

p53-Independent Activities of MDM2 and Their Relevance to Cancer Therapy

Zhuo Zhang and Ruiwen Zhang*

Department of Pharmacology and Toxicology, Division of Clinical Pharmacology, Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, AL 35294, USA

Abstract: The feed-back auto-regulatory loop between p53 and MDM2 has been extensively investigated. MDM2 is under the transcriptional control of p53, and MDM2 acts as a negative regulator of p53. There is increasing evidence, however, supporting the notion that MDM2 has activities independent of p53. In the absence of p53, MDM2 may retain its role in cell cycle control, differentiation, cell fate determination, DNA repair, transcription regulation, signal transduction of steroid receptors, cellular response to hypoxia, internalization of surface receptors, and other processes. MDM2 also has oncogenic transformational activities independent of p53. Moreover, anti-MDM2 antisense oligonucleotides have *in vitro* and *in vivo* antitumor activity and chemosensitizing and radiosensitizing effects in several human cancer models, regardless of their p53 status. In this article, the p53 independent activities of MDM2 and its interactions with various cellular proteins are considered. The studies reviewed provide a basis for developing novel MDM2 inhibitors as a therapy against human malignancies.

INTRODUCTION

MDM2 was initially identified as a protein overexpressed in the murine 3T3DM cell line, and was found to be tumorigenic when the NIH3T3 cells harboring amplified MDM2 were injected into nude mice [1]. Overexpression of the MDM2 protein occurs in many human tumors including sarcomas and cancers of brain, breast, ovarian, cervical, lung, colon, and prostate [2]. Moreover, transgenic mice with overexpression of MDM2 are predisposed to spontaneous tumor formation and demonstrate both the p53-dependent and -independent tumorigenicity of MDM2 [3]. In human cancers, overexpression of MDM2 correlates with a poor prognosis.

The human MDM2 gene is localized to chromosome 12q13-15 [4]. Like the mouse MDM2 gene, the human gene also contains an intronic p53-dependent promoter (P2) [5], which is highly conserved between mouse and human. The P2 structure has two tandem p53 binding elements, and promoter activation requires simultaneous binding of p53 to both of them. This mechanism may prevent premature triggering of the promoter by p53 [4]. The murine full-length MDM2 protein contains 490 amino acids (the human protein contains 491), and migrates as a 90-95 kDa band in SDS-denaturing gel electrophoresis. The MDM2 protein contains a p53 binding domain, a nuclear localization signal (NLS), a nuclear export signal (NES), a central acidic domain, and a C-terminal zinc-finger and RING finger domain [2]. Fig. (1) shows a map of these domains. The major function of MDM2 is believed to be negative regulation of tumor suppressor p53.

The *TP53* gene product, p53, was identified from SV40 transformed rodent cells [6], and murine and human cDNA clones were isolated in the early 1980's [7]. Early studies

demonstrated that p53 was a tumor suppressor [8]. In a wide spectrum of human malignancies, both alleles of the p53 gene were mutated or deleted [9]. Many environmental insults and cancer treatments, including γ -irradiation and chemotherapy, increase the level of p53, leading to G1 arrest or apoptosis [6, 10, 11]. Modulating the p53 induction of cell cycle arrest and apoptosis may lead to the sensitization of tumor cells to DNA damaging treatments like chemotherapy and radiation [3, 12].

A major advancement in understanding the p53 pathway was the discovery of MDM2 and its role in controlling p53 levels. Many reports document the MDM2-p53 autoregulatory loop. MDM2 and p53 regulate each other, and expression of MDM2 is controlled *via* a p53-responsive promoter (P2 promoter). In many studies, the p53-dependence of MDM2 expression is evident and is seen in response to various stress signals, including DNA damage from radiation and cytotoxic agents [13]. MDM2 acts as a negative regulator of p53 by binding to the protein, masking its transcription domain, and keeping it functionally inactive [14]. MDM2 binds p53 through a binding domain contained within its N-terminal region and shuttles the p53 protein out of the nucleus and into the cytoplasm, where it is degraded by the proteasome [15-17]. This degradation is brought about through p53 ubiquitination by the E3 ligase activity of the MDM2 RING-finger domain, which has been mapped to the C-terminal [18]. In cell culture studies, overexpression of MDM2 eliminated the capacity of p53 to induce cell cycle arrest and apoptosis [19], stressing the importance of the p53-MDM2 auto-regulatory loop. Furthermore, knocking out the MDM2 gene in mice led to embryonic lethality that was rescued by deletion of the p53 gene [20]. This observation supports the idea that regulation of p53 is an important function of MDM2.

*Address correspondence to this author at the Department of Pharmacology and Toxicology, University of Alabama at Birmingham, VH 113, 1670 University Blvd., Birmingham, AL 35294, USA; Tel: (205) 934-8558; Fax: (205) 975-9330; E-mail: ruiwen.zhang@ccc.uab.edu

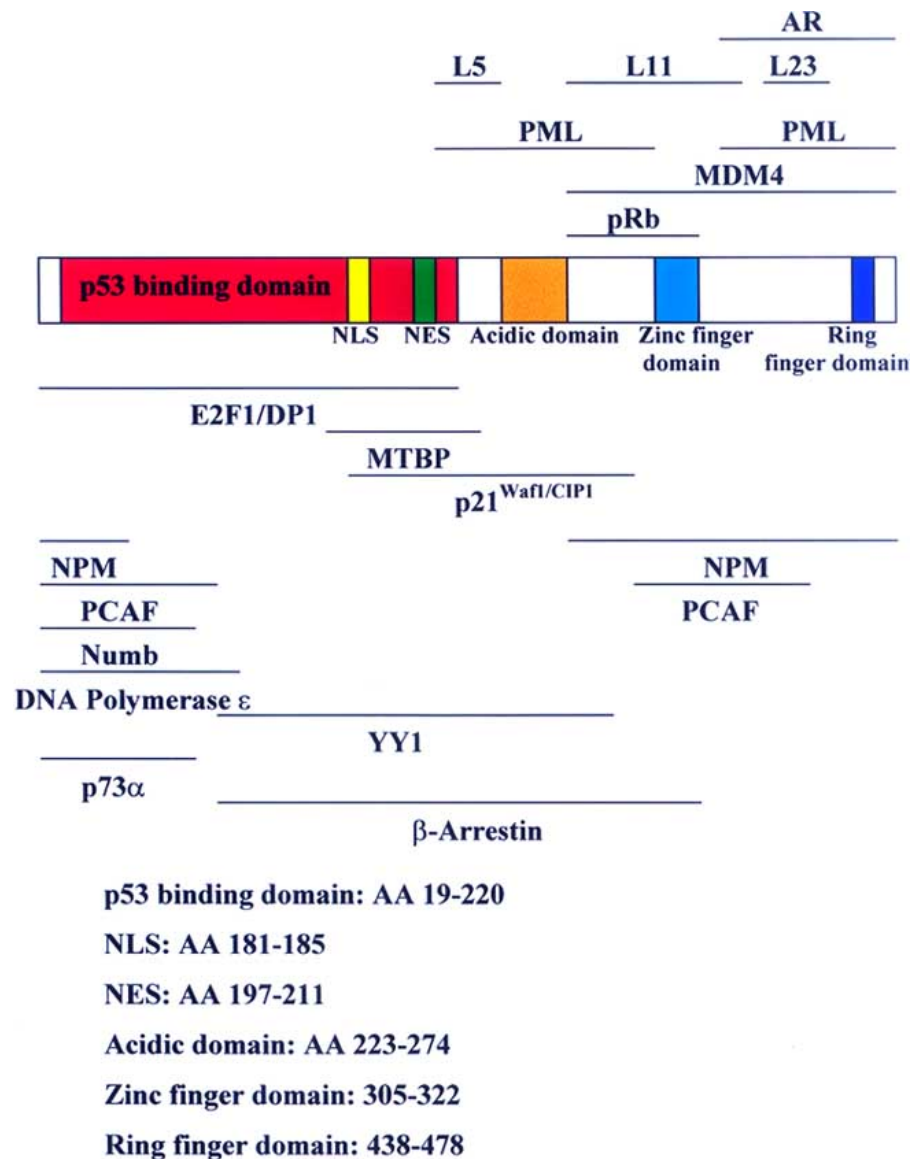


Fig. (1). MDM2 interacting proteins and the binding sites on MDM2.

EVIDENCE FOR THE P53-INDEPENDENT ACTIVITIES OF MDM2

Evidence from *in vitro* Investigations

While the p53-dependent activities of MDM2 are important for a number of cellular processes, MDM2 has p53-independent functions as well. For example, rhabdomyosarcoma cell lines have a recessive or dominant nondifferentiating phenotype, and amplification of MDM2, which interferes with the activities of MyoD, inhibits overt muscle cell differentiation when transferred into C2C12 myoblasts [21]. Whether the cell differentiates or not is decided by the complex interaction of sets of tissue-specific and ubiquitously expressed transcription factors and cell cycle regulatory proteins. The lack of differentiation is a general phenotype of cancer cells. The inhibition of MyoD function is removed by the expression of pRb or Sp1. Recently, the cyclic regulation of MDM2, Sp1, and pRb was demonstrated [22]. MDM2 binds to Sp1 and inhibits Sp1-dependent transcription, which is reversed by pRb displacing

MDM2 from Sp1. These studies indicate that MDM2 has activity in modulating normal muscle cell differentiation, independent of p53.

