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TUMOR SUPPRESSOR P53: REGULATION AND FUNCTION

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TABLE OF CONTENTS

1. ABSTRACT

The p53 protein is a transcription factor involved in maintaining genomic integrity by controlling cell cycle progression and cell survival. Mutations in p53 are the most frequently seen genetic alterations in human cancer. The function of p53 is critical to the way many cancer treatments kill cells because radiotherapy and chemotherapy act in part by triggering programmed cell death in response to DNA damage. Consequently, tumors which bear p53 mutations, are often difficult to treat and their prognosis is poor. Since the underlying feature of tumors with p53 mutations is the absence of functional p53, gene replacement therapy with wild-type p53 gene is being considered as an approach for treating a variety of cancers. In recent years, more information has been obtained regarding various pathways leading to the activation of p53, particularly those involving post-translational modifications of p53. Several new target genes of p53 have been identified. This review will summarize current knowledge on the structure, mechanism of activation and effectors of p53 function.

2. INTRODUCTION

The tumor suppressor p53 is often described as the "guardian of the genome" because it is a critical component of the cellular mechanisms that respond to genotoxic stresses like DNA damage, hypoxia etc. to maintain the genomic integrity in part by arresting cell-cycle progression or by inducing apoptosis (1). Consistent with this view, about 50% of primary human tumors carry mutations in the p53 gene (2, 3). p53 plays no essential role in the normal cell cycle as the p53 knock out mouse develops normally. However, these mice as well as the transgenic mice carrying mutant p53 alleles are highly prone towards developing spontaneous and carcinogen-induced tumors (4, 5).

p53 is a short lived protein that is maintained at very low or undetectable levels under normal circumstances. Upon exposure to stressful stimuli, p53 is activated through post-translational modifications that increase its stability and activity. Activated p53 is a sequence-specific DNA-binding transcription factor. Several down-stream targets genes of p53 have been identified, such *as p21$^{WAF1/CIP1}$*, *MDM2*, *GADD45*, *14-3-3 s*, *bax* and *KILLER/DR5*, whose expression products function as regulators of diverse aspects of cell growth (reviewed by in 1, 6, 7).

3. STRUCTURE OF P53 PROTEIN

The human p53 protein contains 393 amino acids and has been divided structurally and functionally into four domains (Figure 1): an acidic amino-terminal domain (aa 1-43) which is required for transcriptional activation; a central core sequence-specific DNA-binding domain (aa 100-300); a tetramerization domain (aa 324-355) and a C-terminal regulatory domain (aa 363-393), rich in basic amino acids and believed to regulate the core DNA-binding domain.

The N-terminus activation domain interacts with the proteins TFIID (TBP, TAFs),TFIIH which form part of the basal transcriptional machinery and positively regulate gene expression. Amino acids residues F19, L22, and W23 in p53 have been shown to be required for transcriptional activation in vivo (8) and are also involved in the interaction with the TATA-associated factors (9, 10).

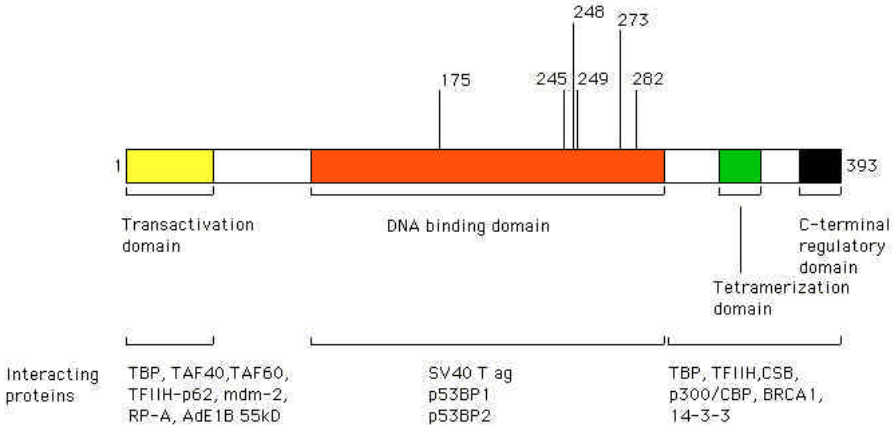

Figure 1. Structure of p53 protein showing the different domains, and hot spots for mutations in human cancer. The 393 amino-acid p53 protein is diagrammed from the amino-terminus (1) to the carboxy-terminus (393) with boundaries for various domains shaded with different colors. The list of different cellular and viral proteins reported to bind to different regions of p53 is given below.

Human MDM2 and adenovirus E1B-55 kDa protein negatively regulate the p53 transcriptional activity through binding to the N-terminus activation domain. The importance of negative regulation of p53 by MDM2 is highlighted by fact that homozygous deletion of mdm-2 in mice results in embryonic lethality, which is rescued by simultaneous homozygous deletion of p53.

