p53 mutations in cancer

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In the past fifteen years, it has become apparent that tumour-associated p53 mutations can provoke activities that are different to those resulting from simply loss of wild-type tumour-suppressing p53 function. Many of these mutant p53 proteins acquire oncogenic properties that enable them to promote invasion, metastasis, proliferation and cell survival. Here we highlight some of the emerging molecular mechanisms through which mutant p53 proteins can exert these oncogenic functions.

The p53 signalling pathway is activated in response to a variety of stress signals, allowing p53 to coordinate transcription programmes that ultimately contribute to tumour suppression[1]. Loss of p53 function, through mutations in p53 itself or perturbations in pathways signalling to p53, is a common feature in the majority of human cancers. More than 75% of the mutations result in the expression of a p53 protein that has — in most cases — lost wild-type functions and may exert a dominant-negative regulation over any remaining wild-type p53 (ref. 2). Most interestingly, however, mutant p53 also acquires oncogenic functions that are entirely independent of wild-type p53 (refs 3–7).

Gain-of-function of mutant p53

The concept that mutant p53 proteins gain tumour-promoting functions was established over two decades ago by showing that mutant p53 has oncogenic effects in the absence of wild-type p53 in tissue culture systems[8,9]. However, the most compelling support for gain-of-function comes from mice engineered to harbour some of the most frequently occurring tumour-associated p53 mutations. In comparison to heterozygous or null ($p53^{+/-}$ or $p53^{-/-}$) mice, animals with one mutant allele show a different and broader tumour spectrum — with the appearance of more carcinomas and sarcomas, in addition to lymphomas. These mutant-p53-driven cancers also showed increased metastasis and genomic instability[10–12]. Many other oncogenic functions of mutant p53 have been characterized in cell culture models, including an ability to promote invasion, migration, scattering, angiogenesis, stem cell expansion, survival, proliferation and tissue remodelling. Enhanced chemo-resistance, mitogenic defects and genomic instability have also been reported. This wide range of different responses is reflected by increasing evidence that mutant p53 can function through multiple different pathways.

The primary alterations in p53 resulting from most tumour-associated mutations are rather modest — a single amino acid substitution in the 393-amino-acid protein. Most of these cluster within the central DNA-binding domain and, although p53 can be mutated at almost any amino acid in this region, a number of hotspots (including R175, G245, R248, R249, R273 and R282) have been identified. The nature of these mutations provides several clues as to how p53 function is affected. The first — and most obvious — is that the small changes in p53 lead to the expression of a protein that may retain at least some wild-type activities. The clustering of mutations indicates that DNA-binding activity is the critical function that is altered — suggesting that changes in transcriptional target genes could be key to the activity of mutant p53. But interestingly, the mutations in the structured core of p53 can also have significant consequences to the folding of the p53 protein. Broadly speaking, the mutations have been divided into two categories: structural mutants that can cause unfolding of the p53 protein, and DNA-contact mutants that change amino acids critical for DNA binding[8,13]. However, even wild-type p53 has an intrinsically unstable structure and it seems that most of the DNA-binding domain mutations serve to unfold p53 to some extent[14]. So although classic structural mutants (such as R175H) are highly unfolded under physiological conditions, even contact mutants (for example, R248Q) are less structurally stable than wild-type p53 (ref. 14). These changes in p53 structural stability may be crucial to the acquisition of the apparent gain-of-function phenotypes.

Mechanisms of mutant p53 function

To clarify the discussion of the molecular mechanisms by which mutant p53 may function, we have divided them into four main categories (Fig. 1), although inevitably there is some overlap between them. These mechanisms reflect either alterations in the DNA-binding ability of mutant p53 (model 1) or changes in the interaction of mutant p53 with other proteins, including other transcription factors (models 2 and 3) or proteins not directly related to the regulation of gene expression (model 4). Although it is convenient to consider these separately, it is clear that the effects of mutant p53 can be strongly context dependent, and interactions that promote activity in some circumstances may be inhibitory in others.