MDM2 also confers a growth advantage to cells without p53 and pRb, overcoming the G1-cell cycle arrest induced by p107 [23]. The p107 binding region maps to the p53 binding domain, and transfection of p53 inhibits the transformation induced by MDM2. Therefore, p53 also serves as an inhibitor of MDM2, which is under the transcriptional control of p53. During tumorigenesis, mutated p53 results in the unmasking of the transformational activities of MDM2, which promotes tumor formation and progression. Another line of evidence comes from a recent study which found that expression of mutant MDM2 without E3 ligase function did not reactivate p53 but promoted MCF7 cell growth through p53-independent mechanisms [24].

Apart from its oncogenic functions, overexpression of MDM2 causes cell cycle arrest in normal cells, and can be

stably expressed only for a few generations in cells with genetic defects [25, 26]. There are three growth-arrest domains on MDM2, and cancer cell lines are resistant to the arrest induced by MDM2, suggesting that inactivation of cell-cycle arrest might contribute to tumorigenesis. Therefore, MDM2 may have cell cycle arrest activities independent of p53.

Evidence from Investigations with Animal Models

Several lines of evidence supporting the existence of p53-independent activities of MDM2 have come from studies involving transgenic animals. Overexpression of MDM2 inhibits differentiation in a p53-independent manner when it is expressed in the differentiating compartment in the epidermis, but does not predispose the tissue to carcinogenesis [27]. When MDM2 is under the control of the K14 promoter targeted to the basal layer, however, mice develop hyperplastic lesions that progress to papillomas and squamous cell carcinomas [28]. In contrast, targeted expression of MDM2 to mammary tissue in mice inhibits the development of the mammary gland and uncouples the S phase from mitosis through a p53-independent mechanism [29]. Transgenic mice with the entire MDM2 gene and a p53^{-/-} background are predisposed to spontaneous tumor formation and have a high incidence of lymphoma and sarcoma [30]. MDM2 overexpression in mice with a p14^{Arf}^{-/-} background display accelerated tumorigenesis, suggesting either that MDM2 promotes tumor formation in ways independent of its facilitating p53 degradation, or that MDM2 attenuates p14-independent pathways of p53 stability [31].

Splice Variants of MDM2

In 1999 Pinkas and colleagues identified three MDM2 mRNA transcripts of 6.7, 4.7 and 1.9 kb in breast cancer cells [32], and Bueso-Ramos and colleagues reported several truncated MDM2 protein isoforms of 85, 76 and 57 kDa with full-length 90 kDa protein in a panel of human breast cancers [33]. To date, more than forty alternative and aberrant splicing variants of MDM2 have been detected in a wide spectrum of human cancers; most of them lack at least part of the p53 binding domain, the NLS, or the acidic domain [34, also see review by Harris in this issue]. Some of these variants transform NIH3T3 cells and are associated with high-grade and late-stage human cancer, suggesting that these variants may play a role in tumorigenesis, independent of p53 [35]. This concept is supported by the observation that MDM2-B, the most prevalent isoform identified in human cancers, causes tumorigenesis in transgenic mice [36]. Moreover, the murine equivalents of the human variants MDM2-B, -D, and -E, but not -A, facilitate lymphomagenesis in an E μ -myc transgenic mouse model [37]. These tumors are aggressive and display pathology comparable to those produced by full-length MDM2. The data suggest that at least some of the variants contribute to tumorigenesis in mouse models in a p53-independent manner. Several variants, including MDM2-B, however, can be identified in normal tissues, indicating that these variants do not always display oncogenic activities. It is important to clarify under what circumstances the variants, including MDM2-B, may be tumorigenic.

Evidence from Investigations with Human Tumors

Although the incidence of human cancers with both nonfunctional p53 and amplification of MDM2 are low, the two genetic changes do occur in some malignancies, for example, soft tissue sarcoma and bladder cancer, and are associated with poorer prognosis compared with tumors with either mutation alone [38]. The existence of amplified MDM2, without it being required for p53 feedback control, suggests that MDM2 has p53-independent functions, which may be important for tumorigenesis.

Evidence from Experimental Therapeutic Applications

Recently, our laboratory developed second-generation antisense oligonucleotides specifically targeting human MDM2. In a wide spectrum of human cancer models, the oligonucleotides have antitumor activity and sensitize tumors to the effects of classical cytotoxic therapies both *in vitro* and *in vivo* [39]. The effectiveness of the MDM2 antisense oligonucleotide in human cancers with both wild type and nonfunctional p53 shows the p53-independent tumorigenicity of MDM2. Data from a different group using the same oligonucleotides indicates that inhibition of MDM2 has a synergistic effect with the specific EGFR inhibitor, gefitinib, on the destruction of hormone-independent prostate cancer, which has nonfunctional p53, further supporting our hypothesis [40].

MDM2 INTERACTS WITH MANY PROTEINS OTHER THAN P53

pRb Family Members

In support of its p53-independent functions, MDM2 interacts with a number of other proteins. One of these, pRb, is a tumor suppressor that, when activated, induces cell cycle arrest and cell death. MDM2 interacts physically with pRb and functionally modulates its inhibitory effects on cell growth [41]. MDM2 binds to pRb *in vivo*, and eliminates the G1 arrest induced by pRb. pRb in turn modulates the functions of MDM2 by forming an *in vivo* complex with it. The tertiary complex of MDM2, pRb and p53 inhibits the degradation of p53, and consequently rescues its pro-apoptotic functions [42]. Binding of pRb with MDM2 also regulates the activity of transcription factor Sp1 [22]. MDM2 binds to Sp1 and inhibits Sp1-dependent transcription, which is reversed by pRb displacing MDM2 from Sp1. MDM2 also confers a growth advantage to cells without p53 and pRb, and overcomes the G1-cell cycle arrest induced by p107 [23].

E2F1/DP1

There is physical binding between MDM2 and E2F1/DP1, a transcription factor promoting G1/S phase transition [43]. The binding of MDM2 to E2F1/DP1 stimulates the activities of the E2F-specific promoter and increases DNA synthesis. E2F1 induces apoptosis, but MDM2 promotes the degradation of E2F1/DP1, thus overcoming apoptosis [44]. Collectively, these data indicate that MDM2 has tumorigenic properties independent of p53. In contrast, other data show that the effects of MDM2 on E2F1 activities depend on p53. E2F1 is induced after DNA

damage in a p53-dependent manner [45], and MDM2 inhibits E2F1-induced apoptosis by inhibition of p53 [46]. In a separate study, overexpression of MDM2 stimulated E2F1 transactivation in a p53-dependent manner [47]. The increased activities of E2F1/DP1 seem to result from the p53-dependent induction of p21, which inhibits the activities of the cyclin-CDK complex. Therefore, the p53-dependent and -independent mechanisms may vary depending on the different cell background and p53 expression level.

MDM4

MDM4 (MDMX), identified in 1996 as a p53-binding protein, is structurally and functionally related to MDM2 [48]. Like MDM2, MDM4 has a p53-binding domain in the N-terminus and a central zinc-finger domain and RING-finger domain in the C-terminus [49]. The embryonic lethality of MDM4 knockout mice can be prevented by p53 deletion, suggesting that MDM4 may also play a role as another negative regulator of p53 [50, 51]. Recent *in vitro* studies suggested that the balance between MDM2 and MDM4 determines the amount of degradation of p53 by MDM2. In non-stressed cells, MDM4 forms a heterocomplex with MDM2, which is stabilized by the binding [52]. Under conditions of stress, however, high levels of MDM4 antagonize the MDM2-dependent degradation of p53 [53, 54]. Although the most recent studies related to MDM4 have focused on its effects on p53, it is possible that MDM4 modulates the p53-independent activities of MDM2.

TGF- β 1

During the early steps of carcinogenesis, TGF- β 1 has a negative regulatory role on cell growth and tumor progression. Resistance to cell cycle arrest induced by TGF- β 1 correlates with tumorigenesis. MDM2 indirectly rescues the sensitivity of mink lung epithelial cells to TGF- β 1. The effects are mediated through MDM2 promotion of the activities of E2F1/DP1, independent of p53 [55].

Another study demonstrates that MDM2 inhibits the transactivation activity of SMAD4 [56]. Nevertheless, increased expression of MDM2 is associated with resistance of human breast tumor cells to TGF- β 1 [57]. Thus, the level and timing of MDM2 expression may be vital to its function.

MTBP

MTBP (MDM2 binding protein) binds to MDM2, as determined by two-hybrid screens. MTBP induces G1 arrest and inhibits cell proliferation in U2OS cells with wild-type p53 and in H1299 cells without p53. These effects were reversed by MDM2 [58]. Further studies are needed to clarify the underlying mechanisms and physiological significance of the MTBP-MDM2 interaction.

