Analysis of Dual MNK/BCR-ABL Inhibitor induced changes in Proteome Phosphorylation for Companion Diagnostics Development

Simone Dorfmueller1, Jianhe Peng1, Li Rong1, Esther Ong1, Boping Liu1, Kassoum Nacro1, Joseph Cherian1, Sharon Lim2, Sin Tiong Ong2, Jeffrey Hill1, Alex Matter1
1Experimental therapeutics Centre, 31 Biopolis Way #03-01, Singapore 138669
2Duke-NUS Graduate Medical School, 8 College Road, Singapore 169657

Introduction

Chronic myeloid leukemia (CML) is characterized by the constitutively active tyrosine kinase Bcr/Ab1. Clinical samples of patients who have entered the blast Crisis (BC) phase of CML however show overexpressed and phosphorylated eIf4e as well as overexpressed and activated mitogen-activated protein (MAP) kinase interacting kinase 1 and 2 (MNK1 and MNK2). EIf4e/pSer209 is a key player of the PI3K/Akt/mTORC1 pathway and is phosphorylated specifically through the serine threonine kinase MNK1/2 (Lim et al, 2013). These reports suggested the value of MNK inhibitors for the treatment of BC patients.

pEIf4e and the BCR-ABL substrate CRKL proved to be valuable molecular markers for assessing the activity of ETCs dual MNK/BCR-ABL and MNK specific inhibitors and could be used to monitor response to therapy. However, little is known about how inhibition of MNK/BCR-ABL will affect global phosphorylation.

Here we describe the evaluation of an ETC MNK (compound D) and three ETC MNK/BCR-ABL (compound A, B and C) specific inhibitor induced changes in the proteome phosphorylation in eIf4e/pEIf4e overexpressing K562 CML cells. By using phosphorylation motif specific antibodies (Cell Signaling Technology) and LC MS/MS we identified proteins whose phosphorylation is directly or indirectly changed by inhibition of MNK.

Methods (PTMScan®)

Phosphorylation pattern analysis

- Protein immuno-purification of SILAC labeled DMSO and compound treated K562 lysate by the previously identified phosphorylation motif antibodies. LC-MS/MS analysis of the immunopurified digested peptides and calculation of H/L ratio for the identified proteins.
- Signaling pathway analysis of the identified proteins by Ingenuity Pathway Analysis and verification of compound induced changes in phosphorylation by specific phospho-antibodies.

Verification of H/L ratios by Western blot

- Compound A and B C and compound treated K562 cells were plated and treated for 24h with the various in DMSO dissolved compounds with only medium and DMSO only. Cells were treated with a serine/threonine and tyrosine phosphorylation motif specific antibody.

Phosphorylation pattern analysis showed that 4 S/T/P/Y motif antibodies picked up compound A induced changes in proteome phosphorylation (Figure 2a). For compound B and C only one S/T/P/Y motif antibody detected differences in band pattern after treatment (Figure 2b). For compound D no induced change in S/T/P/Y phosphorylation was found.

The Y motif antibody detected treatment induced changes in phosphorylation only for the MNK/BCR-ABL compounds A, B and C, but not for the MNK specific compound D (Figure 2c).

The Y motif antibody and a custom made PTMScan® kit of the 4 identified S/T/P/Y motif specific antibodies was used for IP of DMSO and compound treated SILAC K562 cell lysate.

LC MS/MS results and Pathway analysis

<table>
<thead>
<tr>
<th>Compound A</th>
<th>Compound B</th>
<th>Compound C</th>
<th>Compound D</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPT signaling</td>
<td>BPT signaling</td>
<td>Regulated eIf4e and p70S6K signaling</td>
<td>Regulated eIf4e and p70S6K signaling</td>
</tr>
<tr>
<td>n70S6K signaling</td>
<td>n70S6K signaling</td>
<td>mTORC1 signaling</td>
<td>mTORC1 signaling</td>
</tr>
<tr>
<td>MAPK Haplo</td>
<td>Grainsome A signaling</td>
<td>EIf4e signaling</td>
<td>Cancer mediated autophagy</td>
</tr>
<tr>
<td>Mucolipid storage deficiency</td>
<td>Regulated eIf4e and p70S6K signaling</td>
<td>EIf4e signaling</td>
<td>Regulated eIf4e and p70S6K signaling</td>
</tr>
<tr>
<td>EIf4e signaling</td>
<td></td>
<td>Regulated eIf4e and p70S6K signaling</td>
<td></td>
</tr>
<tr>
<td>n70S6K signaling</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Top 3 of the highest scoring canonical IPA® software pathways for proteins whose H/L ratio showed compound induced changes in S/T/P/Y and Y phosphorylation.

Compound treatment affected the phosphorylation of proteins in the EIf2, mTOR and eIf4p/p70S6K signaling pathway (Table 1). There is a higher overlap of identified proteins within the group of dual MNK/BCR-ABL inhibitors (compounds A, B, and C) than to the proteins affected by the MNK specific compound D (Figure 3).

References: