

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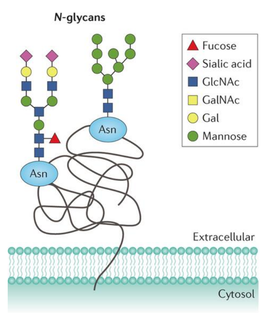
Abstract

Fucosylation is one of the most important glycosylation in the progression of hepatocellular carcinoma (HCC). Recently, fucosylated fraction of alpha-fetoprotein (AFP) has been developed as a serological marker for HCC. In this study, we introduced analytical methods for detecting glycopeptides of AFP using parallel reaction monitoring (PRM) mass spectrometry (MS) coupled with immunoprecipitation. The purpose of this study is to determine fucosylated N-glycopeptides from AFP using human serum. First, desialylation of glycopeptides after immunoprecipitation of AFP was used to improve MS detection limit from 0.1 µL serum. Second, PRM was used to obtain high resolution MS/MS spectra for identifying and quantifying glycopeptides using hybrid quadrupole time-of-flight (Q-TOF) MS. Finally, we compared sera from HCC with liver diseases using relative percentage of fucosylated glycopeptide (AFP-fuc%). Especially, AFP-fuc% showed an area under the ROC curve (AUC = 0.962) to discriminate between early-HCC (grade-1) and cirrhosis.

Introduction

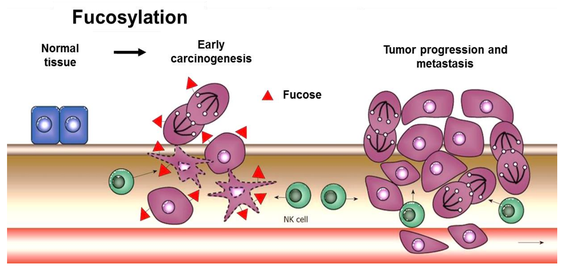
N-glycosylation on proteins



Nat Rev Urol 2016, 13 (6), 324-33

- **N-glycosylation of a protein is the most heterogeneous post-translational modifications (PTMs) and regulates functions, stability, and solubility of glycoproteins.**
- **Alterations of N-glycosylation of proteins have been identified of association with diseases.**

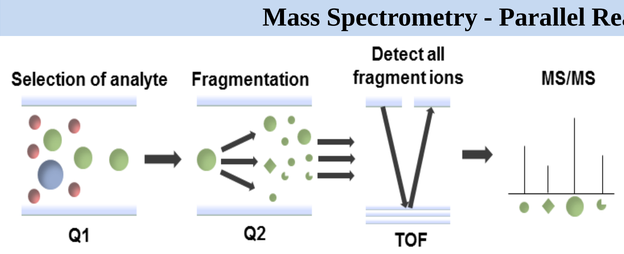
Biological function of fucosylation



World J Hepatol. Apr 27, 2010; 2(4): 151-161

Fucosylation has been associated in tumor progression and metastasis.

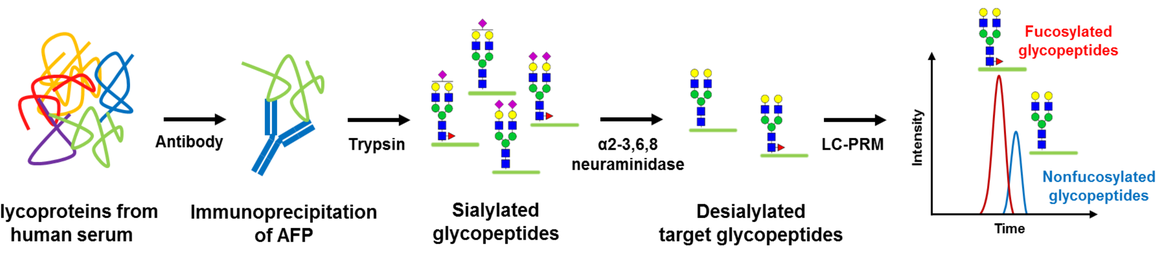
Mass Spectrometry - Parallel Reaction Monitoring (PRM)



- **Parallel Reaction Monitoring (PRM) is based on Time-of-flight or Orbitrap as the representative high resolution mass spectrum platform.**
- **The PRM performs a full scan of each precursor and monitored all fragment ions from the precursor ion.**