PML

The *PML* gene product is a tumor suppressor involved in the tumorigenesis of acute promyelocytic leukemia; it localizes to multi-protein sub-nuclear structures, PML-NBs [59]. The binding of PML (promyelocytic leukemia protein) to MDM2 stabilizes the p53 protein by sequestering MDM2

in the nucleus [60]. PML binds to MDM2 directly *in vitro* and promotes MDM2 accumulation through the inhibition of MDM2 self-ubiquitination. Moreover, there is *in vivo* binding between PML and MDM2, and this binding facilitates the translocation of PML from the nucleus to the cytoplasm [61]. Additionally, the transcriptional activity of a GAL4-CBP protein stimulated by PML is inhibited by MDM2. Further studies of the consequences of MDM2-PML interactions in carcinogenesis are warranted.

p21Waf1/CIP1

Waf1/CIP1 is a p53 target gene, and its product mediates p53-dependent cell cycle arrest and senescence. A role for p21WAF1 in p53-mediated tumor suppression was supported by the capacity of p21WAF1 to block tumor cell growth *in vitro* and *in vivo* [62]. Although initial data on p21 null mice suggested that the loss of p21 did not increase spontaneous tumor frequency [63], a recent study evaluating tumor formation found that p21-null mice do demonstrate an increased incidence of tumor formation when evaluated over two years [64]. In addition to the transcriptional control by p53 and other transcription factors, post-translational turnover is also important for its regulation. Recently, we have found that MDM2 acts as a direct negative regulator of p21 protein, independent of p53, by binding to it and promoting its proteasomal degradation [65]. MDM2 facilitates p21 protein recognition by the proteasome C8 subunit directly, without the prerequisite of being ubiquitinated. The consequences of these activities of MDM2 are important to its tumorigenicity in p53-null cancer cells, and p21 induction is essential for the p53-independent antitumor and sensitization effects induced by MDM2 knockdown in cancer cells. Our results are supported by another group's study, which also demonstrated that the degradative effects of MDM2 on p21 are inhibited by p14^{Arf} [66].

NPM

Nucleophosmin (NPM) is a nucleolar phosphoprotein that acts as a molecular chaperone for ribosome assembly and protein transport and is essential for centrosome duplication [67]. NPM shuttles between the nucleolus and cytoplasm in response to cytotoxic drugs and genotoxic stress [68]. Kurita and colleagues recently demonstrated the p53-independent physical binding between MDM2 and NPM *in vitro* and *in vivo*, and mapped the binding regions on MDM2 to amino acids 1-110, corresponding to the p53-binding domain, and 276-491, which includes the RING-finger domain [69]. As a result, the increased formation of the MDM2-NPM complex after UV stress induces p53 sumoylation, and thus has stabilizing effects on p53 protein. In the same study, NPM increased MDM2 expression in SaOS cells, which do not express p53. NPM interacts with multiple cellular proteins and acts as molecular chaperone, suggesting that it may function as a platform for protein interactions and modifications. It may also modulate other p53-independent activities of MDM2 in cells. Therefore, further studies regarding the interaction between NPM and MDM2 in cancer cells without p53 in the presence/absence of stress are needed.

Merlin

Merlin, the protein product of the *NF2* gene, is involved in regulation of cell growth and proliferation [70]. Germ line heterozygous *NF2* mutant mice develop a variety of aggressive malignancies with metastatic properties, suggesting that the protein is a suppressor of both tumorigenesis and metastasis [71]. Its tumor suppressor function is mediated through inhibition of MDM2-facilitated p53 degradation [71]. The binding between the N-terminal 130 amino acids and MDM2 induces MDM2 degradation, and consequently augments cell death induced by doxorubicin in lung cancer cells with wild-type p53. The possibility that this interaction also modulates the p53-independent activities of MDM2, however, cannot be excluded. In the same study, the cancer cell line H460 (p53-negative) also showed an increased response to doxorubicin after transfection with merlin, compared to an empty control vector.

PCAF

The p300/CREB-binding protein-associated factor (PCAF) is a coactivator that enhances the activity of numerous other activators and proteins involved in transcription. It can function alone or together with p300 CBP coactivators, depending on target promoters [72]. PCAF has an intrinsic histone acetyltransferase that acetylates p53 at lysine 320 after DNA damage, leading to enhanced p53 activity [73]. Based on their initial observation that MDM2 interacts with PCAF and inhibits its acetyltransferase activities, Jin and colleagues found that MDM2 promoted PCAF ubiquitination through its E3 ligase activity [74]. They also observed that ubiquitination of PCAF occurs in the nucleus, because mutant MDM2 lacking the NLS loses this activity. Given the fact that PCAF can form large complexes with more than 20 different proteins to modulate their functions, it is not surprising that MDM2 regulates the expression of genes related to the PCAF-associated complexes through decreasing the PCAF level or facilitating its ubiquitination, even while it is in the protein complex. Moreover, since PCAF is a tissue- and cell-specific protein, the regulation of PCAF by MDM2 may also be tissue- and cell-specific, and depend on certain pathological and physiological conditions. It would be interesting to determine the changes in the PCAF-protein interactions and gene expression during tumorigenesis and progression.

Tip60

Tip60, a histone acetyltransferase (HAT), was identified as a cellular protein that interacts with the HIV Tat protein and is involved in DNA repair and apoptosis after DNA damage [75]. Tip60 can regulate gene transcription positively or negatively, depending on the cell type and the promoter. Tip60 also acts as the HAT subunit of a large complex that readily acetylates histone H4 and H2A assembled in nucleosomes. Tip60 is another ubiquitination target of the MDM2 E3 ligase [76]. Legube and colleagues observed physical binding between Tip60 and MDM2, which is required for the ubiquitination of Tip60 *in vitro* and *in vivo*. The E3 ligase activity of MDM2 is essential for

Tip60 degradation, but the process does not require the presence of p53. These data thus further support the p53-independent tumorigenicity of MDM2, because Tip60 is important for the response to DNA damage. Therefore, it is possible that during the process of tumorigenesis certain transformed cells evade the Tip60-induced apoptosis by overexpressing MDM2.

Ribosomal Proteins

MDM2, which has a NLS, resides in the nucleus, where ribosomes are biosynthesized and assembled. The interactions of ribosomal proteins L5, L11 and L23 with MDM2 [77-79] suggest that it plays a role in the regulation of ribosome assembly or transport, or in RNA synthesis. The physical binding of L5/L11/L23 inhibits the ubiquitination and degradation of p53 induced by MDM2, which binds to these ribosome proteins by its central part: aa 221-274 for L5, aa 284-374 for L11 and aa 384-425 for L23. Because it consumes 80% of the energy in proliferating cells, ribosome assembly is tightly regulated and readily responds to environmental and intracellular stresses [79]. L5, L11 and L23 form complexes with MDM2, independent of the 80S ribosome and polysome, suggesting that inhibition of ribosome biogenesis, which results in the release of these proteins, may alleviate the negative regulation of p53 by MDM2. Releasing the proteins consequently stabilizes p53, which induces cell cycle arrest. Increasingly, the data demonstrate that alterations of ribosome biogenesis may lead to tumorigenesis, and overexpression of ribosome proteins induces cell cycle arrest. Given the p53-independent tumorigenicity of MDM2, it would be useful to determine whether the disturbance of ribosome biogenesis would have consequences in p53-mutant cancer cells.

Numb

Numb was discovered and cloned through use of a yeast two-hybrid system searching for MDM2 binding factors [80]. Numb acts as an antagonist for Notch signaling and is involved in differentiation of neural cells and determination of cell fate. MDM2 binds to Numb by its N-terminal domain, which corresponds to its p53 binding region, and promotes its ubiquitination [81]. The E3 ubiquitin ligase of MDM2 is necessary for the *in vivo* degradation of Numb. Based on the important role of Numb in cellular differentiation, the degradation-promoting activity of MDM2 likely contributes to its p53-independent oncogenicity.

DNA Polymerase ϵ

There is convincing evidence supporting the physical binding of MDM2 and DNA polymerase ϵ [82]. The essential binding region on MDM2 is between aa 1 and 166, and on the C-terminal domain of DNA polymerase ϵ [83]. MDM2 expressed and purified from *E. coli* or insect cells stimulates the activity of DNA polymerase ϵ *in vitro*. In view of the function of DNA polymerase ϵ in DNA repair, recombination, replication and remodeling, it is conceivable that MDM2 may mediate a configuration process responding to DNA damage that promotes DNA polymerase association with repair/recombination proteins.

TSG101

Tumor susceptibility gene 101 (TSG101) encodes a multidomain protein having functions in a variety of biological processes such as protein ubiquitination, regulation of transcription, endosomal trafficking, cell proliferation and cell survival [84]. TSG101 initially was considered a tumor suppressor because its loss in fibroblasts results in transformation, and because of its capacity to form tumors in nude mice [57]. The embryonic lethality of TSG101, accompanied by p53 accumulation, is rescued by the concurrent deletion of p53, suggesting the existence of a functional loop between TSG101 and the p53 pathway [85]. *In vitro* studies have demonstrated that TSG101 modulates MDM2 protein stability and thus indirectly controls the p53 expression level [86]. TSG101 decreases MDM2 ubiquitination, and the stabilized MDM2 in turn promotes p53 and TSG101 degradation. Given the physical binding between MDM2 and TSG101, it is conceivable that this interaction may modulate the activities of MDM2, independent of p53.

