Review Article

p53: Structure, Function and Therapeutic Applications

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Since the *p53* tumor suppressor gene has been found to be mutated in more than 50% of human cancers, it has attracted the interest of numerous researchers. The capacity of p53 for multiple biological functions can be attributed to its ability to act as a sequence-specific transcription factor to regulate expression of over one hundred different targets, and thus to modulate various cellular processes including apoptosis, cell cycle arrest and DNA repair. The p53 protein with its unique C- and N-terminal structures is rigidly modulated by several important biological processes such as phosphorylation, acetylation and ubiquitination, through which it effectively regulates cell growth and cell death. *p53* mutations can lead either to loss or change of p53 binding activity to its downstream targets and may thus induce aberrant cell proliferation, with consequent malignant cellular transformation. Based on p53's critical role in carcinogenesis, scientists have developed multiple effective strategies for treating cancer by enhancing function of wild-type p53 or increasing p53 stability. This review will focus on (i) discussing of the relationship between p53 structure and function, (ii) *p53* mutations, and (iii) recent strategies for improving the efficacy of cancer treatment by therapeutic manipulation of p53.

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Introduction

p53 protein was first identified in 1979 as a transformation-related protein [1] and a cellular protein which accumulates in the nuclei of cancer cells and binds tightly to the simian virus 40 (SV40[2]) large T antigen [2,3]. The gene encoding p53 was initially found to have weak oncogenic activity as the p53 protein was observed to be overexpressed in mouse and human tumor cells [4].

However, almost 10 years later, researchers discovered that it was a missense mutant of p53 which had originally been considered as wild-type p53 (wt p53) in that previous study, and that the oncogenic properties of p53 actually resulted from a *p53* mutation [5,6], which was later called "gain of oncogenic function" [7]. By the early 1990s, data from the first *p53* knockout mice provided inarguable evidence in support of the potent tumor suppressor action of wt p53 [8]. In subsequent studies, p53 became widely recognized as a tumor suppressor, and the *p53* gene became probably the most common site for genetic alterations in human cancers [9,10]. Subsequent research with wt p53 clearly demonstrated that p53 was a major "guardian of the genome" [11]. The biological consequences of p53 activity include cell-cycle regulation, induction of apoptosis, development, differentiation, gene amplification, DNA recombination, chromosomal segregation, and cellular senescence [12]. Presently, p53 is known to play a key role in practically all types of human cancers, and the mutation or loss of the *p53* gene can be identified in more than 50% of all human cancer cases worldwide. This significant involvement in oncogenesis extends far beyond the simple role in viral transformation p53 was suspected of playing in earlier investigation.

p53 belongs to an unique protein family which includes three members: p53, p63 [13] and p73 [14]. Although these proteins are structurally and functionally related to each other, p53 seems to have evolved in higher organisms to prevent tumor development, whereas p63 and p73 have clear roles in normal developmental biology [15].

Because p53 plays a pivotal role in regulation of the cell cycle and induction of apoptosis, there has been enthusiasm about its potential for therapeutic application. It is not surprising that the prominent position p53 plays in tumor development has spurred extensive research into both its basic biologic and clinical aspects. To better understand the relationship between p53 antineoplastic activities and its structure and function, this review focuses on describing biochemical modifications of p53, *p53* mutations and its applicability in cancer treatment.

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[2]**Abbreviations:** SV40, simian virus 40; wt p53, wild-type p53; mt p53, mutated p53; MDM2, murine double minute 2; DSBs, double strand breaks in DNA; ATM, ataxia-telangiectasia mutated protein; ATR, ATM and Rad3-related protein; Gadd45, growth arrest and DNA-damage-inducible protein 45; CDK, cyclin-dependent kinase; Bax, Bcl-2-associated X protein; DR5, death receptor 5; PIG3, p53-inducible gene 3; Puma, p53-upregulated modulator of apoptosis; PIDD, p53-induced protein with death domain; PERP, p53 apoptosis effector related to PMP-22; Apaf-1, apoptotic protease-activating factor-1; p53AIP1, p53-regulated apoptosis-inducing protein 1; Bid, BH3-interacting death agonist; 5-FU, 5-fluorouracil; IAPs, inhibitor of apoptosis proteins; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; ASPP, Apoptotic-Stimulating Protein of p53; HDAC1, histone deacetylase 1; PML, promyelocytic leukaemia protein; YY1, Yin Yang 1; PLD, phospholipase D; HATs, histone acetyltransferases; PCAF, p300/CBP-associated factor; SSDB, sequence-specific DNA binding; APC, adenomatosis polyposis coli protein; HPV, human papilloma virus; PRIMA, p53 reactivation and induction of massive apoptosis.

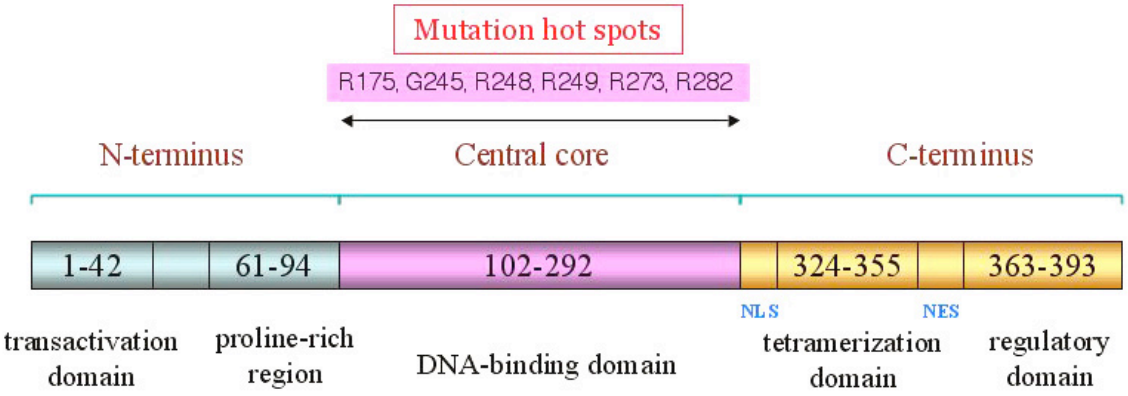

Figure 1: Schematic representation of the p53 structure. p53 contains 393 amino acids, consisting of three functional domains, i.e. an N-terminal activation domain, DNA binding domain and C-terminal tetramerization domain. The N-terminal domain includes transactivation subdomain and a PXXP region that is a proline-rich fragment. The central DNA binding domain is required for sequence-specific DNA binding and amino acid residues within this domain are frequently mutated in human cancer cells and tumor tissues. The Arg175, Gly245, Arg248, Arg249, Arg273, and Arg282 are reported to be mutation hot spots in various human cancers. The C-terminal region is considered to perform a regulatory function. Residues on this basic C-terminal domain undergo posttranslational modifications including phosphorylation and acetylation. Numbers indicate residue number. NLS, nuclear localization signal sequence; NES, nuclear export signal sequence.

The structure of p53

Human p53 is a nuclear phosphoprotein of MW 53 kDa, encoded by a 20-Kb gene containing 11 exons and 10 introns [16], which is located on the small arm of chromosome 17 [17]. This gene belongs to a highly conserved gene family containing at least two other members, *p63* and *p73*.

