48 p53 Tumor Suppressor Opens Gateways for Cancer Therapy

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48.1 INTRODUCTION

The tumor suppressor p53 is one of the most important and intensively studied molecules in biomedical research. Since its discovery 28 years ago, more than 43,000 articles have been published about p53. These studies cover nearly all aspects of biomedical research, encompassing biochemistry, biophysics, molecular biology, cellular biology, genetics, pharmacology, toxicology, metabolism, immunology, bioinformatics, as well as clinical research. Tremendous effort has been spent elucidating the mechanisms underlying p53's tumor suppressive function and how it is regulated. Still, there is much ground to cover before p53 and its signaling pathways are fully understood. However, this fact does not prevent the application of our current knowledge to the development of strategies for treating cancer patients using the p53 pathway as a therapeutic target. Indeed, a number of strategies, such as introduction of functional wild-type p53 into cancer cells and inhibition of MDM2-mediated p53 suppression, have been investigated in recent years. In this chapter, we will review p53's properties and functions, as well as its regulation in response to diverse cellular stressors. We will also briefly describe recent progress in the development of anticancer therapies that target the MDM2-p53 feedback loop. p53 gene delivery-based gene therapy will be discussed in a separate chapter.

The p53 protein is a stress-activated transcription factor; therefore activated p53 can either induce or repress the transcription of many target genes. The proteins encoded by these target genes are involved in the regulation of multiple biological functions, including cell cycle, apoptosis, cell senescence, differentiation, angiogenesis, cell migration, and DNA repair [1]. Diverse stressors, including DNA damage, oncogene activation, hypoxia/anoxia, ribonucleotide depletion, and loss of support/survival signals, stabilize the p53 protein and enhance its activity [2]. The importance of p53 in tumor suppression is highlighted by the fact that more than half of all types of human tumors harbor mutations or deletions in the p53 gene, and the remainder often have impaired function of the p53 pathway through the involvement of direct or indirect p53 regulators [3–6]. Germ-line mutations of p53 have been identified in individuals with the cancer-prone Li-Fraumeni syndrome [7,8]. Similar to human cancers, mice homozygous for inactivated p53 are highly susceptible to spontaneous tumorigenesis [9], and transgenic mice expressing hot-spot gain-of-function p53 mutations develop tumors in various tissues [10,11]. These studies establish p53 as a principal "guardian of the genome" and demonstrate that p53 plays an essential role in protecting the organism from tumorigenesis.

The p53 protein possesses the typical structural domains of a transcription factor, as well as several unique domains. These features include the DNA-binding domain, the transactivation domain, the oligomerization domain, the basic regulatory region, and the proline-rich domain. These features of the p53 protein allow for the dynamic regulation of p53's stability and activity in response to various external and internal cellular stressors. The central DNA-binding domain mediates sequence-specific binding to chromatin [12–14]. The majority of p53 gene mutations, which are found in human cancers occur in this domain, emphasizing the importance of this region for p53's function [15]. These mutations alter the conformation of p53 and effect the folding of the DNA-binding domain, therefore disrupting the capacity of p53 to bind to its DNA target, rendering it inactive. This domain has also been shown to interact with the ASPP (Ankyrin repeat, SH3 domain, and proline-rich domain containing) family proteins ASPP1 and ASPP2, allowing p53 to preferably activate transcription of proapoptotic genes such as *Bax* and *PIG3* [16].

The N-terminal, bipartite acidic transactivation domain makes contacts with basal transcription factors and coactivators, thus initiating transcriptional activation of target genes [17,18]. The C-terminal oligomerization domain allows p53 to form a tetramer and is required for its transcriptional activity [12]. The basic regulatory region at the extreme C-terminus is thought to regulate the sequence-specific binding activity of the central core DNA-binding domain and contributes to p53's ability to recognize several forms of DNA that resemble structures caused by DNA-damaging agents [12,19–24]. Finally, the p53 N-terminal proline-rich domain, containing five copies of the sequence PXXP, has been shown to be important for the p53-induced apoptotic response to DNA-damaging agents [25–30].