Mutant p53 binds to DNA to alter gene expression

As the tumour-derived mutant p53 proteins retain the N-terminal transcriptional transactivation domains, much of the activity of mutant p53 has

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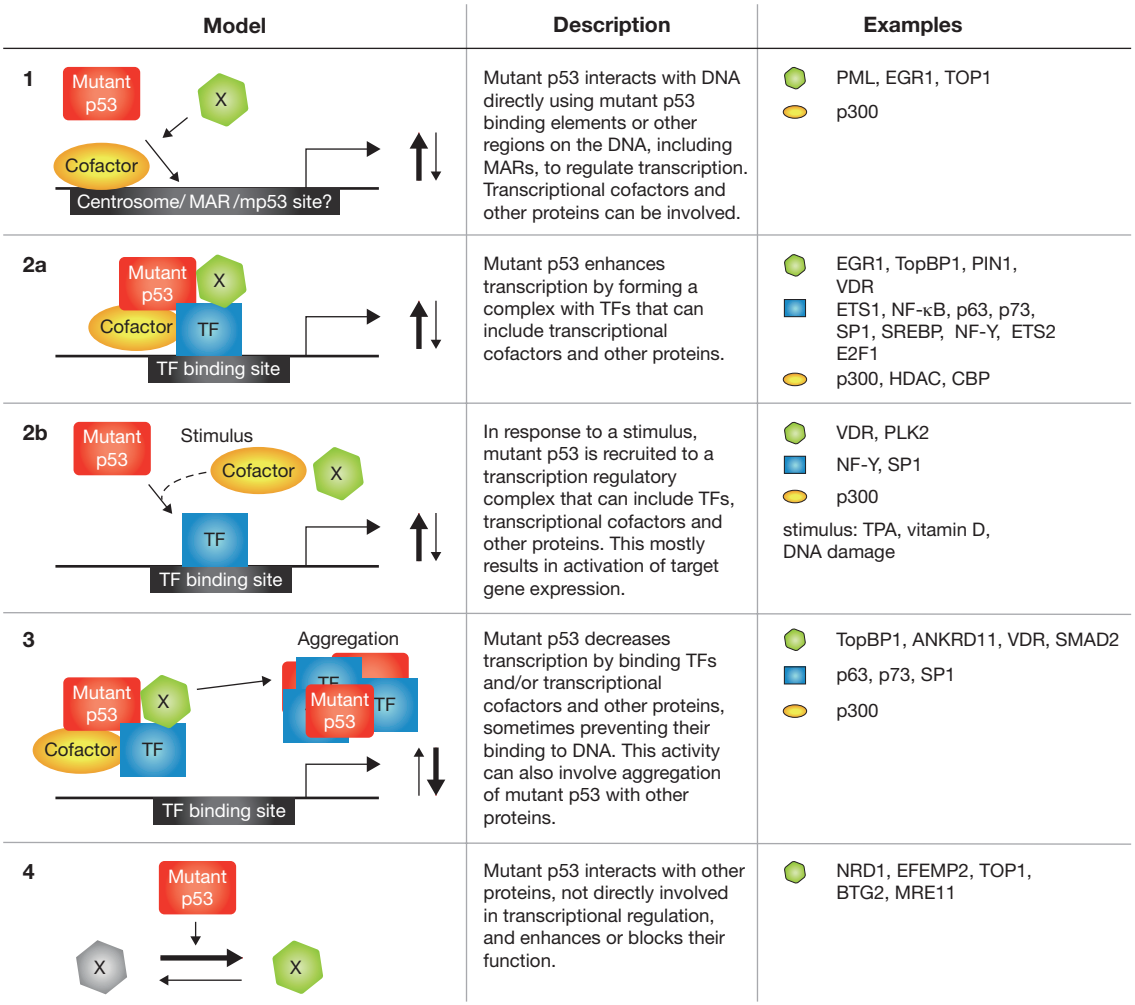

Figure 1 Models of mechanisms through which mutant p53 functions. As part of its gain of function, mutant p53 interacts with different proteins to enhance or inhibit their activities. TF, transcription factor; X, any protein other than a transcription factor or transcriptional cofactor; MAR, matrix attachment region DNA element; mp53, mutant p53.

