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Review

The common and distinct target genes of the p53 family transcription factors

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Abstract
p53 is the most commonly mutated gene in human cancer. After activation by cellular stresses such as DNA damage or oncogene activation, p53, a sequence-specific DNA-binding protein, induces the expression of target genes which mediate tumor suppression. Two recently identified p53 homologues, p63 and p73, appear to function similarly to p53, that is, they both activate target gene expression and suppress cell growth when overexpressed; however, the *p63* and *p73* genes are rarely mutated in human cancer and do not adhere to Knudson's classical model of a tumor suppressor gene. Recently, exciting observations suggest nonoverlapping functions for the family members. Herein, we outline the recent literatures identifying and characterizing both the common and distinct target genes of the p53 family transcription factors in relation to their signaling pathways.

Key words. p53; p63; p73; transcription; cell cycle arrest; apoptosis; development.

Introduction

p53, a tumor suppressor, is the most commonly mutated gene in human cancer. After activation by cellular stresses, p53, a sequence-specific transcription factor, functions to transactivate genes that mediate cell cycle arrest, apoptosis and other p53-dependent activities. Recently, p63 and p73 proteins have been identified and emerged as p53 homologues [1–3]. Like p53, both p63 and p73 bind to the canonical p53 response element, transactivate p53 target gene expression and induce apoptosis when overexpressed. Unlike *p53*, the genes encoding *p63* and *p73* are rarely mutated in human cancer, and neither of the knockout mouse models exhibits a propensity for tumor formation. These animals demonstrate rather discrete developmental defects [4, 5]. Interestingly, the *p53* knockout mouse, which develops multiple tumors at a young age, exhibits few if any of these developmental defects [6]. Thus, these data suggest that while p53 is important for the prevention of cancer, both p63 and p73 are crucial for normal development.

The p53 family proteins contain many functional domains (see fig. 1). p53 family members share significant similarity at the amino acid level within three domains: the activation domain (AD), the DNA binding domain (DBD), and the tetramerization domain (TD). p63 AD (residues 1–59) and p73 AD (residues 1–54) are 22 and 29% identical to the AD1 in p53 (residues 1–45), respectively. p63 DBD (residues 142–321) and p73 DBD (residues 131–310) are 60% and 63% identical to that in p53 (residues 100–300), respectively. p63 TD (residues 353–397) and p73 TD (residues 345–390) are 37 and 38% identical to that in p53 (residues 353–397), respectively. All family members contain at least one proline-rich domain (PRD), containing PXXP motifs. p53 and p73 have a C-terminal basic domain (BD) containing many basic amino acids, while both p63 and p73 contain a C-terminal sterile-α-motif (SAM).

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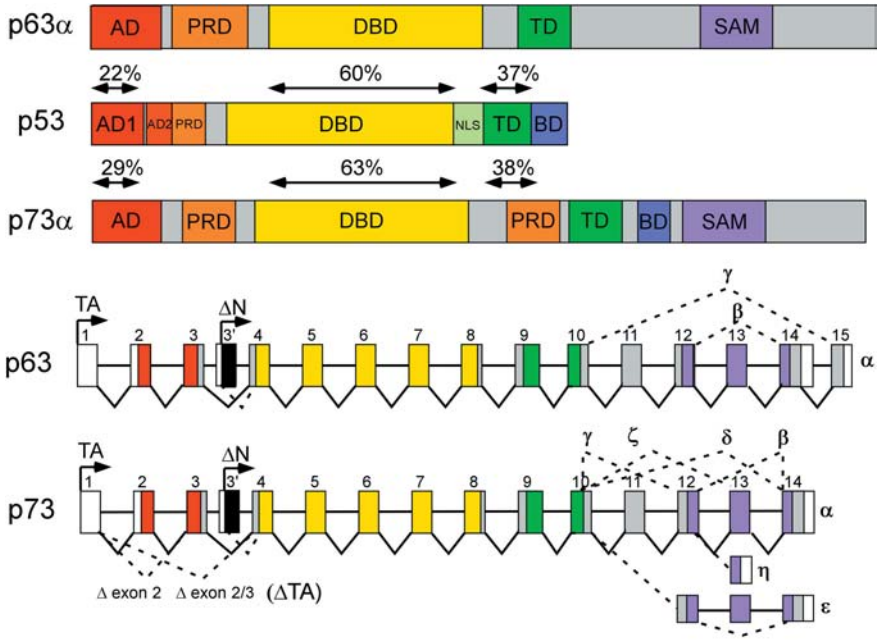

Figure 1. Functional domains and isoforms of the p53 family proteins. AD, activation domain; PRD, proline-rich domain; DBD, DNA binding domain; TD, tetramerization domain; BD, basic domain; SAM, sterile-α-motif domain; TA, isoforms transcribed from the upstream promoter; and ΔN, isoforms transcribed from the cryptic promoter within intron 3. Dotted lines denote alternative splicing. % denotes percent identity.

Although p63 and p73 share features of the p53 tumor suppressor, they are also unique in that many isoforms exist for *p63* and *p73* (see fig. 1). p53 predominantly exists as a single isoform. Both p63 and p73 undergo alternative splicing at their C-termini, resulting in three p63 isoforms ($\alpha-\gamma$) and seven p73 isoforms ($\alpha-\eta$) that differ at their C-termini. These isoforms are transcribed from the upstream promoter, called the TA variants. These isoforms are also transcribed from a cryptic promoter within intron 3, called the ΔN variants. A third group of p73 transcripts has been found, which is transcribed from the 5′ upstream promoter but is aberrantly spliced to either exclude exon(s) 2 or 2 and 3, called the TA variants, or to include and additional exon, exon 3′. Thus, many p63 and p73 proteins are generated through alternative splicing and the use of two transcriptional start sites. For reviews, see [7, 8].

With the expansion of the p53 family and the discovery that the family serves similar, yet nonoverlapping functions, the p53 family network is slowly beginning to unfold. Recent work has focused on the identification of genes regulated by p63 and p73. In order to understand how the family functions during tumor suppression and development, p53 family target genes need to be identified and characterized. This review will provide a current dissection of the role of the p53 family in tumor suppression and development. Herein, we outline the recent literatures identifying and characterizing both the common and distinct target genes of the p53 family transcription factors in relation to their signaling pathways, specifically cell cycle arrest, DNA repair, inhibition of angiogenesis, inhibition of metastasis, apoptosis, regulation of p53 family and development. Due to space limitations, we apologize for not discussing those target genes identified through microarray and bioinformatics approaches [9–11] as well as many other important target genes that have been identified. We note that the discussed unique p53 target genes may be responsive to p63 or p73; however, to date, no supporting evidence exists in the literature.

The p53 family proteins: similar, yet different

The p53 family regulates both common and distinct target genes. Thus, the question arises, How do these homologous proteins differentially regulate gene expression? At least two factors, namely the activation of the p53 family proteins themselves and their mechanism of target gene transactivation, must be involved in facilitating differential gene regulation.

Although the p53 family proteins are collectively activated and stabilized after DNA damage, other signals differentially activate or inhibit the p53 family proteins. For example, BMP signaling induces the expression of ΔNp63α but not other p53 family proteins [12]. The transcriptional repressor ZEB inhibits p73 expression through binding E-boxes located within intron 1 of the *p73* gene

[13]. In addition, Mdm2, a well-characterized negative regulator of p53, functions as a positive regulator of p63 and possibly p73 [14–17]. Thus, the p53 family proteins are activated and stabilized in response to both common and distinct signals, enabling the proteins to function in both common and distinct manners.

As previously discussed, the p53 family proteins are both similar and yet different in composition and organization of functional domains (see fig. 1). These differences may influence the ability of the p53 family proteins to interact with other proteins such as coactivators, corepressors or other regulatory proteins. Protein-protein interactions are important for activation, stabilization and transactivation potential of the p53 family members. In addition to differences in the functional domains, small differences among the domains' amino acid sequences themselves may also influence ability of the p53 family proteins to transactivate certain target genes. The DBDs among the p53 family proteins are similar; however, differing amino acids may alter the ability of the p63 and/or p73 to recognize the canonical p53-responsive element. The canonical p53 responsive element contains two decamers or half-sites, RRRCWWGYYY, which are separated by a spacer of 0–13 bp (R = purine, C = cytosine, W = adenine or thymidine, G = guanine and Y = pyrimidine). Thus, the p53 family proteins may recognize similar but also subtly different DNA sequences. For example, AQP3, a glycerol and water transporter, is induced by p73, but weakly by p53 [18]. Instead of two half-sites, the promoter of AQP3 contains three half-sites. Similarly, JAG1 and JAG2, ligands of the notch receptor, are induced by p63 and p73, but not by p53 [19]. Four half-sites of the p53 responsive element are located within intron 2 of the *JAG1* gene. Thus, both common and distinct regulatory mechanisms and protein sequences are necessary for the response of p53 family proteins to common and different signals.