48.2 BIOLOGICAL FUNCTIONS OF p53

Upon activation, p53 binds to its cognate DNA response elements (p53RE) in the genome and activates or represses the transcription of genes residing in the vicinity of these binding sites. There are over 4000 putative p53-binding sites existing in the human genome [31]. More than 150 p53 target genes have been described and many more will be revealed with the development of advanced molecular technology. The proteins encoded by these genes contribute to diverse biological functions of p53 in multiple ways, including inducing cell cycle arrest, apoptosis, senescence, and angiogenesis [1]. In addition, p53 may facilitate DNA repair directly or indirectly through the induction of genes associated with DNA repair. These cellular responses to p53 activation can be variable and highly dependent on both cell type and the nature of the sustained damage.

Proper cell cycle checkpoints ensure that genomic integrity is maintained throughout cell division. p53 plays a role in both the G1 and the G2 checkpoints of the cell cycle, in part by induction of its target genes $p21^{WAF1/CIP1}$ (p21 will be used hereafter), *14-3-3-σ*, and *GADD45*. The p21 protein inhibits cyclin D-dependent kinases (CDK). CDKs phosphorylate Rb, thereby causing the dissociation of Rb from E2F, allowing E2F to activate the expression of proteins important for the progression of the cell cycle [32]. As a result, p21 maintains the Rb–E2F complex and indirectly inhibits E2F activity, preventing the G1-S transition [15,33]. The 14-3-3-σ and GADD45 proteins inhibit cyclin B-CDC2 kinase activity, which is essential for the G2-M transition. In response to DNA damage, 14-3-3σ binds to phosphorylated Cdc25, a tyrosine protein phosphatase for CDC2, and sequesters Cdc25 in the cytoplasm where it cannot activate CDC2. Then the GADD45 protein dissociates CDC2 from cyclin B, blocking the G2-M phase transition [34,35]. Thus, p53 also mediates the G2 cell cycle arrest [36,37].

Depending on the type and duration of the stress and the cellular growth conditions, p53 can activate different subsets of target genes with proapoptotic activity [1,38]. These genes encode the cell membrane proteins Fas/CD95, KILLER/DR5, and PERP [39–43], the cytoplasmic proteins PIDD and PIG (p53-inducible gene family), and mitochondrial proteins, such as BAX, NOXA [44], PUMA [45], p53AIP1 [46], BID, and others. These proteins trigger the death-receptor-mediated [47,48] and mitochondrial-mediated apoptotic pathways [49,50]. In addition, p53 can interact directly with antiapoptotic proteins, such as Bcl-XL and Bcl-2, to exert its apoptogenic function in the mitochondria, independent of its transcription activity [51–53]. Also, activation of autophagy by the p53-induced protein DRAM has also been described as an important contribution to the apoptotic response [54]. Therefore, primarily by inducing cell cycle arrest or apoptosis, p53 provides a crucial surveillance mechanism for allowing cells to either recover from stress or to be eliminated from the replicative pool, thus preventing growing cells from undergoing malignant transformation.

In addition, p53 plays an important role in maintenance of genomic stability by mediating DNA repair [55–57]. It has been shown that p53 is involved in various types of DNA repair, including nucleotide excision repair (NER), base excision repair (BER), nonhomologous end-joining (NHEJ) and homologous recombination (HR) [58–62]. For example, p53-dependent transcriptional activity is important for regulation of NER by p53 [61]. p53 binds to the NER-associated helicases XPB and XPD and modulates their activities [63,64]. It also regulates the expression of the DDB2 and XPC [65–67], and serves as a chromatin accessibility factor for NER of DNA damage [68]. Further, p53 also binds to RAD51 and RAD54, major components of the HR machinery, and controls the level of HR [69,70]. Therefore, p53 regulates DNA repair as well as the DNA damage response.

In addition to its role in gene maintenance, p53 stimulates the expression of genes important for suppression of blood vessel formation (angiogenesis). Angiogenesis is critical for tumor progression [71]. At least three mechanisms account for this inhibitory effect of p53 on angiogenesis: Interference with the central regulators of hypoxia that mediate angiogenesis, inhibition of the production of proangiogenic factors, and direct increase of the production of endogenous angiogenesis inhibitors. These mechanisms license p53 to shut down the angiogenic potential of cancer cells and prevent tumor growth, progression, and metastasis [71]. Recently, p53 has been shown to inhibit hypoxia-inducible factor-1 (HIF-1) activity; HIF-1 induces angiogenic factors in response to hypoxia and impairs cardiac angiogenesis in response to pressure overload. As a consequence, p53 prevents the development of cardiac hypertrophy and induces systolic dysfunction in response to sustained pressure overload, therefore fulfilling a crucial function in the transition from cardiac hypertrophy to heart failure [72].

