Targeted Degradation of PARP14 Using a Heterobifunctional Small Molecule

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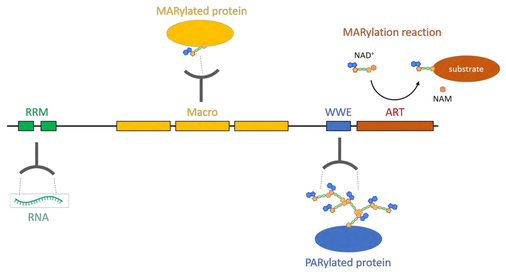
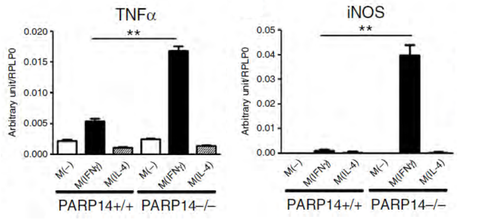
1. Domain Organization and Functions of PARP14



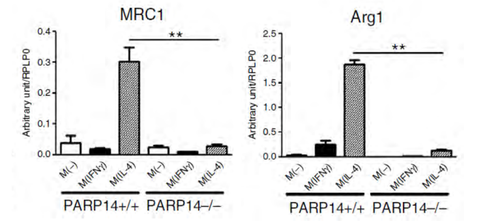
- PARP14 is a 203 kDa protein containing a catalytic domain responsible for the post-translational modification of proteins with mono-ADP-ribose using the substrate nicotinamide adenine dinucleotide (NAD+) and releasing nicotinamide (NAM) during the process.
- It also contains two RNA-recognition motifs which bind to RNA, three macrodomains which bind to mono-ADP-ribosylated proteins, and a WWE domain that binds to poly-ADP-ribosylated proteins.

2. PARP14 Plays a Role in Th1/Th2 Signaling

M1 (IFN-γ)

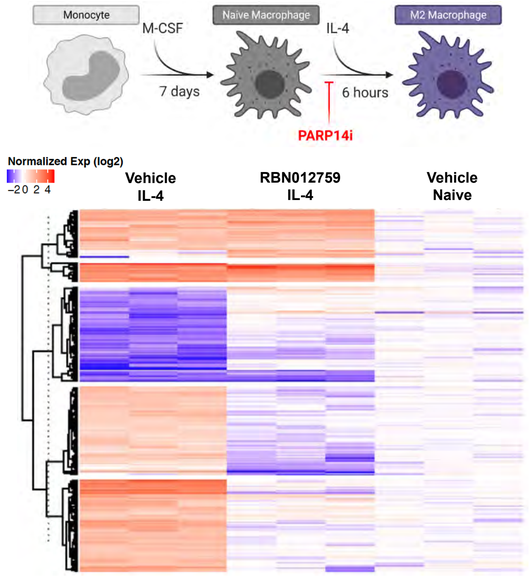


M2 (IL-4)



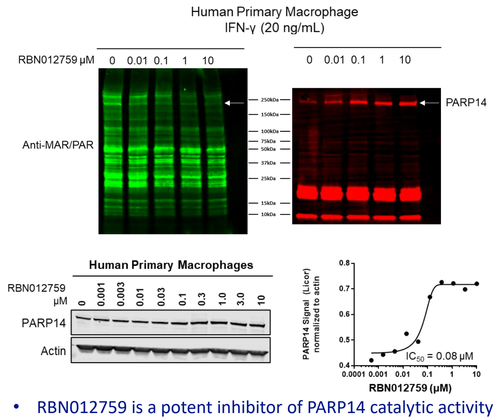
- PARP14 has been shown to be involved in IL-4/STAT6 signaling.
- Primary bone marrow-derived macrophages from PARP14-/- knockout mice show reduced M2 polarization under IL-4 treatment and increased M1 polarization under IFN-γ treatment.

3. PARP14 Inhibition Suppresses IL-4 Signaling in Human Primary Macrophages



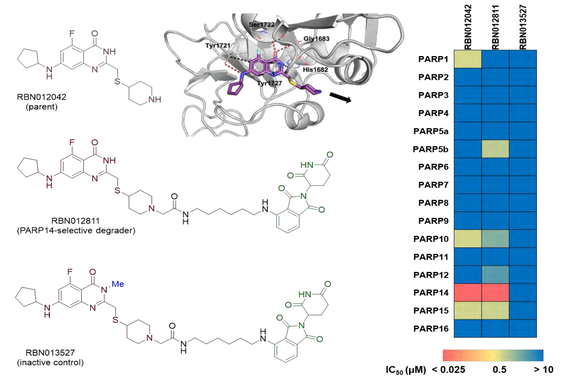
- Catalytic inhibition of PARP14 by RBN012759 suppresses the polarization of primary human macrophages induced by IL-4 treatment.

