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Minireview

Mdm2: The Ups and Downs

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Introduction

Mdm2 was first described 12 years ago. Interest in this oncogene has risen since its identification as a protein that binds and efficiently inactivates the p53 tumor suppressor protein. This interest was further boosted by the discovery that the *mdm2* gene is actually a target for direct transcriptional activation by p53, thus defining an autoregulatory feedback loop for the intracellular modulation of p53 function. Subsequent work has revealed that the Mdm2 protein is engaged in a complex network of regulatory interactions. In addition to p53, these involve several other interesting protein partners, including critical cell cycle regulators such as pRb, E2F1/DP1, and p19ARF. Through these interactions, Mdm2 now appears to exert a host of effects on cell cycle, apoptosis, and neoplastic transformation.

Several recent excellent reviews offer a detailed discussion of the Mdm2 protein and its various functions (1–3). In the present minireview, we will attempt to highlight some exciting new insights into Mdm2, its biochemistry, and its biology.

Mdm2: The Cancer Connection

The *mdm2* gene was originally cloned from a spontaneously transformed mouse 3T3 cell line, where it had been heavily amplified and was present in multiple copies on double minute chromosomes [hence its name: *m*ouse *d*ouble *m*inute; (4)]. Overexpression of the Mdm2 protein was found to confer tumorigenic properties upon rodent fibroblasts, as measured by tumor formation in nude mice (5). Overexpressed Mdm2 was also shown to immortalize primary rat embryo fibroblasts, as well as transform such cells in cooperation with oncogenic ras (6). Moreover, Mdm2 can also overcome suppression of transformed cell growth by wild-type (wt) p53 (6). More recently, targeted expression of Mdm2 to mammary glands was found to result in mammary tumors (7).

Amplification of the *mdm2* gene is observed in a variety of human tumors; an *mdm2* gene amplification database, available on the World Wide Web, has recently been compiled and described by Momand et al. (8). Amplification of the *mdm2* gene is present in a significant percentage of soft tissue sarcomas (9–11) and osteosarcomas (9,12), as well as a smaller subset of esophageal carcinomas (13), gliomas, anaplastic astrocytomas (14,15), and neuroblastomas (16). Interestingly, overexpression of Mdm2 in cancer cells can also be achieved through enhanced translation of the mRNA, without gene amplification (17,18). On average, it is estimated that 5–10% of all human tumors possess deregulated Mdm2 overexpression, due to either gene amplification or transcriptional and post-transcriptional mechanisms.

Several studies have addressed the correlation of *mdm2* deregulation with patient prognosis. *mdm2* gene amplification was detected more frequently in metastatic or recurrent osteosarcomas than in corresponding primary tumors (12). Studies looking in parallel at Mdm2 overexpression and p53 mutation concluded that these are usually mutually exclusive events, supporting the notion that the primary impact of *mdm2* am-

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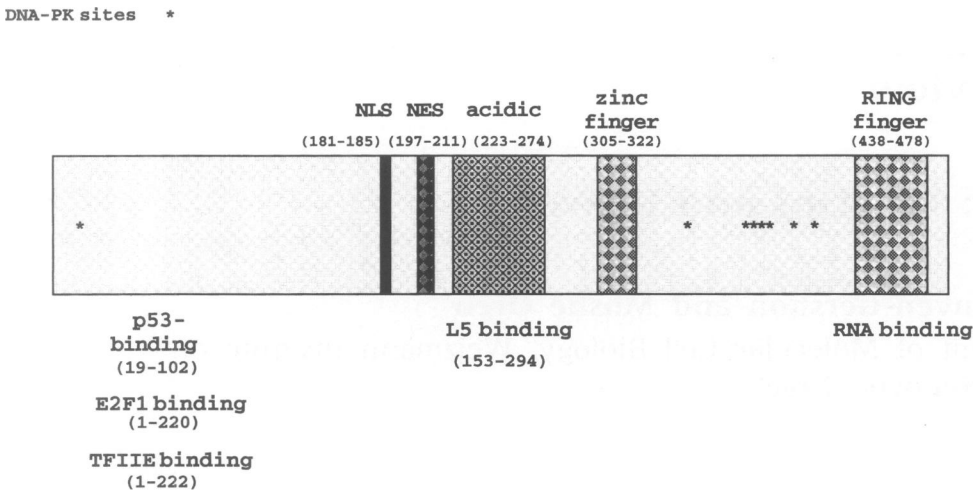

Fig. 1. Schematic map of Mdm2, illustrating functional motifs and protein-interacting domains, as well as potential sites for phosphorylation by DNA-dependent protein kinase (DNA-PK). The N-terminal site (ser17 of human Mdm2) has been shown to be phosphorylated in vitro by DNA-PK. All other DNA-PK sites are based only on sequence predictions. The N terminus harbors binding sites for p53, as well as other proteins (see text). The central region is highly acidic and is capable of binding the ribosomal L5 protein. The C-terminal RING finger has been shown to bind RNA. *, DNA-PK sites; NLS, nuclear localization signal; NES, nuclear export signal.

plification in cancer cells is the inactivation of the resident wt p53 (see ref. 8). Nevertheless, detection of elevated levels of both Mdm2 and p53 proteins, the latter being taken to imply mutational inactivation of p53, was shown to predict particularly poor prognosis and short survival of soft tissue sarcoma patients (11,19). This suggests that, in addition to its well-established ability to inactivate p53, Mdm2 probably possesses p53-independent functions that can further contribute to its oncogenic capacity.

