p53 and MDM2: Antagonists or Partners in Crime?

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Therapeutics that disrupt the p53-MDM2 interaction show promise for cancer treatment but surprisingly have different biological outcomes. A study by Enge et al. in this issue of *Cancer Cell* shows that the ability of MDM2 to target hnRNP K for degradation contributes to the decision to induce apoptosis rather than cell-cycle arrest.

The activities of the p53 tumor suppressor must be carefully controlled in normal cells. If present at high levels, as occurs in response to DNA damage, hypoxia, oncogene activation, or other types of stress, p53 initiates a cell death, cell-cycle arrest, or senescence program. Since these activities prevent damaged cells from dividing, tumor cells often gain a growth advantage by mutating p53 to bypass these terminal phenotypes. p53 functions as a transcriptional activator to induce the expression of genes such as *CDKN1A* (p21), which regulates cell-cycle arrest and senescence, and *BAX*, *Noxa*, and *PUMA*, which regulate apoptosis. p53's choice is dependent on the levels of p53, tissue type, and the presence of other death signals (Vousden and Lu, 2002). A better understanding of how this choice is made will contribute to better treatment of cancers, as death of a tumor cell is preferred to arrest of that cell.

At least two negative regulators, *MDM2* and *MDM4*, are critical inhibitors of p53 in vivo (Iwakuma and Lozano, 2003). Deletion of these genes in mice leads to p53-dependent embryonic lethal phenotypes. The regulation of p53 activity is complex, as p53 can transcriptionally induce its own negative regulator *MDM2*, but not *MDM4*. MDM2 is an E3 ubiquitin ligase that targets p53 and other substrates to the proteasome for degradation. MDM2 is often present at high levels in tumors with wild-type p53 and likely serves as an alternate mechanism to disrupt the p53 pathway in developing cancer cells. However, MDM2 targets numerous other substrates, particularly when present at elevated levels or when it cannot interact with p53 (possibly due to posttranslational modifications of p53) (Iwakuma and Lozano, 2003). These MDM2 substrates have numerous roles in determining cell fate.

Therapeutic agents for the treatment of cancers are being developed to capitalize on the cell-cycle arrest or apoptotic response of the p53 pathway induced by disruption of the p53-MDM2 interaction (Vazquez et al., 2008). Compounds that bind to MDM2 and inhibit its ability to bind to or regulate p53 as well as agents that associate with and activate p53 are in preclinical development. Nutlins, benzodiazepines, spiro-oxindoles, and quinolinols bind to MDM2 and inhibit its p53 association, whereas HL198C is an inhibitor of MDM2 ubiquitin ligase activity (Vazquez et al., 2008). Treatment of cells that express wild-type p53 with any of these compounds induces the stabilization and transcriptional activation of p53, resulting in cell-cycle arrest or apoptosis. Another small molecule called RITA binds p53, inhibits p53-MDM2 interaction, and induces p53-dependent transcription and apoptosis. Although RITA can also alter the cell cycle, it appears to primarily induce apoptosis (Issaeva et al., 2004). A surprising finding has been that although these agents target the same pathway, different biological outcomes can be attained. Determining why one compound induces apoptosis while another results in cell-cycle arrest in a specific tumor cell is the focus of a manuscript by Selivanova and colleagues in this issue (Enge et al., 2009).

To examine the molecular mechanisms of RITA's activity in an unbiased manner, Enge et al. first obtained gene expression patterns for HCT116 cells, which express wild-type *p53*, and their isogenic clone that lacks *p53*. Initial expression arrays revealed some differences in gene expression between HCT116 cells with and without p53, suggesting minor effects of p53 at basal levels. RITA treatment induced significant changes in gene expression in a p53-dependent manner. Interestingly, activation of p53 prominently induced proapoptotic targets but only slightly induced *p21* and other cell-cycle arrest genes in this setting. Since RITA binds p53 specifically as compared to nutlins, which bind MDM2, the authors postulated that the unfettered MDM2 determines the preference for apoptosis.

MDM2, in addition to ubiquitinating p53, also regulates the stability of other proteins, some of which are in the p53 response pathway (Iwakuma and Lozano, 2003). Heterogeneous nuclear ribonucleoprotein K (hnRNP K) is one MDM2 target (Moumen et al., 2005). As part of the hnRNP complex, hnRNP K was originally identified for its role in mRNA biogenesis and maturation, but it is most noted for its involvement in transcription and chromatin remodeling. Upon DNA damage, hnRNP K levels rise due to reduced association with MDM2 (Moumen et al., 2005). hnRNP K is also recruited with p53 to the promoters of p53 transcriptional target genes. p53-dependent transcriptional activation and the resulting cell-cycle arrest, following DNA damage, is impaired in cells depleted of hnRNP K. Moreover, hnRNP K-depleted cells are unable to effectively mount a p53-dependent transcriptional response to nutlin treatment, leading to the conclusion that hnRNP K is required for p53-mediated transcription (Moumen et al., 2005). However, it was unclear whether hnRNP K is necessary for the transcription of all or only a subset of p53 target genes.