been related to a direct or indirect ability to regulate gene expression. Most simply, the amino acid substitutions within the DNA-binding domain of the majority of tumour-derived p53 mutations may change, rather than abolish, sequence-specific DNA binding. It is certainly true that mutant p53 has DNA-binding activity, although in most cases their ability to bind standard p53 response elements is severely impaired[15,16]. This raises the possibility that some mutant p53 proteins may recognise a unique mutant p53 response element, allowing them to function as an oncogenic transcription factor[17–19] (Fig. 1, model 1). However, a consensus mutant-p53-specific DNA response element has so far not been characterized[20–22]. Apart from the possible acquisition of sequence-specific DNA-binding activity, mutant p53 also directly interacts with other parts of the DNA, including sequences that bind to the nuclear matrix (matrix attachment regions), providing another mechanism to regulate gene expression[23].

Mutant p53 binds to transcription factors to enhance their function

The best-described transcriptional functions of mutant p53 relate to its ability to interact with other transcription factors and modulate the expression of their target genes (Fig. 1, models 2 and 3)[24–28]. In some cases, mutant p53 increases the activity of the transcription factor partner (Fig. 2a), with further complexity added by the role of cellular stimuli, transcriptional cofactors and other proteins. For example, the interaction of mutant p53 with nuclear factor Y (NF-Y) deregulates the cell cycle checkpoint following induction of low levels of DNA damage (Fig. 2b)[29,30]. Under these conditions, DNA topoisomerase 2-binding protein 1 (TopBP1) recruits mutant p53 and the transcriptional cofactor p300 to target promoters[30]. At the same time, the PLK2 kinase can phosphorylate mutant p53 and also stimulate the binding of mutant p53 to p300. The phosphorylated mutant p53–p300 complex can subsequently interact with NF-Y to induce transcription[31]. Notably, one of the NF-Y target genes is PLK2 itself, which is subsequently induced by mutant p53 (ref. 32), causing an autoregulatory loop to reinforce mutant p53 activity[31].

Mutant p53 forms a complex with transcription factors to prevent their function

The interaction of mutant p53 with transcription factors can also be inhibitory (Fig. 1, model 3). Probably the best understood of these involve

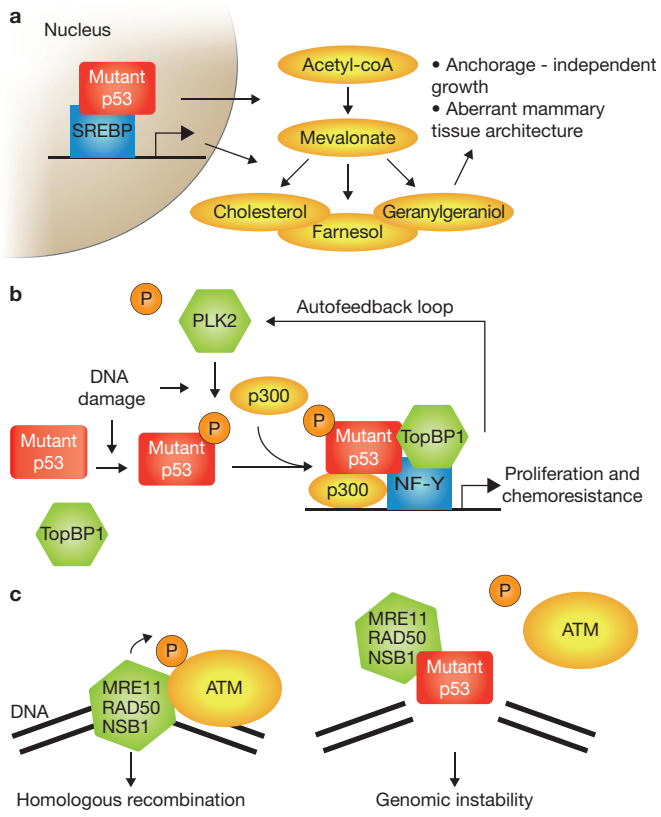

Figure 2 Mutant p53 binds to numerous proteins to enhance or inhibit their function. (**a**) Mutant p53 enhances SREBP function to increase sterol biosynthesis, leading to enhanced anchorage-independent growth and disruption of mammary tissue architecture. (**b**) In response to DNA damage, TopBP1 and PLK2 facilitate the recruitment of mutant p53 to NF-Y, leading to increased expression of genes involved in proliferation and chemoresistance. One of the genes regulated in this way is PLK2, creating an autoregulatory feedback loop. (**c**) Mutant p53 disrupts the function of the MRE11–RAD50–NSB complex, resulting in genomic instability. P, phosphate group.

