A Bespoke Screening Platform to Study Mono-ADP-Ribosylation

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Abstract

1. Mono-ADP-Ribosylation Primer

- The PARP enzyme family is sub-divided based on the type of ADP-ribosylation performed; at least 6 different nucleophilic amino acids can be modified with MAR derived from nicotinamide adenine dinucleotide (NAD+)
- PARPs are activated under conditions of cellular stress such as viral infections and cancer

2. MonoPARP Assay Development Challenges

- Lack of validated substrates for in vitro enzyme assays
- No selective anti-MAR antibodies for assay development
- Unclear how monoPARPs are activated

3. Forced Self-Modification of Immobilized PARPs

- Example of self-modification enzyme inhibition assay development for PARP16
- DELFIA assays developed for all PARPs
- Immobilization overcomes weak KM for self-modification
- Dissociation Enhanced Lanthanide Fluorescence Immunoassay (DELFIA) assays developed for all PARPs
- Example of PARP7 TR-FRET probe displacement assay development
- TR-FRET assays developed for nearly all monoPARPs
- In many cases TR-FRET assays require far less enzyme than DELFIA self-modification assays to observe robust signal

4. NAD1-Competitive Probes for Assay Development

- Pan-PARP inhibitors were modified with a linker + fluorophore or biotin to generate probes for assay development
- Characterization of probe binding by SPR indicates they retain binding affinity

5. In Vitro Probe Displacement Assays

- Cellular probe displacement correlates with enzyme inhibition assays

6. Cellular Probe Displacement Assays

- Multi-step assay development of cellular probe displacement assays successfully applied to multiple PARP enzymes

7. Cellular Inhibitor-Target Residence Time

- Suite of biochemical & cellular assays developed enable family-wide profiling of PARP inhibitors and generation of potent and selective tool compounds for multiple PARP enzymes
- Assays do not rely on knowledge of PARP substrates
- Tool compounds being used at Ribon to investigate role of PARP enzymes in cancer cellular stress response and innate immunity
- RBN-2397, a potent and selective PARP7 inhibitor, was discovered using the assay platform described here. RBN-2397 is in a phase 1 trial in cancer patients.

REFERENCES:

Lu et al., Biochem Pharmacol. (2019)
Wigle et al. SLAS Discovery (2019)