The Mdm2 Protein

The *mdm2* gene can give rise to a series of polypeptides, through the use of multiple initiation codons and alternative splicing (20–22). Unless otherwise stated, when using the term Mdm2 in this review, we refer to the largest, full-length polypeptide.

The N-terminal portion of Mdm2 contains the p53-binding domain (Fig. 1), and can also engage in other protein–protein interactions. Several additional structural motifs, dispensable for p53 binding, exhibit extensive evolutionary conservation and are thus likely to be functionally important (1,23).

Mdm2 contains a central acidic region, capable of interacting with the ribosomal L5 protein (24). Both Mdm2-L5 and Mdm2-L5-p53 complexes can bind specifically to 5SRNA (25). Additional structural hallmarks are a zinc finger motif, as well as a RING finger domain (26). RING finger domains may mediate both protein–protein and protein–nucleic acid interactions; in fact, the Mdm2 RING finger was shown to be capable of sequence-selective RNA binding (24). Along with the above L5 interactions, this raises the interesting possibility that Mdm2 may possess as yet unidentified translational regulatory functions. In addition, the Mdm2 protein also contains a basic nuclear localization signal (5) as well as a closely juxtaposed nuclear export signal (27). These latter elements may allow the Mdm2 protein to shuttle between the nucleus and the cytoplasm, in a manner that may be important for at least some of the biochemical activities of this protein (27).

An interesting mechanism for the intracellular regulation of Mdm2 activity is suggested by the finding that this protein undergoes caspase-mediated proteolytic cleavage during apoptosis (28,29). The resultant major cleavage product misses the C-terminal RING finger, resulting in loss of RNA binding, but retains the N-terminal p53-binding domain and is therefore still capable of inhibiting p53-mediated transactivation. The other, smaller product of the cleavage retains RNA binding ability (28). The biological role of this apoptotic cleavage remains to be unraveled.

The *mdm2*-p53 Autoregulatory Loop

The Mdm2 protein forms a tight specific complex with p53 (30,31), and this interaction results in inhibition of p53-mediated transcriptional activity (30–32). At the same time, expression of the *mdm2* gene is induced by active wt p53, through a p53-responsive promoter (P_2) that resides within intron I of this gene (33–37). In conjunction, this defines an autoregulatory loop whereby the activation of p53 results in enhanced transcription of the *mdm2* gene, which then leads to production of Mdm2 protein and consequent inactivation of p53 through protein–protein interactions (33,35,38).

The inactivation of p53 by Mdm2 is achieved through multiple molecular mechanisms. The fact that the Mdm2-binding domain of p53 overlaps p53's transcriptional activation domain (TAD) results in physical blocking of the interaction between the p53 TAD and critical transcription associated proteins; consequently, the Mdm2-bound form of p53 is expected to be transcriptionally inactive (31,39–41). Moreover, Mdm2 inhibits p53 not only by concealing its TAD, but also through exerting a direct repressor effect on basal transcription from p53-responsive promoters (42), presumably through physical interaction with components of the general transcription machinery (42,43). This dual mechanism probably assures efficient inactivation of p53-dependent transcription by Mdm2.

Further insight into the intricacies of the p53-Mdm2 autoregulatory feedback loop came through the finding that the binding of Mdm2 to p53 results in the targeting of p53 to ubiquitination and subsequent proteasomal degradation (44,45). Thus, Mdm2 can down-regulate p53 function not only through blocking its transcriptional activity, but actually also through directly eliminating it from the cell. Consistent with this conjecture is the finding that interference with p53-Mdm2 interaction through administration of antibodies or peptides that compete with this interaction results in markedly increased steady-state p53 levels in nonstressed cells (46). A possible mechanistic explanation is offered by experiments demonstrating that, at least in vitro, Mdm2 can act as a ubiquitin protein ligase (E3) specific for p53 (47).

