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News and Commentary

p53 downstream target genes and tumor suppression: a classical view in evolution

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The transcription factor p53 is a critical component in the normal cell's response to damage owing to cellular stresses such as DNA damage, oncogenic stimulation, nutrient deprivation, or hypoxia. Its role as a tumor suppressor is exemplified by the fact that many cancers selectively inactivate p53 and/or the p53 pathway. In addition, the loss of p53 in mice leads to spontaneous tumor formation. However, no mouse knockout of an individual p53 target has been able to recapitulate the p53-null mouse phenotype indicating that the loss of p53 has multiple effects on tumor formation.

In the best-characterized model of p53 tumor suppressor activity, p53 becomes activated upon DNA damage incurred from a variety of sources such as UV, γ-irradiation, or from treatment with DNA-damaging chemotherapeutic agents. p53 protein becomes stabilized upon its phosphorylation by ATM/Chk2 or ATR/Chk1/Casein-kinase 1 and accumulates in the nucleus. There, p53 transcribes a network of genes that initiate DNA repair, growth arrest, senescence, and/or apoptosis. In this model, p53 also autoregulates its levels by transcribing its negative regulator, the E3 ligase, Mdm2 which ubiquitinates p53 and targets it to the proteasome for degradation.

The evolving view of p53 function is an increasingly complex one (Figure 1 and Table 1). p53 can be regulated by a variety of post-translational modifications including neddylation, sumoylation, lysine methylation and acetylation, and ubiquitination, in addition to phosphorylation.[1–6] Moreover, a rising number of cofactors have been found to influence the type of targets that are transcribed by p53. This response can be tissue-specific, as in the case of SLUG, a protein upregulated in hematopoietic progenitor cells.[7] In a cell type-specific manner, BCL6 represses p53 leading to the development of lymphoma.[8] Other cofactors appear to alter the p53 target profile to favor either growth arrest or apoptosis. For example, the p53 interacting protein, Muc1, suppresses apoptotic targets while promoting transcription of targets that induce growth arrest.[9] The ASPP1 and ASPP2 proteins have the opposite effect of suppressing growth arrest targets while promoting induction of apoptosis-inducing genes.[10]

Alternatively, the p53 response can be tailored to the type of cellular stress such as the upregulation of *Bnip3L* which, in some cells, is specifically transcribed by p53 under hypoxia, and skews the p53 response towards apoptosis.[11] Because p53 is traditionally thought of as being activated under stress, the targeted degradation of p53 under ER stress by the unfolded protein response is of interest.[12,13]

Newly characterized isoforms of p53 appear to have a distinct, but overlapping, transcriptional profile to full-length wild-type p53.[14–16] Moreover, some of these isoforms can affect the activity of wild-type p53. Mutants of p53 have also been shown to have properties that are distinct from wild-type and that may contribute to tumorigenesis, and polymorphisms of p53 further modify its downstream effects and function.[12,13,17,18]

The model for p53-dependent apoptosis is also evolving. In the classical DNA damage pathway, p53 promotes cell death via the intrinsic pathway. Work with irradiated *DR5*$^{-/-}$ mice suggests that some of the DNA damaged cells may undergo apoptosis via the extrinsic pathway.[19] Moreover, an alternate, cytoplasmic function for p53 has been proposed where p53 may contribute to apoptosis in a transcription-independent mechanism.[20–26]

Recent experiments in mice suggest that novel mechanisms of tumor suppression by p53 remain to be uncovered. The loss of p53 in the stromal mesenchyme supports growth of tumors in adjacent epithelia implying that the stromal cells can communicate with the tumor-epithelial cells in a p53-dependent manner.[27] Novel connections between p53 and mitochondrial respiration may impact on cellular metabolism and tumorigenesis.[28] These data demonstrate the growing complexity of p53 function and its role in cancer.

Novel Modifications of p53 and their Effect on p53 Function

Although the methylation of histone lysines is a common mechanism of regulating transcription through chromatin remodeling, the effect of lysine methylation on transcription factors is less well understood. Methylation of lysine372 in the C-terminal regulatory domain of p53 by SET9 stabilizes p53 in the nucleus,[1] and substitution of Arg for the methylation site Lys in p53 prevents *p21*$^{WAF1/CIP1}$ transcription even upon overexpression of SET9. Although SET9 appears to play a role in p53 activity and DNA damage-induced apoptosis, it is not clear if the methylation of p53 or to the methylation of an

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unknown substrate of SET9 is important. Because p53 transcription of individual targets was not assessed, it is also not clear which apoptosis-related p53 targets are required.

