A small-molecule inhibitor of WRN selectively kills MSI-H cancer cells and phenocopies *WRN* genetic defects

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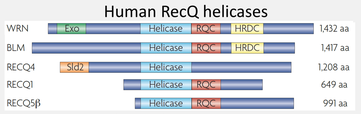
Abstract

Werner syndrome protein (WRN) is a RecQ-family helicase involved in the maintenance of genome integrity. Germline mutations in *WRN* cause premature aging and cancer predisposition. Analysis of systematic RNAi and CRISPR screening data has previously revealed that *WRN* is essential for the survival of cancer cell lines with high microsatellite instability (MSI-H). We have developed potent and selective small-molecule inhibitors of WRN helicase (WRNi) and showed that pharmacological inhibition of WRN causes lethality and induction of DNA damage markers selectively in MSI-H cancer cell lines compared to microsatellite-stable (MSS) cancer cell lines. Screening of WRNi across a large panel of pooled, barcoded cell lines in the PRISM format revealed selective sensitivity in MSI-H cell lines and showed that pharmacological inhibition of WRN is highly correlated with genetic ablation of *WRN* across this panel, confirming selectivity for WRN. *In vivo* evaluation demonstrated robust and MSI-selective tumor regressions. These data provide pharmacological proof-of-concept for the WRN/MSI-H synthetic lethal relationship and support WRN inhibition as a novel therapeutic approach for the treatment of MSI-H cancers.

Background

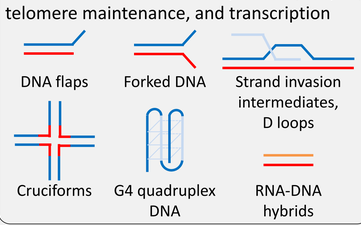
WRN is a human RecQ helicase with 3′-5′ helicase and 3′-5′ exonuclease domains



Exo = Exonuclease domain
RQC = RecQ C-terminal domain
HRDC = Helicase-and-ribonuclease-D C-terminal domain

(Chu and Hickson, 2009)

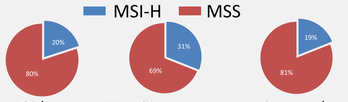
WRN acts on multiple DNA substrates and has roles in DNA replication, repair, telomere maintenance, and transcription

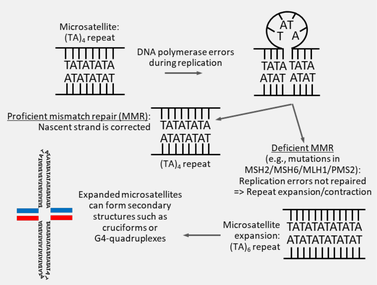


Microsatellite instability (MSI) is prevalent in cancers

- MSI = variable lengths of microsatellites (short segments of repetitive DNA, 1-6 nt per repeat)
- Can lead to the formation of secondary DNA structures, e.g., cruciforms and G4 quadruplexes
- Can be caused be mismatch repair (MMR) defects

~0.25% - 31.4% of cancers have MSI-H





(Bonneville et al, 2017)

Conclusions

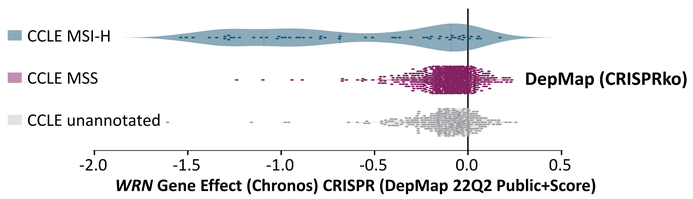
- The synthetic lethal relationship between MSI-H and WRN, observed using genetic knockdown of WRN, is reproducible with small-molecule inhibitors of WRN helicase; IDB-WRNi selectively reduce the viability of MSI-H cancer cells
- Pan-cancer chemical screening of cancer cell lines revealed that IDB-WrNi recapitulates the effect of *WRN* knockdown/knockout; MSI-H colorectal adenocarcinomas are the lineage most sensitive to IDB-WRNi
- Pharmacological inhibition of WRN causes tumor regression specifically in MSI-H but not MSS *in vivo* models, suggesting that WRN inhibition might be a novel therapeutic approach for patients with MSI-H tumors

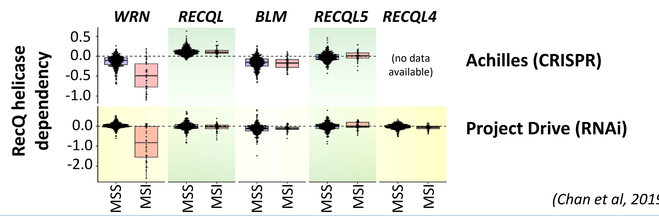
References

1. Chu et al., Nat Rev Cancer 2009, 9:644-54
2. Bonneville et al., JCO Precis Oncol 2017, 2017:PO.17.00073
3. Chan et al., Nature 2019, 568:551-556
4. Kategaya et al., iScience 2019, 293:488-497
5. Sommers et al., PLoS One 2019, 14:e0210525
6. Yu et al., Nat Biotech 2016, 34:419-423

