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FUNCTION AND DYSFUNCTION OF THE HUMAN ONCOPROTEIN MDM2

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1. ABSTRACT

The protein MDM2 coded by the human homologue of mouse double minute-2 (*mdm2*) gene frequently overexpresses in malignant human breast and other tumors. Artificial amplification of mouse *mdm2* gene derived from a transformed murine cell line enhances tumorigenic potential of murine cells. These evidences suggest oncogenic properties of human or mouse MDM2. The tumorigenic property of MDM2 is not unexpected as MDM2 can inactivate several functions of the tumor suppressor p53. The protein also interacts with several cell cycle regulatory proteins that may contribute to its tumorigenic ability. Several spliced forms of MDM2 have been detected in cells that overexpress MDM2. The function of the proteins coded by these spliced forms is not well understood. Overexpression of full-length MDM2 from its cDNA arrests G1 to S phase transition of normal human or murine cells. Elimination of the growth inhibitory domains of the oncoprotein induces tumorigenesis. Some cancer-derived cell lines are partially insensitive to MDM2-mediated growth arrest. Normal cells can induce MDM2 in response to oncogenic challenges such as UV irradiation or estrogen treatment. Normal cells may induce full-length MDM2 in response to oncogenic challenges to protect against premature cell cycle progression. If the oncoprotein is defective in growth arrest or if the cells are insensitive to MDM2 mediated growth arrest, premature progression of cell cycle may lead to tumorigenesis. Elucidation of the growth regulatory functions of MDM2 may help develop new drug design for cancer treatment.

2. INTRODUCTION

One of the greatest developments in cancer research in the past few decades has been the understanding of the nature of genetic damages in cancer cells (1-3). Considering that cancer is caused by multiple genetic damages, and results in genomic instability, some of the genetic damages found in cancer cells should be the consequence of the disease, while some should be the "cause". The marathon drive in quest of this "cause" involved isolation of the genetic damages and introducing them separately or in groups in

non-transformed cells to determine if the product of the damaged gene(s) leads to deregulation of cell growth and eventually tumorigenesis. Although the precise "cause" of carcinogenesis is still unknown, today we have much better understanding of how the genes damaged or abnormally expressed in cancer cells maintain the sophisticated design of normal cell growth regulation.

The biochemical activities of MDM2 in cancer cells have been studied intensely because of its implication in human cancer. Several excellent reviews have been published on many different aspects of mouse and human MDM2 (4-9). Experimental evidences suggest that MDM2 binds and degrades several growth suppressors. However oncogenic challenges such as DNA damage that induce DNA repair induces a form of p53 incapable of interacting with MDM2 and a form of MDM2 inactive for degradation. Thus the function of DNA damage induced MDM2 in normal cells is not clear. Central to the understanding of mechanism of oncogenesis, lays the fact that cancer is caused by accumulation of genetic damages. During cell cycle, growth of cells with genetic abnormalities should be prevented by checkpoint regulation. Thus deregulated cell growth observed in cancer cells must evade the checkpoint regulatory pathways The consequence of chance overexpression of MDM2 in otherwise normal cells is also not clear. This review is focused to discuss the function of MDM2 in normal cells and its role in oncogenesis.

3. MDM2 IS POTENTIALLY ONCOGENIC

3.1. MDM2 overexpresses in tumor cells

The human homologue of the mdm2 gene is frequently overexpressed in many human breast tumors and carcinomas (10-15), soft tissue sarcomas, and other cancers (16-25), suggesting that the genetic alteration may 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 prognosis (11, 26). Amplification of the mdm2 gene leading to overexpression of mdm2 messenger RNA (13, 19, 27-29), and enhanced translation of mRNA has been proposed as mechanisms of MDM2 overexpression (20, 30). Amplification of mdm2 gene is frequent in cancer cells with wild-type p53 (19, 27). Since the oncoprotein can inactivate p53-mediated transcriptional activation, MDM2 is thought induce oncogenesis by inactivating the tumor suppressor p53 (19, 27). Later studies reveal alteration in the expression of both p53 and MDM2 genes ((16). The oncoprotein overexpresses in 41% of benign and 68% of malignant lesions of human breast (10) and is strongly related with the presence of estrogen receptor (10, 14, 15). Elevated expression of estrogen receptor in ER- cells 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 (31). As MDM2 inhibits p53-mediated transcriptional inhibition 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 (32). Since 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 cells