The central core domain of p53 contains the sequence-specific DNA-binding region (11, 12, 13, 14). There are four highly conserved regions within the central core domain, where most of the p53 missense mutations were found (15). The tetrameric p53 protein, which is a dimer of a dimer, binds to four repeats of a consensus DNA sequence 5'-PuPuPuC(A/T)-3' and this sequence is repeated in two pairs, each arranged as inverted repeats (16). The purified core DNA-binding domain can bind co-operatively to DNA (11, 13, 14, 17, 18).

The native p53 protein is a tetramer in solution and the process of tetramerization requires the region of p53 from amino acid 324-355 (19). Oligomerization is essential for the tumor suppressive activity of p53 because oligomerization-deficient mutants of p53 cannot suppress the growth of carcinoma cells (20). X-ray crystallographic studies suggest that a p53 monomer, which consists of a beta strand and an alpha helix, associates with a second monomer across an antiparallel beta sheet and an antiparallel helix-helix interface to form a dimer. Two of these dimers associate across a second and distinct parallel helix-helix interface to form the tetramer (21).

The C-terminal domain regulates the ability of p53 to bind to specific DNA sequences at its central core domain. This extreme C-terminal domain including amino acids 363-393 is rich in basic residues. This domain can bind non-specifically to different forms of DNA such as DNA breaks or internal mismatches (22). Evidence suggests that a structural change at the C-terminal domain is required to activate p53 for sequence-specific DNA-binding. It was found that deletion of this domain, phosphorylation of this domain by protein kinase C or casein kinase II at specific residues or binding of antibody PAB421 can activate sequence-specific DNA-binding by the central core domain (23).

4. MECHANISM OF ACTIVATION OF P53

p53 is a short lived protein with a half life of about 20 minutes and is often undetectable in normal cells. p53 may also exist in a latent inactive form, which could be activated by low doses of UV irradiation without any detectable increase in the p53 protein levels (24). It is believed that ubiquitin-mediated proteolysis plays a role in the rapid turn over of p53 protein (25). *In vitro* studies suggest that p53 may be negatively autoregulated by specifically inhibiting translation of its own mRNA (26).

Several stressful conditions act as the upstream events by signaling to p53 (Figure 2). Many types of DNA damage like double-strand DNA break following ionizing ir-radiation, thymidine dimers produced by ultraviolet irradiation or chemical damage to DNA can lead to p53 activation. In addition to DNA damage, hypoxia, heat-shock, treating the cells with radioactive chemicals, DNA transfection, expression of viral and cellular oncogenes also activate p53 (27-33).

The precise mechanism by which p53 is activated by cellular stress is not yet entirely clear. Evidence suggests that it may involve both an increase in p53 protein levels as well as activation of p53 by post-translational modifications (6, 34). An increase in p53 protein level may be achieved by inhibiting directly or indirectly the degradation of p53, as well as enhancing the rate of translational initiation of p53 mRNA. Post-translational modifications of the C-terminus of p53 including phosphorylation, dephosphorylation, acetylation, antibody binding, deletion of the C-terminus or addition of a C-terminus peptide can induce sequence-specific DNA-binding by p53.