In contrast to the previous data, however, Marissa and colleagues observed that the deletion of TSG101 in both mouse fibroblasts and mammary glands results in cell cycle arrest in G1 and cell death, and that the deletion of p53 or p21 reverses the cell cycle arrest, but not cell death [84]. In addition, deletion of TSG101 does not alter the negative regulation of p53 by activated MDM2 resulting from p19^{Arf} knockdown in mouse fibroblasts. Further, there were no changes in the MDM2 stability or the physical binding between MDM2 and TSG101. This discrepancy might be due to the differences in cell types, experimental conditions or cell states. Therefore, the interaction and the biological consequences of the binding between TSG101 and MDM2 need to be re-examined.

YY1

YY1 (Yin Yang 1) is a multifunctional transcription factor that acts as an activator, repressor, or initiator element binding protein [87]. MDM2 binds to YY1 using a region between aa 150-290 [88]. This binding occurs both *in vitro* and *in vivo*, and the interaction promotes the ubiquitination of p53 induced by MDM2 [88, 89]. Therefore, YY1 is an indirect negative regulator of p53 through MDM2. Blocking the expression of p53 cannot rescue the YY1 deletion phenotype (unpublished data of Y. Shi and colleagues), suggesting that YY1 fulfills multiple, complex functions. Knocking out MDM2 could have an effect on YY1-induced cell cycle arrest or cell death, particularly upon p53 deletion. Such information may provide insight regarding a mechanism for the p53-independent oncogenicity of MDM2.

IGF-1R

The insulin-like growth factor 1 receptor (IGF-1R) is overexpressed in human cancers and is important for tumor transformation, maintenance, promotion of proliferation and prevention of apoptosis [90]. A possible link between the p53 pathway and IGF-1R is suggested by the observation that p53 represses the transcription of the IGF-1R gene, and mutant p53 has the opposite activity [91]. Girnita and colleagues found that MDM2 promotes IGF-1R ubiquitination and proteasomal degradation *in vitro* and *in*

vivo through both its E3 ubiquitin ligase activity and by directly binding to IGF-1R [92]. Wild type and mutant p53 (hot spot mutation) retaining the MDM2 binding capacity can inhibit IGF-1R degradation by sequestering MDM2 in the nucleus. To increase IGF-1R expression, this post-transcription activity is exaggerated in cancer cells with high levels of p53, which in turn favors cancer cell survival and proliferation. Because these data are observed in cancer cells with high levels of p53, it would be appropriate to determine the consequences of this activity in normal cells and in cancer cells without p53.

GR/ER

Glucocorticoids and estrogens regulate a variety of physiological activities through the glucocorticoid receptor (GR) and estrogen receptor (ER), respectively. In tissues expressing both receptors, glucocorticoids and estrogens have opposing functions [93], which suggests the possibility of crosstalk between these two pathways. Estrogen agonists, but not antagonists inhibit GR transcriptional activity and GR-dependent chromatin remodeling in the breast cancer cell line MCF-7 [94]. GR protein stability is decreased after exposure of cells to estrogen, and the GR protein is targeted to proteasomal degradation by MDM2. Moreover, ER is recruited to the MDM2 promoter upon E2 stimulation. Given the important roles of the ER in breast cancer tumorigenesis and the activities of the GR in anti-tumor therapy, it is essential to understand the mechanisms of interplay between the ER and GR pathways, including the role of MDM2, especially in the 50% of tumors with non-functional p53.

AR

Upon binding to its ligand, the androgen receptor (AR) translocates to the nucleus, where it binds to an AR response element and activates target gene expression to regulate diverse cellular functions including cell growth and apoptosis [95]. The possible interaction of MDM2 with the AR was suggested by the observation that MDM2 is upregulated during the development of androgen independence [96]. More recently, it was found that MDM2 forms a complex with the AR *in vitro* and *in vivo*, which facilitates AR ubiquitination and proteasomal degradation [97]. Phosphorylation of the AR and MDM2 by Akt seems essential for its degradation. In the PC-3 prostate cancer cell line, which has no p53 expression, the transcriptional activities of the AR are decreased, and cell death induced by the AR is reversed in the presence of MDM2. These data indicate that the p53-independent oncogenic properties of MDM2 may have selective pressure on prostate cancer cells during the development of androgen independence. Human prostate cancer initially responds to androgen ablation, but then develops androgen independence, making the tumor resistant to further treatment. MDM2 may have an important role in this transition, and only cells that have developed alternative mechanisms to fulfill the functions of the AR can survive.

HIF-1

Hypoxia-inducible factor 1 (HIF-1) is a transcription complex that links hypoxia with tumor angiogenesis and

progression [98]. HIF-1, which is overexpressed in human malignancies, correlates with a poor prognosis [98]. Growth factor stimulation, overexpression of Akt, or loss of *PTEN* result in enhanced expression of MDM2 and HIF-1 [99]. Transient expression of MDM2 induces HIF-1 expression, with no observed change in the half-life of the protein. The induction of HIF-1 and MDM2 in p53^{-/-} HCT116 cells indicates that this occurs in a p53-independent manner. This effect is largely impaired in p53- and MDM2-double-deletion cancer cells, emphasizing the essential role of MDM2. Adaptation to hypoxia provides cancer cells with the capacity to form hypoxic vascular solid tumors, which are aggressive and respond poorly to therapy [100]. When MDM2 is upregulated by hypoxia, there is an increase in the metastatic efficiency of murine KHT fibrosarcoma cells [101]. Further studies of the p53-independent activities of MDM2 in cancer progression and metastasis could provide a basis for the development of novel therapies.

p73

p73, a homologue of p53, also has apoptotic activities and can activate p53 target genes [102]. MDM2 associates with p73 *in vitro* and *in vivo* [103]. The physical binding between the two involves the N-terminal domains of both proteins and does not lead to p73 degradation. Instead, MDM2 inhibits p73 transactivation by acting as a competitor for p300/CBP binding, which is necessary for p73 transactivational activity. Given the fact that p73 is rarely mutated in human cancers, further studies to examine the effects of MDM2 inhibition of p73 and the effect on cell cycle arrest and apoptosis in cancer cells lacking p53 may be instrumental for elucidating the mechanisms of p53-independent tumorigenicity and for developing novel therapies against tumors.

p300

There is evidence for physical binding between the MDM2 protein and p300 [104]. The data regarding the biological consequences of this binding are mostly related to the p53-MDM2 regulatory loop. p300, a transcriptional co-activator, is essential for MDM2 gene induction by p53, and inhibition of p300 leads to p53 stability and increased apoptosis [105]. Overexpression of p300 results in the stabilization of MDM2, perhaps due to the binding, which protects MDM2 from degradation by the proteasome [106]. Given the role that p300 plays in cellular activities, it will be important to examine the consequences of its binding to MDM2, especially in a p53-null background.

NF- κ B

MDM2 has positive effects on the expression of the p65 subunit of NF- κ B [107], which is involved in the regulation of apoptosis, including inhibiting the apoptosis of cancer cells in response to chemotherapy. Overexpression of MDM2 in leukemic bone marrow cells of BCP-ALL patients or in the EU-4 ALL cell line is associated with p65 induction and resistance to doxorubicin. These effects seem to be mediated by the binding of MDM2 to the Sp1 site. Thus, this appears to be another p53-independent function of MDM2. Further studies are needed to clarify whether this activity of MDM2 is important to its oncogenicity.

PSD-95

PSD-95 is a scaffolding protein of the postsynaptic density, which couples the NMDA- and AMPA-type glutamate receptors to signaling molecules and the neuronal cytoskeleton. PSD-95 is regulated through the proteasome pathway mediated by MDM2 [108]. MDM2 binds to PSD-95 and facilitates its ubiquitination and consequent proteasomal degradation. These data indicate that, by promoting PSD-95 degradation, MDM2 is involved in regulating AMPA receptor surface expression during synaptic plasticity.

β 2-Adrenergic Receptor and β -Arrestin

β -Arrestin is a key molecule involved in regulating the recycling of G protein-coupled receptors (GPCRs). Internalized receptors are either recycled back to the cell surface or degraded. MDM2 has inhibitory effects on the internalization of the GPCRs through direct binding to β -arrestin [109]. Moreover, MDM2 promotes β -arrestin ubiquitination through its E3 ligase activity. Therefore, MDM2 may be involved in β 2-adrenergic receptor trafficking by affecting decay of the β -arrestin protein.

CONCLUSION

In our laboratory, MDM2, a potential target for human cancer therapy, has been extensively studied by the use of MDM2-specific antisense oligonucleotides. We have observed that in a variety of human cancer models *in vitro* and *in vivo*, MDM2 knockdown results in inhibitory effects on tumor growth and progression. These anti-tumor effects and the sensitization to cytotoxic therapy are comparable in p53 wild-type and mutant/null cells, indicating that the oligonucleotides are effective against both the p53-dependent and -independent tumorigenic effects of MDM2. These data have raised several questions, such as: What are the mechanisms for the p53-independent anti-tumor effects of MDM2 knockdown? Are these effects specifically from the MDM2 knockdown? Can these effects be confirmed in other cancer models, including transgenic mice? Can these mechanisms be employed to develop novel therapies against human cancers?