Cell cycle arrest

The cell cycle is a tightly controlled process by which a cell replicates its DNA and then divides into two daughter cells. The cell cycle consists of four phases: mitosis phase (M phase), synthesis phase (S phase), Gap1 between M phase and S phase (G1 phase), and Gap2 between S phase and M phase (G2). For a review, see [20]. Progression through the cycle is regulated by a series of checkpoints, the G1/S checkpoint, the S checkpoint and the G2/M checkpoint, that function to prevent the transmission of genetically unstable material to the daughter cells. The G1/S and the G2/M checkpoints mainly center on the regulation and activation of cyclin-dependent kinase (CDK) complexes. Phosphorylation of target proteins by CDKs, which are a serine/threonine kinase, leads to cell cycle progression. Because CDK levels are stable throughout the cell cycle, CDK activity is regulated by their respective cyclins. The p53 family of transcription factors inhibits cell cycle progression by initiating G1, S, and G2 arrest in response to a variety of stress signals (see fig. 2).

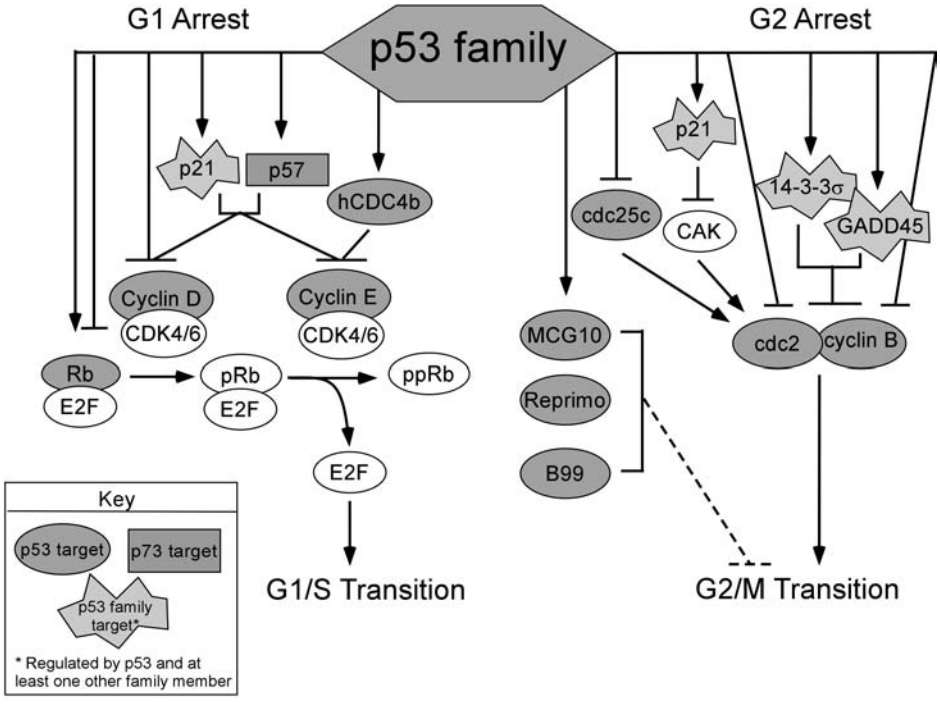

Figure 2. Cell cycle arrest mediated by p53 family target genes.

G1 arrest

The G1 phase of the cell cycle is regulated by the G1 cyclins (D-type and E-type cyclins) and CDKs 2, 4 and 6. In early G1 phase, cyclin D/CDK4/6 phosphorylates the retinoblastoma protein (pRB). pRB binds and inhibits the E2F transcription factor. During late G1 phase, cyclin E/CDK4 hyperphosphorylates pRB (ppRB), facilitating the release of E2F. E2F is then active to induce the expression of genes required for S phase entry. In the event of a stress signal such as DNA damage, G1 arrest is induced by the inhibition of CDKs by cyclin-dependent kinase inhibitors (CKIs). CKIs either bind to cyclins, CDKs or cyclin-CDK complexes. Without cyclin/CDK activity, E2F cannot function to induce expression of genes required for entry into S phase. For a review, see [21].

To date, the only common target of the p53 family involved in G1 arrest is p21, a CKI of the Cip/Kip family. p53, p63 and p73 have all been shown to upregulate endogenous p21 messenger RNA (mRNA) and protein levels [22–24]. p21 binds cyclin-CDK complexes through recognition of a ZRXL motif in the substrate [25]. Overexpression of p21 induces G1 arrest [26]. Interestingly, p21 functions differently at low and high concentrations. At low levels, p21 promotes the association of cyclin D and CDK4 complexes during early G1. However, in response to cellular stresses and perhaps certain developmental signals, p53, p63 and p73 rapidly increase the level of p21 protein. The increase in concentration of p21 stoichiometrically overwhelms and inhibits the activity of cyclin/CDK complexes.

Although p21 is the major effector of p53 family-mediated G1 arrest, p53 regulates a variety of genes that also contribute to G1 arrest. p53 induces and represses the expression of genes that inhibit and promote cell cycle progression, respectively. In addition to the p53-mediated inhibition of cyclinE/cdk2 complexes by p21, p53 also inhibits the activity of cyclin E through the induction of hCDC4b [27]. hCDC4b, an F box protein and component of the SCF ubiquitin ligase complex, targets phosphorylated cyclin E for ubiquitin-mediated degradation.

p57^{KIP}, a paternally imprinted gene, has recently been identified as a unique target of p73 [28]. p57^{KIP}, a member of the Cip/Kip family of CKIs, participates in the induction of G1 arrest by inhibiting cyclin-CDK complexes. To date, unique targets of p63 have not been identified that mediate G1 arrest.

G2/M arrest

The G2/M checkpoint centers on the activation of the cyclin B/cdc2 complex (for a review see [29]). The activation is regulated at many levels. The first level involves formation of the cyclin B/cdc2 complex. Cyclin B synthesis begins during G2 phase, and when protein levels accumulate during late G2 phase, an inactive cyclin B/cdc2 complex is formed. Activation of the complex involves the removal of inhibitory phosphates at T14/Y15 and addition of an activating phosphate at T161 on cdc2 by cdc25c phosphatase and CDK activating kinase (CAK), respectively. The subcellular localization of the cyclinB/cdc2 complex also regulates the activity. Nuclear export inhibits the accumulation of cyclinB/cdc2 in the nucleus until the cell is ready to enter mitosis. Inhibition of nuclear export facilitates accumulation of cyclinB/ cdc2 in the nucleus and the subsequent phosphorylation and activation of proteins required for cell cycle progression.

In addition to G1 arrest, the p53 family contributes to cell cycle regulation by inducing G2/M arrest. The p53 family regulates p21, GADD45 and 14-3-3σ. p21, the major mediator of G1 arrest, also participates in G2 arrest. p21 associates with cyclin B/cdc2 complexes and inhibits the activating T161 phosphorylation of cdc2 by CAK [30]. The growth arrest and DNA damage inducible protein (GADD45) was the first identified p53 target gene. GADD45 binds cdc2, preventing cyclinB/cdc2 complex formation and subsequently inhibiting the kinase activity [31]. *14-3-3σ*, a gene implicated in G2/M arrest, is induced by p53 and p73 and repressed by ΔN p63 [32–34]. Overexpression of 14-3-3σ induces G2 arrest [32]. 14-3-3σ, a scaffold protein, removes cyclin B/cdc2 from the nucleus to physically separate cyclin B/cdc2 from its target proteins. *14-3-3$\sigma^{-/-}$* cells cannot maintain G2/M arrest [35].

p53 also regulates the G2/M transition by inducing the expression of genes that inhibit cell cycle progression and repressing genes that promote progression. p53 induces MCG10, Reprimo and B99 to facilitate G2/M arrest [36–38]. Although the exact mechanisms of these target genes are unknown, these genes induce G2/M arrest when overexpressed. MCG10 is an RNA-binding protein [36]. Reprimo, a highly glycosylated, cytoplasmic protein, may play a role in regulation of cyclin B/cdc2 localization [37]. B99, also known as G- and S-phase specific protein (GTSE-1), localizes to microtubules [38]. Reports also demonstrate that p53 represses the expression of cdc2, cyclin B and cdc25c, each of which plays an important role during the progression from G2 to M phase [39–41].

DNA Repair

DNA repair is a process which enables a cell to maintain fidelity of its genome. There are several pathways for DNA repair, for example excision repair, consisting of both nucleotide excision repair (NER) and base excision repair (BER), mismatch repair (MMR), and double strand break (DSB) repair, reviewed in [42]. Each pathway utilizes unique enzymatic machinery. Bulky lesions

such as ultraviolet (UV)-induced pyrimidine dimers are repaired by NER. NER begins when the repairosome, a multi-enzyme complex, recognizes a bulky lesion. The repairosome cuts the DNA on both sides of the lesion, removes an oligonucleotide stretch, then fills and seals the gap. BER occurs when NER neglects a modified base. In contrast to NER, which removes an oligonucleotide stretch, BER removes only the damaged base. Mismatched bases, as a result of spontaneous hydrolysis or incorrect incorporation during DNA replication, are repaired by MMR. During MMR, a single mismatched nucleotide is replaced with the correct one. Ionizing radiation or oxidative damage can induce DSBs. Depending on cellular circumstances, DSB repair occurs through nonhomologous end joining or homologous recombination mechanisms.

