

Regulation of *p53* downstream genes

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The p53 tumor suppressor is the most commonly mutated gene in human cancer. p53 protein is stabilized in response to different checkpoints activated by DNA damage, hypoxia, viral infection, or oncogene activation resulting in diverse biological effects, such as cell cycle arrest, apoptosis, senescence, differentiation, and antiangiogenesis. The stable p53 protein is activated by phosphorylation, dephosphorylation and acetylation yielding a potent sequence-specific DNA-binding transcription factor. The wide range of p53's biological effects can in part be explained by its activation of expression of a number of target genes including p21^{WAF1}, GADD45, 14-3-3 σ , bax, Fas/APO1, KILLER/DR5, PIG3, Tsp1, IGF-BP3 and others. This review will focus on the transcriptional targets of p53, their regulation by p53, and their relative importance in carrying out the biological effects of p53.

Key words: p53 / tumor suppressor / transcription / apoptosis / growth arrest / cancer

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Activation of p53

IN NORMAL CELLS under physiological conditions the tumor suppressive p53 protein is expressed at low levels and has a short half-life due to rapid turnover mediated by ubiquitination and proteolysis. The p53 protein becomes stabilized and activated in response to a number of stressful stimuli including exposure of cells to DNA damaging agents, hypoxia, nucleotide depletion, or oncogene activation (see ref 1 for recent review and additional references). The activa-

tion of p53 allows it to carry out its function as a tumor suppressor through a number of growth controlling endpoints. These include cell cycle arrest, apoptosis, senescence, differentiation and antiangiogenesis. In its role as a tumor suppressor, p53 serves as a 'guardian of the genome', a 'gatekeeper for growth and division', by regulating critical checkpoints in response to the distinct stresses.

Beginning in 1997, a series of elegant studies (see ref 1 for review) have led to a better understanding of the activation of p53 in response to different signals (Figure 1). There appear to be separate and distinct regulators of p53 protein stability and function including MDM2,²⁻⁴ ARF,⁵⁻¹⁰ p33ING1,¹¹ BRCA1¹², HIF1 α ,¹³ p300¹⁴ and various kinases, such as DNA-PK^{15,16} and ATM¹⁷⁻¹⁹ as well as unknown phosphatases.²⁰

There are two mechanisms that appear to activate p53, one at the amino-terminal end^{15,18,19} and one at the carboxy-terminal end.^{20,21} At the N-terminal amino acids 1–42, resides the transcriptional activation domain which is also where p53 interacts with its negative regulator MDM2 oncoprotein (Figure 2). MDM2 binding to p53 conceals its transactivation domain thereby inhibiting p53-dependent effects in cell cycle inhibition and apoptosis. MDM2 also appears to target p53 protein for ubiquitin-mediated proteolysis.²⁻⁴ It has now become clear that certain stress signals can alter the binding of MDM2 to p53 through apparently different mechanisms. Thus, DNA damage induced by ionizing radiation or the topoisomerase inhibitor etoposide leads to phosphorylation of p53 within its N-terminal region.^{15,17} Two candidate kinases have been identified, ATM and DNA-PK, both of which can phosphorylate p53 on serine 15.¹⁵⁻¹⁹ p53 protein that has been phosphorylated on serine 15 binds poorly to MDM2 and is therefore stabilized through a decrease in its degradation rate. Both ATM and DNA-PK deficient cells have well described defects in p53 activation following DNA damaging ionizing radiation.

For several years it has been known that different

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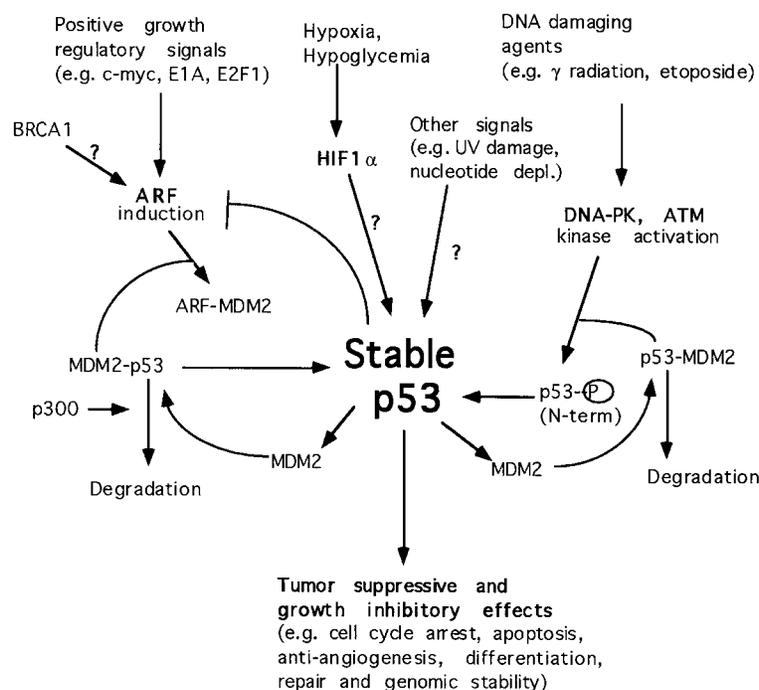


