When mutants gain new powers: news from the mutant p53 field

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Abstract | Ample data indicate that mutant p53 proteins not only lose their tumour suppressive functions, but also gain new abilities that promote tumorigenesis. Moreover, recent studies have modified our view of mutant p53 proteins, portraying them not as inert mutants, but rather as regulated proteins that influence the cancer cell transcriptome and phenotype. This influence is clinically manifested as association of *TP53* mutations with poor prognosis and drug resistance in a growing array of malignancies. Here, we review recent studies on mutant p53 regulation, gain-of-function mechanisms, transcriptional effects and prognostic association, with a focus on the clinical implications of these findings.

Loss of heterozygosity (LOH). A genetic event at a particular locus heterozygous for a mutant allele and a wild-type allele in which the wild-type allele is either deleted (rendering the cell hemizygous for the mutated allele) or mutated (rendering the cell homozygous for the mutant allele).

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Thirty years ago, a ~53 kDa protein that interacts with the viral SV40 T-antigen[1–3] and is frequently detected at high levels in cancer cells[4–6] was discovered, igniting the explosive field of p53 research. For almost a decade, p53 was considered to be a tumour antigen with transforming capabilities. Only during the late 1980s was it revealed that p53 is in fact a tumour suppressor, and that the evidence for its supposed oncogenic functions had been erroneously collected from tumour-derived mutant clones[7]. Thus, the potential of mutant p53 to promote cancer was one of the earliest findings in the field of p53 research.

p53 (encoded by the human gene *TP53*) is a stress response protein that functions primarily as a tetrameric transcription factor which regulates a large number of genes in response to a variety of cellular insults, including oncogene activation and DNA damage. These signals activate p53 primarily through post-translational modifications that result in augmented p53 protein level and transactivation activity. p53 bears the usual hallmarks of a transcription factor, with an amino-terminal transactivation domain, a core DNA-binding domain (DBD) and carboxy-terminal tetramerization and regulatory domains[8]. Activated p53 suppresses cellular transformation mainly by inducing growth arrest, apoptosis, DNA repair and differentiation in damaged cells[9]. Accordingly, p53 function is almost always compromised in tumour cells, usually as a result of somatic mutations, which occur in approximately half of all human cancers and constitute a cornerstone in tumorigenesis[10,11]. The frequencies of reported *TP53* mutations vary considerably between cancer types, ranging from ~10% (for example, in haematopoietic malignancies[12]) to 50–70% (for example, in ovarian[13], colorectal[14] and head and neck[15] cancers). Whereas somatic *TP53* mutations contribute to sporadic cancer, germline *TP53* mutations cause a rare type of cancer predisposition known as Li–Fraumeni Syndrome (LFS)[16] (BOX 1). Importantly, both somatic and germline *TP53* mutations are usually followed by loss of heterozygosity (LOH) during tumour progression, which suggests that a selective force inactivates the remaining wild-type allele. Most *TP53* mutations can be classified into two main categories according to their effect on the thermodynamic stability of the p53 protein[8]. These two mutation categories are commonly referred to as 'DNA-contact' and 'conformational' mutations. The first group includes mutations in residues directly involved in DNA binding, such as R248Q and R273H. The second group comprises mutations that cause local (such as R249S and G245S) or global (such as R175H and R282W) conformational distortions.

The majority of *TP53* mutations observed in human cancers abrogate the sequence-specific DNA-binding activity towards the wild-type p53 responsive element[17]. Moreover, these mutations usually confer the mutant protein with a dominant-negative activity over the remaining wild-type allele, a mechanism that involves hetero-oligomerization of the mutant protein with the wild-type protein[18–20]. However, as the field of p53 research evolves, it is increasingly evident that many mutant p53 forms not only lose their tumour suppressive function and acquire dominant-negative activities, but also gain new oncogenic properties that are independent of wild-type p53. This notion, termed the 'gain-of-function hypothesis', received its first support when transfection of mutant p53 into *TP53*-null cells was shown to enhance their ability to form tumours in mice[21–23]. Since then, numerous studies

At a glance

- The tumour suppressor p53 (encoded by *TP53* in humans) functions primarily as a transcription factor, which, upon cellular stress signals, regulates a plethora of genes that promote cell cycle arrest, senescence, apoptosis, differentiation, DNA repair and other processes.
- *TP53* is somatically mutated in the majority of sporadic human cancers, and mutations in *TP53* are also associated with Li–Fraumeni Syndrome, a familial cancer predisposition syndrome.
- The majority of cancer-associated mutations in *TP53* are missense mutations in its DNA-binding domain. These mutations usually lead to the formation of a full-length mutant protein (mutant p53) incapable of activating p53 target genes and suppressing tumorigenesis. Besides losing their wild-type activities, many p53 mutants also function as dominant-negative proteins that inactivate wild-type p53 expressed from the remaining wild-type allele. Moreover, some mutant p53 forms also acquire new oncogenic properties that are independent of wild-type p53, known as 'gain-of-function' properties.
- In the past three decades ample data were collected in support of the importance of mutant p53 gain-of-function properties for tumorigenesis. These data include cell culture studies that demonstrated the capability of mutant p53 to impinge on pivotal cellular regulatory networks, mouse models that established the ability of mutant p53 to increase tumour aggressiveness and metastatic potential, as well as clinical studies that revealed associations between *TP53* mutations and poor clinical outcome in a variety of malignancies.
- This Review describes recent advances in the research field of mutant p53, with an emphasis on the transcriptional effects of mutant p53, the expression signatures associated with *TP53* mutations *in vitro* and *in vivo* and the diagnostic, prognostic and predictive value of *TP53* mutations in human cancer.

demonstrated a wide range of oncogenic properties for mutant p53 forms and provided key mechanistic insights to explain these phenomena (FIG. 1, TABLE 1). For instance, mutations in *TP53* are associated with drug resistance in several malignancies and cell lines[24–27], a phenomenon that may be partially attributed to transcriptional activation of the multi-drug resistance 1 (*MDR1*; also known as *ABCB1*) gene by mutant p53 (REFS 28,29) and to interference with apoptosis[30]. These and many other oncogenic properties of mutant p53 may underlie the abundant evidence for the association of mutations in *TP53* with poor clinical outcome in a growing array of cancer types.

It is still debatable which property of mutant p53 is primarily selected for during cancer development. Is it the loss of wild-type p53 function, the acquisition of dominant-negative properties, the gain of new oncogenic functions or perhaps a combination of the above? In the following sections we review recent studies that shed light on the relevance of each of the properties of mutant p53 to tumorigenesis.

Lessons from the distribution of *TP53* mutations

Unlike most tumour-suppressor genes, which typically undergo biallelic inactivation during carcinogenesis by deletions or truncating mutations, *TP53* is frequently (74%) inactivated by a single monoallelic missense mutation (FIG. 2) that results in the formation of a stable full-length protein, readily detected in human tumours (BOX 2)[7]. The vast majority of missense mutations are mapped to the DBD and usually abrogate its sequence-specific DNA-binding activity[10,31]. Furthermore, almost a third of all missense mutations arise in six 'hotspot' residues[10,32] (FIG. 2). The high proportion of missense mutations in *TP53*, as well as the existence of hotspot residues, are frequently cited in favour of the gain-of-function hypothesis[33]. However, based on unbiased sequencing of 13,000 genes from human tumour samples[34], it seems that the incidence of missense mutations in *TP53* is equal to the mean frequency of missense mutations in cancer cells[35,36]. Additionally, as the tetramerization domain of p53 resides at its carboxy-terminus and participates in the hetero-oligomerization between mutant and wild-type p53, missense mutations are more likely than truncating mutations to produce a protein capable of binding and inactivating wild-type p53. Hence, a dominant-negative effect may represent an additional selective force for missense mutations.

The range of *TP53* mutations is affected not only by selection processes, but also by intrinsic factors that differentially affect specific nucleotides and regions of the gene. For example, transitions at CpG sites are more frequent due to their high mutagenicity, and represent a key force shaping the distribution of *TP53* mutations[37]. Notably, among all possible missense mutations involving CpG transitions, only the few that result in a loss of transactivation activity are selected in cancers, which leads to the suggestion that loss of function is the dominant selective force in cancers[36]. Nevertheless, systematic data on the effect of CpG transitions on dominant-negative and gain-of-function properties of mutant p53 are scarce, and therefore these properties cannot be excluded as a tumorigenic driving force. Additional complexity is added by environmental carcinogens that leave their fingerprints on *TP53* mutation patterns in several malignancies[37,38]. For example, the V157F and R158L substitutions, which result from G→T transversions, are more frequent in lung cancer compared with other cancers, perhaps due to the mutagenic effect of the tobacco-smoke-derived carcinogen benzo-[a]-pyrene[38]. Similarly, the R249S substitution is extremely common in liver cancer in some developing countries and has been associated with aflatoxin B1 food contamination[38,39]. In sum, the unique range of *TP53* mutations is shaped by intrinsic and environmental factors, as well as complex and not fully understood selection processes in tumours. Additional lines of evidence, such as mouse models and molecular data, should be considered to assess the relative contribution of each property of mutant p53 to tumorigenesis.

Regulation and function of mutant p53

Updates from mouse models. Mutant p53 proteins often accumulate at extremely high levels in tumours[40–42]. In fact, immunohistochemical (IHC) detection of p53 in tumours usually indicates *TP53* missense mutation and can provide prognostic and predictive information[43]. By contrast, wild-type p53 is usually maintained at low levels in normal tissues due to its short half-life[44]. These low levels are primarily maintained by the ubiquitin-mediated degradation of p53, a process regulated by the E3 ubiquitin ligase and the wild-type p53 target gene *MDM2* (also known as *HDM2*)[45,46]. A prominent notion was that the disruption of this negative feedback by mutations that abrogate the

Missense mutation
A single base-pair substitution that results in the translation of a different amino acid at that position.

Transition
A mutation that results in a substitution of a purine for a purine or a pyrimidine for a pyrimidine.

Transversion
A mutation that results in a substitution of a purine for a pyrimidine or vice versa.

Aflatoxin B1
A potent mutagenic fungal-produced toxin. Human exposure to aflatoxin B1 largely stems from *Aspergillus* spp. food contamination, which occurs primarily in eastern Asia and sub-Saharan Africa. Aflatoxin B1 promotes G→T transversions and is associated with a substitution of an arginine (AGG) to a serine (AGT) at *TP53* codon R249, the most common *TP53* mutation in hepatocellular carcinoma.

