

# Tumour suppressor p53: understanding the molecular mechanisms inherent to cancer

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**Tumour suppressor p53 has been the centre of focus of researchers trying to decipher the molecular mechanisms underlying tumorigenesis. In this review, we have summarized the critical role of p53 in tumour suppression through its effects on several cellular processes such as DNA repair, cell cycle and apoptosis. We have also discussed the role of upstream regulators which sensitize p53 to respond to different stress conditions. Post-translational modifications of p53 along with its binding partners have emerged as major determinants of its functional selectivity and specificity. Unravelling the intricacies of p53 functions have been augmented with the development of new mouse models of p53, which also have been discussed in this review.**

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**Keywords:** Cancer, molecular mechanisms, stress signals, tumour suppressor genes.

## Introduction

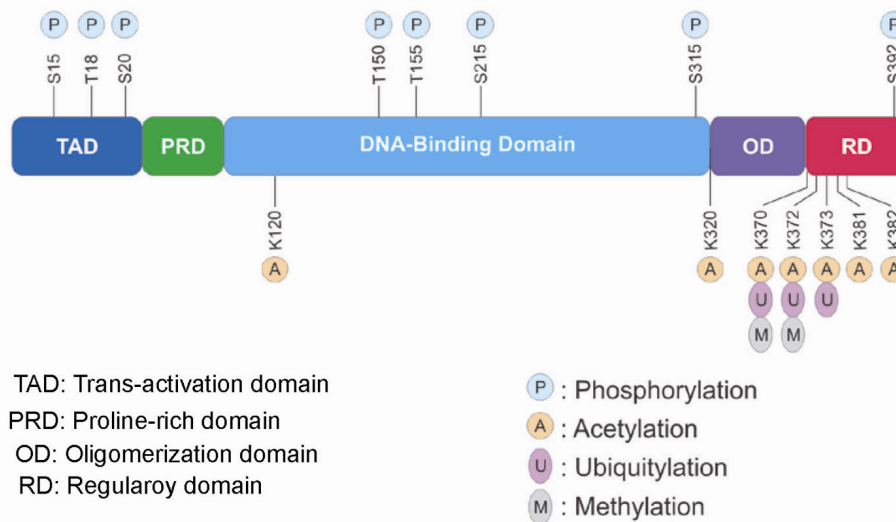
AMONG the tumour suppressor genes, p53 is one of the most well studied. It serves as a master regulator in cancer signalling pathway by sensing diverse cytotoxic and genotoxic stresses which may compromise genomic stability and promote neoplastic transformation. The role played by p53 in tumour suppression is further highlighted by the fact that direct inactivation of this gene is the most common mutation in human cancer, occurring in more than 50% of malignancies<sup>1</sup>. More than 35% of the lung, skin, ovary, pancreas, liver carcinomas and 20% of gliomas, breast carcinomas, cervical carcinoma and breast cancers have p53 mutations as an important deregulation<sup>2</sup>. Once activated by a stress, p53 may mediate a series of cellular outcomes that vary from cell-cycle arrest to DNA-repair, senescence and apoptosis<sup>3</sup>. Moreover, several novel p53 target genes have been identified which play an important role in metabolic processes and have expanded the ways by which p53 can mediate stress response<sup>4</sup>. Apart from this, p53 is commonly regulated by post-translational modifications and by its interaction with other proteins. The focus of this review will be to provide a broad overview about p53 regulation and function.

