Subject Review

Cell Cycle Regulatory Functions of the Human Oncoprotein MDM2

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Abstract

The protein (MDM2) coded by the mouse double minute-2 (mdm2) gene or its human homologue is well known as an oncoprotein. Malignant human tumors particularly breast tumors and soft tissue sarcomas frequently overexpress MDM2. Artificial amplification of mdm2 gene derived from a transformed murine cell line enhances tumorigenic potential of murine cells. Consistent with its tumorigenic property, mouse or human MDM2 can inactivate several functions of the tumor suppressor p53 and can degrade p53. The protein also interacts with other tumor suppressors, and these interactions may contribute to its tumorigenic property. In spite of its oncogenic role, mouse or human MDM2 induces G1 arrest in normal human or murine cells. Some cell lines bearing known genetic mutations are insensitive to MDM2-mediated growth arrest. This review is aimed to collect available information on the functions of MDM2 that could potentially regulate cell cycle and to discuss how this information fit in one model that could explain the two apparently opposite G1 arrest and oncogenic function of MDM2.

Introduction

Selective growth advantage gained by the cancer cells is a result of multiple genetic damages (1–3). Although human cancer cells frequently overexpress MDM2, some experimental evidences suggest that in normal cells, MDM2 overexpression causes G1 arrest, suggesting that MDM2 overexpression requires genetic alterations. The biochemical activities of MDM2 in cancer cells have been studied intensely because of its association with the tumor suppressor p53 and its implication in human cancer. Oncogenic challenges such as DNA damage that induce DNA repair induce a form of p53 incapable of interacting with MDM2 and a form of MDM2 inactive for degradation, suggesting that functionally active p53 and MDM2 are perhaps not degraded. Therefore, MDM2 induced by DNA damage in normal cells may have a role in preventing untimely cell cycle progression. Cancer cells that overexpress MDM2 may evade these checkpoint regulatory pathways by selecting mutated cells. Therefore, MDM2 possibly has dual role in regulating cell growth. The mechanism and significance of the cell cycle regulatory functions of MDM2 in normal and cancer cells thus need to be explored. Several excellent reviews including the present series have been published on many different aspects of mouse and human MDM2 (4–10). Here we discuss how MDM2 modulates cell cycle and how its dysfunction may induce oncogenesis.

MDM2 Is an Oncoprotein

Overexpression of MDM2 Is Capable of Enhancing Tumorigenesis

The mouse double minute-2 gene (mdm2) was discovered as an amplified and overexpressed gene in a spontaneously transformed derivative of mouse BALB/c cell line 3T3DM. To determine if the mdm2 gene has oncogenic potential, a cosmid bearing the mdm2 gene and a dehydrofolate reductase (DHFR) gene was amplified in NIH3T3 cells by methotrexate treatment. In the presence of methotrexate, only the cells that amplified the DHFR gene survived. These cells showed co-amplification of mdm2 gene and overexpression of MDM2. These cells formed tumor in nude mice (11). The cosmid bearing the mdm2 gene also transformed primary rat embryo fibroblasts in the presence of ras oncogene (12). These findings suggest that MDM2 has oncogenic function.

Cancer Cells Overexpress MDM2

Human homologue of the mdm2 gene is frequently overexpressed in many human cancers, particularly in breast tumors and carcinomas (13–18) and soft tissue sarcomas (19–27). These findings suggest that the genetic alteration could be one of the common causes of oncogenesis. Frequent overexpression of MDM2 in advanced breast tumors suggests that the oncoprotein may be used as an indicator for breast cancer (14, 16). Overexpression of MDM2 has been associated with both poor (19) and favorable prognosis (28–31). Amplification of human MDM2 gene is frequent in cancer cells with wild-type p53. Because the oncoprotein can inactivate p53-mediated...
transcriptional activation, MDM2 is thought to induce oncogenesis by inactivating the tumor suppressor p53 (26, 32). However, later studies have reported alteration in the expression of both p53 and MDM2 genes (19).