Focus formation assay
An *in vitro* assay used to measure the oncogenic potential of a gene. Usually, the gene of interest is delivered into animal cells which normally show contact inhibition. A *bona fide* oncogene grants the cells the ability to form areas of multi-layered densely-packed cells (called foci).

Penetrance
A measure of the proportion of individuals carrying a gene variation (for example, a mutation or single nucleotide polymorphism) that also express a phenotypical trait associated with that genetic variation (for example, a disease).

ability of p53 to transactivate *MDM2* underlies the accumulation of mutant p53 (REFS 20,47–49). However, recent *in vivo* data challenge this hypothesis by demonstrating that mutant *Trp53* knock-in mice (*Trp53* encodes mouse p53) do not accumulate mutant p53 in normal tissues[50–52] whereas mutant p53 levels are increased in most tumours, albeit not all[53]. Similarly, mutant p53 does not accumulate in normal tissues from patients with LFS contrary to what occurs in tumours from these same patients[33]. Importantly, *Mdm2*$^{-/-}$ mice harbouring knock-in *Trp53* mutants do accumulate mutant p53 in some normal tissues. Together, these findings demonstrate that *TP53* inactivating mutations alone are insufficient for the accumulation of mutant p53 and that additional events that occur during tumorigenesis are required to release mutant p53 from MDM2-mediated degradation. Furthermore, in the presence of MDM2, the survival of mice homozygous for a hotspot *Trp53* mutant is similar to that of *Trp53*$^{-/-}$ mice[50–52] (although the mice expressing mutant p53 develop more aggressive and metastatic tumours[50]). By contrast, in *Mdm2*$^{-/-}$ mice, the expression of mutant p53 significantly reduces survival[53], which demonstrates that mutant p53 accumulation is important for the execution of its gain-of-function potential. Additionally, although p53 mutants do not display gain-of-function properties in *Mdm2*$^{+/+}$ mice in terms of overall survival, they do have a pronounced effect on the range of tumours that arise in these mice, with each p53 mutant associated with a distinct tumour pattern[50–52]. These data indicate that whereas p53 inactivation is sufficient for tumour initiation at certain tissues, other tissues are perhaps more resistant and require the gain-of-function activities of mutant p53 for their transformation.

Mdm2$^{-/-}$ mice are embryonic lethal, and deletion of both *Trp53* alleles rescues this phenotype[54,55]. Thus, whether a heterozygous p53 mutant can rescue lethality of *Mdm2*$^{-/-}$ mice can be used as an assay to test its dominant-negative activity over the wild-type allele. Arguing against a dominant-negative activity of mutant p53 during development, the mouse hotspot mutant p53^{R172H} failed in this *in vivo* rescue assay[50]. A similar picture is seen in the context of tumour suppression, where *Trp53*$^{+/-}$ mice and *Trp53*$^{+/m}$ mice (where 'm' stands for mouse hotspot mutants p53^{R172H} or p53^{R270H}) display similar survival curves[50,51], which suggests that endogenously expressed mutant p53 is not efficient at inactivating the remaining wild-type allele. The complexity of mutant p53 regulation and function increases when considering experiments that use cultured mouse embryonic fibroblasts (MEFs) derived from mutant *Trp53* knock-in mice. Mutant p53 accumulates in these MEFs although MDM2 levels are not reduced in comparison to MEFs expressing wild-type *Trp53*, which indicates that factors independent of MDM2 levels regulate the accumulation of mutant p53 (REFS 51,52). Accordingly, the ability of MDM2 to bind and ubiquitylate p53 was shown to be reduced or abolished by some hotspot p53 mutations[47,52]. Moreover, although certain conformational p53 mutants are bound by MDM2 as effectively as wild-type p53, MDM2-dependent ubiquitylation is impaired[48]. Importantly, the accumulation of mutant p53 in the MEFs derived from the knock-in mice augments both its dominant-negative and gain-of-function properties (when analyzed in heterozygous and homozygous models, respectively). This is evident by increased growth rate, decreased contact inhibition and increased ability to cooperate with oncogenic *HRAS* in focus formation assays[50]. By contrast, chemotherapy-induced cell-cycle arrest and induction of p53 target genes *Cdkn1a* (which encodes p21) and *Mdm2* were almost unaffected by the expression of mutant *Trp53* in heterozygous MEFs[51,52], arguing against a complete dominant-negative effect. Finally, in support of the gain-of-function hypothesis, stable or conditional knockdown of endogenous mutant p53 in different human cell lines was shown to reduce their proliferation rate and chemoresistance *in vitro* as well as their ability to form tumours in nude mice[56,57].

Therefore, the combined *in vitro* and *in vivo* data suggest that when some mutant p53 forms accumulate, their oncogenic properties are enhanced, prompting the careful consideration of p53-activating drugs when treating tumours that express mutant p53. Furthermore, these data, together with the high incidence of LOH in tumours that harbour mutant p53, challenge the relevance of mutant p53 dominant-negative effects for cancer development and support the gain-of-function hypothesis.

Old and new regulators of mutant p53. As the notion that accumulation of p53 mutants augments their oncogenic potential, the previously unstudied field of molecular modulators of mutant p53 level and activity is gaining interest. Cellular pathways that affect the folding, stability and localization of mutant p53 are being elucidated and their clinical importance appreciated.

One interesting example is the crosstalk between mutant p53 and the pivotal tumour-suppressor PTEN, which enhances wild-type p53 function by indirectly inhibiting MDM2 and directly binding to wild-type p53 (REF. 58). Recently, PTEN was suggested to exert oncogenic functions by enhancing the stability of mutant p53 (REF. 59). In fact, PTEN inhibited growth of glioblastoma cell lines harbouring wild-type p53, whereas in xenografts of cell lines expressing mutant p53, PTEN enhanced proliferation, survival and tumour formation *in vivo*. An opposite example is the tumour-suppressor INK4A (encoded by the *CDKN2A* locus), which reduces wild-type p53 levels through the E2F-Rb axis[60]. When p53^{R172H} knock-in mice were crossed with *Cdkn2a*$^{-/-}$ mice, mutant p53 accumulated in normal tissues and the

Box 1 | **Li–Fraumeni syndrome**

Li–Fraumeni syndrome (LFS) is a rare autosomal-dominant highly penetrant cancer predisposition syndrome, proposed by Li and Fraumeni in 1969 (REFS 168,169). LFS is unique among cancer predispositions as it is not associated with site-specific tumours, but rather with a variety of tumour types occurring at a relatively early age[169]. The underlying genetic defect in most patients with LFS is a germline mutation in one of the alleles that encodes p53 (REF. 16), with an estimated penetrance of 90%–95%. The distribution of germline mutations in patients with LFS reflects that of sporadic tumours, with similar hotspot residues[170]. Another similarity between patients with LFS and individuals with sporadic cancer is the enhanced oncogenic potential of missense *TP53* mutations compared with truncating and inactivating mutations[171].

Autophagy
A cellular catabolic degradation response to stress involving engulfing, degradation and recycling of intracellular material.

mice developed tumours at an earlier age[53]. Therefore, loss of INK4A expression, a common oncogenic event, may also contribute to cancer development by stabilizing mutant p53.

Mutant p53 is known to interact with heat-shock proteins such as HSP90 and HSP70 (REFS 61–64). In this context, some insights into the accumulation of mutant p53 in tumours and its phenotypic consequences were recently obtained. For instance, wild-type and mutant p53 were shown to have opposite dependencies on the molecular chaperone HSP90. Whereas wild-type p53 accumulated following HSP90 inhibition, the protein level of mutant p53 was reduced. In line with the gain-of-function hypothesis, mutant p53 destabilization upon HSP90 inhibition was accompanied by cell death[65]. Additionally, recent studies investigated the involvement of HSP90, HSP70 and the ubiquitin-ligase CHIP (also known as STUB1) in the regulation of mutant p53 stability[49,66], demonstrating that HSP90 inhibition increases the unfolded fraction of the mutant p53 molecules, which are then bound by HSP70 and marked for degradation by CHIP. Because HSP90 is frequently over-activated in tumours[67], the dependency of mutant p53 on HSP90 may account for its tumour-specific accumulation and underlie the therapeutic potential of HSP90 inhibitors[68].

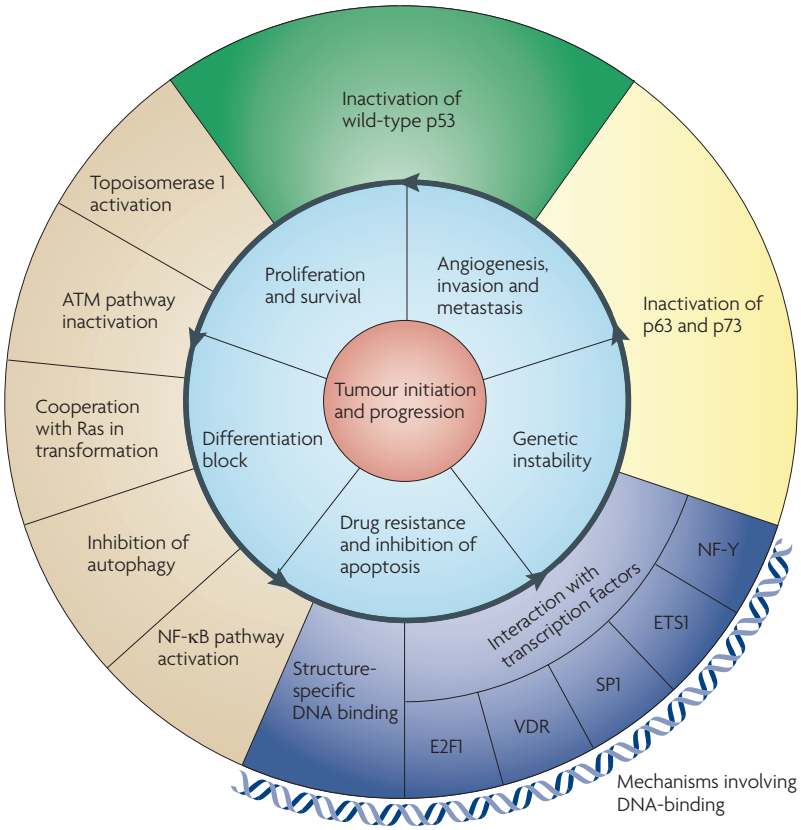


Figure 1 | **Selected oncogenic properties of mutant p53 and their underlying mechanisms.** The inner circle (shaded blue) represents oncogenic phenotypes associated with the activities of mutant p53 proteins. The outer circle depicts key mechanistic properties of p53 mutants that underlie the phenotypes listed in the inner circle. Note that each of the phenotypic effects can be attributed to almost each of the mechanistic properties; hence the inner blue circle can be freely rotated. ATM, ataxia-telangiectasia mutated; NF-κB, nuclear factor-κB; VDR, vitamin D receptor.