There is a large body of evidence supporting MDM2 binding to a number of cellular proteins, besides p53, to target them for proteasomal degradation. Because at least 50% of human cancers harbor p53 mutations, the possibility of using MDM2 as a novel therapeutic agent is appealing. Perhaps more importantly, in light of its many p53-independent activities, MDM2 may prove to be a valuable target in tumors, regardless of the p53 status. In order to facilitate the process of developing novel MDM2 therapies, mechanistic studies will be necessary. Although the exact mechanisms and tumorigenic consequences of MDM2 are not yet clear, some studies have already been accomplished. For example, when we inhibited MDM2 expression, we initially observed p53-independent induction of p21 in human cancer cell lines and *in vivo*, accompanied by antitumor activity and chemosensitization effects. Recently, we elucidated a negative regulatory role for MDM2 in the degradation of p21 protein. MDM2 directly binds to p21 and facilitates its degradation by promoting its interaction with the proteasome. Further, because p21 knockdown cells are

resistant to the inhibition of proliferation and are unresponsive to chemotherapy, we have clarified that the MDM2-induced down-regulation of p21 is independent of p53, and is important for the anti-tumor and chemosensitization effects in p53-null prostate cancer cells. Therefore, future studies aiming to resolve the crystal structure of the binding sites between MDM2 and p21 would be helpful to develop novel small molecule inhibitors that might be useful in human cancers without p53, which are usually in advanced stages and resistant to therapy. Similarly, other mechanistic studies would be instrumental for the development of novel therapies.

In addition to the effects on p21, we have observed that MDM2 knockdown results in a shift of the balance of Bcl2 towards Bax in cancer cells without p53. In order to continue growing, cancer cells must evade the apoptosis induced by internal and external signals. During this evasion, Bax is usually inactivated. From our observations, it might be possible that, during tumorigenesis and cancer progression, cancer cells develop mechanism(s) to down regulate Bax *via* MDM2. These cells would have a growth advantage over others. This would not be surprising, considering that Bax is a substrate of the proteasome, and

MDM2 has E3 ubiquitin ligase activity that facilitates the degradation of several proteins, perhaps including Bax. Mechanistic studies to elucidate the possible mechanisms of MDM2 down-regulation of Bax would be relevant for developing new therapies.

It has also been noted that MDM2 has a functional link with the AR. Prostate cancer, the second leading cause of cancer death in North American men, initially responds to androgen ablation. This treatment, however, is effective for only 1-2 years, after which the cancer finally progresses to an androgen-independent phenotype, which is resistant to the currently available therapies. It is possible that MDM2 has a role in the transition to androgen independence. MDM2 is upregulated during the development of androgen independence, and MDM2 inhibits AR signaling by facilitating AR protein degradation through the proteasome, independent of p53. If indeed MDM2 is involved in this transition, knocking down MDM2 with specific inhibitors might prevent the development of androgen-independence and improve the prognosis for patients with prostate cancer.

In summary, specific inhibitors targeting MDM2 would be promising for the treatment of human cancer. At least

Table 1. Cellular Proteins That Interact with MDM2

Proteins	Binding Sites on MDM2 (aa)	Biological Consequences	Reference
pRb	273-321	Regulates Sp1 activity	[22]
E2F1/DP1	1-220	Increases E2F1 transcriptional activity	[43]
MDM4	276-491	Unknown	[50-52]
MTBP	167-304	Inhibits G1 Arrest Induced by MTBP	[58]
PML	202-304/340-488	Shuttles PML to the cytoplasm	[61]
p21 ^{WAF1/CIP1}	179-299	Promotes p21 degradation by the proteasome	[65]
NPM	1-110/276-491	Unknown	[69]
Merlin	Unknown	Unknown	[71]
PCAF	1-150/294-384	Promotes PCAF degradation by the proteasome	[74]
Tip60	Unknown	Promotes Tip60 degradation by the proteasome	[76]
L5	221-274	Unknown	[77-79]
L11	284-374	Unknown	[77-79]
L23	384-425	Unknown	[77-79]
Numb	1-134	Promotes Numb degradation by the proteasome	[81]
DNA polymerase	1-166	Stimulates the activity of DNA Polymerase	[83]
TSG101	Unknown	Unknown	[86]
YY1	150-290	Unknown	[88]
IGF-1R	Unknown	Promotes IGF-1R degradation by the proteasome	[92]
AR	341-491	Promotes AR degradation by the proteasome	[97]
p73	1-150	Inhibits p73 transactivational activity	[103]
PSD-95	Unknown	Promotes PSD-95 degradation by the proteasome	[108]
-Arrestin	161-321	Promotes -arrestin degradation by the proteasome	[109]

50% of cancers have no functional p53, and these tumors are usually of more advanced stages and are resistant to classical cancer therapies. In tumors expressing functional p53, knocking down MDM2 results in p53-dependent cell cycle arrest and apoptosis. In light of the large number of proteins that interact with MDM2, it is likely that the p53-independent pathways of MDM2 also influence initiation and progression in tumors without functional p53. Table (1) gives a listing of these molecules and a brief description of their relationship with MDM2. If indeed the p53-independent functions of MDM2 are important to tumorigenesis and to determining the course of the disease, knocking down MDM2 would effectively reduce tumor formation, and perhaps prevent the progression of existing tumors. Additionally, while most cytotoxic drugs rely on the existence of functional p53 in order to be effective, knocking down MDM2 could improve the prognosis for patients, independent of the p53 status of their tumors. In order to facilitate the process of developing MDM2 inhibitors for clinical use, mechanistic studies related to the oncogenic MDM2 pathways are necessary.

ACKNOWLEDGEMENTS

This project was supported by grants from the National Institutes of Health/National Cancer Institute (to R.Z., grant numbers R01 CA80698 and R01 CA112029). Z.Z. was supported in part by a post-doctoral fellowship from the USA Department of Defense Prostate Cancer Research Program (grant number W81XWH-04-1-0845). We thank Ms. Elizabeth Rayburn and Dr. Donald Hill for reviewing and editing this manuscript.

ABBREVIATIONS

AMPA	= -amino-3-hydroxy-5-methylisoxazole-4-propionic acid
AR	= Androgen receptor
CIP1	= Cyclin-dependent kinase inhibitor 1A
DP1	= Differentiation related transcription factor 1-polypeptide-1
E2F1	= E2F transcription factor 1
ER	= Estrogen receptor
GPCRs	= G protein-coupled receptors
GR	= Glucocorticoid receptor
HAT	= Histone acetyltransferase
HIF-1	= Hypoxia-inducible factor 1
IGF-1R	= Insulin-like growth factor 1 receptor
MDM2	= Mouse double minute 2
MTBP	= MDM2 binding protein
NES	= Nuclear export signal
NLS	= Nuclear localization signal
NMDA	= N-Methyl-D-aspartate
NPM	= Nucleophosmin

PCAF	= P300/CREB-binding protein-associated factor
PML	= Promyelocytic leukemia protein
pRb	= Hypophosphorylated Rb
SDS	= Sodium dodecyl sulfate
TGF 1	= Transforming growth factor 1
TSG101	= Tumor susceptibility gene 101
Waf1	= Wild-type p53 activated fragment-1
YY1	= Yin Yang 1