MDM2 overexpresses in 41% of benign and 68% of malignant lesions of human breast (13) and is strongly related with the presence of estrogen receptor (13, 15, 18). Elevated expression of estrogen receptor in cells lacking estrogen receptor induces overexpression of MDM2. Overexpression of MDM2 has also been correlated with the cyclin-dependent kinase inhibitor p21. In breast cancer cells, overexpression of MDM2 correlates with lack of p21 expression (33). As MDM2 inhibits p53-mediated transcriptional activation, and p21 is a p53-inducible protein, this correlation is not unexpected. In squamous cell carcinoma, on the other hand, overexpression of MDM2 is associated with higher levels of p21 (34). Because cancer cells select for multiple genetic damages, it is difficult to get significant insight as to the role of MDM2 in tumorigenesis from the pattern of MDM2 overexpression in cancer.

Amplification of the MDM2 gene leading to overexpression of MDM2 mRNA (11, 17, 26, 32, 35), and enhanced translation of mRNA, has been proposed as mechanisms of MDM2 overexpression (25, 36). Recently, the BCR/ABL oncproteins have been shown to activate translation of MDM2 mRNA (37), suggesting that the oncogenic activity of the BCR/ABL oncproteins may be achieved by up-regulating MDM2 expression.

**Targeted Overexpression of MDM2 in Transgenic Mice Inhibits Mammary Gland Development, but Cause Polyploidy in Some Breast Epithelial Cells**

Because most tumor-derived cells harbor multiple genetic defects, and growth regulatory functions of MDM2 have mostly been studied in tumor-derived cells, targeted expression of MDM2 in transgenic mice has been informative. Overexpression of MDM2 in breast epithelial cells inhibited normal development of mammary gland. Transgenic mammary gland showed fewer epithelial cells with no sign of apoptosis. Twelve percent to 27% of leftover epithelial cells were arrested in the S-phase, and 30% to 45% of the cells showed multiple rounds of DNA replication without cell division resulting in polyploidy. Some (16%) of these transgenic mice showed small or rough eye phenotype with disorganization of the bristles. Again, there was no evidence of apoptosis or cell proliferation in MDM2-overexpressing cells (39), suggesting growth arrest properties of MDM2. Targeted overexpression of MDM2 in the basal layer of epidermis increases papilloma formation by chemical carcinogens (40). This finding also suggests the requirement of added genetic damage for MDM2-induced tumorigenesis. Consistent with this hypothesis, a recent report suggests that MDM2 can enhance tumor formation in lethally irradiated Eμ-myc transgenic mice particularly in the absence of p53 (41). This report as well as generation of transgenic mice in the presence or absence of functional p53 demonstrated a p53-independent role of MDM2 in tumorigenesis (42).

Ectopic expression of MDM2 using a retroviral expression vector was reported to rescue transforming growth factor β (TGF-β)-induced growth arrest of mink lung epithelial cells in a p53-dependent manner. This property of MDM2 is thought to be a result of interference of Rb/E2F function by MDM2. Consistent with this finding, some breast tumor cells that overexpress MDM2 show TGF-β resistance (43). However, conditional expression of MDM2 was shown not to overcome TGF-β-mediated growth arrest. It has been suggested that acquisition of additional mutation may be necessary to evade TGF-β-mediated growth arrest (44).

**MDM2 Interacts With Several Growth Suppressors**

Consistent with its oncogenic potential, human or mouse MDM2 has been shown to interact with several growth suppressors. These interactions have been discussed extensively in this issue (44a–45). In this review, only the growth regulatory consequences of these interactions will be discussed briefly. In tumor-derived cells, MDM2 interacts with the tumor suppressor p53, retinoblastoma gene product pRb (45a), the p53-family protein p73 (46, 47), and the growth suppressor p14/p19 coded by the alternate reading frame (ARF) of human/mouse INK4a locus (48–50). These interactions are perceived to be contributing to the tumorigenic role of MDM2.