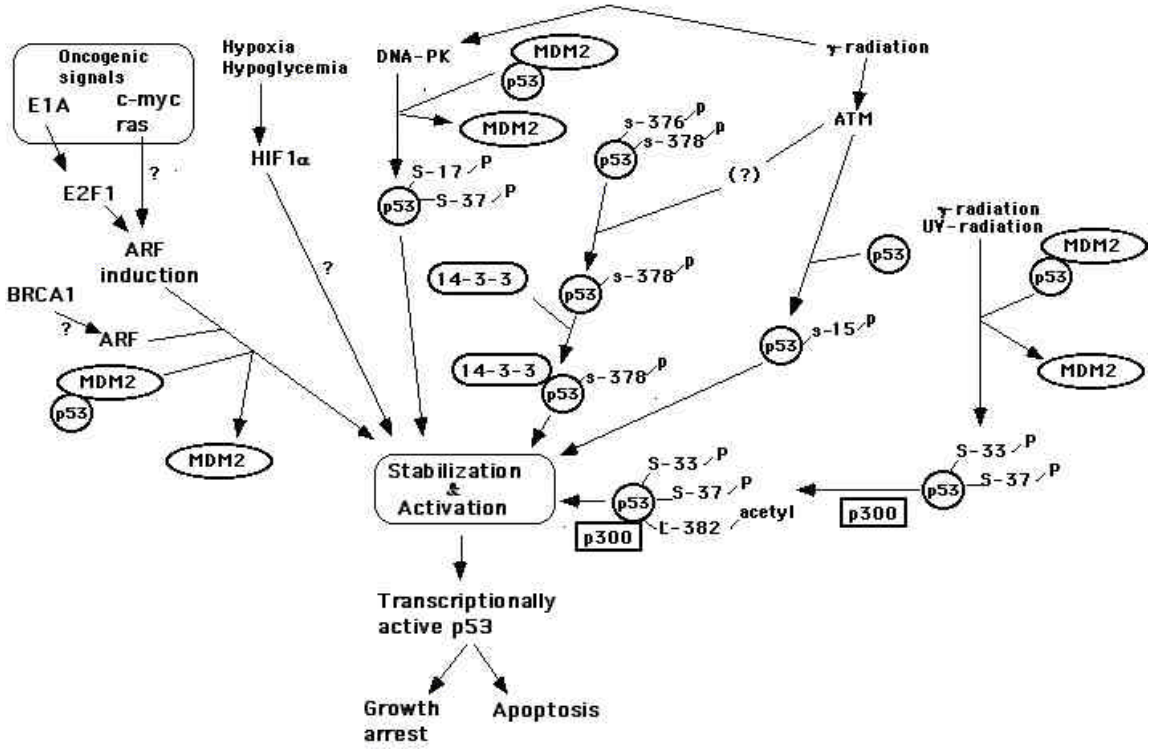

Figure 2. Mechanism of activation of p53. Exposure of cells to a variety of stressful conditions like ionizing radiation or ultraviolet radiation, hypoxia, hypoglycemia or oncogenic activation leads to stabilization of p53. Specific phosphorylations and acetylations involved in the activation of p53 are shown. p53 is subject to feedback regulation through ARF repression and p53-dependent MDM2 transactivation.

A major question is how does the cell sense the DNA damage and transmit a signal to stabilize p53 protein. One model is that there are cellular proteins, which recognize the damaged DNA and communicate with p53 protein. This model is based in part on studies which showed that cells from patients with Ataxia Teleangiectasia have a delayed p53 response to DNA damage. It has been reported that p53 may recognize the damaged DNA through binding to DNA ends, excision-repair damage sites or internal deletion loops (22), as well as to short single-stranded DNA of 16-40 nucleotides long (35).

Specific phosphorylation, dephosphorylation and acetylation events have been reported to activate p53 (reviewed in 7). MDM2 protein, which was originally found to interact with and inhibit p53 transcription activity (36), has recently been found to promote rapid degradation of p53 and it is suggested that this degradative pathway may contribute to the maintenance of low levels of p53 in normal cells (37, 38). In cancer cells with p53 mutation, it is believed that low levels of MDM2 may contribute to p53 stabilization. MDM2 is a target of p53-dependent transcriptional activation, a process which is disrupted by p53 mutation in tumors. Phosphorylation of the amino-terminus of p53, which has been shown to occur after DNA damage, appears to reduce its affinity for binding to MDM2, which may lead to stabilization of p53 (39). Site specific mutational analysis of some phosphorylation sites suggests that certain phosphorylations may not be essential for DNA damage-induced stabilization of p53 (40). DNA damage causes phosphorylation of serine residues in the amino terminus of p53. In particular, serine-15 has been found to be phosphorylated in response to DNA damage by ionizing irradiation (IR) or ultraviolet irradiation (UV). AT (Ataxia telangiectasia) cells show delayed phosphorylation of serine-15 in response to IR but show normal phosphorylation after UV irradiation suggesting that ATM kinase is involved in the serine-15 phosphorylation after IR, although it is not absolutely required (41). Upon DNA damage, ATM kinase phosphorylates the product of c-Abl, which results in the activation of its tyrosine kinase activity (42, 43, 44). Activated c-Abl may bind to p53 and enhance its transcriptional activity (45). Recent experiments have revealed that ATM may associate with p53 and phosphorylate its amino-terminus directly (46). Other kinases which can phosphorylate p53 include cyclin-dependent kinase (CDKs), casein kinase I (CK I), casein kinase II (CK II), protein kinase C (PKC), mitogen activated protein kinase (47), Jun amino-terminal kinase (JNK) (48), Raf kinase (49; reviewed in 50). DNA-dependent protein kinase (DNA-PK), which is activated by DNA strand breaks, may be required to activate sequence-specific DNA-binding by p53 following DNA damage (51).

Serine/threonine protein phosphatase type 5 (PP5) has been shown to modulate the phosphorylation and

DNA binding activity of p53 (52). ATM also appears to be required for IR-induced dephosphorylation of p53 Serine-376 which allows specific binding of 14-3-3 proteins to p53 and leads to an increase in sequence-specific DNA-binding activity of p53 (53).

