Pharmacological Rescue of p53 in Cancer Therapy: Widening the Sensitive Tumor Spectrum by Targeting MDMX

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Targeting p53's main negative regulator MDM2 is a promising strategy for treating cancers that retain wild-type p53. Unfortunately, MDM2 inhibitors are largely ineffective against tumors overexpressing MDMX. In this issue of *Cancer Cell*, Bernal et al. describe a "stapled" peptide that targets MDMX and suppresses the growth of tumors resistant to MDM2 inhibitors.

p53 tumor suppressor function is abrogated by mutations or deletions of the *TP53* locus in about 50% of all human cancers. The remaining cancers often employ alternative mechanisms to subvert the activity of wild-type p53; for example, amplification and overexpression of the p53 negative regulator MDM2 (reviewed in Marine and Lozano, 2010). Ample evidence suggests that transformed cells are more sensitive to p53 activation than their normal counterparts. Accordingly, restoration of p53 activity in mice causes tumor-specific cell death or induction of senescence (Sharpless and DePinho, 2007). Activation of the p53 response becomes, therefore, an attractive therapeutic goal.

Proof-of-concept experiments have demonstrated the feasibility of restoring p53 function in vitro by antagonizing MDM2 as a therapeutic strategy for the treatment of cancers retaining wild-type p53. An elegant structural study had revealed that a hydrophobic cleft on the N-terminal portion of MDM2 engages the amphipathic α-helix of p53 transactivation domain (Kussie et al., 1996). This observation led to the development of potent and selective small molecule inhibitors of MDM2, which disrupt the p53-MDM2 interaction by targeting the hydrophobic groove of MDM2. Nutlin-3, one of these compounds, triggers p53-dependent cell cycle arrest and even apoptosis and exhibits antitumor activities in an osteosarcoma murine xenograft model (Vassilev et al., 2004). However, several caveats have been raised by further basic cancer research studies. Numerous studies in mice indicate that *Mdm2* loss leads to pathologies due to induction of p53-dependent cell death in normal cells, including those that are quiescent and fully differentiated (Marine and Lozano, 2010). Hence, although the use of small molecule inhibitors that have a limited half-life may not have such dramatic effects as genetic ablation of *Mdm2*, toxicity is one potential concern of systemic exposure to potent MDM2 inhibitors. Another major limitation is that, although Nutlin-3 kills several cancer cell lines whether or not MDM2 levels are elevated, other cancer cells are largely immune to it. In particular, tumor cells that overexpress the other key negative regulator of p53, MDMX, only poorly respond to Nutlin-3 (reviewed in Wade and Wahl, 2009). Although the N-terminal regions of MDM2 and MDMX exhibit a high degree of similarity, Nutlin-3's affinity for MDMX is ~40-fold lower compared to MDM2 (reviewed in Marine et al., 2007). This is likely to be due to structural differences within the p53-binding pockets and a region immediately adjacent to it, the "flexible lid" (reviewed in Wade and Wahl, 2009). This is a major hurdle for anti-MDM2-based therapy, since overexpression of MDMX as an alternative route to p53 inactivation is observed in a significant percentage across a wide spectrum of tumors, i.e., 4% in glioblastoma, 19% in breast carcinomas, and up to 65% in retinoblastoma (reviewed in Marine et al., 2007). Consequently, the development of compounds that selectively target the p53-binding domain of MDMX has become a pressing therapeutic goal (reviewed in Wade and Wahl, 2009). Supporting this view are results from mouse models which indicate that ablation of Mdmx is well tolerated in most adult somatic tissues (reviewed in Marine et al., 2007).

In this issue, Bernal and colleagues (Bernal et al., 2010) show that a previously engineered "stabilized alpha-helix" of p53 peptide, SAH-p53-8, preferentially targets MDMX. This peptide was designed using a chemical strategy known as "hydrocarbon stapling" that installs an all-hydrocarbon cross-link within peptides to restore their α-helical structure. SAH-p53-8 is one of the "stapled" peptides that was modeled after the transactivation domain of p53. Coimmunoprecipitation (Co-IP) experiments indicate that this peptide binds to both MDM2 and MDMX; however, in vitro binding and competition assays show a 25-fold greater binding preference for MDMX.

Cell viability assays performed on a series of cell lines expressing varying ratios of the MDM2/MDMX/p53 proteins indicated that in contrast to Nutlin-3, the SAH-p53-8 peptide caused a dose-dependent reactivation of p53 and inhibition of cell viability irrespective of MDMX levels. Cells expressing high levels of MDMX and p53 and low levels of MDM2, which were most resistant to Nutlin-3 treatment, were in fact most sensitive to SAH-p53-8. Importantly, SAH-p53-8 cytotoxicity was specifically dependent on the presence of wild-type p53 and did not significantly affect the viability of normal human diploid fibroblasts. Co-IP and proximity ligation in situ assay (or P-LISA) show that, in contrast to Nutlin-3, SAH-p53-8 efficiently blocked the formation of p53-MDMX complexes. Importantly, the pharmacological disruption of the

p53-MDMX complexes by the peptide coincided with significant transcriptional activation of endogenous p53-target genes and induction of p53-dependent reduced tumor cell viability.