Figure 1. Biochemical signals leading to stabilization of p53. p53 protein is stabilized following exposure of mammalian cells to a variety of stresses including DNA damaging agents, hypoxia, UV damage, nucleotide depletion or oncogene activation. Cellular proteins, such as ARF, HIF1 α , DNA-PK and ATM appear to be required for transduction of the various signals as indicated. In the case of oncogenic stimuli involving c-myc, E1A, ras, and E2F1 the ARF protein appears to mediate p53 stabilization in part through an interaction between ARF and MDM2. p300 may be involved in coordinating the MDM2-dependent degradation of p53. p53 is subject to feedback regulation through ARF repression and p53-dependent MDM2 transactivation. Following exposure to γ -radiation or etoposide p53 becomes phosphorylated in an ATM and DNA-PK-dependent manner. Serine-15-phosphorylated p53 is stabilized through a mechanism that involves decreased binding to MDM2. The mediators of p53 stabilization following UV exposure or nucleotide depletion are unknown. The mechanism of p53 stabilization by BRCA1 and the hypoxia inducible factor HIF1 α is unknown.

oncogenic stimuli can stabilize p53 protein leading to p53-dependent apoptosis. It has now become clear that signaling between oncogenes and p53 stabilization can be mediated by the p14ARF protein encoded by the alternative reading frame (ARF) at the INK4a familial melanoma locus on chromosome 9p.⁷⁻¹⁰ ARF appears to stabilize p53 by binding MDM2 and may also involve the induction of MDM2 degradation.²² A number of positive regulatory or oncogenic signals have been found to induce ARF expression, including c-myc, adenovirus E1A, ras and E2F1.⁷⁻¹⁰ Interestingly, the overexpression of ARF in the absence of oncogenic stimuli leads to cell cycle arrest in G1 and G2 which is p53-dependent. However, in response to oncogenic stimuli associated with increased ARF expression cells undergo apoptosis,

suggesting that a second signal is required to induce apoptosis. The signal transduction between UV damage and p53 stabilization is not presently known; however, it does not require ATM.²³ During exposure to hypoxia, the hypoxia inducible factor HIF1 α appears to be required for p53 stabilization although the precise mechanism is not yet known.¹³ Other regulators of p53 function including BRCA1 and p33ING1 respond to unknown stimuli and regulate p53 through unclear mechanisms.^{11,12}

Sequence-specific DNA-binding activity of p53

p53 is a nuclear phosphoprotein with the potential to bind DNA both non-specifically and specifically. Non-specific DNA binding may be involved in DNA

