The MDM2-p53 Interaction

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Abstract
Activation of the p53 protein protects the organism against the propagation of cells that carry damaged DNA with potentially oncogenic mutations. MDM2, a p53-specific E3 ubiquitin ligase, is the principal cellular antagonist of p53, acting to limit the p53 growth-suppressive function in unstressed cells. In unstressed cells, MDM2 constantly monoubiquitinates p53 and thus is the critical step in mediating its degradation by nuclear and cytoplasmic proteasomes. The interaction between p53 and MDM2 is conformation-based and is tightly regulated on multiple levels. Disruption of the p53-MDM2 complex by multiple routes is the pivotal event for p53 activation, leading to p53 induction and its biological response. Because the p53-MDM2 interaction is structurally and biologically well understood, the design of small lipophilic molecules that disrupt or prevent it has become an important target for cancer therapy.

Introduction

The p53 tumor suppressor is a principal mediator of growth arrest, senescence, and apoptosis in response to a broad array of cellular damage (1, 2). Rapid induction of high p53 protein levels by various stress types prevents inappropriate propagation of cells carrying potentially mutagenic, damaged DNA. p53 can kill cells via a dual transcription-dependent and -independent function in the nucleus and at the mitochondria (3, 4). It has been conclusively demonstrated that cellular p53 protein levels are the single most important determinant of its function. In normal unstressed cells, p53 is a very unstable protein with a half-life ranging from 5 to 30 min, which is present at very low cellular levels owing to continuous degradation largely mediated by MDM2[1] (1, 2). Conversely, a hallmark of many cellular stress pathways such as DNA damage, hypoxia, telomere shortening, and oncogene activation is the rapid stabilization of p53 via a block of its degradation. During the past decade, MDM2 has emerged as the principal cellular antagonist of p53 by limiting the p53 tumor suppressor function (5–7).

p53 and MDM2 Are Linked Through an Autoregulatory Loop

The *MDM2* gene was originally identified on double-minute chromosomes of spontaneously transformed mouse 3T3 fibroblasts (mouse double minute; 8), and the MDM2 protein was later found to be physically associated with p53 (7). Since then, compelling evidence has emerged for MDM2 to have a physiologically critical role in controlling p53. MDM2-null mouse embryos die early after implantation (before E6.5), but are fully rescued if they are co-deficient for p53 (9, 10). This provides compelling genetic evidence that the most important role of MDM2 is the physiological regulation of p53 function, at least in early development (9, 10). Importantly, MDM2 itself is the product of a p53-inducible gene (2, 11–13). Thus, the two molecules are linked to each other through an autoregulatory negative feedback loop aimed at maintaining low cellular p53 levels in the absence of stress (Fig. 1). Moreover, this relationship limits the duration and severity of various p53-mediated biological responses after a non-lethal stress response (2, 14, 15). By the same token, mutant p53 proteins in tumor cells are stable because they are deficient in transactivating MDM2—hence they have a defective negative feedback loop (reviewed *e.g.* in 16). Principally, MDM2 is an E3 ligase and promotes p53 degradation through a ubiquitin-dependent pathway on nuclear and cytoplasmic 26S proteasomes (14, 17–20). Protein modification by ubiquitin conjugation is a general intracellular targeting mechanism and covalently attached polyubiquitin chains on lysine residues target proteins to proteasomes for degradation. Recent studies using various MDM2 and p53 mutants elucidated structural requirements for the MDM2-mediated destruction of p53. Combined, these results revealed the importance of several regions on both proteins and showed that in order for p53 to accumulate in response to stress, the regulatory mechanism has to be interrupted so that p53 can escape the degradation-promoting effects of MDM2.

