#5786 Synthetic-Lethality Screening with PARP1 inhibition and Novel Non-CRBN Based Degrader Identification via High-throughput Screen and Target Deconvolution by Proximity Labeling

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Abstract

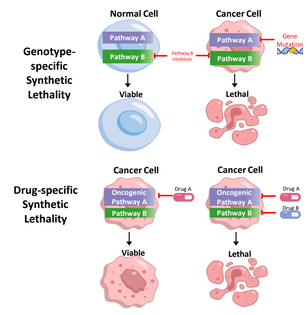
Targeted Protein Degradation (TPD) is considered one of the new drug discovery strategies that could address previously "undruggable" disease targets through the ubiquitin-proteasome system (UPS). Although the current Target Protein Centric approach has been proven successful in many cases, especially with well-validated E3 ligases such as CRBN and VHL, there is still a strong need to identify potential target protein degraders (PROTACs, molecular glues, etc.) through both target-centric and target-agnostic approaches. This would not only explore and identify potential tissue-specific novel E3 ligases but also greatly expand the range of potential degradable disease targets.

In this study, we first developed a molecular glue-focused library consisting of approximately 5,000 compounds through various medicinal chemistry evaluations. A GSPT1-HiBit cell line and a GSPT1-CRBN-KO counter cell line were developed for primary hit screening purposes. More than 100 potential hits were identified through the initial screening, which included both CRBN-dependent and CRBN-independent hits. A GSPT1-based proximity labeling cell line was then constructed for further target deconvolution purposes via proteomics.

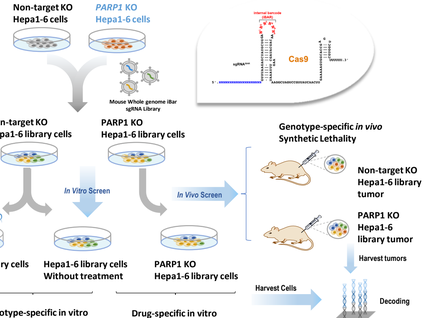
Additionally, the synthetic-lethality mechanism can achieve specific killing of tumors with specific mutation background, laying the foundation for the development of precision treatment strategies based on molecular adhesives. We tried *in vitro* and *in vivo* synthetic-lethality screen to explore the sensitivity mechanism of liver cancer to PARP1 inhibition. We plan to future use the Molecular glue-focused library to identify novel degraders of PARP1 and other co-lethal genes, then combined both transcriptomics and proteomics assays to evaluate potential related E3 ligase. To sum up, our study demonstrates that integrated synthetic-lethality target discovery and target deconvolution strategies could be useful for TPD drug, realizing a closed loop from mechanism discovery to intervention strategies.

Synthetic Lethal Target Discovery Design

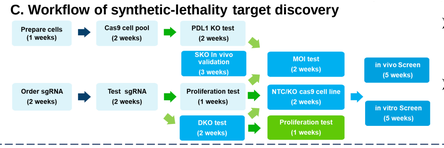
A. Introduction of synthetic-lethality strategy



B. Experiment design of synthetic-lethality screen



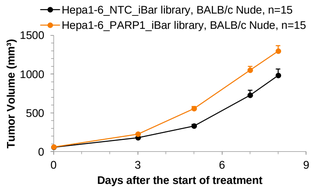
C. Workflow of synthetic-lethality target discovery



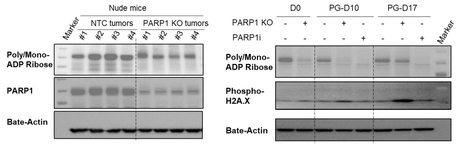
- The synthetic-lethality mechanism can achieve specific killing of tumors with specific mutation background[1]
- A whole-genome library of mouse with barcodes[2] (~80k) for parallel *in vivo* and *in vitro* screen in liver cancer

Synthetic Lethal Target Discovery Process

A. Tumor volume cure for *in vivo* screen

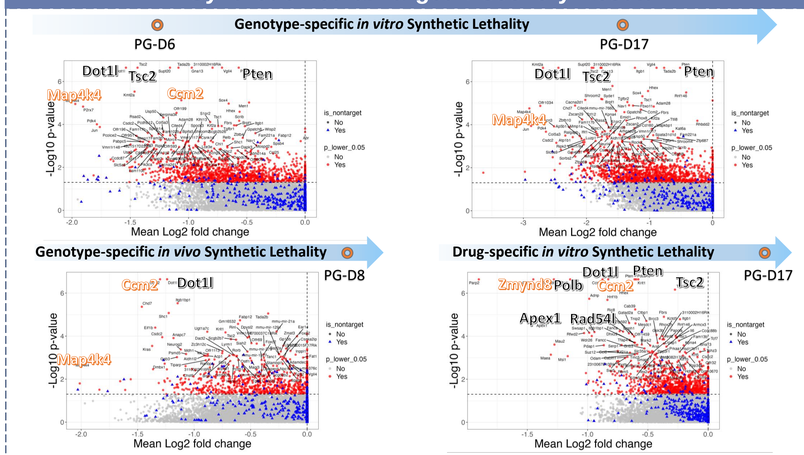


B. Biomarker validation of screen samples



- The changes of biomarker PAR indicated the PARP1 inhibit effect caused by Genotype-KO or Saruparib treatment.