Wild-type p53 protein contains 393 amino acids and is composed of several structural and functional domains (Figure 1): a N-terminus containing an amino-terminal domain (residues 1-42) and a proline-rich region with multiple copies of the PXXP sequence (residues 61-94, where X is any amino acid), a central core domain (residues 102-292), and a C-terminal region (residues 301-393) containing an oligomerization domain (residues 324-355), a strongly basic carboxyl-terminal regulatory domain (residues 363-393), a nuclear localization signal sequence and 3 nuclear export signal sequence [18-20]. The amino-terminal domain is required for transactivation activity and interacts with various transcription factors including acetyltransferases and MDM2 (murine double minute 2, which in humans is identified as Hdm2)[21,22]. The proline-rich region plays a role in p53 stability regulated by MDM2, wherein p53 becomes more susceptible to degradation by MDM2 if this region is deleted [23]. The central core of this protein is made up primarily of the DNA-binding domain required for sequence-specific DNA binding (the consensus sequence contains two copies of the 10-bp motif 5'-PuPuPuC(A/T)-(T/A)GPyPyPy-3', separated by 0-13 bp)[24]. The basic C-terminus of p53 also functions as a negative regulatory domain [20], and has also been implicated in induction of cell death [25]. According to the allosteric model, in which C-terminal tail of p53 was considered as a negative regulator and may regulate the ability of its core DNA binding domain to lock the DNA binding domain as an latent conformation. If the interaction between the C-terminus and the core DNA binding domain is disrupted by posttranslational modification (such as phosphorylation and acetylation), the DNA binding domain will become active, thus induce an enhanced transcriptional activity. The central region of p53 is its most highly conserved region, not only when p53 is compared with its homologues from *Drosophila* and *Caenorhabditis elegans*, but also as compared with its mammalian family members, p63 and p73 [26]. Structural studies of p53 have revealed that the majority of *p53* mutations found in cancers are missense mutations that are mostly located in the central DNA-binding domain, and more than 80% of *p53* mutation studies have focused on residues between 126–306 [27]. In the p53 family, both p73 and p63 show considerable homology with p53 and have similar domain structures including an oligomerization domain, with over 60% amino acid identity within the DNA binding region [28], and all three of these proteins can induce apoptosis [29]. However, at the same time there are many structural and functional differences between p53 and its other two family members. Because space in this review is limited, a description of the differences between p53 and its homologues will not be detailed in this review.

The physiological functions of p53

As a tumor suppressor, p53 is essential for preventing inappropriate cell proliferation and maintaining genome integrity following genotoxic stress [20,30]. Following various intracellular and extracellular stimuli, such as DNA damage (by means including ionizing radiation, UV radiation, application of cytotoxic drugs or chemotherapeutic agents, and infectious virus), heat shock, hypoxia, and oncogene overexpression, wt p53 is activated and emerges as a pivotal regulatory protein which triggers diverse biological responses, both at the level of a single cell as well as in the whole organism [20,30,31]. p53 activation involves an increase in overall p53 protein level as well as qualitative changes in the protein through extensive posttranslational modification, thus resulting in activation of p53-targeted genes [32]. For example, in response to DNA damage which leads to double strand breaks in DNA (DSBs), ATM (ataxia-telangiectasia mutated) protein kinase is activated which in turn activates Chk2 kinase [33]. ATM and Chk2 then both phosphorylate p53 at distinct sites leading to p53-dependent cell cycle arrest or apoptosis [34,35]. In addition, DNA damage can also lead to replication blockage, thus activating the ATR (ATM and Rad3-related) kinase. Consequently, both activated ATR and subsequently activated Chk1 phosphorylate and activate p53 [36,37]. Genes activated by wt p53 are functionally diverse and constitute downstream effectors of signaling pathways that elicit diverse responses such as cell-cycle checkpoints, cell survival, apoptosis, and senescence [38]. Many of the multiple functions of p53 including

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the primary role of p53 in tumor suppression, can be attributed to its ability to act as a sequence-specific transcription factor which regulates expression of different cellular genes to modulate various cellular processes [39], although protein-protein interactions may also play a role. In response to various types of stress, p53 is accumulated in the nucleus and binds to specific sites in the regulatory regions of p53-responsive genes, and then strongly promotes the transcription of such genes [24]. The p53 downstream targets are differentially activated depending on the cell type, extent of the damage which has influenced p53 activation, and various other as yet unidentified parameters [40].

Many approaches have been employed to identify the targets of p53 in various experimental systems [41]. As a result of these efforts, hundreds of physiologically p53 responsive genes have been reported. These genes include genes involved in cell cycle arrest and DNA repair, as well as apoptosis and senescence-related genes (Figure 2), such as genes for $p21^{Waf1/Cip1}$, Gadd45 (growth arrest and DNA-damage-inducible protein 45) and genes of the Bcl-2 family [20,42].

Besides leading to direct or indirect transcriptional activation, elevated levels of wt p53 also cause repression of gene expression [43]. Genes which may be repressed by p53 include *bcl-2, bcl-X, cyclin B1, MAP4* and *survivin*, some of them are negative regulators of apoptosis [20,44]. Intriguingly, using ovarian cancer cells infected with p53-expressing adenovirus indicated that approximately 80% of the putative p53-responsive genes are in fact repressed by p53 [45]. The functions of p53 target genes are diverse, corresponding to p53's activity as a multifunctional protein.

Cell-cycle regulation

Among various cellular responses induced by p53, most notable are the induction of cell cycle arrest and apoptosis. It appears that the ability of p53 to prevent cell growth is pivotal to its tumor suppressor functions. p53 can induce cell cycle arrest in the G_1, G_2 and S phases of the cell cycle [46]. The induction of cell cycle arrest at G_1 and G_2 by p53 provides additional time for the cell to repair genomic damage before entering the critical stages of DNA synthesis and mitosis. The arrested cells can be released back into the proliferating pool through p53's biochemical functions that facilitate DNA repair including nucleotide excision repair and base excision repair [47].

A cyclin-dependent kinase (CDK) inhibitor, $p21^{waf1/Cip1}$ is perhaps the best known downstream target of p53 among the various p53 target gene products identified. $p21^{waf1/Cip1}$ is a primary mediator of p53-dependent G_1 cell cycle arrest following DNA damage [48-50]. In response to cellular stresses, p53 upregulates endogenous $p21^{waf1/Cip1}$ mRNA and protein levels [49]. $p21^{waf1/Cip1}$ binds cyclin-CDK complexes through the ZRXL motif [51]. Overexpression of $p21^{waf1/Cip1}$ induces G_1 arrest by blocking cyclin E/CDK2-mediated phosphorylation of Rb and release of E2F which functions to induce expression of genes required for S phase entry [25]. This response is also governed by other p53 target gene products, as seen for example, in the increased expression of Gadd45 and 14-3-3δ that participate in p53-driven G_2 arrest [52,53]. Gadd45 binds to CDC2 (i.e. CDK1), preventing cyclin B/CDC2 complex formation and subsequently inhibiting the kinase activity [52]. A scaffold protein 14-3-3δ removes cyclin B/CDC2 from the nucleus to physically separate cyclin B/CDC2 from its target proteins. Overexpression of 14-3-3δ induces G_2 arrest [54].