3.2. Amplification Of MDM2 Gene Enhances Tumorigenic Potential Of Murine Cells

The mouse double minute-2 (mdm2) gene was identified as an amplified and overexpressed gene in a spontaneously transformed derivative of mouse BALB/c cell line 3T3DM (28). The protein is coded in the second reading frame of the double minute chromosome. Introduction and amplification of a cosmid bearing the entire mdm2 gene in NIH3T3 cells caused enhanced tumorigenic potential of NIH3T3 cells (28). The cosmid bearing the mdm2 gene also transformed primary rat embryo fibroblasts in the presence of ras oncogene (33). These findings suggest that MDM2 has oncogenic function.

Ectopic expression of MDM2 using a retroviral expression vector was reported to rescue transforming growth factor β (TGF β) induced growth arrest 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 shows TGF β resistance (34). However a later report suggests that MDM2, expressed conditionally cannot overcome TGF ß-mediated growth arrest (35).

3.3. Targeted overexpression of MDM2 in transgenic mice may potentially cause polyploidy

Since most tumor-derived cells harbor multiple genetic defects, targeted expression of MDM2 in transgenic mice have been more 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. A significant percentage of cells were arrested in the S phase, and induced multiple rounds of DNA replication without cell division resulting in polyploidy. Some of these transgenic mice showed tumor formation later in life (36). These observations strongly suggest that overexpression of MDM2 although do not induce direct cell proliferation, can induce multiple rounds of DNA replication. These cells, as a result, may become prone to tumorigenesis with further accumulation of genetic damages. Targeted overexpression of MDM2 in the basal layer of epidermis increases papilloma formation by chemical carcinogens (37). This finding also suggests the requirement of added genetic damage for MDM2 induced tumorigenesis. Generation of transgenic mice in the

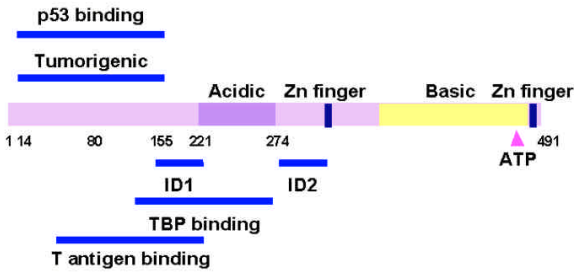

Figure 1. Functional domains of MDM2. The acidic, basic, Zn-finger and ATP-binding domains have been predicted by computer analysis. The rest of the functional domains are according to our studies (46,58,60,94). Growth inhibitory domain 1 and 2 are shown as ID1 and ID2 respectively.

presence or absence of functional p53 demonstrated a p53-independent role of MDM2 in tumorigenesis (38).

4. SPLICE VARIANTS OF MDM2

The mdm2 gene is evolutionarily conserved among eukaryotes (28). At least five to seven MDM2-related polypeptides have been found in cultured mouse (39, 40) or human (14, 41) cells that overexpress the protein. Two (p90, the full length protein and p58, which harbors a deletion at the C-terminus) of these five forms are capable of binding p53. UV irradiation of cells induces multiple MDM2 proteins including the forms that can and cannot bind the tumor suppressor p53 (42). The presence of MDM2-related polypeptides that cannot bind p53 suggests p53-independent biochemical function(s) of MDM2. Several splice variants of MDM2 mRNA have been detected in brain, ovarian and bladder carcinomas along with the full-length cDNA (43-45). These forms have been isolated and cloned. Many of these forms show large deletions in the MDM2 reading frame. Some of the splice variants show shift in the reading frame. These forms may potentially code for MDM2 with inactive C-terminal domains. It is not clear whether these mRNA are translated in the cancer or normal cells or what their comparative levels are. Interestingly most of the splice variants of MDM2 mRNA reported in cancer cells have deleted or mutated growth inhibitory domains of MDM2 (46).