As a guardian of the genome, it is not surprising that the p53 family plays a role in DNA repair (see fig. 3). The p53 family participates in NER by inducing the expression of GADD45, xeroderma pigmentosum group E gene [XPE] and XPC (43–45). *GADD45*$^{-/-}$ murine keratinocytes show impaired thymidine dimer repair and increased sensitivity to UV radiation [46]. GADD45 has also been shown to interact with the core histones and facilitate topoisomerase relaxing of chromatin [47].

Defective NER is associated with xeroderma pigmentosum (XP), an autosomal recessive disorder characterized by excessive skin cancers caused by an extreme sensitivity to UV light. The p53 target gene *XPE* is a subunit of the UV-damaged DNA-binding protein (UV-DDB). XPE was implicated in NER when XPE cells were found to lack a nuclear factor that binds to DNA [48]. XPE localizes to damaged DNA and enhances the removal of UV photoproducts such as cyclobutane pyrimidine dimmers (CPDs) and 6-4 photoproducts (6-4PP) [49]. The p53 target gene *XPC* is also implicated in NER. XPC localizes to areas of UV-induced damage, and its interaction with damaged DNA is enhanced by XPE [49]. *XPC* mutant mice show reduced DNA repair following UV irradiation and an inability to repair 6-4PPs [50].

Interestingly, the p53 protein itself has been implicated in both NER and BER of the excision repair pathway. p53 interacts with several factors that play a role in NER, namely TFIIH, and the helicases XPB and XPD [51]. p53 also positively influences BER; overexpression of transactivation-deficient p53 mutants but not core domain mutants augments BER activity [52]. p53 has been shown to interact with DNA pol β to stabilize the interaction between damaged DNA and BER machinery [53].

The mismatch repair pathway is also influenced by the p53 family. p53 and p73 induce the expression of *p53R2*, a gene with homology to the R2 regulatory subunit of ribonucleotide reductase (RNR) [54]. RNR consists of an R1 catalytic subunit and an R2 regulatory subunit. p53R2 functions in a general manner to increase the pool of free dNTPs when repair is needed. Although p53R2 and R2 are similar, they differ in their N-terminal amino acid sequence and regulation. p53R2 is induced by p53 and p73, while R2 synthesis occurs during S phase. p53R2 and R1 complex functions as an active RNR [55].

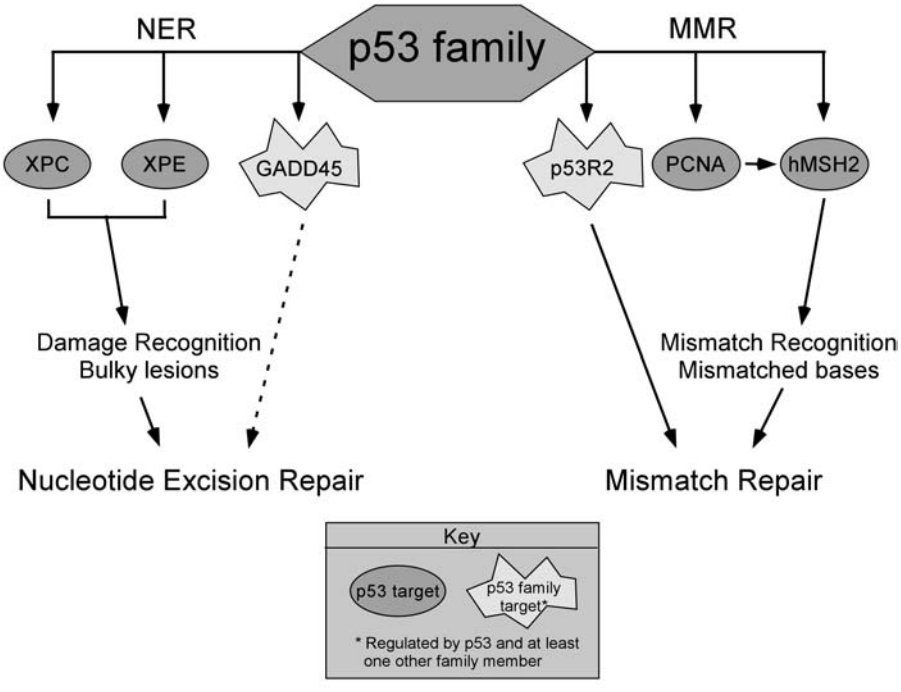

Figure 3. DNA repair mediated by p53 family target genes.

p53 upregulates two very important proteins in the MMR pathway: human MutS homologue 2 (hMSH2) and proliferating cell nuclear antigen (PCNA) [56, 57]. Mutations of *hMSH2* result in the colorectal cancer syndrome hereditary nonpolyposis colorectal cancer (HNPCC). Thus, *hMSH2* is also known as *HNPCC type 1* (HNPCC-1). hMSH2 functions in mismatch recognition and binds mismatched bases [58]. Interestingly, the binding of MMR machinery to DNA appears to be regulated by the binding of ADP or ATP. An ADP-bound hMSH2-hMSH6 complex binds DNA, whereas an ATP-bound complex dissociates from DNA [59]. *PCNA*, a cofactor for DNA polymerase δ, is another p53 target gene and has been shown to interact with hMSH2 to facilitate hMSH2 transfer to mismatched bases [60].

Inhibition of angiogenesis

An important step in the growth of any tumor beyond a few millimeters is the generation of new blood supplies that feed and nurture the malignant cells. Angiogenesis is a multi-step process, involving degradation of the endothelial cell basement membrane, endothelial cell migration to the perivascular stroma and capillary sprouting (for a review see [61]). Previously, the tumor suppressor p53 was understood to regulate the process of angiogenesis through the activation of genes that inhibit neovascularization and the repression of genes that promote vessel growth. With the identification of p63 and p73, p53 family regulation of angiogenesis has broadened and become more complex (see fig. 4). The p53 family differentially regulates thrombospondin-1 (TSP-1), an anti-angiogenic molecule, and vascular endothelial growth factor (VEGF), a promoter of angiogenesis. p53 also regulates unique target genes that function to inhibit vessel growth. TSP-1 is activated by p53 and repressed by p73 [62, 63]. TSP-1, an extracellular matrix glycoprotein, is secreted by many cell types and binds several cellular receptors. TSP-1 inhibits angiogenesis through the binding of its type I repeat with the CD36 LIMP II Emp sequence homology (CLESH-1) domain of the CD36 transmembrane receptor on microvascular endothelial cells [64]. The CD36 receptor is a member of the class B scavenger receptor family. TSP-1 binding to CD36 ultimately leads to sequential activation of the Src-tyrosine kinase family member Fyn, p38MAPK and caspase 3, culminating in apoptosis [65]. What is the function of p53 induction and p73 repression of TSP-1? Perhaps an explanation lies in the functional domains of the TSP-1 molecule itself. The N-terminal domain of TSP-1 is pro-angiogenic in certain models, such as corneal neovascularization, and the central region of TSP-1 containing the type I repeat is anti-angiogenic [64, 66]. Moreover, TSP-1 has been implicated in both abrogating and enhancing endothelial cell migration. In addition to TSP-1, VEGF is differentially regulated by the p53 family. VEGF binds to VEGF receptor on endothelial cells and enhances endothelial cell

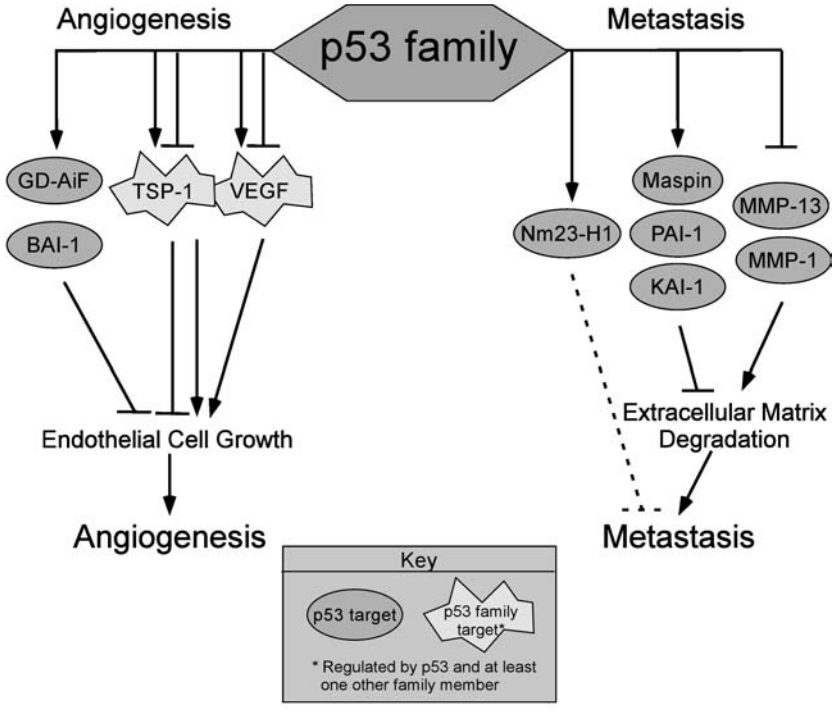

Figure 4. Inhibition of angiogenesis and metastasis by p53 family target genes.

growth. p53 represses VEGF expression, while p63 and p73 have been shown to both repress and induce the expression of VEGF [63, 67–69].

p53 functions to inhibit angiogenesis in the brain by promoting the expression of both glioma-derived angiogenesis inhibitory factor (GD-AiF) and brain specific angiogenesis inhibitor-1 (BAI-1) [70, 71]. GD-AiF is a secreted inhibitor of angiogenesis. BAI-1 is a seven transmembrane protein with the extracellular domain containing an RGD integrin binding domain and five TSP type I repeats [71]. In vitro models suggest that restoring expression of BAI-1 can suppress tumor angiogenesis without affecting cell proliferation [72].

