A WRN screening cascade to facilitate novel drug discovery

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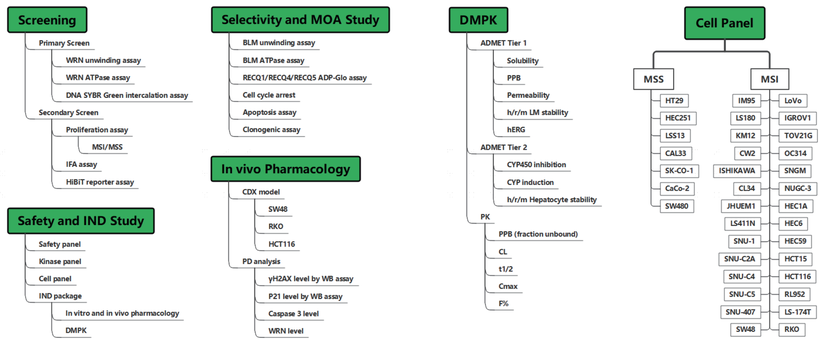
Abstract #5609

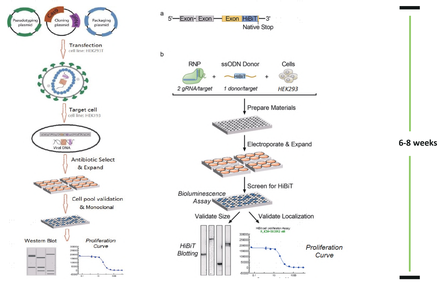
Introduction

Human RecQ deconjugating enzyme WRN is involved in DNA replication, DNA repair, recombination, transcription and telomere stabilization[1]. It plays a key role in nucleic acid metabolism as well. WRN defects lead to premature aging, type II diabetes, osteoporosis, atherosclerosis and cancer[2]. Hence it is of great interest of both pharmaceutical and academic field to develop the WRN inhibitors. Here we constructed an integrated experimental cascade, which contains both in vitro and in vivo assays, to conduct the high throughput hit-to-lead compound screen. WRN proteins of different length have been successfully purified and utilized to develop multiple biochemical assays such as unwinding assay and ATPase assay. We have also validated different cellular assays, including proliferation and immunofluorescence, to assess the cytotoxicity and the influence of downstream biomarkers of WRN inhibitors. A WRN knock-out cell line has been generated to better appreciate the inhibition mechanism. In addition, we have generated a WRN-HiBiT knock-in cell line to evaluate WRN degraders or target-compound interactions. Lastly, multiple CDX models utilizing different MSI or MSS cell lines have been validated to help determine the efficacy of WRN inhibitors thus shed light on the drug indications. Together our WRN screening cascade can provide comprehensive compound evaluation across in vitro and in vivo platforms, thus serve as an efficient screening platform for new drug discovery.

WRN Screening Cascade

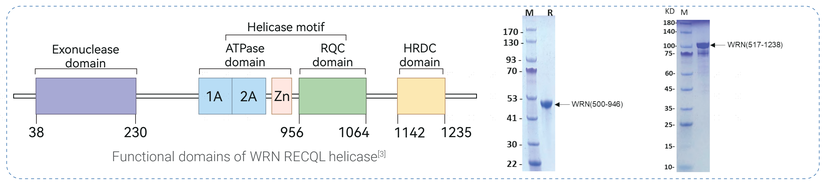


WRN KO & HiBiT KI Cell Line Generation

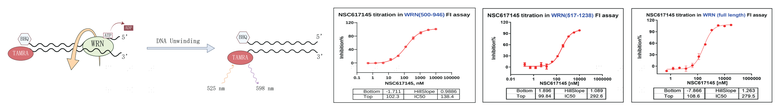


Results

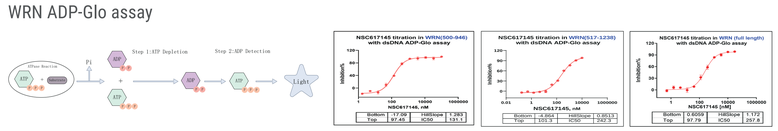
1.Biochemical Assays



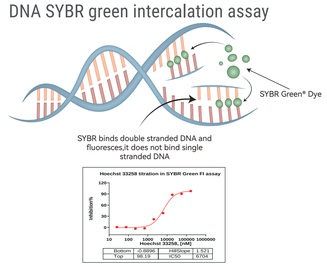
WRN unwinding assay



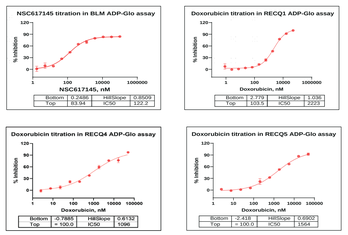
WRN ADP-Glo assay



DNA SYBR green intercalation assay



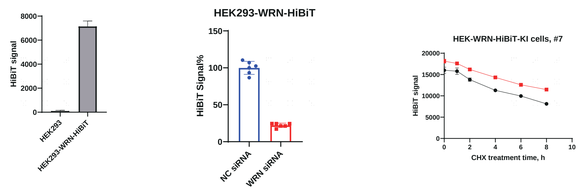
WRN selectivity assay



We have successfully purified WRN proteins of different length. We have developed WRN-related unwinding assay, ATPase assay, DNA SYBR Green interaction and other assays to assess the enzymatic activity. These assays can be used for rapid and efficient screening of WRN inhibitors. Representative data are shown above.