Recently, acetylation of the C-terminus of p53 by CREB-binding protein (p300/CBP) was shown to enhance sequence-specific DNA-binding by p53 (54). p300/CBP are closely related histone acetyl transferases (HATs) (55, 56) that interact with p53 and function as coactivators for p53-mediated transcription (57-59). The activation of sequence-specific DNA-binding by p53 following DNA damage may involve sequential amino-terminal phosphorylation followed by carboxy-terminal acetylation by the coactivator p300 following DNA damage (60). With the use of phosphorylated or acetylated peptide specific antibodies, it has been shown that p300 acetylates Lys-382 while the p300/CBP-associated factor (PCAF) acetylates Lys-320 of p53 and that either acetylation leads to enhanced sequence-specific DNA-binding in-vitro (60). p53 was found to be acetylated at Lys-382 and phosphorylated at Ser-33 and Ser-37 in vivo after exposure of cells to UV light or ionizing radiation. Interestingly, acetylation of p53 by p300 and PCAF was strongly inhibited by phosphopeptides corresponding to the amino terminus of p53 phosphorylated at Ser-37 and/or Ser-33 suggesting that phosphorylation in response to DNA damage may enhance the interaction of p300 and PCAF, thereby driving p53 acetylation.

Several viral and cellular oncogenes have been shown to stabilize p53. Viral oncogenes including SV40 T antigen, adenovirus E1A and Human papillomavirus 16 E7 stabilize p53 (31, 32). Why should viral oncogenes, whose main purpose is to induce DNA replication, stabilize p53 which would result in growth arrest or apoptosis? One possibility may be that p53 stabilization in response to viral oncogene expression is a cellular response to viral infection. As might be expected viruses have developed additional mechanisms to overcome this cellular response. For example, adenovirus has other cooperating oncogenes like E1B and E4, which bind to p53 and inhibit apoptosis and/or growth arrest thereby leading to successful replication and cellular transformation. E1A can also inhibit transcriptional activation by p53 which may inhibit p53-mediated growth arrest and/or apoptosis (61, 62). The E6 gene product encoded by HPV16 binds to p53, which results in degradation of p53 and suppression of negative growth signals from p53. The mechanism of p53 stabilization in response to viral oncogene expression has not been clearly understood until recently when $p19^{ARF}$($p14^{ARF}$ in human), a product of INK4a/ARF locus translated in an alternate reading frame (63, 64) was identified. It was found that the ability E1A to induce p53 is severely compromised in $p19^{ARF}$-null cells (65). $p19^{ARF}$ is a tumor suppressor, which can induce cell-cycle arrest in a p53-dependent manner. $p19^{ARF}$ can physically associate with p53 itself and/or Mdm2 to alter the p53 levels and activity (66, 67, 68). E2F1, which was found to activate $p14^{ARF}$ transcriptionally, linked E1A to $p14^{ARF}$ and then to p53 (69). Certain cellular oncogenes like c-myc and ras have also been shown to stabilize p53 in a $p19^{ARF}$ -dependent manner (70, 71). Interestingly, the tumor suppressors WT1 and BRCA1 also appears to stabilize p53 and modulate p53 mediated transcription (33, 72, 73).

5. EFFECTORS OF P53 FUNCTION

The principal function of p53 appears to be in mediating a response to DNA damage and thereby preventing accumulation of potentially oncogenic mutations and genomic instability (74). Activation of p53 leads to suppression of cell growth. Two mechanisms have been identified that, either individually or in combination, could account for the growth-suppression function of p53: cell cycle arrest and apoptosis (1). p53 has also been implicated in differentiation (75), senescence (76), inhibition of angiogenesis (77). Although we do not yet fully understand how p53 elicits its effects upon cells, it is clear that the transcriptional activation function of p53 is a major component of its biological effects (78, 79). Activated p53 binds to a specific DNA sequence and activates transcription. The importance of the DNA-binding is underscored by the fact that the vast majority p53 mutations derived from tumors usually map within the domain required for sequence-specific DNA-binding (2, 6). p53 normally recognizes a 20 base-pair response element that has an internal symmetry. The consensus sequence is 5'-PuPuPuC(A/T-A/T)GPyPyPy- N(0-13) -PuPuPuC(A/T-A/T)GPyPyPy-3' with the third C and seventh G being highly conserved in the ten base-pair half-sites (16). Identification of transcriptional targets of p53 has been critical in dissecting pathways by which p53 functions (80). A growing number of genes have been found to contain p53-binding sites and/or response elements and thus to have the potential to mediate the effects of p53 on cells, through upregulation of their expression and function (Table 1).