Mdm2 expression is induced in a p53-dependent manner in response to a variety of stress signals, including DNA damage (48–52). Extensive DNA damage may sometimes result in delayed induction of Mdm2 protein expression (48,49). This delay is believed to provide p53 with an extended time window, in which it can continue to function without being subject to inhibition by the induced Mdm2. At later times, presumably when the stress has been successfully resolved (e.g., through DNA repair), Mdm2 eventually becomes induced, leading to inactivation of the accumulated p53 and termination of the p53 signal (48,51). Although this model is very appealing, it holds only for a subset of circumstances. In other situations of extended stress Mdm2 does get induced rapidly; yet p53 remains constitutively active under those conditions, presumably through post-translational mechanisms that render it immune to Mdm2-mediated inhibition (see below).

Tight temporal and spatial regulation of cellular p53 activity may further be achieved through the fact that Mdm2 itself is a short-lived protein (20), also subject to degradation by the ubiquitin-proteasome pathway (53). Thus, once the stress signal has been successfully taken care of, both p53 and Mdm2 can be rapidly cleared through a similar proteolytic mechanism. This will allow a prompt return of the cell to its nonstressed ground state, without compromising the ability of the p53 response to be triggered immediately again should another stress signal be delivered.

It is of note that the *mdm2* transcripts induced by activated p53, which originate in the P2 promoter, differ from those expressed from the P1 promoter under basal conditions in a p53-independent manner (36,50). The P2-derived transcripts have a shorter 5′ UTR and are more efficiently translated, which may facilitate the rapid inactivation of p53 once *mdm2* gene expression is induced.

The crystal structure of the N-terminal domain of Mdm2 bound to a short p53 segment reveals that Mdm2 has a deep hydrophobic cleft into which p53 binds as an amphipathic α-helix (54). The interface relies on van der Waals interactions involving primarily the hydrophobic and aromatic amino acids Phe19, Trp24, and Leu26 of p53, and on the steric complementarity between the Mdm2 cleft and the p53 helix. The nature of these tight interactions, also supported by earlier mutational analysis (40,41), offers clues to potential therapeutic intervention and p53 activation.

Mdm2 binds to p53 preferentially when the latter is present as a tetramer (55). This may serve to ensure that in the absence of stress

Mdm2 will not cause a complete elimination of cellular p53 and that there will always be a small reservoir of (possibly monomeric) p53 molecules ready to be rapidly activated by incoming signals. Recently, it was shown that the C-terminal part of p53, including the dimerization domain, is important for Mdm2-targeted degradation (56). This pertains not only to the p53 oligomerization domain itself, predicted to be required for efficient Mdm2 binding, but also to the more extreme C-terminal portion of p53, which is not part of the oligomerization domain. The latter might be due to the presence of multiple lysine residues within this region of p53, which may serve as sites for Mdm2-directed ubiquitination. Alternatively, since the extreme C terminus has a critical role in the allosteric regulation of p53 (reviewed in ref. 57), it is conceivable that such regulation may be an important determinant in the ability of p53 to serve as a target for Mdm2-promoted degradation.

As mentioned earlier, Mdm2 was shown capable of shuttling between the nucleus and the cytoplasm. This shuttling, while p53-independent, appears to be required for the ability of Mdm2 to promote the degradation of p53 (27). Recent work has demonstrated directly that treatment of cells with leptomycin B (LMB), which blocks nuclear export and prevents the shuttling of Mdm2 from the nucleus into the cytoplasm, effectively abrogates Mdm2-mediated p53 degradation and leads to nuclear accumulation of active p53 (58). This finding supports the importance of Mdm2 nucleocytoplasmic shuttling for p53 proteolysis. Nevertheless, this does not necessarily prove that p53 can be exported from the nucleus only in complex with Mdm2. In fact, LMB is expected to block the nuclear export of many proteins. Hence, it is equally likely that p53 can also translocate into the cytoplasm without the help of Mdm2, either on its own or in complex with other proteins, and become targeted for proteolysis upon encountering Mdm2 in the cytoplasm.

In conclusion, Mdm2 can inactivate p53 both in the nucleus and in the cytoplasm—in the former compartment through blocking p53's transcriptional capacity and in the latter through direct down-modulation of cellular p53 levels.

As expected, interaction of Mdm2 with p53 can result not only in inhibition of p53-mediated transactivation but also in inhibition of p53-mediated G_1 arrest and apoptosis (51,59,60). The actual fraction of total cellular p53 that is bound by Mdm2 may be an important determinant of the eventual growth characteristics of the cell (61).

The in vivo importance of Mdm2–p53 interactions is underscored by the finding that whereas *mdm2* null mice die early in development, mice lacking both *p53* and *mdm2* ("double knock-outs") are viable and develop quite normally (62,63). This implies that Mdm2 plays a critical role in development through the negative regulation of p53 activity; in the absence of Mdm2 function, p53 presumably becomes aberrantly activated, giving rise to a lethal embryonic phenotype.