## Domain organization of the p53 protein

The p53 protein, like many other transcription factors, has a modular structure characterized by the presence of evolutionarily conserved functional domains (Figure 1). The N-terminal acidic domain of p53 is responsible for its transactivation function<sup>5</sup>. This domain interacts with components of the basal transcriptional machinery such as the TATA-binding protein (TBP) and TBP associated factors (TAFs) as well as with p300/CBP (ref. 6). Adjacent to the transactivation domain there is a proline-rich domain (PRD, aa 61–94) containing five repeats of the amino acid motif PXXP (where P designates proline and X any other amino acid)<sup>7</sup>. This region is thought to be important for p53 regulation as PXXP motifs provide binding sites for Src homology 3 (SH3) domain containing proteins that partake in signal transduction. The central part of p53 protein (aa 100–300) contains the sequence-specific DNA-binding domain. The canonical p53-responsive element contains two decamers or half sites, PuPuPuC(A/T)(A/T)GPyPyPy (Pu = purine, Py = pyrimidine), which are separated by a spacer of 0–13 base pairs<sup>8</sup>. The significance of sequence-specific DNA-binding activity for p53 to function as a tumour suppressor is highlighted by the fact that 97% of tumour-associated mutations cluster in this domain<sup>9</sup>. Moreover, many carcinogens specifically target this domain. For example, hepatocarcinogen aflatoxinB1 causes G to T transversion at codon 249 of p53 (Arg to Ser), which completely abolishes the transactivation function of p53 and hence inhibits p53-induced apoptosis. About 5–25% of hepatocellular carcinoma incidence results from dietary intake of aflatoxinB1 (ref. 10). Furthermore, several studies have reported the use of aflatoxinB1 for selective targeting of p53 mutational hotspots<sup>11</sup>. p53 tetramerization, required for its transactivation function, is mediated through the oligomerization domain (aa 326–356). Tetramerization appears to be essential for p53 tumour suppressor activities. The last 30 amino acids of p53 constitute a basic C-terminal domain (CT, aa 364–393) that has been regarded as a regulatory domain due to its ability to influence p53 activity upon stress signalling. Several lysine residues in this domain undergo various post-translational modifications which regulate p53 stability and function.

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**Figure 1.** Domain organization and post-translational modifications of p53 protein.

### Upstream events activating the p53 pathway

The tumour suppressor p53 responds to a plethora of stress signals, including DNA damage, hypoxia, starvation and aberrant oncogenic events (Figure 2). Some of them are discussed below.

#### DNA damage

Genome integrity is constantly threatened by various intrinsic and extrinsic genotoxic agents, including metabolic by-products and radiation. These agents can cause many different modifications in the physico-chemical structure of DNA leading to alterations in base pairing, deletions, mutations and chromosomal aberrations<sup>12</sup>. Accumulation of multiple genomic insults can result in altered gene function, including loss of tumour suppressor genes and enhanced expression of oncogenes which facilitate cancer development. Thus, exposure of cells to DNA damaging agents of different types, including gamma irradiation, UV irradiation, oxidative free radicals, etc. triggers various sensory serine/threonine kinases that play key roles in p53 activation by mediating the post-translational phosphorylations necessary to promote p53 stabilization and transcriptional activity. ATR and ATM, the two DNA damage sensor kinases and their respective downstream kinases Chk1 and Chk2, phosphorylate p53 at different sites. Specifically, ATM and Chk2 act in response to ionizing radiation leading to phosphorylation of p53 at Ser15, Thr18 and Ser20. ATR and Chk1 appear to be required in UV damage response<sup>13-15</sup>. Upon activation, ATR phosphorylates p53 at Ser15 and Ser37 while Chk1 at Ser6, Ser9 and Ser20. Ionizing radiations can also activate DNA-dependent protein kinase (DNA-PK). DNA-PK has been shown to phos-

phorylate p53 at Ser15 and Ser37 and to interact with it at sites of DNA damage<sup>16</sup>. Another kinase p38 is activated in response to genotoxic stresses and has been shown to phosphorylate p53. Activation of p38 upon UV irradiation or nitric oxide treatment leads to apoptosis by phosphorylation at Ser46 that is abrogated upon treatment with p38 inhibitors<sup>17</sup>.

#### Aberrant oncogenic events

Oncogenic signalling activates p53 not only through the DNA damage response pathways, but also through the transcriptional activation of p14ARF (ARF)<sup>18,19</sup>. The levels of ARF protein are found to be increased upon aberrant expression of oncogenes such as *Ras* and *Myc*<sup>20</sup>. One of the key roles of the ARF protein is to bind to Mdm2 and inhibit its ubiquitin ligase activity, thus promoting p53 stabilization<sup>21</sup>. In human tumours, ARF is inactivated with an extraordinarily high frequency due to genetic deletion at p14ARF locus or mutation in the gene and miRNAs which degrade p14ARF protein. Decreased ARF protein in the cell leads to lesser p53 levels in response to damage and hence interferes with cell-cycle control in several tumours.

