SRM-based LC-MS Method for Detection of Hemoglobin Variants at Hemoglobinopathies Diagnostics

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Overview

Introduction

The variant-specific tryptic peptides and their normal analogs (Fig. 3) were selected to explore the potential diagnostic value of the peptides. These peptides were validated for the specificity of their detection. The results of the in-silico tryptic digestion of human globin revealed that the peptides are specific to the disease. The results of the in-silico digestion of the intact proteins revealed that the peptides are specific to the disease. The in-silico digestion of the intact proteins revealed that the peptides are specific to the disease.

Methods

The assay development workflow is shown in Fig. 3.

Results

To predict the SRM transitions for the light and heavy hemoglobin variants and to explain the theory of SRM, a series of iterative experiments was conducted using Prophesy software and the Xcalibur software (Thermo Fisher Scientific). The optimized transition list was then used in the SRM assay.

Conclusions

We have developed an SRM-based LC-MS/MS method for the detection of hemoglobin variants. The method is superior to current methods because it combines high sensitivity and specificity with the ability to perform a quantitative assay in a single run. The method also allows for the detection of single-nucleotide polymorphisms in a single run. Further work is needed to validate the method in clinical samples.

References


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FIGURE 3. Hemoglobinopathy-specific tryptic peptides and their normal analogs.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Mass/Charge</th>
<th>MRM Transition</th>
<th>Retention Time</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>VHLTPEEK</td>
<td>974.0 → 1261.0</td>
<td>195.0 → 1261.0</td>
<td>3.0 min</td>
<td>Normal</td>
</tr>
<tr>
<td>VHLTPEEK (Hb E specific)</td>
<td>974.0 → 1261.0</td>
<td>195.0 → 1261.0</td>
<td>3.0 min</td>
<td>Hb E</td>
</tr>
<tr>
<td>VHLTPEEK (Hb S specific)</td>
<td>974.0 → 1261.0</td>
<td>195.0 → 1261.0</td>
<td>3.0 min</td>
<td>Hb S</td>
</tr>
<tr>
<td>VHLTPEEK (Hb D-Punjab specific)</td>
<td>974.0 → 1261.0</td>
<td>195.0 → 1261.0</td>
<td>3.0 min</td>
<td>Hb D-Punjab</td>
</tr>
<tr>
<td>VHLTPEEK (Hb O-Arab specific)</td>
<td>974.0 → 1261.0</td>
<td>195.0 → 1261.0</td>
<td>3.0 min</td>
<td>Hb O-Arab</td>
</tr>
<tr>
<td>VHLTPEEK (normal)</td>
<td>974.0 → 1261.0</td>
<td>195.0 → 1261.0</td>
<td>3.0 min</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Data acquisition parameters on the TSQ Vantage are shown in Fig. 5.