Krummel *et al*.[29] attempted to address the significance of lysine-modifications of p53. They generated mice containing knockin mutations of p53 at seven lysines in the C-terminal regulatory domain. Equivalent protein levels of the C-terminal lysine mutant were observed as compared to the wild-type under normal or stressful conditions suggesting that they were expressed and regulated similarly. The mutant mice had apoptotic rates comparable to wild-type following exposure to low levels of ionizing radiation or adriamycin. When the mice were irradiated and their thymi examined for induction of p53 targets, Krummel *et al*.[29] observed a modest increase in the transcripts of $p21^{WAF1/CIP}$, *Puma*, and *Mdm2* genes with the largest difference for the cell cycle inhibitor $p21^{WAF1/CIP}$ that had an approximately threefold increase. Because no large defect in apoptosis was observed, perhaps these lysine modifications are involved in non-apoptotic functions of p53. Indeed when the authors attempted to immortalize MEFs expressing the lysine mutant p53, the cells stopped proliferating after a few passages and acquired a senescent-like morphology whereas the wild-type and null counterparts could be immortalized.

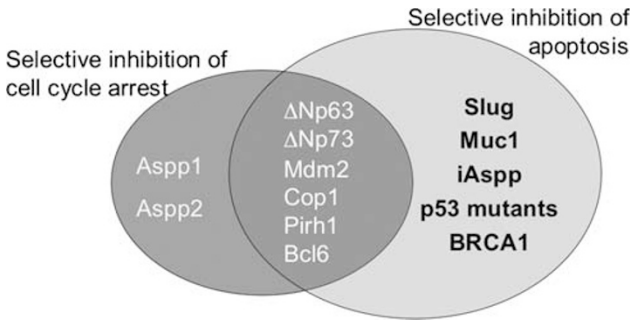

Figure 1 The modulation of p53 transcriptional target profile. A number of recently identified p53-binding proteins and modifications differentially alter the transcriptional profile of p53 towards either cell cycle arrest and repair or apoptosis. Mechanisms are emerging that explain how the cell can control the multiple functions of p53, especially the seemingly conflicting ones of cell cycle arrest and repair and cell death. The proteins listed in the intersection inhibit p53 transcription in general

Similarly, Feng *et al*.[30] generated murine ES cells, MEFs, and thymocytes which contained either wild-type p53 or p53 with six of the lysines in the C-terminus mutated to arginines (p53K6R). These lysines are conserved from mouse to human, and overlap with the residues that were mutated by Krummel *et al*. Although no significant differences were observed in the transcriptional profile of the ES cells or MEFs expressing wild-type *versus* mutant p53 after exposure to UV irradiation, there was a 50% increase in the transcripts of $p21^{WAF1/CIP}$ and *Mdm2* after treatment with adriamycin. Wild-type and p53K6R thymocytes were similarly sensitive to γ-irradiation even though the p53K6R thymocytes transcribed less *DR5* and *Puma*. These data are consistent with the minimal changes in the global transcriptional profile that Krummel *et al*. observed in their C-terminal lysine mutant p53 mice. However, the differences in *DR5* and *Puma* transcript levels in the irradiated p53K6R thymocytes suggest that perhaps modifications at these lysines may make some contribution to p53 transcriptional activity in a tissue-specific manner.

The canonical view of p53 regulation consists of p53 ubiquitination by MDM2 and its subsequent degradation. Xirodimas *et al*.[2] found a new wrinkle in this classic paradigm in that MDM2 is also able to neddylate p53. Lysines 370, 372, and 373 in the C-terminus of p53 were mapped as neddylation attachment sites. Using a temperature-sensitive cell line for neddylation and a p53 luciferase reporter, Xirodimas *et al*.[2] observed that neddylation of p53 corresponded to a decrease in the general transcriptional activity of p53. They observed transient neddylation of p53 in cells overexpressing NEDD8 that were exposed UV light. The role of neddylation, in general, is far from understood apart from its regulation of the cullins. Neither the effects of neddylation on the apoptosis or DNA damage signaling functions of p53 nor the physiological circumstances under which it may regulate p53 have been addressed. It is also not known if p53 mutants that are unable

Table 1 p53 is involved in many cellular processes and some seemingly contradictory ones

Cell survival		Cell death pathways	
Pathway	**p53 target**	**Pathway**	**p53 target**
Classical:			
DNA repair	*PCNA*	Apoptosis	*PUMA*, *BAX*, *DR5*, and *PIG3*
Cell cycle arrest	*p21*		
	CYCLING		
Evolving:			
Mitochondrial respiration	*SCO2*	Autophagy	*DRAM1*
Inhibit glycolysis	*TIGAR*		
Senescence	*PAI-1*		
Antiapoptotic	*Slug*		

The classical view of p53 function focuses on the balance between cell cycle arrest and apoptosis. The evolving view includes more recent connections between p53 and mitochondrial respiration, glycolysis, and active self-regulation of apoptosis. Although senescence has long been known to be a p53-dependent function, the molecular mechanism of how p53 contributes to senescence is beginning to emerge. Thus, p53-dependent senescence is a classic concept, but the downstream targets that achieve senescence are part of the evolving view of p53 function

to be neddylated confer any particular growth advantage or what triggers and/ or regulates the removal of NEDD8 from p53.