the p53 family members p63 and p73 (refs 33,34). Although mutant p53 can prevent p63 or p73 from binding DNA[30,35], recent work has found that mutant p53 is frequently tethered to DNA through p63, albeit at sites distinct from those that p63 would normally bind — thus effectively preventing normal p63 function[36]. Understanding the exact consequences of the interaction between mutant p53 and p63 or p73 is complicated by the existence of multiple, functionally distinct p53 family member isoforms. The TA isoforms of p63 and p73 (containing the full-length sequence) and the ΔN forms (lacking the N-terminus) are both transcriptionally active, but each regulates a different group of genes. Intriguingly, although mutant p53 inhibits the transcriptional activity of the TA isoforms, in some systems the mutant p53–p63 interaction was found to enhance — rather than repress — the expression of some p63-regulated genes[32]. This may reflect a different effect of mutant p53 on TA and ΔNp63, and indicates that, as is also the case for vitamin D receptor (VDR) and the SP1 transcription factor, mutant p53 might act as both an activator (Fig. 1, model 2) and repressor (Fig. 1, model 3) of p63 function[24,25,28,37,38].

The ability of mutant p53 to form a complex with p63 is itself regulated at various levels, with many proteins reported to influence this interaction (Fig. 3). TopBP1and PIN1 promote binding of mutant p53 to p63 (refs 30,35,39), whereas ankyrin repeat domain 11 (ANKRD11) disrupts this interaction[40]. In response to TGF-β treatment, SMAD2 promotes the complex between mutant p53 and p63 (ref. 39), leading to the inhibition of p63-driven gene expression. These observations highlight the complexity of the transcriptional activity of mutant p53, and illustrate how the consequences of mutant p53 expression depend on cellular context.

Recently, certain mutant p53 proteins were shown to form prion-like aggregates[41], which may contribute to the binding and inhibition of p53 family members[42]. Further studies will determine to what extent this mode of function underlies mutant p53 activity, and whether mutant p53 aggregates with other transcription factors.

Mutant p53 interacts with proteins to change their function directly

Although there has been much focus on the role of mutant p53 in affecting transcriptional programmes, mutant p53 also binds and modulates the function of proteins that are not directly involved in transcription (Fig. 1, model 4). For example, by interacting with MRE11, a DNA nuclease required for DNA repair, mutant p53 prevents the MRE11–RAD50–NSB1 complex from phosphorylating ATM, leading to impaired homologous recombination (Fig. 2c)[12,43]. Furthermore, the structural mutant p53 proteins interact with BTG2, a cell cycle regulator, preventing it from de-activating H-Ras — with the potential for a number of oncogenic outcomes[44].

An interesting example of a protein whose function is enhanced by mutant p53 binding is topoisomerase 1 (Top1), which maintains topology of DNA. Whereas wild-type p53 both promotes and counteracts Top1 function, mutant p53 has specifically lost the negative regulation of Top1, resulting in hyper-recombination and genomic instability[45]. Mass spectroscopy analyses of the mutant-p53-specific interactome have provided many more possible targets for mutant p53 function[46]. Amongst these, nardilysin 1 (NRD1) was shown to bind a subset of mutant p53 proteins and so contribute to the ability of these proteins to drive invasion towards heparin-binding epidermal growth factor (HB-EGF) in a p63-independent manner[46].