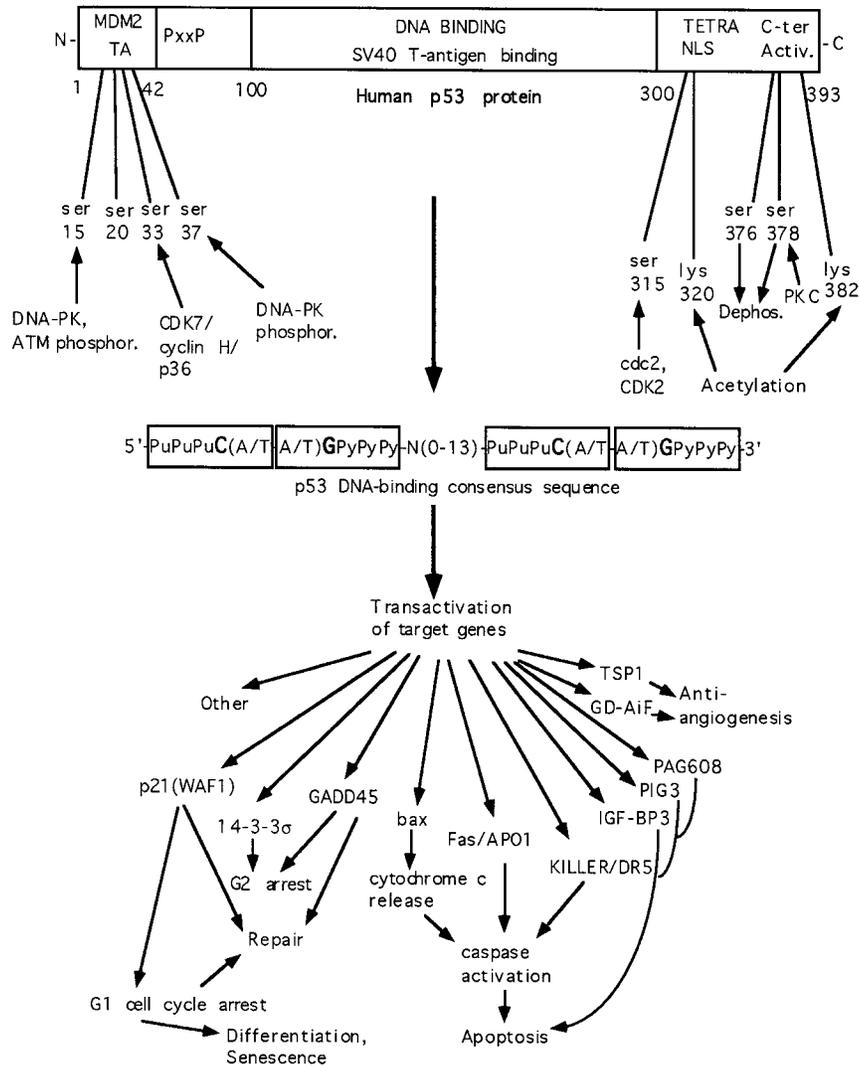


Figure 2. Schematic diagram showing p53 protein domain structure, specific amino acid residues that are subject to phosphorylation, dephosphorylation, or acetylation following DNA damage as well as downstream targets of p53 that mediate its biological effects. The 393 amino-acid p53 protein is diagrammed from the amino-terminus (N) to the carboxy-terminus (C) with boundaries on various domains identified with amino acid numbers below the domains. The transactivation domain which also includes the MDM2 interacting domain resides between p53 amino acids 1–42. PxxP refers to the polyproline-rich domain of p53 that has been implicated in transcription-independent apoptosis. The DNA-binding domain resides between p53 amino acids 100–300 and this contains the SV40 T-antigen binding domain. Specific amino acid residues in human p53 that become phosphorylated following DNA damage include serine 15 and serine 37. DNA-PK can phosphorylate p53 at both serine 15 and serine 37. ATM phosphorylates p53 at serine 15. When the N-terminus of p53 is phosphorylated following DNA damage, p53 interacts poorly with MDM2 and becomes stabilized. The C-terminus of p53 is also subject to phosphorylation and dephosphorylation events following DNA damage. The dephosphorylation of p53 at serine 376 creates a consensus binding site for interaction with 14-3-3 σ which enhances the ability of p53 to bind DNA. p53 can also be acetylated by p300 at lysine 382 and by PCAF at lysine 320. Acetylation of the C-terminal region activates p53 in terms of DNA binding. It is believed that *in vivo*, following DNA damage, N-terminal phosphorylation directs C-terminal acetylation to activate p53. The DNA-binding consensus sequence for p53 is shown. Although DNA binding occurs with up to a 13 bp spacer region (N:0–13), usually for transcriptionally active binding sites, the spacer between the two 10-bp half sites contains less than three nucleotides. p53 is a sequence-specific

damage recognition. The sequence-specific DNA-binding mediates p53-specific transactivation. Although p53 can also repress gene expression, this does not appear to involve sequence-specific DNA binding, at least of its classical response element. The DNA-binding central domain of p53 is the target for mutational activation in most human cancers. The crystallization of p53 revealed that the most common mutation hotspots are involved in direct contact with the p53 DNA-binding site.²⁴

The DNA-binding activity of p53 is subject to regulation by several mechanisms. The first mechanism involves stabilization of p53 protein which can occur through N-terminal phosphorylation and decreased MDM2 binding, for example after exposure to DNA-damaging ionizing radiation. The second mechanism involves a conformational change in p53 at the C-terminus. The C-terminal region of p53, which also encodes its nuclear localization signal and its tetramerization domain, can allosterically inhibit the central DNA-binding domain. It now appears that acetylation of the C-terminal domain, which occurs on lysine 382 by p300 or lysine 320 by PCAF, activates p53-dependent DNA binding.²⁵ There is evidence to support a phosphorylation-acetylation cascade model which suggests that *in vivo*, following DNA damage, N-terminal phosphorylation directs C-terminal acetylation to activate p53.²⁵ A third mechanism involves C-terminal dephosphorylation of p53 at serine 376 which creates a consensus binding site for interaction with 14-3-3 which enhances the ability of p53 to bind to DNA.²⁰

In addition to 'activation' (or inhibition) of p53 in terms of DNA-binding, the post-translational modifications, either through direct effects on p53 conformation or perhaps through altered protein-protein interactions, may affect or selectively alter the affinity of p53 for particular DNA-binding sites.²⁶ Such regulation suggests the possibility that in response to certain signals, different downstream programs of expressed genes may be directed leading to variable outcomes in a given cell or even cell-type-specific

biological effects. It is therefore critically important to understand the upstream regulation of p53 in order to understand its downstream effects.

p53 transcriptional target genes

Analysis of the degeneracy of the p53 DNA-binding site suggests that there may be as many as 200–400 p53 target genes or perhaps even more.²⁷ Despite this staggering number it has been possible to identify certain critical targets but additional targets continue to be discovered. Most of the current understanding of how p53 achieves its biological effects has been derived from the identification of effector genes whose expression is upregulated by p53.

Downstream mediators of p53-dependent cell cycle arrest

There appears to be a variable pattern concerning the mechanism of p53 gene action through specific target gene activation. For example, with respect to cell cycle arrest in G1 the single target p21(WAF1) CDK inhibitor appears to be a critical component, whereas for G2 arrest or apoptosis induction multiple effector target genes may be required.