Even the classical notion that MDM2 polyubiquitination targets p53 for proteasomal degradation has been evolving. Li *et al.*[31] found that when MDM2 levels were low, MDM2 was able to monoubiquitinate p53 leading to its export from the nucleus, and a monoubiquitin-p53 fusion protein accumulated in the cytoplasm. At higher levels, MDM2 is able to polyubiquitinate nuclear p53 leading to its degradation, and this polyubiquitination function of MDM2 was facilitated by p300.[32] The differential control of p53 levels and localization is consistent with current models: under cellular stress, p53 is localized to the nucleus and activates transcription of MDM2 as part of its well-known negative feedback loop. When cells are not under stress, steady state MDM2 levels are relatively low. Perhaps the ability of MDM2 to monoubiquitinate p53 under these conditions prevents aberrant activation of p53 that could be detrimental to a healthy cell. Along with the already finely tuned oscillation of p53 and MDM2 protein levels with DNA damage, monoubiquitination of p53 by MDM2 is an additional layer of regulation that is required to keep p53 in check.[33]

The model of p53 degradation by MDM2 also needs to be revised to include new regulators of MDM2 activity. Both gankyrin and Ying Yang 1 (YY1) enhance the ability of MDM2 to polyubiquitinate p53.[34,35] Nucleophosmin (NPM) may have a more complex effect because it appears to regulate both MDM2 and its antagonist, ARF.[36,37] NPM transiently binds to MDM2 upon DNA damage and, at later time points, re-associates with and inhibits ARF.[38,39] This model was supported when knockdown of NPM was sufficient to increase the MDM2–ARF interaction and to concurrently activate p53.[40] Other E3 ligases can also degrade p53 including the p53 targets COP1 and PIRH2.[41,42] COP1 activity is regulated by the DNA damage signaling kinase, ATM.[43] Upon phosphorylation, COP1 undergoes autoubiquitination and degradation thus, increasing the stability of p53. Another E3 ligase, MULE/ ARF-BP1 polyubiquitinates p53 *in vitro*.[44]

Post-translational modifications of the proline-rich region can modulate downstream effects of p53. For example, the modification of the proline-rich region by the prolyl isomerase PIN1 regulates p53 activity. PIN1 binds to phosphorylated p53 after DNA damage and changes its conformation.[45–47] The modification of p53 by PIN1 increases the stability of p53 and is required for the transcription of p53 targets such as *BAX*, *DR5*, $p21^{WAF1/CIP1}$, and *MDM2*. The current evidence suggests that the proline isomerization inhibits the interaction of MDM2 and p53 and promotes the interaction between CHK2 and p53.[45,48] Yet, the conformational change in p53 caused by prolyl isomerization may also affect interactions with other p53 binding partners resulting in impaired transcription of various targets. The importance of the poly-proline-rich region in p53 was highlighted by the phenotype of a mouse expressing a knocked-in p53 mutant lacking the conserved proline-rich region ($p53^{\Delta P}$). The $p53^{\Delta P}$ protein was more sensitive to MDM2 degradation and the $p53^{\Delta P}$ expressing cells also exhibited impaired apoptosis and cell cycle arrest upon DNA damage.[49] Further studies are needed to characterize the physiological relevance of prolyl isomerization as well as other modifications of the proline-rich region and their effect on p53 transcription.

Modulation of p53 Specificity

Even though p53 has the potential to induce apoptosis in all cells, not all cellular stresses result in p53-dependent apoptosis. The response appears to be, in part, tissue-specific. For example, hematopoietic progenitors cells undergo cell cycle arrest and DNA repair preferentially to apoptosis. In these cells, p53 specifically transcribes *Slug* which is a selective repressor of *Puma* transcription, a p53-dependent apoptosis effector.[7] Thus, p53 appears to use one of its survival-associated transcriptional targets to regulate one of its apoptosis-related targets. In this way, the DNA repair pathways are favored, and the cells are refractory to cell death.

