A WRN Screening Cascade to Facilitate Novel Drug Discovery

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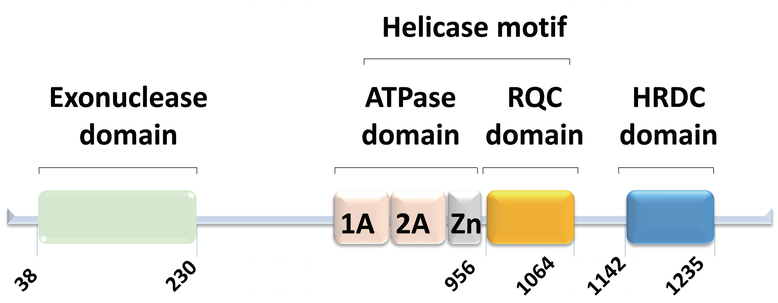
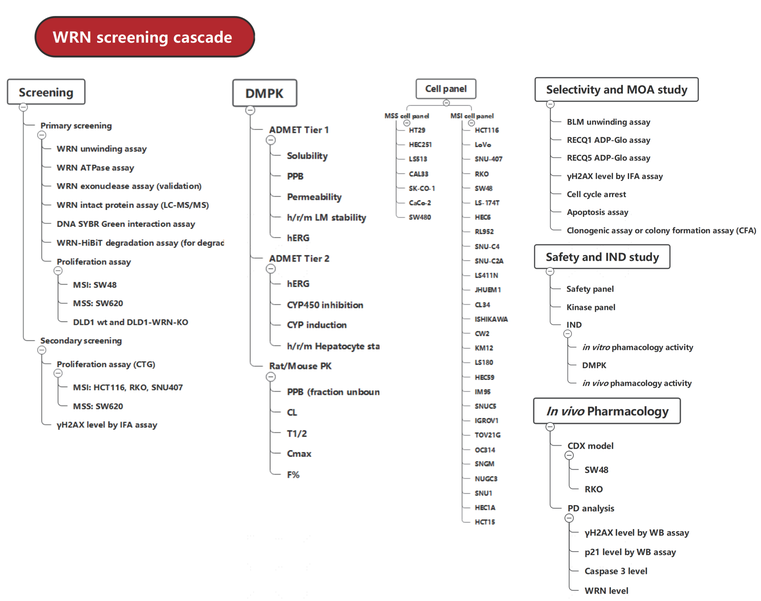
Introduction

WRN is a nuclear protein with two known enzymatic functions: a 3'–5' exonuclease activity residing in the amino-terminal region and ATP-dependent 3'–5' DNA helicase unwinding activity, which has roles in various cellular processes that are crucial for the maintenance of genome stability.
Cancers with microsatellite instability (MSI) result from mutations in mismatch repair genes, leading to broadly distributed insertions and/or deletions of TA-dinucleotide repeats within microsatellite regions across the genome.
WRN is required for unwinding problematic DNA structures found in MSI-H cells, and the loss of WRN during cell replication leads to dsDNA breaks and the initiation of a DNA repair response[3-4]. WRN emerges as a promising synthetic lethal target in MSI-H cancers.

WRN Screening Cascade



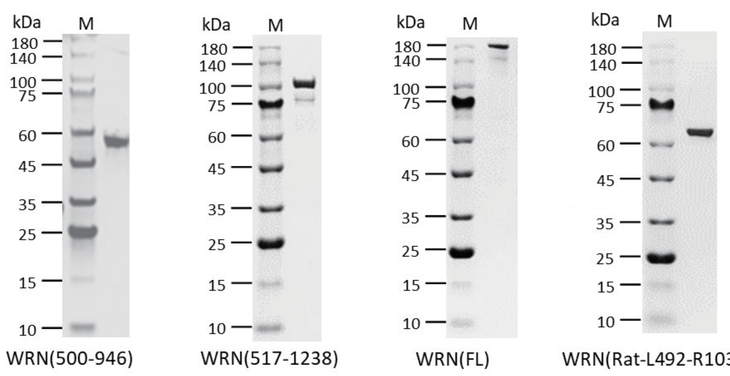
Functional domains of WRN



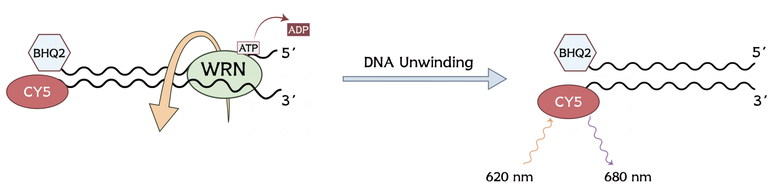
1. Biochemical Assays

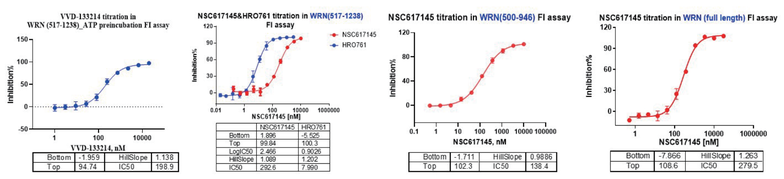
We have successfully purified WRN proteins. We have developed WRN unwinding assay and DNA intercalation assay to screen the inhibitors of WRN. We also have developed ADP-Glo assay to assess its ATPase activity. SPR assay was developed to measure the binding interactions between WRN and potential inhibitors. Our high-quality, detailed kinetic data help in understanding the mechanisms of action of these compounds, supporting the development of targeted therapies for conditions associated with WRN dysfunction.