5. MDM2 FAMILY OF PROTEINS

Presence of a protein, structurally related to MDM2 has been identified and has been described as MDMX. The p53-binding domain and the C-terminal metal binding domain of MDM2 are conserved in MDMX (47, 48). MDMX interacts with p53 and inhibits p53-mediated transactivation, although the protein is not induced by DNA damage or cannot compensate for the lack of MDM2 during embryogenesis (49). MDM2 can bind with MDMX through its ring finger domain (50, 51). This interaction stabilizes MDM2. MDMX expresses in all tissues, particularly in growing and transformed cells (52). Interestingly, transformed cells expresses a short form of MDMX transcript with an internal 68 base pair deletion generating a shift in the reading frame after codon 114 and a stop signal at codon 127. The tumorigenic domain of MDM2 maps to a homologous domain of MDMX-short form (46).

6. LOCATION

MDM2 is a nuclear phosphoprotein (7, 9). The protein has two presumptive nuclear localization signals (43). However, deletion of both nuclear signals does not abolish nuclear localization of the protein (46). It is not clear whether MDM2 uses redundant pathways to enter nucleus. Human and mouse MDM2 also harbors a nuclear export signal (53). Absence of the nuclear export signal captures MDM2 in the nucleus. It has been proposed that the nuclear export signal of MDM2 regulates its ability to degrade p53 (54). Thus MDM2 shows a dynamic role in different intracellular organelle.

7. FUNCTIONS AND FUNCTIONAL DOMAINS

7.1. Interaction with the tumor suppressor p53

Among all cellular and viral proteins that MDM2 interacts with its association with p53 is considered to be the most important. The mdm2 gene product was originally detected in a complex with the tumor suppressor p53 (55). MDM2 recognizes the transactivation domain of p53 and inactivates p53-mediated transcriptional activation (5, 7, 9, 56). The p53-inactivating function of MDM2 argues for the hypothesis that MDM2 induces tumorigenesis by inactivating the tumor suppressor p53 (19, 27). Consistent with this hypothesis, amplification of MDM2 gene is more frequent in cancer cells with wild-type p53 than in cells with mutant or deleted p53 (9, 16, 45, 57).

Our laboratory as well as others have shown that human or mouse MDM2 can interact with the tumor suppressor p53 in cell free systems (58, 59) or in the whole cell (39, 58, 60, 61) and inhibit transactivation by wild-type p53. Work from our laboratory showed that the interaction of MDM2 with p53 is needed for inhibition of p53-mediated transactivation (58) and only 127 amino acids (amino acids 28 to 154) of MDM2, out of a total of 491, are sufficient for this interaction (60) (Figure 1). The crystal structure of a complex formed between 109 N-terminal residues of MDM2 and 15-residue transactivation domain of p53 has been analyzed (62). 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 (63-65). These p53-inhibitory functions of MDM2 thus could account for MDM2-mediated oncogenesis. To inhibit p53-mediated apoptosis, MDM2 requires its N-terminal amino acid residues essential to inhibit p53-mediated transactivation. The ability of MDM2 to inhibit p53-mediated growth suppression seems to be cell-type specific (65). This observation is consistent with the cell-type specific nature of p53-dependent apoptosis. MDM2 can also inhibit cisplatin-mediated apoptosis (66).

The p53-regulatory function of MDM2 is required for embryonic development (67, 68). It was shown in two independent studies that mdm2-null mice die early during embryogenesis, whereas mice deficient for both p53 and mdm2 develop normally. This observation argues that p53-inhibitory role of MDM2 is critical for embryogenesis. 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 suggesting that apoptosis inhibitory role of MDM2 is indispensable during embryogenesis (69).