MDM2 harbors a self- and p53-specific E3 ubiquitin ligase activity within its evolutionarily conserved COOH terminal RING finger domain (Zinc-binding), and its RING finger is critical for its E3 ligase activity (21). MDM2 transfers monoubiquitin tags onto lysine residues mainly in the COOH terminus of p53 (21–24). MDM2-mediated p53 ubiquitination takes place in the nucleus in a complex with the p300/CREB-binding protein (CBP) transcriptional coactivator proteins,

[1]The abbreviations used are *MDM2*, human gene and oncogene; MDM2, human protein and isoform; *mdm2*, mouse gene; Mdm2, mouse protein.
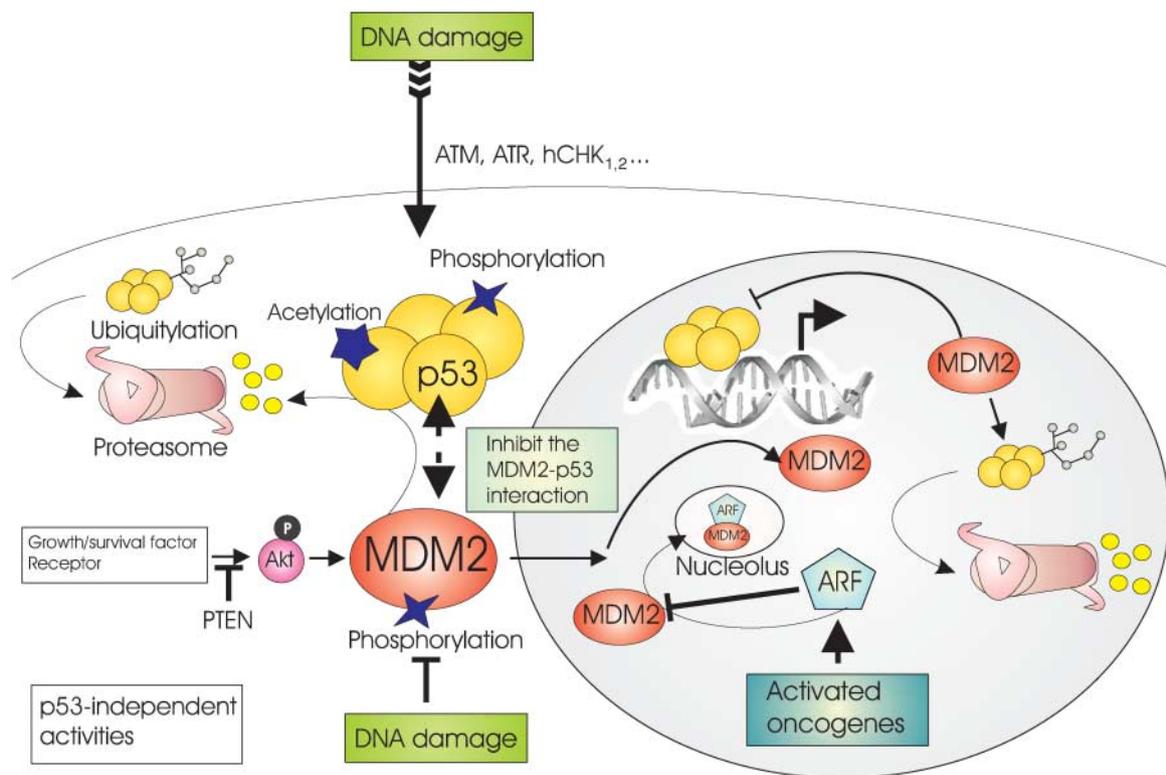
FIGURE 1. Regulation of p53 by MDM2. p53 and MDM2 form an autoregulatory feedback loop. p53 stimulates the expression of MDM2; MDM2, in turn, inhibits p53 activity because it stimulates its degradation in the nucleus and the cytoplasm, blocks its transcriptional activity, and promotes its nuclear export. A broad range of DNA damaging agents or deregulated oncogenes induces p53 activation. DNA damage promotes phosphorylation of p53 and MDM2, thereby preventing their interaction and stabilizing p53. Likewise, activated oncogenes induce ARF protein, which sequesters MDM2 into the nucleolus, thus preventing the degradation of p53. Conversely, survival signals mediate nuclear import of MDM2 via Akt activation, which destabilizes p53.