5.1. Cell cycle Regulation

Expression of p53 in many cell types results in arrest of progression through the cell cycle, with evidence for both a G_1 and G_2/M checkpoint function (1, 81). The ability of p53 to induce a growth arrest is correlated with its ability to function as a sequence-specific transcriptional coactivator (Figure 3). In particular, G_1 arrest induced by p53 has been well studied. The $p21^{WAF1/CIP1}$, which encodes a cyclin dependent kinase inhibitor (CKI) (82), is a critical target of p53 (83) in facilitating G_1 arrest (84). p21 binds to a number of cyclin and Cdk complexes: cyclin D1-Cdk4, cyclin E-Cdk2, cyclin A-Cdk2, cyclin A-cdc2, and cyclin B-cdc2 which results in the inhibition of their kinase activity (85). This allows accumulation of hypophosphorylated Rb, which remains associated with transcription factors such as E2F and the resultant failure to activate E2F-responsive genes results in G_1 arrest. $p21^{WAF1/CIP1}$ also binds to PCNA and blocks its role in DNA replication at least *in vitro*. Thus, p21 inhibits DNA replication by binding to cyclin-Cdk complexes and PCNA (86). Mice with homozygous deletion of p21 gene develop normally but the mouse embryo fibroblast derived from these mice are only partially deficient in their ability to arrest cells in G_1 in response to DNA damage, which

Table 1.Transcriptional targets of p53

Gene	Interacting molecule/Signaling pathway	Effect
p21$^{WAF1/CIP1}$	Cyclin-CDks and PCNA	G1 arrest and G2/M arrest
MDM2	p53	Inhibition of p53 transactivation; Targets p53 for degradation
GADD45	Cdc2 and PCNA	G2/M arrest and DNA repair
14-3-3s	Phosphorylated Cdc25	Inhibits dephosphorylation of Cdc25 and activation of Cdc2 by Cdc25; G2/M arrest
B99	N/A	G2 arrest
Cyclin G	N/A	Growth suppression
Bax	Translocation to mitochondria and release of cytochrome C	Apoptosis
IGF-BP3	Binds to IGF1 and inhibits growth factor signaling	Apoptosis
FAS/APO1	Uses FADD as an adaptor molecule to activate caspase cascade	Apoptosis
KILLER/DR5	Activates caspase cascade	Apoptosis
PIG1-PIG14	Oxidative stress response	Apoptosis
p85	Oxidative stress response	Apoptosis
PAG608	N/A	Apoptosis
TSP1	N/A	Inhibits angiogenesis
TSP1	N/A	Inhibits angiogenesis
BAI1	N/A	Inhibits angiogenesis
GD-AIF	N/A	Inhibits angiogenesis

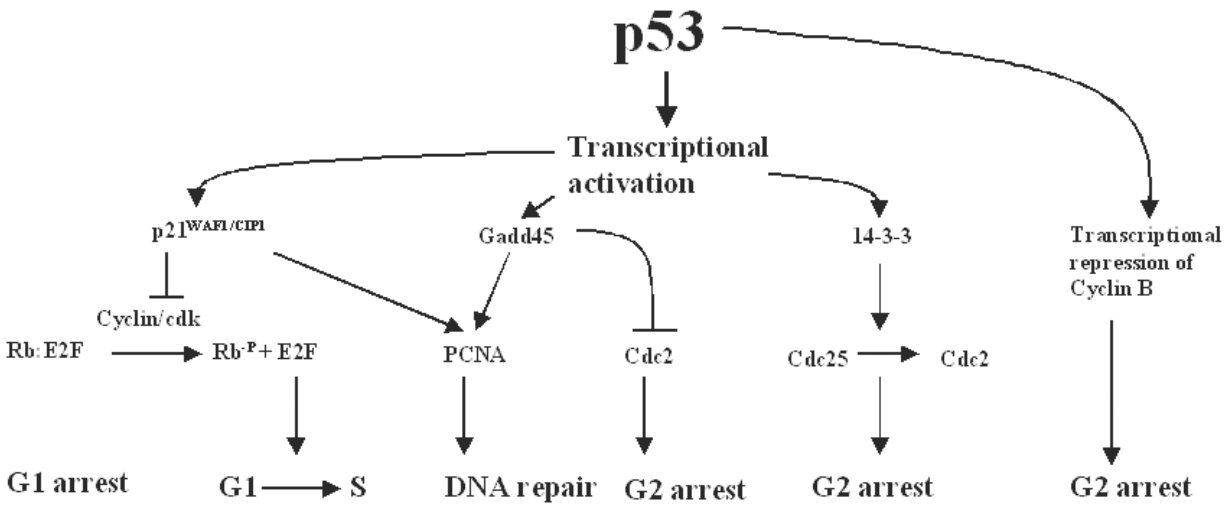

Figure 3. Cell cycle regulation by p53. Transcriptional activation of p53 results in both G1 and G2 arrest. p53 mediated transcriptional induction of p21WAF1/CIP1 plays a major role in mediating the G1 arrest. p21WAF1/CIP1 binds to and inhibits cyclin/cdk complexes resulting in the accumulation of hypophosphorylated Rb leading to G1 arrest. Gadd45 and 14.3.3σ have been found to mediate the G2 arrest. A direct transcriptional repression of cyclin B by p53 also results in G2 arrest. Both Gadd45 and p21WAF1/CIP1, through their interaction with PCNA, may play a role in DNA repair.