#### Other stress signals

p53 can also be activated by paucity of nutrients, energy or oxygen availability<sup>22</sup>. Reduced nutrient or energy levels result in the activation of AMPK, which leads to p53 induction by direct phosphorylation of Ser15 that stabilizes p53 (refs 23, 24), contributing to the induction of a reversible cell cycle arrest. mTOR signalling can inhibit p53, wherein a phosphatase PP2A dephosphorylates p53 (ref. 25).

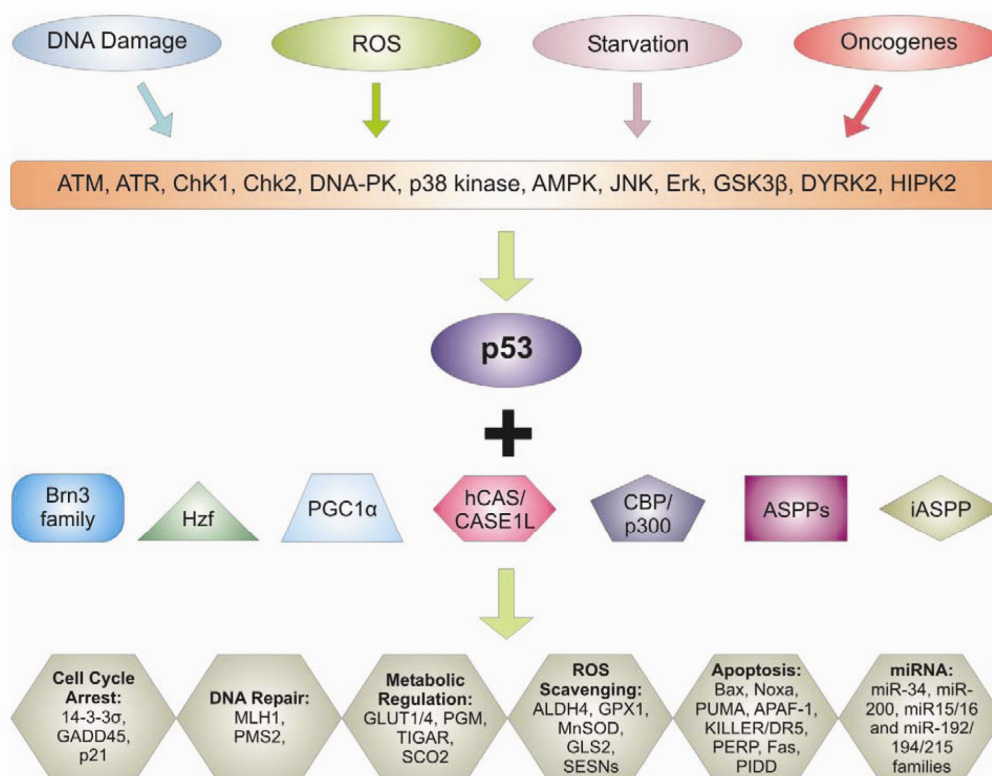


Figure 2. Schematic overview of p53 pathway.

## Outcomes of p53 activation

Once the p53 protein is activated, the ultimate outcome can be quite diverse, ranging from the induction of reversible cell-cycle arrest, apoptosis and senescence to protective antioxidant activities and DNA repair (Figure 2). The best understood way by which p53 mediates its response is to act as a transcription factor with sequence-specific DNA-binding ability and the potential to induce the expression of a large number of genes.

### Cell-cycle arrest

The ability of p53 to induce cell-cycle arrest mostly depends on three critical target genes: *p21*, *14-3-3σ* and *GADD45* (refs 26–28). The cyclin-dependent kinase inhibitor *p21* was the first transcriptional target identified and its transactivation results in cell-cycle arrest in G1 phase due to inhibition of cyclinE/CDK2, cyclinA/CDK2 and cyclinD/CDK4 (ref. 29). *p21* expression directly correlates with p53 status in most of the cancers, but p53-independent deregulation of *p21* expression also has been implicated in some of the cancers, e.g. HCC, AML, gliomas, etc.<sup>30</sup>. This is achieved by transcriptional repression of *p21* by oncogenes such as *HRAS* and *myc*. The p53-induced G2 arrest is mostly mediated by the activation of genes such as *14-3-3σ* and *GADD45*. *GADD45* abrogates