serving as a scaffolding. The majority of endogenous MDM2 is bound to p300/CBP in the nucleus. However, whereas MDM2 catalyzes p53 monoubiquitination (24), which in itself is not a substrate for proteasome degradation, p300/MDM2 complexes are mediating the final p53 polyubiquitination (25, 26). *In vitro*, p300 with MDM2 catalyzed p53 polyubiquitination, whereas MDM2 alone only catalyzed p53 monoubiquitination. Thus, generation of the degradable polyubiquitinated forms of p53 requires the intrinsic E3- and the E4-type ubiquitin ligase activities of both MDM2 and p300/CBP. In addition, MDM2 present in the ternary complex of MDM2-p53-p300 in HeLa cell nuclear extracts inhibits p300-mediated p53 acetylation and transcriptional activation (27, 28).

Structural Requirements for MDM2-p53 Interaction

Human MDM2 is a 491-amino acid (aa)-long phosphoprotein that interacts through its NH_2 terminal domain with an α-helix present in the NH_2 terminal transactivation domain of p53 (29). This entails several negative effects on p53. MDM2 binding to the NH_2 terminal transactivation domain of p53 blocks its transcriptional activity directly (12, 30). More importantly, MDM2 functions as the E3 ligase that ubiquitinates p53 for proteasome degradation (14, 18). The crystal structure of the p53-MDM2 complex has been solved (29). The

biochemical basis of MDM2-mediated inhibition of p53 function was further elucidated by crystallographic data that showed that the amino terminal domain of MDM2 forms a deep hydrophobic cleft into which the transactivation domain of p53 binds, thereby concealing itself from interaction with the transcriptional machinery (29). This has been confirmed by biochemical analysis. The direct interaction between the two proteins has been localized to a relatively small (aa 25–109) hydrophobic pocket domain at the NH_2 terminus of MDM2 and a 15-aa amphipathic peptide at the NH_2 terminus of p53 (12, 29). The minimal MDM2-binding site on the p53 protein was subsequently mapped within residues 18–26 (12, 17, 31, 32). Site-directed mutagenesis has shown the importance of p53 residues Leu14, Phe19, Leu22, Trp23, and Leu26, of which Phe19, Trp23, and Leu26 are the most critical (12, 31). Accordingly, the MDM2-binding site p53 mutants are resistant to degradation by MDM2 (14, 15, 18). Similarly, mutations of MDM2 at residues Gly58, Glu68, Val75, or Cys77 result in lack of p53 binding (33). The interacting domains show a tight key-lock configuration of the p53-MDM2 interface. The hydrophobic side of the amphipathic p53 α-helix, which is formed by aa 19–26 (with Phe19, Trp23, and Leu26 making contact), fits deeply into the hydrophobic cleft of MDM2. Thr18 is very important for the stability of the p53 α-helix (34). The MDM2 cleft is formed by the aa 26–108 and consists of two structurally similar portions that fold up into a deep groove

lined by 14 hydrophobic and aromatic residues (29). Experimental measurements of the strength of the p53-MDM2 bond range from a K_d of 60 to 700 nM, depending on the length of the p53 peptide (34–37). Phosphorylation of S15 and S20 does not affect binding, but T18 phosphorylation weakens binding 10-fold, indicating that phosphorylation of T18 only is responsible for abrogating p53-MDM2 binding (35). DNA damage-mediated disruption of this complex *in vivo* also requires only T18 phosphorylation. However, stabilization of p53 after ionizing radiation results from an inhibition of MDM2 binding through a phosphorylation-phosphorylation cascade that first requires phosphorylation of p53 S15, which is required for subsequent phosphorylation of T18 (37). Nuclear magnetic resonance studies revealed global conformational changes of the overall structure of MDM2, stretching far beyond the binding cleft on p53 peptide binding (35, 37). In conclusion, conformation and hydrophobicity appear to be two critical requirements for the interaction between MDM2 and p53.