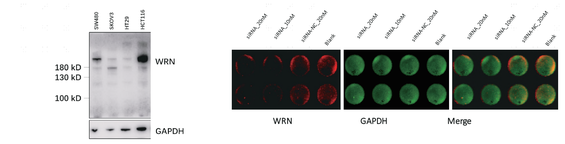
2. HiBiT KI Cell Line Generation

We have successfully constructed HiBiT KI cell lines and validated them using HiBiT reporter assay and siRNA assay. A decreased HiBiT signal has been observed upon cycloheximide (CHX) treatment. The constructed HiBiT cells can be utilized for the screen of WRN inhibitors and the subsequent MOA studies.

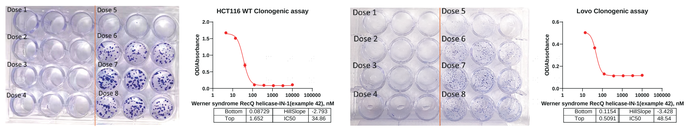


3. Cellular Assays in WRN-KO cell line

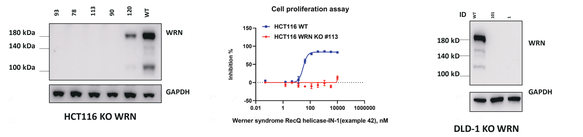
We have developed multiple cellular assays to evaluate DNA damage biomarkers, cell viability in various cell lines. These assays provide broad range of service for the screening and development of WRN inhibitors.



Traditional Western Blot and In-Cell Western to evaluate WRN expression level across different cell lines.



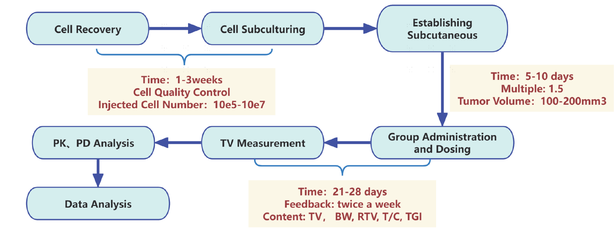
Clonogenic assay serves as a method to assess the viability of drug-treated cells with high sensitivity.

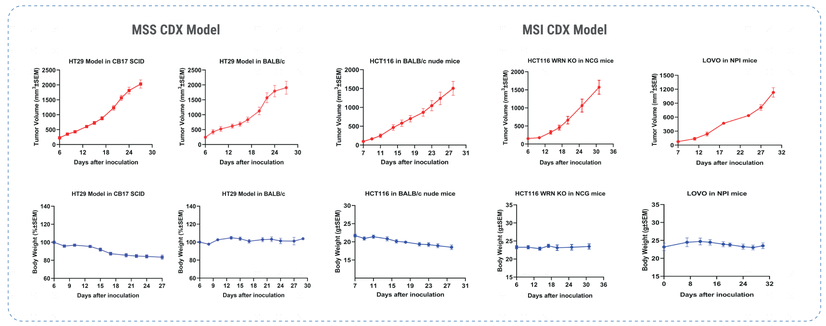


Selected cell proliferation data of both parental and WRN KO cells are shown above. Werner syndrome RecQ helicase-IN-1 (example 42) exhibits inhibitory effect in HCT116 WT but not in DLD-1 WT and WRN-KO cells. This result is in line with the published data[4], [5].

4. Animal Modeling

CDX modeling utilizing different cell lines has been established for the efficacy study as shown below to expand the drug discovery cascade to the in vivo experiments.





Besides in vivo CDX modeling, we can also provide PK/PD study to in-depth evaluate the efficacy of the potential drug compounds. Furthermore, our safety and kinase panel screening capabilities can provide the pre-clinical Adverse Drug Reactions (ADRs) evaluation and prediction.

Summary

When WRN is inhibited, it impairs the growth of tumor cells as well as the integrity and stability of the genome. Therefore, the development of WRN inhibitors has received great attention from academia field and pharmaceutical companies. Our WRN screening cascade can provide comprehensive compound evaluation across in vitro and in vivo platforms, thus serve as an efficient screening platform for new drug discovery.

References

[1] Kitano K. Structural mechanisms of human RecQ helicases WRN and BLM. Front Genet. 2014 Oct 29;5:366.
[2] Hussain, et al. "Skin Abnormalities in Disorders with DNA Repair Defects, Premature Aging, and Mitochondrial Dysfunction." The Journal of investigative dermatology vol. 141,4S (2021): 968-975.
[3] Gupta, et al. "Enigmatic role of WRN-RECQL helicase in DNA repair and its implications in cancer." J. Transl. Genet. Genom, 6, 147-156 (2022).
[4] Hao, et al. "Synthetical lethality of Werner helicase and mismatch repair deficiency is mediated by p53 and PUMA in colon cancer." Proc Natl Acad Sci U S A. 2022 Dec 20;119(51):e2211775119.
[5] Lieb, et al. "Werner syndrome helicase is a selective vulnerability of microsatellite instability-high tumor cells." Elife. 2019 Mar 25;8:e43333.