Enge et al. (2009) now show that RITA treatment increases MDM2 levels and MDM2-hnRNP K association, resulting in

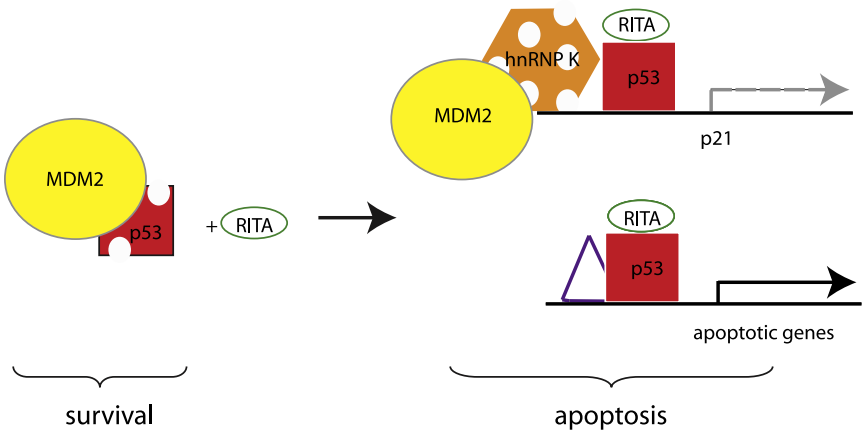

Figure 1. MDM2 Aids in Mediating RITA-Induced Apoptosis
MDM2 binds and targets p53 (mottled to show degradation) for proteasome-mediated degradation. The small molecule RITA binds p53 and disrupts the p53-MDM2 interaction. The released MDM2 then targets hnRNP K (mottled) for proteasomal degradation, dampening expression of the cell-cycle inhibitor p21 but not the expression of proapoptotic genes. The triangle represents other transcription factors that cooperate with p53 to induce transcription of apoptotic genes.

MDM2-mediated degradation of hnRNP K and, consequently, reduced transcriptional upregulation of *p21*, but not the apoptotic gene *Noxa* (Figure 1). Nutlin, on the other hand, did not alter hnRNP K levels and preferentially induced *p21* over *Noxa* transcription. In addition to a reduction in *p21* transcription, p21 half-life was also decreased following RITA treatment. Since MDM2 has been reported to induce p21 degradation independent of its ubiquitin ligase activity and independent of p53 by facilitating p21 association with the proteasome (Jin et al., 2003; Zhang et al., 2004), MDM2 was postulated to be responsible for the decreased p21 protein levels after RITA treatment. Together, the results suggest that RITA preferentially induces an apoptotic response in part due to MDM2-mediated degradation of p21 and hnRNP K, which reduces transcription of *p21* and permits transcription of *Noxa*. In contrast, nutlins, by binding to MDM2, prevent hnRNP K degradation, allowing for efficient transcription of *p21*, which induces cell-cycle arrest. Notably, hnRNP K was necessary for *p21* transcription but appeared dispensable for *Noxa* transcription. Lastly, overexpression of p21 experimentally dampened the apoptotic response of cells treated with RITA, and depletion of *p21* enhanced apoptosis in nutlin-treated cells, further documenting an important role of p21 in the decision between cell-cycle arrest and apoptosis.

In summary, the work of Enge et al. shows that when it cannot bind p53, MDM2 is able to target hnRNP K, the depletion of which decreases levels of *p21* and pushes cells toward an apoptotic response (Figure 1). Thus, in this scenario, MDM2 is forced to help p53 eliminate tumor cells. MDM2 likely has other targets that assist in determining the cellular response, as it binds a multitude of other proteins (Iwakuma and Lozano, 2003). In contrast, if MDM2 is bound to p53, then p53 cannot function and cells have a growth advantage. Many other studies have shown that elevated levels of MDM2 are oncogenic; additional studies will be necessary to understand the mechanisms behind the different cellular outcomes following disruption of the p53-MDM2 interaction. Importantly, since MDM2 also regulates mutant p53 levels resulting in gain-of-function phenotypes, the ability of RITA to bind mutant p53 must be examined, and caution may need to be exercised to treat only tumors with wild-type p53 (Terzian et al., 2008). These results suggest that p53 does not function alone in making the choice between apoptosis and cell-cycle arrest. Treatment of tumors with drugs that decrease the expression of p53 targets such as *p21* may cooperate with drugs such as nutlins to swing the balance toward cell death.

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