Inhibition of Metastasis

Tissue invasion and metastasis involves a multi-step process of invasion, intravasation, extravasation and growth at a secondary site. Invasion and metastasis occur when genes that normally function to hold a cell in place lose their ability to do so. For a review on the extracellular matrix milieu, see [73]. Cell-cell adhesion is mediated by cadherins, transmembrane proteins that mediate Ca^{++}-dependent adhesion and cell-adhesion molecules (CAMS), cell surface proteins. Integrins, a family of transmembrane proteins, mediate adhesion of cells to the extracellular matrix (ECM). Altered gene expression or targeted degradation leads to the disruption of cell-cell and cell-matrix interactions. Tumor cells then lose contact with each other and migrate. Alteration in extracellular protease activity is the other major mediator of invasive potential and metastasis. Cells with high metastatic potential have increased protease activity and decreased protease inhibitor activity. ECM-degrading proteases are the matrix metalloproteinases (MMPs) and the serine proteases. MMPs are regulated by tissue inhibitors of MMPs (TIMPs). Serine proteases are regulated by serpins, inhibitors of serine proteases.

To date, neither p63 nor p73 is known to regulate inhibitors of metastasis, thus this section of the review will focus on p53-mediated inhibition of metastasis (see fig. 4). p53 inhibits metastasis by inducing genes that block ECM degradation and by repressing genes that degrade the ECM. p53 induces the expression of at least two serpins, plasminogen activator inhibitor-1 (PAI-1) and maspin [74, 75]. PAI-1 functions to inhibit urokinase-type plasminogen activator (u-PA). u-PA initiates a cascade of cleavages that ultimately result in the activation of plasmin. Plasmin degrades a wide variety of ECM proteins such as fibrin, fibronectin and laminin. By inducing PAI-1, ECM degradation is limited, and the metastatic potential of tumor cells is decreased. Maspin, a classified serpin, does not utilize its protease inhibitor activity to inhibit migration or metastasis [76]. Overexpression of maspin in a highly invasive mouse mammary tumor inhibits tumor growth and metastasis [77]. In addition, the tumors are more encapsulated with less necrosis. Maspin has been shown to interact with collagen type I and III and to increase cell adhesion to the ECM [78, 79].

KAI-1/CD82, a p53 target, is a member of the transmembrane 4 superfamily (TM4SF) [80]. Members of the TM4SF are implicated in inhibiting cell motility and metastatic potential. KAI-1/CD82 has been shown to inhibit metastasis of prostate cancer cells and to induce a homotypic aggregation of prostate cancer cells in a Src-dependent manner [81]. Overexpression of KAI-1/CD82 has been associated with an increase in focal adhesion kinase (FAK) and Lyn, a Src kinase, and a decrease in p130CAS expression. Restoration of p130CAS restores cell mobility [82].

p53 has recently been shown to induce the expression of the metastasis suppressor gene *Nm23-H1*, a nucleoside diphosphate kinase [83]. High levels of Nm23-H1 correlate with low metastatic potential and reduced levels correlate with increased metastatic potential. The mechanism by which Nm23-H1 inhibits metastasis is yet unknown and may not be associated with its known function as a nucleoside kinase [83].

Apoptosis

Apoptosis, or programmed cell death, has been extensively studied and is a highly complex process. Apoptosis occurs when a cell is triggered, from either outside or within, to undergo a type of cell death that is undetected by the immune system; thus apoptosis occurs without inflammation. The characteristics of an apoptotic cell are nuclear condensation and fragmentation, membrane blebbing and mitochondrial swelling. In vast contrast, a cell can also die by necrosis, where the cell swells and bursts. Because the p53 family proteins act as stress sensors, it is not surprising that the p53 family plays a role in inducing apoptosis. There are several pathways by which a cell can initiate apoptosis. Two well-studied pathways are the extrinsic or death receptor pathway and the intrinsic or mitochondrial pathway. For reviews, see [84, 85]. Briefly, the extrinsic pathway is activated when an extracellular ligand binds a cell surface death receptor, initiating a signal to activate the initiator caspase 8. The intrinsic pathway involves the alteration of the mitochondrial membrane potential, facilitating the release of cytochrome c and the eventual activation of the initiator caspase 9. The intrinsic and extrinsic pathways then converge with initiator caspases 8 and 9 activating effector caspases 3, 6 and 7. The cleavage of nuclear and cytoskeletal structural proteins by activated effector caspases triggers cellular events that culminate in apoptosis. A less well characterized apoptotic pathway involves the

production of reactive oxygen species (ROS), for a review, see [86]. ROS are generated during normal mitochondrial physiology, and ROS levels increase during p53-mediated apoptosis. Although the exact mechanism by which ROS facilitate apoptosis is yet unknown, ROS may act as mediators of other pro-apoptotic proteins. The p53 family plays a role in all three of these apoptotic pathways and most likely other pathways that have not been fully established.

While many p53 target genes have been identified to play a role in p53-mediated apoptosis, no single gene that is absolutely required for apoptosis has been identified. Much effort is dedicated to the identification of this gene. However, since p53 regulates all known apoptotic pathways, it is likely that a given apoptotic target gene is only necessary for a specific apoptotic pathway. Because many p53 pro-apoptotic target genes have been identified, this section will highlight pro-apoptotic target genes regulated by the p53 family and focus on recently identified pro-apoptotic target genes, see figure 5 and table 1.

Apoptosis: the extrinsic pathway

The death receptor pathway is a means of triggering cell death after an extrinsic signal. To date, the p53 family does not regulate common genes that participate in the extrinsic apoptotic pathway. p53, however, does induce many genes that participate in this process. p53 upregulates two cell surface death receptors, Fas/CD95 and KILLER/DR5, and the ligand for Fas, namely FasL or CD95L [87–89]. Both Fas and KILLER/DR5 are members of the tumor necrosis factor (TNF)/nerve growth factor receptor superfamily. FasL/CD95L is a member of the TNF family. Ligand binding induces receptor trimerization and clustering of the intracellular death domain (DD) region of the receptor. The DD recruits the adaptor protein, Fas-associated death domain (FADD), through homotypic DD interactions. The death effector domain (DED) of FADD recruits pro-caspase-8 through homotypic DED interactions, forming the death-inducing signaling complex (DISC). Activation of the initiator *caspase-8*, another p53 target gene, eventually leads to activation of effector caspases and induction of apoptosis [90]. As more pro-caspase-8 molecules accumulate at the DISC, pro-caspase-8 undergoes transcatalysis, resulting in activation of a large pool of the initiator caspase-8. p53 is also shown to induce the expression of caspase-1 and an effector caspase-6 [91, 92].

