Introduction

Molecular features were identified using an algorithm that finds the mass peaks in all mass spectra, removes chemical background, clusters by RT and m/z, calculates and determines a peak volume. Related peaks (isotopes, adducts, elution, isomers, multiple charge states) are combined and assigned a neutral mass and total volume. Extracted features were then evaluated using software that identifies common features and calculates cross-sample response values. Preliminary data from the model study shows that the features can be used to identify patterns on both the 25 and 45 replicate regulated features. Combined and assigned a neutral mass and total volume. Extracted features were then evaluated using software that identifies common features and calculates cross-sample response values. Preliminary data from the model study shows that the features can be used to identify patterns on both the 25 and 45 replicate regulated features.

Table 2: Treatment of CASM cells

<table>
<thead>
<tr>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>400</td>
<td>500</td>
<td>600</td>
</tr>
<tr>
<td>2</td>
<td>400</td>
<td>500</td>
<td>600</td>
</tr>
<tr>
<td>3</td>
<td>400</td>
<td>500</td>
<td>600</td>
</tr>
<tr>
<td>4</td>
<td>400</td>
<td>500</td>
<td>600</td>
</tr>
</tbody>
</table>

Table 2: CASM Sample Preparation

CASM cultures were obtained, grown, and treated as previously described. Table 2 (above) shows the various treatments done. After treatment, cells were lysed in 90% buffer which contained 8.5 M Tris-HCl, 1 M NaCl, 2 M dithiothreitol, 15 mM EDTA, and 15% NP-40. NP-40 is a non-ionic detergent which aids in lysing and solubilizing the protein. An detergent can interfere with MS analysis, so an attempt was made to remove the detergent prior to digestion by diluting and filtering in a 5kDa MWCO filter. The sample was then digested using 2.2 µl trypsin/mass to desalting and desalting proteins as previously described. However, LC/MS analysis showed both signal suppression and a dramatic decrease in signal at m/z 140. The low critical mass concentration (0.50 µM) of NP-40 resulted in the results shown during chromatography in the MWCO filters. However, using a 5 µM trypsin at digestion and retaining the peptide containing flow-through was effective in removing the detergent.

Experimental

Model Study

Initial studies were done using a trypsin digest of E. coli lysate (Bluff) spiked with both isoforms known and identified in this study. Samples were prepared at various levels of up- and down-regulated. Samples were prepared three times to model variability. The samples were prepared such that 3 replicates were run in duplicate. The samples were then run in duplicate. The samples were then run in duplicate.

Table 1: Composition of model study samples.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>400</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>TGF beta</td>
<td>400</td>
<td>400</td>
<td>400</td>
</tr>
</tbody>
</table>

Table 3: Reproducibility of mass and intensity from an earlier model study performed by HP1/HPLC/TOF MS.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Reproducibility</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.8</td>
<td>1.0</td>
</tr>
<tr>
<td>TGF beta</td>
<td>0.9</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Results and Discussion

Molecular features were identified using an algorithm that finds the mass peaks in all mass spectra, removes chemical background, clusters by RT and m/z, calculates and determines a peak volume. Related peaks (isotopes, adducts, elution, isomers, multiple charge states) are combined and assigned a neutral mass and total volume. Extracted features were then evaluated using software that identifies common features and calculates cross-sample response values. Preliminary data from the model study shows that the features can be used to identify patterns on both the 25 and 45 replicate regulated features. Combined and assigned a neutral mass and total volume. Extracted features were then evaluated using software that identifies common features and calculates cross-sample response values. Preliminary data from the model study shows that the features can be used to identify patterns on both the 25 and 45 replicate regulated features.

Figure 7. BPG for control (top), oxidized LDL treated (middle) and TGF beta treated (bottom) cultures.

Figure 6. Fragment ion assignments for transferrin peptide.

Figure 5. Apo A mass spectrum for targeted species.

Figure 4. Clustering of features that were significantly different by ANOVA by sample (left) and by level (right).

Figure 3. Contour plots of raw (left) and processed (right) data.

Figure 2. TIC of Sample 2 (on left) from the model study of spiked E. coli lysate.

Figure 1. HP1/Chip interfaced to an Agilent 6100 Q-TOF MS.

Figure 2. TIC of Sample 2 (on left) from the model study of spiked E. coli lysate.

Figure 1. HP1/Chip interfaced to an Agilent 6100 Q-TOF MS.

Figure 2. TIC of Sample 2 (on left) from the model study of spiked E. coli lysate.

Figure 1. HP1/Chip interfaced to an Agilent 6100 Q-TOF MS.

Figure 2. TIC of Sample 2 (on left) from the model study of spiked E. coli lysate.

Figure 1. HP1/Chip interfaced to an Agilent 6100 Q-TOF MS.

Figure 2. TIC of Sample 2 (on left) from the model study of spiked E. coli lysate.

Figure 1. HP1/Chip interfaced to an Agilent 6100 Q-TOF MS.

Figure 2. TIC of Sample 2 (on left) from the model study of spiked E. coli lysate.

Figure 1. HP1/Chip interfaced to an Agilent 6100 Q-TOF MS.

Figure 2. TIC of Sample 2 (on left) from the model study of spiked E. coli lysate.

Figure 1. HP1/Chip interfaced to an Agilent 6100 Q-TOF MS.

Figure 2. TIC of Sample 2 (on left) from the model study of spiked E. coli lysate.

Figure 1. HP1/Chip interfaced to an Agilent 6100 Q-TOF MS.

Figure 2. TIC of Sample 2 (on left) from the model study of spiked E. coli lysate.

Figure 1. HP1/Chip interfaced to an Agilent 6100 Q-TOF MS.

Figure 2. TIC of Sample 2 (on left) from the model study of spiked E. coli lysate.

Figure 1. HP1/Chip interfaced to an Agilent 6100 Q-TOF MS.

Figure 2. TIC of Sample 2 (on left) from the model study of spiked E. coli lysate.

Figure 1. HP1/Chip interfaced to an Agilent 6100 Q-TOF MS.