SDS-PAGE

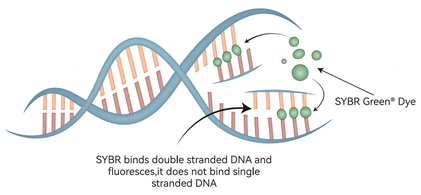


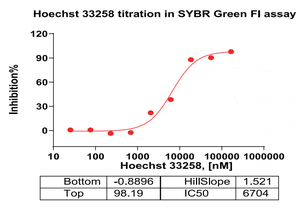
WRN unwinding assay



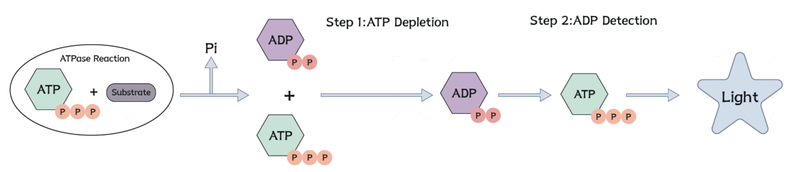


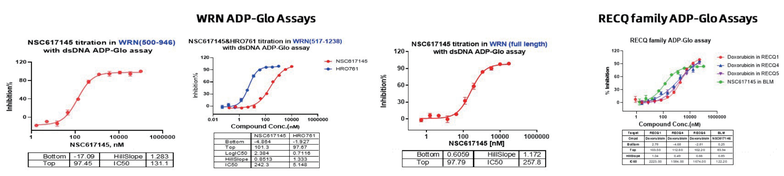
DNA SYBR green intercalation assay



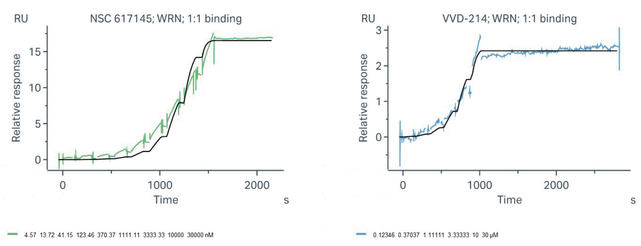


WRN ADP-Glo assay





WRN SPR assay

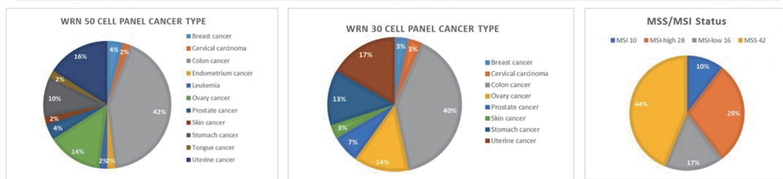


Immobilized ligand	Injection variables Single cycle kinetics 1 Solution	General Kinetics model	1:1 binding ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Quality Kinetics Chi² (RU²)	U-value
WRN	NSC617145	1:1 binding	1.08e+03	8.39e-07	7.80e-10	16.6	9.46e-01	95
WRN	VVD-214	1:1 binding	5.64e+02	7.66e-08	1.36e-10	2.5	1.43e-02	95

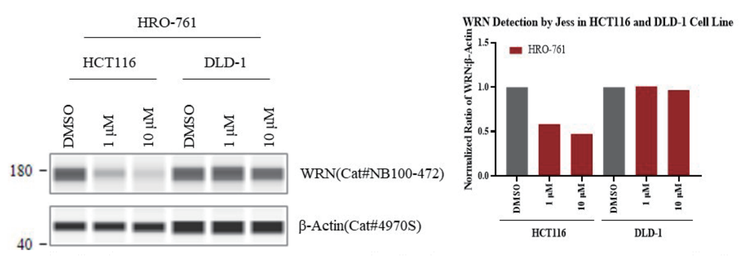
2. Cellular Assays

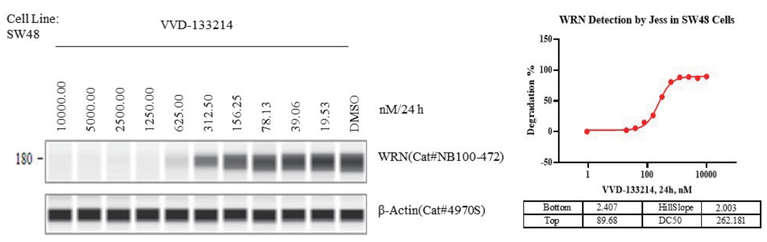
WRN cell panel: A customizable WRN cell panel for assessing cell viability and proliferation is ready which includes 96 tumor cell lines with various MSI, MSS, and MSI-high statuses, allowing for compounds screening.