To regulate the amplitude of the apoptotic signal, p53 induces the expression of proteins that inhibit the extrinsic apoptotic pathway, namely TRUNDD/TRAIL-R4, TRID/TRAIL-R3 and cFLIP [93–95]. TRUNDD/TRAIL-R4 and TRIDD/TRAIL-R3 are TRAIL decoy receptors. The decoy receptors lack the DD. Therefore, upon ligand binding and receptor trimerization, the decoy receptors cannot recruit DED containing proteins required to initiate the caspase cascade. Cellular FLICE-like inhibitory protein (cFLIP) binds and inhibits caspase-8, preventing formation of an active DISC. Recently, cFLIP-L, but not cFLIP-S, was shown to inhibit p38MAPK activation and induction of apoptosis by in-

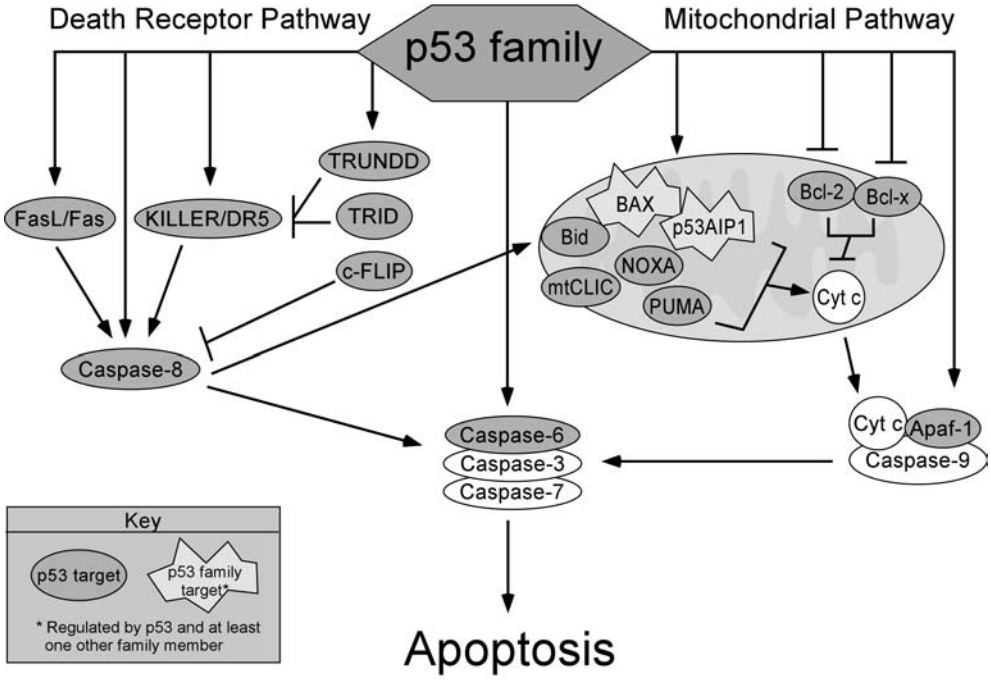

Figure 5. Apoptosis mediated by p53 family target genes.

Table 1. Transcriptional targets of the p53 family involved in apoptosis.

Gene	Regulation	Function	Ref.
Apaf-1	p53+	component of the apoptosome; intrinsic apoptotic pathway	[116]
APC	p53+	participates in β-catenin degradation	[180]
BAX	p53+, p63+, p73+	pro-apoptotic Bcl-2 family member; intrinsic apoptotic pathway	[108]
Bcl-2	p53–	anti-apoptotic Bcl-2 family member; inhibits Cyt c release from mitochondria	[181]
Bcl-x	p53–	anti-apoptotic Bcl-2 family member; inhibits Cyt c release from mitochondria	[109]
BID	p53+	pro-apoptotic Bcl-2 family member; intrinsic apoptotic pathway	[107]
BIK/NBK	p53+	BH3-only pro-apoptotic Bcl-2 family member that localizes to the ER	[114]
Caspase-1	p53+	caspase	[91]
Caspase-6	p53+	effector caspase; functions during the extrinsic and intrinsic apoptotic pathways	[92]
Caspase-8	p53+	initiator caspase; functions during the extrinsic apoptotic pathway	[90]
Cathepsin D	p53+	lysosomal proteinase	[182]
cFLIP	p53+	inhibitor of caspase-8	[95, 96]
c-Fos	p53+/–	transcription factor; oncoprotein	[183, 184]
COX-2	p53+/–, p73+	catalyzes the rate-limiting step of prostaglandin synthesis; inhibitor of apoptosis	[185, 186]
DAN	p73+	secreted inhibitor of BMP signaling; increased expression during cell death	[174, 179]
DDA3	p53+, p73+	overexpression inhibits cell growth	[187]
DR5/KILLER	p53+	death receptor; functions during the extrinsic apoptotic pathway	[88]
DRAL	p53+	LIM domain containing protein; promotes apoptosis	[188]
EF-1α	p53+	translation factor	[189]
EphA2	p53+, p63+, p73+	receptor tyrosine kinase; overexpression of EphA2 promotes apoptosis	[190]
Fas/Fas L	p53+	death receptor and ligand; functions during the extrinsic apoptotic pathway	[87]
FDXR	p53+, p63+, p73+	ferrodoxin reductase; functions in electron transfer, ROS production	[119]
GML	p53+	GPI-anchored molecule-like protein; overexpression promotes apoptosis	[191]
GPX	p53+	antioxidant; scavenges ROS	[126]
HSP27	p53+	heat shock protein; inhibitor of apoptosis; decreases levels of ROS	[125]
HTRA2	p53+	pro-apoptotic serine protease; inhibits an inhibitor of apoptosis (IAP)	[192]
IGFBP3	p53+	secreted IGF-binding protein; promotes apoptosis	[193]
mRTVP-1	p53+	pro-apoptotic putative transmembrane protein	[194]
mtCLIC	p53+	organellular chloride channel protein; localizes to mitochondria	[104]
NOXA	p53+	BH3-only pro-apoptotic Bcl-2 family member; intrinsic apoptotic pathway	[105, 110]
p21B	p53+	variant of the CKI p21; overexpression of p21B promotes apoptosis	[195]
p53AIP1	p53+, p73+	pro-apoptotic; localizes to mitochondria; intrinsic apoptotic pathway	[102]
p53DINP1	p53+	overexpression enhances phosphorylation of p53 and promotes apoptosis	[196]
p53RDL1	p53+	transmembrane receptor containing a cytoplasmic death domain	[197]
p85	p53+	role in the response to ROS	[128]
PAC1	p53+	dual T/Y phosphatase that specifically inactivates MAP kinases	[198]
PAG608	p53+	zinc finger protein; overexpression promotes apoptosis	[199]
PERP	p53+	tetraspan transmembrane protein; overexpression promotes apoptosis	[200]
PIDD	p53+	pro-apoptotic protein containing death and LIM domains	[201]
PIG3	p53+	oxidation-reduction reactions; ROS production	[123, 124]
PIG6,	p53+	proline oxidase; generation of proline-dependent ROS	[202]
PRG3	p53+	oxidation-reduction reactions; homology to apoptosis inducing factor (AIF)	[122]
PUMA	p53+	BH3-only pro-apoptotic Bcl-2 family member; intrinsic apoptotic pathway	[106]
Pw1/Peg3	p53+	interacts with Siah-1 and mediates BAX translocation to the mitochondria	[203]
Reaper	p53+	*Drosophila* protein; potent activator of caspase-dependent apoptosis	[204]
REDD1	p53+, p63+	role in the production of and response to ROS	[120]
Scotin	p53+	localizes to ER and nuclear membranes; caspase-dependent apoptosis	[205]
Siah	p53+	pro-apoptotic; interacts with APC facilitating degradation of β-catenin	[206]
SIP	p53+	stress induced protein; nuclear protein; promotes cell death when overexpressed	[207]
TP53TG5	p53+	suppresses cell growth	[208]
TRAF4	p53+	zumor necrosis receptor associated factor 4; overexpression promotes apoptosis	[98]
TRID	p53+	decoy TRAIL receptor lacking a death domain; inhibits extrinsic pathway	[94]
TRUNDD	p53+	decoy TRAIL receptor lacking a death domain; inhibits extrinsic pathway	[93]
Wig-1	p53+	zinc-finger protein; overexpression inhibits cell growth	[209]

+, Activation; –, inhibition.

hibiting its phosphorylation [96]. In further support of cFLIP's anti-apoptotic role, increased levels of cFLIP correlate with resistance to apoptosis by the death receptor pathway in bladder cancer cells [97].

p53 was recently shown to induce the expression of tumor necrosis receptor associated factor 4 (TRAF4) [98]. TRAF4 contains an N-terminal RING domain and a C-terminal TRAF domain. Overexpression of TRAF4 induces apoptosis and inhibits colony formation [98]. The mechanism by which TRAF4 induces apoptosis is unknown, and TRAF4 differs from other TRAFs in that it has not been identified to interact with any of the TNF receptors or IL-1R/Toll-like receptors. *TRAF4* mutant mice show axial skeletal malformations, impaired neural tube closure and tracheal malformations [99]. Thus far, no unique genes for p63 or p73 have been identified to play a role in the extrinsic pathway.

Apoptosis: the intrinsic pathway

In addition to the extracellular trigger, apoptosis is also triggered by an internal mechanism, namely through the mitochondrial pathway. Briefly, the mitochondrial membrane potential is altered, leading to the release of cytochrome c from the mitochondrial membrane. The apoptosome, consisting of cytochrome c, APAF-1 and caspase 9, activates effector caspases that mediate cellular events, leading to apoptosis.

