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

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

Fucosylation has been associated in tumor progression and metastasis. Fucosylation has been identified in tumor proteins. Recent studies indicate that fucosylation can be a promising method for detection of liver diseases. For example, fucosylated glycopeptides of AFP have been identified in early-HCC (grade-1) and cirrhosis. In this study, we introduced analytical methods for detecting glycopeptides of AFP using parallel reaction monitoring (PRM) mass spectrometry (MS) coupled with immunoprecipitation.

Abstract

The current study introduces a novel method for the identification and quantification of fucosylated glycopeptides of AFP in human serum using parallel reaction monitoring (PRM) mass spectrometry (MS) coupled with immunoprecipitation. The aim 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.

Methods

Selection of target glycopeptides

<table>
<thead>
<tr>
<th>Target glycopeptides</th>
<th>Release %</th>
<th>Tandem ion, m/z</th>
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<tbody>
<tr>
<td>VNFTEIQK_5_4_0_1</td>
<td>100.76 (69)</td>
<td>1181.41 (DPF)_1_1_1_1, 1187.46 (DPF)_2_0_0_0, 1191.49 (DPF)_2_0_0_0</td>
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<tr>
<td>VNFTEIQK_5_5_1_1</td>
<td>100.62 (65)</td>
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<td>VNFTEIQK_5_5_3_1</td>
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<td>VNFTEIQK_5_5_3_2</td>
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<td>VNFTEIQK_5_5_3_3</td>
<td>100.40 (53)</td>
<td>1181.41 (DPF)_1_1_1_1, 1187.46 (DPF)_2_0_0_0, 1191.49 (DPF)_2_0_0_0</td>
</tr>
</tbody>
</table>

Results

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

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

References