Induction of apoptosis

As a cellular gatekeeper [31,55], one of roles of p53 is to monitor cellular stress and to induce apoptosis as necessary [38]. In tissues where stressors generate severe and irrevocable damage, p53 can initiate apoptosis, thereby eliminating damaged cells. Studies with flies and nematodes have shown that induction of cell death following genotoxic challenges appears to be a function of p53, whereas in higher organisms p53 activity is acquired for cell growth arrest [56,57].

Apoptotic gene products which are induced by p53 include Bax (Bcl-2-associated X protein)[58,59], DR5/KILLER (death receptor 5)[60], DRAL [61], Fas/CD95 (cell-death signaling receptor)[62], PIG3 (p53-inducible gene 3)[63], Puma (p53-upregulated modulator of apoptosis)[64,65], Noxa (from the Latin word for "harm" or "damage")[66], PIDD (p53-induced protein with death domain)[67], PERP (p53 apoptosis effector related to PMP-22)[68], Apaf-1 (apoptotic protease-activating factor-1)[69], Scotin [70], p53AIP1 (p53-regulated apoptosis-inducing protein 1)[71], and others. The p53 associated apoptotic targets can be divided into several groups based on their functions and their executed pathways (Figure 3). The products of these genes may induce apoptosis through either an extrinsic pathway or an intrinsic pathway, namely the death receptor pathway and the mitochondrial pathway, respectively. The intrinsic apoptotic pathway is engaged when cells are challenged by stress and is dominated by the Bcl-2 family proteins [72,73]. The Bcl-2 family proteins are composed of three classes: anti-apoptotic proteins Bcl-2 and Bcl-X_L, pro-apoptotic proteins Bax, Bak and Bcl-XI , and pro-apoptotic "BH3-only" proteins Bid (BH3-interacting death agonist), Bad, Noxa, and Puma [74]. In the regulation of the intrinsic pathway, pro-apoptotic gene products such as Bax, Bid, Puma, Noxa, and p53AIP1 localize to the mitochondria and promote the loss of mitochondrial membrane potential and release of cytochrome *c*, resulting in the formation of the apoptosome complex with Apaf-1 and caspase 9 [64-66,71].

These apoptosis-related gene products mentioned above are closely associated with p53 function. Bax was the first identified p53-regulated pro-apoptotic Bcl-2 family member [58], and p53-responsive elements have been unequivocally identified in the *bax* gene [59]. Bax is specificlly required for Puma-mediated apoptosis, and it also participates in the death response as an indirect target of p53 through Puma [75]. The requirement for Bax in p53-mediated apoptosis appears to be cell-type dependent. Loss of Bax is responsible for nearly half of the accelerated tumor growth in brain tumors that is related to loss of p53 function [76,77]. Bax also accounts for nearly half of p53-dependent apoptosis induced by 5-fluorouracil (5-FU) in colorectal cancer cells [78]. On the other hand, Bax is dispensable for the apoptosis induced by irradiation in thymocytes and intestinal epithelial cells [79]. The first evidence which suggested that mitochondria might be involved in p53-dependent apoptosis was the observation that Bcl-2 protected cells from p53-dependent apoptosis [80]. Several Bcl-2 family proteins and mitochondrial proteins such as Puma, Noxa, p53AIP1, and PIGs are implicated in p53-dependent apoptosis. They are activated in a p53-dependent manner following DNA damage. Puma induces very rapid apoptosis, which occurs within hours following its expression [64-66]. p53AIP1 can cause mitochondrial membrane potential dissipation by interacting with Bcl-2 [71]. p53 also regulates the genes encoding Apaf-1, a key component of the apoptosome [69], and PIG3, which may cause mitochondrial depolarization [63]. Nevertheless, activated p53 can directly or indirectly modulate the expression of its targeted proteins and other proteins that control mitochondrial membrane permeability, and can therefore modulate the release of mitochondrial proteins which further carry out apoptosis.

Another p53-related class of pro-apoptotic gene products is the components of the death receptor-mediated extrinsic pathway. In this cell death pathway, p53 can promote apoptosis through activation of the death receptors located at the

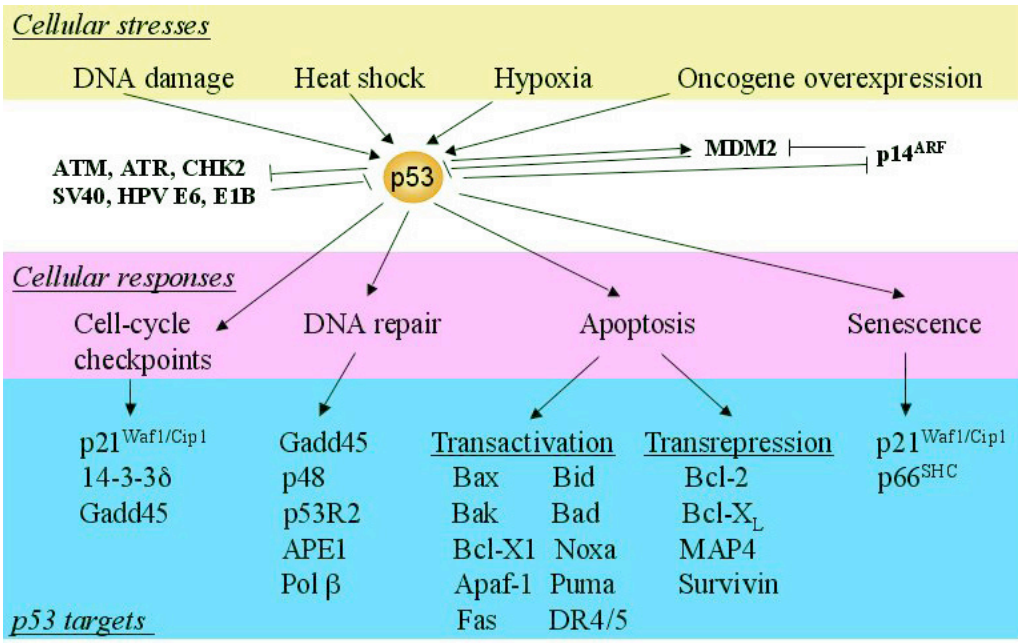

Figure 2: p53 locating at the crossroads of complex networks of stress response pathways. Various intercellular or extracellular stresses elicit cellular responses directly or indirectly through p53 activation. p53 activates its downstream targets to perform various functions including cell cycle arrest, DNA repair, apoptosis, and senescence.

plasma membrane, including Fas/CD95 [62], DR4 [81] and DR5 [60], and lead to inhibition of the production of IAPs (inhibitor of apoptosis proteins)[60]. Both DR5 and DR4 can trigger or induce apoptosis by TRAIL (tumor necrosis factor-related apoptosis-inducing ligand), Fas ligand and chemotherapeutic agents [81,82], and Fas is indispensable for p53-dependent apoptosis in most tissues [62]. p53 may also induce apoptosis via an endoplasmic reticulum-dependent mechanism by transactivating the expression of Scotin, a protein located in the endoplasmic reticulum and in the nuclear membrane [70]. It has been suggested that the intrinsic apoptotic pathway is primarily utilized in p53-mediated apoptosis, whereas the extrinsic pathway is used to augment the apoptotic response [42].

p53 can also promote apoptosis through transcription independent mechanisms (including direct shuttling of p53 to the mitochondrial membrane)[74]. p53 probably has direct apoptotic activity in the absence of transcription or protein synthesis under certain conditions and in certain cell types [83,84]. In mitochondria, p53 directly binds to Bcl-X_L/Bcl-2 to displace Bax or BH3 domain-only pro-apoptotic proteins, and thus facilitates Bax-dependent mitochondrial apoptotic changes [85]. p53 can also bind to mitochondrial Bak and induce Bak oligomerization, which facilitates the release of cytochrome *c* after permeabilization of the mitochondrial membrane [85]. This is a very rapid response (30 min), which precedes the transcriptional response (taking at least 2 h)[86]. This direct response is tissue-specific and appears to be limited to radiosensitive tissues.

