WRN suppresses p53/PUMA-induced apoptosis in colorectal cancer with microsatellite instability/mismatch repair deficiency

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Colorectal cancer (CRC) initiates in the large intestine (colon or rectum) and is a leading cause of cancer-related deaths in the United States in both males and females and across all racial and ethnic groups (1). As much as 16% of these patients harbor a cancer susceptibility gene pathogenic variant, such as mutations in the adenomatous polyposis coli gene, the DNA polymerase genes POLE or POLD1 or the base excision repair genes MUTYH or NTHL1 (2). The most prominent of these hereditary CRC syndromes include pathogenic mutations in genes of the mismatch repair (MMR) pathway, including MLH1, MSH2, MSH6, and PMS2 (2). Patients with MMR deficiency are classified with Lynch syndrome (3) and have a high incidence of many cancers in addition to CRC, including cancer of the stomach, endometrium, and ovaries, among others (2, 3). As such, there is a dire need to uncover new therapies that might be selective for MMR-deficient cancers such as CRC. In PNAS, Hao et al. (4) uncover the mechanism that leads to apoptosis for a recently identified targeted therapy approach for MMR-deficient CRC (Fig. 1).

An active area of discovery for tumor targeted therapeutic approaches relies on synthetic lethality, whereby treatments are designed to exploit compensatory (synthetic lethal) relationships among biological pathways essential for tumor growth, one of which is uniquely defective in the tumor (5, 6). Such an approach, for example, has been highly effective for the treatment of breast cancer with BRCA1/BRCA2 deficiency or with defects in other homologous recombinant genes, shown to be selectively sensitive to inhibitors of the DNA damage response (DDR) signaling enzyme, PARP1, such as olaparib (7) or talazoparib (8). Recent efforts (6, 9–13) have indicated that MMR-deficient CRC tumor cells are highly sensitive to loss of expression of WRN, a RecQ-family ATP-dependent helicase/bifunctional 3′-5′ exonuclease, pointing to a synthetic lethal relationship between WRN and the MMR pathway in CRC. Hao et al. (4) build on their lab's expertise on the mechanism of p53-dependent cell death, further documenting the significance of the WRN/MMR synthetic lethal relationship. Importantly, they find that the loss of or inhibition of WRN, in MMR-defective cells, triggers DNA damage that leads to p53-dependent and p53-independent PUMA activation that precipitates the onset of mitochondria-mediated apoptosis (Fig. 1).

MMR is a post-replicative DNA repair pathway that recognizes and repairs base-base mis-pairs and DNA strand misalignments that arise during DNA replication (14, 15). Such lesions or DNA replication errors are recognized by the MSH2/MSH6 heterodimer (the MutSα complex) that in turn recruits the MLH1/PMS2 heterodimer (the MutLα complex) (16). The base-base or strand misalignment error is then corrected by excision of the 'error-containing' DNA strand followed by gap-filling DNA synthesis, improving the overall fidelity of DNA replication by ~1,000-fold (14, 16, 17). As such, pathogenic defects in MMR lead to elevated mutation rates (14, 18) and genetic variability characterized by microsatellite instability (MSI) (19, 20). Regions of the genome encoding microsatellites or tracts of short (2 to 4 base) tandem repeats are highly unstable when MMR is defective, giving rise to either expansions or contractions of these microsatellites (19, 20). Close to 15% of CRC is classified by high levels of MSI (also called MSI-high), whereas 85% are found to have chromosomal instability but with genetically stable microsatellite regions, defined as microsatellite stable (MSS) (21).