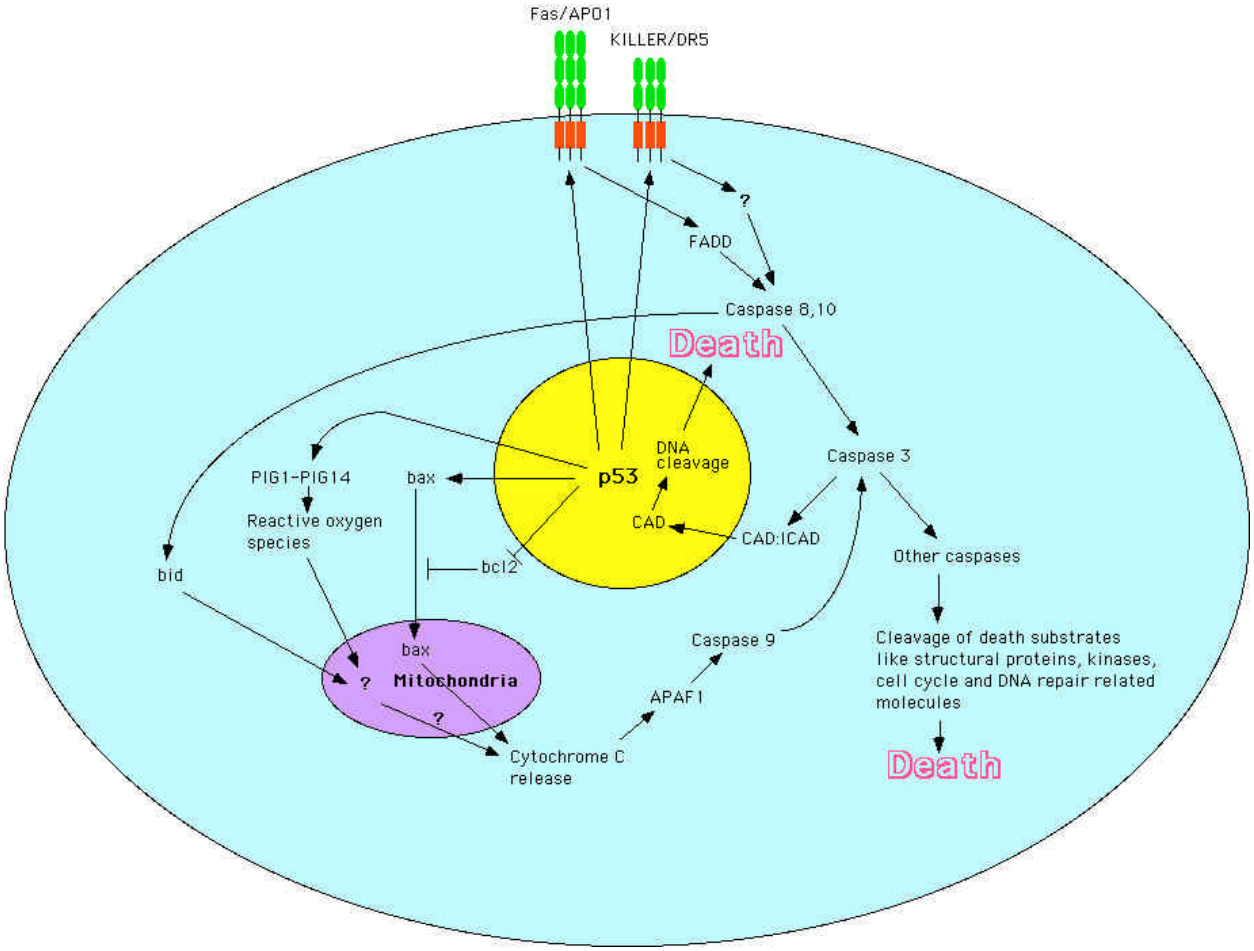

Figure 4. Apoptosis induction by p53. p53 transcriptionally activates bax, Fas/APO1, KILLER/DR5, and PIG genes and represses bcl2 expression which results in the activation of the caspase cascade leading to apoptosis. Fas/APO1 and KILLER/DR5 are death receptors located on the cell membrane, activation of which results in activation of the caspase cascade which is initiated through specific adapter molecules. The transfer of Bax to the mitochondria results in the release of cytochrome C, which in turn activates the caspase casade through activation of APAF1. Bcl2 inhibits apoptosis by preventing Bax function. The PIG genes are also believed to act on mitochondria to induce apopotosis. Caspase activated endonuclease CAD (caspase activated Dnase) mediates DNA cleavage. CAD is normally found in the cytoplasm in complex with ICAD (inhibitor of CAD), but upon caspase 3 activation, ICAD becomes cleaved so that CAD can enter the nucleus and cleave DNA.