Consequences of mutant p53 expression and pathways through which mutant p53 functions

Most of our understanding of pathways mediating mutant p53 function has been derived from exploring the consequences of the mutant p53–p63/p73 interaction. Mice that are heterozygous for p63 and p73 ($p63^{+/-}$ and $p73^{+/-}$) develop spontaneous metastatic tumours[47] very similar to those seen in mutant-p53-expressing mice, supporting a model in which mutant p53 functions by inhibiting p63/p73. A more detailed analysis of isoform-specific deletion in mice revealed that loss of the TA variants of p63 or p73 caused spontaneous tumour formation[48,49], and modulation of TAp63 in cell lines enhanced invasive behaviour and cell scattering in a manner similar to that seen following expression of mutant p53 (refs 39,50). In contrast, animals lacking the ΔNp73 variant did not show any signs of tumourigenesis[51], whereas the contribution of ΔNp63 could not be evaluated as the knockout mice suffered from embryonic lethality due to skin epithelial defects[52]. Nevertheless, both ΔNp63 and ΔNp73 have pro-survival and anti-apoptotic roles in cells[51,53,54]. Taken together, these observations suggest that the TA forms of p63 and p73 harbour tumour suppressor activities, whereas the ΔN variants are more likely to be oncogenic. Although most studies so far have not clearly distinguished between the different p63 and p73 isoforms, it is tempting to speculate

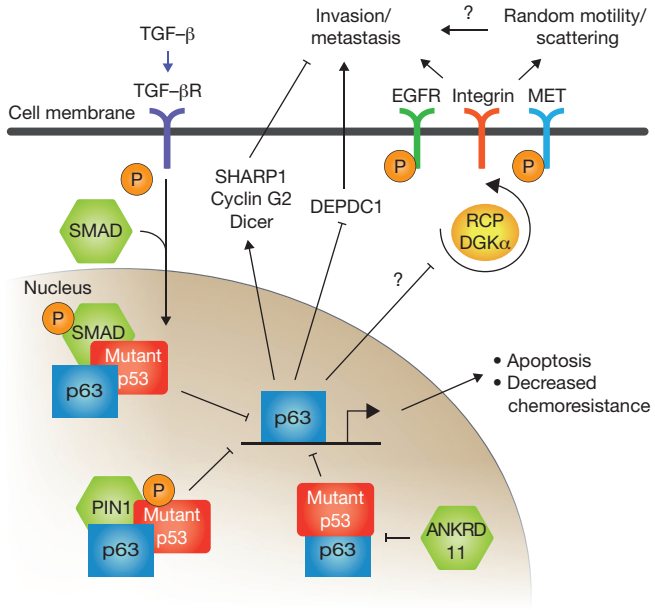

Figure 3 Mutant p53 inhibits the function of p63. p63 inhibition by mutant p53 leads to changes in the expression of genes such as Dicer, DEPDC1, SHARP1 and Cyclin G2, and enhances the recycling of integrins and growth factor receptors, resulting in invasion and metastasis. Various proteins, including SMAD2, PIN1 and ANKRD11, can impinge on the binding capacity of mutant p53 to p63.

that mutant p53 inhibits the tumour suppressor functions of TAp63/p73, and enhances the oncogenic activities of ΔNp63/p73. This idea would fit nicely with an ability of mutant p53 to inhibit TAp63 while promoting ΔNp63, but it remains to be investigated.

As expected, mutant-p53-dependent modulation of the expression of several p63 target genes has been associated with enhanced invasive behaviour (Fig. 3). The expression of SHARP1 and Cyclin G2 is modulated by a complex containing mutant p53, p63 and phospho-SMAD2, explaining how mutant p53 and TGF-β might cooperate to promote metastases in some systems[39], although mutant p53 can also inhibit TGF-β signalling[55]. Pin1 is required for mutant p53 to prevent the p63-mediated expression of Dicer[35], resulting in increased metastases in an *in vivo* tumour model[48,56]. Mutant p53 can also counteract p63-mediated repression of genes such as DEPDC1 (DEP domain containing 1)[35]. Although SHARP1, Cyclin G2, Dicer and DEPDC1 have all been implicated in invasion and metastasis, the mechanisms through which these proteins confer these effects are mostly unknown.

The interaction of mutant p53 with TAp63 also enhances the recycling and signalling of cell surface receptors, by engaging the RAB11 effector, RAB coupling protein (RCP) (Fig. 3). Indeed, expression of mutant p53, or inhibition of TAp63, promotes RCP-mediated recycling of integrins and growth factor receptors such as the EGFR (epithelial growth factor receptor) and MET (also known as HGFR, hepatocyte growth factor receptor)[50,57]. Furthermore, diacylglycerol kinase (DGKα) was required for mutant p53/p63/RCP-dependent invasion by promoting the translocation of RCP to the invadopodia of migrating cells[58]. These findings open the possibility that the mutant p53–p63 complex can regulate multiple receptor tyrosine kinases to control different facets of invasion and migration.