WRN is essential in microsatellite-unstable (MSI-H) cancers but not in microsatellite-stable (MSS) cancers

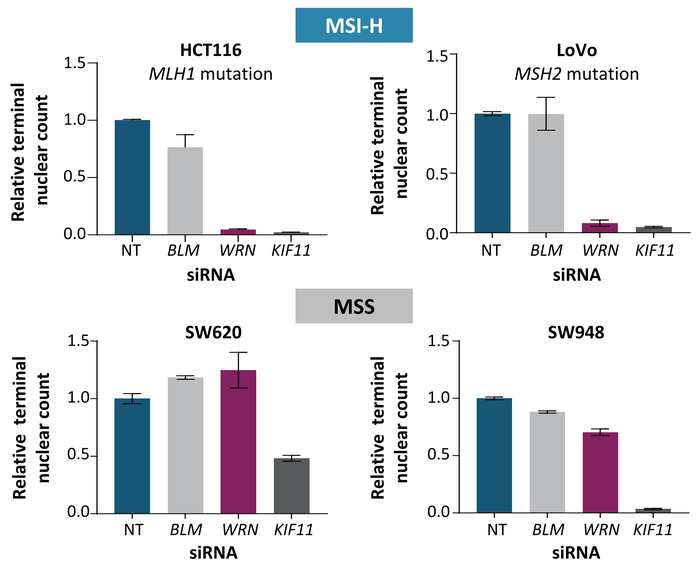
Analysis of pan-cancer screen datasets reveals *WRN* essentiality in MSI-H cancer cell lines





(Chan et al, 2019)

WRN knockdown selectively causes synthetic lethality in combination with mismatch repair (MMR) defects

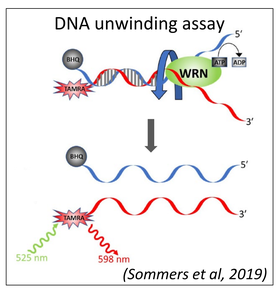


- WRN helicase domain is sufficient to restore viability to WRN-depleted MSI-H cells
- Rescue with a WRN WT or helicase construct also reduced Caspase 3/7 activation

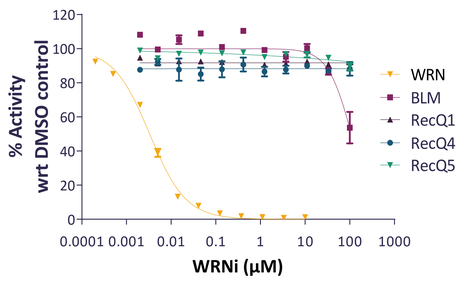
(Kategaya et al, 2019)

WRN-selective chemical inhibitors phenocopy genetic knockdown of *WRN*

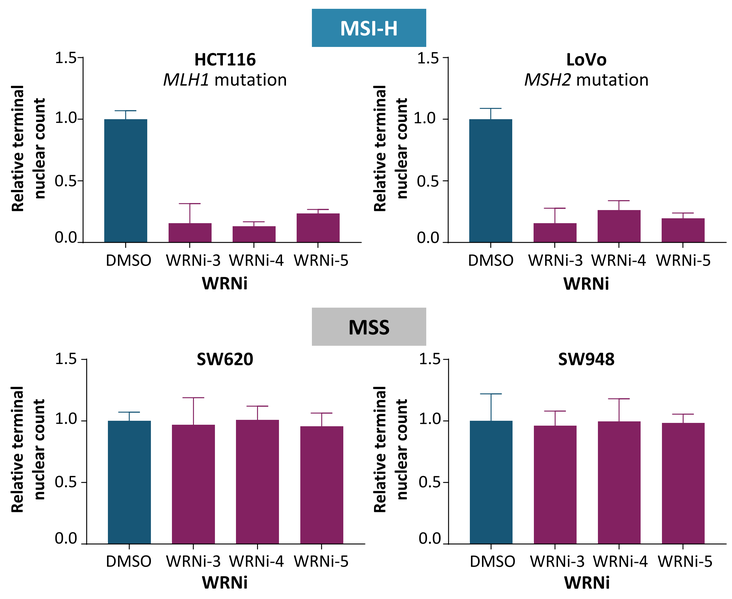
WRNi selectively inhibits WRN vs other human RecQ helicases