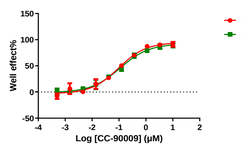
Synthetic Lethal Target Discovery Result



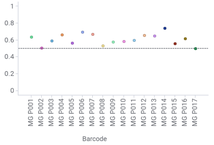
- The screening results identified parp1 co-lethal genes that had been reported in other tumor models: Dot1l (Ovarian carcinoma[3]), Apex1 (Gastric cancer[4]), Pten (Prostate cancer[5], colorectal cancer[6]), Tsc2 (Leiomyoma[7]), and also genes have not be reported before: Map4k4, Ccm2, Zmynd8…

Molecular Glue-focused Library Screen

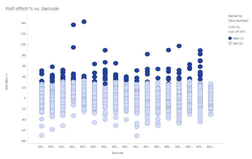
A. Dose-response and uniformity test of ref compound



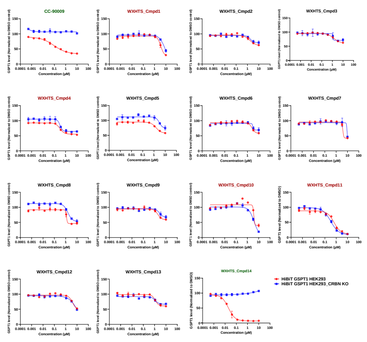
B. Z prime > 0.5 for all screening plates in the HTS campaign

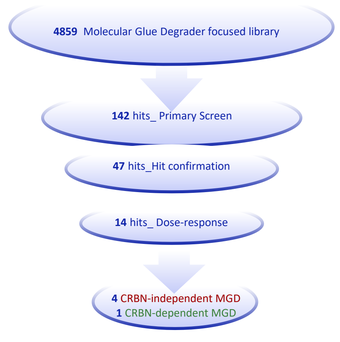


C. Overview of tested compound



D. Dose-response of hit compounds across both target and counter cell lines

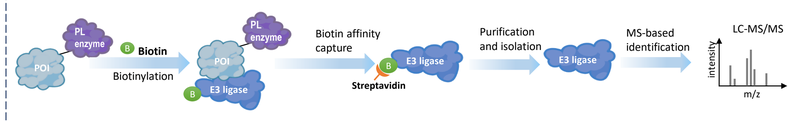




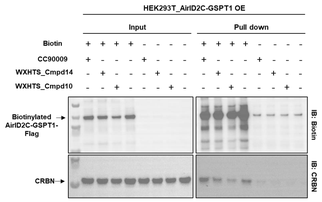
- Nano-Glo® HiBiT assay at 384 well format was developed with robust performance.
- Screened 4.8K compounds with hit rate 2.92%.
- Hits identified over the counter screen include both CRBN-dependent and CRBN-independent degraders.

Biotin Ligase-based Proximity Labeling

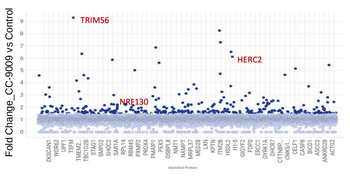
A. Proximity Labeling E3 deconvolution strategy[8]



B. Biotinylation and pull down



C. LC-MS for E3 identification and ranking



Genes	Fold Change
TRIM56	9.31
HERC2	6.12
RNF130	2.29
SH3RF1	1.89
ZBED1	1.55
UBE2E1	1.51
UBE2K	1.45
MARCHF5	1.43
PIAS2	1.41
CRBN	1.33

- CRBN could be captured and detected after GSPT1 degrader treatment for 4 hrs.
- The LC-MS analysis revealed multiple E3 ubiquitin ligases with a fold change exceeding 1.3.

Conclusion and Future Perspective

Conclusion

- Utilizing the construction of counter cell lines combined with high-throughput screening, CRBN-dependent and CRBN-independent degraders were successfully identified from the WuXi MGD focused library.
- Successfully established proximity labeling system combined with proteomics strategies to facilitate the deconvolution of E3 ligase.
- The iBar technology was utilized to achieve whole-genome *in vivo* screening, expanding the range of reported synthetic-lethality genes of PARP1 to liver cancer and identifying new synthetic-lethality genes.

Future Perspective

- We plan to future use the Molecular glue-focused library to identify novel degraders of PARP1 and other co-lethal genes, then combined both transcriptomics and proteomics assays to evaluate potential "degradable" targets.
- Potential candidate E3 ligases could be validated using a newly established array-based expression manipulation strategy.

References

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