The subcellular localization of mutant p53 is another parameter that affects its oncogenic properties. Although mutant p53 usually accumulates in the nucleus of cancer cells[43,69,70], in some cases it localizes to the cytoplasm, depending on the type of mutant, the cellular context and a variety of stress signals that modulate p53 localization[48,69]. As with wild-type p53, MDM2-dependent and MDM2-independent ubiquitylation seems to regulate nuclear export of p53 mutants, and especially of conformational mutants, probably by exposing their C-terminal nuclear export signal[48,71]. Interestingly, a recent study demonstrated that cytoplasmic p53 mutants can inhibit autophagy in cancer cells[72]. For each of 22 mutant p53 forms analyzed, a unique localization pattern was observed, from almost exclusively nuclear (for example, $p53^{R282W}$) to almost exclusively cytosolic (for example, $p53^{R273H}$). On the single-cell level, all p53 mutants were competent at inhibiting autophagy when localized to the cytosol, although the inhibitory effect on the entire cell population was much more pronounced for mutants with predominantly cytosolic localization. Chronic suppression of autophagy was demonstrated to facilitate tumorigenesis, and several proteins that promote autophagy are considered tumour suppressors[73]. Hence, inhibition of autophagy may constitute a new oncogenic property of p53 mutants, and highlights the importance of subcellular localization of p53 mutants in tumours.

Mutant p53 gain-of-function properties

Interaction with p63 and p73. Since the discovery of mutant p53 oncogenic potential[21–23,74,75], numerous gain-of-function properties were demonstrated and a variety of underlying mechanisms were proposed[7,20,76,77] (FIG. 1). However, recent findings not only broaden the array of gain-of-function properties, but also highlight their relevance to tumorigenesis. A pivotal gain-of-function mechanism is the ability of common p53 mutants to bind and inactivate p53 family members, p63 and p73 (REFS 78,79). These transcription factors have key roles during development and can be expressed as several splice variants with distinct and even antagonistic functions[80]. Importantly, there is a substantial amount of data to support the role of the transactivation-potent variants of p63 and p73 in suppressing tumorigenesis[80]. Moreover, analysis of mouse models demonstrated that p63 and p73 can partially compensate for deletion of *Trp53* as $Trp53^{+/-}Trp63^{+/-}$ mice and $Trp53^{+/-}Trp73^{+/-}$ mice have reduced survival and increased metastatic rate compared with $Trp53^{+/-}$ mice[81]. Therefore, inhibition of p63 and p73 function is considered a key mechanism for mutant p53 gain of function[82,83]. Evidence supporting this notion has come from the recently developed knock-in mouse model in which $p53^{R172H}$ was shown to bind p63 and p73 in tumour-derived cell lines, consequently inhibiting their abilities to induce cell-cycle arrest and suppress focus formation[50]. The capacity of different p53 mutants to bind p73 was shown to be significantly influenced by the site of mutation as well as by the

Table 1 | **Selected list of genes that are transcriptionally regulated by mutant p53**

Phenotypic effects	Corresponding gene(s)	Effect	Refs
Enhanced proliferation	NF-Y target genes (such as *CCNA*, *CCNB2*, *CDK1*, *CDC25C*)	Upregulated	92
	MAP2K3 (mitogen-activated protein kinase kinase 3)	Upregulated	57
	MAD1L1 (MAD1 mitotic arrest deficient-like 1)	Upregulated	172
	FOS , also known as *c-fos*	Upregulated	173
	PCNA (proliferating cell nuclear antigen)	Upregulated	174
	MYC, also known as *c-myc*	Upregulated	100
	E2F5	Upregulated	95
	ASNS (asparagine synthetase)	Upregulated	108
	ARHGEF2 (Rho/Rac guanine nucleotide exchange factor (GEF) 2; also known as GEF-H1)	Upregulated	175
	ID2 (inhibitor of DNA binding 2)	Downregulated	176
	MCM6 (minichromosome maintenance complex component 6)	Upregulated	95
	IGF1R (insulin-like growth factor 1 receptor)	Upregulated	177
	CXCL1 (CXC-chemokine ligand 1; also known as *GRO1*)	Upregulated	178
Inhibition of apoptosis and/or chemoresistance	*EGR1 (early growth response 1)*	Upregulated	179
	ATF3 (activating transcription factor 3)	Downregulated	180
	LGALS3 (lectin, galactoside-binding, soluble; also known as Galectin-3	Upregulated	181
	FAS , also known as *CD95/APO-1*	Downregulated	182
	MST1 (macrophage stimulating 1; also known as MSP)	Downregulated	183
	BCL2L1, also known as *bcl-xL*	Upregulated	57
	DHCR24 (24-dehydrocholesterol reductase; also known as seladin-1)	Upregulated	57
	NFKB2 (nuclear factor-κB2)	Upregulated	95
	ABCB1 (ATP-binding cassette, sub-family B (MDR/TAP), member 1; also known as MDR1)	Upregulated	28
	IGF2 (insulin-like growth factor 2)	Upregulated	184
	BAG1 (BCL2-associated athanogene)	Upregulated	185
	DUT (deoxyuridine triphosphatase ; also known as dUTPase)	Upregulated	186
Other effects	*TGFBR2* (transforming growth factor, beta receptor II)	Downregulated	187
	ARHGDIA (Rho GDP dissociation inhibitor (GDI) α)	Upregulated	57
	RANGAP1 (Ran GTPase activating protein 1)	Upregulated	57
	PXN (paxillin)	Upregulated	57
	KIF20A (kinesin family member 20A)	Upregulated	57
	ALOX15 (arachidonate 15-lipoxygenase)	Upregulated	188
	ribosomal proteins *RPL37*, *RPLP1*, and *RPS2*	Upregulated	189
Limitless replication	*TERT (telomerase reverse transcriptase)*	Upregulated	108
Invasiveness, inflammation and angiogenesis	NFκB target genes (such as *CXCL1*, interleukin 1β (*IL1B*), *IL6*, *IL8*, *MMP3*)	Upregulated	*
	WISP2 (WNT1 inducible signalling pathway protein 2; also known as cyclin 5)	Upregulated	190
	VEGFA (vascular endothelial growth factor A)	Upregulated	191

*Rotter V. *et al.*, unpublished data. CDC25C, cell division cycle 25C; CDK1, cyclin dependent kinase 1; MMP3, matrix metalloproteinase 3.

single nucleotide polymorphism at codon 72 (REF. 84). Moreover, the p73-binding capacity is correlated with the ability of p53 mutants to protect cells from chemotherapeutic agents, and, accordingly, with less favourable response to chemo-radiotherapy in patients with head and neck cancer[84]. Therefore, targeting the interaction of mutant p53 with p63 and p73 seems a promising strategy for cancer therapy. Indeed, short peptides that disassemble $p53^{R175H}$ from p73 restore the activity of p73 and re-sensitize cells that harbour mutant p53 to chemotherapy[85]. Similarly, the small molecule RETRA, which interferes with mutant-p53–p73 interaction, hinders the growth of cells that express mutant p53 and their ability to form tumours in mice[86].

Single nucleotide polymorphism
(SNP). A germline variation in a single nucleotide that exists at a frequency of at least 1% in the general population.

DNA binding, transcriptional regulation and more. Inactivation of p63 and p73 seems to account for a large proportion of the oncogenic properties of p53 mutants. Yet, there are other well-characterized gain-of-function

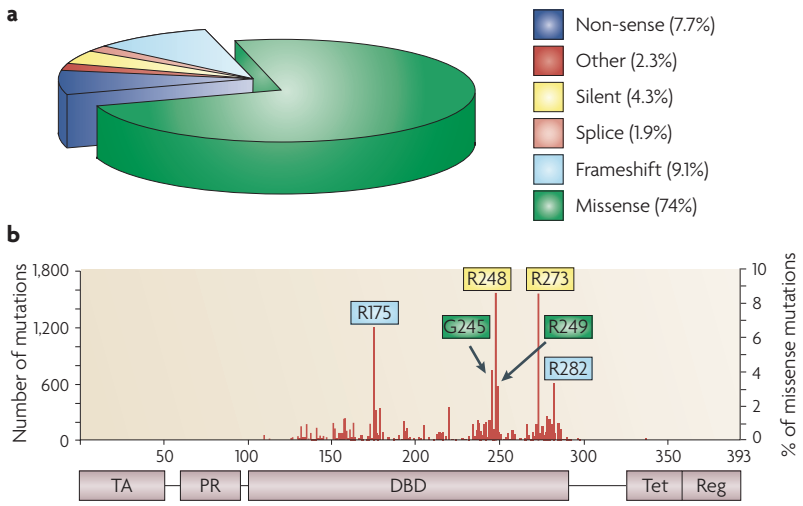


Figure 2 | **Distribution of *TP53* somatic mutations according to the IARC TP53 Mutation Database. a** | Pie chart representing the different tumour-derived mutation types reported in the IARC TP53 Mutation Database. **b** | The distribution of reported missense mutations along the 393 amino-acid sequence of p53. The six most common hotspot mutations are highlighted in yellow for DNA-contact mutations, green for locally distorted mutants and blue for globally denatured mutants. The domain architecture of p53 is aligned below. Note that the depicted enrichment of mutations in the DNA-binding domain (DBD) is probably an overestimation as in many studies only the core domain exons of *TP53* are sequenced, thus, mutations outside this region are overlooked. PR, proline-rich domain; Reg, carboxy-terminal regulatory domain; TA, transactivation domain; Tet, tetramerization domain. Data derived from the IARC TP53 Mutation Database version R13 (November 2008)[32].

mechanisms, which can be roughly categorized into two groups, depending on whether they involve DNA binding and modulation of gene transcription by mutant p53.