(Sommers et al, 2019)

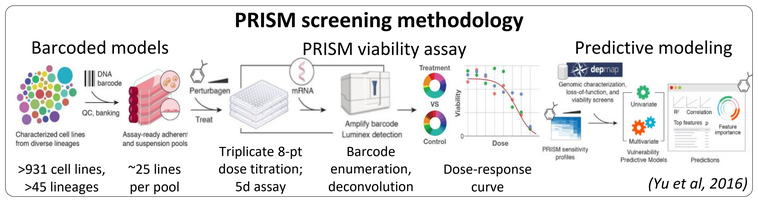


WRNi selectively inhibit the growth of MSI-H cell lines



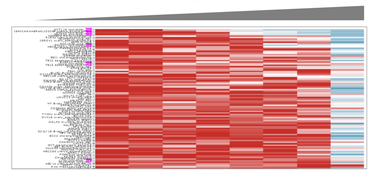
High-throughput screening of >900 cell lines supports the MSI-H selectivity and on-target effect of IDB-WRNi

PRISM screening methodology

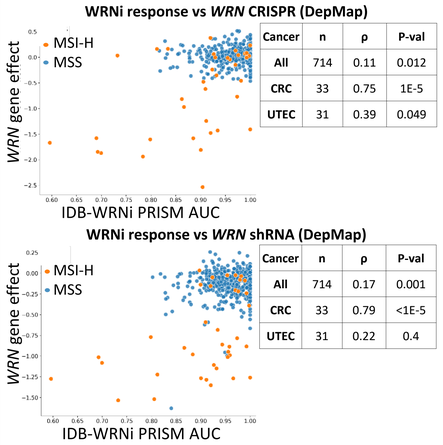


(Yu et al, 2016)

MSI-H CRC lines show the greatest sensitivity to WRNi



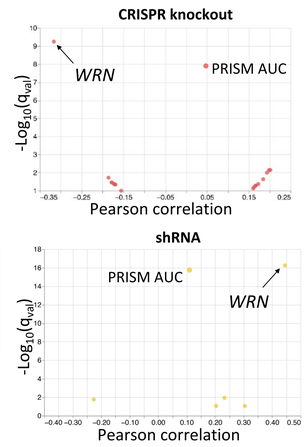
Chemical screening with WRNi recapitulates the genetic *WRN*-dependence of MSI-H cancers



Cancer	n	ρ	P-val
All	714	0.11	0.012
CRC	33	0.75	1E-5
UTEC	31	0.39	0.049

Cancer	n	ρ	P-val
All	714	0.17	0.001
CRC	33	0.79	<1E-5
UTEC	31	0.22	0.4

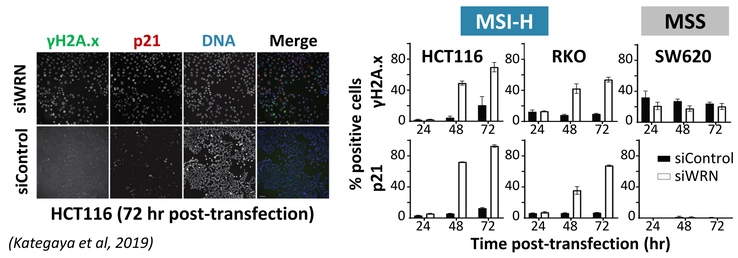
Sensitivity to WRNi correlates most strongly with *WRN* gene dependency



WRN is the strongest correlate between sensitivity to WRNi and gene dependency in DepMap CRISPR and shRNA datasets, supporting an on-target effect of IDB-WRNi

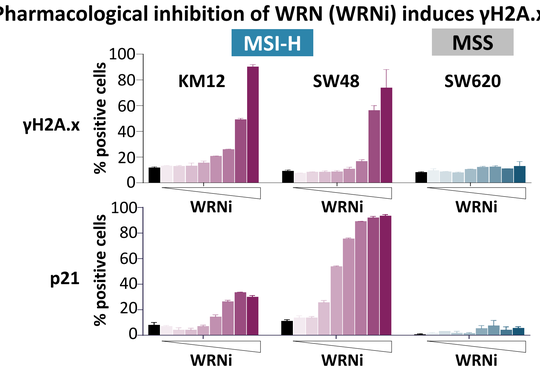
WRN inhibition or depletion induces DNA damage response markers selectively in MSI-H cancer cells

Genetic depletion of *WRN* (siWRN) induces γH2A.x and p21



(Kategaya et al, 2019)

Pharmacological inhibition of WRN (WRNi) induces γH2A.x and p21



WRNi shows pharmacological activity in MSI-H colorectal cancer models

IDB-WRNi show *in vivo* efficacy (tumor regression) in MSI-H xenograft models