Methods

Strategy for analysis of glycopeptides from AFP by PRM



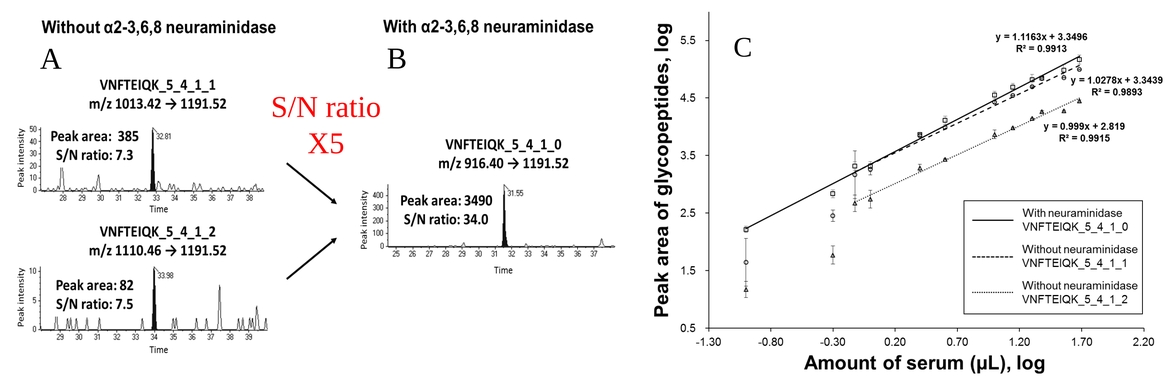
Selection of target glycopeptides

	Target glycopeptides	Precursor ion, m/z (charge)	Transition ions, m/z
Sialylated glycopeptides	VNFTEIQK_5_4_0_1	964.74 (+3)	1181.61 (PEP_0_1_0_0), 1037.46 (PEP_3_3_0_0), 1118.49 (PEP_4_3_0_0)
	VNFTEIQK_5_4_1_1	1013.42 (+3)	1181.61 (PEP_0_1_0_0), 1110.49 (PEP_3_3_1_0), 1191.52 (PEP_4_3_1_0)
	VNFTEIQK_5_4_0_2	1061.77 (+3)	1181.61 (PEP_0_1_0_0), 1118.49 (PEP_4_3_0_0), 1264.04 (PEP_4_3_0_1)
	VNFTEIQK_5_4_1_2	1110.46 (+3)	1181.61 (PEP_0_1_0_0), 1191.52 (PEP_4_3_1_0), 1337.07 (PEP_4_3_1_1)
Desialylated glycopeptides	VNFTEIQK_5_4_0_0	867.71 (+3)	1181.61 (PEP_0_1_0_0), 1037.49 (PEP_3_3_0_0), 1118.46 (PEP_4_3_0_0)
	VNFTEIQK_5_4_1_0	916.40 (+3)	1181.61 (PEP_0_1_0_0), 1110.52 (PEP_3_3_1_0), 1191.49 (PEP_4_3_1_0)

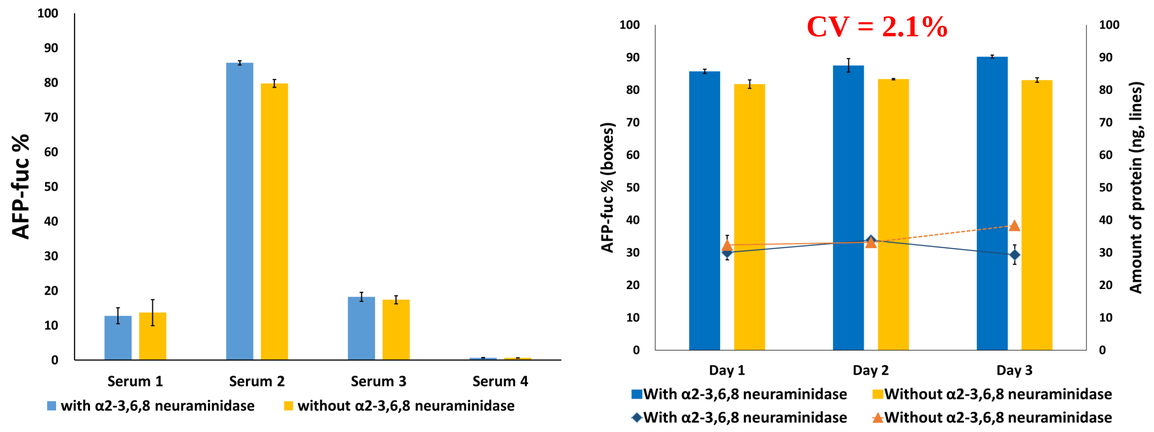
$$\text{AFP-fuc\%} = \frac{\text{peak area of fucosylated glycopeptides}}{\text{peak area of (fucosylated+nonfucosylated)}\,glycopeptides} \times 100$$