CDC2-cyclinB complex<sup>31,32</sup>. Significance of *GADD45a* is highlighted by the promoter hypermethylation-mediated silencing of *GADD45a* observed in breast and prostate cancer. Mutations in *GADD45a* have also been reported in pancreatic cancer. Many of these events are independent of p53 inactivation<sup>33</sup>. *14-3-3σ* inhibits nuclear import of cyclin B1 and CDC2 through cytoplasmic sequestration<sup>34</sup>. *14-3-3σ* protein levels are undetectable in several breast carcinoma patients and its gene also has been seen to be hypermethylated in those patients and breast cancer cell lines, stressing the fact that *14-3-3σ* silencing is a primary event for breast carcinogenesis. Although expression of *14-3-3σ* is not strictly regulated by p53, some basal level of protein is maintained following DNA damage in cells<sup>35</sup>.

### Senescence

p53 tumour suppressor activity is partly mediated through the induction of senescence, a programme leading to irreversible arrest of cell growth accompanied by a characteristic set of phenotypic changes. In both replicative and premature senescence, a key role is mediated by tumour suppressor pathways involving p53 and p16-pRB (refs 36, 37). Consistently, in models of cellular senescence induced by DNA damaging agents causing double-strand breaks, ATR/ATM mediates the activation of cell-cycle

checkpoints via CHK1/CHK2 and p53, with the participation of p21, p16 and Rb (ref. 38). Senescence is an important component of p53 anti-tumour repertoire as reactivation of p53 in tumours of murine carcinoma models elicits robust tumour regression mediated by induction of senescence<sup>39</sup>. While p53 involvement in senescence is well established, the underlying molecular mechanism is still poorly understood. Among the many target genes of p53, p21 plays a pivotal role in triggering senescence<sup>40</sup>.

### Apoptosis

To facilitate the removal of irreparably damaged cells or in response to prolonged stress conditions, p53 induces the expression of genes such as *Bax*, *Noxa* and *PUMA* that promote the release of cytochrome *c* into cytoplasm from mitochondria to initiate the intrinsic apoptotic pathway. *Bax* was the first identified p53 proapoptotic target gene of the Bcl-2 family<sup>41</sup>. *PUMA* and *Noxa* are induced in a p53-dependent manner upon genotoxic stress<sup>42,43</sup>. Moreover, p53 contributes to the formation of the apoptosome through the transcriptional activation of APAF-1 (refs 44, 45) and is also involved in the more downstream phases of apoptosis by transactivating caspase-6 (ref. 46). p53 can promote apoptosis via the extrinsic pathway by activating the transcription of the death receptors such as *KILLER/DR5* and *Fas*<sup>47,48</sup>. *PERP* is induced by p53 upon genotoxic stress and plays an important role in p53-mediated apoptosis<sup>49</sup>. *PIDD* (p53-induced protein with death domain) was identified as a p53 target gene induced by ionizing radiation<sup>50</sup>. Loss of *PIDD* expression significantly attenuated p53-induced apoptosis. Under genotoxic stress, *PIDD* has also been shown to act as molecular switch between cell survival and cell death by regulation of two different pathways, i.e. NF- $\kappa$ B and caspase-2 due to phosphorylation by ATM<sup>51</sup>.

### DNA repair

p53 can also exert its function as a tumour suppressor by preventing propagation of deleterious mutations arising from DNA damage. p53 regulates the DNA repair by either inducing the expression of repair proteins or through interactions with the repair machinery. Two mismatch repair genes, *MLH1* and *PMS2*, have been shown to be responsive to p53 activation after DNA damage. These two genes act as DNA repair sensors and are a critical determinant of p53-mediated cell fate decisions<sup>52</sup>. *DDB2*, a key player in nucleotide-excision repair is also a p53 target gene. p53 can bind several key repair proteins, including Rad51, RPA, BRCA1 and BRCA2, Bloom's syndrome protein and Werner's syndrome protein to facilitate the repair process. Moreover, p53 also plays an indirect role in DNA repair through the induction of ribonucleotide reductase subunits<sup>53,54</sup>. Studies with p53

null mice and tumour cells differing in p53 status have revealed that impaired p53 function is associated with its inability to prevent genetic instabilities, which results in aneuploidies, allelic losses as well as increase in gene amplification rates<sup>55</sup>. Thus p53 plays an essential role in maintaining genomic integrity, thereby counteracting the multistep process of tumorigenesis.