Although two thirds of missense mutations in the DBD, including all hotspot mutations, abrogate the ability of p53 to activate target genes[17], modulation of gene transcription by mutant p53 is well documented as an important gain-of-function mechanism[7,76,77]. The earliest evidence emerged with the demonstration that a functional transcription-activation domain of p53 is required for mutant-p53-dependent activation of *MDR1* and protection from drug-induced apoptosis[87,88]. Since then, the transcriptional regulation of numerous genes has been implicated in mutant p53 gain of function (for reviews see REFS 7,76,77) (TABLE 1). However, a unifying mechanism for the selectivity of mutant p53 to certain genes is still missing owing to, among other reasons, the lack of consensus DNA sequence among genes regulated by mutant p53 and the variability in the identity of genes affected by different p53 mutants. A possible solution is provided by recent *in vitro* data, demonstrating that several p53 mutants, although defective in sequence-specific DNA binding, retain the ability to bind specific non-B DNA structures with high affinity[89]. Consistent with the diverse gene specificity associated with different p53 mutants, various DNA structures are bound by different mutants through distinct mechanisms and with different affinities. Supporting these data, DNA sequences bound by mutant p53 are rich in repetitive elements and other sequences with a high likelihood of adopting non-B conformations[90,91]. Therefore, the specificity of mutant p53 to certain regulatory sequences is perhaps mediated through preferential binding to structural DNA motifs and not consensus sequences.

Another well-established mechanism for gene-specific transcriptional regulation is the interaction of mutant p53 with sequence-specific transcription factors, which results in either augmentation or attenuation of their activity. Besides p63 and p73, well-characterized transcription factors that interact with mutant p53 are SP1 and ETS1, which also interact with wild-type p53. Interestingly, the effects of mutant and wild-type p53 on SP1 and ETS1 are antagonistic, suggesting that additional but distinct co-factors are recruited by mutant p53 and wild-type p53 (REF. 76). Similarly, the transcription factor NF-Y, which interacts with wild-type p53, was recently demonstrated to interact with the $p53^{R175H}$ and $p53^{R273H}$ hotspot mutants[92]. NF-Y binds the CCAAT motif and regulates numerous cell-cycle-associated genes. Mutant-p53–NF-Y complexes are recruited to CCAAT-box-containing promoters upon treatment with adriamycin (also known as doxorubicin), and this results in activation of NF-Y target genes such as cyclins and cyclin-dependent kinases (CDKs), and, consequently, increased DNA synthesis. As opposed to wild-type p53, which binds the histone deacetylase HDAC1 in adriamycin-treated cancer cells, mutant-p53–NF-Y promoter-bound complexes switch binding partners from HDAC1 to the histone acetyltransferase p300 following adriamycin treatment, and this results in increased histone acetylation and transcription of NF-Y target genes[92]. In this example, therefore, gene-specific regulation by mutant p53 is achieved through interaction with NF-Y, and the opposite effects of wild-type and mutant p53 on NF-Y target genes are explained by recruitment of antagonistic epigenetic modifiers. Importantly, mutant-p53–NF-Y interactions may underlie the growth-promoting properties of mutant p53 as well as the insensitivity of some mutant-p53-expressing tumours to DNA damage[24,25,27].

Another fascinating example is the interaction of $p53^{R175H}$ with the transcription factor vitamin D receptor (VDR). By analyzing chromatin immunoprecipitation (ChIP)-on-chip data, the VDR response element was found to be over-represented in promoters bound by mutant p53. Apparently, $p53^{R175H}$ physically binds VDR and is recruited onto VDR target gene promoters, resulting in their deregulation. Accordingly, vitamin D treatment, which leads to cell death in cells that express wild-type p53, induces survival in cells expressing mutant p53 (REF 93). Because vitamin D analogues are being tested as cancer therapeutic agents, this observation underlies the importance of identifying p53 mutations in tumours, which may reverse the therapeutic effect of vitamin D.

Finally, recent data implicate mutant p53 in activating genes involved in inflammation such as those encoding cytokines, chemokines and extracellular matrix modulators. Interestingly, whereas conformational mutants only inhibit the ability of wild-type p53 to repress the expression of these genes, DNA-contact mutants display a gain of function, whereby they induce the transcription of inflammatory genes through the activation of the nuclear factor-κB (NF-κB) pathway (V.R. unpublished data).

Similarly, mutant p53 was shown to enhance the NF-κB response to tumour necrosis factor α (TNFα) in cancer cells[94], and to transcriptionally activate *NFKB2* (REF. 95). These data combined suggest that *TP53* mutations, by antagonizing the ability of wild-type p53 to inhibit the expression of inflammatory genes and by activating the pro-inflammatory NF-κB pathway, may promote tumorigenesis in the context of chronic inflammation.

As mentioned earlier, several gain-of-function properties of p53 mutants are mediated not through DNA binding, but rather through modulation of non-transcriptional processes. A recent study revealed that the hotspot DNA-contact mutants $p53^{R248W}$ and $p53^{R273H}$ can bind MRE11, an upstream component of the ataxia-telangiectasia mutated (ATM)-dependent DNA-damage response pathway and, consequently, inhibit the cellular response to DNA double-stranded breaks[52]. The apparent phenotypes in mutant *Trp53* knock-in mice are augmented genetic instability, increased levels of interchromosomal rearrangements in pre-malignant thymocytes and development of lymphomas, which were not observed in *Trp53*-null mice[52]. The clinical implications of this finding are perhaps the enhanced resistance of some tumours harbouring mutant p53 to cancer therapies that induce double-stranded breaks[27] and the observed association between mutant p53 and chromosomal instability in human cancers[96,97].

Taken together, it seems that modulation of gene transcription and interference with pivotal signalling pathways are important mechanisms by which p53 mutants exert their oncogenic functions. As discussed in the following section, the interaction of mutant p53 with DNA, with DNA-binding proteins and with the DNA-damage response network may account for the emerging transcriptional signatures associated with *TP53* mutations in human malignancies.

Gene signatures associated with *TP53* mutations

Gene-expression signatures can be used to develop genomic tests that may provide better predictions of clinical outcome than the traditional clinical and pathological standards[98]. Whole-genome expression profiles of tumours provide more information than histopathological examination and other classical biomarkers, and enables the sub-typing of tumours into distinct classes with different prognostic characteristics, and, importantly, with varying responses to therapeutic drugs. Therefore, expression signatures afford opportunities to match therapies to individual patients[99].

When analyzing genome-wide expression profiles across different *in vitro* studies, it is hard to find common signatures associated with *TP53* mutations, probably owing to variations in the type of mutant analyzed, the cellular system and other technical variables. The expression profiles of human tumours are even more heterogeneous owing to variability in the patients' background, type of *TP53* mutation, *TP53* LOH and the proportion of contaminating stroma. Accordingly, widely recognized mutant p53 target genes (such as *MDR1* (REF. 28) and *MYC*[100]) are rarely found in these signatures. However, some patterns begin to emerge, even when the effects of different p53 mutants are collectively evaluated. For example, an expression signature consisting of 95 genes that were universally modulated by four different hotspot mutants in prostate cancer cells was recently discovered[101]. Similarly, three hotspot mutants ectopically expressed in *TP53*-null lung cancer cells induce a common gain-of-function transcriptional signature comprising more than 100 genes[95,102]. These results indicate common transcriptional activities for different p53 mutants, and provide a basis for the association of *TP53* mutations with transcriptional signatures in human tumours. Accordingly, expression signatures associated with *TP53* mutations were recently identified using large sets of breast cancer samples. Specifically, strong association was found between *TP53* mutations and expression signatures that were previously demonstrated to predict patient survival[103–105]. Tumours with *TP53* mutations were mostly classified into the basal-like or ERBB2-like subgroups. Tumours from the basal-like subgroup display an expression profile characteristic of breast basal epithelium, including high expression of keratin 5 and laminin, and are usually oestrogen receptor- and progesterone receptor-negative. High expression of genes associated with oncogenic *ERBB2* amplification characterizes tumours from the ERBB2-like category. Importantly, the basal-like and ERBB2-like subgroups are associated with

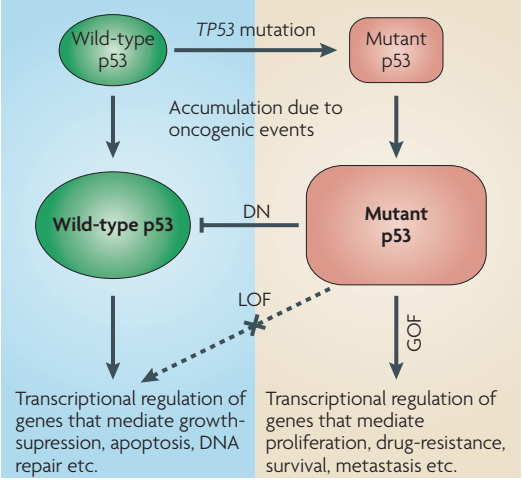
Box 2 | **Functional impact of *TP53* mutations**

The phenotypic effects of *TP53* mutations can be classified into three non-mutually exclusive groups[7,35]:

First, most mutations observed in human tumours abrogate or attenuate the binding of p53 to its consensus DNA sequence and, consequently, impede the transcriptional activation of p53 target genes[17]. In genetics, these mutations can be defined as hypomorphic or amorphic, for partial or complete loss of function, respectively. Loss of function (LOF) is frequent among missense mutants, but is particularly relevant to truncating, splicing and nonsense mutations, as well as to gene deletions.

Second, most missense mutations, but usually not the other types of mutations, also produce a full-length mutant p53 capable of inhibiting, to varying degrees, the function of the wild-type protein encoded by the second allele. This (antimorphic) dominant-negative (DN) effect is achieved by oligomerization of the mutant and wild-type proteins, forming a heterotetramer defective in sequence-specific DNA binding[18–20].