Results

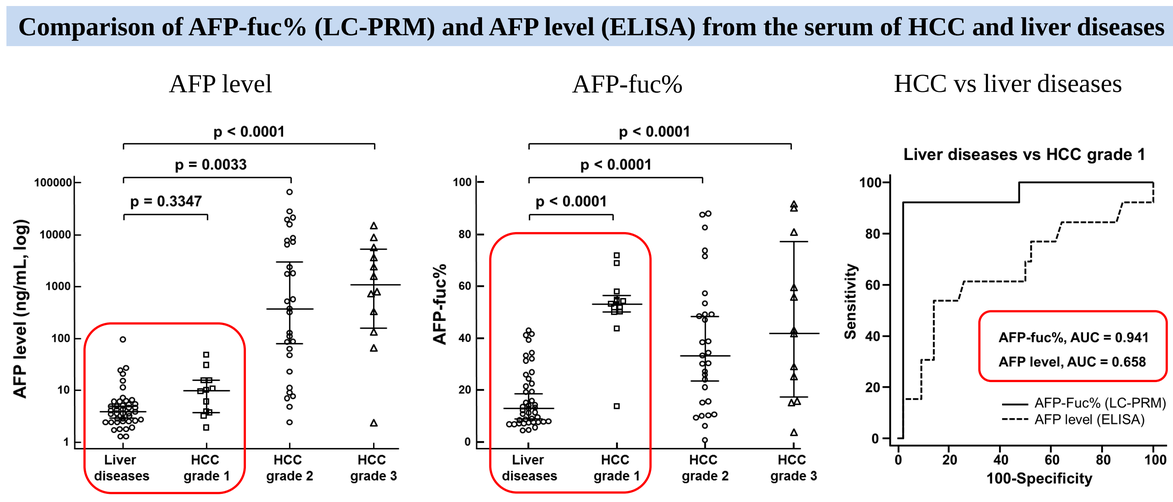
Comparison of sensitivity and reproducibility after α2-3,6,8 neuraminidase treatment



Extracted ion chromatograms for (A) glycopeptides (VNFTEIQK_5_4_1_1 and VNFTEIQK_5_4_1_2) and (B) desialylated glycopeptides (VNFTEIQK_5_4_1_0) for a cirrhosis patient serum. C) Measurement of these glycopeptides using HCC serum by LC-PRM.

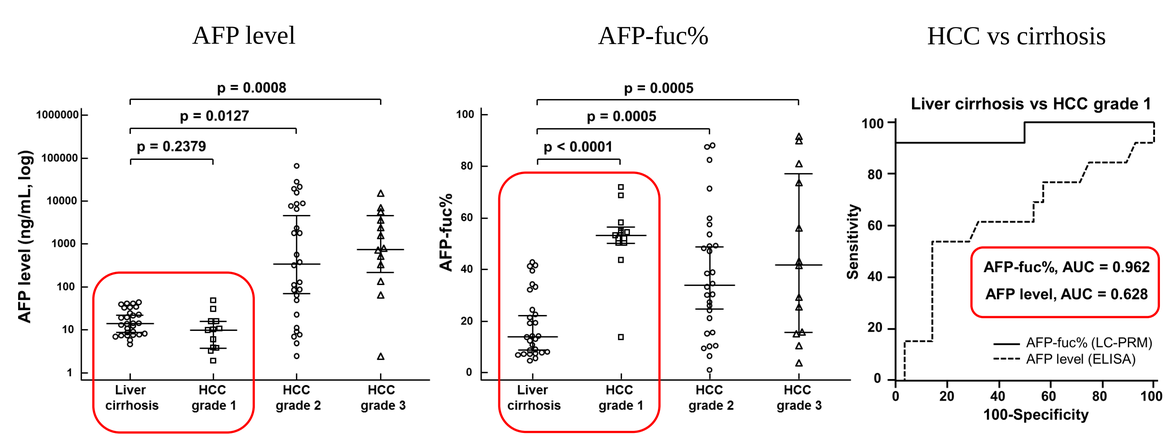


Comparison of AFP-fuc% (LC-PRM) and AFP level (ELISA) from the serum of HCC and liver diseases



