Investigating the Mechanism of PARP7 Inhibition in Type I Interferon Signaling by Arrayed CRISPR Screening

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Summary

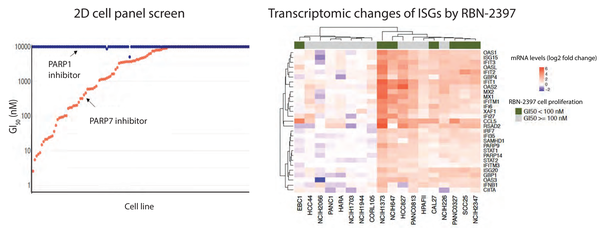
Genomic instability in cancer cells leads to cellular stress through the accumulation of aberrant nucleic acid species in the cytosol. We have shown that PARP7, a monoPARP, is a negative regulator of cytosolic nucleic acid sensing in cancer cells. RBN-2397 is a potent and selective PARP7 inhibitor that induces antitumor immunity in preclinical models and is currently being evaluated in a Phase I clinical trial.

In our preclinical investigations, we found that in a subset of cancer cell lines, such as NCI-H1373, inhibition of PARP7 triggers Type I IFN release, STAT1 phosphorylation, and growth arrest. In contrast, other cell lines, for example, HARA, do not mount an IFN-response upon PARP7 inhibition. To investigate the underlying mechanism of PARP7 inhibition and to determine the drivers of the differential sensitivity across cell lines we performed arrayed CRISPR knockout screens, targeting approximately 240 genes in the nucleic acid sensing and IFN signaling pathways, in the presence and absence of RBN-2397.

Our arrayed screens revealed depletion of cGAS/STING pathway conferred resistance to RBN-2397 in the NCI-H1373 responder cells, suggesting a critical dependence on this sensing pathway. In the RBN-2397-resistant HARA cells, deletion of components of innate immune-signaling (such as AIM2), and the NF-κB pathway sensitized the cells to PARP7 inhibition.

With our work, we shed light on the mechanism by which PARP7 acts as a critical suppressor of the innate immune response in tumor cells. Our findings demonstrate both redundancy and crosstalk between different nucleic acid-sensing pathways and may explain why some cell lines are resistant to RBN-2397.

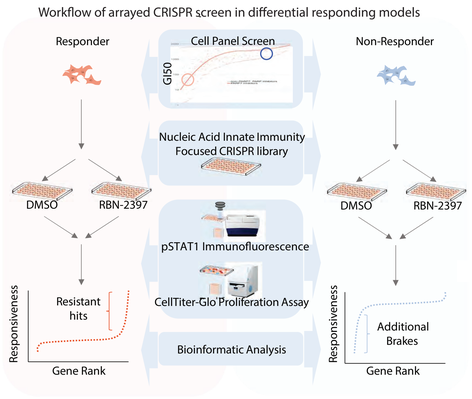
A Subset of Cancer Lines Is Sensitive to PARP7 Inhibition



- RBN-2397 is a potent and selective PARP7 inhibitor showing antiproliferative activity and activation of Type I IFN signaling in a subset of cancer cell lines
- Reactivation of tumor intrinsic Type I IFN signaling is a major determinant of the antitumor activity of RBN-2397 in vitro and in vivo

Results

1. Arrayed CRISPR Screen Performed to Explore the Mechanism of Action of PARP7 Inhibition

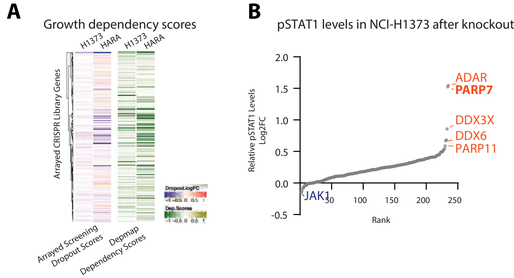


2. NCI-H1373 and HARA Cells Exhibit Differential Sensitivity to RBN-2397

Cell Line	Cancer Type	In vitro GI50 (nM)	In vivo TGI	pSTAT1 induction	PARP7 activity	Nucleci Acid Sensing
NCI-H1373	Lung Adenocarcinoma	40	100% TR	Y	Y	Intact
HARA	Squamous cell lung carcinoma	> 10,000	0% TGI	N	Y	Intact