Of further interest, p53 also can activate the transcription of some noncoding RNAs, resulting in cell growth inhibition and apoptosis. For example, p53 induces the expression of miRNA-34a, which also contributes to p53-mediated cell cycle arrest and apoptosis [73–76]. Moreover, p53 represses RNA polymerase (Pol I)-mediated transcription of precursor rRNAs and Pol III-mediated transcription of tRNAs and 5S rRNA, leading to inhibition of ribosomal biogenesis [77].

p53 has been shown to repress the Pol II-mediated transcription of U1 snRNA [78] and Pol III-mediated transcription of U6 snRNA [79,80]. Therefore, there are many layers to p53's role in cell growth.

The tumor suppressive function of p53 is validated concretely by several in vivo mouse models. It is firmly established that p53 knockout mice die within 10 months due to a variety of spontaneous tumors [9]. Remarkably, restoration of endogenous p53 expression in p53-deficient tumors leads to complete regression of these tumors due to cell cycle arrest, apoptosis, senescence, and initiation of an innate immune response [81,82]. These studies place important emphasis on the fact that, although cancer arises from a combination of mutations in oncogenes and tumor suppressor genes, p53 deficiency is required for maintenance of aggressive tumors. Also, these in vivo studies provide an incredibly solid foundation for cancer therapeutic strategies aimed at reintroduction of p53's function.

48.3 MDM2: A FEEDBACK INHIBITOR OF p53

The ability of p53 to induce apoptosis or cell cycle arrest can be detrimental to normal cell growth if left uncontrolled. Therefore, it is essential for a cell to tightly control p53 activity during normal development and cell growth. Under physiological conditions, p53 is maintained at an extremely low and inert level with a half-life of approximately 30 min. This rapid turnover of p53 is due to its ubiquitylation-mediated proteasomal degradation. Although a number of ubiquitin ligases, such as Pirh2, COP1, and alternative reading frame (ARF)-BP1, have been shown to ubiquitylate p53 [83], the central and most extensively studied ubiquitin ligase is the oncoprotein MDM2. The *mdm2* gene was originally identified on a mouse double minute chromosome in the 3T3DM cell line [84]. It can immortalize and, in cooperation with Ras, transform rat embryonic fibroblasts [85]. Consistent with this study, overexpression or gene amplification of *mdm2* has been shown in a variety of human tumors, particularly in soft tissue sarcomas, carcinomas, leukemias, lymphomas, breast and lung cancers [86–91]. More recent data have shown that a naturally occurring polymorphism (SNP309) within the *mdm2* promoter leads to an increase in *mdm2* mRNA and protein in human populations [92], which may be related to higher incidence of cancers.

MDM2 is a nuclear phosphoprotein, which possesses several important functional domains, including the p53-binding domain, a central acidic region with a C4 zinc finger, and a C-terminal RING domain, which confers MDM2's E3 ligase activity. MDM2's N-terminal p53-interacting domain mediates MDM2's binding to the N-terminal transcriptional activation domain of p53, thus interfering with p53's ability to interact with the transcription machinery [93,94]. The central acidic domain of MDM2 is pivotal for MDM2-mediated p53 degradation, but not p53's ubiquitylation [95,96]. A number of proteins, such as the ribosomal proteins L5 and L23, and ARF, bind to this domain, leading to inhibition of MDM2-mediated p53 degradation. The C-terminal side of the acidic domain contains a C4 zinc finger domain, which has recently been shown to mediate the binding of MDM2 to ribosomal protein L11. Mutation of residue Cys 305 to either Phe or Ser resulted in the loss of L11 binding to MDM2 and stabilization of p53, indicating this region may also play an important role in controlling p53 degradation [97]. The C-terminal RING finger domain is required for the E3 ligase activity of MDM2 [98]. MDM2 also contains a nuclear localization signal (NLS) and a nuclear export signal (NES), which mediates the shuttling of MDM2 between the cytoplasm and the nucleus and also provides a mechanism to regulate p53's activity [99,100]. Further, within the RING domain, amino acids 464–471 can function as a nucleolar localization signal (NoLS) [101]. All of these domains in MDM2 are crucial for regulating p53's stability and activity.

