A WRN screening cascade to facilitate novel drug discovery

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Introduction

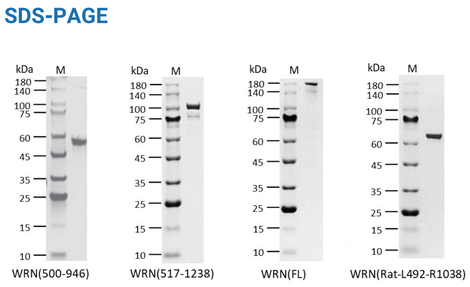
Werner Syndrome (WS) is an autosomal-recessive genetic disorder characterized by premature ageing and DNA repair defects because of mutations in the WRN gene. WRN is a RecQ family protein with helicase, strand annealing and exonuclease activities. WRN localizes to the sites of damaged DNA, interacts with several DNA repair pathways including base excision DNA repair, non-homologous end-joining (NHEJ), homologous recombination (HR) and replication re-start after DNA damage.

Here, we constructed an experimental cascade from in vitro to in vivo, which is composed of protein production, biochemical assays, cell line construction, cellular assays, and animal modeling. This WRN screening cascade can satisfy the mechanism study of WRN as well as efficient and comprehensive screen of WRN inhibitor, thus accelerate the novel drug discovery.

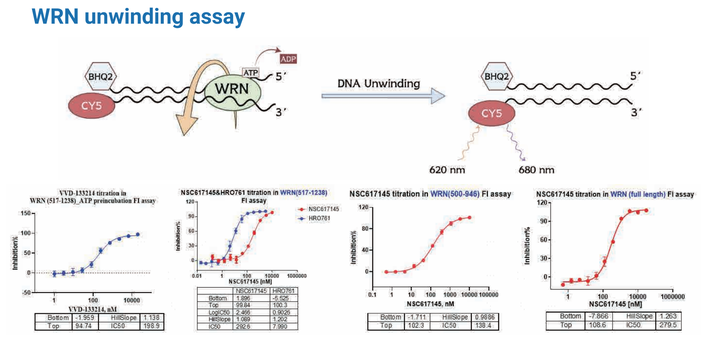
1. Biochemical Assays

We have successfully purified WRN proteins. Three WRN biochemical assays, WRN unwinding assay, DNA-intercalation assay and ADP-Glo assay, are developed to screen the inhibitors of WRN. SPR assay was developed to measure the binding interactions between WRN and potential inhibitors.