Mature B cells within the germinal center have evolved to evade both p53-dependent DNA repair and apoptosis. These cells overexpress *BCL6*, which binds to the *p53* promoter and suppresses p53-mediated transcription.[8] The authors suggest that preventing a p53-dependent DNA repair response is necessary to allow the DNA double-strand breaks that are required for immunoglobulin class switching. In this case, the p53-mediated downstream response is abrogated in a cell type-specific manner.

p53 cofactors also alter the specificity profile of target genes. For example, MUC1 favors p53-induced transcription of the cell cycle arrest gene, $p21^{WAF1/CIP}$ while inhibiting the induction of the proapoptotic target *BAX*.[9] Likewise, BRCA1 promotes transcription of DNA repair gene while suppressing the transcription of apoptosis-associated genes.[50] Conversely, expression of the ASPP1 and ASPP2 cofactors enable p53 to favor apoptotic transcripts over those that induce cell cycle arrest.[51] ASPP1 and ASPP2 bind to p53 directly and promote transcription of proapoptotic *BAX* and *PIG-3* while inhibiting $p21^{WAF1/CIP}$ and *MDM2*. ASPP1 and ASPP2 similarly promote upregulation of *BAX* and *PIG* genes by the p53 family members, p63 and p73.[10] An inhibitory member of the ASPP proteins, iASPP, interacts with p53 and squelches its apoptotic function.[52] However, the effect of iASPP1 on p53-activated non-apoptotic targets such as $p21^{WAF1/CIP}$ following exposure DNA damage was not investigated. Because ASPP1/2 and iASPP are predicted to compete for p53 binding and thereby regulate p53 activity, elucidating how the balance between ASPP1/2 and iASPP expression is maintained could provide further insight into understanding what preferentially signals the proapoptotic program as compared to cell cycle arrest.

The histone acetyl transferase (HAT) complex containing TIP60 has also been shown to modulate p53 transcriptional activity and to be necessary for G1 arrest and apoptosis after double-stranded breaks.[53–55] In a series of experiments examining the mRNA expression, knockdown of *Tip60* impaired the upregulation of $p21^{WAF1/CIP}$ and *FAS* after UV irradiation, or treatment with adriamycin or cisplatin.[56] Using chromatin immunoprecipitation, the authors demonstrated that the occupancy of p53 was decreased at the p53 binding sites of *FAS* and $p21^{WAF1/CIP}$ when *Tip60* is knocked down. Although p53 and TIP60 can physically interact, TIP60 has not

been colocalized to *p53* binding sites with p53 protein. At present, the exact mechanism of theTIP60 complex's ability to affect p53 transcriptional activity is unknown, but it may be due to acetylation of ATM or another TIP60 target.[57] The importance of TIP60's HAT activity was addressed by treating 293 cells that overexpressed both p53 and a catalytically inactive form of TIP60 with a high-dose γ-irradiation. Transcripts of $p21^{WAF1/CIP}$ were not induced when the HAT-inactive mutant was expressed, suggesting that the acetylase activity may be required for p53 induction of $p21^{WAF1/CIP}$ mRNA.[54]

p53 can also transcribe a subset of genes that are specific to a particular stress as is observed with hypoxia. In some cells, p53 is activated and transcribes a hypoxia-specific target, *Bnip3L* which encodes a protein that promotes apoptosis.[11] Surprisingly, one type of cellular stress, endoplasmic reticulum stress from an accumulation of unfolded proteins, can lead to the specific degradation of p53.[12]

Transcriptional Control of p53 by p53 Family Members

With two different promoters and multiple splice variants, the p53 family members, p63 and p73, exist in many different isoforms possessing potentially different functions.[58,59] Heterogeneous C-termini permit unique protein–protein interactions.[59] N-terminally deleted (ΔN) isoforms of p63 and p73 which lack their transactivation domain(TA) act as dominant-negative inhibitors of transcriptionally active p53 and its family members.[58,60,61] In the absence of p53, p73 can transcribe a subset of p53 targets after DNA damage or after activation by small molecules.[62,63] The transcription *p53* mRNA is also directly affected by p73 and *p53* itself.[64]

The phenotypes of p63 and p73 heterozygous mice demonstrated that these p53 family members have roles in tumor formation beyond inhibiting p53 transcription and, in fact, have inherent tumor suppressive properties. Heterozygous mice for *p63* and *p73* exhibited some spontaneous tumor formation including squamous cell carcinomas and histiocytic sarcomas whereas $p73^{+/-}$ mice develop lung adenocarcinomas, thymic lymphoma, and hemangiosarcomas.[65] Moreover, loss of heterozygosity (LOH) was observed at a high frequency in both $p63^{+/-}$ and $p73^{+/-}$ tumors.