MDM2 inhibits p53's function through several mechanisms. MDM2 binds p53 specifically, linking their N-terminal domains. This binding conceals the N-terminal transcription activation domain of p53 at its target promoters, preventing the interaction of p53 with the basal transcription machinery. Also, by occupying at p53's target promoters with p53, MDM2 can also interact with histones and promote monoubiquitylation of histone H2B in the vicinity of a p53-binding site [102–105]. These actions lead to the inhibition of p53's transcriptional activity [94,106]. In addition, this binding initiates the ubiquitylation of p53 at several C-terminal lysine residues, catalyzed by the C-terminal RING-finger domain of MDM2; this ubiquitylation results in p53's degradation by the 26S proteasome [98,107]. MDM2 was recently found to differentially catalyze monoubiquitylation and polyubiquitylation of p53 in a dosage-dependent manner [108]. As a consequence, low levels of MDM2 activity induce monoubiquitylation and nuclear export of p53, whereas high levels promote polyubiquitylation and nuclear degradation of p53. It seems likely that these distinct mechanisms are employed under different physiological settings. For example, MDM2-mediated polyubiquitylation and nuclear degradation may play a critical role in suppressing p53's function during the later stages of a DNA damage response, or when MDM2 is malignantly overexpressed [109,110]. On the other hand, MDM2-mediated monoubiquitylation and subsequent cytoplasmic translocation of p53 may represent an important means of p53 regulation in unstressed cell, where MDM2 maintained at low levels [111–114]. Moreover, MDM2 was also reported to promote NEDD8 conjugation of p53. The C-terminal glycine residue of the ubiquitin-like protein NEDD8 can be covalently linked to Lys 370, 372, or 373 of p53. This modification inhibits p53's transcriptional activity without affecting p53's protein stability [115]. The lysine residues modified by neddylation are three of the six lysines also targeted by ubiquitylation. Whether neddylation augments ubiquitylation is not yet clear. Interestingly, the *mdm2* gene is a downstream target gene of p53 [116,117], thus forming a negative feedback loop [118,119]. Indeed, genetic disruption of *p53* rescues the lethal phenotype of *mdm2* knockout mice [120,121], firmly validating that MDM2 is a critical inhibitor of p53.

48.4 p53 STRESS RESPONSE

To activate p53, cells must overcome the MDM2-p53 negative feedback circuit. Multiple pathways can lead to activation of p53 in response to a wide variety of cellular stressors, including DNA damage, oncogenic stress, ribosomal stress, and others, such as those induced by hypoxia, reactive oxygen species, telomere erosion, and the loss of survival signals [122,123]. All of these stressors lead to disruption of the negative control of p53 imposed by MDM2 through shared or distinct pathways or cellular components.

DNA damage triggers an Ataxia telangiectasia mutated kinase (ATM) or ataxia telangiectasia RAD3-related kinase (ATR) kinase-dependent phosphorylation cascade and results in p53 activation. In response to ionizing radiation (IR), p53 is phosphorylated at Ser 15 by ATM kinase [124–127] and at Ser 20 by Chk2, which is phosphorylated by ATM [128–130]. In response to UV damage, p53 is phosphorylated at Ser 15 by ATR kinase [131,132] and at Ser 20 by Chk1, which is phosphorylated and activated by ATR [133]. Although phosphorylation of Ser 15 and Ser 20 did not diminish the binding of an N-terminal p53 peptide to MDM2, subsequent phosphorylation of Thr 18 drastically reduced p53-MDM2 binding [134]. Since phosphorylation of Thr 18 requires prior phosphorylation on Ser 20, DNA damage-induced phosphorylation of p53 at the N-terminal residues within the MDM2 binding region impairs the binding of MDM2 to p53 and blocks its inhibitory effect on p53. Similar to p53, phosphorylation of MDM2 also plays a role in p53's activation during a DNA damage response. Most MDM2 phosphorylation sites are clustered within MDM2's N-terminal p53-binding domain and the central acidic domain. For example, MDM2 is phosphorylated by DNA-PK at Ser 17. This phosphorylation might play a role in blocking the MDM2—p53 interaction [135]. ATM phosphorylates MDM2 at Ser 395 and impairs MDM2's ability to promote p53 degradation, possibly through phosphorylation-dependent inhibition of p53's nuclear export by MDM2 [136–138]. In addition to the regulation of p53's stability upon DNA damage, phosphorylation also regulates the recruitment of transcriptional coactivators such as p300/CBP to p53, thus enhancing p53's transcriptional activity [139]. Taken together, DNA damage triggers the activation of p53 through phosphorylation of both p53 and MDM2, impairing MDM2's ability to bind to p53, therefore relieving its inhibitory effect on p53.

