Protein–protein interactions for cancer therapy

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The p53 tumor suppressor network is frequently disabled by mutation of its molecular node, the *p53* gene (1–3). However, the p53 gene is wild type in ≈50% of human cancers. In the early 1990s, the p53–MDM2 autoregulatory feedback loop was discovered (reviewed in refs. 4 and 5) (Fig. 1). MDM2 (murine double minute 2; also termed HDM2 for its human equivalent) is an oncoprotein. In response to cellular stress, p53 transcriptionally transactivates the *MDM2* gene, and then the MDM2 protein binds to and transports the p53 to the cytoplasm where MDM2, an E3 ubiquitin ligase, promotes p53 ubiquitination and degradation by the proteasome. Increased expression of MDM2 in human cancer involves four mechanisms: gene amplification, increased expression by activated p53, stabilization by an aberrantly spliced form of HMDX, or augmented translation (4, 6). In addition to these cancer-related mechanisms, functional single-nucleotide polymorphisms (SNP) may modulate MDM2 expression. For example, increased p53-mediated expression of MDM2 due to an SNP at nucleotide 309 (SNP309) in the MDM2 gene occurs in the germ line of the population that can increase tumor progression (7). Because MDM2 is overexpressed in certain cancers (8) and may reduce the effectiveness of p53-dependent cancer therapies, the disruption of the p53–MDM2 autoregulatory feedback loop emerged as a molecular targeting strategy. Although the interactive binding sites on p53 and MDM2 were well defined by crystallography (9), the strategy of using drug-like small molecules to block such protein–protein interactions was not considered as attractive by both academia and the pharmaceutical industry as were inhibitors of key cancer-related enzymes such as kinases. Challenging this widely held dogma, Vassilev *et al.* (10) developed a class of small molecules, the nutlins, that occupy the p53-binding pocket in MDM2, prevent its binding to p53, and, thus, facilitate the p53 tumor suppressor network to inhibit human cancer cell lines *in vitro* and as xenografts *in vivo*. Vassilev and coworkers (11), in this issue of PNAS, extend these initial observations by demonstrating that the nutlin-3, a tetra-substituted imidazoline, induces apoptosis most robustly in cancer cell lines with increased MDM2 expression, and this response correlates with the *in vivo* antitumor efficacy of nutlin-3.

Functional single-nucleotide polymorphisms may modulate MDM2 expression.

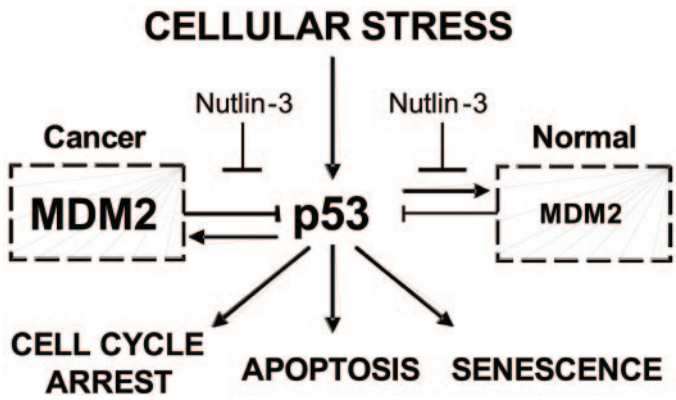

Fig. 1. Cellular stress, e.g., DNA damage, telomere erosion, hypoxia, or oncogene expression, activates the p53 response pathway. The p53–MDM2 autoregulatory feedback loop governs p53 amounts. Overexpression of MDM2 in human cancer, e.g., gene amplification of MDM2, targets p53 for ubiquitin-dependent protelytic degradation to disable the p53 network. Nutlin-3 complexes with MDM2 and inhibits its interaction with p53.

p53-Independent Activities of MDM2

MDM2 also has p53-independent activities (12, 13). MDM2 physically and/or functionally interacts with many proteins involved in the controlling of cell proliferation and survival, including the Rb tumor suppressor, E2F1 transcription factor, PML tumor suppressor, and $p21^{Waf1}$ cyclin-dependent kinase inhibitor. For example, MDM2 acts as a direct negative regulation of p21 by enhancing its recognition by the proteasome C8 subunit (14). Several proteins, e.g., E2F1, p73α, and PCAF, bind amino-terminus MDM2 near the p53-binding site. Are the functions of these proteins affected by nutlin-3? Would small-molecule antagonists targeting the carboxyl terminus of MDM2-binding sites of proteins, e.g., Rb, HMDX, and PML, enhance the antitumor properties of nutlin-3? Although only a limited number of human cancer cell lines have been examined, the current evidence indicates that p53 is the major downstream pathway enhanced by nutlin-3.

Implications for Cancer Prevention and Therapy

The studies of Vassilev and coworkers (10, 11) demonstrate *in vivo* proof of principle that inhibitors of protein–protein interactions can be efficacious anticancer drugs. Therapy could be individualized to tumors with cancer-related MDM2 overexpression. Human cancers in individuals with MDM2 overexpression due to the functional germ-line SNP309 may be more responsive to MDM2 antagonists. In addition to apoptosis, nutlins may induce a p53-meditated permanent cellular arrest, senescence, an intrinsic cancer therapeutic endpoint (15). The therapeutic index between cancer and normal cells will need to be determined. Nutlin-3 and other MDM2 antagonists (5, 16, 17) may be active without the genotoxicity of traditional cancer chemotherapy or radiation therapy. This lack of genotoxicity may reduce both DNA damage in normal cells and the potential of inducing mutant clones of cancer cells that are resistant to cancer therapy. Nutlins and other MDM2 antagonists are still in preclinical development. The therapeu-

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tic utility of these antagonists will become evident only when they are evaluated in the clinic.

Nutlin-3 and other MDM2 antagonists are also potential cancer chemopreventive drugs. This potential and their chronic toxicity can initially be examined in animal models of human cancer. Previous studies of p53 and MDM2 in murine models provide some insights. For example, the fine control of p53 is needed to maintain the balance between premature aging and cancer proneness. Constitutive modest overexpression of p53, either as a transgene or as a germ-line hypomorph, causes premature aging with reduced spontaneous tumor formation (18, 19). MDM2 antagonists, including those with favorable pharmacodynamic properties after oral administration, e.g., nutlin-3, offer an opportunity to determine whether intermittent overexpression of p53 retains the cancer-preventive properties without the induction of premature aging. Haploinsufficiency of p53 increases spontaneous tumorigenicity in a murine model of the cancer-prone Li–Fraumeni syndrome (20). Would chronic administration of nutlin-3, which increases p53 to normal levels, prevent tumor formation? If so and if the chronic toxicity is acceptable, individuals with the Li–Fraumeni syndrome could benefit from treatment with a MDM2 antagonist.

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