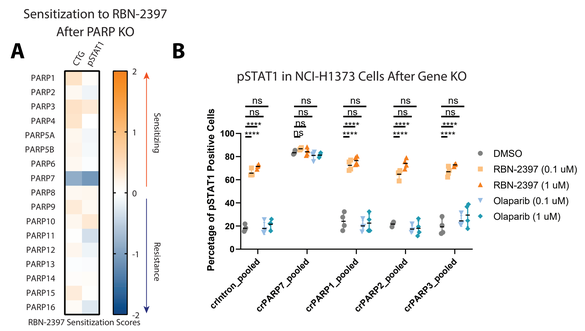
- NCI-H1373 (responder) and HARA (non-responder) both have an intact nucleic acid sensing pathway and PARP7 is enzymatically activated
- NCI-H1373 and HARA cells exhibit differential dependencies on PARP7

3. Arrayed Screening Successfully Performed With Known Regulators of Nucleic Acid Innate Immunity Identified as Hits



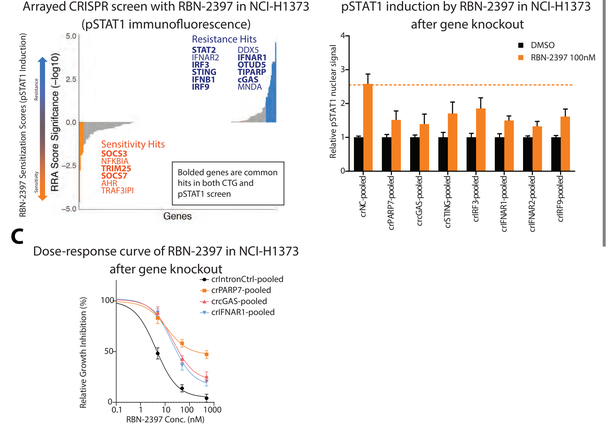
- The growth dependency scores from arrayed screening match published datasets.
- Knockout of known negative regulators of innate immunity, as well as PARP7, induce STAT1 phosphorylation in responder model

4. PARP-focused Arrayed CRISPR Screening Confirmed the PARP7 Dependency of RBN-2397



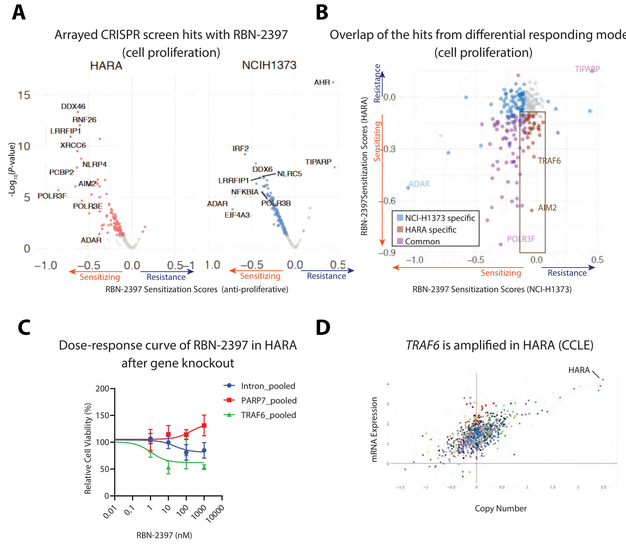
- PARP7, but not other PARP family members, is indispensable for the antiproliferative and IFN-I-inducing activities of RBN-2397
- The mechanisms of RBN-2397 is distinct from PARP1/2 inhibition

5. cGAS/STING Signaling Is Required for the Antiproliferative and IFN-I-inducing Activities of RBN-2397



- Arrayed screening in responding model (NCI-H1373) identified hits that sensitize (orange) or render resistance (blue) to PARP7 inhibition
- Knockout of cGAS/STING pathway attenuates the antiproliferative and IFN-I-inducing activities of RBN-2397

6. Activity of the NF-κB Pathway Contributes to the Resistance to RBN-2397



- Deletion of components of innate immune signaling (such as AIM2 and ADAR1), and the NF-κB pathway, sensitized cells to RBN-2397
- Unique sensitizing hits in non-responder cell line HARA (such as TRAF6) suggest potential de novo resistance mechanisms
- TRAF6 is amplified in HARA, depletion of which sensitizes cells to RBN-2397

Conclusion

- Arrayed CRISPR screening performed in differential responding models to explore mechanism of action of PARP7 inhibition
- RBN-2397 is a potent and selective PARP7 inhibitor that suppresses cell proliferation and activates Type-I IFN pathway in tumor cells
- The underlying mechanism by which RBN-2397 induces antitumor immunity involves cGAS/STING pathway
- Activation of additional nucleic acid sensing pathways modulates the dependency on PARP7