Finally, several mutations were shown to confer mutant p53 with new functions that are independent of wild-type p53. These (neomorphic) gain-of-function (GOF) properties can be experimentally demonstrated in the absence of a functional wild-type p53. The first such experimental settings used overexpression of mutant p53 in *TP53*-null cells[21–23]. More advanced and physiological systems include knockdown of endogenous mutant p53 in cell lines that do not express wild-type p53 (REFS 56,57) or comparison of mutant *Trp53* knock-in mice with *Trp53*-null mice[50–52]. Most gain-of-function properties are believed to stem from binding of mutant p53 to cellular proteins such as transcription factors and, consequently, alteration in their activity.



a significantly higher relative risk of mortality compared with the luminal group, which includes almost exclusively tumours expressing wild-type *TP53* (REF. 104). These findings suggest that p53 mutants can directly or indirectly induce specific gene signatures with significant prognostic value. Moreover, based on large datasets from breast cancer samples, expression signatures that distinguish between tumours that express wild-type p53 and mutant p53 were identified[105,106]. These signatures outperform sequence-based assessment of *TP53* mutations in predicting survival and therapeutic response. They can also reliably distinguish between tumours with wild-type *TP53* and mutant *TP53* in independent datasets derived from breast or liver cancer samples. Therefore, expression signatures associated with mutant p53 may be useful clinical tools for prognosis and for prediction of treatment response.

A common denominator of mutant-p53-associated expression signatures both in cell lines and in human cancers is the high percentage of proliferation-associated genes[95,102–110]. This finding probably reflects an increased proliferation rate of cells expressing mutant p53 compared with wild-type *TP53* and *TP53*-null cells. In support of this notion is the observation that in numerous cancer types, mutations in *TP53* are strongly associated with a high Ki-67 score[37] — a commonly used marker of proliferation. Importantly, high expression of the proliferation-associated gene cluster, known as the proliferation signature[111], is the most frequent observation made when comparing expression profiles of tumour samples with normal tissues or when comparing high-grade tumours with low-grade ones[111]. Additionally, in the vast majority of cases, high expression of the proliferation signature is associated with poor clinical outcome[111]. The proliferation signature is comprised mainly of proliferation-associated genes that participate in the core processes of the cell cycle, including DNA replication, spindle assembly and spindle checkpoint, chromosome segregation and other mitotic processes. Accordingly, many of these genes are regulated by cell-cycle-associated transcription factors such as the E2f family and NF-Y[111–113].

Several mechanisms could underlie the association between *TP53* mutations and high expression of the proliferation signature in tumours. First, wild-type p53 is probably inactivated in most mutant-p53 tumours, either by a dominant-negative mechanism or by LOH. As wild-type p53 can repress the expression of the proliferation signature by inhibiting the activity of E2F1 and NF-Y[113–115], its inactivation should alleviate this effect and, consequently, upregulate the proliferation signature. A recent study supported this mechanism by establishing isogenic colorectal cancer cell lines differing in their *TP53* status[110]. Microarray analysis revealed that upon γ-irradiation, a large set of proliferation-associated genes was downregulated in wild-type *TP53* cells; whereas in cells expressing mutant p53 and in *TP53*-null cells, this downregulation was abolished. Similarly, Troester *et al.* identified a gene signature enriched with proliferation-associated genes that is associated with both *TP53* mutations in primary breast cancers and wild-type p53 knockdown in breast cancer cell lines[105]. These data imply that the upregulation of the proliferation signature in mutant p53 tumours stems from wild-type-p53 loss of function. Alternatively, induction of cell-cycle genes may result from mutant p53 gain-of-function properties, including the ability of several mutants to enhance the activity of NF-Y[92] and E2F1 (REF. 116), and to transcriptionally activate various proliferation-associated genes (TABLE 1). Accordingly, in addition to their ability to interfere with wild-type-p53-mediated cell-cycle arrest, many p53 mutant proteins can also actively promote proliferation[7,56,108]. Finally, several hotspot p53 mutants were recently shown to repress wild-type p53 target genes in *TP53*-null cells, demonstrating a new gain-of-function activity[117]. As several of these repressed genes encode pivotal cell-cycle inhibitors (such as p21 and growth arrest and DNA-damage-inducible α (GADD45α)), their repression may increase proliferation rate and, in turn, induce the proliferation signature. In sum, the induction of the proliferation signature may account, at least partially, for the increased aggressiveness of tumours expressing mutant p53. However, to elucidate the mechanistic association between *TP53* mutations and the proliferation signature, large-scale studies combining gene profiling, IHC, patient data and molecular investigations of crucial players in the p53 pathway should be performed.

TP53 and prognosis, prediction and diagnosis

Inconsistent data regarding the association of *TP53* mutations with survival and drug response have led to a debate over the prognostic and predictive values of *TP53* status in cancer, and delayed the translation of the assessment of *TP53* status into the clinic[37]. A main reason for the inconsistency is that until recently most studies used IHC detection of p53 accumulation in tumour samples as a marker for *TP53* mutations. However, the assignment of *TP53* status to a tumour sample is often inaccurate when IHC is solely used, as many tumours with *TP53* mutations do not accumulate mutant p53; this is especially the case for frameshift, nonsense and splicing mutations[37,43]. Additionally, not all tumours with missense *TP53* mutations are IHC positive[50,104,118]. Moreover, few tumours accumulate a functional wild-type p53 due to persistent stress signals[37], and some tumours inactivate wild-type p53 function by mutation-independent mechanisms, such as *MDM2* amplification or deregulation of upstream or downstream components of the p53 pathway[11,33,37,43]. Finally, lack of standard protocols and cut-off thresholds for IHC detection of p53 increases the inter-study variability[33,37].

In the past decade, more and more studies have accumulated, in which *TP53* status was accurately assessed by gene sequencing or related methods. This trend generated more reliable associations between *TP53* mutational status and clinical properties, with the general trend being that *TP53* mutations are associated with poor overall and disease-free survival, as well as with poor drug response[37]. However, many studies still report lack of such associations, and few report opposite trends. As depicted in FIG. 3, it seems that in malignancies involving the breast, head and neck, bladder, colorectum and the haematopoietic system, 65%–90% of studies find

TP53 mutations associated with poor prognosis; whereas in brain, lung and ovarian cancers the picture is more complex, since in roughly half of the studies no significant association between *TP53* mutations and clinical outcome is found and in rare cases *TP53* mutations are associated with good prognosis. Similar data are available for additional malignancies in the IARC TP53 database[32].

Several clinical studies that compared IHC-based to sequencing-based assessment of *TP53* status found significant prognostic values only for the sequencing-based classification[119,120]. However, given that accumulation of mutant p53 can enhance both its dominant-negative and gain-of-function properties, combining IHC and gene-sequencing analysis may be a more reliable prognostic tool[121,122]. Indeed, recent studies revealed that mutant *TP53* tumours that are IHC positive have a worse prognosis than IHC-negative mutant p53 tumors[122,123]. In addition, some studies could demonstrate better prognostic values when their data were stratified not only according to the mere presence of *TP53* mutations, but also according to the predicted or experimentally measured effect of these mutations on p53 structure or function[124–126], the exon or functional domain in which the mutation resides[37,118,127–132] and even the specific mutated residue[37,129,131]. Similarly, a number of studies used multi-parameter analyses integrating, in addition to *TP53* mutation analysis, other data such as the status of additional tumour suppressors and oncogenes, global or local measurement of genomic aberrations, *TP53* and *MDM2* single nucleotide polymorphisms, and other clinicopathological factors[129,133–139]. These types of analyses can sometimes generate better and more translatable prognostic tools and, in addition, may shed light on the molecular mechanisms that underlie the oncogenic effect of *TP53* mutations.

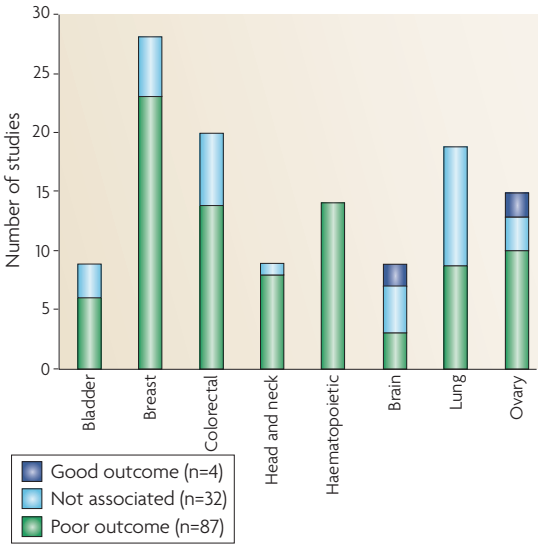


Figure 3 | **Association of *TP53* mutations and clinical outcome in selected cancer types.** The chart summarizes the main conclusions of studies that assess the association of *TP53* mutations and clinical outcome (overall survival, disease-free survival or drug response). Only studies that analyzed *TP53* mutations by gene sequencing or related methods and with cohorts > 50 patients were considered. Only cancer types for which > 8 studies were available are presented. See Supplementary information S1 (table) for detailed list of references. Data derived from the IARC TP53 Mutation Database version R13 (November 2008)[32].

Regarding the question raised earlier concerning which of the properties of mutant p53 is selected during tumorigenesis it seems that the clinical data do not clearly support one specific mechanism, perhaps implying that mutant p53 loss-of-function, dominant-negative and gain-of-function properties are all important for tumorigenesis in humans. For example, in a large-scale study that analyzed 1,794 patients with breast cancer, evidence for all three mechanisms were found[129]. First, specific hotspot mutations (such as R248W and mutations at codon 179) were associated with worse prognosis compared with other DBD mutations, suggesting the involvement of a gain-of-function mechanism. However, DBD missense mutations were associated with a slightly better prognosis than truncating mutations, implying the selection of loss of function and opposing the notion of gain of function. Finally, DBD missense mutations had worse prognosis than non-DBD missense mutations. Complementary yeast-based data demonstrated that DBD mutants are more likely to inhibit wild-type p53 than non-DBD mutants[140], which leads to the suggestion that selection of a dominant-negative trait is in play[36]. Additional large-scale and meta-analysis studies are necessary to provide sufficient statistical power to assess the prognostic value of individual *TP53* mutations in breast cancer, as well as in other malignancies.