WRN loss (via RNA interference or CRISPR/cas9-mediated gene knockout, KO) in MMR-deficient and MSI-high cells (9–13) leads to elevated DNA damage (DNA double-strand breaks, DSBs) and cell death (9), not seen in MSS cells (10). Interestingly, it is the helicase function of WRN that is required for viability of MMR-deficient cells, not the WRN exonuclease activity (9–11). WRN may help resolve abnormal genomic structures, such as long $(TA)_n$ repeat expansions (13), that accumulate in MMR-deficient and MSI-high cells (10, 11, 13). Upon loss of WRN in MMR-deficient/MSI-high cells, such genomic structures are likely not resolved, leading to an increase in DNA damage and the activation of the DDR signaling kinases ATM and CHK2. The increase in DSBs upon loss of WRN in MSI-high cells (10, 11, 13), with high prevalence of end-resected breaks (13), is in-line with an increase in ATM/CHK2 activation. Hao et al. (4) show in MSI CRC, but not MSS CRC, that WRN depletion triggers an increase in DNA damage, as measured by phosphorylation (activation) of ATM(Ser1981) and CHK2(Thr68). Further, they find that ATM inhibition suppresses the WRN loss-induced phenotype, highlighting the significance of DNA damage induced apoptosis following WRN inhibition in MMR-deficient CRC cells (Fig. 1).

WRN dependency in MMR-deficient/MSI-high cells has been documented in over 60 preclinical models (12), and loss

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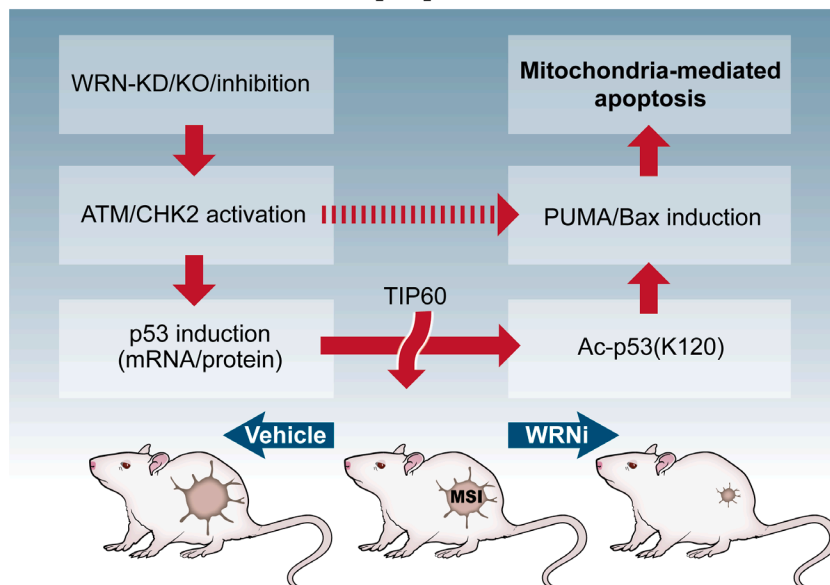
WRNi-Induced Apoptosis in MSI CRCs

Fig. 1. Loss of WRN expression via RNA interference or gene KO, as well as inhibition of WRN's helicase activity, triggers an increase in DNA damage accompanied by ATM and CHK2 activation. This initiates the induction of p53 expression and activation followed by TIP60-mediated p53 acetylation that triggers induction of PUMA and Bax, while DNA damage can also directly lead to PUMA induction. Once activated, PUMA selectively triggers the onset of mitochondrial-mediated apoptosis in colorectal cancer cells/tumors with microsatellite instability/mismatch repair deficiency. The onset of WRN inhibition (WRNi) induced apoptosis is demonstrated in cell lines, tumor models, and patient-derived xenograft (PDX) tumors.

of WRN in MMR-deficient cells induces p53 activation and increased p21 levels (11). While this might suggest a role for PUMA, Noxa, or Bax, the mechanism of DNA damage induced apoptosis upon WRN loss or inhibition in MMR-deficient/MSI-high cells had not been explored. However, the increase in DSBs would be predictive for induction and/or stabilization of p53 to trigger either cell cycle arrest or apoptosis. Here, Hao et al. (4) show that WRN loss in MMR-defective cells (MSI), but not MSS cells, selectively induces activation of apoptosis, specifically by an increase in Annexin V, the release of cytochrome *c,* and cleavage of caspases 3 and 9 (Fig. 1).