**Interaction With the Tumor Suppressor p53**

Among all cellular proteins that interact with MDM2, its association with p53 has been studied most intensely. The mdm2 gene product was originally detected in a complex with the tumor suppressor p53 (51). MDM2 recognizes the transactivation domain of p53 and inactivates p53-mediated transcriptional activation (4–8). The p53-inactivating function of MDM2 argues for the hypothesis that MDM2 induces tumorigenesis by inactivating the tumor suppressor p53 (26, 32). Consistent with this hypothesis, amplification of MDM2 gene is more frequent in human cancer cells with wild-type p53 than in cells with mutant or deleted p53 (26, 32).

Human or mouse MDM2 can interact with the tumor suppressor p53 in cell-free systems (52, 53) or in the whole cell (52, 54–56) and inhibit transactivation by wild-type p53. The interaction of MDM2 with p53 is needed for inhibition of p53-mediated transactivation (52) and only 127 amino acids (amino acids 28 to 154) of MDM2, out of a total of 491, are sufficient for this interaction (55). The crystal structure of a complex formed between 109 NH2 terminal residues of MDM2 and
15-residue transactivation domain of p53 has been analyzed (57). According to this study, p53 contacts MDM2 by three hydrophobic and aromatic amino acids, Phe-19, Trp-23, and Leu-26. This interaction fills up a complementary hydrophobic pocket of MDM2. The three amino acids that are involved in the interaction are essential for transactivation of p53. Thus, the crystal structure supports the earlier reports that MDM2 conceals the transactivation domain of p53.

Consistent with its ability to inhibit p53-mediated transactivation, human or mouse MDM2 regulates several functions of p53. In tumor-derived cells, MDM2 inhibits p53-mediated growth suppression and apoptosis (58–60). Both of these p53-inhibitory functions of MDM2 seem to be cell-type specific. Interestingly, a recent report (39) suggests that overexpression of MDM2 in the wing imaginal disc of transgenic drosophilae induces caspase activity to degrade MDM2, induces apoptosis in the wing imaginal disc, and generates either a weaker and blistered phenotype or a gnarled phenotype of the wing. No growth proliferating activity of MDM2 was detected in these cells. Because the apoptotic activity was not detected in the eye imaginal disc, it is possible that the wing cells induced degradation of ectopically expressed MDM2 and discarded MDM2-expressing cells by inducing apoptosis. However, MDM2 did not induce any growth proliferative effects.

These p53 inhibitory functions of MDM2 thus could account for MDM2-mediated oncogenesis. To inhibit p53-mediated apoptosis, MDM2 requires its NH2 terminal amino acid residues essential to inhibit p53-mediated transactivation (60). Overexpression of p53 using a retroviral expression vector carrying temperature-sensitive p53 induces apoptosis rather than growth arrest in mouse embryo fibroblast lacking mdm2 and p53. Cells deficient in p53 alone did not show this phenotype (61). This observation suggests that MDM2 prevents p53-mediated apoptosis. Also, the presence of MDM2 may be necessary for p53-mediated growth arrest. This observation is consistent with the cell-type specific nature of p53-dependent apoptosis. MDM2 can also inhibit cisplatin-mediated apoptosis, suggesting that the G1 arrest function of MDM2 may induce antiapoptotic effect (62).

MDM2 Is an E3 Ubiquitin Ligase and Can Degrade p53

MDM2 degrades p53 by targeting p53 to ubiquitination (63–66). At least three different domains of MDM2 seem to be necessary for this function. The p53 interaction domain is necessary to locate p53, the ring finger domain is required for monoubiquitination, whereas the p300 interaction domain is required for polyubiquitination (67–71). Different domains of MDM2 are shown in Fig. 1 in the review by Ganguli and Wasylyk in this issue (45). Because the p53 interaction domain of MDM2 is within the NH2 terminal 130 amino acids and the ring finger domain is toward the COOH terminus (residues 438–478), mutants of MDM2 lacking the E3 ubiquitin ligase activity efficiently bind with wild-type p53 and inhibit p53-mediated transcriptional activation (55). Therefore, inhibition of p53-mediated transactivation and degradation of p53 by MDM2 perhaps are designed to regulate p53 in two different situations.