While cell cycle arrest can function to inhibit the growth of normal cells, it seems that cells which have attained oncogenic activation are less susceptible to such inhibition [87]. The ability of p53 to induce apoptosis appears to be well correlated with its ability to suppress malignant transformation. Loss of p53-dependent apoptosis accelerates mouse brain tumorigenesis [76]. Similarly, the observation that mice harboring the p53 R172P mutant develop many tumors may be due to lack of p53-induced apoptosis [88]. These results reveal that regulation of apoptosis is an important and evolutionarily conserved tumor suppressor function of p53.

It appears that transcriptional factors such as c-Myc, JMY (junction-mediating and regulatory protein), ASPP (Apoptotic-Stimulating Protein of p53) family, p63, and p73 can influence the balance between cell cycle arrest and apoptosis [89]. A crucial balance between Puma and $p21^{Waf1/Cip1}$ has been identified which determines the onset of arrest or death in response to exogenous p53 expression in human colorectal cancer cells [74]. Growth arrest through activation of $p21^{Waf1/Cip1}$ is the normal response to p53 expression in these cells. If *$p21^{Waf1/Cip1}$* is disrupted, cells die through apoptosis. However, when *Puma* is disrupted, apoptosis is prevented. Cell cycle arrest is not only a positive element of the p53 response, but is also a negative element for p53-dependent apoptosis in some situations. Induction of apoptotic genes alone is sometimes not sufficient to induce apoptosis, as the high levels of cell cycle inhibitors may dominant and lead to cell cycle arrest [89]. Apoptotic response can be enhanced through abolition of cell cycle arrest (for instance by suppression of $p21^{Waf1/Cip1}$ or 14-3-3δ). Following p53 expression or DNA damage in colorectal cancer cells, apoptosis is inhibited through cell cycle arrest mediated by $p21^{Waf1/Cip1}$ and/or 14-3-3δ [90]. If $p21^{Waf1/Cip1}$ or 14-3-3δ is removed from these cells, cell death rather than cell cycle arrest may result. When Puma is removed, these cells become resistant to apoptosis [75]. Under certain conditions, cell cycle arrest protects cells from apoptosis. However, under other circumstances, cells undergo apoptosis.

The regulation of p53 level and activity

Under normal circumstances, wt p53 is maintained at very low concentrations within the cells and exists mainly in an inactive latent form [31]. During the cell cycle progression, the low basal level of wt p53 has to be precisely controlled [91]. In normally growing cells, the half-life of p53 is limited to minutes, whereas cellular stress or exposure to DNA-damaging agents prolongs it to hours [92]. Increased levels

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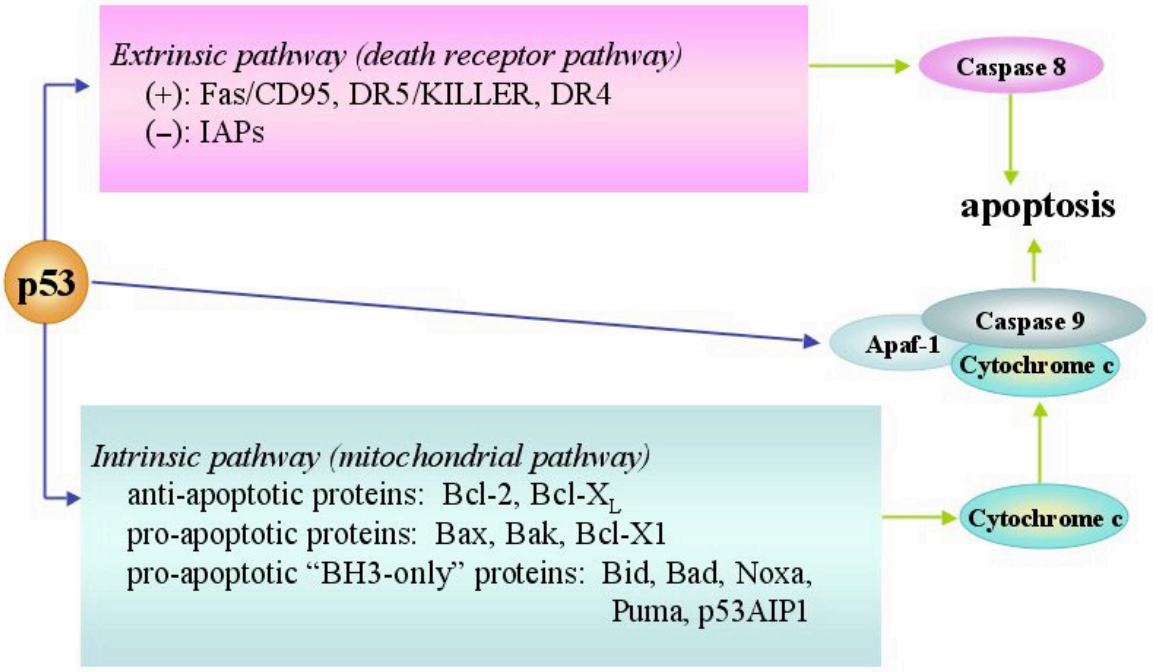

Figure 3: p53-associated genes and pathways involved in apoptotic cell death. p53 induces apoptosis mainly via two pathways: extrinsic and intrinsic pathways. The p53-associated extrinsic pathway is mainly executed by activating caspase 8 to induce apoptosis, whereas the p53-associated intrinsic pathway is almost executed by influencing mitochondrial proteins, by which activate caspase 9 to induce apoptosis. In addition, p53 may directly activate Apaf-1 to induce apoptosis.

of the p53 protein are primarily regulated through lengthening of its half-life. The level of p53 and its activities in the cell depend on the cell's situation and extracellular stimuli.

Genes involved in regulating p53 level and activity

The regulation of p53 level and activity involves a complex network of a multitude of cellular proteins including HPV16 E6 [93], WT-1 [94], E1B/E4 (95), SV40 T-antigen [96,97], MDM2 [98,99], JNK [100], Pirh2 [101,102], and PARP-1 [20,103]. Moreover, wt p53 can switch between a latent and an active form in its function as a transcription factor. The binding of SV40 T antigen, WT1 or E1B/E4 with p53 increases its stability, whereas the association of E6 or MDM2 with p53 accelerates its degradation. MDM2 is an important related protein, which is the product of a p53 inducible gene. The importance of MDM2 in the regulation of p53 levels is demonstrated by the fact that disruption of the *MDM2* gene is lethal in early embryos, whereas the concurrent inactivation of the *p53* gene rescues the animal from a lethal consequence [104]. MDM2 inhibits p53 activity by blocking its transcriptional activity, favoring its nuclear export and stimulating its degradation. MDM2 protein has been found to play an additional role in blocking the interaction of p53 with the transcriptional apparatus by binding to and shielding the transactivation domain of p53 within its N-terminus [99,105]. MDM2 protein possesses intrinsic E3 ubiquitin-ligase activity and mediates both the ubiquitylation and proteasome-dependent degradation of p53 [106]. Ubiquitinated p53 is exported to the cytoplasm, thereby moving it away from its site of action and promoting its rapid degradation by the proteosome [98]. MDM2 can also recruit the histone deacetylase 1 (HDAC1) to deacetylate key lysine residues in the C-terminus of p53, thus making them available for ubiquitination. The *MDM2* gene itself also contains a p53-dependent promoter and is transcriptionally regulated by p53 following challenge of the cell by various stresses [107]. In this fashion, the p53 protein regulates the *MDM2* gene at the level of transcription and the MDM2 protein regulates the p53 protein at the level of its activity, and an autoregulatory feedback loop is established that regulates both the activity of the p53 protein and the expression of the *MDM2* gene. In this sequence of events, it is wt p53 that is targeted by MDM2 for degradation, whereas mt p53 is outside of this negative feedback loop and accumulates to high levels in cancer cells (as will be discussed later).

