O-Glycosylation of Transcription Factor BCL11b: Truth or Myth?

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T-cell Leukemia

- T-cell Leukemia: Cancer of the white blood cells, or T-cells.
  - Affects approximately 50,000 people per year
  - About 4,000 people die from it annually
- Recovery Rate: ~90% in children
- Relapse Rate: ~50% in adults
- Looking for potential drug targets

BCL11b: a haploinsufficient tumor suppressor

- Loss or mutation of BCL11b results in T-cell leukemia
- BCL11b mutation is associated with ~15% of T-cell leukemia cases
Bcl11b is upregulated at DN2 stage

Bcl11b needed for proliferation of immature myeloid T cell progenitors

Bcl11b needed for progression into CD4 or CD8 SP cells

Bcl11b known to be modified by phosphorylation and sumoylation

Scheme c/o Ellen Rothenberg, Cal Tech
Transcription Factors – Control gene expression by activating or inhibiting transcription of DNA sequences

Regulated by localization, synthesis/degradation, and post-translational modifications.
**Post Translational Modification**

<table>
<thead>
<tr>
<th>PTM</th>
<th>Major Targeted Amino Acids</th>
<th>Modifies BCL11b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphorylation</td>
<td>Ser, Thr, Tyr</td>
<td>Yes</td>
</tr>
<tr>
<td>Sumoylation</td>
<td>Lys</td>
<td>Yes</td>
</tr>
<tr>
<td>O-Glycosylation</td>
<td>Ser, Thr</td>
<td>?</td>
</tr>
</tbody>
</table>
Methods for Detecting O-Glycosylation

- **Antibodies** targeted to O-glycosylation residues
- **Beta-N-Acetylhexosaminidase** to remove glycosylated residues
- **Wheat germ agglutinin** to precipitate glycosylated proteins
- **Mass Spectrometry** to characterize location on protein
Inhibits $O$-$\text{GlcNAc-}$$\beta$-$N$-acetylglucosaminidase (the enzyme for deglycosylation) in the cells

Hypothesis: If BCL11b is O-glycosylated, PUGNAc treatment will cause a significant increase in O-glycosylation signal.
HEK293T Cells
Human Embryonic Kidney Cells
Transformed

- Can easily be transfected to express BCL11b
- Can also easily be treated with PUGNAc
Experimental Methods

- Cell Culture
- Immunoprecipitation
- Western Blot

Changes in O-Glycosylation caused by PugNAc In BCL11b-Transfected HEK cells
Beta-N-acetylhexosaminidase

- An enzyme that removes an O-linked oligosaccharide from a glycoprotein
- Designing a negative control for the O-glycosylation antibodies
Beta-N-acetylgalactosaminidase

**IB:** BCL11b

**IB:** O-Glycosylation

**Overlay**

**HEK 203T cells Treated with Beta-N-acetylgalactosaminidase over time**

<table>
<thead>
<tr>
<th>Treatment Type (min)</th>
<th>Ratio of Glycosylation to Total BCL11b</th>
</tr>
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<tbody>
<tr>
<td>Beta0</td>
<td>0.9</td>
</tr>
<tr>
<td>Beta5-</td>
<td>0.8</td>
</tr>
<tr>
<td>Beta5+</td>
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<tr>
<td>Beta10-</td>
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<tr>
<td>Beta10+</td>
<td>0.9</td>
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<tr>
<td>Beta30-</td>
<td>0.8</td>
</tr>
<tr>
<td>Beta30+</td>
<td>0.9</td>
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</tbody>
</table>
Mouse Thymocytes

Why are they a better model?

Natively express BCL11b

Phosphorylation and sumoylation change dynamically in thymocytes upon stimulation

Is BCL11b glycosylated?

Does it also change?
Mimics T-cell receptor activated signaling and phosphorylation cascades
- MAPK pathway stimulated by Phorbol ester
- Ca2+ pathway stimulated by A23187
Changes in Glycosylation (P/A) On BCL11b in Thymocytes

- Non-specific binding of the antibody
Wheat Germ Agglutinin (WGA)

- Binds to N-acetyl-D-glucosamine
- Procedure is identical to the immunoprecipitation described previously
- Pulls down all glycosylated proteins
- Gel is then scanned for specific proteins
- AKT1 is used as a positive control, as it is known to be glycosylated in mouse thymocytes
Wheat Germ Agglutinin Blot

Result: WGA did not bind BCL11b
AKT1 was present in the WGA lane

Conclusion: BCL11b is not directly glycosylated
Result: Phosphorylation levels of BCL11b decreased when PUGNAc was added.

Conclusion: Glycosylation levels affect the activity of BCL11b, but not directly.
Changes in Phosphorylation caused by PUGNAc In BCL11b-Transfected HEK293T cells

IB: BCL11b Signal

Phosphorylation Signal

Overlay

Phosphorylation of HEK cells Treated with PUGNAc

<table>
<thead>
<tr>
<th></th>
<th>0 min</th>
<th>20 min</th>
<th>45 min</th>
</tr>
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<tbody>
<tr>
<td>Series1</td>
<td>0.338500714</td>
<td>0.302533413</td>
<td>0.437811187</td>
</tr>
</tbody>
</table>
Next Up:

- Determining the effects of glycosylation on other proteins in the NuRD complex and along the MAPK and Ca2+ dependent pathways.
Special Thanks

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- College of Pharmacy
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