A basic but insufficient requirement for p53 degradation is the direct interaction between p53 and MDM2 through their NH_2 termini. Additional requirements on p53 include its homo-tetramerization region, which enhances degradability possibly via improved MDM2 binding, because a monomeric p53 mutant has lost sensitivity to degradation by MDM2 (23, 38). As stated above, lysine residues, mainly in the COOH terminus of p53, are required anchors for mono-ubiquitination (21–24, 39). Also, the extreme COOH terminus of p53 (aa 363–393) is required (23), presumably again due to its lysine residues.

Because the hydrophobic p53-MDM2 interaction is structurally and biologically well understood, the design of small lipophilic molecules that disrupt or prevent the interaction of p53 and MDM2 in wild-type p53 harboring tumors is currently a hotly pursued therapeutic strategy. Of note, only the MDM2 partner has structurally well-defined binding sites, while p53 undergoes phosphorylation-induced re-conformation. This implies that inhibitors should mimic p53 rather than MDM2. Another reason for this design limit is that only the p53 interface is composed of a single short contiguous stretch of amino acids. The bond consists of only three hydrogen bonds, and the most buried one is contributed by Trp23 on p53. The contact surface between both partners is rather small. All these features favor the possibility that a small inhibitory molecule might work, which in turn favors an oral administration route. Early studies using phage-display peptide libraries yielded natural peptides that bound to MDM2 even more avidly than the native p53 sequence, while a lead peptide of eight non-natural amino acids induced a full p53 response in the absence of any other signal (17, 31, 32). Remarkably, the four invariate residues from the minimum phage consensus peptide, FxxL/YWxxL, were all facing to one side of an amphipathic α-helix and fit into the hydrophobic groove on the surface of MDM2. Moreover, one of the phage peptides (called 12/1, MPRFMDY-WEGLN) bound to p53 about 50 times more avidly than the natural p53 peptides. To work in tissue culture, these peptides needed to be conformationally stabilized, which was achieved by cloning them in frame into the active site loop of the bacterial Thioredoxin protein (called TIP proteins). Sole microinjection of the 12/1 TIP protein plasmid, but not of an Ala substitution plasmid, induced massive p53 accumulation and a strong p53 reporter response in normal and MDM2-overexpressing cell lines (40). Whether TIP proteins can induce cell cycle arrest or apoptosis in cells is currently under study. In an effort to optimize the 12/1 peptide further, Ac-FMDY-WEGLN was identified as the minimal sequence retaining micromolar MDM2-binding affinity (IC_{50} = 8.9 ± 0.6 μM). Subsequent substitutions by non-natural amino acids and peptide cyclization to reduce their flexibility and enhance their pre-organization eventually led to a lead peptide with an increased potency of 1780-fold (reviewed in 17).

Another promising approach, albeit mechanistically not understood, reactivates both cellular wild-type and mutant p53 with a styrylquinazoline compound named CP-31398 (41). CP-31398 blocks ubiquitination and degradation of p53 without altering phosphorylation at serine 15 or 20 or MDM2 binding. Instead, it restores a wild-type-associated epitope (positive for monoclonal antibody 1620) on the DNA-binding domain of p53 and leads to high levels of transcriptionally active p53, independent of mutant or wild-type, endogenous or ectopic p53, and independent even of MDM2 (41). Further elucidation of this unusual mechanism seems a worthwhile undertaking.