Post-translational Regulation of Mdm2

There is growing evidence that many aspects of p53 activity are regulated through post-translational modifications, including phosphorylation and acetylation (reviewed in ref. 57). In particular, phosphorylation of p53 on serine 15 was shown to inhibit its interaction with Mdm2 in vitro (64), and this mechanism may be responsible in part for the accumulation of stabilized p53 in cells exposed to DNA damage (65). Recent evidence indicates that this site is phosphorylated by the DNA damage-induced ATM kinase (66,67). An involvement has also been suggested for the DNA-dependent protein kinase [DNA-PK; (68)], which very effectively phosphorylates this residue in vitro (64).

Mdm2 is also a phosphoprotein (30,69). Hence, it is conceivable that its interaction with p53 may be regulated through phosphorylation not only of p53 but also of Mdm2 itself. In fact, Mdm2 harbors many putative phosphorylation sites for a variety of different protein kinases (5). Moreover, Mdm2 was shown to be phosphorylated in vitro by casein kinase 2 [CK2; (70)], as well as DNA-PK (71). Importantly, in vitro phosphorylation of Mdm2 by DNA-PK prevents it from interacting with p53 (71). This is in line with a model where, upon cellular exposure to stress, both p53 and Mdm2 become modified in a manner that interferes with their ability to bind each other, thus leading to stabilization and biochemical activation of p53. The model predicts that upon recovery from stress, the relevant protein kinases will lose their activity and thereby allow Mdm2 to bind efficiently to p53 and promote its inactivation and rapid degradation. It should be stressed, however, that there is presently no evidence that DNA-PK indeed phos-

phorylates Mdm2 in vivo. It is also worth noting that Mdm2 was shown to be stabilized and acquire an altered phosphorylation pattern in cells infected or transformed by SV40 (72).

In conclusion, the nature of the kinases that actually modify Mdm2 within living cells and the regulatory significance of such modifications remain to be elucidated. Given the obvious importance of this issue, significant progress is likely to be achieved within the near future.

Mdm2-p53 Interactions: Potential Target for Therapeutic Intervention?

Many years ago, Oliner et al. (31) suggested that disruption of p53–Mdm2 interactions may bear potential therapeutic promise. In principle, when achieved in tumor cells expressing excess Mdm2 together with endogenous wt p53, such intervention may release p53 from the inhibitory action of Mdm2 and lead to reconstitution of cancer-inhibitory p53 function. This notion has been explored recently by Lane and co-workers, employing primarily synthetic Mdm2-binding peptides (46,73,74). One successful approach was based on the insertion of such peptide, through recombinant DNA manipulation, into the active site of thioredoxin. When introduced into wt p53-containing cells, the recombinant protein led to accumulation of endogenous p53, activation of p53-dependent transcription, and eventually cell cycle arrest (46). Another approach aimed at inhibition of Mdm2–p53 interactions employed *mdm2* antisense oligonucleotides (75). In that case down-modulation of Mdm2 resulted in p53-inducible gene expression, followed by apoptosis. Moreover, this activation of p53 was further enhanced by exposure to DNA damage (75). This provides a rational basis for hopes to increase the response of tumor cells to cancer therapy through elimination of the p53-inhibitory effects of their endogenous Mdm2.

Similarly, microinjection of an anti-Mdm2 antibody that disrupts Mdm2–p53 interaction gave rise to increased p53 protein levels in a tumorigenic cell line expressing functional wt p53 (76). However, a similar study with the same antibody revealed that normal cells also responded by activation of their endogenous p53 (77). This raises a concern that complete abrogation of Mdm2–p53 interactions may also achieve the apoptotic demise of critical stem cell populations—a problem routinely encountered with conventional anti-cancer therapy. The extent of potential risk can be assessed properly only through studying the effect of Mdm2 inactivation in a wide range of normal tissues under physiological conditions.

An alternative experimental approach is suggested by the observation that a synthetic peptide derived from Mdm2 can stimulate autoreactive cytotoxic T lymphocytes (CTL) that recognize cells expressing endogenous Mdm2 (78). If in vivo such CTLs can preferentially target cells that overexpress Mdm2, this could provide a means for the selective killing of certain types of tumor cells.

Protein Partners of Mdm2: Who Else Besides p53?

Although p53 remains the most prominent Mdm2 interaction partner, both biochemically and biologically, there is a growing list of additional proteins that Mdm2 can bind and possibly regulate through this binding. The study of such interactions and of their interplay with Mdm2-p53 interactions is still at an early stage.

Mdm2 inhibits not only the antiproliferative effect of p53 but also that of the retinoblastoma gene product, pRb. Mdm2 can bind pRb and prevent the induction of pRb-mediated G_1 cell cycle arrest (79). In addition, Mdm2 can also interact directly with the pRb-regulated transcription factor E2F1/DP1, resulting in stimulation of S-phase progression (80). Mdm2 can thus augment E2F1/DP1-dependent transactivation through multiple mechanisms. The consequent enhancement of S-phase entry is in line with the oncogenic nature of Mdm2.