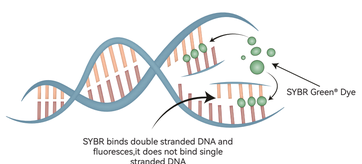
SDS-PAGE

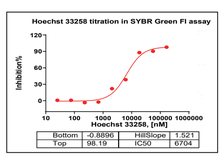


WRN unwinding assay

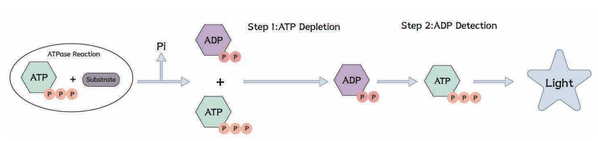


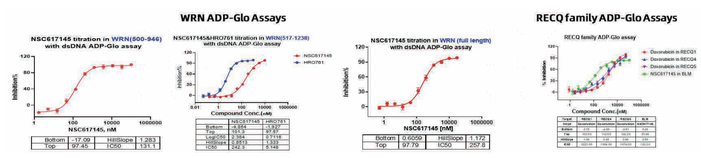
DNA SYBR green intercalation assay



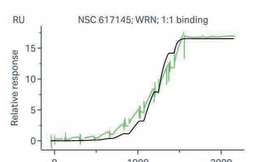


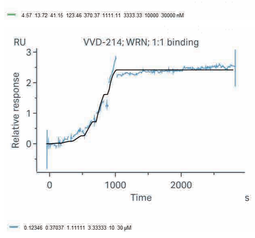
WRN ADP-Glo assay





WRN SPR assay





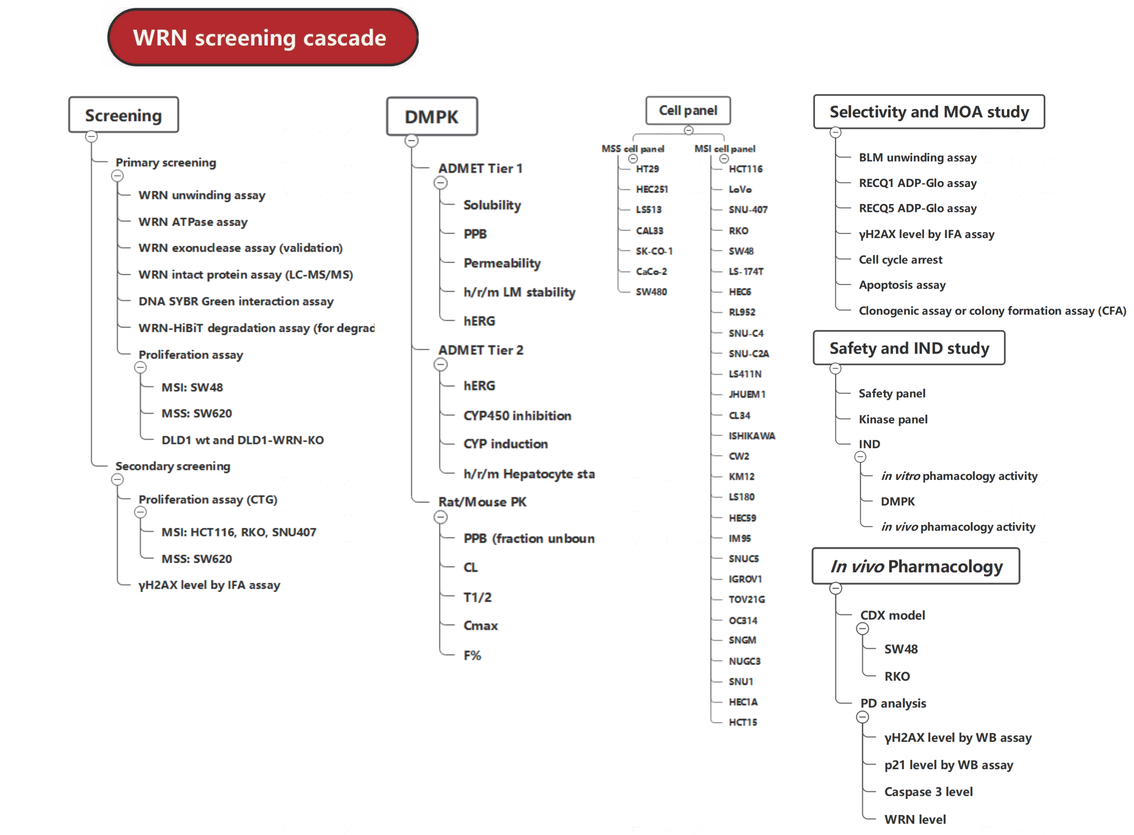
Helicase panel

Target	Substrate	Target	Substrate
WRN	DNA	DDX5	RNA
BLM	DNA	DHX58/LGP2	RNA
RECQ1	DNA	DDX6	RNA
RECQ4	DNA	DDX20	RNA
RECQ5	DNA	DDX17	RNA
HELQ	DNA	DDX21	RNA
DHX9	RNA	DDX39B	RNA
POLQ	DNA	DDX49	RNA
MDA5/IFIH1	RNA	DDX43	RNA
FANCJ	DNA	DDX53	RNA
RIG-1/DDX58	RNA	DDX3X	RNA
DDX48(eIF4a3)	RNA	DDX3Y	RNA

Note: blue represents now avilable and light blue represents under development

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

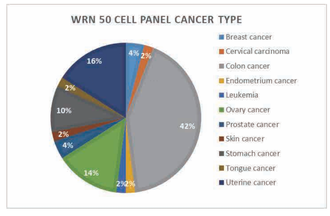
WRN Screening Cascade

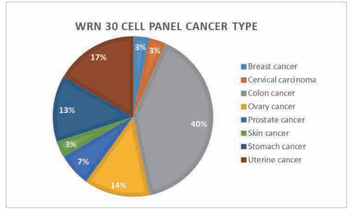


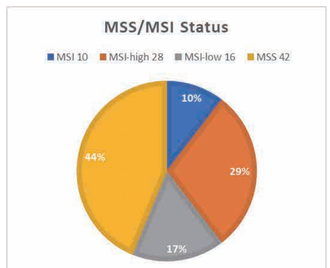
2. Cellular Assays

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.

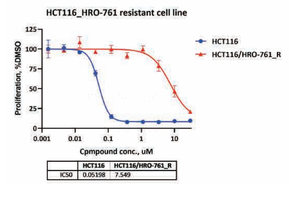
WRN Cell panel List				
#	Cell panel name	Fixed/Customized type	Cell line number	Cancer type number
1	WRN 50 2D cell panel	Fixed	50	11
2	WRN 30 2D cell panel	Fixed	30	8
3	WRN customized cell panel	Customized	96	\