Given that the p53-mediated transactivation should be a nuclear event and p53 degradation is a cytoplasmic event, the ubiquitin ligase function of MDM2 could be a cellular mechanism for turnover of p53-MDM2 complexes after their functions are completed and after they are exported from the nucleus. This view is consistent with the fact that the nuclear export signal of MDM2 is required for p53 degradation (72). The nuclear export signal of MDM2 is needed for MDM2-mediated degradation of p53 (72). The drug leptomycin B, which blocks formation of nuclear export complexes, prevents nuclear-cytoplasmic shuttling of MDM2 and p53, and stabilizes p53. This report is supported by the observation that p53, if sequestered in the cytoplasm, is resistant to degradation by MDM2 (73). Therefore, transport of p53 from cytoplasm to nucleus and back to cytoplasm seems to be needed for its degradation. This requirement of cytoplasmic-nuclear-cytoplasmic shuttling of MDM2 and p53 may prevent premature degradation of MDM2 or p53 before conferring their cell growth regulatory function in the nucleus. Also, MDM2 can degrade many other proteins. MDM2 promotes its own degradation (70) and degradation of mutant p53 (74) and growth suppressor p19 (50). MDM2 binds and degrades the cell-fate protein numb (75).

Interaction With the Tumor Suppressor p14/p19 Coded by ARF-INK4a Locus

Interaction of MDM2 with the growth suppressor p14/p19 has been studied in detail. The growth suppressor p14/p19 interacts with MDM2 and stabilizes p53 (48–50, 76). Because p14/p19 can interact with p53 in the absence of MDM2, and is suggested to form a trimeric complex (48, 76), the consequence of the interactions seems to be complex. However, most of the studies show that p14/p19 inhibits MDM2-mediated ubiquitination and degradation of p53 (65, 76). Two binding domains of MDM2 (residues 154–221 and residues 271–342) have been implicated in this interaction in three independent studies (48, 50). These interaction domains are dispensable for MDM2-mediated tumorigenesis. At least one of the p14 interaction domain overlaps with one (ID1) of the G1 arrest domains of MDM2 (Table 1, Fig. 1 in the review in this issue by Ganguli and Wasylyk; 45).

Interaction With the Retinoblastoma Gene Product pRb

MDM2 has been shown to enhance E2F-mediated transcriptional activation and release growth arrest of an osteogenic sarcoma cell line (U2-OS) mediated by a phosphorylation-resistant mutant of pRb (45, 77). Because MDM2 can interact with both p53 and Rb, it has been suggested that Rb can indirectly regulate the stability and apoptotic function of p53 (78). Recently, it has been reported that MDM2 interacts with transcription factor sp1 and blocks its DNA-binding activity. Tumor suppressor Rb can replace sp1 from this complex, restoring its DNA-binding activity (79).

Role of MDM2 in Growth Regulation

MDM2 Induces G0/G1 Arrest

In view of the oncogenic functions of MDM2, one would expect that overexpression of MDM2 would confer growth advantage in cultured cells. In contrast, MDM2 can only be stably expressed in cells harboring genetic defects (80), and
often for only few generations. Overexpression of MDM2 from its full-length cDNA efficiently arrests normal human diploid cells at G1. Even 2.5-fold increase in MDM2 expression over the endogenous levels induces G1 arrest in cells that are not tumor derived, whereas many cancer cells including the cells that overexpress MDM2 show poor G1 arrest after 20- to 50-fold increase in MDM2 expression over their endogenous levels (80). Thus, tumor-derived cells are rather insensitive to MDM2-mediated growth arrest.

Consistent with the growth arrest properties of MDM2, ectopic overexpression of MDM2 in the compound eyes of transgenic drosophilae showed small or rough eye phenotype with disorganization of the bristles. There was no evidence of apoptosis or cell proliferation in MDM2-overexpressing cells (39). Apart from these reports, the ring domain of MDM2 has been shown to induce growth arrest (81).