It is becoming evident that a number of mechanisms exist to abolish MDM2-mediated degradation of p53, thereby allowing the maintenance of a p53 response initiated by various genotoxic stimuli [20,30]. Under stress conditions, distinct signaling pathways can be activated to prevent p53 from ubiquitylation and degradation through posttranslational modifications and abolishment of MDM2 activity. Several regulators of p53 have been identified recently, such as the positive regulator PML (promyelocytic leukaemia protein)[108,109] and the negative regulators YY1 (Yin Yang 1)[110], survivin [111] and PLD (phospholipase D)[112]. All these regulators appear to influence p53 through MDM2, and they all appear to affect the transcriptional activity of p53.

Posttranslational modifications of p53

In response to stress, p53 activity and its stabilization are also highly governed through complex networks of post-translational modifications [113], including phosphorylation, acetylation, ADP-ribosylation, ubiquitylation, sumoylation, neddylation, and cytoplasmic sequestration [19,20,30]. Most of these modifications occur in the N- and C-terminal regions of p53. Posttranslational phosphorylation and acetylation are the main modifications enhancing the transcription activating ability of p53 because these modifications generally result in p53 stabilization and accumulation in the nucleus, where p53 interacts with sequence-specific sites of its target genes [114,115]. Posttranslational modifications also prevent p53 from being targeted for degradation as these modi-

fications of p53 at distinct sites may abolish the interaction between MDM2 and p53 [116].

Up to this point, phosphorylation has been regarded as the most commonly reported protein modification that occurs in mammalian cells, although it appears that the frequency and importance of acetylation may actually rival phosphorylation as a crucial posttranslational modification. Phosphorylation of p53 generally results in its stabilization and has also been shown to increase its sequence-specific DNA binding [117]. Twenty serine and threonine sites on p53 have been identified to be phosphorylated in human cells following DNA damage induced by ionizing radiation or UV irradiation [19,118]. Most of these sites are located in the N-terminal region at the Ser6, Ser9, Ser15, Thr18, Ser20, Ser33, Ser37, Ser46, Thr55, and Thr81 residues, but there are also other sites in the C-terminal domain at Ser315, Ser366, Ser371, Ser376, Ser378, Thr387, and Ser392, and also in the central core at Ser149, Ser150, Ser155 [19,118]. Phosphorylation at most of these sites is induced by DNA damage. However, there are some sites such as Thr55 and Ser376, which are repressed upon genotoxic stress [114].

Many protein kinases have been implicated in phosphorylating p53. Different protein kinases phosphorylate several sites on p53, and in some instances the same site can be phosphorylated by more than one protein kinase. For example, phosphorylation at Ser15 is mediated by ATM/ATR, either directly or through Chk1/Chk2, or at Ser20 by Chk1/Chk2 [35-37].

Expression of oncogenic *ras* in primary human cancer cells causes phosphorylation of p53 at Ser33 and Ser46 by $p38^{MAPK}$. It is of interest that the activity of $p38^{MAPK}$ is negatively regulated by PPM1D phosphatase, whose expression is upregulated by p53 [119]. This forms a negative feedback loop to modulate p53 activity in normal cells. It has been shown that inactivation of $p38^{MAPK}$ or overexpression of PPM1D significantly reduces p53-dependent transactivation. Phosphorylation of p53 at Ser33, Thr81 and Ser315 gives rise to binding sites for Pin1, a peptidylprolyl isomerase that recognizes the phosphorylated Ser/Thr-Pro motif [120,121]. Overexpression of Pin1 enhances p53-dependent transactivation, gene expression and apoptosis in an enzyme activity-dependent manner. In a Pin1-inducible cell line, the expression of Pin1 does not change the level of p53, but nevertheless increases the expression of p53 target genes [121], suggesting that Pin1 directly affects the transactivation ability of p53 through inducing conformational change.

Stress-induced N-terminal phosphorylation increases p53 stability by dissociating the negative regulator MDM2. For example, Ser15, Thr18, and Ser20, which are located in the MDM2-binding site, are phosphorylated in response to DNA damage. This phosphorylation alleviates the inhibition or degradation of p53 by MDM2, leading to p53 stabilization and activation [122]. Phosphorylation of Ser20 by Chk1 and Chk2 in response to ionizing radiation is important in abolishing the p53-MDM2 interaction [37,123]. In addition, phosphorylation at Ser33 and Ser46 by p38, and phosphorylation of Thr81 by JNK can lead to p53 stabilization [124].

Much less is known about the biological consequences of p53 dephosphorylation. It has been reported that Ser376 [125] and Thr55 are dephosphorylated in cells exposed to ionizing radiation, indicating that dephosphorylation may also contribute to the activation of p53. By contrast, a recent study showed that dephosphorylation of p53 was associated with increased $p21^{Waf1/Cip1}$ expression together with increased caspase 3 activity and induction of apoptosis, presumably because of p53 transcriptional activity [126]. However, this was not demonstrated by direct evidence.

Acetylation may also have a crucial role in stabilization of p53 [127] and p53 transcriptional activation [128]. Almost every type of cellular stress increases acetylation levels of p53 in a range of cell types. Several lysines can be acetylated on p53 and all are located in the C-terminal region, including Lys305, Lys320, Lys372, Lys373, Lys381, Lys382, and Lys386. These acetylated residues are located in the regulatory domains adjacent to the tetramerization domain. Two histone acetyltransferases (HATs) are known to acetylate p53: p300 or/and CBP acetylates the C terminus of p53 at Lys305, Lys372, Lys373, Lys381, and Lys382, whereas PCAF (p300/CBP-associated factor) acetylates Lys320 [129-132]. The consensus is that the recruitment of co-activators CBP and p300 stabilizes p53 and augments sequence-specific DNA binding following DNA damage [129,133-135]. Moreover, the acetylation of p53 can dramatically stimulate its sequence-specific DNA binding activity, possibly as a result of an acetylation-induced conformational change [129,136,137]. It has been shown that acetylation by p300/CBP and PCAF enhances the transactivation activity of p53 in cells, whereas deacetylation of p53 suppresses such activity [19]. Our recent study has shown that depsipeptide, an inhibitor of HDAC, can induce $p21^{Waf1/Cip1}$ expression through acetylation of p53 at Lys373/Lys382 [138]. Another study suggests that HDAC inhibitors TSA and butyrate both activate p53 by acetylation of Lys320 and Lys373, upregulate *PIG3* and *Noxa* expression, and also induce apoptosis in cancer cells with both wild and pseudo-wild-type *p53* genes [139]. Another p53-binding protein SIRT1 (human Sir2) is a deacetylase that can specifically deacetylate p53 at Lys382 and attenuate p53 transcriptional activity [140,141]. Deacetylation of p53 compromises its ability to induce cell cycle arrest and apoptosis [142]. Significantly, inhibition of SIRT1 activity can increase p53 acetylation but does not alter cell survival following DNA damage in primary human mammary epithelial cells and certain cell lines [143].