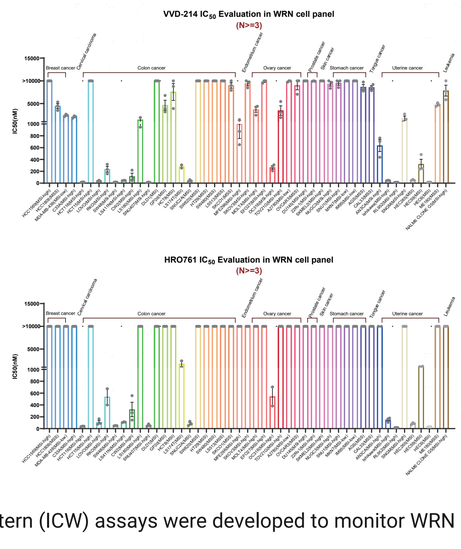




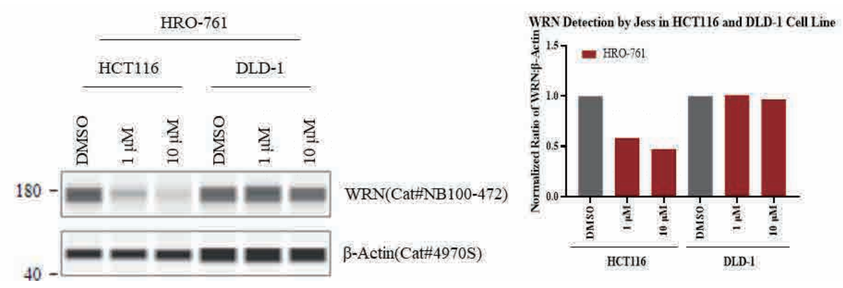


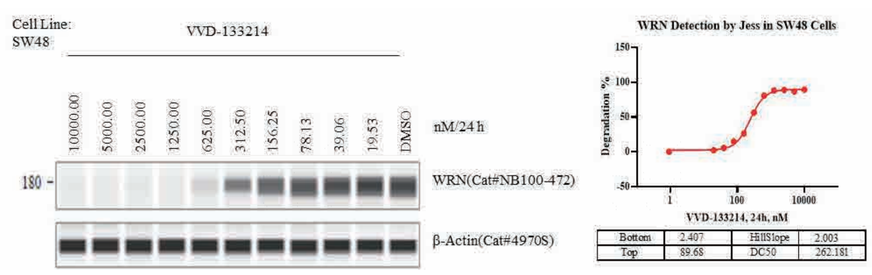
WRNi resistant cell line





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.





Conclusions

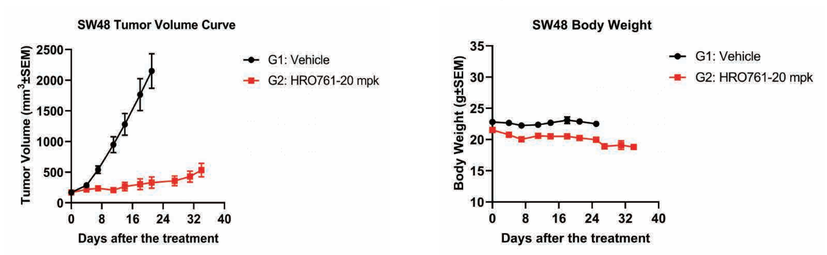
WRN helicase is involved in DNA repair pathways, especially in DSB repair. Consequently, WRN deficiency is causally associated with genomic instability and cancer predispositions. Therefore, extensive work is required to glean an in-depth understanding of the molecular mechanisms of the involvement of 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.

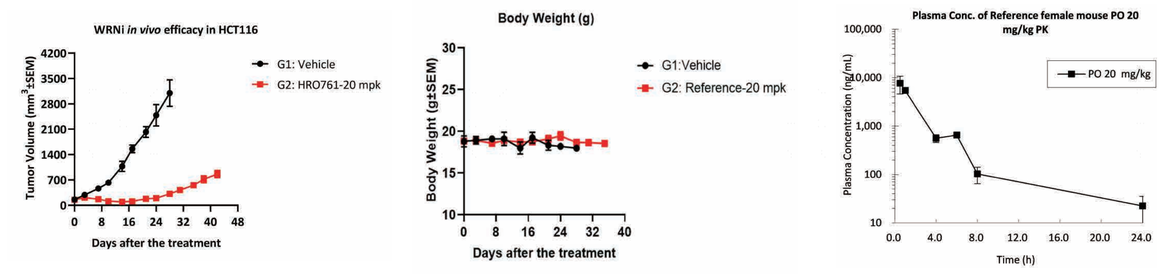
3. CDX Models and Drug Resistance Studies

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.

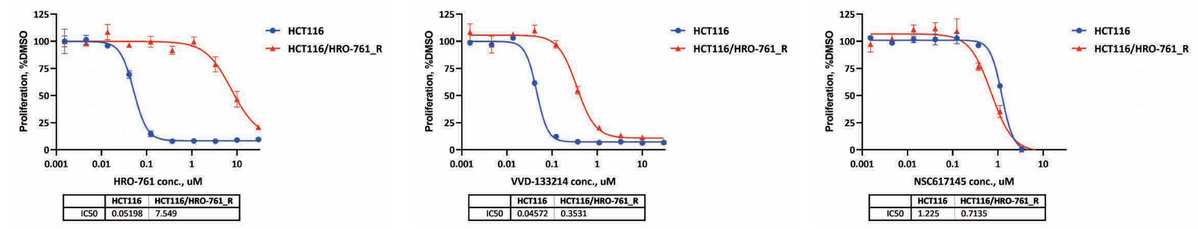
SW48 in vivo efficacy



HCT116 in vivo efficacy



Cell line isolation and expansion



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

References

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