Screening of fermented microbial extracts led to the identification of chlorofusin, a natural inhibitor of the p53-MDM2 interaction in ELISA-based assays with an IC_{50} of 4.6 mM. Chlorofusin is a secondary metabolite from the fungus *Fusarium* sp., which antagonizes the interaction between p53 and MDM2 by direct binding to the NH_2 terminal domain of MDM2 (42, 43). In addition, polycyclic compounds and chalcone derivatives (44) are being actively pursued as small non-peptidic inhibitors of this interaction. If one or several of these approaches will be successful, another potential bystander benefit might be the disruption of structurally related protein complexes between MDM2 and p63 and p73 as well as between MDMX and p53, p63, and p73.

Subcellular Sites of MDM2-Mediated p53 Degradation: Nucleus and Cytoplasm

In the past, it was assumed that the cytoplasm is the exclusive site of p53 degradation, thus nuclear export of p53 is a prerequisite for its delivery to cytoplasmic proteasomes (45–47). This export model was based on two pieces of evidence: First, Leptomycin B (LMB), a specific inhibitor of the CRM1 nuclear export receptor, induced nuclear accumulation of p53 (1, 45, 48). Second, MDM2 shuttles between nucleus and cytoplasm which was interpreted to mean that MDM2 carries p53 out of the nucleus for cytoplasmic degradation (46). After recognizing that p53 itself possesses nuclear export signals (NES), self-transported p53 was also accommodated into the model. The significance of its two NESs, one COOH terminal and one NH_2 terminal, was interpreted to indicate obligatory export for the sole purpose of degradation (48, 49). Later it was found that the MDM2 RING finger domain, but not the MDM2 NES, is required as a prerequisite for efficient export of p53 to the cytoplasm (50, 51). MDM2 monoubiquitinates all available lysine residues in the COOH terminus of p53 (24, 39), thereby revealing the

NES in the adjacent tetramerization domain and allowing interaction with the CRM1 export machinery (39). However, one caveat of this 'obligatory export model' was that it is largely based on studies using ectopic p53 proteins, often as GFP-p53 fusion proteins, expressed at excessively high levels. The second caveat was that the interpretation of the LMB results was complicated by the fact that LMB is not only a powerful export blocker of CRM1 with respect to Rev-like NES proteins such as p53, but is also a specific stress signal for p53, which in itself would induce marked p53 accumulation in the nucleus and transcriptional activation (52, 53).

This model has now been corrected to include the nucleus as a physiological site of p53 degradation. Importantly, 26S proteasomes are equally abundant in both cytosol and nucleus (54–57). Moreover, ubiquitination of p53—the precondition for its degradation—clearly occurs in the nucleus (58), and in fact the nucleus is probably the exclusive site for this modification (50, 51). Thus, there is no *a priori* biological necessity why nuclear export should be required for p53 degradation. Consistent with this notion, it was recently shown that the nucleus constitutes a significant proteasomal compartment for MDM2-mediated degradation *in vivo* and operates in parallel to the cytoplasmic compartment (20, 59). Using overexpression of MDM2 in mutant p53 tumor cells in the presence of the CRM1 inhibitors LMB or HTLV1-Rex, it was initially found that p53 degradation occurs both in the nucleus and in the cytoplasm on a single cell level. Also, p53-null cells co-expressing nuclear export-defective mutants of both wtp53 and MDM2 retain partial competence for p53 degradation, challenging the obligatory export model (20). Importantly, in untransfected cells subjected to nuclear export blockade, nuclear degradation of endogenous wtp53 and MDM2 occurs during down-regulation of the p53 response, supporting the physiological significance of this regulation. This was seen in cells recovering from all major forms of DNA damage, including UV, γ-IR, Camptothecin, or Cisplatinum (59). Also, significant nuclear degradation of endogenous p53 and MDM2 occurs in isolated nuclear fractions prepared from these recovering cells. Furthermore, nuclear proteasomes efficiently degrade ubiquitinated p53 *in vitro* (59). Thus, in non-lethal outcomes of cellular stress, when DNA damage has been successfully repaired and the active p53 response needs to be down-regulated quickly to resume normal homeostasis, both nuclear *and* cytoplasmic proteasomes are recruited to efficiently degrade the elevated p53 and MDM2 protein levels. Local nuclear destruction adds tighter control and speed to switching the p53 pathway off.