Mouse genetic experiments have highlighted nonoverlapping and synergistic p53-inhibitory activities of Mdm2 and Mdmx throughout embryonic development (reviewed in Marine et al., 2006). Moreover, Chen and colleagues showed that simultaneous disruption of p53 binding to MDM2 and MDMX using a peptide that exhibits dual MDM2/MDMX specificity results in efficient p53 activation and apoptosis of tumor cells overexpressing MDM2 and MDMX (Hu et al., 2007). Consistently, SAH-p53-8 treatment sensitized Nutlin-3-resistant cells to Nutlin-3-mediated p53 upregulation and functional activation. This experiment was conducted in cells in which high levels of MDMX are expressed and the p53-MDMX complexes are readily detectable by Co-IP experiments. Nutlin-3 and SAH-p53-8 synergistically enhanced cytotoxicity, which was correlated with a blockade of the p53-MDMX complex formation. Importantly, this synergism is observed only in cells with relatively low basal levels of p53 and not in cells expressing very high p53 levels. In cells with low MDMX, treatment with the peptide provides no added benefit to Nutlin-3 treatment (Figure 1).

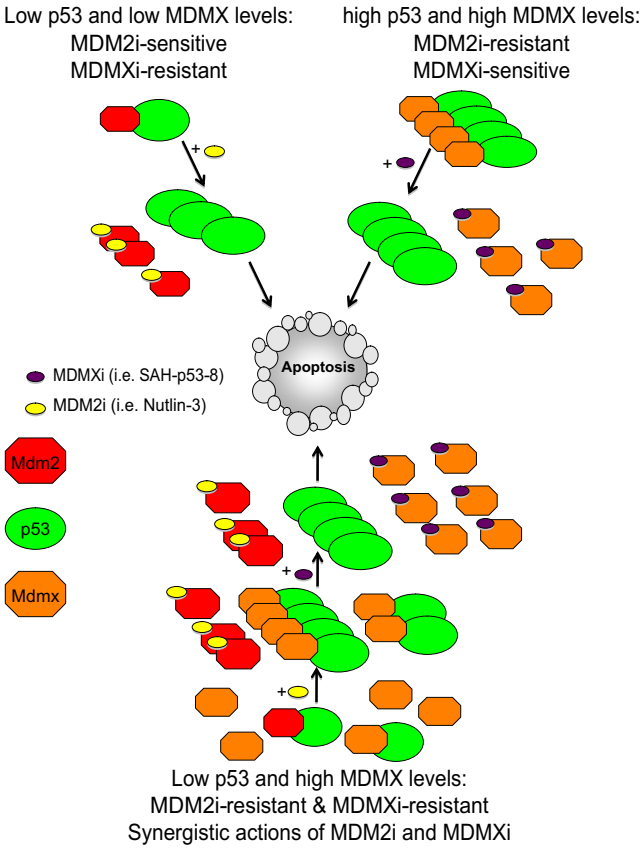

Figure 1. The p53/MDM2/MDMX Ratio Determines Cancer Susceptibility to Pharmacologic Inhibition of MDM2 and/or MDMX
MDMX inhibitors, such as the "stapled" SAH-p53-8 peptide, trigger beneficial p53 tumor-suppressor responses in cells expressing high levels of MDMX and p53. If basal p53 levels are low, maximal rescue of p53 function is achieved by combined exposure to an MDM2 inhibitor (MDM2i), which elevates p53 levels, and an MDMX inhibitor (MDMXi), which blocks the formation of inhibitory MDMX-p53 complexes.

Finally, the authors assessed the pharmacological potential of intravenous administration of the peptide to suppress tumor growth in a murine xenograft model. In this experimental setting, SAH-p53-8 induced a p53-response in the tumor cells and significantly suppressed tumor growth. The classical problems associated with peptide-based therapy such as poor solubility and cellular uptake, short-half life in vivo due to protease-dependent degradation seemed therefore to be overcome by the chemical design of the "stapled" peptides, which confers protease resistance and favors cellular uptake. Crucially, no obvious toxicity of the peptide to normal tissues was observed.

The work of Bernal et al. therefore identifies one of the long-awaited, specific MDMX inhibitors. Notably, a high throughput screening has recently been used to identify a small molecule MDMX antagonist (SJ-172550), which also binds selectively to MDMX and kills cells expressing high levels of MDMX (Reed et al., 2010). Importantly, SAH-p53-8 is the first compound shown to efficiently induce a p53 tumor-suppressive response in vivo. The authors also provide a framework for determining how to optimally apply MDM2 and MDMX inhibitors to rescue p53 tumor suppressor function in cancer. The data predict that MDMX targeting will be maximally effective when p53 levels are either naturally high or pharmacologically elevated, for example, as a result of Nutlin-3 treatment, and when the p53-MDMX complexes can be readily detected (Figure 1). The authors therefore suggest that detection of such complexes could in principle be used as a valuable biomarker for predicting therapeutic efficacy.

The ability of SAH-p53-8, either alone or in combination with MDM2 inhibitors, to kill cancers with elevated MDMX levels widens very significantly the spectrum of tumors that are expected to be sensitive to MDM2 and/or MDMX blockade. This study therefore opens up new therapeutic opportunities and further strengthens the concept that pharmacological rescue of p53 is a viable alternative to current cytotoxic chemotherapy. The field now awaits full validation of this concept in clinical settings.

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