4. Catalytic PARP14 Inhibition Stabilizes PARP14 in Human Macrophages



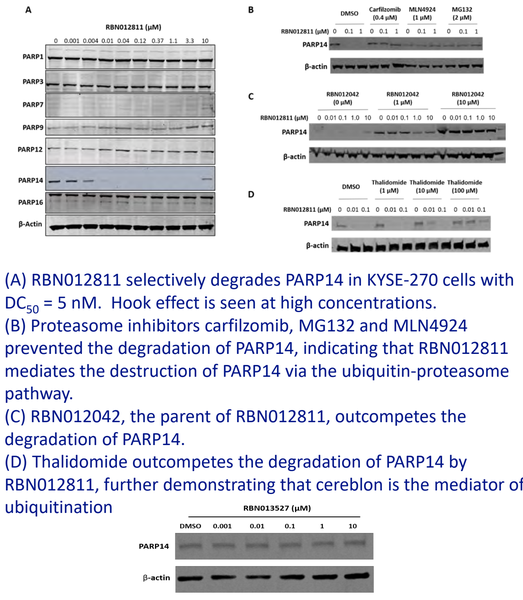
- RBN012759 is a potent inhibitor of PARP14 catalytic activity at nanomolar doses.
- At similar doses, the catalytic inhibitor also increases the levels of PARP14 protein by stabilizing it. This effect is similar to cellular thermal shift assays (CETSA), however no hear needs to be applied to de-stabilize PARP14.
- This suggests that PARP14 functions beyond the catalytic activity could affect the observed phenotypes.

5. Design and Biochemical Characterization of a PARP14 Degrader



- RBN012042 is a potent and selective inhibitor of PARP14
- An X-ray structure of RBN012042 (purple) bound to PARP14 (grey ribbon) (PDB ID: 7L9Y) indicated a vector (black arrow) that could tolerate a linker and thalidomide moiety to generate the degrader, RBN012811, that would lead to cereblon-mediated ubiquitination and destruction of PARP14.
- RBN012811 is potent against PARP14 and is at least 200-fold selective over all other PARPs tested.
- RBN013527 is an N-methylated version of RBN012811 that has no measurable inhibition of all PARP enzymes, including PARP14.

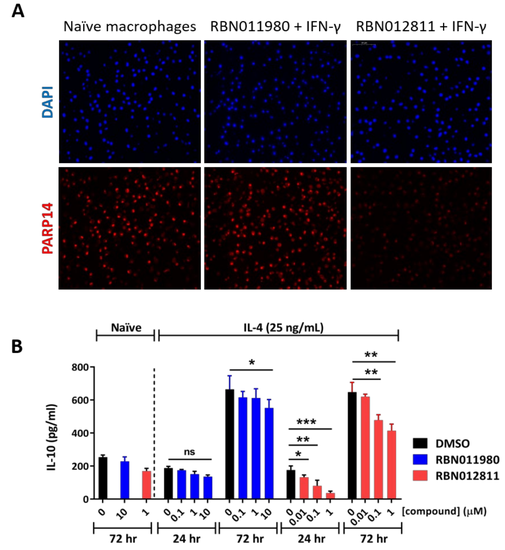
6. Characterization of a PARP14 Degrader in KYSE-270 Cells



- (A) RBN012811 selectively degrades PARP14 in KYSE-270 cells with DC_{50} = 5 nM. Hook effect is seen at high concentrations.
- (B) Proteasome inhibitors carfilzomib, MG132 and MLN4924 prevented the degradation of PARP14, indicating that RBN012811 mediates the destruction of PARP14 via the ubiquitin-proteasome pathway.
- (C) RBN012042, the parent of RBN012811, outcompetes the degradation of PARP14.
- (D) Thalidomide outcompetes the degradation of PARP14 by RBN012811, further demonstrating that cereblon is the mediator of ubiquitination

- RBN013527 does not degrade PARP14 and serves as a close-in analog inactive control

7. RBN012811 Degrades PARP14 in Primary Human Macrophages and Causes a Dose-Dependent Reduction in IL-10 Levels



- (A) Human primary macrophages were treated with DMSO (naïve), RBN011980 (catalytic inhibitor of PARP14), or RBN012811 for 24 h, then were stimulated with IFN-γ to induce polarization to an M1-like state or left unstimulated (naïve state). RBN012811 led to a significant decrease in PARP14 levels as seen by the red staining.
- (B) Human primary macrophages were stimulated with IL-4 to induce polarization to an M2-like state or left unstimulated (naïve state), then were treated with RBN012811 or RBN011980 for 24 or 72 h, and the culture supernatants were analyzed for IL-10. The level of IL-10 in M2-like human primary macrophages decreased in a dose-dependent manner upon RBN012811 treatment, and to a lesser extent upon RBN011980 treatment.

8. Conclusions

- In many cancer indications, tumor-associated M2-like macrophages (TAM) are associated with poor prognosis and outcome.
- Multiple strategies to affect TAM recruitment and polarization are currently under investigation in clinical trials.
- PARP14 catalytic inhibitors have been shown to regulate macrophage polarization; however, stabilization of protein by catalytic inhibitors makes it unclear if enzymatic activity or another function of PARP14 is responsible.
- RBN012811 recruits cereblon to selectively ubiquitinate and degrade PARP14, and RBN013527 serves as a close-in analog negative control.
- RBN012811 degrades PARP14 in KYSE-270 cells and human primary macrophages, as well as JJN-3, MDA-MB-231 and 293T cells (data for latter three not shown).
- Degrader causes a reduction in IL-10 levels in M2-polarized macrophages similar to catalytic inhibitor treatment.

REFERENCES:
Iwata et al., *Nature Comm* (2016) Wigle et al., *SLAS Discovery* (2019)
Wigle et al., *Cell Chem Bio* (2020) Schenkel et al., *Cell Chem Bio* (2021)