Despite its similarity to p63, the role of p73 in invasion and metastasis remains more elusive, although the role of p73 in enhancing apoptosis, cell senescence and chemosensitivity has been well characterized[59–61]. Consistently, mutant p53 prevents p73-dependent apoptosis in response to chemotherapeutic treatment[30,62–64], an activity that requires TopBP1 to prevent binding of p73 to target gene promoters[30]. Although mutant p53 likely functions by modulating p63 and p73 activity, further studies are required to clarify effects on different isoforms and different outcomes.

Besides regulating the transcription of many different protein-coding genes, mutant p53 has also been shown to regulate microRNAs and thereby alter the stability of various microRNA target transcripts. Examples include miR-130b, miR-155 and miR-205, each of which can influence important invasive and metastatic pathways through the regulation of transcripts such as *ZEB1* and *ZNF652* (refs 65–67). Notably, although mutant p53 regulated miR-155 and miR-205 in a p63-dependent manner, the regulation of miR-130b was p63-independent[65–67].

As well as binding to p63 and p73, mutant p53 functions through multiple other interactions. For example, by binding sterol regulatory element binding proteins (SREBPs), mutant p53 enhances the expression of various enzymes that regulate the mevalonate pathway (Fig. 2a) and thereby contributes to tissue remodelling through an activity that requires geranylgeranylation, rather than cholesterol synthesis[68]. An exciting suggestion from this study is that very commonly used inhibitors of this pathway, such as statins, may help to limit the SREBP-mediated activity of mutant p53, and so could be used for cancer therapy.

The multitude of mutant p53 interaction partners and the diversity in functional consequences of mutant p53 expression suggests that specific protein domains may play different roles in its various gain-of-function activities. The interaction with p63 and p73 requires the mutant p53 DNA-binding domain[33,34], and the C-terminus of mutant p53 is also necessary to inhibit p63 function (although it is dispensable for p63 binding) and for the modulation of invasion or apoptosis[40,50,69]. This region of p53 has been shown to bind to many other proteins[27,28,31,40], and the interaction of the C-terminus of mutant p53 with ANKRD11, for example, disrupts the p63–mutant-p53 interaction[40]. By contrast, binding of proteins such as PLK2, ETS2 and VDR to the C-terminus of mutant p53 promotes gain of function[27,28,31]. Although not required for all gain-of-function activities[29,50], the N-terminal transactivation domain of mutant p53 is essential for some activities — including the induction of target genes such as GRO1, the modulation of transcription factors such as the SREBPs, and for interference with drug-induced apoptosis[68,70,71].

Are all p53 mutants different in the same way?

Most studies so far have indicated that cancer-associated p53 mutations result in a broadly similar gain of invasive and metastatic activity, raising the question of why there are so many different mutations. One explanation is that the enrichment for certain mutations in some tumour types, for example aflatoxin-induced liver cancers or smoking-related lung cancers, is a reflection of the mutational stress that contributed to these cancers. But there is also evidence that the mutations are not all functionally equivalent. Most obviously, as discussed above, mutations in the DNA-contacting residues of p53 have a less dramatic effect on the folding of the p53 protein than the structural mutants. The native and denatured forms of p53 can be differentiated using conformation-specific antibodies, suggesting that the structural switch could reveal or obscure epitopes for binding to different partner proteins. This effect is seen in