The MDM2–p53 feedback loop is also subjected to regulation through protein–protein interaction. One critical player of this regulatory mechanism is ARF ($p14^{ARF}$ in human, $p19^{ARF}$ in mouse) that is encoded by the INK4a locus and translated in an ARF, when compared to the reading frame for the CDK inhibitor p16 [140]. ARF activates p53 in response to aberrant growth and proliferation signals, such as those induced by the overexpression of the oncogenes Ras [141], c-Myc [142], E2F [143], E1A [144], or β-catenin [145]. It binds to the central acidic domain of MDM2 and directly inhibits MDM2 ubiquitin ligase activity, both in vitro and in cells [146], thus leading to stabilization and activation of p53 [146–150]. Because of this function, ARF also acts as an important tumor suppressor [137,151,152].

Another group of proteins, which activate p53 through direct interaction with MDM2 and suppression of MDM2's activity, are ribosomal proteins. Recently, at least four ribosomal proteins, including L11, L5, L23, and S7 [83,153–158], have been shown to interact with MDM2 in response to ribosomal stress caused by perturbation of ribosomal biogenesis.

The ribosome is a finetuned cellular machine that translates cellular mRNA through a static, higher-ordered cellular process, into proteins [159,160]. To produce a ribosome, eukaryotic cells must assemble about 79 ribosomal proteins with four different ribosomal RNA (rRNA) species (28S, 18S, 5.8S, and 5S) into ribosomal subunits in the nucleolus [161,162]. Notably, all three RNA polymerases (I, II and III) are involved in this process and are coordinated to ensure the high efficiency and accuracy of ribosome production. Together, these complex processes are called ribosomal biogenesis and of fundamental importance for normal cell growth and proliferation. Therefore, it is also perfectly coupled with cell growth and proliferation. Illustrating this point are studies showing that interference with ribosome production severely retards animal growth and development, at both the cellular level and the organism level.

Since ribosomal biogenesis occurs primarily in the nucleolus and many external and internal stimuli lead to the disruption of the nucleolus, it is understandable that perturbation of the nucleolus or nucleolar protein production would be linked to p53 activity along with other types of stress [163], This specific type of stress is often referred to ribosomal (or nucleolar) stress, and can be triggered by actinomycin D or 5-fluorouracil (5-FU) treatment [164–166], serum starvation [167], the expression of dominant-negative Bop1 [168], or the genetic disruption of ribosomal protein S6 and TIF-IA [169,170]. In response to ribosomal stress, free L5, L11, L23, and S7 may be released to the nucleus or the cytoplasm where they bind to MDM2 and inhibit MDM2-mediated p53 suppression [83,153–158]. These studies suggest that p53-dependent cell cycle checkpoints monitor the malfunction ribosomal biogenesis. Interestingly, like ARF, these individual ribosomal proteins are small basic proteins. They also bring up several important questions. Why do so many basic nucleolar proteins bind to and inhibit MDM2's function? Do these nucleolar proteins collaborate to produce an optimal stress response? Would they play a role in response to different nucleolar stressors remain? Finally, how might the regulation of ribosomal proteins play a role in preventing tumorigenesis remains?

48.5 OTHER REGULATORS OF THE MDM2–p53 FEEDBACK LOOP

Besides the aforementioned proteins, the MDM2–p53 feedback loop is also subjected to regulation by many other proteins. The transcriptional coactivators p300 and CBP appear to exert a dual function on this loop [171]. p300/CBP acetylates

p53 and stimulates its activity. This acetylation can be inhibited by MDM2 [172,173]. Additionally, p300/CBP interacts with MDM2 in nuclear body-like structures, where MDM2 might be protected from proteasomal degradation [174] and cooperates with MDM2 to degrade p53 [171,175,176]. Consistently, MDM2 mutants lacking the p300/CBP-binding domain within MDM2's central acidic domain failed to degrade p53, but still promoted monoubiquitylation of p53 [177,178]. More recently, p300/CBP was shown to act as an E4 enzyme to assist MDM2 in polyubiquitylation of p53 [179]. It is yet unclear what physiological conditions may cause p300 to regulate this portion of the feedback loop and if the overall outcome of this stimulus would be the positive or negative regulation of p53.