WRN Cell panel List				
#	Cell Panel Name	Fixed/Customized Type	Cell Line Number	Cancer Type Number
1	WRN 50 2D cell panel	Fixed	50	11
2	WRN 30 2D cell panel	Fixed	30	8
3	WRN customized cell panel	Customized	96	\

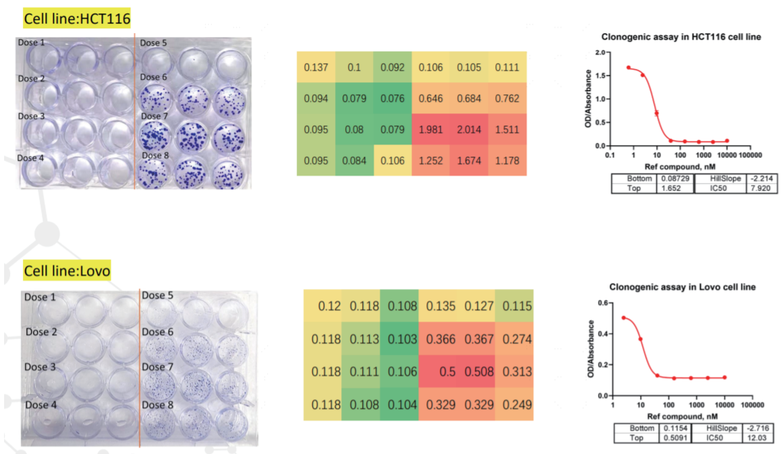


Protein Detection and Analysis: The HiBit, Western Blot (WB), and In-Cell Western (ICW) assays were developed to monitor WRN expression and degradation in response to treatments.



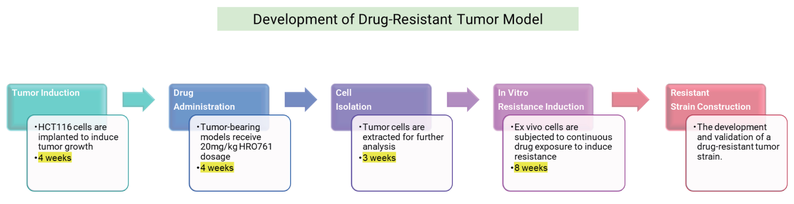


Clonogenic assay: Our clonogenic assay evaluates the ability of cells to form colonies, providing insights into the long-term effects of WRN inhibitors on cell survival and proliferation.

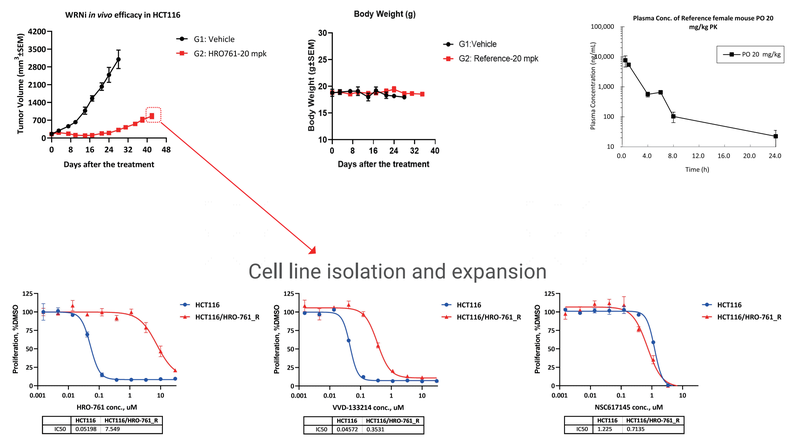


3. CDX Models and Drug Resistance Studies

We have constructed multiple cell-derived xenograft (CDX) models, such as HCT116, for WRN drug research. Using WRN inhibitors (WRNi), we have induced resistance in vivo, isolated resistant cells from mice, and established resistant cell lines in vitro. These models are crucial for studying drug resistance mechanisms and developing more effective treatments.



HCT116 in vivo efficacy



IC_{50},uM	HCT-116	HCT-116/HRO761_R
HRO-761	0.052	7.5
VVD-133214	0.045	0.35
NSC-617145	1.2	0.71

Conclusions

The helicase activity of WRN plays an essential and non-redundant role in the resolution of DNA secondary structures formed by expanded TA-dinucleotide repeats in MSI-H cancers. WRN deficiency is causally associated with genomic instability and cancer predispositions.
Therefore, extensive work is required to glean an in-depth understanding of the molecular mechanisms of the involvement of WRN in various DNA repair and cellular processes.
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

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