Scatter plot of AFP-fuc% values from 42 liver disease patients and 54 HCC patients

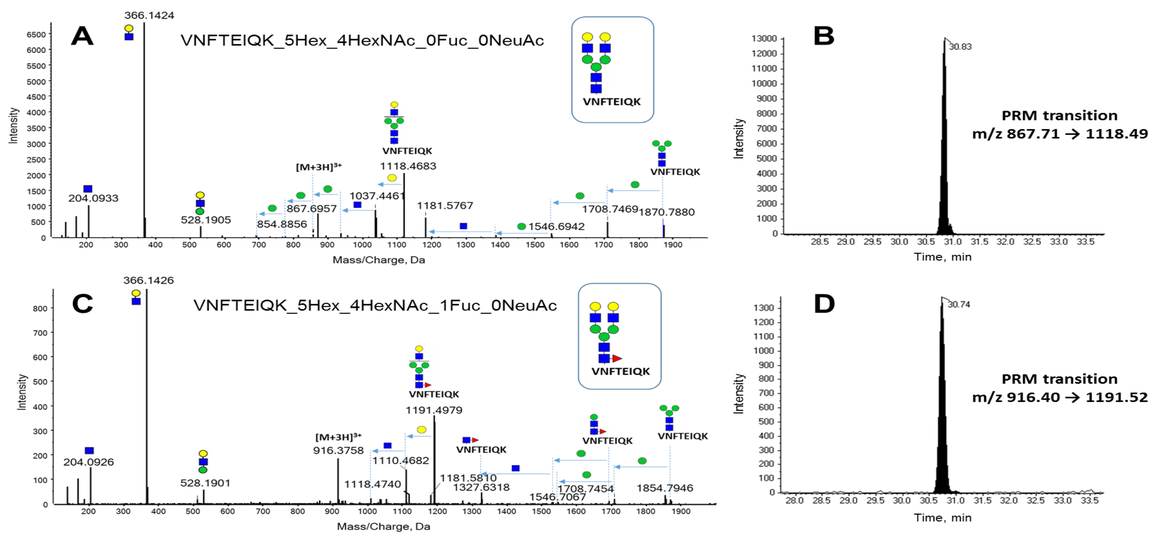
Comparison of AFP-fuc% (LC-PRM) and AFP level (ELISA) from the serum of HCC and cirrhosis



Scatter plot of AFP-fuc% values from 28 liver cirrhosis patients and 54 HCC patients

Results

MS/MS spectra of target glycopeptides from AFP



(A) The MS/MS spectrum and (B) extracted ion chromatogram of VNFTEIQK 5Hex 4HexNAc 0Fuc 0NeuAc (VNFTEIQK_5_4_0_0) obtained from LC-PRM analysis. (C) The MS/MS spectrum and (D) extracted ion chromatogram of VNFTEIQK 5Hex 4HexNAc 1Fuc 0NeuAc (VNFTEIQK_5_4_1_0).

Conclusions

1. LC-PRM combined with immunoprecipitation has the potential to become a promising method for detection of low abundant AFP glycopeptides in human serum.
2. Glycopeptides-based AFP-fuc% by LC-PRM could be a useful diagnostic parameter for detection of HCC in comparison to serum AFP levels by ELISA (AUC = 0.941 vs 0.658).
3. The diagnostic performance of AFP-fuc% for differentiating between liver cirrhosis and early-HCC by LC-PRM was better than that of serum AFP levels only by ELISA (AUC = 0.962 vs 0.628).
4. This study suggests that further validation of our LC-PRM methods is needed for a larger cohort sample set of HCC patients.

References

1. Kim, K. H., Lee, S. Y., Hwang, H., Lee, J. Y., Ji, E. S., An, H. J., Kim, J. Y., Yoo, J. S. (2018). Direct Monitoring of Fucosylated Glycopeptides of Alpha-fetoprotein in Human Serum for Early Hepatocellular Carcinoma by Liquid Chromatography–Tandem Mass Spectrometry with Immunoprecipitation. PROTEOMICS–Clinical Applications, 1800062.
2. Kim, K. H., Park, G. W., Jeong, J. E., Ji, E. S., An, H. J., Kim, J. Y., & Yoo, J. S. (2019). Parallel reaction monitoring with multiplex immunoprecipitation of N-glycoproteins in human serum for detection of hepatocellular carcinoma. Analytical and bioanalytical chemistry, 1-11.