REFERENCES

- [1] Fakharzadeh, S. S.; Trusko, S. P.; George, D. L. Tumorigenic potential associated with enhanced expression of a gene that is amplified in a mouse tumor cell line. *EMBO J.* **1991**, *10*, 1565-1569.
- [2] Iwakuma, T.; Lozano, G. MDM2, an introduction. *Mol. Cancer Res.* **2003**, *1*, 993-1000.
- [3] Jones, S. N.; Hancock, A. R.; Vogel, H.; Donehower, L. A.; Bradley, A. Overexpression of Mdm2 in mice reveals a p53-independent role for Mdm2 in tumorigenesis. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 15608-15612.
- [4] Oliner, J. D.; Kinzler, K. W.; Meltzer, P. S.; George, D. L.; Vogelstein, B. Amplification of a gene encoding a p53-associated protein in human sarcomas. *Nature* **1992**, *358*, 80-83.
- [5] Zauberman, A.; Flusberg, D.; Haupt, Y.; Barak, Y.; Oren, M. A functional p53-responsive intronic promoter is contained within the human mdm2 gene. *Nucleic Acids Res.* **1995**, *23*, 2584-2592.
- [6] Linzer, D. I.; Levine, A. J. Characterization of a 54K dalton cellular SV40 tumor antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells. *Cell* **1997**, *17*, 43-52.
- [7] Oren, M.; Levine, A. J. Molecular cloning of a cDNA specific for the murine p53 cellular tumor antigen. *Proc. Natl. Acad. Sci. USA* **1983**, *80*, 56-59.
- [8] Finlay, C. A.; Hinds, P. W.; Levine, A. J. The p53 proto-oncogene can act as a suppressor of transformation. *Cell* **1989**, *57*, 1083-1093.
- [9] Bartek, J.; Bartkova, J.; Vojtesek, B.; Staskova, Z.; Lukas, J.; Rejthar, A.; Kovarik, J.; Midgley, C. A.; Gannon, J. V.; Lane, D. P. Aberrant expression of the p53 oncoprotein is a common feature of a wide spectrum of human malignancies. *Oncogene* **1991**, *6*, 1699-1703.
- [10] Kastan, M. B.; Onyekwere, O.; Sidransky, D.; Vogelstein, B.; Craig, R. W. Participation of p53 protein in the cellular response to DNA damage. *Cancer Res.* **1991**, *51*, 6304-6311.
- [11] Clarke, A. R.; Purdie, C. A.; Harrison, D. J.; Morris, R. G.; Bird, C. C.; Hooper, M. L.; Wyllie, A. H. Thymocyte apoptosis induced by p53-dependent and independent pathways. *Nature* **1993**, *29*, 849-852.
- [12] Zhang, R. W.; Wang, H. MDM2 Oncogene as a Novel Target for Human Cancer Therapy. *Curr. Pharma. Design* **2000**, *6*, 393-416.
- [13] Chen, C. Y.; Oliner, J. D.; Zhan, Q.; Fornace, A. J. Jr.; Vogelstein, B.; Kastan, M. B. Interactions between p53 and MDM2 in a mammalian cell cycle checkpoint pathway. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 2684-2688.
- [14] Momand, J.; Zambetti, G. P.; Olson, D. C.; George, D.; Levine, A. J. The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. *Cell* **1992**, *69*, 1237-1245.
- [15] Chen, J.; Marechal, V.; Levine, A. J. Mapping of the p53 and mdm-2 interaction domains. *Mol. Cell Biol.* **1993**, *13*, 4107-4114.
- [16] Freedman, D. A.; Levine, A. J. Nuclear export is required for degradation of endogenous p53 by MDM2 and human papillomavirus E6. *Mol. Cell Biol.* **1998**, *18*, 7288-7293.
- [17] Fuchs, S. Y.; Adler, V.; Buschmann, T.; Wu, X.; Ronai, Z. Mdm2 association with p53 targets its ubiquitination. *Oncogene* **1998**, *17*, 2543-2547.
- [18] Haupt, Y.; Maya, R.; Kazaz, A.; Oren, M. Mdm2 promotes the rapid degradation of p53. *Nature* **1997**, *387*, 296-299.

- [19] Chen, J.; Wu, X.; Lin, J.; Levine, A. J. mdm-2 inhibits the G1 arrest and apoptosis functions of the p53 tumor suppressor protein. *Mol. Cell Biol.* **1996**, *16*, 2445-2452.
- [20] Jones, S. N.; Roe, A. E.; Donehower, L. A.; Bradley, A. Rescue of embryonic lethality in Mdm2-deficient mice by absence of p53. *Nature* **1995**, *378*, 206-208.
- [21] Fiddler, T. A.; Smith, L.; Tapscott, S. J.; Thayer, M. J. Amplification of MDM2 inhibits MyoD-mediated myogenesis. *Mol. Cell Biol.* **1996**, *16*, 5048-5057.
- [22] Guo, C. S.; Degnin, C.; Fiddler, T. A.; Stauffer, D.; Thayer, M. J. Regulation of MyoD activity and muscle cell differentiation by MDM2, pRb, and Sp1. *J. Biol. Chem.* **2003**, *278*, 22615-22622.
- [23] Dubs-Poterszman, M. C.; Tocque, B.; Wasylyk, B. MDM2 transformation in the absence of p53 and abrogation of the p107 G1 cell-cycle arrest. *Oncogene* **1995**, *11*, 2445-2449.
- [24] Swaroop, M.; Sun, Y. Mdm2 ligase dead mutants did not act in a dominant negative manner to re-activate p53, but promoted tumor cell growth. *Anticancer Res.* **2003**, *23*, 3167-3174.
- [25] Brown, D. R.; Thomas, C. A.; Deb, S. P. The human oncoprotein MDM2 arrests the cell cycle: elimination of its cell-cycle-inhibitory function induces tumorigenesis. *EMBO J.* **1998**, *17*, 2513-2525.
- [26] Deb, S. P. Cell cycle regulatory functions of the human oncoprotein MDM2. *Mol. Cancer Res.* **2003**, *1*, 1009-1016.
- [27] Alkhalaf, M.; Ganguli, G.; Messaddeq, N.; Le Meur, M.; Wasylyk, B. MDM2 overexpression generates a skin phenotype in both wild type and p53 null mice. *Oncogene* **1999**, *18*, 1419-1434.
- [28] Ganguli, G.; Abecassis, J.; Wasylyk, B. MDM2 induces hyperplasia and premalignant lesions when expressed in the basal layer of the epidermis. *EMBO J.* **2000**, *19*, 5135-5147.
- [29] Lundgren, K.; Montes de Oca Luna, R.; McNeill, Y. B.; Emerick, E. P.; Spencer, B.; Barfield, C. R.; Lozano, G.; Rosenberg, M. P.; Finlay, C. A. Targeted expression of MDM2 uncouples S phase from mitosis and inhibits mammary gland development independent of p53. *Genes Dev.* **1997**, *11*, 714-725.
- [30] Vargas, D. A.; Takahashi, S.; Ronai, Z. Mdm2: A regulator of cell growth and death. *Adv. Cancer Res.* **2003**, *89*, 1-34.
- [31] Moore, L.; Venkatachalam, S.; Vogel, H.; Watt, J. C.; Wu, C. L.; Steinman, H.; Jones, S. N.; Donehower, L. A. Cooperativity of p19ARF, Mdm2, and p53 in murine tumorigenesis. *Oncogene* **2003**, *22*, 7831-7837.
- [32] Pinkas, J.; Naber, S. P.; Butel, J. S.; Medina, D.; Jerry, D. J. Expression of MDM2 during mammary tumorigenesis. *Int. J. Cancer.* **1999**, *81*, 292-298.
- [33] Bueso-Ramos, C. E.; Manshouri, T.; Haidar, M. A.; Yang, Y.; McCown, P.; Ordonez, N.; Glassman, A.; Sneige, N.; Albitar, M. Abnormal expression of MDM-2 in breast carcinomas. *Breast Cancer Res. Treat.* **1996**, *37*, 179-188.
- [34] Bartel, F.; Harris, L. C.; Wurl, P.; Taubert, H. MDM2 and its splice variant messenger RNAs: expression in tumors and down-regulation using antisense oligonucleotides. *Mol. Cancer Res.* **2004**, *2*, 29-35.
- [35] Liang, H.; Atkins, H.; Abdel-Fattah, R.; Jones, S. N.; Lunec, J. Genomic organisation of the human MDM2 oncogene and relationship to its alternatively spliced mRNAs. *Gene* **2004**, *338*, 217-223.
- [36] Steinman, H. A.; Burstein, E.; Lengner, C.; Gosselin, J.; Pihan, G.; Duckett, C. S.; Jones, S. N. An alternative splice form of Mdm2 induces p53-independent cell growth and tumorigenesis. *J. Biol. Chem.* **2004**, *279*, 4877-4886.
- [37] Fridman, J. S.; Hernando, E.; Hemann, M. T.; de Stanchina, E.; Cordon-Cardo, C.; Lowe, S. W. Tumor promotion by Mdm2 splice variants unable to bind p53. *Cancer Res.* **2003**, *63*, 5703-5706.
- [38] Cordon-Cardo, C.; Latres, E.; Drobnjak, M.; Oliva, M. R.; Pollack, D.; Woodruff, J. M.; Marechal, V.; Chen, J.; Brennan, M. F.; Levine, A. J. Molecular abnormalities of mdm2 and p53 genes in adult soft tissue sarcomas. *Cancer Res.* **1994**, *54*, 794-799.
- [39] Wang, H.; Oliver, P.; Zhang, Z.; Agrawal, S.; Zhang, R. Chemosensitization and radiosensitization of human cancer by antisense anti-MDM2 oligonucleotides: *in vitro* and *in vivo* activities and mechanisms. *Ann. NY Acad. Sci.* **2003**, *1002*, 217-235.
- [40] Bianco, R.; Caputo, R.; Caputo, R.; Damiano, V.; De Placido, S.; Ficorella, C.; Agrawal, S.; Bianco, A. R.; Ciardiello, F.; Tortora, G. Combined targeting of epidermal growth factor receptor and MDM2 by gefitinib and antisense MDM2 cooperatively inhibit hormone-independent prostate cancer. *Clin. Cancer Res.* **2004**, *10*, 4858-4864.
- [41] Xiao, Z. X.; Chen, J.; Levine, A. J.; Modjtahedi, N.; Xing, J.; Sellers, W. R.; Livingston, D. M. Interaction between the retinoblastoma protein and the oncoprotein MDM2. *Nature* **1995**, *375*, 694-698.
- [42] Hsieh, J. K.; Chan, F. S.; O'Connor, D. J.; Mittnacht, S.; Zhong, S.; Lu, X. RB regulates the stability and the apoptotic function of p53 via MDM2. *Mol. Cell* **1999**, *3*, 181-193.
- [43] Martin, K.; Trouche, D.; Hagemeyer, C.; Sorensen, T. S.; La Thangue, N. B.; Kouzarides, T. Stimulation of E2F1/DP1 transcriptional activity by MDM2 oncoprotein. *Nature* **1995**, *375*, 691-694.
- [44] Loughran, O.; La Thangue, N. B. Apoptotic and growth-promoting activity of E2F modulated by MDM2. *Mol. Cell Biol.* **2000**, *20*, 2186-2197.
- [45] Blattner, C.; Sparks, A.; Lane, D. Transcription factor E2F-1 is upregulated in response to DNA damage in a manner analogous to that of p53. *Mol. Cell Biol.* **1999**, *19*, 3704-3713.
- [46] Kowalik, T. F.; DeGregori, J.; Leone, G.; Jakoi, L.; Nevins, J. R. E2F1-specific induction of apoptosis and p53 accumulation, which is blocked by Mdm2. *Cell Growth Differ.* **1998**, *9*, 113-118.
- [47] Wunderlich, M.; Berberich, S. J. Mdm2 inhibition of p53 induces E2F1 transactivation via p21. *Oncogene* **2002**, *21*, 4414-4421.
- [48] Shvarts, A.; Steegenga, W. T.; Riteco, N.; van Laar, T.; Dekker, P.; Bazuine, M.; van Ham, R. C.; van der Houven, van Oordt, W.; Hateboer, G.; van der Eb, A. J.; Jochemsen, A. G. MDMX: a novel p53-binding protein with some functional properties of MDM2. *EMBO J.* **1996**, *15*, 5349-5357.
- [49] Shvarts, A.; Bazuine, M.; Dekker, P.; Ramos, Y. F.; Steegenga, W. T.; Merckx, G.; van Ham, R. C.; van der Houven van Oordt, W.; van der Eb, A. J.; Jochemsen, A. G. Isolation and identification of the human homolog of a new p53-binding protein, Mdmx. *Genomics* **1997**, *43*, 34-42.
- [50] Finch, R. A.; Donoviel, D. B.; Potter, D.; Shi, M.; Fan, A.; Freed, D. D.; Wang, C. Y.; Zambrowicz, B. P.; Ramirez-Solis, R.; Sands, A. T.; Zhang, N. mdmx is a negative regulator of p53 activity *in vivo*. *Cancer Res.* **2002**, *62*, 3221-3225.
- [51] Migliorini, D.; Denchi, E. L.; Danovi, D.; Jochemsen, A.; Capillo, M.; Gobbi, A.; Helin, K.; Pelicci, P. G.; Marine, J. C. Mdm4 (Mdmx) regulates p53-induced growth arrest and neuronal cell death during early embryonic mouse development. *Mol. Cell Biol.* **2002**, *22*, 5527-5538.
- [52] Kawai, H.; Wiederschain, D.; Kitao, H.; Stuart, J.; Tsai, K. K.; Yuan, Z. M. DNA damage-induced MDMX degradation is mediated by MDM2. *J. Biol. Chem.* **2003**, *278*, 45946-45953.
- [53] Linares, L. K.; Hengstermann, A.; Ciechanover, A.; Muller, S.; Scheffner, M. HdmX stimulates Hdm2-mediated ubiquitination and degradation of p53. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 12009-12014.
- [54] Mancini, F.; Gentiletti, F.; D'Angelo, M.; Giglio, S.; Nanni, S.; D'Angelo, C.; Farsetti, A.; Citro, G.; Sacchi, A.; Pontecorvi, A.; Moretti, F. MDM4 (MDMX) overexpression enhances stabilization of stress-induced p53 and promotes apoptosis. *J. Biol. Chem.* **2004**, *279*, 8169-8180.
- [55] Sun, P.; Dong, P.; Dai, K.; Hannon, G. J.; Beach, D. p53-independent role of MDM2 in TGF-beta1 resistance. *Science* **1998**, *282*, 2270-2272.
- [56] Yam, C. H.; Siu, W. Y.; Arooz, T.; Chiu, C. H.; Lau, A.; Wang, X. Q.; Poon, R. Y. MDM2 and MDMX inhibit the transcriptional activity of ectopically expressed SMAD proteins. *Cancer Res.* **1999**, *59*, 5075-5078.
- [57] Ganguli, G.; Wasylyk, B. p53-independent functions of MDM2. *Mol. Cancer Res.* **2003**, *1*, 1027-1035.
- [58] Boyd, M. T.; Vlatkovic, N.; Haines, D. S. A novel cellular protein (MTBP) binds to MDM2 and induces a G1 arrest that is suppressed by MDM2. *J. Biol. Chem.* **2000**, *275*, 31883-31890.
- [59] Salomoni, P.; Pandolfi, P. P. The role of PML in tumor suppression. *Cell* **2002**, *108*, 165-170.
- [60] Bernardi, R.; Scaglioni, P. P.; Bergmann, S.; Horn, H. F.; Vousden, K. H.; Pandolfi, P. P. PML regulates p53 stability by sequestering Mdm2 to the nucleolus. *Nat. Cell Biol.* **2004**, *6*, 665-672.
- [61] Wei, X.; Yu, Z. K.; Ramalingam, A.; Grossman, S. R.; Yu, J. H.; Bloch, D. B.; Maki, C. G. Physical and functional interactions between PML and MDM2. *J. Biol. Chem.* **2003**, *278*, 29288-29297.