In the past decade considerable progress has been made in the development of detection methods for *TP53* mutations and in their practical implementation (for comparison of *TP53* mutation screening and identification see REF 33). For instance, several oligonucleotide microarray-based methods were developed for rapid and accurate detection of *TP53* mutations[93,141–144]. Using these technologies, together with the advancement made in DNA-sequencing applications, large-scale mutational studies are becoming more feasible, and may facilitate bench-to-bedside transition. Additionally, many studies have demonstrated the possibility to obtain clinical data when analyzing *TP53* status using patients' blood, either by sequencing circulating free DNA from the plasma[37], or by detection of the p53 protein or p53-specific antibodies in the serum[145–148]. Moreover, *TP53* mutations have been detected in additional types of body fluids and excretions such as saliva (in oral cancer), urine (in bladder cancer), sputum (in lung cancer)[37] and others[149,150]. Therefore, in addition to the prognostic value of *TP53* mutations, these possibilities may help to translate the assessment of *TP53* status into diagnostic applications. This is particularly relevant for early diagnosis of malignancies in which *TP53* mutations occur relatively early during tumorigenesis, which is the case with many carcinogen-induced cancers, as well as for detection of tumour relapse[37]. Notably, whether mutations in *TP53* represent early or late events during tumorigenesis is still debatable, and probably depends primarily on the cancer type. Nevertheless, *TP53* aberrations can be detected in pre-malignant lesions found in, for example, breasts[151,152]

and oral cavities[153,154], demonstrating the role of mutant p53 in tumour initiation. An apparent controversy is the lack of mutant p53 gain-of-function effect on survival and its moderate influence on the overall tumour incidence in knock-in mice (when comparing *Trp53*$^{+/m}$ mice with *Trp53*$^{+/-}$ mice)[50–52], which may imply that *TP53* mutations are not a major driving force in tumour initiation, at least in most tissues. However, as these mice grow in an environment almost free of mutagens, they may not constitute a flawless model for human tumorigenesis. As discussed earlier, an acquired *TP53* mutation may not have a strong oncogenic effect without the conditions required for accumulation of mutant p53. In humans, who are exposed to a variety of environmental mutagens, mutant p53 is more likely to accumulate, and may better fulfil its oncogenic potential in initiating tumours. This is supported by the finding that germline *TP53* missense mutations in LFS patients are associated with an earlier age of tumour onset than loss-of-function *TP53* alterations (such as deletions)[155].

A large proportion of clinical studies that assessed *TP53* status by gene sequencing have focused only on the central part of the coding region (the DBD). Although almost all missense mutations are mapped to this area, a few missense mutations occur outside the DBD, and other types of mutations (such as nonsense, frameshift and splicing mutations) are more equally distributed, leading to misclassification of 10%–20% of cases[37,43]. Notably, the carboxy- and amino-termini may be important for the oncogenic functions of mutant p53, as they regulate its accumulation, transactivation activity[87], structure-specific DNA-binding[89], subcellular localization[70–72] and gain-of-function properties[88,156]. Moreover, with the appreciation of the importance of post-transcriptional gene regulation in tumorigenesis, and, specifically, the key roles of microRNAs in cancer development[157], previously ignored mutations in untranslated regions of *TP53* should be analyzed for their potential effect on the protein level of mutant p53.

Conclusions and some unanswered questions

Despite the immense knowledge on p53 involvement in tumorigenesis, its translation to clinical use has yet to be accomplished. Nevertheless, it seems that recently collected data may facilitate the implementation of *TP53* mutational analysis into clinical practice as the significance of this analysis for early tumour diagnosis, relapse detection, prognosis and prediction of drug response is gradually realized. Furthermore, in recent years, several p53-based therapeutic approaches have been developed, including compounds that reactivate specific p53 mutants, non-genotoxic p53-activating drugs, p53 gene-delivery approaches and more[158]. New high-resolution structural studies on the deleterious effects of cancer mutations and their reversal by suppressor mutations[159,160] may also facilitate the design of newer and more effective p53-based therapeutic modalities.

Tumour genomic expression signatures represent a feasible and reliable platform for the development of accurate prognostic and predictive tools for personalized medicine. However, efforts to correlate expression signatures with *TP53* status and clinical outcome were performed almost exclusively for breast cancer. Similar studies of additional cancer types will improve our understanding of p53 function in these malignancies, and may offer reliable and practical tools for predicting clinical outcome. Moreover, microRNA profiles associated with mutant p53 were demonstrated only in breast cancer[114], and need to be explored further. Similarly, association of *TP53* status with additional data types such as whole-genome DNA methylation, histone modification and genomic aberrations may promote our understanding of how *TP53* mutations affect cancer.

Several key issues are still not fully explored in the field of mutant p53. First, the prognostic, predictive and diagnostic value of *TP53* mutations is still unknown for the majority of the less-common cancer types. Similarly, the effect of specific *TP53* mutations in each cancer type is far from resolved. In this context, numerous *in vitro* and *in vivo* studies demonstrated that different p53 mutants are distinct in their abilities to bind other proteins, regulate gene expression, protect cells from chemotherapeutic drugs and, generally, exert various degrees of gain of function. However, there are currently no published experimental systems that address this issue in a systematic manner. Such studies, combined with large-scale clinical analyses may provide the data needed to enrich our understanding of individual mutants in the context of gain of function. Second, recent findings indicate that *TP53* mutations occur not only in cancer cells, but also in their supporting stroma[161], and may have significant prognostic value[162]. However, the credibility of these findings is controversial[163,164]. Another long-standing enigma is the molecular mechanisms underlying the tumour-specific accumulation of mutant p53, although some clues begin to emerge with the unveiling of the roles of MDM2 (REFS 47,48,53), INK4A[53], promyelocytic leukaemia (PML)[165], heat-shock proteins, ubiquitin ligases[49,65] and other proteins in regulating mutant p53 level and activity in tumour cells.

Finally, considering recent *in vitro*, *in vivo* and clinical data, it seems that the gain-of-function properties of specific p53 mutants are important for their oncogenic properties, and that their accumulation in normal tissues and in tumours further augments their tumorigenic potential. Therefore, tumour *TP53* status should not be considered a binary variable, but rather as multi-parameter data, consisting of the type of mutation, the level and subcellular localization of the mutant protein, as well as the status of *TP53* LOH, codon 72 single nucleotide polymorphism[166,167], and other pathway components. Similarly, treatment approaches that result in p53 accumulation should be carefully considered when dealing with tumours that express mutant p53 as they may represent a double-edged sword. In fact, preliminary data suggest that chemotherapy may decrease the survival of patients with lung cancer with mutated *TP53* (REF. 123). Moreover, based on mouse models[56,57,86], inhibition of gain-of-function mutants in tumours may represent an attractive therapeutic strategy.