7.2. The autoregulatory feedback loop

Wild-type p53 induces MDM2 expression by recognizing a response element situated downstream of the first exon of the oncogene (70-72). The p53-inducible promoter (P2) generates a transcript that lacks the first exon and a few nucleotides of second exon. Another upstream constitutive promoter (P1) that can generate the full transcript is minimally affected by p53. Since the first in-frame translation initiation codon of MDM2 is located within exon-3, both the transcripts should generate the same protein. However in vitro studies show that the P1-transcript prefers translation from an internal initiation site generating a protein that cannot interact with p53. The P2 - transcript can generate both the full-length and the shorter protein. Analysis of MDM2 RNA in transformed 3T3DM cells that overexpress MDM2, on the other hand, shows that the cell line almost exclusively contains the full-length transcripts and proteins that are capable and incapable of binding p53. Presence of multiple promoters, various transcripts generating functionally differing translation products of MDM2 suggest that the protein expression is regulated by multiple factors at the level of transcription as well as translation.

Several laboratories (64, 73-75) have shown that ionizing irradiation induces MDM2 expression in a p53-dependent manner. Overexpression of the oncoprotein (human or mouse MDM2) reduces the ability of the tumor-derived RKO or U2-OS cells to arrest in the G1 phase following p53-induction (63, 64). These observations suggest the presence of an autoregulatory feedback loop in which a higher level of p53 expression will arrest the cell cycle at the G1 phase and, at the same time, will induce MDM2 expression, which will in turn inactivate p53 (76). UV irradiation also induces p53-nonbinding forms of MDM2 (42). It has also been reported that DNA damaging agents including γ- and UV-irradiation induce phosphorylation of p53 at Serine 15 and 37. This phosphorylation alleviates inhibition of p53 by MDM2 (77). Recently ATM-dependent phosphorylation of MDM2 has been demonstrated (78). ATM phosphorylates MDM2 on serine 395 prior to accumulation of p53 (79). This phosphorylation may stabilize p53 by preventing its degradation. These findings argue against the presence of an autoregulatory feedback loop during γ- and UV-irradiation.

7.3. MDM2 is an E3 ubiquitin ligase and can degrade p53

MDM2 degrades p53 by targeting p53 to ubiquitination (80-83). Although interaction of p53 with MDM2 is needed for this degradation, the ring finger domain of MDM2 seems to be essential for its ubiquitin ligase activity (84, 85). Since the p53-interaction domain of MDM2 is within the N-terminal 130 amino acids and the ring finger domain is towards the C-terminus (438-478), mutants of MDM2 lacking the E3 ubiquitin ligase activity would efficiently bind with wild-type p53 and inhibit p53-mediated transcriptional activation (60). This “survivors will be prosecuted” attitude of MDM2 towards p53 could be a cellular mechanism for turnover of p53-MDM2 complexes. This view is also consistent with the facts that MDM2 can degrade many other proteins. MDM2 promotes its own degradation (86), degradation of mutant p53 (87) and the growth suppressor p19 (88). MDM2 binds and degrades the cell-fate protein “numb” (89).

The nuclear export signal of MDM2 is needed for MDM2-mediated degradation of p53 (90). The drug leptomycin B that 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 (91). This requirement of 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. Degradation of MDM2 is regulated by SUMO-1 modification. Sumoylation prevents self-ubiquitination of MDM2 and increases p53-degradation (86).