The p53 family commonly upregulates at least two proteins that play a role in the mitochondrial pathway of apoptosis: Bax and p53-apoptosis inducing factor 1 (p53AIP1) [100–103]. Bax is a pro-apoptotic Bcl-2 family protein. In general, all Bcl-2 family members contain Bcl-2 homology (BH) domains that are similar to four of the seven α-helices of Bcl-X_L, another Bcl-2 family member. Bax contains a BH1, BH2 and BH3 domain. The mechanism of Bax-induced apoptosis is complex. Bax translocates from the cytosol to the inner mitochondrial membrane and facilitates the release of cytochrome c from the mitochondria. In the cytosol, cytochrome c combines with Apaf-1 and caspase-9 to form the apoptosome, which activates effector caspases. Both p53 and p73 induce the expression of p53AIP1 [102, 103]. p53AIP1 localizes to the mitochondria and interacts with Bcl-2 to facilitate the release of cytochrome c from the mitochondria. Interestingly, both p53 and p73 require posttranslational modifications to induce the expression of p53AIP1. p53 requires S46 phosphorylation, while p73 requires acetylation by p300/CBP [102, 103]. Perhaps this is evidence of a cellular switch or mechanism by which p53 and p73 are activated to induce apoptotic target genes.

Although only a few target genes have been positively identified for the entire p53 family, many genes have been identified that are regulated by p53. Many of these proteins localize to the mitochondria and regulate the release of cytochrome c. These mitochondrial localizing proteins include Bcl-2 family proteins as well as non-Bcl-2 family members. Two non-Bcl-2 family members include p53AIP1 and mtCLIC/CLIC4 [104]. mtCLIC, an organellular chloride channel protein of the CLIC family of intracellular chloride channels, reduces mitochondrial membrane potential [104]. The Bcl-2 family proteins include Bax, Noxa, p53 upregulated modulator of apoptosis (PUMA), BID, Bcl-2 and Bcl-x [105–109]. Noxa and PUMA are BH3-only containing Bcl-2 proteins. Noxa localizes to the mitochondria [110]. Through homotypic BH3 domain interactions, PUMA binds to Bcl-2 and Bcl-XL and induces cytochrome C release [111]. Recently, BID was identified to bridge the extrinsic and intrinsic pathways for apoptosis. Cleavage of BID by caspase-8 exposes an N-terminal glycine that is subject to myristoylation [112, 113]. The cleaved, modified BID translocates to the mitochondria to participate in the intrinsic apoptotic pathway. p53 represses the anti-apoptotic Bcl-2 family members Bcl-2 and the minimal promoter of Bcl-x [109]. These proteins function to protect the cell from apoptosis by stabilizing the mitochondrial membrane potential. p53 upregulates one other BH3 only Bcl-2 family member, BIK/NBK [114]. Interestingly, this protein localizes to the endoplasmic reticulum and not the mitochondria.

Recently, studies have shown that in addition to p53 target genes, the p53 protein itself localizes to the mitochondria and perhaps alters the mitochondrial membrane potential [115]. Thus, both the p53 protein and p53 target genes facilitate the induction of the intrinsic pathway.

In addition to genes localized to organelles, p53 also induces the expression of intracellular regulators of the intrinsic pathway, namely apoptosis protease-activating factor 1 (Apaf-1) and the effector caspase-6 [92, 116]. Apaf-1 combines with cytochrome c and caspase-9 to form the apoptosome. *Zac1*, a potential p53 target gene, has been shown to upregulate Apaf-1. Thus, a feedforward loop for the induction of apoptosis arises: p53 upregulates Zac1, then Zac1 and p53 collectively upregulate Apaf-1 [117].

Reactive oxygen species

Reactive oxygen species (ROS), such as superoxide anions, radicals and hydrogen organic peroxides, are highly toxic molecules that are detoxified by the body under normal conditions. Detoxification proteins include glutathione, thioredoxin and superoxide dismutase (SOD). During oxidative stress, a large accumulation of ROS, the body is unable to detoxify the molecules, and oxidative damage, such as protein crosslinking through sulfhydryl

groups and peroxidation of lipids, occurs. ROS are generated as a byproduct of respiration during electron transport in the mitochondria. Inhibition of electron transport increases ROS levels. Increased ROS levels correlate with the induction of apoptosis. High levels of ROS have been detected before mitochondrial membrane permealization and the release of cytochrome c. For reviews of mitochondrial physiology and the role of ROS during apoptosis, see [84, 86].

ROS have been correlated with p53-mediated apoptosis. Upon overexpression of p53, levels of ROS rise, and inhibition of ROS with antioxidants inhibits apoptosis in smooth muscle cells [118]. The p53 family commonly upregulate at least two proteins that participate in ROS-mediated apoptosis: ferrodoxin reductase (FDXR) and REDD1/HIF-1 [119, 120]. FDXR is localized to the mitochondria and functions to transfer an electron from NADPH (the reduced form of nicotinamide adenine dinucleotide phosphate) to cytochrome P450 during oxidative phosphorylation. Overexpression of FDXR sensitizes cells to 5-flurouracil, doxorubicin and H_2O_2-mediated apoptosis. Because inhibition of the electron transport chain significantly increases ROS production, FDXR possibly functions in a positive feedforward loop to enhance the generation of ROS during ROS-mediated p53-dependent apoptosis. *REDD1* or *hypoxia inducible factor (HIF-1)* is a recently identified target of both p53 and p63 [120]. The role of REDD1 in this pathway is complex. Recent reports demonstrate that REDD1/HIF-1 decreases ROS in MCF7 and PC12 cells but also increases susceptibility to ROS-induced cell death in more differentiated neuron-like PC12 cells.

Unique p53 target genes play a role in generating and inhibiting ROS formation. The p53 inducible genes (PIGs) 3 and 6 and p53-responsive gene 3 (PRG3) are implicated in ROS generation [121, 122]. PIG3 and PRG3 share homology with oxidoreductases, which catalyze oxidation-reduction reactions. PIG3 localizes to the cytoplasm [123]. Interestingly, p53 binds a pentanucleotide microsatellite sequence within the *PIG3* promoter instead of the consensus responsive element [124]. The finding that the microsatellite sequence is polymorphic suggests that inheritance of the sequence may be more protective against cancer. PIG6, a proline oxidase, catalyzes the generation of proline-dependent ROS. To regulate the levels of ROS, p53 activates the promoters of glutathione peroxidase (GPX), an antioxidant, and heat shock protein 27 (HSP27) [125, 126]. HSP27, a stress response protein and apoptosis inhibitor, may reduce ROS levels by increasing glutathione levels [127].

In addition to the generation of ROS, p53 induces the expression of p85, which may function as a signaling molecule during ROS-mediated p53-dependent apoptosis. p85 is a known regulator of phosphatidyl inositol-3 kinase (PI3K); however, its function during ROS-induced apoptosis is independent of PI3K [128]. $p85^{+/+}$ MEFs undergo cell death after H_2O_2 treatment, while $p85^{-/-}$ cells are highly resistant to cell death. To date, no unique p63 or p73 targets have been identified that mediates ROS-induced p53-mediated apoptosis.

Regulation of the p53 family

A large portion of research on the p53 family is dedicated to studying the regulation of p53 family members. Regulation of the p53 family involves both activation and inhibition. The p53 family is activated mainly by posttranslational modifications, such as phosphorylation, acetylation and protein-protein interactions. For reviews, see [129, 130]. For example, ataxia telangiectasia mutated (ATM), DNA dependent protein kinase (DNA-PK), and Chk2 phosphorylate and stabilize p53 in response to ionizing radiation (IR). ATR, Chk1 and homeodomain-interacting protein kinase 2 (HIPK2) phosphorylate and stabilize p53 in response to UV. p53 and p73 are both acetylated by p300/CBP [103, 131]. Acetylation of p53 inhibits its degradation by Mdm2 and activates its DNA binding activity [132]. Acetylated p73 preferentially binds to promoters of pro-apoptotic genes [103]. The p53 family is also regulated by protein-protein interactions. Many proteins that interact with p53 family members influence the transactivation of target genes or stabilization of protein levels.

In addition to the many mechanisms of regulation mentioned above, the p53 family also activates the expression of genes that in turn regulate the level and activities of the p53 family proteins themselves. Because this review focuses on p53 family target genes, this section of the review will focus on those genes that are regulated by the p53 family and also regulate the p53 family (see fig. 6). The p53 family regulates *Mdm2*, *high mobility group 1 (HMG-1),* and *insulin-like growth factor type I receptor (IGF-IR)*. In addition, both p53 and p73 regulate unique genes that affect the stability and function of the p53 family.

p53, p63 and p73 induce the expression of Mdm2, a regulator of the p53 family proteins [14, 24, 133]. Mdm2 is a well-characterized and very potent negative regulator of p53; however, the activity of Mdm2 toward p63 and p73 is somewhat different, albeit less well characterized. Upon induction of Mdm2 expression by the p53 family proteins, Mdm2 regulates the activity, subcellular localization and stability of p53 family members. Mdm2 functions in a negative feedback loop to regulate p53. Through binding to the N-terminus of p53 between residues 17 and 27, Mdm2 directly obscures activation domain 1 (AD1) of p53 and interferes with p53's ability to transactivate target genes. The nuclear export signal (NES) and the RING domain of Mdm2 are required for Mdm2-mediated nuclear export and nuclear exclusion of

