Selective, Rapid and Optically Switchable Regulation of Protein Function in Live Mammalian Cells

Burke Group Literature Seminar
6/6/2015
Adam Hill
Chemical genetics and the value of small molecule inhibitors

**RNA Interference**

- siRNA Gene Silencers
- shRNA Plasmid DNA
- shRNA Lentiviral Particles
- Transcription of shRNA
- shRNA
- Dicer
- Processing by Dicer into siRNA
- siRNA
- RISC
- siRNA unwinding
- Activated RISC
- Association with target mRNA
- Target mRNA cleavage

**Chemical Genetics**

<table>
<thead>
<tr>
<th>Wild-type kinase</th>
<th>Drug-sensitive kinase</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP1 inhibitor</td>
<td>1NM-PP1 inhibitor analog</td>
</tr>
</tbody>
</table>

- Temporal control of protein function
- Differential degrees of protein inactivation
- Better model for pharmacological intervention

Designing Selective Inhibitors is a Significant Challenge

Inhibitors through classic medicinal chemistry:

Stopped in early clinical trials for malignant cancer due to neurological side effects

- hydroxamate linkage was metabolically unstable
- crossed the BBB leading to neurotoxicity

**Pfizer/Warner Lambert MEK inhibitor**

In early stage clinical trials for malignant cancer

- metabolically stable
- no neurological toxicity

**Exelxis MEK-1 inhibitor**

- diphenyl amine necessary for binding
- 5 & 6 member carboxamide rings not tolerated
- piperidine was necessary for binding MEK-1 and ATP and for oral bioavailability

Chemical Genetics Approach to Selective Inhibitors

bump-hole method for selective inhibition of BET bromodomain proteins

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Kd (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT+I-BET</td>
<td>1.8</td>
<td>360</td>
</tr>
<tr>
<td>WT+ ET</td>
<td>n.d</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>LA/WT+ET</td>
<td>1.0</td>
<td>150</td>
</tr>
<tr>
<td>WT/LA+ET</td>
<td>1.0</td>
<td>140</td>
</tr>
<tr>
<td>LA/LA+ET</td>
<td>1.9</td>
<td>24</td>
</tr>
</tbody>
</table>

Mutagenesis of Brd2 allows for the development of a selective inhibition of Brd2 over highly similar Brds 1-4.

Unnatural Amino Acid Mutagenesis by Amber Codon Suppression

requires a novel tRNA which recognizes the “UAG” codon, and a novel aminoacyl tRNA synthetase which charges the UAG tRNA with the unnatural amino acid

2 rounds of selection:
1) select for a tRNA which reads stop codon “UAG”
2) Select for an aminoacyl tRNA synthetase which incorporates the unnatural amino acid

Used for incorporation of >100 unnatural amino acids
>99% fidelity for unnatural AA incorporation

Chin group used the pyrrolysyl-tRNA synthetase/tRNA$_{CUA}$ with relatively high efficiency in mammalian cells

Selective and Rapid Inhibition of Protein Function

requires a rapid and irreversible bioorthogonal reaction

Tsai, T.; Essig, S.; James, J.R.; Lang, K.; Chin, J.W. Nat. Chem. 2015 AOP, DOI: 10.1038/NCHEM.2253
Inhibitors based on the non-selective diphenyl amine scaffold

Inverse Electron Demand Diels-Alder Conjugation

unnatural AA with cyclooctyne

control probe
Inhibition by Bioorthogonal Ligand Tethering (iBOLT)

<table>
<thead>
<tr>
<th></th>
<th>MEK2</th>
<th>MEK1</th>
<th>INH (1 µM)</th>
<th>IB: p-ERK</th>
<th>IB: HA</th>
<th>IB: ERK</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>wt</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>73-1</td>
<td></td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>76-1</td>
<td></td>
<td>4</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>103-1</td>
<td></td>
<td>3</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>104-1</td>
<td></td>
<td></td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Phosphorylated substrate

HA tagged MEK

Non-phosphorylated substrate

iBOLT inhibitor (3)

Control probe (4)

Tsai, T.; Essig, S.; James, J.R.; Lang, K.; Chin, J.W. *Nat. Chem.* 2015 AOP, DOI: 10.1038/NCHEM.2253
iBOLT is Selective for Modified MEK1

