PURDUE INNOVATES | Office of Technology Commercialization

Inhibitor against FicD/HYPE mediated AMPylation

High-throughput FP assay identifies selective inhibitors of FicD/HYPE AMPylation linked to neurodegeneration.

Fic enzymes are known for catalyzing AMPylation, a posttranslational modification essential for cell signaling, involving the addition of adenosine monophosphate (AMP) to serine, threonine, or tyrosine residues on proteins. This process is facilitated by a conserved Fic motif within the enzyme's active site, where a histidine residue initiates the transfer of AMP from adenosine triphosphate (ATP) to the target protein. The sole human Fic protein, HYPE, located in the endoplasmic reticulum, specifically AMPylates the chaperone protein Binding Immunoglobulin Protein (BiP), regulating its activity. Under normal conditions, AMPylated BiP is inactive, but it becomes active (deAMPylated) in response to misfolded proteins in the ER. HYPE has additionally been found to AMPylate alpha-synuclein, which leads to a reduction in harmful phenotypes linked to Parkinson's disease and affects the aggregation of proteins associated with various neurodegenerative diseases. Following this, more potential substrates of HYPE-mediated AMPylation have been identified, implicating HYPE in a variety of diseases including cancer, diabetes, and neurodegeneration.

Purdue researchers have developed a method for screening pharmaceutical compositions for treating neurodegenerative diseases that are impacted by HYPE-mediated AMPylation. Researchers were able to complete a high throughput small molecule screen, which included over 30,000 compounds, to identify activators and inhibitors of HYPE. This developed method can be used for optimizing and scaling dual screening assays for the identification of novel modulators of in vitro HYPE AMPylation. Furthermore, this has allowed for the discovery of a novel inhibitor of HYPE-directed AMPylation in I2.10. I2.10's specificity for HYPE was confirmed through validations, showing that the inhibitor was effective without affecting other cellular targets. Additionally, the inhibitor demonstrated minimal toxicity in human cell lines, showing viable candidacy for further development.

Technology ID
2023-MATT-70064

Category
Pharmaceuticals/Drug Discovery & Development

Authors
Seema Mattoo

Further information
Clayton Houck
CJHouck@prf.org

View online

Technology Validation:

- Fluorescence polarization (FP) of Fl-ATP was used to monitor HYPE-mediated AMPylation

- FP AMPylation assay was performed to confirm both WT HYPE activator and inhibitor validation

- In-gel autoAMPylation assay with WT HYPE and E234G HYPE validated WT HYPE activators and showed fluorescence for direct AMPylation and Coomassie staining for protein loading

- Fluorescence quantification was normalized to DMSO controls with data from four experiments; showing direct protein AMPylation

- Radioactive in-gel AMPylation assay used WT IbpA-Fic2, Cdc42, and α-32P-ATP to assess cross-reactivity and control activation with T229A BiP-AMP

- Phosphor screen and Coomassie staining confirmed direct AMPylation and protein loading.

- MTT cell viability assay of HeLa cells were incubated with inhibitor compounds demonstrating low cellular toxicity

Advantages:

- High-throughput screening allows for rapid identification of potential inhibitors or activators

- Precise and scalable method to measure enzyme activity

- High-quality and reproducible selection process for inhibitors and activators

- Low cytotoxicity of discovered compounds

Applications:

- Developing therapies for neurodegenerative diseases such as Parkinson's, Alzheimer's, and Huntington's by targeting protein misfolding

- Identifying novel compounds that can serve as either activators or inhibitors of HYPE-mediated AMPylation

- Potentially discovering treatments for diseases that involve proteostasis imbalances, like certain cancers and diabetes

TRL: Biotechnology

Intellectual Property:

Provisional-Gov. Funding, 2023-05-16, United States

Utility-Gov. Funding, 2024-05-15, United States

Keywords: Alzheimer's, Biomedical Engineering, Health, Medical, Therapeutics