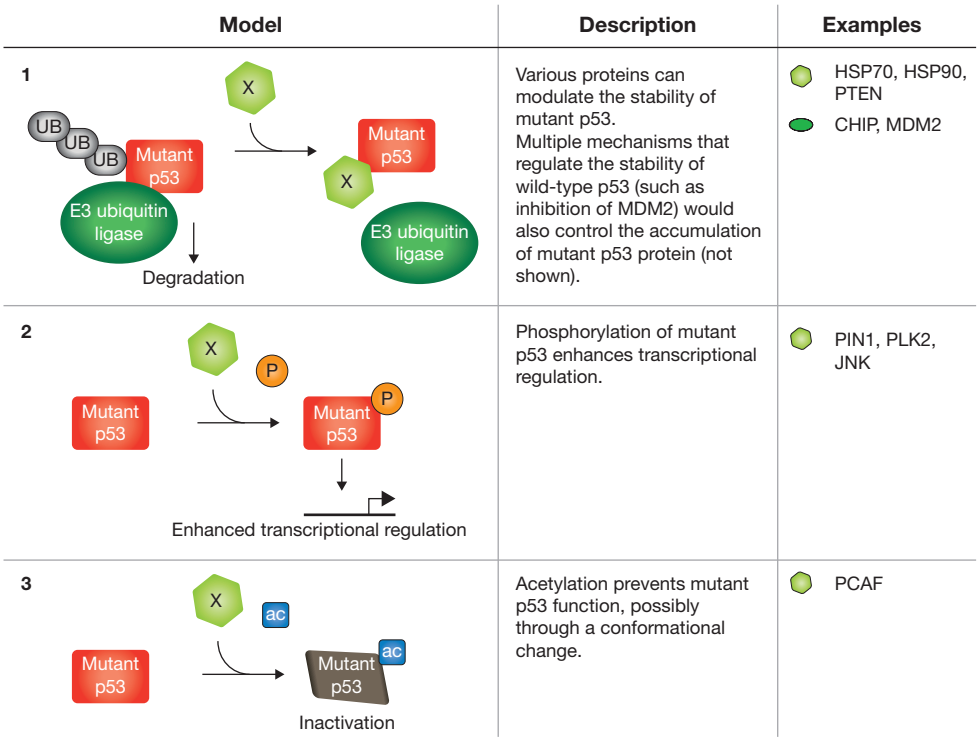

Figure 4 Modifications of mutant p53. Various modifications of mutant p53 have been described that affect its gain of function. TF, transcription factor; X, any protein other than a transcription factor or transcriptional cofactor.

the interaction of mutant p53 with p63 or p73 (refs 33,34,60), where the structural mutants bind p63 or p73 with a much higher affinity than the contact mutants, although —somewhat surprisingly — both groups of mutations seem equally capable of inhibiting p63 and/or p73 to promote invasion and metastasis or to prevent apoptosis[30,39,50,72]. Different mutant p53 proteins also show varying abilities to bind NRD1, which differentiate their ability to promote invasion towards HB-EGF compared to EGF or HGF (refs 46,57,73). Other examples include the observation that only the structural mutants activate H-Ras through BTG2 inactivation to induce a specific set of genes. Remarkably, although the DNA-contact mutants do not inactivate BTG2, they cooperate with NF-κB transcription to induce expression of the same set of genes[44]. These studies suggest that although the two groups of p53 mutants act in a mechanistically different manner, they converge on the same pathways to elicit similar outcomes.

Gain of function or subversion of function?

Mutant p53 clearly functions differently from wild-type p53, but there are some activities —such as the regulation of autophagy — that are displayed by both wild-type and mutant proteins[74]. Potentially, therefore, the point mutations do not disrupt all normal p53 activity but retain functions that might contribute to tumour development.

A further, intriguing possibility is that even wild-type p53 may, under some conditions, behave like mutant p53. Our understanding that the gain of function displayed by some mutant p53 proteins may reflect a difference in conformation allows for some speculation — can wild-type p53 adopt a misfolded conformation under certain conditions, so allowing for the manifestation of mutant-p53-associated activities? The detection of wild-type p53 in a mutant conformation has been reported in hypoxic cells[75] and binding of MDM2 (a ubiquitin ligase that targets p53 for degradation) to wild-type p53 can promote a conformational change to resemble mutant p53 (refs 76,77). Intriguingly, wild-type p53 was also shown to adopt a mutant conformation in cells that were serum-stimulated to enter the cell cycle[78]. Could a change in wild-type p53 conformation contribute to conditions where enhanced cell survival, invasion and motility are important — during normal development, for example? These ideas are speculation, at present, and must be put into context with the observation that p53 null mice can develop normally. However, it seems possible that the mutant protein represents an inappropriate and sustained signalling of normal p53 function.