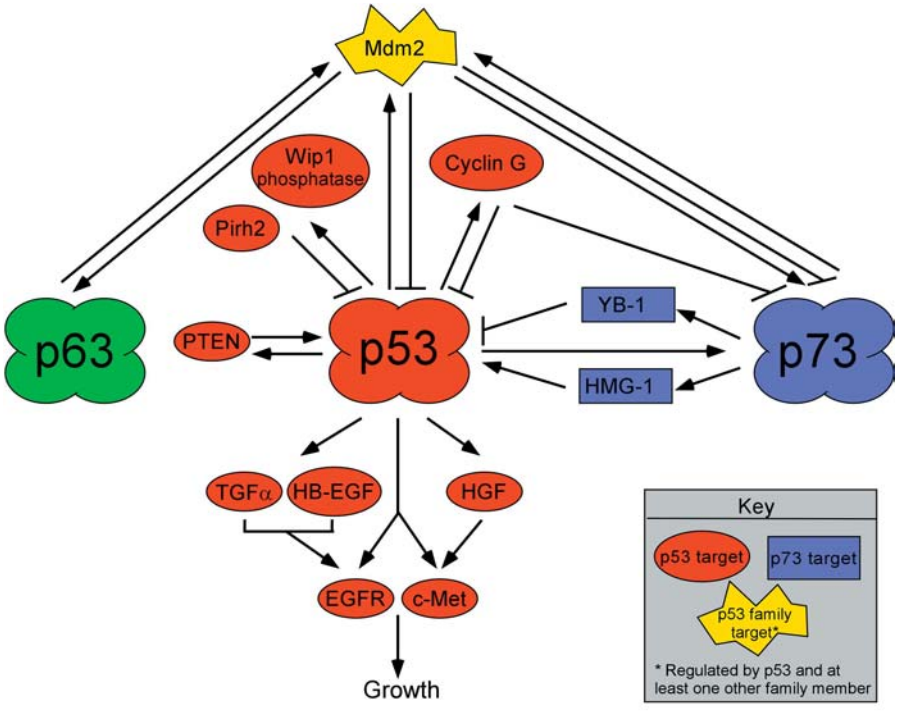

Figure 6. Regulation of the p53 family by p53 family target genes.

p53 [134]. The exclusion of p53 from the nucleus physically separates p53 from DNA, thus inhibiting its ability to function as a transcription factor. Once the Mdm2-p53 complex is located in the cytoplasm, Mdm2 functions as a p53-specific ubiquitin ligase, targeting p53 C-terminal lysine residues [135]. Ubiquitinated p53 is targeted to the proteosome for degradation. Interestingly, p53 is specifically degraded by cytoplasmic and not nuclear proteosomes, suggesting the requirement of Mdm2-mediated nuclear export prior to Mdm2-mediated degradation of p53 [136]. In some cell types, Mdm2 requires p300/CBP, a histone acetylase, as a cofactor for ubiquitination. In vitro data demonstrate that p53, Mdm2 and p300/CBP do form a trimeric complex. Moreover, Mdm2-mediated degradation of p53 is abrogated if either p53 or Mdm2 is unable to bind p300/CBP regardless of their ability to bind one another [137].

Although p63 induces Mdm2, the function of Mdm2 for the regulation of p63 has not been determined. Reports indicate that Mdm2 does not interact with p63 and that overexpression of Mdm2 increases cellular levels of p63 and enhances p63 transcriptional activity [14, 15].

In a manner consistent with the Mdm2-p53 interaction, Mdm2 binds the N-terminus of p73 and interferes with the ability of p73 to induce the expression of certain target genes [138]. Similarly to p53, Mdm2 binds and reduces the transcriptional capacity of p73; however, Mdm2 appears to regulate the protein level and subcellular location of p73 quite differently from p53. Mdm2 is unable to induce proteosome degradation of p73 and potentially stabilizes p73 protein levels [16, 139]. Mdm2 induces nuclear aggregation of p73 [17]. Thus, Mdm2 does not shuttle p73 out of the nucleus, and inhibits the interaction between p73 and cytosolic proteosomes. In addition, Mdm2-bound p73 does not interact with p300/CBP [140]. Without this interaction, Mdm2 may be unable to ubiquitinate p73 and is therefore unable to target p73 for degradation.

Both p53 and p73 regulate the promoters of *HMG-1*, a member of the chromatin-associated nucleoprotein family, and *IGF-IR*, a transmembrane heterotetramer that mediates growth and differentiation signals from insulin-like growth factors I and II (IGF-1 and IGF-II). Reports indicate the p73 activates while p53 represses the promoters of both HMG-1 and the IGF-IR [141, 142]. HMG-1 has been shown to upregulate p53 by activating DNA binding [143]. With the knowledge that p53 can induce the expression of p73 [144], a potential positive feedforward loop may be hypothesized: p73 upregulates HMG-1, HMG-1 activates p53 DNA binding activity, p53 induces the expression of p73.

Unique target genes of p53 have been identified that both positively, such as *PTEN*, and negatively, namely *Cyclin G, Pirh2* and *Wip1*, influence the protein levels of p53 [145–148]. The PTEN tumor suppressor is a protein tyrosine and lipid phosphatase that negatively regulates the PI3K/AKT survival pathway. Recently, PTEN has been identified as a positive regulator of p53 stability and

function. PTEN has been shown to physically associate with p53 and to modulate p53 DNA binding. *PTEN*$^{-/-}$ mice show dramatically decreased p53 protein levels. Reintroduction of wild-type PTEN or even a mutated, phosphatase dead PTEN results in p53 protein stabilization [149].

The interesting story of cyclin G, one of the earliest identified p53 target genes, has recently unfolded. Cyclin G functions as a negative regulator of p53 by activating Mdm2-mediated p53 degradation, reviewed in [150]. Through interaction with the B′ subclass of PP2A, cyclin G recruits PP2A phosphatase to dephosphorylate Mdm2 at S166 and T216, thus enhancing Mdm2 binding to and targeted degradation of p53. In addition to modulating the activity of p53, cyclin G has been shown to interact with both p53 and p73 and induce Mdm2-independent degradation of p73.

Pirh2 and *Wip1*, both of which are p53 target genes, negatively regulate p53. Pirh2, a RING-H2 domain containing protein with ubiquitin ligase activity, binds to the central region of p53 (residues 82–292), ubiquitinates p53 in an Mdm2-independent manner, represses transcriptional activity of p53 and reduces p53's growth arrest potential [147]. Wip1, a type 2c phosphatase (PP2C), participates in a negative feedback loop to attenuate UV-induced p53-mediated apoptosis [151]. Wip1 phosphatase negatively regulates p53 by dephosphorylating S33 and S46, thus removing the activating phosphates added by active p38MAPK. Wip1 also attenuates the signal by dephosphorylating a conserved threonine on p38MAPK.

In addition to the induction of genes that specifically inhibit p53 function or reduce p53 protein level, p53 induces a set of genes that counteract p53-dependent growth inhibition by activating growth-promoting pathways. p53 induces epidermal growth factor receptor (EGFR) and two of its ligands: heparin-binding EGF-like growth factor (HB-EGF) and transforming growth factor α (TGFα) [152–154]. p53 induces the cell surface receptor c-met and its ligand hepatocyte growth factor (HGF) [155, 156]. Both of these receptor-ligand systems stimulate growth.

Y-box binding protein 1 (YB1) has been identified as a unique p73 target [157]. Interestingly, YB1 is implicated as a negative regulator of p53 [157, 158]. YB1 represses the *p53* promoter and decreases endogenous levels of p53. Inhibiting YB1 leads to p53-mediated apoptosis. p53 interacts with YB1 and nuclear translocation of YB1 is dependent on wild type p53 [159].

Development

The requirement of the p53 family during development was not elucidated until the identification of *p53* homologues *p63* and *p73* and the generation of the respective knockout mouse models. Although p53 is not thought to be essential to life, as live-born *p53* knockout mice are developmentally normal, there is a subset of p53-deficient female embryos that exhibit exencephaly [160]. The majority of *p53*$^{-/-}$ mice develop multiple spontaneous tumors by the age of 6 months [6]. *p53* germline mutations result in Li Fraumeni syndrome (LFS). LFS is characterized by the development of a variety of tumors at a young age [161].

p63 has been shown to be essential during development [4]. *p63*$^{-/-}$ mice die several hours post birth and show striking developmental abnormalities, such as limb, craniofacial and epithelial defects. Absent or truncated limbs are due to the failure of differentiation or maintenance of the apical ectodermal ridge (AER), a stratified epithelium essential to development. Lack of epithelial stratification and all squamous epithelia are due to the inability of *p63*$^{-/-}$ cells to maintain a specific population of epithelial stem cells. Due to the failure of ectodermal formation, *p63*$^{-/-}$ mice also lack structures that require ectodermal/mesenchymal signaling, such as hair follicles and mammary glands.