Acetylation may also regulate the stability of p53 by inhibiting its ubiquitylation induced by MDM2 [144]. The ubiquitylation and acetylation of p53 occur at the same sites in the C terminus, suggesting that these modifications may compete for the same residues. The acetylation sites of p53 are essential for ubiquitylation and subsequent degradation of p53 by MDM2. That is to say, p53 acetylation is directly involved in the regulation of its ubiquitylation and subsequent proteolysis induced by MDM2 [144]. Inhibition of p53 deacetylation leads to a longer half-life of endogenous p53 [128,138,145], suggesting that acetylation of p53 may also contribute to p53 stabilization. On the other hand, in response to a variety of stress-inducing agents, MDM2 can inhibit acetylation of p53 mediated by p300/CBP *in vitro* and *in vivo* [145,146].

Phosphorylation and acetylation of p53 are interrelated [19,131,147]. For example, in response to UV or ionizing irradiation, the N terminus of p53 first becomes phosphorylated at Ser33 and Ser37, and in turn phosphorylated p53 activates p300 and PCAF to induce p53 acetylation at Lys373/Lys382 and Lys320, respectively [131,148]. In some cases, phosphorylation might be required for subsequent acetylation of p53. Phosphorylation of p53 at certain sites such as Ser15 and Ser20 enhances its interaction with p300/CBP and potentiates p53 acetylation [131,148], suggesting that acetylation may have an important role in activating p53-responsive genes by DNA-damaging agents, which is induced by phosphorylation of p53 at multiple sites. One recent study has demonstrated that p53 C-terminal phosphorylation by Chk1 and Chk2 may also modulate the level of p53 C-terminal acetylation [149]. These data indicate that p53 modulation is a complex process, and the biological consequences of p53 activation induced by certain stimuli may be dependent on p53 posttranslational modifications at multiple sites.

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Another mechanism allowing p53 to overcome targeting by MDM2 is modification-independent and involves the upregulation of the human $p14^{ARF}$ (mouse $p19^{ARF}$) protein. The ARF protein binds directly to MDM2 in a region distinct from the p53 binding region and prevents degradation of p53 [150].

Mutation of p53

The p53 gene is often found to be genetically altered in tumors, and is one of the most frequently inactivated genes in human cancers [9,151]. Aberrant stimulation of cell proliferation leads to DNA replication stress, DNA DSBs, genomic instability, activation of the DNA damage checkpoint, and ultimately p53-dependent apoptosis. p53-dependent apoptosis suppresses expansion of pre-cancerous lesions (p53 tumor suppressor function) and provides selective pressure for p53 inactivation [152,153]. The function of p53 tumor suppressor in cancers can be lost by various mechanisms, including lesions that prevent activation of p53, mutations within the *p53* gene itself or mutations of downstream mediators of p53's function [20]. Acquired mutations (more than 18,000 mutations have been identified) in the *p53* gene are found in all major types of human cancers. Approximately half of all human tumors have a mutation or loss in the *p53* gene leading to inactivation of its function [19,154]. For example, p53 mutation frequency is 70% in lung cancer, 60% in cancers of colon, head and neck, ovary, and bladder, and 45% in stomach cancer. In many of the other approximately 50% human tumors in which p53 is not functionally inactive, p53 function is impaired owing to mutations in proteins operating either upstream or downstream of p53 targets, such as MDM2 or the E6 protein of HPV, or deletion of key p53 co-activators such as the ARF gene [9,30,155]. Genes other than *p53* are also frequently mutated in human cancers, such as $p16^{INK4}$ and $p14^{ARF}$ [155,156]. However, there are no gene mutations as pervasive in multiple types of human cancers as the mutations in p53. Although the wt *p53* gene is a tumor suppressor gene, some mutants of *p53* (mt *p53*) can be considered to be oncogenes. Patients with the Li–Fraumeni syndrome, who have an inherited germline mutation in one of the two *p53* alleles, are at very high risk of developing cancer throughout their lifetimes [157,158]. The subsequent loss of the wt *p53* allele leads to tumors of the brain, breast, connective tissue, hematological system and adrenal gland. p53 inactivation also leads to cancer development in tumors without p53 mutation. For example, mice deficient in wt *p53* are susceptible to spontaneous tumorigenesis [8]. In tumors without p53 mutation, the p53 pathway is frequently inactivated by oncoproteins or by defective upstream signals [20,30]. Mutations in the *p53* gene can result in abolition of protein function and this loss of function may be linked to tumor progression and genetic instability. Clearly, inactivation of p53 is a key event in carcinogenesis. The presence of mt p53 protein, rather than the complete lack of wt p53 activity may confer a selective advantage to evolution of tumor cells [159]. Indeed, it has been demonstrated that mt p53 can be a causative factor in tumor progression, because expression of some mt p53 proteins in tumor cells with a p53-null background leads to an increase of their tumorigenic potential *in vitro* and *in vivo* [7].

The realization that some mt p53 proteins most likely play important roles as oncogenic factors in cancer progression led to the formulation of the "gain-of-function by mt p53" supposition. According to this hypothesis, mt p53 proteins unable to prevent uncontrolled growth and protect cells from genomic alterations may acquire novel activities that actually promote cell growth and survival [160]. For example, the role of wt p53 as a transcription factor is vital for its ability to induce apoptosis and inhibit tumor development [18], whereas mt p53 suppresses the expression of *CD95 (Fas/APO-1)* gene, which encodes a death receptor implicated in a variety of apoptotic responses [161]. This activity of mt p53 may be contributed to its "gain of function" effect in oncogenesis.

Mutant p53 proteins generally show significant phosphorylation and acetylation at sites that are well known to stabilize wt p53, thus potentially facilitating accumulation of dysfunctional mt p53 in the nucleus, where it can act as an oncogene. In tumor cells, phosphorylation of p53 at Ser46 and/or functional interaction with apoptotic cofactors (such as ASPP, JMY and p63/p73) allows for the activation of apoptotic target genes. These cofactors can bind p53 directly or indirectly, and assist p53 DNA binding by directly interacting with p53-responsive promoters. It is possible that phosphorylation alters the conformation of p53 to either enhance interaction with apoptotic cofactors, or allow binding to apoptotic target promoters [20].

Although mt p53 proteins are often referred to as being transcriptionally inactive, some mt p53 proteins that are unable to activate transcription of wt p53-inducible genes can potentially activate transcription of genes associated with growth or survival-promoting activities [7]. Some p53 mutants are able to bind the promoters of some p53 target genes such as $p21^{waf1/cip1}$ and *MDM2*, but are not able to bind promoters of proapoptotic genes like *bax* and *PIG3* [162]. Consequently, this class of mt p53 can induce cell cycle arrest as effectively as wt p53, but are unable to induce apoptosis [163,164]. Inactivation of p53 allows cancer cells to escape apoptosis, while still retaining the ability through Chk1 and Chk2 to arrest in G_2 until all DSBs are repaired [165]. This indicates that the transactivation deficiency of such mt p53 proteins applies only to a specific set of genes. Moreover, most promoters activated by mt p53 do not contain sequences resembling the wt-p53 consensus, suggesting that mt p53 may regulate transcription via response elements that are distinct from wt-p53 response elements [160]. It is interesting that different promoters activated by mt p53 show no sequence homology, suggesting that sequence-specific recognition is unlikely to be a parameter determining the specificity of mt-p53 DNA binding.