The biochemical interaction of Mdm2 with E2F1 prompted attempts to look for a possible effect of Mdm2 on E2F1-induced apoptosis. While E2F1-induced apoptosis was not affected by Mdm2 in a *p53*−/−, *Rb*−/− background (81), such apoptosis was indeed inhibited by Mdm2 when the process was p53-dependent (82). However, conclusive interpretation of these data is hampered by the fact that E2F1 and DP1 were reported to interact not only with Mdm2 and pRb but also with p53 itself (83,84).

Another recently described provocative interaction of Mdm2 is with the cell-fate regulator protein Numb (85). This association promotes translocation of Numb into the nucleus and leads

to a reduction in Numb steady-state levels, through a mechanism that appears to be similar to Mdm2-mediated p53 degradation (85). This raises the possibility that Mdm2 may regulate the stability of a larger panel of regulatory proteins; identification of additional targets for Mdm2-directed proteolysis might provide new insight into the biological roles of Mdm2.

Unlike p53, the degradation of its homolog p73 is not promoted by Mdm2; in fact, Mdm2 overexpression may even lead to an increase in cellular p73 levels (86). Nevertheless, Mdm2 does bind to p73 and inhibits its transcriptional activity. This is apparently achieved through interference with the binding between p73 and p300, a critical co-activator required for optimal transcriptional activity of both p53 and p73 (86). Hence, the autoregulatory loop appears to apply to at least several members of the recently enlarged p53 family, although the mechanisms may be different in each case. In any event, the outcome of deregulation is likely to be similar: abrogation of proper growth arrest and of apoptotic responses (86), facilitating the development of cancer.

Perhaps the most revealing Mdm2-related finding in 1998 relates to the p19ARF (mouse)/p14ARF (human) tumor suppressor protein. In earlier work, it was found that this Alternative Reading Frame product of the p16 tumor suppressor locus is a potent cell cycle inhibitor (87). Subsequent work revealed that ARF can in fact bind Mdm2 (88–91). In addition, ARF can bind directly to p53, and formation of p53/Mdm2/ARF ternary complexes has also been described (89,90). The physical interaction of ARF with Mdm2 blocks Mdm2-mediated p53 degradation and restores p53-mediated transactivation (88). Hence, through its effects on Mdm2 (as well as directly through p53 binding) ARF can serve as an upstream activator of p53. In line with the model where p19 acts upstream of p53, its overexpression results in growth arrest on a wt p53 background but not in p53 null cells (90).

The ARF-binding domain of Mdm2 does not overlap the p53-binding domain (88–90); this might explain the ability to form ternary complexes, presumably assembled around Mdm2. An enigmatic issue is how such complexes retain the biochemical activities of p53, given that the p53 TAD is expected to be blocked by the associated Mdm2.

Like with many other aspects of p53-related regulatory circuits, the ARF-Mdm2 story is not so simple. While being up-regulated by ARF, p53 itself actually down-regulates ARF expression (91). This implies the existence of an additional negative autoregulatory loop, somewhat akin to the p53-Mdm2 loop. Furthermore, production of ARF mRNA is positively regulated by the E2F1 transcription factor (92). Since Mdm2 has been proposed to induce E2F activity (see above), this raises the testable prediction that Mdm2 should also augment ARF expression (see Fig. 2).

Several recent reports open the road to a better understanding of how aberrant oncogene activation triggers p53 activation. The cellular myc protein, as well as the adenovirus E1A oncoprotein and an oncogenic activated ras, are potent inducers of cell transformation but also activators of p53. As it turns out, the three oncoproteins induce strongly ARF expression and p53 stabilization (93–95). The positive regulation of p53 through ARF may serve as a fail-safe device for counteracting the harmful effects of unscheduled oncogene activation. The molecular mechanism for oncogene-initiated, ARF-mediated p53 activation is clearly distinct from that utilized by DNA damage; this is indicated by the fact that, unlike signals emanating from damaged DNA, activation of p53 by the oncoprotein/ARF upstream pathway does not involve phosphorylation of p53 on serine 15 (93).

Like ARF, the transcriptional coactivator p300 is also capable of direct interaction with both Mdm2 and p53 (96). p300 and the closely related CBP bind p53 and are required for its efficient transcriptional activity (97–100). On the other hand, the interaction of Mdm2 with p300 contributes to the p53-destabilizing action of Mdm2. Thus, an Mdm2 mutant capable of binding p53 but not p300 is defective in p53 degradation (96). Similarly, a p53 mutant unable to bind p300 is resistant to Mdm2-mediated degradation. This suggests that the interplay between Mdm2, p53, and p300 can regulate p53 activity by either allowing effective p300-dependent p53 transcriptional activity or targeting p53 for rapid proteasomal degradation.