<table>
<thead>
<tr>
<th>3 (µM)</th>
<th>MEK1(wt)</th>
<th>MEK2(wt)</th>
<th>MEK1(76-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td><img src="image" alt="IB: p-ERK" /></td>
<td><img src="image" alt="IB: p-ERK" /></td>
<td><img src="image" alt="IB: p-ERK" /></td>
</tr>
<tr>
<td>0</td>
<td><img src="image" alt="IB: HA" /></td>
<td><img src="image" alt="IB: HA" /></td>
<td><img src="image" alt="IB: HA" /></td>
</tr>
<tr>
<td>0</td>
<td><img src="image" alt="IB: ERK" /></td>
<td><img src="image" alt="IB: ERK" /></td>
<td><img src="image" alt="IB: ERK" /></td>
</tr>
<tr>
<td>10</td>
<td><img src="image" alt="IB: HA" /></td>
<td><img src="image" alt="IB: HA" /></td>
<td><img src="image" alt="IB: HA" /></td>
</tr>
<tr>
<td>10</td>
<td><img src="image" alt="IB: ERK" /></td>
<td><img src="image" alt="IB: ERK" /></td>
<td><img src="image" alt="IB: ERK" /></td>
</tr>
<tr>
<td>10</td>
<td><img src="image" alt="IB: HA" /></td>
<td><img src="image" alt="IB: HA" /></td>
<td><img src="image" alt="IB: HA" /></td>
</tr>
<tr>
<td>10</td>
<td><img src="image" alt="IB: p-ERK" /></td>
<td><img src="image" alt="IB: p-ERK" /></td>
<td><img src="image" alt="IB: p-ERK" /></td>
</tr>
</tbody>
</table>

Selective inhibition of modified MEK1 over wt MEK1 or MEK2

- **IB: p-ERK**: phosphorylated substrate
- **IB: HA**: HA tagged MEK
- **IB: ERK**: non-phosphorylated substrate

**iBOLT inhibitor (3)**

**control probe (4)**

Tsai, T.; Essig, S.; James, J.R.; Lang, K.; Chin, J.W. *Nat. Chem.* 2015 AOP, DOI: 10.1038/NCHEM.2253
iBOLT Inhibits Protein Function in under 30 minutes

<table>
<thead>
<tr>
<th>MEK1</th>
<th>wt</th>
<th>76-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (min)</td>
<td>0</td>
<td>180</td>
</tr>
<tr>
<td>IB: p-ERK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IB: HA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IB: ERK</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

inhibition as a function of time

Phosphorylated substrate
HA tagged MEK
Non-phosphorylated substrate

iBOLT inhibitor (3)
control probe (4)

Tsai, T.; Essig, S.; James, J.R.; Lang, K.; Chin, J.W. Nat. Chem. 2015 AOP, DOI: 10.1038/NCHEM.2253
Photoswitchable iBOLT

MEK1

State
12 (5 μM)

IB: p-ERK
IB: HA
IB: ERK

trans-state

cis-state

unnatural AA
ligation handle
inhibitor
inhibitor conjugate
photoswitch

photo-iBOLT (12)
iBOLT of Lymphocyte Specific Kinase (LCK)

<table>
<thead>
<tr>
<th>LCK</th>
<th>wt</th>
<th>247-16</th>
<th>250-16</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 (0.1 mM)</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>15 (1 µM)</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

IB: pZAP70  
- phosphorylated substrate (1)

IB: pTCR    
- phosphorylated substrate (2)

IB: GFP     
- GFP tagged LCK

iBOLT works on a structurally distinct kinase (LCK has 26% sequence homology to MEK1)

Unnatural AA (16)

iBOLT inhibitor (15)

Tsai, T.; Essig, S.; James, J.R.; Lang, K.; Chin, J.W. Nat. Chem. 2015 AOP, DOI: 10.1038/NCHEM.2253
iBOLT Rapidly Inhibits Kinase Activity

LCK tagged with GFP

ZAP70-mCherry

merge

Tsai, T.; Essig, S.; James, J.R.; Lang, K.; Chin, J.W. *Nat. Chem.* 2015 AOP, DOI: 10.1038/NCHEM.2253
Selective, Rapid and Optically Switchable Regulation of Protein Function

Chemical genetics has been previously used to design selective kinase inhibitors.

This work represents a new approach taking advantage of both unnatural amino acid incorporation and a bioorthogonal ligation reaction for rapid and selective protein inhibition.

I will be interested to see if the Chin lab will follow up this paper and use the technology to further understand unknown protein function.

Tsai, T.; Essig, S.; James, J.R.; Lang, K.; Chin, J.W. Nat. Chem. 2015 AOP, DOI: 10.1038/NCHEM.2253