### Metabolism

New functions of p53 are reshaping various aspects of metabolism. One of the key metabolic changes associated with proliferating tumour cells is augmentation of glycolysis. p53 can inhibit glycolysis by regulating the expression of its target genes such as *GLUT1/4*, *PGM* and *TIGAR*. GLUT1 and GLUT4 are transmembrane glucose transporter proteins which facilitate glucose uptake in cells. p53 represses the expression of GLUT1 and GLUT4 and hence limits the transport of glucose into cells<sup>56</sup>. PGM (phosphoglycerate mutase) is a glycolytic enzyme that is repressed by p53 (ref. 57). Aberrant expression of PGM increases glycolytic rate, permits unchecked proliferation and promotes resistance to oncogene-induced senescence. p53 can also induce the expression of *TIGAR* (TP53-induced glycolysis and apoptosis regulator). *TIGAR* suppresses glycolysis by lowering the levels of fructose-2,6-bisphosphate, an allosteric regulator of glycolytic enzyme 6-phosphofructo-kinase-1 (PFK-1) by acting as a fructose-2,6-bisphosphatase<sup>58</sup>. Similar to glycolysis, TCA cycle intermediates can be used in anabolic pathways to promote cell growth and proliferation. Thus p53 also promotes oxidative phosphorylation (OXPHOS) by inducing its target gene *SCO2* (synthesis of cytochrome *c* oxidase 2), which maintains COX complex and enhances OXPHOS<sup>59</sup>. Thus p53 promotes TCA cycle for energy production. Consequently, loss of p53 function in cancers results in impaired mitochondrial respiration, thereby provoking a shift to glycolysis.

Reactive oxygen species (ROS) are generated during normal metabolism and excess ROS can cause cellular damage and hence can contribute to ageing, cancer and other pathologies. While promoting mitochondrial respiration, p53 neutralizes the deleterious effect of ROS by inducing the expression of several genes encoding antioxidant enzymes, including Glutaminase 2 (*GLS2*), aldehyde dehydrogenase (*ALDH4*), glutathione peroxidase (*GPXI*), Mn-superoxide dismutase (*MnSOD*) and sestrins (*SESN1* and *SESN2*). p53 target gene *GLS2* increases the levels of reduced glutathione (GSH), which has antioxidant activities<sup>60,61</sup>. *ALDH4* is a direct p53 transcriptional target that helps in clearance of ROS through regulation of amino acid metabolism<sup>62</sup>. Another p53 induced gene, *GPXI* can convert H<sub>2</sub>O<sub>2</sub> to H<sub>2</sub>O and O<sub>2</sub> (ref. 63). p53 can induce *MnSOD* levels thereby promoting scavenging of free radicals<sup>63</sup>. *SESN1* and *SESN2* generate the reduced

form of peroxiredoxin proteins, which neutralize the peroxides produced during oxidative stress<sup>64</sup>. Thus p53 acts as a key mediator between energy producing and antioxidant pathways and promotes cell survival under stress conditions.

### *Role of p53-induced miRNAs*

MicroRNAs (miRNAs) are small non-coding RNAs, about 20–24 nucleotides in length, that play an important role in post-transcriptional regulation of gene expression. p53 induces the expression of several genes encoding miRNAs such as miR-34, miR-200, miR15/16 and miR-192/194/215 families. These miRNAs play an important role in p53-mediated tumour suppression and stress response by regulating several key cellular processes, including cell cycle, cell survival and metabolism. miR-34 induces G1 arrest by directly downregulating CDK4, CDK6 and cyclinE2 (ref. 65). miR-15 and miR-16 trigger apoptosis by targeting Bcl-2 (ref. 66). miR-34 targets lactate dehydrogenase A, thereby inhibiting glycolysis<sup>67</sup>. Several studies have also reported potential p53-regulated miRNAs employing array-based expression profiling in clinical samples. For example, reduced expression of miR-34a, miR-29c and miR-17-5p were detected in chronic lymphocytic leukaemia patients with p53 abnormalities<sup>68</sup>. Similarly, the decreased p53 expression was found to be linked with downregulation of miR-195 and miR-497 in primary peritoneal carcinoma<sup>69</sup>. Thus miRNAs add another layer to molecular mechanisms through which p53 governs cell fate.