Another facet of this story is provided by the observation that while p300 is generally required for optimal p53-mediated transactivation, it is particularly crucial for the activation of the *mdm2* gene (101). Consequently, the viral E1A oncoprotein, which sequesters p300, interferes selectively with *mdm2* induction by p53. The failure to produce Mdm2 protein results in p53 stabiliza-

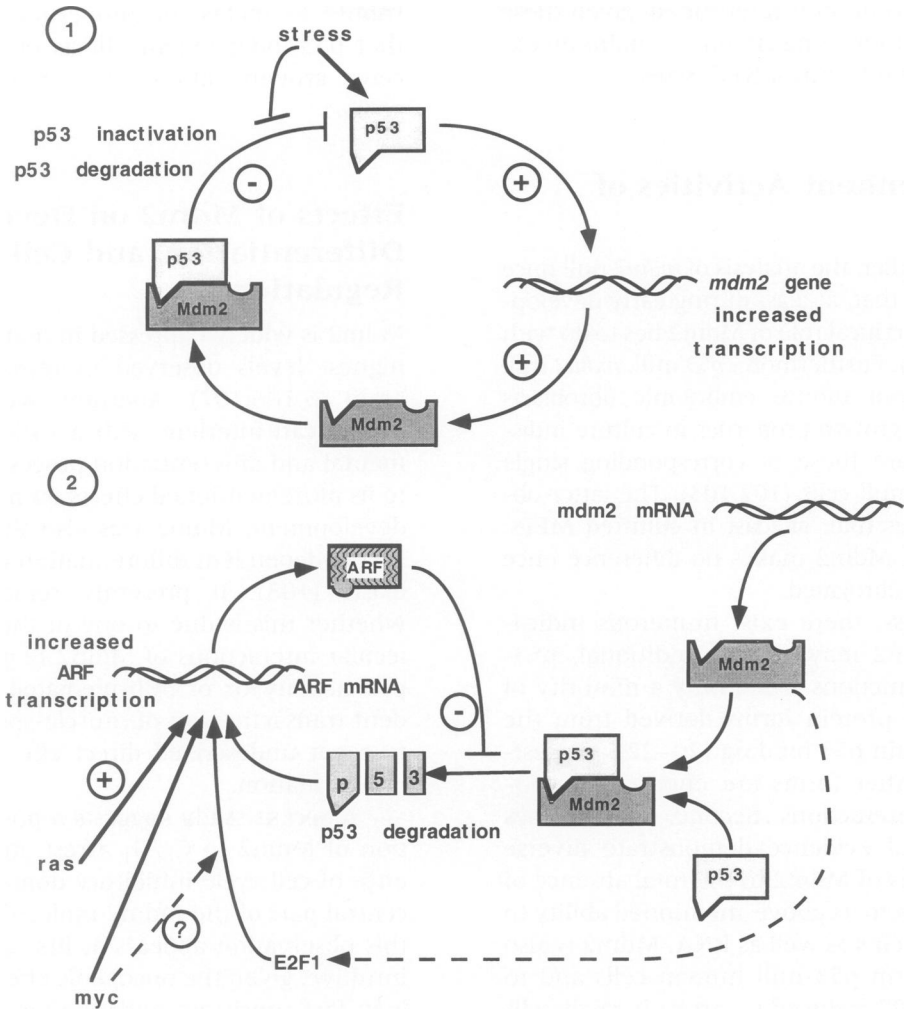

Fig. 2. The "Mdm2 loops." Depicted are two possible autoregulatory loops whose existence is supported by the data discussed in this minireview. These loops are not mutually exclusive; in fact, they most probably are integrated into each other, and are depicted as separate only for the sake of simplicity. In loop 1 (the "traditional" loop), activation of p53 is proposed to induce *mdm2* transcription and production of Mdm2 protein, which then binds to p53, inactivates it, and targets it for degradation, thereby restraining excess p53 activity. Stress signals may allow p53 activation and accumulation through interfering with the inhibitory effects of Mdm2 on p53. In loop 2, augmented Mdm2 protein production (e.g., through gene amplification, translational mechanisms, etc.) is proposed to lead to down-regulation of p53 protein. This will relieve the inhibitory effect of p53 on ARF transcription and, in conjunction with the Mdm2-mediated augmentation of E2F1 activity, will result in enhanced ARF expression, and consequently in inhibition of Mdm2 function through ARF-Mdm2 protein interactions. The nonoverlapping binding sites for p53 and for ARF on the Mdm2 molecule are indicated by different shapes. See text for further details.

tion and accumulation, culminating in p53-dependent apoptosis (101). In this context, it was indeed noted that expression of extra p300 could rescue cells from p53-mediated apoptosis, presumably through induction of Mdm2 production and subsequent p53 inactivation (101).