suggest the existence of p21-independent pathway that contributes to the p53-mediated G_1 arrest (87). However, the removal of both alleles of p21 from a colon cancer cell line, which harbors wild-type p53, results in complete elimination of the DNA damage-induced G1 arrest in these cells (84).

p53 has also been implicated in the control of a G_2/M checkpoint. Transcriptional down-regulation of cyclin B1 by p53 (88, 89) may be involved in p53-mediated G2/M arrest as cyclin B1/Cdc2 complex is the major regulatory factor required for entry into mitosis (90, 91). Recently, the cyclin B1 promoter has shown to be down regulated by p53 (92). Gadd45, another p53-induced gene, may be involved in G_2/M arrest as Gadd45 overexpression results in a G2/M arrest, which can be attenuated by overexpression of cyclin B1 and Cdc2, and Gadd45 inhibits the activity of the cyclin B1/Cdc2 complex *in vitro* possibly through disruption of the complex or by direct binding and inhibition of cyclin B/cdc2 kinase activity (93). 14-3-3σ,another IR-inducible p53-target gene, may also be involved in G_2/M arrest (94) and it appears to act by binding and sequestering phosphorylated Cdc25C in the cytoplasm, thereby preventing its phosphorylation and activation of Cdc2 (95). A number of other of p53-induced genes like $p21^{WAF1/CIP1}$ and BTG2 have also been implicated in G2/M arrest (81, 96-99).

5.2. Apoptosis induction by p53

p53 plays a major role in triggering apoptosis under many different physiological conditions (100-102). Unlike the cell cycle arrest function by p53, a dependence of apoptosis on the transcription activity of p53 is not entirely clear. Evidence suggests that apoptosis could be induced by p53 by both transcription dependent and independent mechanisms (Figure 4). For example,

apoptosis is induced by p53 in the presence of actinomycin D or cycloheximide, which block RNA or protein synthesis, respectively (103-104). Similarly, p53 mutants which are deficient in transcription activation have been shown to activate apoptosis in Hela cells (105). However, in another report, one of mutants used in the above study was found to be defective for inducing apoptosis in baby rat kidney cells (106). Hence p53 may induce apoptosis by both transcription dependent and independent mechanisms and cell type might be one of the important factors determining the mechanism of apoptosis induction.

p53 target genes such as bax and Fas/APO1, have been implicated in apoptosis signaling (107, 108). Bax signaling involves steps that involve translocation of bax to the mitochondria and release of cytochrome C from the mitochondria, which triggers activation of caspase 9 and the downstream caspase cascade (109, 110). The Fas/APO1 signal is transmitted through the FADD (Fas-associated death domain) adapter which recruits initiator caspases 8 and 10 resulting in the activation of the caspase cascade. Although these data suggest that bax or Fas/APO1 may be involved in p53-mediated apoptosis, neither of them are absolutely required for p53 to induce apoptosis because cells which are deficient for bax or Fas/APO1 are proficient in inducing apoptosis upon p53 activation (111-113).

Other p53 target genes, which are believed to be involved in inducing apoptosis are KLLER/DR5 (114), PAG608 (115), PIG's (p53-induced genes) (116), p85 (117), and IGF-BP3 (118, 119). KILLER/DR5 is a death-domain containing pro-apoptotic member of a recently discovered family of TRAIL (TNF-realted apoptosis inducing ligand) receptors (120). Expression of KILLER/DR5 appears to be increased following exposure of wild-type p53-expressing cells to cytotoxic DNA damaging agents such as γ-radiation, doxorubicin or etoposide (114). Like the Fas/APO1 receptor, signaling through pro-apoptotic TRAIL receptors involves downstream caspase activation (121, 122). Induction of apoptosis by PIG's involves formation of reactive oxygen species (116). IGF-BP3 (Insulin like growth factor-binding protein 3) may induce apoptosis by inhibiting growth factor-associated signaling (118). Repression of apoptotic protectors such as Bcl-2 may also contribute to apoptosis induction by p53 (123). A novel pathway of p53-induced apoptosis which ivolves direct binding of p53 to TFIIH DNA helicases XPB or XPD has also been identified (124).