A. p21(WAF1)

p53 regulates cell cycle checkpoints in response to DNA damage, hypoxia or oncogene activation. Expression of the p21 CDK inhibitor appears to be directly controlled by p53 through two specific p53 DNA-binding response elements located within the p21 promoter.^{28,29} Recent studies have suggested the possible existence of distinct signalling pathways that may preferentially utilize one or the other p53-binding elements within the p21 promoter.²⁶ Once activated, p21 protein binds to cyclin-CDK complexes and inhibits their kinase activity (see ref 30 for recent

DNA-binding transcription factor that upregulates expression of a number of target genes that in part mediate its effects in cell cycle arrest, apoptosis, differentiation, senescence and antiangiogenesis. p53 targets serve as effectors of various p53-dependent checkpoints. In addition to targets that are transcriptionally upregulated p53 also represses expression of a number of genes (see text). Upregulated genes contain p53 DNA-binding sites whereas repressed genes generally do not contain p53 DNA-binding sites. A single target may primarily account for a given phenotype, e.g. p21(WAF1) and DNA damage-induced p53-dependent G1 arrest, or multiple targets may contribute to a given phenotype. The latter appears to be the case in apoptosis induction where several targets including bax, KILLER/DR5, Fas/APO1, IGF-BP3 and PIG3 may be involved. In other cases such as G2 arrest or antiangiogenesis more than one target has been implicated (see text) and it remains to be seen which are critically required.

review). Because CDK kinase activity is required for various cell cycle transitions, p21 is a potent cell cycle inhibitor. p21 binds with its greatest affinity to G1 cyclin-CDK complexes, and binds poorly to cyclin B/cdc2. p21 is a member of the p27 and p57 KIP/CIP family of universal cell cycle inhibitors which can inhibit cyclin D/CDK4/6 complexes which are also specifically inhibited by the INK4 family (p16, p15, p18, p19) of CDK inhibitors. p21 also binds to the proliferating cell nuclear antigen (PCNA) and can inhibit the processivity of DNA replication. p21 is the only CDK inhibitor whose expression is directly regulated by the p53 tumor suppressor. p21 expression can be regulated by a number of growth inhibitory differentiation-associated transcription factors independent of p53 leading to cell cycle arrest in some differentiating cells. p21 expression is also subject to regulation by other candidate tumor suppressors, such as IRF1, p73, WT1, and BRCA1. BRCA1 can regulate p21 expression through p53-independent and p53-dependent mechanisms.¹² p21 appears to be required for G1 arrest following DNA damage, although it is possible that there may be other unknown mediators of this checkpoint.³¹⁻³³

B. 14-3-3 σ :

While it is clear that p53-deficiency leads to defective cell cycle arrest in G1, the role of p53 in G2 arrest is less clear. Although wild-type p53-deficient cells can arrest in G2 following exposure to ionizing radiation, it appears that p53 may contribute to G2 arrest when it is present and not mutated. In this regard, the three p53-target genes 14-3-3 σ ³⁴, GADD45³⁵ and B99³⁶ have emerged as potential mediators of p53-dependent G2 arrest. In the case of 14-3-3 σ , it has been suggested that following exposure to γ -radiation phosphorylated cdc25c becomes bound by cytoplasmic 14-3-3 σ and its phosphatase activity becomes sequestered from activation of nuclear cyclin B/cdc2³⁴.

C. GADD45 (growth arrest and DNA-damage inducible gene #45)

GADD45 was originally identified through a subtractive hybridization screen as a gene whose expression is increased in chinese hamster ovary cells exposed to ultraviolet radiation. In the case of GADD45, recent evidence has suggested that cyclin B/cdc2 may be bound and inhibited by GADD45 thereby leading to

G2 arrest.³⁵ Both 14-3-3 σ and GADD45 contain p53 DNA-binding response elements in their regulatory regions. In the case of GADD45, there is evidence for modulation of expression by p53 through non-p53 response elements.³⁷

D. B99

A recently identified DNA damage-inducible p53-target gene is B99.³⁶ B99 gene expression is specifically upregulated in the G2-phase where its protein product specifically localizes to the microtubule network. Ectopic overexpression of B99 in p53-null fibroblasts leads to G2 arrest.

Downstream mediators of p53-dependent apoptosis

The picture that has emerged is that multiple downstream targets of p53 may be involved in mediating its apoptotic effects. The earliest mediators of p53-dependent apoptosis to be identified were bax³⁸ and Fas/APO1.³⁹ More recently identified targets include KILLER/DR5⁴⁰, the PIG genes,⁴¹ IGF-BP3⁴² and PAG608⁴³ (Figures 2, 3). It is clear that no one target of p53 will be sufficient to recapitulate the p53-dependent apoptosis phenotype under all physiological circumstances. It is not clear, however, if in certain tissues or under certain conditions of stress or animal development some p53 targets may be critically required for apoptosis.

