Identification of Phospho-tyrosine Biomarkers for Hepatocellular Carcinoma

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Overview
- The general 3-year survival rate for hepatocellular carcinoma (HCC) is < 20%.
- Time to detection is one of the greatest obstacles for effective treatment of HCC, and advanced cases of HCC have fewer available treatment options.
- Proteins expressed by cancerous liver cells are modified differently than those expressed by healthy liver cells.
- Kinase dysregulation and altered phosphorylation are common in HCC tumors.
- Tyrosine phosphorylation provides an important mechanism in cellular communication and is commonly disrupted in cancer.
- New phosphotyrosine-based protein biomarkers could provide clinicians with a means of early HCC detection.
- In this project, 2 candidate phospho-proteins were identified by computer-aided analysis of a phospho-proteome database.
- Overall expression levels of the candidates in malignant and adjacent healthy liver tissue samples were then quantified.

Methods
Global Phospho-proteome Analysis
- PhosphoSitePlus contains information on the expression of thousands of phosphorylated proteins in 51 HCC tissue/adjacent non-tumor tissue pairs.
- After analysis with a custom python script, 6 candidate proteins were chosen based on their ability to distinguish between tumor and non-tumor tissue (Table 1).
- Of the 6 candidates, BHMT (betaine–homocysteine S-methyltransferase 1) and GNMT (glycine N-methyltransferase) were found to be suitable for further investigation based on evidence from a previous publication implicating these proteins in biochemical pathways that are disrupted in HCC.

Total Protein Quantification
- Tissue homogenates were made from 24 tumor tissue and 24 corresponding adjacent non-tumor tissue samples.
- Total BHMT and GNMT (combined phosphorylated and unphosphorylated) were quantified by western blot using a β-actin loading control.
- Samples were made available to the Snider Lab by the UNC Tissue Procurement Facility and were described in a previous publication.

Table 1. Candidate phospho-proteins from computer-aided analysis

<table>
<thead>
<tr>
<th>Protein</th>
<th>Incidence in tumor tissue (%)</th>
<th>Incidence in healthy tissue (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHMT*</td>
<td>7.843</td>
<td>62.745</td>
</tr>
<tr>
<td>PKHB</td>
<td>11.765</td>
<td>66.667</td>
</tr>
<tr>
<td>GNMT*</td>
<td>1.961</td>
<td>41.176</td>
</tr>
<tr>
<td>PTS</td>
<td>9.804</td>
<td>47.059</td>
</tr>
<tr>
<td>MYH9</td>
<td>49.020</td>
<td>19.608</td>
</tr>
<tr>
<td>PIK3R2</td>
<td>50.980</td>
<td>15.686</td>
</tr>
</tbody>
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For each candidate protein listed, the incidence reflects the proportion of tumor tissue or adjacent, healthy tissue samples that the protein appeared in. Asterisks indicate candidates that were chosen for further analysis.

Figure 1. Models of tertiary structure for (A) human BHMT and (B) human GNMT.

Figure 2. Total protein levels from quantification experiments. Whiskers indicate a difference of 1 standard deviation. Protein levels exhibited statistically significant increases (via unpaired, two-tailed t tests) from tumor to non-tumor tissue samples for both GNMT (p = 0.0080) and BHMT (p = 0.0095).

Results

Future Directions and Conclusions
Future Directions
- Investigate the state of phosphorylation in tumor to non-tumor tissue:
  1) Immunoprecipitate samples with phospho-tyrosine antibody to isolate all proteins phosphorylated on tyrosine residues
  2) Quantify the phosphorylated GNMT and BHMT in the phospho-protein isolates
- Investigate the potential for these proteins to act as accurate serum biomarkers for HCC:
  1) Screen patient serum samples for the presence of both the total and phospho-specific forms of GNMT and BHMT

Conclusions
- Total GNMT and BHMT decreased in expression from healthy to malignant tissues (Figure 2).
- These results provide a strong starting point for further work dealing with HCC biomarker identification and the development of a noninvasive blood test for HCC that minimizes time to detection.

References