- [62] Weinberg, W. C.; Denning, M. F. P21Waf1 control of epithelial cell cycle and cell fate. *Crit. Rev. Oral. Biol. Med.* **2002**, *13*, 453-464.
- [63] Deng, C.; Zhang, P.; Harper, J. W.; Elledge, S. J.; Leder, P. Mice lacking p21CIP1/WAF1 undergo normal development, but are defective in G1 checkpoint control. *Cell* **1995**, *82*, 675-684.
- [64] Martín-Caballero, J.; Flores, J. M.; García-Palencia, P.; Serrano, M. Tumor susceptibility of p21(Waf1/Cip1)-deficient mice. *Cancer Res.* **2001**, *61*, 6234-6238.
- [65] Zhang, Z.; Wang, H.; Li, M.; Agrawal, S.; Chen, X.; Zhang, R. MDM2 is a negative regulator of p21WAF1/CIP1, independent of p53. *J. Biol. Chem.* **2004**, *279*, 16000-16006.
- [66] Jin, Y.; Lee, H.; Zeng, S. X.; Dai, M. S.; Lu, H. MDM2 promotes p21waf1/cip1 proteasomal turnover independently of ubiquitylation. *EMBO J.* **2003**, *22*, 6365-6377.
- [67] Okuda, M.; Horn, H. F.; Tarapore, P.; Tokuyama, Y.; Smulian, A. G.; Chan, P. K.; Knudsen, E. S.; Hofmann, I. A.; Snyder, J. D.; Bove, K. E.; Fukasawa, K. Nucleophosmin/B23 is a target of CDK2/cyclin E in centrosome duplication. *Cell* **2000**, *103*, 127-140.
- [68] Rubbi, C. P.; Milner, J. Disruption of the nucleolus mediates stabilization of p53 in response to DNA damage and other stresses. *EMBO J.* **2003**, *22*, 6068-6077.
- [69] Kurki, S.; Peltonen, K.; Latonen, L.; Kiviharju, T. M.; Ojala, P. M.; Meek, D.; Laiho, M. Nucleolar protein NPM interacts with HDM2 and protects tumor suppressor protein p53 from HDM2-mediated degradation. *Cancer Cell* **2004**, *5*, 465-475.
- [70] Morrison, H.; Sherman, L. S.; Legg, J.; Banine, F.; Isacke, C.; Haippek, C. A.; Gutmann, D. H.; Ponta, H.; Herrlich, P. The NF2 tumor suppressor gene product, merlin, mediates contact inhibition of growth through interactions with CD44. *Genes Dev.* **2001**, *15*, 968-980.
- [71] Kim, H.; Kwak, N. J.; Lee, J. Y.; Choi, B. H.; Lim, Y.; Ko, Y. J.; Kim, Y. H.; Huh, P. W.; Lee, K. H.; Rha, H. K.; Wang, Y. P. Merlin neutralizes the inhibitory effect of Mdm2 on p53. *J. Biol. Chem.* **2004**, *279*, 7812-7818.
- [72] Zhao, L. Y.; Liu, Y.; Bertos, N. R.; Yang, X. J.; Liao, D. PCAF is a coactivator for p73-mediated transactivation. *Oncogene* **2003**, *22*, 8316-8329.
- [73] Liu, L.; Scolnick, D. M.; Trievel, R. C.; Zhang, H. B.; Marmorstein, R.; Halazonetis, T. D.; Berger, S. L. p53 sites acetylated *in vitro* by PCAF and p300 are acetylated *in vivo* in response to DNA damage. *Mol. Cell Biol.* **1999**, *19*, 1202-1209.
- [74] Jin, Y.; Zeng, S. X.; Lee, H.; Lu, H. MDM2 mediates p300/CREB-binding protein-associated factor ubiquitination and degradation. *J. Biol. Chem.* **2004**, *279*, 20035-20043.
- [75] Ikura, T.; Ogryzko, V. V.; Grigoriev, M.; Groisman, R.; Wang, J.; Horikoshi, M.; Scully, R.; Qin, J.; Nakatani, Y. Involvement of the TIP60 histone acetylase complex in DNA repair and apoptosis. *Cell* **2000**, *102*, 463-473.
- [76] Legube, G.; Linares, L. K.; Lemercier, C.; Scheffner, M.; Khochbin, S.; Trouche, D. Tip60 is targeted to proteasome-mediated degradation by Mdm2 and accumulates after UV irradiation. *EMBO J.* **2002**, *21*, 1704-1712.
- [77] Dai, M. S.; Zeng, S. X.; Jin, Y.; Sun, X. X.; David, L.; Lu, H. Ribosomal protein L23 activates p53 by inhibiting MDM2 function in response to ribosomal perturbation but not to translation inhibition. *Mol. Cell Biol.* **2004**, *24*, 7654-7668.
- [78] Dai, M. S.; Lu, H. Inhibition of MDM2-mediated p53 ubiquitination and degradation by ribosomal protein L5. *J. Biol. Chem.* **2004**, *279*, 44475-44482.
- [79] Zhang, Y.; Wolf, G. W.; Bhat, K.; Jin, A.; Allio, T.; Burkhart, W. A.; Xiong, Y. Ribosomal protein L11 negatively regulates oncoprotein MDM2 and mediates a p53-dependent ribosomal-stress checkpoint pathway. *Mol. Cell Biol.* **2003**, *23*, 8902-8912.
- [80] Juven-Gershon, T.; Shifman, O.; Unger, T.; Elkeles, A.; Haupt, Y.; Oren, M. The Mdm2 oncoprotein interacts with the cell fate regulator Numb. *Mol. Cell Biol.* **1998**, *18*, 3974-3982.
- [81] Yogosawa, S.; Miyauchi, Y.; Honda, R.; Tanaka, H.; Yasuda, H. Mammalian Numb is a target protein of Mdm2, ubiquitin ligase. *Biochem. Biophys. Res. Commun.* **2003**, *302*, 869-872.
- [82] Vlatkovic, N.; Guerrero, I.; Li, Y.; Linn, S.; Haines, D. S.; Boyd, M. T. MDM2 interacts with the C-terminus of the catalytic subunit of DNA polymerase epsilon. *Nucleic Acids Res.* **2000**, *28*, 3581-3586.
- [83] Asahara, H.; Li, Y.; Fuss, J.; Haines, D. S.; Vlatkovic, N.; Boyd, M. T.; Linn, S. Stimulation of human DNA polymerase epsilon by MDM2. *Nucleic Acids Res.* **2003**, *31*, 2451-2459.
- [84] Carstens, M. J.; Krempler, A.; Triplett, A. A.; Van Lohuizen, M.; Wagner, K. U. Cell cycle arrest and cell death are controlled by p53-dependent and p53-independent mechanisms in Tsg101-deficient cells. *J. Biol. Chem.* **2004**, *279*, 35984-35994.
- [85] Ruland, J.; Sirard, C.; Elia, A.; MacPherson, D.; Wakeham, A.; Li, L.; de la Pompa, J. L.; Cohen, S. N.; Mak, T. W. p53 accumulation, defective cell proliferation, and early embryonic lethality in mice lacking tsg101. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 1859-1864.
- [86] Li, L.; Liao, J.; Ruland, J.; Mak, T. W.; Cohen, S. N. A TSG101/MDM2 regulatory loop modulates MDM2 degradation and MDM2/p53 feedback control. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 1619-1624.
- [87] Shi, Y.; Lee, J. S.; Galvin, K. M. Everything you have ever wanted to know about Yin Yang 1. *Biochim. Biophys. Acta.* **1997**, *1332*, F49-F66.
- [88] Sui, G.; Affar el, B.; Shi, Y.; Brignone, C.; Wall, N. R.; Yin, P.; Donohoe, M.; Luke, M. P.; Calvo, D.; Grossman, S. R.; Shi, Y. Yin Yang 1 is a negative regulator of p53. *Cell* **2004**, *117*, 859-872.
- [89] Gronroos, E.; Terentiev, A. A.; Punga, T.; Ericsson, J. YY1 inhibits the activation of the p53 tumor suppressor in response to genotoxic stress. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 12165-12170.
- [90] Werner, H.; Le Roith, D. The insulin-like growth factor-I receptor signaling pathways are important for tumorigenesis and inhibition of apoptosis. *Crit. Rev. Oncog.* **1997**, *8*, 71-92.
- [91] Werner, H.; Karmeli, E.; Rauscher, F. J.; LeRoith, D. Wild-type and mutant p53 differentially regulate transcription of the insulin-like growth factor I receptor gene. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 8318-8323.
- [92] Girnita, L.; Girnita, A.; Larsson, O. Mdm2-dependent ubiquitination and degradation of the insulin-like growth factor I receptor. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 8247-8252.
- [93] Sutherland, R. L.; Prall, O. W.; Watts, C. K.; Musgrove, E. A. Estrogen and progesterin regulation of cell cycle progression. *J. Mammary Gland Biol. Neoplasia* **1998**, *3*, 63-72.
- [94] Kinyamu, H. K.; Archer, T. K. Estrogen receptor-dependent proteasomal degradation of the glucocorticoid receptor is coupled to an increase in mdm2 protein expression. *Mol. Cell Biol.* **2003**, *23*, 5867-5881.
- [95] Heisler, L. E.; Evangelou, A.; Lew, A. M.; Trachtenberg, J.; Elsholtz, H. P.; Brown, T. J. Androgen-dependent cell cycle arrest and apoptotic death in PC-3 prostatic cell cultures expressing a full-length human androgen receptor. *Mol. Cell Endocrinol.* **1997**, *126*, 59-73.
- [96] Grossmann, M. E.; Huang, H.; Tindall, D. J. Androgen receptor signaling in androgen-refractory prostate cancer. *J. Natl. Cancer Inst.* **2001**, *93*, 1687-1697.
- [97] Lin, H. K.; Wang, L.; Hu, Y. C.; Altuwajri, S.; Chang, C. Phosphorylation-dependent ubiquitylation and degradation of androgen receptor by Akt require Mdm2 E3 ligase. *EMBO J.* **2002**, *21*, 4037-4048.
- [98] Harris, A. L. Hypoxia--a key regulatory factor in tumour growth. *Nat. Rev. Cancer*, **2002**, *2*, 38-47.
- [99] Bardos, J. I.; Chau, N. M.; Ashcroft, M. Growth factor-mediated induction of HDM2 positively regulates hypoxia-inducible factor 1alpha expression. *Mol. Cell Biol.* **2004**, *24*, 2905-2914.
- [100] Semenza, G. L. HIF-1 and tumor progression: pathophysiology and therapeutics. *Trends Mol. Med.* **2002**, *8*, S62-S67.
- [101] Zhang, L.; Hill, R. P. Hypoxia enhances metastatic efficiency by up-regulating Mdm2 in KHT cells and increasing resistance to apoptosis. *Cancer Res.* **2004**, *64*, 4180-4189.
- [102] Melino, G.; De Laurenzi, V.; Vousden, K. H. p73: Friend or foe in tumorigenesis. *Nat. Rev. Cancer* **2002**, *2*, 605-615.
- [103] Zeng, X.; Chen, L.; Jost, C. A.; Maya, R.; Keller, D.; Wang, X.; Kaelin, W. G. Jr.; Oren, M.; Chen, J.; Lu, H. MDM2 suppresses p73 function without promoting p73 degradation. *Mol. Cell Biol.* **1999**, *19*, 3257-3266.
- [104] Grossman, S. R.; Perez, M.; Kung, A. L.; Joseph, M.; Mansur, C.; Xiao, Z. X.; Kumar, S.; Howley, P. M.; Livingston, D. M. p300/MDM2 complexes participate in MDM2-mediated p53 degradation. *Mol. Cell* **1998**, *2*, 405-415.