Another key regulator of MDM2 is its homolog, MDMX, which assists MDM2 in downregulating the p53 function [180]. MDMX shares significant homology with MDM2 in its N-terminal p53-binding domain and its C-terminal RING-finger domain [181]. Like MDM2, MDMX binds p53 and inhibits its function [182–184]. As in the case of MDM2, genetically targeting the p53 gene also rescues the lethal phenotype of *mdmx* knockout mice, suggesting that MDMX is critical for MDM2-p53 feedback regulation as well [185–187]. Increased expression of MDMX is frequently observed in human tumors [188–190]. However, unlike MDM2, the expression of MDMX is not regulated by p53 [180], and MDMX alone does not ubiquitylate p53 [182,186,191,192]. Also distinct from MDM2, MDMX appears to reside mostly in the cytoplasm [186,193], but can be recruited to the nucleus by MDM2 [186,194]. The nuclear import of MDMX is also induced by DNA damage signals, such as γ irradiation [195]. In the absence of MDMX, MDM2 is relatively ineffective at downregulating p53 because of its extremely short half-life. MDMX aids MDM2 through an interaction between MDM2 and MDMX's RING-finger domains. This interaction sufficiently stabilizes MDM2 and enables it to degrade p53 at its optimal turnover rate [194]. MDMX is also degraded by MDM2 [196,197]. Moreover, ARF prevents MDM2 from degrading p53 and shifts MDM2 activity to degrade MDMX instead [197]. Therefore, MDM2 and MDMX may have different roles in inhibiting p53. MDMX is thought to enhance MDM2-mediated p53 ubiquitylation and degradation [198,199], consequently repressing p53's function. Interestingly, in response to ionizing or UV irradiation, MDMX is phosphorylated at Ser 376 by ChK2 or ChK1 and this phosphorylation leads to the interaction of MDMX with 14-3-3 proteins. As a result, MDMX loses its ability to suppress p53, thus leading to p53 activation [200,201]. Therefore, to activate p53, stress signals must turn on cellular mechanisms that surmount the negative control by MDM2 and MDMX.

Finally, the MDM2–p53 feedback loop is regulated by deubiquitylation. Herpes virus-associated ubiquitin-specific protease (HAUSP), an ubiquitin hydrolase, was shown to be a direct antagonist of MDM2 activity and acts by specifically deubiquitylating p53 after stimulation by DNA damage, thus protecting p53 from MDM2-mediated degradation [202]. However, HAUSP was also shown to bind and to deubiquitylate MDM2 and MDMX, thus stabilizing both proteins [203,204]. This effect appears to be more dominant, as knockdown or knockout of HAUSP activates p53 function [203]. In contrast to HAUSP, another deubiquitylation enzyme called USP2a has recently been shown to specifically bind to and deubiquitylate MDM2, but not p53, thus enhancing MDM2-mediated p53 degradation. Consistently, reduction of USP2a levels destabilizes MDM2 and causes the accumulation and activation of p53 [205]. These studies suggest that deubiquitylation also regulates the MDM2-p53 feedback loop. Whether these deubiquitylases play a role in tumorigenesis would be an interesting and critical question for future investigation.

The above-discussed and other p53 regulators not discussed are listed in Table 48.1, highlighting the extreme complexity of p53 regulation in cells.

TABLE 48.1
Upstream Regulators of p53

Protein	Type of Molecule	Role	References
(A) Enzymatic activators			
E4F1	Atypical ubiquitin ligase	Ubiqutylation	[224]
p300/CBP	Acetyltransferase	Acetylation	[171,179,225]
PCAF	Acetyltransferase	Acetylation	[102,226,227]
PML/p300	Tumor suppressor/acetyltransferase complex	Transcription	[228]
Set7/9	Lysine methyltransferase	Methylation	[269]
NQO1	NADH oxidioreductase	20S proteasome associated factor	[230]
Pin 1	Prolyl isomerase	Phosphorylation alteration/enhancement	[231,232]
p38	Ser/Thr kinase	Phosphorylation	[233–235]
ATM/ATR	Ser/Thr kinases	Phosphorylation	[4,236]
CK1	Ser/Thr kinase	Phosphorylation	[134,237]
Chk ½	Ser/Thr kinases	Phosphorylation	[238]

(*continued*)