### **Regulation of p53**

The activation and stabilization of p53 is regulated by multiple proteins in response to diverse stress conditions. These include proteins which can modify p53 for both stabilization and increased transcriptional activity, reverse these modifications and regulate the translation of p53 mRNA.

#### *Regulation by E3 ligases*

In the absence of stress, p53 protein is maintained at very low levels. This is achieved by ubiquitin–proteasome-mediated degradation of p53 protein. Among the E3 ubiquitin ligases, Mdm2 plays a major role in regulating p53 stability. This is highlighted by the fact that altered expression of Mdm2 is a feature of many tumours. Elevated Mdm2 levels have been reported in 70% of liposarcoma, 37% of melanoma and 31% of breast carcinoma, which also correlates with reduced p53 levels in these cancers<sup>70</sup>. Increased expression of MDM2 in these cancers is due to a combination of gene amplification, high rate of transcription and altered post-translational modifi-

cations of Mdm2. Molecular mechanism underlying Mdm2-mediated regulation of p53 levels is well understood. Mdm2 upon binding to p53 catalyses polyubiquitylation of p53 leading to proteasomal degradation<sup>71</sup>. Under stress conditions, p53 activity increases and an early step in this process is the abrogation of Mdm2-mediated degradation. ATM-mediated phosphorylation of p53 at Ser15 inhibits the interaction between p53 and Mdm2, allowing the stabilization of p53 (ref. 72). The p53–Mdm2 interplay is tightly regulated in a negative feedback loop, wherein p53 induces Mdm2 expression while Mdm2 inhibits p53 activity. E3 ligase Pirh2 binds to p53 and triggers p53 ubiquitination and degradation<sup>73</sup>. Like Mdm2, Pirh2 is also a p53 target gene and partakes in an auto-regulatory negative feedback loop. Another E3 ligase, COP1 is also a p53 target gene that ubiquitylates and degrades p53 (ref. 74). RNAi-mediated depletion of COP1 promotes p53-mediated G1 arrest in response to ionizing radiation. Together, Mdm2, COP1, Pirh2 and other such proteins represent an array of E3 ligases that act to regulate and maintain p53 levels. This redundancy suggests that multiple mechanisms act synergistically for tight p53 regulation.

#### *Post-translational modifications*

p53 undergoes a great variety of post-translational modifications that influence its stability and its transcriptional activity. These include phosphorylation, acetylation, ubiquitination, sumoylation, neddylation and methylation of specific amino acids spanning throughout p53 protein (Figure 1). The actual pattern of post-translational modifications is complicated since the same residue might be modified in different ways by different enzymes.

**Phosphorylation:** Many kinases, including ATM, ATR, Chk1, Chk2, c-JUN NH2-terminal kinase (JNK), Erk, p38, Aurora kinase A, glycogen synthase kinase-3 $\beta$  (GSK3 $\beta$ ), AMPK, HIPK2 and DYRK2, phosphorylate p53 upon stress. There are 17 phosphorylation sites in p53 protein which are targeted by these kinases in response to different kinds of stresses. In human p53 these residues are serines 6, 9, 15, 20, 33, 37 and 46, and threonines 18 and 81 in the amino-terminal region; Ser315 and Ser392 in the C-terminal domain; and Thr150, Thr155 and Ser215 in the central DNA-binding domain. The phosphorylation of the N-terminal domain amino acid residues Ser15, Thr18 and Ser20 has been extensively studied. Ser15 is phosphorylated by ATM kinase rapidly in response to  $\gamma$ -irradiation, but not in response to UV radiation<sup>13</sup>. Glucose starvation is also known to induce Ser15 phosphorylation in an AMPK-dependent mechanism<sup>23,24</sup>. Phosphorylation at Ser15 along with other phosphorylations at Ser20 and Thr18 interferes with the interaction of p53 with Mdm2, thus

promoting p53 stabilization<sup>14,75</sup>. Phosphorylation of Ser46 by the kinases HIPK2 and p38MAPK promotes selective binding of p53 to the promoters of proapoptotic targets such as p53-regulated apoptosis-inducing protein 1 (p53AIP1)<sup>76,77</sup>.