1. Lane, D. P. & Crawford, L. V. T antigen is bound to a host protein in SV40-transformed cells. *Nature* **278**, 261–263 (1979).
2. Linzer, D. I. & Levine, A. J. Characterization of a 54K dalton cellular SV40 tumor antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells. *Cell* **17**, 43–52 (1979).
3. Kress, M., May, E., Cassingena, R. & May, P. Simian virus 40-transformed cells express new species of proteins precipitable by anti-simian virus 40 tumor serum. *J. Virol.* **31**, 472–483 (1979).
4. Rotter, V., Witte, O. N., Coffman, R. & Baltimore, D. Abelson murine leukemia virus-induced tumors elicit antibodies against a host cell protein, P50. *J. Virol.* **36**, 547–555 (1980).
5. DeLeo, A. B. *et al.* Detection of a transformation-related antigen in chemically induced sarcomas and other transformed cells of the mouse. *Proc. Natl Acad. Sci. USA* **76**, 2420–2424 (1979).
6. Rotter, V., Boss, M. A. & Baltimore, D. Increased concentration of an apparently identical cellular protein in cells transformed by either Abelson murine leukemia virus or other transforming agents. *J. Virol.* **38**, 336–346 (1981).
7. Weisz, L., Oren, M. & Rotter, V. Transcription regulation by mutant p53. *Oncogene* **26**, 2202–2211 (2007).
8. Bullock, A. N. & Fersht, A. R. Rescuing the function of mutant p53. *Nature Rev. Cancer* **1**, 68–76 (2001).
9. Oren, M. Decision making by p53: life, death and cancer. *Cell Death Differ.* **10**, 431–442 (2003).
10. Hollstein, M., Sidransky, D., Vogelstein, B. & Harris, C. C. p53 mutations in human cancers. *Science* **253**, 49–53 (1991).
The first comprehensive analysis of *TP53* mutations in various cancers, including identification of hotspot residues and aetiological factors that shape the distribution of *TP53* mutations.
11. Vogelstein, B., Lane, D. & Levine, A. J. Surfing the p53 network. *Nature* **408**, 307–310 (2000).
12. Peller, S. & Rotter, V. TP53 in hematological cancer: low incidence of mutations with significant clinical relevance. *Hum. Mutat.* **21**, 277–284 (2003).
13. Schuijer, M. & Berns, E. M. TP53 and ovarian cancer. *Hum. Mutat.* **21**, 285–291 (2003).
14. Iacopetta, B. TP53 mutation in colorectal cancer. *Hum. Mutat.* **21**, 271–276 (2003).
15. Blons, H. & Laurent-Puig, P. TP53 and head and neck neoplasms. *Hum. Mutat.* **21**, 252–257 (2003).
16. Malkin, D. *et al.* Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science* **250**, 1233–1238 (1990).
This study reveals the association between germline mutations in *TP53* and LFS.
17. Kato, S. *et al.* Understanding the function-structure and function-mutation relationships of p53 tumor suppressor protein by high-resolution missense mutation analysis. *Proc. Natl Acad. Sci. USA* **100**, 8424–8429 (2003).
A yeast-based assay for the transactivation ability of 2,314 *TP53* mutants (representing all possible non-synonymous point mutations) towards several p53 target genes.
18. Milner, J., Medcalf, E. A. & Cook, A. C. Tumor suppressor p53: analysis of wild-type and mutant p53 complexes. *Mol. Cell Biol.* **11**, 12–19 (1991).
19. Milner, J. & Medcalf, E. A. Cotranslation of activated mutant p53 with wild type drives the wild-type p53 protein into the mutant conformation. *Cell* **65**, 765–774 (1991).
20. Sigal, A. & Rotter, V. Oncogenic mutations of the p53 tumor suppressor: the demons of the guardian of the genome. *Cancer Res.* **60**, 6788–6793 (2000).
21. Wolf, D., Harris, N. & Rotter, V. Reconstitution of p53 expression in a nonproducer Ab-MuLV-transformed cell line by transfection of a functional p53 gene. *Cell* **38**, 119–126 (1984).
The earliest direct demonstration of mutant p53 gain of function: when injected into mice, virally transformed L12 cells (that do not produce p53) normally cause local tumours that later regress. Following transfection with mutant p53, these cells caused lethal tumours in mice.
22. Shaulsky, G., Goldfinger, N. & Rotter, V. Alterations in tumor development *in vivo* mediated by expression of wild type or mutant p53 proteins. *Cancer Res.* **51**, 5232–5237 (1991).
23. Dittmer, D. *et al.* Gain of function mutations in p53. *Nature Genet.* **4**, 42–46 (1993).
24. Bergh, J., Norberg, T., Sjogren, S., Lindgren, A. & Holmberg, L. Complete sequencing of the p53 gene provides prognostic information in breast cancer patients, particularly in relation to adjuvant systemic therapy and radiotherapy. *Nature Med.* **1**, 1029–1034 (1995).
25. Aas, T. *et al.* Specific P53 mutations are associated with *de novo* resistance to doxorubicin in breast cancer patients. *Nature Med.* **2**, 811–814 (1996).
26. Blandino, G., Levine, A. J. & Oren, M. Mutant p53 gain of function: differential effects of different p53 mutants on resistance of cultured cells to chemotherapy. *Oncogene* **18**, 477–485 (1999).
27. Lu, C. & El-Deiry, W. S. Targeting p53 for enhanced radio- and chemo-sensitivity. *Apoptosis* **14**, 597–606 (2009).
28. Chin, K. V., Ueda, K., Pastan, I. & Gottesman, M. M. Modulation of activity of the promoter of the human MDR1 gene by Ras and p53. *Science* **255**, 459–462 (1992).
29. Bush, J. A. & Li, G. Cancer chemoresistance: the relationship between p53 and multidrug transporters. *Int. J. Cancer* **98**, 323–330 (2002).
30. Li, R. *et al.* Mutant p53 protein expression interferes with p53-independent apoptotic pathways. *Oncogene* **16**, 3269–3277 (1998).
References 26 and 30 demonstrate in cancer cell lines gain of function for p53 mutants in apoptosis inhibition.
31. Kim, E. & Deppert, W. The versatile interactions of p53 with DNA: when flexibility serves specificity. *Cell Death Differ.* **13**, 885–889 (2006).
32. Petitjean, A. *et al.* Impact of mutant p53 functional properties on TP53 mutation patterns and tumor phenotype: lessons from recent developments in the IARC TP53 database. *Hum. Mutat.* **28**, 622–629 (2007).
An overview of the data and lessons from the IARC TP53 Mutation Database.
33. Soussi, T. in *25 Years of p53 Research* (eds P. Hainaut & K. G. Wiman) 255–292 (Springer, 2005).
34. Sjoblom, T. *et al.* The consensus coding sequences of human breast and colorectal cancers. *Science* **314**, 268–274 (2006).
35. Soussi, T. p53 alterations in human cancer: more questions than answers. *Oncogene* **26**, 2145–2156 (2007).
36. Petitjean, A., Achatz, M. I., Borresen-Dale, A. L., Hainaut, P. & Olivier, M. TP53 mutations in human cancers: functional selection and impact on cancer prognosis and outcomes. *Oncogene* **26**, 2157–2165 (2007).
37. Olivier, M., Hainaut, P. & Borresen, A. L. in *25 Years of p53 Research* (eds P. Hainaut & K. G. Wiman) 321–338 (Springer, 2005).
38. Shi, H., Le Calvez, F., Olivier, M. & Hainaut, P. in *25 Years of p53 Research* (eds P. Hainaut & K. G. Wiman) 293–319 (Springer, 2005).
39. Aguilar, F., Hussain, S. P. & Cerutti, P. Aflatoxin B1 induces the transversion of G--> T in codon 249 of the p53 tumor suppressor gene in human hepatocytes. *Proc. Natl Acad. Sci. USA* **90**, 8586–8590 (1993).
40. Rotter, V. p53, a transformation-related cellular-encoded protein, can be used as a biochemical marker for the detection of primary mouse tumor cells. *Proc. Natl Acad. Sci. USA* **80**, 2613–2617 (1983).
41. Iggo, R., Gatter, K., Bartek, J., Lane, D. & Harris, A. L. Increased expression of mutant forms of p53 oncogene in primary lung cancer. *Lancet* **335**, 675–679 (1990).
42. Bartek, J. *et al.* Aberrant expression of the p53 oncoprotein is a common feature of a wide spectrum of human malignancies. *Oncogene* **6**, 1699–1703 (1991).
A large scale IHC analysis of human tumour samples demonstrating tumour-specific accumulation of mutant p53.
43. Soussi, T. & Beroud, C. Assessing TP53 status in human tumours to evaluate clinical outcome. *Nature Rev. Cancer* **1**, 233–240 (2001).
44. Reich, N. C. & Levine, A. J. Growth regulation of a cellular tumour antigen, p53, in nontransformed cells. *Nature* **308**, 199–201 (1984).
45. Barak, Y., Juven, T., Haffner, R. & Oren, M. Mdm2 expression is induced by wild type p53 activity. *EMBO J.* **12**, 461–468 (1993).
46. Haupt, Y., Maya, R., Kazaz, A. & Oren, M. Mdm2 promotes the rapid degradation of p53. *Nature* **387**, 296–299 (1997).
This study describes the negative-feedback loop between p53 and MDM2.
47. Midgley, C. A. & Lane, D. P. p53 protein stability in tumour cells is not determined by mutation but is dependent on Mdm2 binding. *Oncogene* **15**, 1179–1189 (1997).
48. Lukashchuk, N. & Vousden, K. H. Ubiquitination and degradation of mutant p53. *Mol. Cell Biol.* **27**, 8284–8295 (2007).
49. Muller, P., Hrstka, R., Coomber, D., Lane, D. P. & Vojtesek, B. Chaperone-dependent stabilization and degradation of p53 mutants. *Oncogene* **27**, 3371–3383 (2008).
50. Lang, G. A. *et al.* Gain of function of a p53 hot spot mutation in a mouse model of Li-Fraumeni syndrome. *Cell* **119**, 861–872 (2004).
51. Olive, K. P. *et al.* Mutant p53 gain of function in two mouse models of Li-Fraumeni syndrome. *Cell* **119**, 847–860 (2004).
52. Song, H., Hollstein, M. & Xu, Y. p53 gain-of-function cancer mutants induce genetic instability by inactivating ATM. *Nature Cell Biol.* **9**, 573–580 (2007).
53. Terzian, T. *et al.* The inherent instability of mutant p53 is alleviated by Mdm2 or p16INK4a loss. *Genes Dev.* **22**, 1337–1344 (2008).
References 50–53 describe knock-in mouse models for different hotspot p53 mutants and their associated gain-of-function properties *in vivo*.
54. Jones, S. N., Roe, A. E., Donehower, L. A. & Bradley, A. Rescue of embryonic lethality in Mdm2-deficient mice by absence of p53. *Nature* **378**, 206–208 (1995).
55. Montes de Oca Luna, R., Wagner, D. S. & Lozano, G. Rescue of early embryonic lethality in mdm2-deficient mice by deletion of p53. *Nature* **378**, 203–206 (1995).
56. Bossi, G. *et al.* Mutant p53 gain of function: reduction of tumor malignancy of human cancer cell lines through abrogation of mutant p53 expression. *Oncogene* **25**, 304–309 (2006).
57. Bossi, G. *et al.* Conditional RNA interference *in vivo* to study mutant p53 oncogenic gain of function on tumor malignancy. *Cell Cycle* **7**, 1870–1879 (2008).
58. Trotman, L. C. & Pandolfi, P. P. PTEN and p53: who will get the upper hand? *Cancer Cell* **3**, 97–99 (2003).
59. Li, Y. *et al.* PTEN has tumor-promoting properties in the setting of gain-of-function p53 mutations. *Cancer Res.* **68**, 1723–1731 (2008).
60. Zhang, J., Pickering, C. R., Holst, C. R., Gauthier, M. L. & Tlsty, T. D. p16INK4a modulates p53 in primary human mammary epithelial cells. *Cancer Res.* **66**, 10325–10331 (2006).
61. Hinds, P. W., Finlay, C. A., Frey, A. B. & Levine, A. J. Immunological evidence for the association of p53 with a heat shock protein, hsc70, in p53-plus-ras-transformed cell lines. *Mol. Cell Biol.* **7**, 2863–2869 (1987).
62. Blagosklonny, M. V., Toretsky, J., Bohen, S. & Neckers, L. Mutant conformation of p53 translated *in vitro* or *in vivo* requires functional HSP90. *Proc. Natl Acad. Sci. USA* **93**, 8379–8383 (1996).
63. Sepehrnia, B., Paz, I. B., Dasgupta, G. & Momand, J. Heat shock protein 84 forms a complex with mutant p53 protein predominantly within a cytoplasmic compartment of the cell. *J. Biol. Chem.* **271**, 15084–15090 (1996).
64. Whitesell, L., Sutphin, P. D., Pulcini, E. J., Martinez, J. D. & Cook, P. H. The physical association of multiple molecular chaperone proteins with mutant p53 is altered by geldanamycin, an hsp90-binding agent. *Mol. Cell Biol.* **18**, 1517–1524 (1998).
65. Lin, K., Rockliffe, N., Johnson, G. G., Sherrington, P. D. & Pettitt, A. R. Hsp90 inhibition has opposing effects on wild-type and mutant p53 and induces p21 expression and cytotoxicity irrespective of p53/ATM status in chronic lymphocytic leukaemia cells. *Oncogene* **27**, 2445–2455 (2008).
66. Esser, C., Scheffner, M. & Hohfeld, J. The chaperone-associated ubiquitin ligase CHIP is able to target p53 for proteasomal degradation. *J. Biol. Chem.* **280**, 27443–27448 (2005).
67. Kamal, A. *et al.* A high-affinity conformation of Hsp90 confers tumour selectivity on Hsp90 inhibitors. *Nature* **425**, 407–410 (2003).
68. Solit, D. B. & Rosen, N. Hsp90: a novel target for cancer therapy. *Curr. Top. Med. Chem.* **6**, 1205–1214 (2006).
69. Rotter, V., Abutbul, H. & Ben-Ze'ev, A. P53 transformation-related protein accumulates in the nucleus of transformed fibroblasts in association with the chromatin and is found in the cytoplasm of non-transformed fibroblasts. *EMBO J.* **2**, 1041–1047 (1983).