- [105] Thomas, A.; White, E. Suppression of the p300-dependent mdm2 negative-feedback loop induces the p53 apoptotic function. *Genes Dev.* **1998**, *12*, 1975-1985.
- [106] Zeng, S. X.; Jin, Y.; Kuninger, D. T.; Rotwein, P.; Lu, H. The acetylase activity of p300 is dispensable for MDM2 stabilization. *J. Biol. Chem.* **2003**, *278*, 7453-7458.
- [107] Gu, L.; Findley, H. W.; Zhou, M. MDM2 induces NF-kappaB/p65 expression transcriptionally through Sp1-binding sites: a novel, p53-independent role of MDM2 in doxorubicin resistance in acute lymphoblastic leukemia. *Blood* **2002**, *99*, 3367-3375.
- [108] Colledge, M.; Snyder, E. M.; Crozier, R. A.; Soderling, J. A.; Jin, Y.; Langeberg, L. K.; Lu, H.; Bear, M. F.; Scott, J. D. Ubiquitination regulates PSD-95 degradation and AMPA receptor surface expression. *Neuron.* **2003**, *40*, 595-607.
- [109] Shenoy, S. K.; McDonald, P. H.; Kohout, T. A.; Lefkowitz, R. J. Regulation of receptor fate by ubiquitination of activated beta 2-adrenergic receptor and beta-arrestin. *Science* **2001**, *294*, 1307-1313.