**Acetylation:** p53 is acetylated at several lysine residues by different acetyltransferases. p300/CBP and PCAF acetylate p53 in response to genotoxic stress such as UV- and  $\gamma$ -irradiation at lysine residues in the C-terminal domain (Lys370, 372, 373, 381, 382)<sup>78</sup>. Moreover, Lys320 and Lys305 are acetylated by PCAF and p300 respectively<sup>79</sup>. Also, the MYST family acetyltransferases, hMOF and Tip60, acetylate p53 at Lys120 in the DNA-binding domain<sup>80,81</sup>. These acetylations play a critical role in mediating p53 binding to DNA and also potentiate the recruitment of cofactors, thus favouring p53 transcriptional activation. Acetylation of Lys320 favours p53 recruitment to high-affinity binding sites in target genes which promote cell survival and cell-cycle arrest. On the other hand, acetylation of Lys373 promotes recruitment to low-affinity binding sites, which are found in proapoptotic target genes which trigger cell death<sup>82</sup>. Acetylation on Lys120 by Tip60 has also been shown to modulate p53 transcriptional activity and to be necessary for apoptotic response.

**Other modifications affecting lysine residues:** Specific lysine residues in C-terminal domain of p53 also undergo methylation. Methylation of p53 at Lys372 by the methyltransferase Set9, increases p53 stability and transcriptional activity<sup>83</sup>. Methylation of Lys370 by Smyd2 represses p53 transcriptional activity<sup>83,84</sup>. The lysine-specific demethylase LSD1 binds to p53 and inhibits p53-mediated transactivation and apoptosis<sup>84</sup>. *In vitro* both monomethylation (K370me1) and dimethylation (K370me2) at K370 are targeted by LSD1, while *in vivo* it removes dimethylation (K370me2). Thus p53 is dynamically regulated by lysine methylation/demethylation and the methylation status at a single lysine residue triggers different functional outputs.

Lysine residues are also subjected to other post-translational modifications such as neddylation and sumoylation. Three lysines targeted for ubiquitination (Lys370, Lys372 and Lys373) are also subjected to neddylation and inhibit p53 transactivation function<sup>85</sup>. Sumoylation was reported to positively modulate p53 transcriptional activity<sup>86</sup>.

### Regulation of translation

Regulation of p53 protein depends mostly on its stabilization as a consequence of reduced ubiquitination and proteasome degradation. However, regulation of p53 mRNA translation has also been shown to play a role in determining p53 expression levels. A negative auto-regulation

of p53 mRNA translation is mediated by both a secondary structure of the 5'UTR in p53 mRNA and an element within its 3'UTR<sup>87,88</sup>. Further studies showed that the translation of p53 is regulated by the ribosomal protein L26 (RPL26) in response to DNA damage<sup>89</sup>. RPL26 preferentially binds to the 5'UTR after DNA damage and increases the rate of p53 translation and promotes p53-mediated apoptosis.