Because MDM2 induces G1 arrest, cells that overexpress MDM2 do not multiply. This property prevents any clonal selection of MDM2-expressing cells. Development of an inducible expression system is possible in cell lines that are less sensitive to MDM2-mediated G1 arrest, as most of the available inducible systems are often leaky. Therefore, in a mixed population of MDM2-overexpressing and non-overexpressing cells, the latter population gets a selective growth advantage over MDM2-expressing cells. To detect MDM2-mediated G1 arrest, it is thus essential to identify MDM2-overexpressing cells rather than checking average expression of a mixed population (82).

MDM2 harbors three growth inhibitory domains (Fig. 1 in the review by Ganguli and Wasylyk in this issue; 45). One of the growth suppressor domains of MDM2 is nonfunctional in many tumor-derived cells. This observation suggests that the tumor-derived cells that are insensitive to the normal cell-specific G1 arrest domain of MDM2 have lost a function that would sense the G1 arrest function of MDM2. The mutations that confer insensitivity to MDM2-induced G0/G1 arrest in cancer cells are not known. Because most of the tumor-derived cells are insensitive to G1 arrest mediated by this domain, it should be a pathway frequently mutated in cancer cells.

The other two growth suppressor domains (ID1 and ID2) of MDM2 function in most cell lines although less efficiently in tumor-derived cells (80). Most tumor-derived cells require higher levels of MDM2 to induce G1 arrest (80). In view of these growth suppressive effects of ID1 and ID2, MDM2 should have suppressed growth of cancer cells where it overexpresses. Mutation or deletion in the growth suppression domains of MDM2 could be an explanation. However, recent studies agree that the overexpressed MDM2 is primarily unmutated (83–85).

These observations suggest that the ID1 and/or ID2 growth arrest domains of MDM2 perhaps function by inactivating a factor frequently overexpressed in cancer cells. Depending on the endogenous level of the overexpressed MDM2 and the interacting factor, the cancer cells can disable growth arrest function of ID1 and ID2 up to a certain extent. Because overexpression of growth suppressors would inhibit multiplication of normal cells, MDM2 may indirectly select for defective cells that would evade growth suppression. Cancer cells overexpressing MDM2 may be a result of such selection (Fig. 1). Thus, identification of the mechanism of MDM2-induced G1 arrest should not only unravel novel growth regulatory pathways, but also allow us to predict the gene mutation that would disable growth arrest function of MDM2.

Apart from its inability to induce G1 arrest in genetically defective cells, MDM2 seems to harbor a dominant tumorogenic domain at its NH2 terminus (80). Therefore, MDM2 may be potentially oncogenic in a cell that is defective in sensing the G1 arrest domains of MDM2. The presence of a dominant tumorogenic domain would imply that the tumorogenic domain might have a growth proliferating function in normal cells, which is contained in the G1 phase by the built-in G1 arrest devices. Because targeted overexpression of MDM2 in breast epithelial cells of transgenic mice causes polyploidy (38), the predicted growth proliferating function of MDM2 could be initiation of DNA replication. The pre-replication complex is assembled during G1, MDM2 may induce the pre-replication factors or may help complex formation. This possibility underscores the importance of built-in mechanisms to induce G1 arrest to prevent chromosomal abnormalities. If this hypothesis were true, MDM2 would keep initiating complex formation inducing more than one round of DNA replication per cell cycle in the absence of G1 arrest. Thus, the three G1 arrest domains of MDM2 would provide three layers of protection against aneuploidy. Disruption of these G1 arrest functions would lead to aneuploidy and oncogenesis.

This hypothesis is consistent with the observation that targeted overexpression of MDM2 in breast epithelial cells of transgenic mice causes polyploidy, yet inhibits normal development of mammary gland (38). Perhaps the tumorogenic domain of MDM2 induces polyploidy in cells that disable MDM2-mediated G1 arrest, while most of the breast epithelial cells overexpressing MDM2 undergo G1 arrest. Requirement of chemical carcinogens for papilloma formation in the basal layer of epidermis after targeted overexpression of MDM2 (40) argues for this hypothesis.