In comparison to the singly mutated mice, a more severe tumorigenic phenotype was observed when combinations of $p53^{+/-}$, $p63^{+/-}$, and $p73^{+/-}$ mice were generated.[65] $p63^{+/-}$;$p73^{+/-}$ mice exhibited a broader tumor spectrum than the single mutants and LOH for either $p63^{+/-}$ or $p73^{+/-}$ or both. $p53^{+/-}$;$p63^{+/-}$ and $p53^{+/-}$;$p73^{+/-}$ mice had shorter lifespans as compared to the $p53^{+/-}$ mice (7 and 6 months *versus* 10 months, respectively) owing to high tumor burden and metastatic disease. As with the $p63^{+/-}$;$p73^{+/-}$ mice, the tumors excised from the $p53^{+/-}$ combination mice frequently lost one or both wild-type copies of the heterozygous *p53* family members. These data further suggest a synergy between p53 and its family members in tumor suppression.

Differential Transcriptional Activity of p53 Isoforms

A series of alternatively spliced forms of *p53* have been recently identified.[14,16] One variant that excludes exon8, Δp53, appears to preferentially transcribe cell cycle arrest targets such as $p21^{WAF1/CIP}$ and *14-3-3*σ and not the downstream targets associated with apoptosis such as *BAX* and *PIG3*.[14] Moreover, this variant is activated in S phase whereas full-length p53 functions at G1 and G2. Bourdon *et al*.[16] describe several other isoforms which are derived primarily from a second p53 transcriptional start site within intron 3. Although they demonstrate that one isoform, p53β, is unable to induce transcription of a $p21^{WAF1/CIP}$ or a *BAX* promoter-luciferase reporter on its own, it seemed to enhance wild-type p53-mediated transcription of the *BAX* reporter in the presence of Actinomycin D. Because these variants contain the p53 DNA-binding domain, one would expect some transcriptional activity. A more comprehensive assessment of transcriptional induction of p53 targets remains to be determined for these particular isoforms.

A p53 mutant which has a deletion in both its N-terminal activation domain 1 and C-terminal basic domain was found to differentially regulate a previously described p53 target, *IGFBP3*.[15,66,67] Overexpression of this mutant and other naturally occurring C-terminal truncations of p53, p63, and p73 permitted induction of *IGFBP3* whereas full-length p53 protein did not.[15] Only when the cells were treated with histone deacetylase (HDAC) inhibitors in conjunction with DNA-damaging agents was full-length p53 able to transcribe the *IGFBP3* gene. Although these experiments suggest a role for truncated p53, the physiological relevance of the truncated protein and the circumstances of HDAC inhibition require further investigation.

Gain-of-function of p53 Mutants

p53 is often mutated rather than deleted in cancer, and a range of mutations have been found including amino-acid changes within the DNA-binding domain. Moreover, many of these mutant p53 proteins are overexpressed and can stabilize wild-type p53.[68] One systematic analysis of 56 colorectal carcinoma cell lines found that almost 77% of the cell lines had mutated p53.[69] Most of these mutations resulted in truncated p53 transcripts, that is, lacking some or all of their DNA-binding domains.

The apparent selection of mutations in p53 has led to the hypothesis that these mutations may exert differential effects in the development and/or maintenance of the tumor. Typically, these experiments consist of exogenously overexpressing mutants in p53-null cell lines such as H1299, Saos2, or PC3 cells. In this way, mutant p53s have been shown to induce transcription of the oncogenes *c-Myc*, *EGFR*, and *hTERT* in a manner distinct from wild-type.[70–72] A series of p53 mutants that have been previously identified in human tumors were screened for their ability to upregulate $p21^{WAF1/CIP}$, *MDM2*, *BAX*, and *MSH2*.[73] Five unique transcription profiles were observed that were distinct from wild-type and each other. Even two different amino-acid changes at the same position, G279E and G279R, transformed a non-transcriptional p53 mutant into one that could selectively transcribe $p21^{WAF1/CIP}$ and *MDM2*, but not *BAX*, or *MSH2*. Conversely, $p53^{R175H}$ has been shown to repress *MSP* resulting in resistance to etoposide and *CD40/FAS*, a gene that is normally a target for wild-type p53.[74,75] Expression of

siRNA against endogenous mutant p53 in the cell lines SKBR3, HT29, and SW480 made the cells less resistant to long-term treatment with chemotherapy agents and reduced tumorigenic potential.[76] Taken together, these data provide evidence that certain mutant p53 proteins are actively functioning to promote tumor growth as compared to simple inactivation or deletion of wild-type p53. One caveat is that many of the studies utilized human tumor cell lines that are considered null for p53, and the expression of p53 isoforms has not been characterized for many cell lines.