### Regulation by cofactors

A diverse array of cofactors influence p53 activity in different ways (Figure 2). Coactivators and corepressors may affect p53 transcriptional activity by inducing modifications in chromatin surrounding the p53 binding site, by mediating or preventing the assembly of the transcriptional machinery, or by directing p53 activation towards a particular subset of target genes thus leading to a specific response. An important role in regulating the apoptotic functions of p53 at the transcriptional level is played by the highly conserved ASPP (Ankyrin repeats, SH3 domain, proline-rich protein) family of proteins, composed of three members, ASPP1, ASPP2 and the inhibitory iASPP. ASPP1/2 bind preferentially to the DBD, while iASPP binds to the PRD of p53 (ref. 90). When ASPP1 or ASPP2 binds to p53, it promotes p53-mediated apoptosis, but not cell-cycle arrest, while iASPP acts as p53 inhibitor. The Brn3 family of transcription factors also modulate p53 target selection. The POU domain of Brn-3a and Brn-3b interacts with the DNA-binding domain of p53. While Brn-3a promotes growth arrest leading to increased cell survival and differentiation, the other member Brn-3b promotes apoptosis<sup>91,92</sup>. The role of CBP/p300 in promoting p53-dependent transcription is well established<sup>78,93</sup>. Another coactivator, hCAS/CSE1L, binds to p53 and facilitates the formation of active chromatin at selective target promoters such as PIG3, p53AIP1 and p53R2 (ref. 94). Under metabolic stress conditions, PGC-1 $\alpha$  binds to p53 and promotes prosurvival and metabolic functions of p53 (ref. 95). Taken together all these evidences suggest that many regulatory cues are involved in p53 transcriptional activation.

### Mouse models of p53

p53 knockout mice were first reported in the early nineties<sup>96-99</sup>. p53<sup>-/-</sup> mice had normal prenatal and postnatal development. However, p53<sup>-/-</sup> mice exhibited spontaneous tumorigenesis at a very early age. They succumbed to cancer by 10 months of age. The tumour spectrum observed in p53-null mice also varies with thymic T-cell lymphomas and different types of soft-tissue sarcomas prevailing more frequently.

Since post-translational modifications of p53 are known to modulate its stability and activity, mutant mice with

p53 alterations at specific amino acid residues corresponding to such PTMs have also been generated<sup>100,101</sup>. These changes disrupt PTMs from occurring, but have subtle effects on p53 function. Most of these mice do not have major tumour suppressor defects, suggesting that upstream signals synergize to regulate p53. Thus mutant mice in which the seven C-terminal lysines of p53 were mutated to arginine residues (referred to as p53<sup>7KR</sup>) showed similar phenotype as that of wild-type p53 mice. MEFs from these mice showed normal cell-cycle regulation and apoptotic responses. However, these MEFs were resistant to spontaneous immortalization and p53<sup>7KR</sup> was activated more robustly than wild-type p53 upon irradiation<sup>102</sup>. These results indicate that lysine residues in the C-terminal domain are not essential for p53 function, but are required for fine-tuning of stress response<sup>102</sup>. Serine 392 is a highly conserved residue in human p53 which is phosphorylated by several kinases. Mice with the equivalent mutation (S389A) had a slightly attenuated p53 apoptotic response. Moreover, p53<sup>S389A/S389A</sup> mice did not show spontaneous tumorigenesis, but had a mild predisposition to UV-induced skin tumours<sup>103</sup>. Again, the mutation of this residue had a modest phenotypic response.

The p53 mouse models discussed so far are germline models. These studies have now been supported by the newer conditional p53 mice models that allow more precise regulation of p53 functions. Thus transgenic mice were generated having a knock-in wild-type p53 allele fused in frame with the hormone-binding domain of the modified estrogen receptor (p53ER(TAM) mice) that is activated by the estrogen analog 4-hydroxytamoxifen (4-OHT). In these mice p53 is activated only by 4-OHT administration. Using this model it was shown that lymphomas which arose in response to ionizing radiation could be delayed by 4-OHT treatment<sup>104</sup>. Similar conditional p53 mice models have also been used to demonstrate p53-mediated tumour suppression *in vivo*<sup>39,105,106</sup>. As these newer models are developed and characterized, further insights will be obtained about the mechanistic aspects of p53-mediated tumour suppression.

### Concluding remarks

Although p53 was discovered as an oncogene, later research revealed its importance as one of the crucial tumour suppressor genes. Intense research on p53 has revealed a complex network wherein diverse stress signals get transduced to p53, which in turn regulates diverse cellular processes to determine cell fate. Further studies would be required to integrate the p53 network with other regulatory events for a more comprehensive understanding of tumorigenesis. High-throughput approaches to quantitatively assess gene expression signatures as well as functional readouts such as metabolite profiling would be necessary to unravel tumour cell vulnerabilities which could be exploited for therapeutic intervention.

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