TABLE 48.1 (continued)
Upstream Regulators of p53

Protein	Type of Molecule	Role	References
DNAPK	Ser/Thr kinase	Phosphorylation	[239,240]
ERK	Ser/Thr kinase	Phosphorylation	[235,241,242]
MAPK	Ser/Thr kinase	Phosphorylation	[242,243]
JNK	Ser/Thr kinase	Phosphorylation	[244–246]
Daxx/Axin/HIPK2	Ser/Thr kinase complex	Phosphorylation (UV response)	[247]
FACT (SSRP1/SPT 16)/CK2	Ser/Thr kinase/cofactor complex	Phosphorylation	[248,249]
c-Abl	Tyr kinase	p53 binding and Phosphorylation of MDM2	[250,251]
(B) Enzymatic Repressors			
HDAC	Deacetylase	Deacetylation	[252–254]
Sir2α	Deacetylase	Deacetylation	[255]
FBX011	NEDD ligase	Neddylation	[256]
Set8/PR-Set7	Lysine methyltransferase	Methylation	[257]
Smyd2	Lysine methyltransferase	Methylation	[258]
Pias (1, xβ, y)	SUMO ligase	Sumoylation	[259,260]
Sumo 1	SUMO ligase	Sumoylation	[261]
ArfBP1 (HECTH9/MULE)	Ubiquitin ligase	Ubiquitylation	[262]
Carps	Ubiquitin ligase	Ubiquitylation	[263,264]
CHIP	Ubiquitin ligase	Ubiquitylation	[263,265]
E6AP	Ubiquitin ligase	Ubiquitylation	[266]
Mdm2	Ubiquitin ligase	Ubiquitylation/Neddylation	[98,107]
PIRH2	Ubiquitin ligase	Ubiquitylation	[267]
WWP1	Ubiquitin ligase	Ubiquitylation	[268,269]
Daxx/HAUSP/MDM2/MDMX	Ubiquitin ligase complex	Ubiquitylation	[270]
LAMA/EC5S/VHL	Ubiquitin ligase complex	Ubiquitylation	[271]
YY1/MDM2	Ubiquitin ligase complex	Ubiquitylation	[272,273]

Protein	Type of Molecule	Role	p53's Fate	References
(C) Nonenzymatic Interactors				
ASPP1/2	Binding protein	Cell cycle/apoptosis	Activation	[16,274,275]
VHL	Binding protein	Hypoxia/tumor suppressor	Activation	[276]
Topors	RING family zinc finger Protein	Binding protein	Activation	[277]
WRN	Helicase	Binding protein	Activation	[152,278]
Ribosomal proteins (L5, L11, L23, S7)	Ribosomal subunits	Binding proteins to MDM2	Activation	[83,153–158]
Sp1	Transcription factor	Transcription	Activation	[279]
p14/p19Arf	Tumor suppressor	Cell Cycle/MDM2 inhibitor	Activation	[280,281]
iASPP	Binding protein	Cell cycle/apoptosis	Inactivation	[275,282]
Hsp 90	Chaper one	Conformation	Inactivation	[283]
Jab-1	Shuttling factor	Cell cycle	Inactivation	[284,285]

48.6 STRATEGIES FOR TARGETING p53 IN CANCER THERAPY

The understanding of p53's biological function and its regulation provides a basis for targeting p53 for anticancer drug development. Over the past decade, a number of attempts have been made to develop drugs that either rescue p53's activity by overexpressing its wild-type form in cancers, or enhance p53's activity by interfering with the MDM2–p53 interaction or MDM2's ubiquitin ligase activity (Table 48.2).

Some of the approaches currently explored to activate or rescue the wild-type function of p53 are the small molecules cp-31398, PRIMA-1, and MIRA-1, and the recombinant adenoviral p53, known as Gendicine. CP-31398, PRIMA-1, and MIRA-1 were developed as chaper one molecules to aid in refolding of mutant p53 in cancer tissue so that it can assume a proper wild-type conformation. CP-31398 had the disadvantage in that it could only chaper one the newly translated p53 protein. However, recent tests with PRIMA-1 and MIRA-1 are very promising and demonstrate that these compounds can not only chaper one the folding of the newly produced p53 but also refold the mutant p53 already present in the cells [206–208]. The description of Gendicine, a recombinant adenovirus encoding the human *p53* tumor suppressor gene (rAd-p53), and its clinical studies are discussed by Dr. Zhaohui Peng and his