A. p21(Bax)

The Bax gene encodes a 21-kD protein pro-apoptotic member of the Bcl-2 family.⁴⁴ The ratio of p21(Bax):Bcl-2 has been likened to a rheostat that determines whether cells live or die. Bcl-2 and p21(Bax) appear to control apoptosis at the level of mitochondrial cytochrome *c* release; p21(Bax) promotes release whereas Bcl-2 blocks release of cytochrome *c* from the mitochondria.^{45,46} Cytosolic cytochrome *c* in concert with APAF1 appears to mediate activation of initiator caspase 9 which triggers a caspase cascade leading to apoptosis.⁴⁷ p21(Bax) protein appears to be a target for binding and inactivation by the 19-kDa anti-apoptotic E1B adenoviral protein.⁴⁸ Bax is a p53-target gene whose expression is upregulated through a p53 DNA-binding response element located within the Bax promoter⁴⁹. Bax-

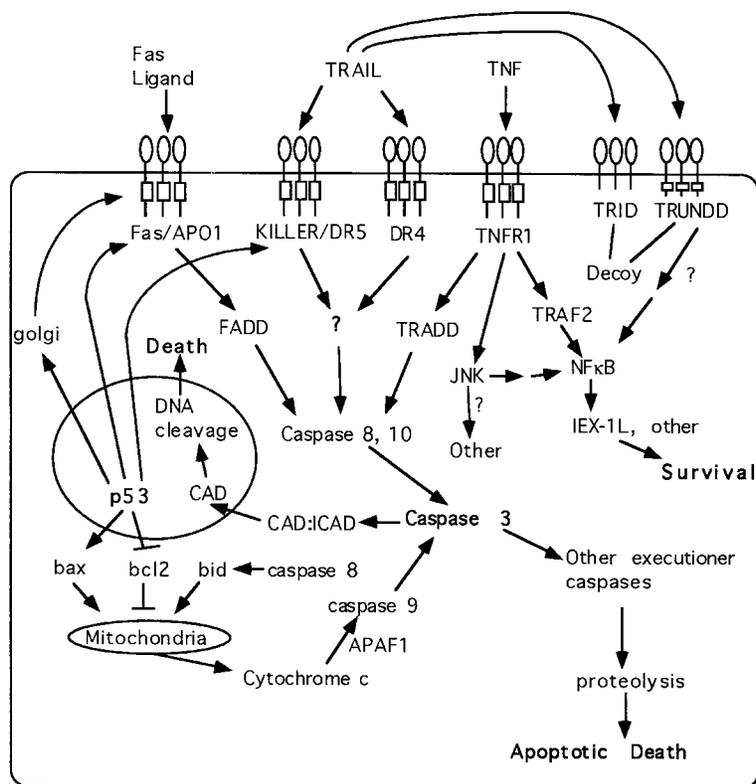


Figure 3. A model for p53-dependent apoptosis involving caspase activation. p53 upregulates expression of bax, Fas/APO1 and KILLER/DR5 and represses bcl2 expression thereby impacting on multiple steps that initiate the caspase cascade. Bax and bcl2 act at the level of mitochondrial cytochrome *c* release which through an interaction with APAF1 leads to caspase 9 activation which then triggers a cascade involving downstream caspases 3, 6 and 7. The death receptor Fas/APO1 signals caspase activation by a mechanism involving the death domain containing adaptor molecule FADD which in turn recruits caspase 8 (also known as FLICE) which acts as an initiator caspase. Other death receptors such as the TNF receptor signal death through the TRADD adaptor but can also simultaneously signal survival through JNK and TRAF2. IEX-1L was recently identified as a transcriptional target of NF κ B which can enhance survival. The downstream signaling from the p53-regulated KILLER/DR5 TRAIL death receptor or the other pro-apoptotic TRAIL death receptor DR4 is not entirely clear. It is known that FADD is not required for signaling death by DR4. In addition to the pro-apoptotic members of the TRAIL receptor family, two anti-apoptotic decoy receptors TRID (truncated intracellular domain) and TRUNDD (truncated death domain) are shown. Decoy receptors compete for TRAIL ligand binding and thereby inhibit death signaling by this cytokine. Endonucleolytic cleavage of DNA, one of the hallmarks of apoptosis, is catalyzed by the caspase-activated endonuclease CAD. 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. A recently identified mechanism of p53-dependent apoptosis that is transcription-independent appears to involve Fas/APO1 translocation from the golgi to the cell surface.

knockout mice do not display the same spectrum of tumors as p53-knockout mice,⁵⁰ although certain Bax-deficient transgenic mice appear to be predisposed to tumor development.⁵¹ The Bax gene can be a target for mutational inactivation in tumors with microsatellite instability although it is not clear that

such mutations represent initiating events in tumor development.⁵² Bax knockout thymocytes, unlike p53-knockout thymocytes, are not deficient in γ -irradiation-induced apoptosis.⁵⁰ However, Bax-knockout fibroblasts appear to be compromised in DNA-damage-induced apoptosis.⁵³ Thus there is evi-

dence that under certain conditions p21(Bax) may be an important target mediator of p53-dependent apoptosis.