To better define the WRN-dependent apoptotic pathway in MSI cells, Hao et al. (4) used gene set enrichment analysis to evaluate the changes in mRNA species (RNA-seq) in an MMR-deficient/MSI cell line (HCT116) and after chromosome 3+5 complementation to revert to MMR proficiency and MSS status. Consistent with the prediction that WRN-KO-induced DNA damage may activate p53, they found that the predominant gene changes were downstream targets of the p53 pathway, including p21, PUMA, and Noxa, among others (4). In cells displaying MSI, WRN loss (via RNA interference) induced elevated protein expression levels for p53, p21, PUMA, and Noxa. PUMA is directly induced at the mRNA level by p53 via binding to the PUMA promoter and is required for WRN-KO-induced apoptosis (4). Further, viability of the WRN-depleted cells can be

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rescued by CRISPR/cas9-mediated PUMA or Bax gene KO but not by KO of Bim, Noxa, or BAK (4). This apoptotic signature (genotype) can be blocked by the pan caspase inhibitor z-VAD

and is indicative of mitochondrial-mediated apoptosis via the p53/PUMA/Bax axis (Fig. 1) (4).

In PNAS, Hao et al. define the mechanism of PUMA-induced activation of the mitochondria-mediated apoptosis pathway upon WRN loss or inhibition of the helicase activity of WRN in MMR-deficient/MSI-high CRC cells and tumor/PDX models.

Specific post-translational modifications (PTM) of p53 define cell fate, with different PTMs driving the onset of either cell cycle arrest or apoptosis. Upon loss of WRN in MSI cells, Hao et al. (4) show that the induction of apoptosis is dependent on p53(K120) acetylation, likely via TIP60 (4), and blocking p53(K120) acetylation prevents both PUMA induction and apoptosis (Fig. 1). This study, using a series of isogenic and genetically defined cell lines, documents that PUMA is the key facilitator in WRN loss-induced apoptosis in MSI CRC cells (4). There was evidence previously (11), and as shown here by Hao et al. (4), for p53-independent cell death/apoptosis upon WRN-KO in MSI cells, complicating the mechanistic clarity of the response. However, here Hao et al. demonstrate that while most of the phenotype is p53-dependent PUMA induction, there is clear evidence for p53-independent PUMA induction (4), likely via DNA damage (Fig. 1).

Finally, Hao et al. (4) use PDX tumor models to evaluate the role for PUMA in the response to WRN loss on MSI CRC tumors. As with the cell line models, loss of WRN (via RNA interference) shows an increase in DNA damage in the MSI tumors, but not MSS tumors, and the onset of apoptosis is dependent on p53 and PUMA (4). The essential role for PUMA observed here may be anticipated, as PUMA is a BH3-only Bcl-2 family member that is important for apoptosis induction in CRC (4). Earlier reports suggested that only the helicase

activity of WRN is essential for viability of the MSI CRC cells (9–11). Similarly, Hao et al. show that inhibition of the helicase activity of WRN, with the small molecule inhibitors NSC617145 and ML216, selectively kills MSI CRC cells and demonstrates ML216 selective efficacy in MSI CRC PDX tumors (4).

CRC with MMR deficiency and the resulting MSI or MSI-high genotype show poor treatment outcomes and resistance to therapy that have necessitated more selective therapeutic approaches. Such targeted therapies that exploit the unique genetic defects of CRC tumors have been used to great effect to selectively suppress tumor growth (22). The increased mutational load in MMR-deficient CRC (18) suggested that such tumors may encode a high level of 'non-self' antigens that could be exploited by immune checkpoint inhibition, such as anti-PD-1, shown to have significant clinical benefit (23). Although immune checkpoint monotherapies such as cytotoxic T-cell lymphocyte-4 inhibitors (ipilimumab) and PD-1 inhibitors (pembrolizumab, nivolumab) have shown benefit in MSI CRC, especially regarding metastatic disease,

acquired or intrinsic resistance is quite prevalent (>60%) and reliable biomarkers have not yet been defined that can help predict responsiveness (24). In this study (4), Hao et al. reinforce the findings that MMR-deficient CRC cells and tumors are dependent on WRN expression and WRN helicase activity and define this mechanistically as being a p53/PUMA-dependent mechanism. Interestingly, p53 mutations in MSI CRC are rare (<20%) (4), suggesting that most MSI CRC may be responsive to WRN helicase inhibition (12, 25) to induced PUMA and the onset of apoptosis (4) (Fig. 1).

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