Mapping the Palmitoylation Sites of MBLAC2 by Click Chemistry

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What is palmitoylation?

- Palmitate: A 16-C fatty acid with a reactive carbonyl group
- Cysteine: An amino acid with a reactive thiol group

A 16-C fatty acid with a reactive carbonyl group

Amino acid with a reactive thiol group
MBLAC2: Metallo-beta-lactamase-domain-containing-protein-2

- 280 amino acid protein
- Enzyme of unknown function

**KNOWN:**
MBLAC2 is predicted to be palmitoylated

**UNKNOWN:**
Which of the three cysteines is/are palmitoylated
Mapping the Palmitoylation Sites of MBLAC2

**WT**
If palmitoylation occurs, one, two, or all of the Cys are palmitoylated.

**C175A**
If palmitoylation occurs, C211 and/or C253 are palmitoylated.

**C211A**
If palmitoylation occurs, C175 and/or C253 are palmitoylated.

**C253A**
If palmitoylation occurs, C175 and/or C211 are palmitoylated.

**3CA**
Negative control, no Cysteines, therefore no palmitoylation occurs.

**Palmitate**

**Cysteine**

C175A means that the Cysteine at the 175th position is mutated to an Alanine.
Outline of Experiment

Transfection ➔ 17-ODYA Labeling ➔ Cell Lysis

Gel Analysis ← Click Chemistry ← GFP Immuno-precipitation
1. Transfection

To monitor transfection efficiency, each construct was tagged with GFP (green fluorescent protein).
2. Labeling MBLAC2 with 17-ODYA

- Incubate the transfected cells with 17-ODYA for 6 hours
- A palmitate analog - has similar reactivity towards cysteines as palmitate
- Exchanges with palmitate, important for click chemistry

1. Transfection
2. 17-ODYA Labeling
3. Cell lysis
4. GFP Immuno-precipitation
5. Click Chemistry
6. Gel Analysis
3. Lysing the HEK cells

- Release cell contents
- Lysis Buffer
  - Use detergent to disrupt cell membrane
    - Disrupts hydrophobic-hydrophilic interactions of the membrane bilayer, breaking down the membrane
4. Pulldown of MBLAC2 using GFP-Affinity Beads

1. Transfection
2. 17-ODYA Labeling
3. Cell lysis
4. **GFP Immuno-precipitation**
5. Click Chemistry
6. Gel Analysis
• We have isolated the GFP-tagged, ODYA-labeled MBLAC2 from HEK cells.
• The alkyne group of 17-ODYA is critical for reacting with an azide group that allows for the attachment of a fluorescent reporter group.

Reporter group has a fluorescence at 647nm
5. Click Chemistry

- Energetically favorable reaction used to see ODYA labeling
- Contents:
  - **Alexa 647**: reporter group with reactive azide group, reacts with ODYA’s alkyne group
  - **CuSO\(_4\)**: catalyzes the reaction
  - **TCEP**: reducing agent, keeps the cysteines from getting oxidized
  - **TBTA**: ligand of Cu\(^{2+}\) to stabilize CuSO\(_4\)
  - **PBS**: maintains physiological pH and salt concentration
6. Gel Analysis

- The MBLAC2 protein was eluted from the beads and analyzed by gel electrophoresis.
- What we can learn from the gel:
  - MBLAC2 size
  - Palmitoylation (in-gel fluorescence at 647 nm)
  - Confirm MBLAC2-GFP expression using a Western Blot
Results

Length of MBLAC2 with tags ~ 56 kDa

Western Blot (α-GFP)

647 nm
Preliminary Conclusion and Future Directions

- C253 is the main site of palmitoylation
- Characterize MBLAC2:
  - Cellular localization
  - Enzyme interactions – determine what enzyme palmitoylates MBLAC2
  - Confirm predicted activity as a hydrolase
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