B. Fas/APO1

The Fas receptor (also known as APO1) is a potent inducer of apoptosis in hematopoietic or liver cells exposed to Fas ligand (see ref 54 for review). In fact, some tumor cells have been found to express Fas ligand and to use it as a means to evade the killing effects of cytotoxic T cells.⁵⁵ Following exposure to Fas ligand, the Fas receptor trimerizes and through its cytoplasmic death domain recruits the adaptor FADD (Fas-associated Death Domain). Through the DED (Death Effector Domain) of FADD, the pro-initiator caspase FLICE (caspase 8) is recruited to the DISC (Death Inducing Signalling Complex). Through autocatalytic cleavage releasing the pro-domain and the two-subunit mature form, a chain of downstream events is triggered to amplify the apoptotic signal and activate downstream executioner caspases leading to apoptosis. Although Fas/APO1 expression appears to be regulated by p53,³⁹ there is evidence that Fas-deficient cells can still undergo p53-dependent DNA-damage-induced apoptosis.⁵⁶ However, Fas/APO1 may contribute to p53-dependent apoptosis. There is evidence that FADD is required for mediating the Fas-dependent death signal and that FADD is required for embryonic development.⁵⁷ Recent evidence has implicated Fas/APO1 receptor translocation from the golgi to the cell surface as a potential mechanism of p53-dependent transcription-independent apoptosis.⁵⁸

C. Killer / DR5:

KILLER/DR5 is a death-domain containing pro-apoptotic member of a recently discovered family of TRAIL (TNF-related apoptosis inducing ligand) receptors (see ref 59 for review). The family includes another pro-apoptotic member called DR4 and two decoy receptors called TRID (Truncated Intracellular Domain) and TRUNDD (Truncated Death Domain). 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.⁴⁰ KILLER/DR5 was identified as a p53-regulated DNA-damage inducible death receptor gene through a subtractive hybridization screen aiming to identify genes whose transcription is upregulated in chemosensitive but not

chemoresistant cells.⁴⁰ Like the Fas/APO1 receptor and the TNF receptor, signalling through pro-apoptotic TRAIL receptors involves downstream caspase activation.^{60,61} However, unlike Fas and TNFR1, FADD does not appear to be required for TRAIL receptor-mediated apoptosis.⁵⁷ It is not yet known if KILLER/DR5 is required for p53-dependent apoptosis under any circumstances, including following exposure of mammalian cells to DNA-damaging chemotherapy. However, KILLER/DR5 is a potent inducer of apoptosis when overexpressed^{40,60,61} and thus represents a potential target linking p53-dependent apoptotic signals to the caspase cascade, especially in cells where the Fas receptor is not expressed. Mutations in the KILLER/DR5 receptor have been described in head and neck cancer leading to loss-of-function.⁶² KILLER/DR5, as well as other TRAIL receptor genes, is located on human chromosome 8p21 which is a hotspot for translocations and a location where tumor suppressors for colon, head and neck and prostate cancer are believed to reside. Thus, like bax, KILLER/DR5 may be rarely involved through mutational inactivation in human cancer. The mechanism by which p53 regulates KILLER/DR5 expression as well as the mechanism of KILLER/DR5-dependent apoptosis remains unclear.

D. PIGs (p53-induced genes)

A group of PIG's has been recently identified through SAGE (Serial Analysis of Gene Expression) screening for p53 target genes.⁴¹ PIG genes are involved in the generation of toxic reactive oxygen species. At least one PIG gene, PIG3, appears to be directly regulated by p53 through a p53 DNA-binding response element located in the PIG3 gene promoter.⁴¹ The coordinated control of PIG gene expression under apoptotic signalling as well as their precise role and mechanism in p53-dependent apoptosis is not yet clear. However, it is clear that toxic reactive oxygen species are generated with kinetics which follow the p53-dependent induction of PIGs in cells undergoing p53-dependent apoptotic death.⁴¹

E. p85

p53 appears to be required for apoptotic death in response to oxidative stress.⁶³ p53 can upregulate expression of p85, an SH2/SH3 domain-containing protein that regulates PI(3)-kinase. However, regulation of PI(3)-kinase activity does not mediate the apoptotic effects of p85 as PI(3)-kinase activity does

not change in response to oxidative stress.⁶³ p85^{-/-} MEFs are resistant to apoptosis induced by oxidative stress, such as following exposure to Hydrogen Peroxide.⁶³ The mechanism of upregulation of p85 protein expression by wild-type p53 and the mechanism by which p85 transduces an apoptotic signal following oxidative stress remains unclear.

F. PAG608

PAG608 is a nuclear zinc finger protein that was isolated using a differential display technique and found to be induced by DNA damage in a p53-dependent manner.⁴³ PAG608 localizes to nucleoli following exogenous transfection into mammalian cells. The overexpression of PAG608 leads to morphological changes characteristic of apoptosis.⁴³ The mechanism by which p53 regulates PAG608, as well as the signals downstream of PAG608 that promote its pro-apoptotic effects remain unclear.