Certain work suggests that the interaction between mt p53 and DNA could be related to the conformation and structure of the DNA [166,167]. One recent study has shown that some inhibition of transcription by mt p53 requires binding of the protein to a different promoter site, independent from the presence of canonical p53 binding sites [161]. Sequence-specific DNA binding (SSDB) of wt p53 is not only sequence-specific, but also DNA structure-dependent, and is regulated by the non-SSDB activity of the p53 C-terminus [168]. In contrast to wt-p53-SSDB, the specificity of mt-p53 DNA binding is determined exclusively by DNA topology, due to the impairment of sequence-specific recognition affected by mutations in the core domain [160].

A characteristic feature of the p53 mutational map is the frequency of missense point mutations. Unlike many other tumor suppressor genes, more than 80% of p53 mutations result in single amino-acid substitutions that lead to the synthesis of a stable full-length protein rather than deletions, frameshifts or nonsense mutations like those found in most other tumor suppressor genes, such as *APC* (adenomatosis polyposis coli) [9,169]. These missense mutations lead to the synthesis of a protein that lacks specific DNA binding function and accumulates in the nucleus of tumor cells. The maintenance of mt p53 in tumor cells is believed to be required for both a dominant negative activity to inhibit wt p53 expressed by the remaining allele, and for a gain of function that transforms mutant p53 into a dominant oncogene [169]. Structural studies have revealed that the amino acid resi-

dues in the mutation hot spots of p53 are within the central region (residues 102-292), encoding the central DNA binding domain of the protein, whereas few *p53* mutations are found in the regulatory domains (N terminus, residues 1–99; C terminus, residues 301–393). Certain *p53* codons show an unexpectedly high mutation frequency, with 28% of the mutations affecting only six residues of p53, namely Arg175, Gly245, Arg248, Arg249, Arg273, and Arg282 [18](Figure 1). Among these six residues, the most frequently mutated sites are Arg248 and Arg273 that contact both DNA and ASPP2. The result of mutational inactivation of p53 by single amino acid substitutions is that many tumor cells retain the ability to express the mt p53 protein. These proteins are often more stable than wt p53, and are present at very high levels in tumor cell [20]. The higher proportion of missense mutations in p53 suggests that expression of mt p53 may confer some selective advantage to the cells expressing the mutant protein over cells null in p53 or expressing the wt form [170]. In a previous study, we have demonstrated that the human lung cancer cell line H719 lacks p53-dependent $p21^{Waf1/Cip1}$ expression due to a missense mutation at codon 242 of the *p53* gene (Cys→Trp)[171]. In contrast, $p21^{Waf1/Cip1}$ expression in response to treatment with γ irradiation or etoposide in wt p53-harboring A549 cells is correlated with an increase in wt-p53 [171]. It has been demonstrated that residues Leu22/Trp23 (human) and Leu25/Trp26 (mouse) within the transactivation domain in the N-terminus of p53 are indispensable for the transcriptional activity of p53 [22]. In p53 mutants in which residues Leu22/Trp23 or Leu25/Trp26 are replaced with Gln22/Ser23 or Gln25/Ser26, there was complete loss of ability to activate or repress the expression of p53 target genes [172]. Examination of the mutation frequency of p53 in over 10,000 tumors has shown that the probability of p53 acquering a mutation varies dramatically depending on the tissue in which the tumor originates. In lung cancer, for example, the p53 mutation frequency is as high as 75%, whereas only around 30% of breast tumors and as few as 5% of leukemias have acquired mutations in p53 [18].

Several models have been proposed to explain the high frequency of p53 inactivation in human cancer, such as the "guardian of the genome" hypothesis [11] and the "resistance to apoptosis" hypothesis [76], and the former has attracted a good deal of attention. According to this hypothesis, p53 can guard the genome against accumulation of oncogenic mutations. The strongest evidence in support of this hypothesis comes from analysis of *p53* knockout mice [165]. Since these mice develop tumors with 100% penetrance, the absence of p53 function must be conducive to the accumulation of oncogenic mutations and, by inference, the normal function of p53 may be to preserve genomic integrity.

Therapeutic applications of p53

The insights which have been provided by p53 research over the years have been for improvement of diagnostic techniques, accuracy of prognosis, and treatment of cancer. Since about half of all human tumors have an abnormal p53 which can occur early in carcinogenesis, and since post-translational modifications of p53 can reflect the type and magnitude of cellular stress [114], p53 can be a useful biomarker in carcinogenesis. Indeed, p53 has been used as a molecular signature to study both target tissues and surrogate fluids such as blood in high-risk cancer populations [173]. Mutated p53 protein accumulation and posttranslational modification endpoints could also prove useful in studying the efficacy of chemopreventive agents [38].

As p53 plays a key role in the cellular response to stress, it serves as a major barrier to tumorigenesis. This obstacle has to be removed in order for tumor development to proceed and restoration of wt p53 function is thus a potential key in anticancer therapies. Since MDM2 is an important negative regulator of p53, MDM2 hyperactivity may inhibit the function of p53 and lead to the development of a wide variety of cancers. For example, 30% of human sarcomas show no p53 mutations, but have an overexpressed *MDM2* gene [174]. It is believed that inhibiting the E3 activity of MDM2 and blocking the interaction of p53 with MDM2 are potential effective strategies for killing certain tumor cells selectively by restoring the function of wt p53 [41]. Therefore, many studies have focused on the p53-MDM2 interaction as the basis of a drug development strategy. A series of small molecule inhibitors have been developed, and some of these can bind to MDM2 and block its interaction with p53, including peptides that have been shown to elevate the levels of p53 protein and its transcriptional activity and trigger p53-dependent apoptosis in tumor cells [175,176]. A class of small molecules named *nutlins* have been identified to block p53/MDM2 interaction *in vitro* and *in vivo*. Treatment of tumor cells with *nutlins* results in induction of p53 and its target genes and triggering of apoptosis. Recently, a novel series of benzodiazepinedione antagonists of the p53/MDM2 interaction have been discovered which increase the transcription of p53 target genes and decrease proliferation of tumor cells expressing wt p53 [177]. One study suggests that antisense oligodeoxynucleotides targeted against MDM2 and $p21^{Waf1/Cip1}$ could be employed in a potential therapeutic strategy sensitizing tumor cells to certain antineoplastic agents [178]. One of the major concerns about blocking the p53/MDM2 interaction for use in treatment of cancer was the idea that activation of p53 might be toxic to normal tissues. However, certain data suggest that the mechanisms governing p53 activity in tumor cells and normal cells are quite different, so the different effects of p53 in reactivating different molecules in tumor cells and normal cells might provide a molecular basis for a therapy without the need for tumor targeting [179].