Figure 2 depicts two possible feedback circuits suggested by these recent findings. It is clear that the situation is not as simple as depicted; within a living cell, these and additional "cycles" are part of one complex network, which most certainly includes many other critical regulatory proteins.

Altogether, one starts to get a glimpse of a complex network of closely interwoven, regulatory feedback loops involving as central players p53, Mdm2, and ARF, as well as several of their interaction partners. How the actual cellular

phenotype is eventually determined, given these sometimes counteracting circuits, remains an extremely important, unresolved issue.

p53-Independent Activities of Mdm2

As discussed earlier, the analysis of *mdm2* null mice argues strongly that, at least during early development, the most critical role of Mdm2 has to do with p53 modulation. Furthermore, *p53* null, *mdm2* null double knock-out mouse embryonic fibroblasts (MEFs) exhibit growth properties in culture indistinguishable from those of corresponding single knock-out *p53* null cells (102,103). The latter observation implies that, at least in cultured MEFs, the presence of Mdm2 makes no difference once p53 function is abrogated.

Nevertheless, there exist numerous indications that Mdm2 may possess additional, p53-independent functions. First, only a minority of the alternative protein forms derived from the *mdm2* gene retain p53 binding (20–22), suggesting that the other forms are engaged in p53-independent interactions. Second, several lines of experimental evidence demonstrate diverse biological effects of Mdm2 in the total absence of p53. In addition to its above-mentioned ability to bind other proteins as well as RNA, Mdm2 is also able to transform p53-null human cells and to overcome a p107-induced G_1 arrest in such cells (104). Moreover, the effects of overexpressed Mdm2 on mammary gland development and tumorigenicity observed by Lundgren et al. (7) could be seen even on the background of *p53*-null mice.

The potential importance of p53-independent activities of Mdm2 is highlighted by the recent work of Sun et al. (105). A screen for genes capable of conferring resistance to the antiproliferative effects of transforming growth factor β (TGF-β) yielded repeatedly cDNA clones corresponding to Mdm2. Furthermore, overexpression of Mdm2 could rescue cells from TGF-β-mediated growth inhibition. Importantly, this effect did not require the presence of functional p53; rather, it was exerted through inactivation of pRb and augmentation of the activity and overall protein levels of E2F1 (105). Abrogation of TGF-β responsiveness may thus contribute to the oncogenic activation of Mdm2. Moreover, since enhanced TGF-β resistance is frequently encountered as tumors become metastatic, it is conceivable that Mdm2 expression may also contribute to metastatic properties. It is expected that p53-independent effects of Mdm2 will receive growing attention within the next few years.

Effects of Mdm2 on Development, Differentiation, and Cell Cycle Regulation

Mdm2 is widely expressed in many tissues, with highest levels observed in testis, muscle, and brain (5,106,107). Aberrant overexpression of Mdm2 can interfere with a variety of developmental and differentiation processes. In addition to its aforementioned effects on mammary gland development, Mdm2 was also shown to inhibit MyoD-dependent differentiation of mouse myoblasts (108). It presently remains unknown whether this is due to one of the reported molecular interactions of Mdm2 (e.g., inhibition of p53 activity or of pRb-mediated MyoD-dependent transactivation of muscle-specific genes) or to a yet undescribed direct effect of Mdm2 on differentiation.

A recent study suggests a positive contribution of Mdm2 to G_0/G_1 arrest, due to the presence of cell cycle inhibitory domains within the central part of the Mdm2 molecule (109). While this observation appears at first glance counterintuitive, given the oncogenic effects of Mdm2, it is in fact consistent with the fact that many investigators have encountered great difficulties in obtaining Mdm2 overexpression through stable transfection of a variety of cell types. The intact Mdm2 protein may thus contain a "self-restraining" domain [mapped by Brown et al. to the central portion of the protein (109)] which restricts its potential oncogenic effects in case it becomes aberrantly overexpressed. This possibility echoes a familiar theme, already introduced in the context of other, better studied oncogenes: the existence of protective "fail-safe" mechanisms that couple a growth-inhibitory (e.g., in the case of ras) or pro-apoptotic (e.g., myc) effect with deregulated oncogene action. It is tempting to speculate that the Mdm2 "self-restraining" domain is the same one that is responsible for ARF binding, so that its loss now renders Mdm2 immune to inhibition by ARF and thus constitutively active and more effectively oncogenic. The existence of a plethora of alternative Mdm2 polypeptides may be one way in which forms with different degrees of built-in "restraint" are generated. This notion is supported by the data of