TABLE 48.2
Chemothereputic Agents Targeting p53 Pathways

Compound	Form	Pathway Target	Trials	References
Chalcone derivatives	Flavonoid intermediate	Disruption of p53/MDM2 interaction—activity questionable	Cells, animals	[208,220]
Chlorofusin	Fungal metabolite	Disruption of p53/MDM2 interation—activity questionable	Cells, animals	[208,219]
CP-31398	Small molecule	Reactivation of endogenous mutant p53—only newly synthesized	Mouse	[206,208]
Gendicine	Recombinant adenovirus	Direct expression of wild-type p53	Progress to clinical trials	See chapter
HLI98	Small molecule	MDM2 E3 ligase inhibition	Cells	[215]
MDM2 silencing	Oligonuclotides	MDM2 downregulation	Cells, mouse, human	[209–211,213]
MIRA-1	Small molecule	Reactivation of endogenous mutant p53	Mouse	[206–208]
Nutlin	Small molecule	MDM2/binding E3 ligase inhibition	Cells, mouse	[208,222]
PRIMA-1	Small molecule	Reactivation of endogenous mutant p53	Mouse	[206–208]
RITA	Small molecule	Disruption of p53/MDM2 interaction	Cells, mouse	[208,286]

AQ2 colleagues in Chapter 49, and represents a paradigm for clinical application p53 as an anticancer agent.

Since aberrant overexpression of MDM2 occurs in subset of tumors with wild-type p53, it is also necessary to overcome MDM2's inhibition of p53 by downregulating its expression, either by directly inhibiting its ubiquitin ligase activity or compromising its interaction with p53 to restore p53 function in some tumors. Over past years, several strategies that target MDM2 for inhibition have been explored: (1) Inhibition of MDM2 expression by antisense oligonucleotides has been shown to activate p53 in various wild-type p53-containing tumor cell lines and has antitumor activity in xenograft tumor models in nude mice [209–211]. These antisense oligonucleotides synergistically enhance the antitumor effect of chemotherapeutics and radiation therapy [212–214]. Interestingly, the antisense MDM2 inhibitors also have antitumor activities in human cancers with p53 deficiency, reflecting their inhibitory effect on p53-independent function of MDM2 [210,213].

As noted above, MDM2 is the central negative regulator of p53, acting as an ubiquitin ligase to target p53 for proteasome-mediated degradation. Thus inhibition of MDM2's E3 ligase activity would stabilize p53 for activation. Recently, small molecule inhibitors have been identified to possess such an inhibitory effect on MDM2. One of such compounds, named HLI98, inhibits MDM2-mediated p53 ubiquitylation and induces p53-dependent apoptosis in cancer cells [215]. The major drawback for this class of compounds is their low selectivity and potency. To screen more selective small molecules for the desired specificity would increase the feasibility of using them in cancer therapy.

Finally, a potential way to activate p53 is through inhibition of the MDM–p53 binding. MDM2 contains a well-defined, relatively deep hydrophobic pocket in its the N- terminus (residues 25–109) where the transactivational domain of p53 binds, thereby concealing p53 from interacting with the transcriptional machinery [216]. The minimal MDM2-binding site on p53 was subsequently mapped to residues 18–26 [93,217,218]. This pocket is filled by three primary side chains (Phe 19, Trp 23, and Leu 26) from the helical region of the p53 peptide [216,217]. Therefore, it is possible to design small molecules to mimic p53's binding to MDM2. A number of such molecules have been investigated, including chalcone derivatives, cholorofusin, nutlin, and RITA. Chalcone derivatives are present in AQ3 many antioxidant-rich foods and are intermediates in the production of flavanoids. They were the first inhibitors found of the MDM–p53 interaction, as was cholorofusin, a fungal metabolite [208, 219,220]. However, their activity and cell and animal models are currently unconfirmed. The small molecule inhibitors nutlin and RITA are potent and selective MDM2 antagonists, which bind to MDM2, blocking its suppression of p53 [221,222] in vitro and in vivo tumor models and are promising for future study [223].

In summary, p53 and the MDM2–p53 feedback loop are highly relevant to cancer formation and progression. Hence, using p53 as an anticancer gene therapy or targeting this loop for anticancer therapy presents a very promising approach. Other alternative strategies could be designed by either disrupting MDMX–p53 binding or screening compounds that target the central domain of MDM2 or MDMX, thus inhibiting their ability to inactivate p53. Although we have a long path to march in order to develop strategies stemmed from these concepts for effective cancer treatment, such a triumphant day is within reach, given that tremendous effort will continuingly be spent expanding upon the wealth of knowledge already established in this exciting and advancing arena.

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