Two recent reports have examined mutations in p53 that are associated with Li–Fraumeni syndrome, *R175H*, and *R273H*, by knocking-in the analogous mutations into mice (*R172H* and *R270H*, respectively). This approach permits the investigation of the mutants under the regulation of the endogenous p53 promoter. In this way, Lang *et al*.[77] and Olive *et al*.[78] were able to follow tumor progression *in vivo*. Both groups generated heterozygous mice containing the murine equivalent of the Li–Fraumeni mutation of $p53^{R172H^+}$ in the DNA-binding domain of p53 in different genetic backgrounds and found similar results. In both instances, the $p53^{R172H^+}$ mice exhibited the appearance of tumors with the same frequency as $p53^{+/-}$ mice, but they also observed numerous metastases of these tumors. Although the average lifetimes of the mutant mice were comparable to the analogous heterozygous and *p53-null* mice, the mutant mice exhibited a range of carcinomas that were invasive and metastatic whereas the *p53-null* mice developed mostly sarcomas and lymphomas, not carcinomas. As is often observed for Li–Fraumeni tumors, some tumors in the mutant mice lost the wild-type allele. Olive *et al*.[78] made a second mutation at murine $p53^{R270H}$.[68] $p53^{R270H^-}$ gave rise to more carcinomas and endothelial-derived cancers than was observed for *p53-null* or the $R172H^+$ mice. In other examples, a mouse expressing a mammary-specific $p53^{R270H}$ allele developed breast tumors at a high frequency, and expression of the $p53^{R270H}$ allele in a lung-specific *K-Ras* mouse aided sino-nasal adenocarcinoma initiation.[79,80]

These mutant mice also provided *in vivo* evidence for the dominant-negative and gain-of-function effects of *R172H* and *R270H*. The *R172H* mutation made p53 heterozygous mice refractory to apoptosis of the embryonic hypothalami when treated with IR. This result indicated that the mutant functioned as a dominant-negative inhibitor of wild-type p53 activity. The simultaneous gain-of-function was apparent in that the mutant MEFs proliferated at a faster rate than even *p53-null* MEFs. Lang *et al*.[77] were able to demonstrate that the gain-of-function phenotype was due, at least in part, to effects of the $p53^{R172H/-}$ mutant binding and inactivating p63 and p73.[81] Indeed, inhibition of p73 activity by p53 mutants in head and neck squamous carcinoma cells decreased their sensitivity to cisplatin.[17]

The preference for some tumors to mutate p53 rather than to delete it, coupled with the mounting evidence that p53 gain-of-function mutations affect tumor growth supports the idea that some of the p53 mutants confer an advantage for the tumor. The p53 mutants may transcribe beneficial and/or repress detrimental genes to promote tumor progression. The differential downstream target profiles of the mutant p53 proteins are beginning to be elucidated and may provide a comprehensive mechanism for the function of mutant p53 in tumor progression. When the transcription of recently identified and known p53 transcriptional target genes were compared from a series of tumors with wild-type or mutant p53, the resulting target profiles had predictive value in patient prognosis.[82] Microarray experiments comparing prostate cancer cell lines expressing gain-of-function p53 mutants yielded 95 differentially expressed genes where 50 targets were preferentially induced and 45 were repressed.[83] These mutants may lead to increased genetic instability and actively inhibit p53-dependent and -independent apoptosis while promoting proliferation.

Non-apoptotic Functions of p53

Expression of p53 and its downstream targets promotes non-apoptotic effects including survival mechanisms. Senescence is well known as p53-dependent process and is characterized by an irreversible growth arrest of cells that remain metabolically active. However, the molecular targets of p53 that induce senescence have not been convincingly identified. Knockout of the most obvious candidate, $p21^{WAF1/CIP1}$, is surprisingly ineffective at immortalizing cells.[84] Unexpectedly, another p53 transcriptional target, *plasminogen activator inhibitor-1 (PAI-1)* was found to be sufficient to induce senescence.[85] Moreover, $p21^{WAF1/CIP1}$ levels were unchanged in cells expressing the siRNA against *PAI-1*, yet these *PAI-1* knockdown cells were able to bypass p53-induced senescence further suggesting that $p21^{WAF1/CIP1}$ is not a limiting factor.