G. IGF-Bp3 (insulin growth factor-binding protein #3)

IGF-BP3 binds IGF1 and inhibits growth factor signalling. IGF-BP3 appears to be directly regulated by wild-type p53 through sequence-specific DNA-binding and transactivation.⁴² There is some evidence that overexpression of IGF-BP3 may have pro-apoptotic effects independent of its ability to bind IGF1.⁶⁴

p53 targets that inhibit angiogenesis

Overexpression of p53 has been found to inhibit angiogenesis possibly through upregulation of expression of Thrombospondin 1,⁶⁵ BAI1⁶⁶ or GD-AiF.⁶⁷ The effects of p53 on anti-angiogenesis may provide a mechanism for a bystander effect of p53 in tumor suppression *in vivo*. It is not yet clear however, if anti-angiogenesis is required for p53-dependent tumor suppression and it is also not clear if any target of p53 is critically required for its anti-angiogenic effect.

A. Tsp1 (Thrombospondin 1)

Wild-type p53-deficient (late passage) Li-Fraumeni fibroblasts were found to undergo an 'angiogenic switch' associated with their immortalization and loss or mutation of the second allele of p53⁶⁵. Early passage Li-Fraumeni cells were found to secrete large amounts of a potent angiogenesis inhibitor, Tsp1,

which no longer occurs with late passage angiogenically-prone cells.⁶⁵ The Tsp1 promoter appears to be positively regulated by wild-type p53, although specific binding sites have not been identified.⁶⁵

B. BAI1 (Brain-specific angiogenesis inhibitor 1)

Using a known p53-specific DNA-binding site as a probe, a cosmid library was screened in search of genes that may be nearby.⁶⁶ BAI1 was isolated as a brain-specific transcript that can be upregulated by wild-type p53 in glioblastoma cells. The BAI1 gene encodes a 1584-amino acid polypeptide that contains seven hydrophobic segments suggestive that it is a membrane spanning protein.⁶⁶ BAI1 also contains five Tsp1/Tsp2 repeats in its extracellular region. Recombinant BAI1 was found to be a potent inhibitor of neovascularization in the rat cornea.⁶⁶

C. GD-AiF

Glioblastoma cells engineered to express wild-type p53 were found to secrete a factor called GD-AiF that appears to neutralize the angiogenic factors produced by the parental cells.⁶⁷ It remains unclear whether GD-AiF and BAI1 are the same or different factors.⁶⁶

Feedback regulation by MDM2, a p53-target gene

Some targets of p53 appear to negatively regulate its function through regulation of the half-life of p53. The MDM2 gene encodes a p53-binding protein that conceals its transactivation domain thereby inhibiting its function as a transcriptional activator. MDM2 expression is increased in some tumor cells which as a consequence inactivate p53 function in cell cycle arrest and apoptosis without the apparent need to mutate p53. MDM2 appears to play a role in targeting p53 for degradation through a mechanism that may involve p300¹⁴ and which appears to be inhibitable by the ARF protein⁵⁻¹⁰ or phosphorylation of p53 at its amino-terminal end.^{15,17} MDM2 is a gene whose expression is transcriptionally upregulated by p53. Therefore induction of MDM2 is believed to feedback regulate p53 function in cell cycle arrest and apoptosis induction. In another negative feedback loop, p53 appears to repress ARF expression thereby indirectly regulating p53 protein stability.⁶

p53-upregulated targets of unknown function

Cyclin G

Cyclin G was isolated as a p53-regulated transcript from mouse cells expressing temperature-sensitive p53 and was found to be upregulated following γ -irradiation in a wild-type p53-dependent manner.⁶⁸ Cyclin G expression appears to be directly regulated through a p53 DNA-binding site in the upstream regulatory region of the cyclin G gene.⁶⁸ Overexpression of cyclin G leads to growth suppression, but the precise role of cyclin G as a p53 downstream target gene remains unknown.⁶⁸

GML (GPI-anchored molecule-like protein)

Using previously isolated human genomic p53 DNA-binding sites as probes of a cosmid library, it was possible to isolate GML as a p53-regulated target gene.⁶⁹ GML appears to be directly regulated by p53 and its expression correlates with p53 status in esophageal cancer.⁶⁹ GML is a growth inhibitory p53-target gene whose expression appears to enhance the cytotoxic effects of several anti-neoplastic drugs including bleomycin, and cisplatin.⁶⁹ The mechanism of growth inhibition by GML and how it contributes to drug-induced apoptosis remains unclear.

Wip1

Differential display was used to isolate Wip1 as a γ -irradiation-induced transcript in wildtype p53-expressing WMN Burkitt lymphoma cells.⁷⁰ Wip1 has strong homology to type 2C protein phosphatases and appears to be a target for p53-dependent upregulation following γ -irradiation of multiple cell lines.⁷⁰ Wip1 can also be upregulated by UVC exposure. Wip1 suppresses cancer colony growth in a manner similar to p21(WAF1). The mechanism of Wip1 induction by p53, growth inhibition following Wip1 overexpression and the cellular substrates for the phosphatase activity of Wip1 remain unknown.