Regulation of Ubiquitination and Degradation of p53

Disruption of the p53-MDM2 complex, by whichever route, is the pivotal event during the induction of p53, leading to the accumulation of active p53 in the cell (reviewed in 60). Interestingly, different activators of the p53 checkpoint response target the MDM2 degradation pathway by completely different mechanisms. Consequently, p53 half-life prolongs from minutes to hours. Ionizing radiation acts through a cascade of so-called stress kinases, with the ATM kinase signaling to the

checkpoint kinases hCHK1 and hCHK2 to phosphorylate p53 at several NH_2 terminal serine residues (61–63). The overall effect of these modifications may be a reduced affinity of the p53-MDM2 complexes. One of these p53 residues, Ser20, lies directly within the MDM2-binding domain and its phosphorylation may interfere with the proper fit into the MDM2 binding pocket. However, measurements by fluorescence anisotropy determined that phosphorylation of Ser15 and Ser20 did not affect the binding of an NH_2 terminal p53 peptide to MDM2. Thr18 phosphorylation, on the other hand, did reduce the binding by at least 20-fold, suggesting that phosphorylation of Thr18 could be a regulatory mechanism that disrupts the p53-MDM2 complex, thus activating p53 in response to DNA damage (64). In contrast, both UV radiation and hypoxia reduce the levels of MDM2 transcripts and protein (65, 66), hence reducing p53 degradation. Moreover, UV damage blocks ubiquitination and instead favors sumoylation of p53 on Lys386, which promotes its transcriptional activity (67).

Thus, initial attempts to understand the mechanism of p53 induction focused on the role of a complex series of phosphorylation and acetylation modifications of p53 which accompany p53 accumulation mediated by stresses such as DNA damage. However, while such modifications may contribute to weakening the p53-MDM2 interaction, phosphorylation of p53 is not essential for DNA damage-induced stabilization (63, 68, 69), as testified by the oncogene-mediated pathway of p53 activation (70) that is independent of p53 phosphorylation.

For this reason, MDM2 protein itself then became the focus of attention and is now also recognized as a principal target of signals that lead to p53 stabilization. MDM2 undergoes multisite phosphorylation *in vivo*, with most of the modification sites clustered within the p53-binding domain and the central acidic domain that is required for degradation of p53 (71). Moreover, MDM2 is a rapid, DNA damage/ATM-dependent target for phosphorylation, an event which is thought to contribute to the p53 activation mechanism (63).

The balance between self- and p53-targeted ubiquitination of MDM2 is modulated by posttranslational modifications including phosphorylation. Phosphorylation of p53 by the ataxia telangiectasia-mutated kinase (ATM) renders p53 more resistant to inhibition by MDM2 and enhances its transcriptional activity (61, 72, 73), whereas phosphorylation of MDM2 by ATM largely impairs the ability of MDM2 to promote p53 degradation (74). The finding that the same kinases can phosphorylate both MDM2 and p53 adds further complexity to the closely interrelated regulation between the two proteins. On the other hand, current evidence suggests that the initially implicated SUMO-1 modification of MDM2 does not play a role in regulating this balance (75).