Another factor in p53 inactivation is the presence of the human papilloma virus (HPV). In cervical carcinomas, p53 is targeted by HPV encoded E6 protein, which potentiates p53 degradation and inactivates its function in 90% of cervical cancers [180]. Drugs that inhibit E6 should promote p53 reactivation and thus have selective therapeutic effect. It has been reported that leptomycin B and actinomycin D inhibit E6 expression, stabilize p53 and induce apoptosis in a model system of cultured cells [181]. Interestingly, both drugs can inhibit MDM2-mediated inactivation of p53, possibly via inhibition of p53 ubiquitylation (leptomycin B) or by decreasing *MDM2* gene transcription (actinomycin D)[182]. Although these specific molecules *per se* have limited therapeutic effect, these data serve to encourage a search for other compounds with similar effects.

Because the apoptotic function of p53 is critical for tumor suppression, induction of apoptotic pathways through p53-induced apoptotic targets may be an attractive strategy for anti-cancer treatment. Furthermore, the p53 apoptotic targets, unlike p53, are rarely mutated in human cancers [30]. Some of the p53 apoptotic targets, such as *bax*, *Puma*, *p53AIP1*, *Noxa* and others could potentially be used as targets for gene therapy [183]. For example, adenoviral gene transfer of *bax* can act synergistically with chemotherapy to induce apoptosis in tumors [184]. A recent study has demonstrated that siRNA targeting of survivin, a negative regulator of apoptosis which is downregulated by p53, could be potentially useful for increasing sensitivity to anticancer drugs, especially in drug-resistant cells with mutated p53 [185]. However, the effects of p53-dependent apoptosis are not always favorable for clinical use, and so the inhibitors of p53-mediated apoptosis might be used to transiently de-

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crease apoptosis in normal tissues when patients are receiving high doses of radiation or chemotherapy [89].

In addition to reactivation of wt p53 in tumors, introduction of the wt *p53* gene into tumors is also an important therapy. This is based on several observations including the fact that *p53*-null thymocytes and intestinal stem cells are more resistant to radiation-induced apoptosis than their normal counterparts, and *p53*-null mouse embryonic fibroblast cells are resistant to apoptosis induced by oncogene overexpression and chemotherapeutic agents [186-188]. *p53* null colorectal cancer cells are resistant to apoptosis induced by the anticancer agent 5-FU [189]. Expression of wt p53 was found to cause rapid loss of cell viability with morphological characteristics of apoptosis [190]. Delivery of the wt *p53* gene by replication defective adenoviruses in *p53*-null tumors can directly induce apoptosis and restore sensitivity to chemotherapeutic drugs. Irreversible cell cycle arrest is sufficient to elicit tumor regression after transfer of the *p53* gene in p53-deficient tumor cells [191]. Gene therapy based on the introduction of wt *p53* has been undergoing clinical trials, and some of the results of the clinical trials have been promising, such as in non-small cell lung cancer and in ovarian cancer [192]. Introduction of *p53*, *p73* and *p63* into colorectal cancer cell lines via adenoviral vectors results in adenovirus-mediated *p73* and *p63γ* transfer, suggesting a potential novel approach for the treatment of human cancers, particularly for tumors that are resistant to *p53* gene therapy [193]. However, improvement of the efficiency of gene therapy treatment is required, including development of the new generation vectors, since application of gene therapy strategy is limited at the moment largely by the low efficiency of infection by existing viral vectors *in vivo* and the robust immune response. New forms of p53 that have increased DNA binding to promoters of apoptosis-inducing genes, resistance to degradation, and enhanced thermodynamic stability will likely be more therapeutically active [194]. A strategy that improves the antitumor efficacy using an adenovirus expressing p53 fusion to VP22 protein of herpex simplex virus type 1 has already been developed [195].

There is a clear consensus that restoration of the function of mt p53 in tumor cells would also be of potential therapeutic benefit. This strategy should be specific to cancer cells, as normal cells contain no mt p53. Mutant p53 can be thought of as a "loaded gun", present in abundance in tumor cells – but with a jammed trigger [179]. Tumor cells should be sensitive to the restoration of the p53 pathway, because of specific suppression of this pathway mutation in neoplasia. Since mt p53 is unable to perform its function due to the defect in its folding which is produced by any one of many single amino acid substitutions, several approaches aimed at reversing this defect and restoring the function of mt p53 have been tried during the past few years. One such potential approach is the use of several peptides and small molecule compounds that can act to stabilize the structure of mt p53, and thus restore the specific DNA-binding, transcription and apoptosis functions to mt p53. They include synthetic peptides derived from the C-terminus of p53 [196], as well as peptides such as CDB3, and compounds isolated from chemical library screening such as CP-31398 and PRIMA-1 (p53 reactivation and induction of massive apoptosis). CDB3 stabilizes the structure of mt p53 proteins [197], and it binds mt p53 and efficiently induces the refolding of two hot spot p53 mutants, His273 and His175, in cancer cells. The transactivation activity of p53 can also be rescued by CDB3 [198]. PRIMA-1 selectively inhibits the growth of tumor cells by provoking apoptosis in a transcription-dependent fashion through conformational manipulation of p53 mutants to restore sequence-specific DNA binding [199]. CP-31398 is a small synthetic molecule [200] with the capacity to restore wt p53 function to mutants [201]. It has been suggested that it triggers apoptosis of human cancer cells through the intrinsic Bax/mitochondrial/caspase-9 pathway [202] and can stabilize wt p53 protein [203]. However, the exact molecular mechanism by which these molecules act upon the mis-folded mt p53 to restore its activity is as yet unclear.

The biochemical study of p53 may help us clarify its mechanisms of action for use in anticancer therapy. Data has suggested that the mechanism by which some chemotherapeutic agents confer antineoplastic activity is through inducing DNA damage, thereby activating *p53*, which in turn increases downstream target expression, leading to the inhibition of cell proliferation. In one previous study, we found that anticancer agent 5-aza-2'-deoxycytidine induces inhibition of cell proliferation by increasing p53-dependent $p21^{Waf1/Cip1}$ expression following DNA damage, and the resultant data have provided useful clues for judging the therapeutic efficacy of 5-aza-2'-deoxycytidine in the treatment of human cancer cells [204]. Another study also showed that cisplatin, widely used in cancer chemotherapy, can trigger cell cycle arrest and apoptosis following DNA damage through two distinct pathways, one involving p53, the other mediated by the p53-related protein p73. As such, a better understanding of the signaling networks involved in cisplatin toxicity would likely provide a rational basis for the development of new therapeutic strategies [205].

Concluding remarks

p53 was originally viewed as an oncogene, but during the past several decades it has come to be understood to be a tumor suppressor gene. During this time, many p53 family transcriptional targets have been identified as having the capacity to modulate various cellular processes including growth arrest, apoptosis, senescence, differentiation, and DNA repair. In fact, it has become evident that this small 53-kDa tumor suppressor is a molecular node at the crossroads of an extensive and complex network of stress response pathways. Deregulation of p53 has enormous influence on carcinogenesis as mt p53 can induce an increased epigenetic instability of tumor cells, facilitating and accelerating the evolution of the tumor. Understanding the mechanisms of p53's function is currently a major challenge in p53 research, and such knowledge may ultimately provide novel targets and approaches to therapeutic manipulation of the p53 pathway in the treatment of cancer. The challenge in the future will be to use our knowledge of p53 to develop more highly effective strategies and novel drugs for cancer prevention and treatment with fewer side effects.

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