Five human syndromes are associated with *p63* mutations. These syndromes are limb-mammary syndrome (LMS), split hand/foot malformation (SHFM4), Hays-Wells syndrome or ankyloblepharon-ectodermal dysplasia clefting syndrome (AEC), Acro-dermato-ungual-lacrimal tooth syndrome (ADULT), and ectrodactyly, ectodermal dysplasia and cleft lip/palate (EEC) syndrome, reviewed in [162]. The common characteristics of these syndromes include limb abnormalities, ectodermal dysplasia and facial clefts. Interestingly, EEC syndrome is associated with DNA binding mutations, while the other syndromes are associated with alterations in the C-terminal SAM domain [162].

The *p73* knockout mouse demonstrates the important role of p73 during development [5]. *p73*$^{-/-}$ mice show neurological, pheromone and inflammatory defects. The mice show a runting phenotype and have high mortality rates. Less than 25% of *p73*$^{-/-}$ mice in the SJ129 background survive longer than 30 days, while *p73*$^{-/-}$ mice in a mixed background live longer. Death usually occurs as a result of a massive gastrointestinal hemorrhage, but intracranial bleeding also occurs. The GI hemorrhage is due to the erosion and loss of enterocytes. The intestines show substantially increased mucosecretions. Neurological deficits include hippocampal dysgenesis and hydrocephalus. In the *p73*$^{-/-}$ mice, the hydrocephalus is communicating, indicating that the cause may lie in the overproduction or underabsorption of cerebrospinal fluid. Inflammatory defects include chronic inflammation and infection. Abnormalities in pheromone or sensory signaling pathways are exemplified by *p73*$^{-/-}$ mouse behavior. Both males and females lack sexual interest, and males also lack aggressive behavior towards other males [5]. To date, no human syndrome has been attributed to *p73* germline mutations.

Consistent with the role of p53 in the development of female embryos [160], p53 has been implicated in the regulation of target genes involved in development. Perhaps these genes may also be regulated by the p53 family, possibly contributing to the explanation of the *p53*, *p63* or *p73* knockout mouse phenotype. p53 has been shown to regulate genes that are involved in development, namely *dickkopf-1 (Dkk-1)*, *Apaf-1 semaphorin3B (Sema3B)*, *hypermethylated in cancer-1 (HIC-1)* and *Wip1 phosphatase* (see table 2).

Dkk-1, a p53 target gene, is a secreted cysteine-rich glycoprotein that inhibits the Wnt3b signaling pathway [163]. Briefly, Wnt signaling is involved in short-range signaling during embryonic patterning. Generally, Wnt signals are posteriorizing and Wnt inhibitors such as Dkk-1 are anteriorizing. Although the exact mechanism of Dkk-1 inhibition of Wnt signaling is unknown, evidence suggests a role for the trimeric complex containing Dkk-1, LDL-receptor related protein 6 (LRP6), and Kremen. LRP6 and Kremen, both transmembrane receptors that interact with Dkk-1, function to synergize and antagonize Wnt signaling, respectively [164, 165]. Posteriorizing Wnt signals inhibit the spemann head organizer. Thus, inhibition of Wnt by Dkk-1 facilitates head development. Overexpression of Dkk-1 induces head formation in *Xenopus,* and *Dkk-1*$^{-/-}$ mice show lack of anterior head structures. Interestingly, Dkk-1 is also involved in limb morphogenesis; *Dkk-1*$^{-/-}$ mice demonstrate duplications and fusions of limb digits [166].

The search for unique target genes involved in p63-mediated development has begun. The *p63* knockout mouse demonstrates that p63 target genes are essential for the proper development of ectodermal structures [167]. During *Xenopus* development, high levels of p63 are expressed in the neural crest and its derivatives such as branchial arches and tail fin [168]. Thus, two p63 target genes, *JAG1* and *JAG2*, will be discussed in relation to their roles during development.

Both p63 and p73 regulate the ligands of the notch receptor, Jagged 1 (JAG1) and Jagged 2 (JAG2) [19]. During development, notch signaling mediates many processes, including the segregation of neural and epidermal lin-

Table 2. Transcriptional targets of the p53 family involved in development.

Gene	Regulation	Function	Ref.
Apaf-1	p53+	component of the apoptosome; mutant mice show craniofacial malformation, brain overgrowth and persistence of interdigital webs	[210]
Aquaporin-3	p73+	glycerol and water transporter; mutant mice show defective skin hydration, elasticity and barrier function	[211]
DAN	p73+	member of the DAN family of BMP inhibitors; overexpression enhances neuronal differentiation	[179]
Dickkopf-1	p53+	secreted inhibitor of Wnt signaling; functions during head and limb development; mutant mice show lack of anterior head structures	[166]
HIC-1	p53+	transcriptional repressor; heterozygous mutant mice show propensity toward tumor formation; mutant mice show acrania, exencephaly, cleft palate, limb abnormalities and so on	[212]
Involucrin	p63+, p73+	late epidermal differentiation marker	[213]
JAG1	p63+, p73+	transmembrane ligand for the notch receptor; associated with Alagille syndrome, characterized by abnormalities of the heart, liver, eye, skeleton and facial structure	[170, 214]
JAG2	p63+, p73+	transmembrane ligand for the notch receptor; mutant mice show defects in limb, craniofacial and thymic development	[171, 172]
Loricin	p63+, p73+	late epidermal differentiation marker	[213]
MCK	p53+	muscle creatine kinase	
N-CAM	p73+	neural cell adhesion molecule; deficient mice show size reduction of the olfactory bulb and a spatial learning deficit	[175, 176]
$p57^{KIP2}$	p73+	member of the CIP/KIP family of CKIs; mutant mice show cleft palate, abdominal muscle defects, skeletal abnormalities and adrenocortical dysplasia	[215]
REDD1/HIF-1	p53+, p63+	expression pattern mirrors that of p63 in the mouse	[120]
Semaphorin 3B	p53+	secreted ligand; binds to plexin and neuropilin receptors; functions in the repulsion of axons across the midline	[216]
TRAF4	p53+	member of the TNFR-associated protein family; mutant mice show axial skeletal malformations, impaired neural tube closure and tracheal malformations	[99]
Wip1 phosphatase	p53+	phosphatase; mutant mice show defects in reproductive organs, immune function and cell cycle control	[217]

+, Activation; –, inhibition.

eages. In general, ligand binding induces intracellular cleavage of the notch receptor. The cleavage product goes to the nucleus to activate CBF-1, which induces the expression of basic helix-loop-helix (bHLH) transcription factors that then induce the expression of effector target genes. For a review of notch signaling, see [169]. Interestingly, mutations or deletions of *JAG1* or *JAG2* yield developmental defects that are reminiscent of the *p63* and *p73* knockout mouse phenotypes. Mutations in the extracellular domain of JAG1 are implicated in human Alagille syndrome (AGS) [170]. AGS is characterized by abnormalities of the heart, liver, skeleton, eye and facial structure. Interestingly, JAG2 is expressed in the AER and sites of epithelial-mesenchymal interactions [171]. *JAG2* mutant mice show a similar phenotype to $p63^{-/-}$ mice, with defects in limb, craniofacial and thymic development [172].

The search for unique target genes involved in p73-mediated development has begun. Here, two p73 target genes, neural cell adhesion molecule (*N-CAM*) and *DAN* will be discussed in relation to their roles during development [173, 174].

N-CAM is a transmembrane receptor with five immunoglobulin domains and two fibronectin type III repeats. N-CAM is expressed in neurons, glial cells and skeletal muscle cells. N-CAMs mediate cell adhesion through homotypic interactions between neighboring cells and function in axon growth and guidance. Interestingly, *N-CAM* knockout mice show a striking similarity to $p73^{-/-}$ mice. *N-CAM*-deficient mice display a 36% reduction in the size of the olfactory bulb and deficient spatial learning [175]. N-CAM is also important for the correct formation of the mossy fiber tract in the hippocampus [176]. Thus, the p73-dependent N-CAM-mediated role in development may explain the pheromone deficits and hippocampal dysgenesis in the $p73^{-/-}$ mouse. For a review of N-CAMs, see [177].

DAN is a member of the DAN family of BMP inhibitors. In general, BMPs induce the differentiation of mesenchymal cells to osteoblastic cells. DAN inhibition of BMP signaling induces neuralization in *Xenopus* ectodermal explants [178]. Overexpression of DAN enhances retinoic acid induced-neuronal differentiation detected by neurite outgrowth in a neuroblastoma cell line [179].

Concluding remarks

While many p53 family transcriptional targets have been identified, future research is needed to identify the remaining p53 family target genes. These putative target genes must undergo stringent analysis to verify that they are true transcriptional targets of the p53 family. For example, chromatin immunoprecipitation (ChIP) assay is needed to determine the in vivo binding of the p53 family proteins to the p53/p63/p73 responsive elements within the target gene. Induction of the target gene by p53 family proteins needs to be verified through detection of both increased mRNA and protein levels, using Northern and Western analyses, respectively. Once a putative target gene is identified as a true target, the function of the gene must be characterized. With the identification and characterization of all the p53 family target genes, we will eventually uncover the complex signaling pathways of the p53 family proteins in tumor suppression and development.

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