This hypothesis is also consistent with the ubiquitin ligase function of MDM2 to degrade p53 and itself. This function may be necessary to prevent growth proliferating function of MDM2 when the cells exit from G1 phase. Degradation of MDM2 and p53 would ensure absence of MDM2 and thus untimely growth proliferating functions. Absence of p53 would ensure lack of MDM2 expression. Thus, the ubiquitin-mediated proteolysis induced by MDM2 is perhaps needed to maintain genomic integrity rather than inducing oncogenesis by degrading p53 in normal cells. This hypothesis is supported by the fact that the ubiquitin ligase domain is dispensable for MDM2-mediated tumorigenesis (68, 80).

Consistent with the complexity of its normal function, MDM2 has been reported to interact with a number of factors. To understand which of these interactions could be relevant for the G1 arrest or growth proliferating functions of MDM2, the
The following table (Table 1) provides a comparison of the functional domains of MDM2 along with its interaction domains. The mechanism of tumor formation could be a result of inactivation of the tumor suppressor p53 as the domain overlaps with the p53-interaction domain. One of the suggested p14/p19 interaction domains of MDM2 overlaps with one of the growth inhibitory domains (48). However, MDM2-mediated growth inhibition is probably independent of this interaction because MDM2 can induce G1 arrest in NIH3T3 cells, which have deleted p16/p19 gene (86, 87).

The two apparently opposite functions, tumorigenesis and growth arrest, of MDM2 are intriguing and suggest the presence of an as-yet unknown cell cycle regulatory mechanism. As mentioned earlier, many oncogenic challenges such as ionizing radiations, aryl hydrocarbons, or oncogenic ras induce MDM2 overexpression in a p53-dependent or -independent pathway (58, 88–93). Because MDM2 induces growth arrest in normal diploid cells, the consequence of this overexpression should be G1 arrest unless the cells bear mutation to disable the growth inhibitory domains of MDM2. Thus, the cell cycle inhibitory function of MDM2 could be a protective mechanism of normal cells to prevent untimely growth proliferation normally or in response to abnormal tumorigenic signals. This normal growth arrest function of MDM2 is disabled in genetically damaged cells to an extent that the regulatory molecule turns into an oncoprotein generating further chromosomal abnormalities and oncogenesis (Fig. 1).

It is well known that oncogenesis is a result of multiple genetic damages (2). The sophisticated and often redundant network of gene regulation in higher eukaryotes is equipped to

<table>
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<td>l.5</td>
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FIGURE 1. Consequences of MDM2 overexpression in response to oncogenic challenges in normal cells and in cells defective in sensing the growth inhibitory domains of MDM2. Cells defective in sensing the growth inhibitory domains (normal cell-specific ID, ID1, and ID2) of MDM2 acquire a selective growth advantage.
handle errors. Because MDM2 has three G1 arrest domains, it may take at least two and perhaps three errors to turn the growth regulatory molecule into an oncoprotein. Yet, this highly improbable event is fairly frequent in cancer patients, because the errors enable the damaged cell to evade the regulatory mechanisms and confer selective growth advantage.

This phenomenon is not unique for MDM2. In recent years, several oncoproteins have been shown to possess growth inhibition function. Activated Ras induces senescence, and cells with defects in p53 and p16 pathway disable this function (93, 94). Raf-1 induces growth arrest that is overcome in immortal cells (95). The cell death inhibitor Bcl-2 and its homologues show cell growth inhibitory properties (96). These observations suggest that the cancer cells that overexpress these oncoproteins must have acquired genetic damages to evade the growth arrest function of the overexpressed oncoproteins. Also, cancer cells often disfunction of known growth suppressors such as Rb. Rb cannot induce growth suppression in C33a (cervical carcinoma), U2-OS (osteosarcoma), or SW13 (adenocarcinoma) cells (97). Thus, MDM2 may belong to a group of proteins required for growth proliferation in normal cells that are equipped with growth suppressor activities to strictly regulate their own function.

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