7.4. MDM2 interacts with p53 mutants

MDM2 interacts with the tumor-derived mutants of p53 and inhibits mutant p53-mediated transcriptional activation (92, 93). This interaction involves two domains of both proteins (92). The two mutant p53-binding domains of MDM2 are able to function independent of each other. Interaction with either of the domains is sufficient for inhibition of

mutant p53-mediated transactivation. One of these domains is the same as the wild-type p53 interaction domain of MDM2, whereas a second domain is situated within amino acid 190 and 276 residues and is specific for mutant p53 (Figure 1). The transactivation and the oligomerization domains of mutant p53 are dispensable for its interaction with MDM2. Thus, both MDM2 and p53 recognize each other through unique domains. These observations suggest that forms of MDM2 incapable of interacting with the wild-type p53, and are often expressed in transformed cells, would inhibit mutant p53-mediated transactivation and antagonize the tumorigenic function of mutant p53. This inhibitory function of MDM2 may account for infrequent co-occurrence of p53 mutation and MDM2 overexpression in cancer cells.

7.5. MDM2 associates with cellular and viral proteins

MDM2 can bind with an ever-increasing number of proteins. MDM2 interacts with TATA-binding protein (94, 95) and TATA associated factors (96), SV40 T antigen (58), ribosomal L5 protein (97), the retinoblastoma gene product pRb (98), the transcription factors E2F1/DP1 (99) the p53-family protein p73 (100, 101), the transcription factor sp1 (102), cell fate protein numb (89), the growth suppressor p19 (88, 103-105) and DNA polymerase ε (106). We have shown that MDM2 interacts with the human TATA-binding protein (TBP). A domain (221-276) rich in acidic amino acids is needed for interaction with TBP (94) (Figure 1). MDM2 can act as a transcriptional inhibitor for promoters that recruit the protein (95). 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 (98, 99). Since MDM2 can interact with both p53 and Rb, it has been suggested that Rb can indirectly regulate the stability and apoptotic function of p53 (107). 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 (102).

Interaction of MDM2 with the growth suppressor p19 has been studied in detail. The growth suppressor p19 interacts with MDM2 and stabilizes p53 (88, 103, 104). Since p19 can interact with p53 in the absence of MDM2, and suggested to form a trimeric complex (105), the consequence of the interactions seems to be complex. However, most of the studies show that p19 inhibits MDM2-mediated ubiquitination and degradation of p53 (54, 83, 88, 104). Two binding domains of MDM2 (residues 154-221 and residues 271-342) have been implicated in this interaction in three independent studies (88, 104).

Three different mechanisms have been proposed for stabilization of p53. p19 have been shown to promote degradation of MDM2 resulting in p53 stabilization (88). The stabilization of p53 may also be a result of p19-mediated inhibition of ubiquitin ligase activity of MDM2 (83). It has been shown that p19 localizes MDM2 in the nucleolus stabilizing p53 (108, 109). p19 also rescues p300/CBP-mediated acetylation of p53 from inhibition by MDM2 (110). However stabilized p53 is transcriptionally active and induces MDM2 as a result (105).

7.6. Role of MDM2 in growth regulation

In view of all 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. Overexpression of MDM2 from its full-length cDNA efficiently arrests normal human diploid cells at G0/G1. Tumor-derived cells are rather insensitive to MDM2-mediated growth arrest. MDM2 harbors two growth inhibitory domains (Figure 1). Elimination of these growth inhibitory domains releases MDM2-mediated growth suppression. Deletion mutants of MDM2 that lack growth inhibitory property can induce tumor formation in NIH3T3 cells (Figure 1). Thus MDM2 harbors a potentially tumorigenic domain (46). Therefore MDM2 may be potentially oncogenic in a cell that is defective in sensing the growth inhibitory domains of MDM2, or if the overexpressed MDM2 lacks a functional growth inhibitory domain. One of the growth inhibitory domains of MDM2 overlaps with one of its suggested p19/p14 interaction domain (104). However, MDM2-mediated growth inhibition is probably independent of this interaction as MDM2 can induce G0/G1-arrest in NIH3T3 cells, which have deleted p16/p19 gene (111,112).

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. The cell cycle inhibitory function of MDM2 could be a protective mechanism of normal cells against growth proliferation in response to abnormal tumorigenic signals. It is possible that both the functions are necessary at different stages of normal cell growth and a balance of the two functions is maintained in normal cells.

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