Other protein kinases that have been implicated in regulating MDM2 phosphorylation and function include AKT, p38 mitogen-activated kinase (MAPK), DNA-dependent protein kinase (DNA-PK), and cyclin A-dependent kinases 1 and 2 (CDK1 and CDK2) (76–79). AKT phosphorylation consensus sites were identified in MDM2 (80). Entry of MDM2 into the nucleus is dependent on its phosphorylation by the phosphatidylinositol 3-kinase (PI3K)/AKT kinases (81, 82) and activation of AKT, for example, by growth factors or activated

Ras is sufficient to promote nuclear entry of MDM2. On phosphorylation of these sites by AKT, MDM2 translocates from the cytoplasm to the nucleus where it ubiquitinates p53 and mediates its degradation (81). For example, HER-2/*neu* induces p53 ubiquitination via AKT-mediated MDM2 phosphorylation (83). On the other hand, pharmacological inhibition of PI3K activity (*e.g.*, Wortmannin), expression of dominant-negative AKT mutants, or mutation of the AKT phosphorylation sites in MDM2 blocks nuclear entry of MDM2 (81). Because PTEN (phosphatase and tensin homologue deleted on chromosome 10) phosphatase is the major antagonist of the AKT pathway, it follows that PTEN protects p53 from degradation by inhibiting PI3K and is therefore an important component of the p53 stress response (83, 84). Entirely consistent with this notion, the onset of tumor development in p53 (+/−);PTEN(+/−) mice is similar to p53(−/−) animals, and p53 protein levels are dramatically reduced in PTEN(−/−) cells and tissues (84). Reintroducing wild-type or, surprisingly, phosphatase-dead PTEN mutants leads to a significant increase in p53 stability. PTEN is also a p53 target gene and PTEN protein physically associates with endogenous p53, hence, this is another p53 autoregulatory feedback loop (84). While there is agreement that PTEN indirectly protects p53 from degradation, it is unclear whether the phosphatase activity is dispensable for this function, because Mayo and Donner (80) found that only wild-type PTEN, but not a phosphatase-dead mutant, protects p53 from degradation and promotes p53 transcription function by inhibiting PI3K.

Yet another important level of p53 regulation is linked to its aberrant posttranslational modification present in certain human tumors such as neuroblastoma. This was found by studying unstressed human neuroblastoma cells with constitutively stabilized wild-type p53 sequestered in the cytoplasm, which is functionally compromised for growth arrest and transcription (85). Endogenous and exogenous p53 in these cells is resistant to MDM2-mediated degradation despite normal levels of MDM2 protein and normal p53 ubiquitination patterns. Instead, this sequestered p53 protein is aberrantly modified with an acidic shift of its charge isoform profile that correlates with a masked COOH terminal p53 epitope covering aa 371–381 (85). Parc, a Parkin-like protein and a non-p53 ubiquitin ligase, was recently identified to function as a cytoplasmic anchor protein for p53. Abnormal cytoplasmic sequestration of wtp53 in several neuroblastoma cell lines correlated with elevated Parc levels, while RNAi-mediated reduction of endogenous Parc relocated p53 to the nucleus and sensitized these cells for a p53-mediated DNA damage response (86).

The MDM2-ARF Connection

Deregulated oncogenes, such as oncogenic Ras mutants, c-*myc*, or viral E1A, use yet another way of interfering with MDM2 regulation to stabilize and activate p53. This mechanism is largely independent of p53 modifications. Ras stimulates the production of p14ARF protein (p19ARF in the mouse), the alternate product of the INK4A tumor suppressor locus. ARF is a direct inhibitor of the E3 activity of MDM2 (87). Specifically, ARF binds to the RING finger domain of MDM2, which houses the E3 ligase activity, and sequesters