Regulation of mutant p53

Wild-type p53 can be regulated through various mechanisms, many of which also control mutant p53 (Fig. 4). Key to the control of wild-type p53 function is the regulation of protein half-life — with p53 rapidly degraded in normal tissue, but stabilized in response to stress. The turnover of wild-type p53 is largely determined by the activity of the p53-targeting ubiquitin ligase MDM2 (refs 79,80). Mutant p53 expressed in normal tissues is also kept at low levels through the action of MDM2 (refs 81,82), although it often accumulates to high levels in tumour cells[83]. Since the mutant p53 proteins are not intrinsically resistant to degradation, it would seem that tumour-associated stress that normally stabilizes wild-type p53 also provokes a futile accumulation of mutant protein. Heat shock proteins, activated RAS and PTEN have all been implicated in the stabilization of mutant p53 (ref. 81), and targeting some of these pathways may be useful in preventing mutant p53 function in tumours. On the other hand, wild-type p53 stabilizing therapies can lead to the accumulation of mutant p53 in mice, resulting in a worse

outcome[81]. These are observations that must give pause for thought about any long-term or systemic use of such therapies.

Like wild-type p53, mutant p53 undergoes several post-translational modifications, some of have been shown to affect mutant p53 function. Phosphorylation by PLK2 or JNK enhances mutant p53 function[31,35,84], whereas phosphorylation at Ser 392 has been linked with poorer prognosis as well as promotion of MDM2-mediated degradation and reduced transforming activity[85–87]. Acetylation of some mutant p53 proteins by PCAF resulted in the restoration of partial wild-type DNA-binding activity and growth suppression[88]. The modulation of mutant p53 function through control of post-translational modifications is an underexplored, but potentially very interesting, area for future studies.

Therapeutic avenues to target mutant p53

Mutant p53 proteins are highly expressed in many cancers, making them extremely attractive targets for therapy. Strategies have focused on destabilization or inactivation of mutant p53, or reactivation of wild-type function in the mutant p53 protein. The latter strategy is difficult to achieve, but particularly appealing in light of mouse models showing that the activation of wild-type p53 in established tumours can lead to efficient tumour regression[89]. Drugs that inhibit aggregation of certain p53 mutants have also been described[90].

Destabilization of mutant p53 has been addressed mainly by targeting heat shock proteins through histone deacetylases to rescue MDM2-dependent degradation of mutant p53 (refs 91,92), whereas disruption of mutant p53 function may be achieved by preventing its interaction with other transcription factors. To this end, the molecule RETRA has been shown to inhibit the mutant p53–p73 interaction and to restore p73 function[93]. A number of compounds or peptides that result in the reactivation of wild-type function in mutant p53 have also been described[94–101]. Some of these compounds bind to grooves in the mutant p53 proteins and readjust the folding into a wild-type conformation, but for many the exact mechanism of function is unknown. In some cases, the reactivating compound appears to be specific for a certain mutation (Y220C)[101] or for a group of mutations (conformational mutants)[98–10094]. Peptides corresponding to the C-terminus of p53 have been shown to restore apoptosis induction by both structural and contact p53 mutants[96,102]. This is interesting given the importance of the C-terminus for mutant p53 function[40,50,103], but further studies are necessary to explore the mechanisms involved.

A more accessible approach may be to target the downstream pathways mediating mutant p53 activity. Mutant p53 has multiple functions — so it is unclear how effective the modulation of only one of these will be. Nevertheless, the ability of mutant p53 to engage in signalling pathways for which established, clinically approved drugs are already available (such as EGFR, MET and cholesterol synthesis pathways) gives hope that rapid progress may be made in translating such approaches into the clinic.

Conclusions

The growing understanding of mutant p53 functions has already led to the identification of some interesting molecules with the potential for clinical development. However, there is much left to learn. The fact that mutant p53 seems to play a role in promoting metastasis — the principal cause of cancer-related death — is particularly attractive in terms of possible therapeutic applications. However, although many cancers express mutant p53, it seems very unlikely that the different mutations of this versatile protein will have equivalent activities, and we may face the prospect of tailoring therapies not on the basis of mutant versus wild-type p53, but on which mutation is present. These challenging questions will provide exciting research avenues to be explored in the coming years.

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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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