5.3. Inhibition of Angiogenesis by p53

Overexpression of p53 has been found to inhibit angiogenesis possibly through the upregulation of Tsp1 (77), BAI1 (125), and/or GD-AiF (126). It is not clear why anti-angiogenesis is required for p53-dependent tumor suppression. One explanation could be that tumor suppression by p53 is achieved by inhibiting the angiogenesis because as the tumor cells progress toward malignancy they must switch to an angiogenic phenotype to attract the nourishing vasculature for their growth. Early passage Li-Fraumeni cells which carry one wild-type p53 allele secrete large amounts of a potent angiogenic inhibitor, TSP1 (77). However, the late passage cells undergoes an angiogenic switch associated with their immortalization, loss or mutation of the wild type p53 allele and reduced expression of TSP-1 (77). It was also shown that the promoter of TSP1 is regulated positively by p53 (77).

5.4. p53 and genomic stability

p53 has been proposed to be involved in maintaining stability of the genome (127, 128). Both cell cycle arrest and apoptosis functions of p53 could be involved in maintaining the genomic stability. At early passages, fibroblast from p53-/- mice developed several chromosomal abnormalities (129). Tumors from p53-/- mice also showed aneuploidy and evidence of chromosomal instability (130, 131). Fibroblasts from p53-/- mice showed tetraploidy and octaploidy after exposure to spindle inhibitors (132). Inactivation of p53 by T antigen leads to formation of a tetraploid cell-intermediate that is predisposed to chromosome segregation abnormalities and the development of multiple aneuploid cell populations (133). Inhibition of homologous recombination by p53 through a putative interaction with Rad51 may be involved in the maintenance of genomic stability (134, 135).

5.5. p53 and Senescence

There is some evidence that p53 may be involved in cellular senescence. Evidence of a role in senescence for p53 came from the finding that increased sequence-specific DNA-binding in vitro and transcriptional activity *in vivo* with increased levels of $p21^{WAF1/CIP1}$ in high-passage human diploid fibroblasts undergoing replicative aging (76). Expression of oncogenic ras in primary human or rodent cells resulted in a permanent G1 arrest, accumulation of p53 and p16, and features of senescence (136). In another study, introduction of a transdominant-acting p53 mutant rescued the senescing human diploid fibroblasts with a concomitant decrease in p53 transcriptional activity (137). A mutant p53 containing EJ bladder carcinoma cell line underwent G1, G2/M arrest, p21WAF1/CIP1 upregulation and senescence when wild-type p53 was introduced (138). The maintenance of fibroblast senescence may be critically dependent on functional p53 because inhibition of p53 activity by microinjection of a p53 N-terminus specific antibody PAB1801 or DO-1 results in reinitiation of DNA synthesis and cell division (139).

Inhibition of p53 function by the addition of a transdominant-acting p53 mutant did not result in complete elimination of senescence and it only resulted in increased life span, undergoing senescence at a later passage level (137). This suggests the existence of other molecules involved in senescence. Indeed, the skin fibroblasts from patients with Li-Fraumeni syndrome lose the wild-type p53 allele during culture but ultimately undergo senescence (140). Increased p53 transcriptional activity in cell undergoing senescence suggests that $p21^{WAF1/CIP1}$ might be one of the mediators of senescence. In fact, $p21^{WAF1/CIP1}$ was isolated independently as SDI1 (Senescent cell-derived inhibitor 1), which was found to be overexpressed in human diploid fibroblasts undergoing senescence (141).

Consistent with view, disruption of both alleles of p21WAF1/CIP1 in normal human diploid lung fibroblasts resulted in extended life span (142).

6. P53 FAMILY

Several p53 family members have been identified recently. They are p73 (143), p51 (144), p63 (145), and p40 (146). The p53 family members seem to share many properties with p53. All of them have considerable homology with p53 in particular with the conserved domains of p53. p73, p51 and p63 activate the p53 target gene p21WAF1/CIP1 and induce apoptosis when overexpressed (144, 145, 147).

p53 is an ideal choice for gene replacement therapy because it is a potent inducer of cancer cell apoptosis and is effective despite the presence of multiple genetic changes in the cancer cells (148). However, p53-gene therapy has important limitations that must be considered for its clinical development as an anticancer agent despite its strengths (149). For example, cells transformed by HPV type 16 or 18 are resistant to p53 mediated killing because E6 oncoprotein encoded by the virus targets p53 for degradation (150). Human cancer cell lines, which over express MDM2 are resistant to the growth inhibitory effects of Ad-p53 (151). Similarly, cancer cells which express viral oncogenes like SV40 T antigen, hepatitis B virus X gene, the products of which have been shown to inactivate p53, are resistant to p53 gene therapy (152, 153). Despite the high degree of similarity between p53 and p73, p73 has been shown to be resistant to many viral oncogenes. p73β has been shown to be resistant to degradation by HPV E6 and can suppress growth and induce apoptosis in HPV E6-expressing cancer cells (154). Subsequently, it was found that the viral oncogenes adenovirus E1B 55K, SV40 T antigen, and human papillomavirus E6 do not physically interact with p73 (155). These findings raise the possibility of using p73 in gene therapy of cancer cells which are resistant to p53 based gene therapy.

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