MDM2. A model has been proposed in which the ARF protein binds MDM2 and sequesters it into the nucleolus, away from p53 that remains in the nucleoplasm (88, 89). Sequestration requires the combined nucleolar localization signals (NoLS) of ARF and MDM2 (87, 90). There is some disagreement, however, as to whether sequestration of MDM2 by ARF takes place in the nucleolus or in the nucleoplasm (88, 89, 91). Whatever the actual mechanism of MDM2 inactivation by ARF, the major consequence of this interaction lies in the stabilization of nuclear p53 levels. Thus, the oncogenic pathway neutralizes MDM2 function by enzymatic inactivation and physical removal of MDM2 via a specific capture protein, rather than by modifying the substrate, p53. This ARF-MDM2-p53 relationship appears to be an integrated part of several cellular networks involving complex mitogenic signaling pathways, such as Wnt (via β-catenin), Myc, and pRb-E2F (92, 93). Of note is that while Ras induces ARF expression, it also increases the levels and activity of MDM2, both through elevated MDM2 transcription and activation of the AKT pathway, thus generating a ying-yang situation (94–96). However, while the role of ARF in oncogene-mediated p53 activation is very strong, there has been evidence that it is not absolute in some viral and cellular contexts, where it is in fact dispensable (97, 98).

Opposite to ARF is the effect of MDMX (MDM4), a recently discovered MDM2 homologue that associates with p53 and MDM2. MDMX, like MDM2, is also an essential negative regulator of p53 *in vivo* and is capable of attenuating p53 transcriptional activities *in vitro*. However, MDMX itself is not an E3 ligase (99, 100) nor does it have nuclear localization and export signals, although MDMX binds to p53 with similar requirements as MDM2. Instead, MDMX cooperates with MDM2 biochemically. In the absence of MDMX, MDM2 is relatively ineffective in down-regulating p53 because of its extremely short half-life. MDMX renders MDM2 protein sufficiently stable to function at its full potential for p53 degradation by interacting through their RING finger domains (101). On the other hand, Hdmx protein is degraded by the ubiquitin ligase activity of MDM2 (102, 103). Moreover, ARF blocks MDM2 from degrading p53 and shifts MDM2 into degrading MDMX instead (103). MDM2 promotes ubiquitination and degradation of MDMX. The importance of MDMX as a negative regulator of p53 in cellular growth functions is best illustrated by the finding that MDMX knock-out mice fail to develop, and can be completely rescued by crossing with p53-deficient mice, thereby recapitulating some of the major characteristics of MDM2 knock-out mice (99, 104, 105). This is also reflected in the fact that many human tumors with wild-type p53 overexpress MDMX, reminiscent of MDM2 overexpression in soft tissue sarcomas and some leukemias (106). Also, MDMX mediates immortalization of primary mouse embryo fibroblasts and alleviates the pressure to lose ARF or p53 function.

Similar to ARF, the ribosomal protein L11 can interact with MDM2 and inhibit MDM2 function, thus leading to p53 stabilization and a p53 response. Overexpression of L11 activates p53. This MDM2/L11 interaction might represent a pathway for p53 stabilization in response to perturbations in ribosome biogenesis (107).

MDM2-Independent Regulation of p53 Function

Because tight regulation of p53 is so crucial for maintaining normal cell growth, cells are unlikely to rely solely on MDM2. At least in mouse cells, Pirh2 appears to also participate in an autoregulatory feedback loop that antagonizes p53 function, analogous to MDM2. Pirh2, a gene regulated by p53, encodes a RING-H2 domain-containing protein with intrinsic ubiquitin-protein ligase activity. Pirh2 physically interacts with p53, promotes ubiquitination of p53 independently of MDM2, and represses p53-dependent transactivation and growth inhibition (108).

On the other hand, HAUSP is a direct antagonist of MDM2 activity and acts by specifically deubiquitinating p53 after stimulation by DNA damage (109). HAUSP can protect p53 from MDM2-mediated degradation. Whether HAUSP acts as a tumor suppressor gene and is inactivated in tumors remains to be studied.

In response to stress, p53 stabilization may also occur in an MDM2-independent manner. JNK (110), β-catenin (111), and calpain-1 (15) overexpression all stabilize p53 in MDM2-null cell lines. Their physiological relevance remains to be elucidated.

Addendum

The crystal structure of the p53-MDM2 complex can be found at http://www.rcsb.org/pdb/cgi/explore.cgi?pid=138061037348961&pdbId= 1YCR.

Acknowledgments

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