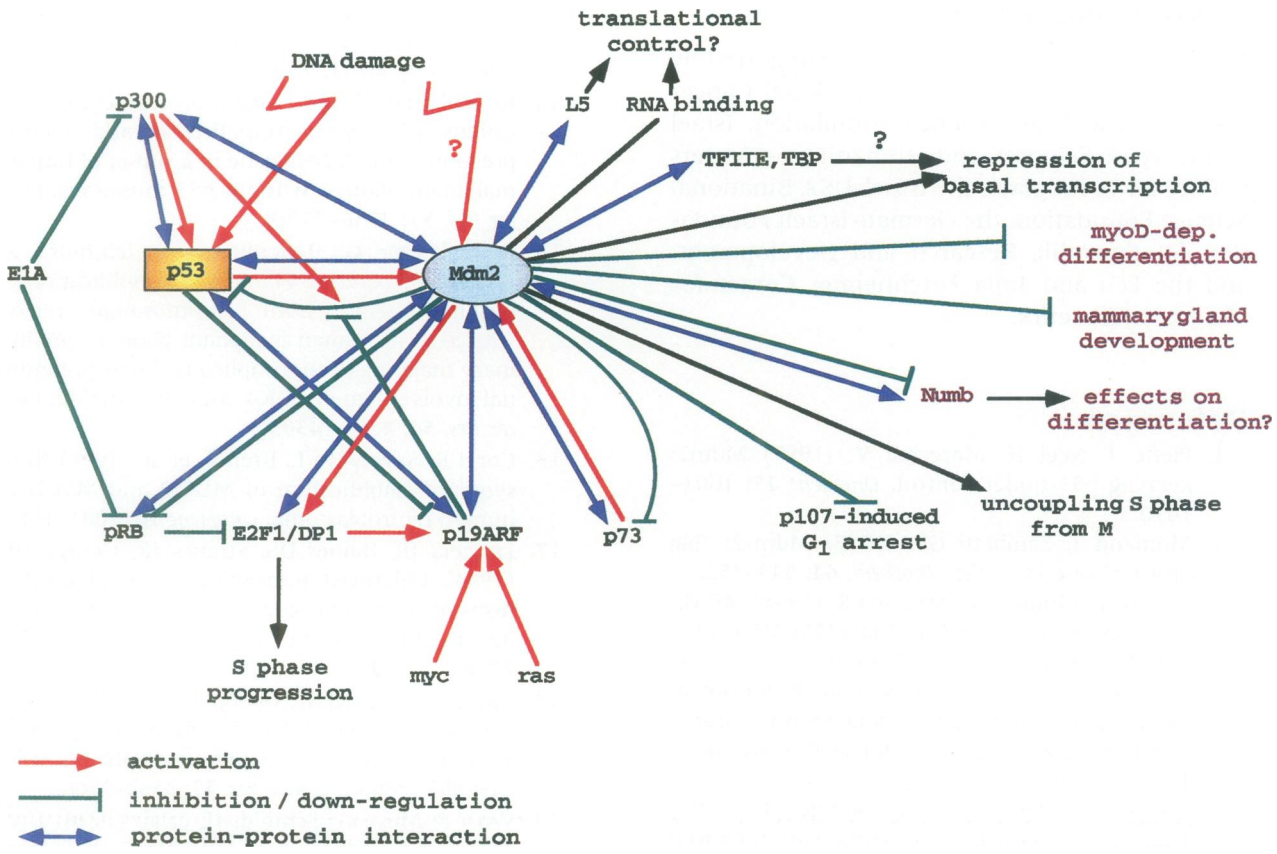

Fig. 3. Schematic model depicting regulatory interactions between Mdm2 and other proteins, as well as possible functional consequences of such interactions. See text for details.

Sigalas et al. (22), based on the analysis of human tumors.

Family Connections

A recent excitement in the p53 field has been the discovery of several related genes and proteins, which together now comprise a small p53 gene family. In the case of *mdm2*, a "cousin" has already been described earlier and given the name *mdmx* (106). The Mdmx protein is structurally similar to Mdm2, especially in the N-terminal p53-binding domain and in the C-terminal part. Identified by virtue of its association with p53 (106), Mdmx resembles Mdm2 in that it can bind p53 and inhibit p53-mediated transactivation. However, Mdmx expression is not induced by DNA damage (106). It remains to be established whether Mdmx can also target p53 for proteasomal degradation. It is of note that in both mouse and humans, *mdmx* mRNA is expressed in all tissues tested (106,110), raising the possibility that Mdmx may provide a constitutive "buffer" for p53, minimizing the latter's biochemical effects as long as p53 is not induced well above its low basal levels. Obviously, a better understanding of the roles of Mdmx, along with the possible existence of additional *mdm2* family members, awaits further studies.

Conclusions

Until recently, the interplay between p53 and Mdm2 was perhaps the only part in the complex p53 picture that was believed to be simple and satisfactorily understood. Not so anymore. Figure 3 depicts the complex network of regulatory interactions in which Mdm2 is engaged. As Mdm2 is becoming implicated in more and more pathways, and on its way is picking a growing company of new protein partners, there is an increased realization that the Mdm2 story is likely to be as complicated as that of p53, and, we hope, equally exciting.

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