Another non-apoptotic function of p53 appears to be the regulation of glycolysis and oxidative phosphorylation. Under conditions of low glucose, p53 engages a reversible cell cycle checkpoint.[86] p53 can also inhibit glycolysis through its transcriptional target, TIGAR, which lowers the levels of fructose 2,6-bisphosphate, a molecule that promotes glycolysis.[87] Expression of TIGAR also reduced the amount of reactive oxygen species and protected cells from apoptosis in some cell types. In this way, p53 appears to promote cell survival during glucose deprivation. Expression of p53 also promotes oxidative phosphorylation over glycolysis via its transcriptional target, *SCO2* (Synthesis of Cytochrome *c* Oxidase 2).[28] SCO2 is an assembly factor for the cytochrome *c* oxidase complex and links p53 to mitochondrial respiration. This evidence suggests that the loss of p53 may contribute to the Warburg effect, which describes the preferential use of glycolytic pathways by cancer cells.[88]

p53 as a Transcriptional Repressor

Although the bulk of research on p53 has focused on its ability to upregulate transcriptional targets, p53 has been shown to repress the transcription of a variety of genes including *c-MYC*, *Cyclin B*, *VEGF*, *RAD51*, and *hTERT*. This function was recently reviewed in this journal.[89] Briefly, several mechanisms of p53-dependent repression have been proposed. One mechanism suggests that p53 binds directly to specific G2/M-regulated promoters in conjunction with the NFY CCAAT box-associated factor to inhibit activation.[90] In this case and in the case of *c-MYC* repression, p53 recruits HDACs to targets,

thus altering their chromatin structure and preventing transcriptional activators from binding to the target promoter.[90–92] p53 has also been implicated in repressing genes by interacting directly with the transcriptional machinery such as the TATA-binding protein.[91] Whether the mechanism of p53 repression is direct or indirect, it is clear that p53 can affect the downregulation of some genes and warrants consideration when the impact of p53 in different cellular contexts is evaluated.

p53 Induces Apoptosis Via the Intrinsic and Extrinsic Pathways

During the classic p53-dependent DNA damage response, cell death is mediated by the intrinsic pathway, which includes permeablization of the mitochondrial outer membrane and release of cytochrome *c* and Smac/Diablo to initiate the downstream caspase cascade. However, experiments described by Finnberg *et al*.[19] suggest that p53 can also exert apoptosis via the extrinsic pathway through DR5 in a tissue-specific manner in mice. Irradiated $DR5^{-/-}$ mice exhibited reduced amounts of apoptosis in the thymus, spleen, Peyer's patches, small intestinal villi and in the white matter of the brain as compared to wild-type $DR5^{+/+}$ mice. Perhaps an additional layer of regulation exists for these tissues where p53-mediated upregulation of DR5 is coupled to TRAIL in an effort to sensitize some cell types to apoptosis.

Non-transcriptional Functions of p53

Although p53 acts primarily as a transcription factor, recent observations suggest that p53 may also function in a transcriptionally independent manner in the cytoplasm. Chipuk *et al*.[93] made a p53 construct consisting of the TA and proline-rich domains and a major deletion of the DNA-binding domain. Unexpectedly, this p53 deletion mutant was able to induce apoptosis when expressed in the p53-deficient cell line H1299, and the apoptosis was blocked by overexpression of BCL-xL. To further test this apoptotic mechanism, they pretreated MEFs with wheat germ agglutinin to block nuclear import of p53 upon UV irradiation.[20] In this system, cell death was observed for $p53^{+/+}$ MEFs, but not $p53^{-/-}$, or $Bax^{-/-}$ MEFs. *In vitro* experiments using purified proteins and mitochondria corroborated the requirement for both p53 and BAX for permeabilization of the mitochondria and subsequent release of cytochrome *c*.

Based upon these *in vitro* experiments, Chipuk *et al*. have proposed a mechanism wherein p53 binds to BCL-xL thus displacing BAX and freeing it to initiate cell death. However, in their initial deletion mutants of p53, the DNA-binding domain was deleted as well as the oligomerization domain. Furthermore, the estrogen receptor-p53 fusion protein used in some of the experiments to prevent p53 from entering the nucleus may or may not have permitted tetramer formation of p53. Also, the most prevalent mutations of p53 that are found in tumors occur in the DNA-binding domain. Many of these mutant p53 proteins are transcriptionally inactive and act in a dominant-negative manner to prevent wild-type transcription of downstream target genes. Mutant p53 proteins also tend to be overexpressed as compared to wild-type endogenous, unstressed levels. An open question is how do cancer cells, which accumulate p53 with intact proline-rich domains, bypass this pathway. One answer might be that a critical concentration of p53 must be present in the cytoplasm, and another is that these cancer cells block the pathway downstream of p53 by overexpressing Bcl-xL, for example.