EI24 (Etoposide-Induced #24)

Differential display was used to isolate genes whose expression was increased in NIH3T3 cells exposed to the cytotoxic chemotherapeutic drug etoposide.⁷¹ EI24 is not only inducible by etoposide but also by ionizing irradiation or following exogenous overexpression of wild-type p53.⁷¹ The function of EI24

remains unknown, although it may be structurally related to the human PIG8 gene (T. Maclachlan and W.S.E.-D., unpublished observation).

EF-1 α (Elongation Factor-1 alpha):

An erythroleukemia cell line that expresses temperature-sensitive p53 was used to isolate EF-1 α through a differential display technique.⁷² The EF-1 α promoter appears to contain multiple p53-responsive elements that are conserved between the human, rat and frog EF-1 α genes.⁷² In addition to being an essential component of the eukaryotic translation machinery, EF-1 α is a microtubule-severing protein. It has been hypothesized that the microtubule-severing activity of EF-1 α may contribute to p53-dependent cell death.⁷²

HIC-1 (Hypermethylated In Cancer #1)

A novel zinc finger protein encoding gene was found to be underexpressed in cancer when hypermethylated.⁷³ The gene appears to be regulated by p53 and suppresses growth when overexpressed in a variety of human cancer cell lines. The HIC-1 gene which is located on human chromosome 17p is a candidate tumor suppressor that appears to be inactivated by hypermethylation as a late event in tumor evolution.⁷⁴

RTP/rit42 (reduced in tumor, 42 kDa)

Using a differential gene expression screening technique comparing normal versus tumor cells, rit42 was isolated as a gene whose expression is reduced in tumor versus normal cells.⁷⁵ rit42 is the same as RTP, a homocysteine-inducible gene which also bears similarity to an androgen-responsive gene in the mouse. RTP/rit42 is induced by DNA-damaging agents in a p53-dependent manner.⁷⁵ Overexpression of RTP/rit42 appears to suppress cell growth and tumorigenicity in nude mice.⁷⁵ The precise function of RTP/rit42 *in vivo* and the mechanism by which p53 regulates its expression remains unclear.

TP53TGI

Use of a differential display technique and an inducible wild-type p53 in colon cancer cells led to the isolation of TP53TGI, a novel 90 amino acid polypeptide encoding gene whose expression is increased in

a wild-type p53-dependent manner in response to UV radiation, bleomycin or cisplatin.⁷⁶ The function of TP53TGI downstream of p53 remains unclear.

Cathepsin D

A subtractive hybridization approach led to the isolation of Cathepsin D as a p53-upregulated transcript in chemosensitive cancer cells exposed to doxorubicin.⁷⁷ There is some evidence that the Cathepsin D promoter may be directly regulated by p53 through DNA-binding and transactivation.⁷⁷ Cathepsin D also appears to be involved in IFN- γ and TNF- α -induced apoptosis. The p53-dependent upregulation of Cathepsin D and its role as a potential inducer of apoptosis appears to be restricted to certain cell types, such as hematopoietic cells.⁷⁷ In epithelial cells the Cathepsin D promoter is strongly inhibited by p53. Because Cathepsin D overexpression has been associated with a metastatic phenotype, repression of Cathepsin D expression may be a mechanism by which p53 suppresses metastasis development.⁷⁷ It is of interest that the Cathepsin D promoter contains p53 DNA-binding sites and appears to be upregulated by p53 under some circumstances and repressed by p53 in a cell-type-specific manner.

wig-1 (wild-type p53-induced gene 1)

A novel three-zinc-finger containing protein (WIG-1) is encoded by a gene whose 7.6-kb and 2.2-kb transcripts are both upregulated by ts p53 as well as ionizing radiation in a wide array of tissues.⁷⁸ The cell line from which the wig-1 gene was isolated undergoes both cell cycle arrest and apoptosis in response to wild-type p53.⁷⁸ The function of wig-1 remains unclear.

p53-repressed genes

Although the main focus of this review is the growing list of p53-upregulated genes, a brief mention should be made that some of the biological effects of p53 may be mediated in part through repression of gene expression. The regulatory regions of repressed genes do not in general contain p53 DNA-binding sites.

p53 is known to repress expression from serum-inducible promoters including *c-fos*.^{79,80} p53 represses expression of a number of promoters involved in promoting growth including the IL6 gene promoter,⁸¹ the *c-myc* promoter,⁸² the insulin receptor

promoter,⁸³ the IL2 and IL4 promoters.⁸⁴ p53 represses the activity of a number of transcription factors including TBP,⁸⁵ SP1,⁸⁶ the thyroid hormone receptor,⁸⁷ the estrogen receptor,⁸⁸ the hypoxia-inducible factor⁸⁹ and STAT5.⁹⁰ p53 repression of Bcl-2⁹¹ and re1A⁹² may contribute to its proapoptotic effects. Other genes that have been reported to be repressed by p53 include hsp70,⁹³ MDR1,⁹⁴ MAP4,⁹⁵ and actin.⁹⁶ The C-terminal domain of p53 may be involved in mediating repression.⁹⁷ The mechanism of p53-mediated repression of gene expression is not clear and the contribution of repression to the biological effects of p53 is still under investigation.

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