In their most recent paper, their data suggests that PUMA releases p53 from its interaction with BCL-xL. Chipuk *et al*.[94] propose that when p53 is stabilized, it accumulates both in the cytoplasm and in the nucleus. Nuclear p53 transcribes *Puma*, and cytoplasmic p53 binds to BCL-xL, presumably in a simultaneous manner. The newly expressed PUMA then displaces p53 from BCL-xL so that p53 may activate BAX even though *in vitro* experiments described in their previous paper suggested that the activation of BAX by p53 does not require p53 transcription of its downstream targets, including *Puma*[20,93] However, apoptosis is impaired in *Puma*-deficient MEFs. Although many intriguing connections between p53, PUMA, BCL-xL, and BAX have been raised, the physiological relevance of this model remains unclear and needs further investigation.

Additional evidence suggests that p53 interacts with BCL-xL and its family members.[22,26] In one case, the initial observation was made that a fraction of the total p53 was localized to the mitochondria during p53-dependent apoptosis, and recently, mitochondrial p53 was detected specifically in the thymus, spleen, testis, and brain of mice treated with γ-irradiation.[23,25] When p53 was specifically targeted to the mitochondria, it interacted with BCL-xL and BCL-2, and these interactions were verified in mitochondria purified from RKO and ML-1 cells treated with camptothecin.[22] An alternative proposal has suggested that p53-mediated mitochondrial apoptosis may be enhanced by an R72 polymorphism *versus* proline at this position and functions through BAK, but not BCL-xL, or BCL-2.[18,26] Murphy and George[95] propose that the localization of p53 to the mitochondria may fully commit the cell to apoptosis. That p53 promotes cells death both indirectly through its transcription of its proapoptotic targets and directly at the mitochondria is intriguing and requires further investigation *in vivo* in particular with regards to the R72 polymorphism of p53.

Is There a Stromal Tumor Suppressive Function of p53?

Most research regarding the role of p53 as a tumor suppressor has focused upon loss of p53 function in the epithelial-derived tumor cells themselves. Hill *et al*.[27] provide evidence to expand the function of p53 in tumorigenesis by proposing that p53 activity in stromal cells can influence tumor progression. They generated mice that express a 121 amino-acid N-terminal fragment of SV40 large T-antigen in the prostate epithelium ($TgAPT_{121}$), which induces murine prostatic intraepithelial neoplasia. When this mouse was crossed into a p53-deficient background, epithelial apoptosis and proliferation in the prostate were unaffected.[96] However, the $TgAPT_{121}$; $p53^{+/-}$ mice developed an aberrant mesenchyme and rapid growth of tumors (up to 2 cm^3 by 19 weeks).[27] In $TgAPT_{121}$; $p53^{-/-}$ mice, these tumors appeared by 4 weeks, and in addition, poorly differentiated adenocarcinomas were observed by 22 weeks. Unlike the p53 wild-type and hetero-

zygous counterparts, the $TgAPT_{121}$; $p53^{-/-}$ mice were not able to be maintained beyond 24 weeks owing to severe tumor burden. Loss of p53 expression was observed in fibroblasts associated with both $p53^{+/+}$ and $p53^{+/-}$ tumors that were histologically similar. As the stromal cells preferentially lose *p53* in a $p53^{+/-}$ background, presumably, a p53 downstream signaling pathway in these cells that inhibits tumor progression exists that remains to be uncovered.

Conclusion and Future Directions

After over 20 years of study, our understanding of p53 biology continues to evolve in new and exciting directions. More layers of regulation to prevent aberrant function of p53 continue to be uncovered and may lead to a better understanding of tissue or stress-specific p53 response. The emerging transcriptional profiles for the activation and repression of targets by the various p53 isoforms and tumor-associated mutants may shed light on the tuning of p53 activity and its potentially active role in tumorigenesis. The recent evidence that p53 in stromal cells may affect adjacent epithelial tumor cells suggests another network of p53 downstream targets and consequences. Moreover, the link between p53 activation and an increase in autophagy through its transcriptional target, *DRAM*, necessitates considering multiple p53 cell death pathways that might affect cell fate in parallel.[97,98] Perhaps the analysis of the p53 target profiles under different circumstances will elucidate a question that has long plagued the p53 field: what is the tipping point between p53-dependent DNA repair and cell cycle arrest and cell death? Understanding this balance, as well as the complexities of p53 regulation, will continue to point the way towards new therapies targeting the p53 pathway in cancer.

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