70. Shaulsky, G., Goldfinger, N., Ben-Ze'ev, A. & Rotter, V. Nuclear accumulation of p53 protein is mediated by several nuclear localization signals and plays a role in tumorigenesis. *Mol. Cell Biol.* **10**, 6565–6577 (1990).
71. Nie, L., Sasaki, M. & Maki, C. G. Regulation of p53 nuclear export through sequential changes in conformation and ubiquitination. *J. Biol. Chem.* **282**, 14616–14625 (2007).
72. Morselli, E. *et al.* Mutant p53 protein localized in the cytoplasm inhibits autophagy. *Cell Cycle* **7**, 3056–3061 (2008).
73. Maiuri, M. C. *et al.* Control of autophagy by oncogenes and tumor suppressor genes. *Cell Death Differ.* **16**, 87–93 (2009).
74. Eliyahu, D., Raz, A., Gruss, P., Givol, D. & Oren, M. Participation of p53 cellular tumour antigen in transformation of normal embryonic cells. *Nature* **312**, 646–649 (1984).
75. Parada, L. F., Land, H., Weinberg, R. A., Wolf, D. & Rotter, V. Cooperation between gene encoding p53 tumour antigen and ras in cellular transformation. *Nature* **312**, 649–651 (1984).
76. Kim, E. & Deppert, W. Transcriptional activities of mutant p53: when mutations are more than a loss. *J. Cell Biochem.* **93**, 878–886 (2004).
77. Strano, S. *et al.* Mutant p53: an oncogenic transcription factor. *Oncogene* **26**, 2212–2219 (2007).
78. Di Como, C. J., Gaiddon, C. & Prives, C. p73 function is inhibited by tumor-derived p53 mutants in mammalian cells. *Mol. Cell Biol.* **19**, 1438–1449 (1999).
79. Gaiddon, C., Lokshin, M., Ahn, J., Zhang, T. & Prives, C. A subset of tumor-derived mutant forms of p53 down-regulate p63 and p73 through a direct interaction with the p53 core domain. *Mol. Cell Biol.* **21**, 1874–1887 (2001).
80. Deyoung, M. P. & Ellisen, L. W. p63 and p73 in human cancer: defining the network. *Oncogene* **26**, 5169–5183 (2007).
References 79 and 80 demonstrate mutant p53 gain of function in inactivating p63 and p73.
81. Flores, E. R. *et al.* Tumor predisposition in mice mutant for p63 and p73: evidence for broader tumor suppressor functions for the p53 family. *Cancer Cell* **7**, 363–373 (2005).
82. Irwin, M. S. Family feud in chemosensitvity: p73 and mutant p53. *Cell Cycle* **3**, 319–323 (2004).
83. Li, Y. & Prives, C. Are interactions with p63 and p73 involved in mutant p53 gain of oncogenic function? *Oncogene* **26**, 2220–2225 (2007).
84. Bergamaschi, D. *et al.* p53 polymorphism influences response in cancer chemotherapy via modulation of p73-dependent apoptosis. *Cancer Cell* **3**, 387–402 (2003).
85. Di Agostino, S. *et al.* The disruption of the protein complex mutantp53/p73 increases selectively the response of tumor cells to anticancer drugs. *Cell Cycle* **7**, 3440–3447 (2008).
86. Kravchenko, J. E. *et al.* Small-molecule RETRA suppresses mutant p53-bearing cancer cells through a p73-dependent salvage pathway. *Proc. Natl Acad. Sci. USA* **105**, 6302–6307 (2008).
87. Lin, J., Teresky, A. K. & Levine, A. J. Two critical hydrophobic amino acids in the N-terminal domain of the p53 protein are required for the gain of function phenotypes of human p53 mutants. *Oncogene* **10**, 2387–2390 (1995).
88. Matas, D. *et al.* Integrity of the N-terminal transcription domain of p53 is required for mutant p53 interference with drug-induced apoptosis. *EMBO J.* **20**, 4163–4172 (2001).
89. Gohler, T. *et al.* Mutant p53 proteins bind DNA in a DNA structure-selective mode. *Nucleic Acids Res.* **33**, 1087–1100 (2005).
90. Koga, H. & Deppert, W. Identification of genomic DNA sequences bound by mutant p53 protein (Gly245-->Ser.) *in vivo*. *Oncogene* **19**, 4178–4183 (2000).
91. Brazdova, M. *et al.* Modulation of gene expression in U251 glioblastoma cells by binding of mutant p53 R273H to intronic and intergenic sequences. *Nucleic Acids Res.*, **37**, 1486–1500 (2009).
92. Di Agostino, S. *et al.* Gain of function of mutant p53: the mutant p53/NF-Y protein complex reveals an aberrant transcriptional mechanism of cell cycle regulation. *Cancer Cell* **10**, 191–202 (2006).
This study demonstrates mutant p53 gain of function in the NF-Y pathway.
93. Olivier, M. *et al.* Recent advances in p53 research: an interdisciplinary perspective. *Cancer Gene Ther.* **16**, 1–12 (2009).
94. Weisz, L. *et al.* Mutant p53 enhances nuclear factor kappaB activation by tumor necrosis factor alpha in cancer cells. *Cancer Res.* **67**, 2396–2401 (2007).
95. Scian, M. J. *et al.* Tumor-derived p53 mutants induce NF-κB2 gene expression. *Mol. Cell Biol.* **25**, 10097–10110 (2005).
96. Jain, A. N. *et al.* Quantitative analysis of chromosomal CGH in human breast tumors associates copy number abnormalities with p53 status and patient survival. *Proc. Natl Acad. Sci. USA* **98**, 7952–7957 (2001).
97. Kleivi, K. *et al.* TP53 mutations are associated with a particular pattern of genomic imbalances in breast carcinomas. *J. Pathol.* **207**, 14–19 (2005).
98. Sotiriou, C. & Pusztai, L. Gene-expression signatures in breast cancer. *N.Engl. J. Med.* **360**, 790–800 (2009).
99. Potti, A. & Nevins, J. R. Utilization of genomic signatures to direct use of primary chemotherapy. *Curr. Opin. Genet. Dev.* **18**, 62–67 (2008).
100. Frazier, M. W. *et al.* Activation of c-myc gene expression by tumor-derived p53 mutants requires a discrete C-terminal domain. *Mol. Cell Biol.* **18**, 3735–3743 (1998).
101. Tepper, C. G. *et al.* Profiling of gene expression changes caused by p53 gain-of-function mutant alleles in prostate cancer cells. *Prostate* **65**, 375–389 (2005).
102. Scian, M. J. *et al.* Modulation of gene expression by tumor-derived p53 mutants. *Cancer Res.* **64**, 7447–7454 (2004).
103. Sorlie, T. *et al.* Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc. Natl Acad. Sci. USA* **98**, 10869–10874 (2001).
104. Langerod, A. *et al.* TP53 mutation status and gene expression profiles are powerful prognostic markers of breast cancer. *Breast Cancer Res.* **9**, R30 (2007).
References 103 and 104 provide comprehensive analyses of breast cancer expression profiles, their association with *TP53* mutations, and their prognostic value.
105. Troester, M. A. *et al.* Gene expression patterns associated with p53 status in breast cancer. *BMC Cancer* **6**, 276 (2006).
106. Miller, L. D. *et al.* An expression signature for p53 status in human breast cancer predicts mutation status, transcriptional effects, and patient survival. *Proc. Natl Acad. Sci. U S. A* **102**, 13550–13555 (2005).
107. Brosh, R. & Rotter, V. Transcriptional control of the proliferation cluster by the tumor suppressor p53. *Molec. Biosyst.* (in the press).
108. Scian, M. J. *et al.* Tumor-derived p53 mutants induce oncogenesis by transactivating growth-promoting genes. *Oncogene* **23**, 4430–4443 (2004).
109. Sanchez-Carbayo, M. *et al.* Genomic and proteomic profiles reveal the association of gelsolin to TP53 status and bladder cancer progression. *Am. J. Pathol.* **171**, 1650–1658 (2007).
110. Sur, S. *et al.* A panel of isogenic human cancer cells suggests a therapeutic approach for cancers with inactivated p53. *Proc. Natl Acad. Sci. USA* **106**, 3964–3969 (2009).
111. Whitfield, M. L., George, L. K., Grant, G. D. & Perou, C. M. Common markers of proliferation. *Nature Rev. Cancer* **6**, 99–106 (2006).
112. Salvatore, G. *et al.* A cell proliferation and chromosomal instability signature in anaplastic thyroid carcinoma. *Cancer Res.* **67**, 10148–10158 (2007).
113. Tabach, Y. *et al.* The promoters of human cell cycle genes integrate signals from two tumor suppressive pathways during cellular transformation. *Mol. Syst. Biol.* **1**, 2005.0022 (2005).
114. Brosh, R. *et al.* p53-repressed miRNAs are involved with E2F in a feed-forward loop promoting proliferation. *Mol. Syst. Biol.* **4**, 229 (2008).
115. Imbriano, C. *et al.* Direct p53 transcriptional repression: *in vivo* analysis of CCAAT-containing G2/M promoters. *Mol. Cell Biol.* **25**, 3737–3751 (2005).
116. Donzelli, S. *et al.* Oncogenomic approaches in exploring gain of function of mutant p53. *Curr. Genomics* **9**, 200–207 (2008).
117. Vikhanskaya, F., Lee, M. K., Mazzoletti, M., Broggini, M. & Sabapathy, K. Cancer-derived p53 mutants suppress p53-target gene expression--potential mechanism for gain of function of mutant p53. *Nucleic Acids Res.* **35**, 2093–2104 (2007).
118. Alsner, J. *et al.* A comparison between p53 accumulation determined by immunohistochemistry and TP53 mutations as prognostic variables in tumours from breast cancer patients. *Acta Oncol.* **47**, 600–607 (2008).
119. Mollevi, D. G. *et al.* Mutations in TP53 are a prognostic factor in colorectal hepatic metastases undergoing surgical resection. *Carcinogenesis* **28**, 1241–1246 (2007).
120. Ahmed, I. A. *et al.* The predictive value of p53 and p33(ING1b) in patients with Dukes'C colorectal cancer. *Colorectal Dis.* **10**, 344–351 (2008).
121. Ryu, M. H. *et al.* Prognostic significance of p53 gene mutations and protein overexpression in localized gastrointestinal stromal tumours. *Histopathology* **51**, 379–389 (2007).
122. Bartel, F. *et al.* Both germ line and somatic genetics of the p53 pathway affect ovarian cancer incidence and survival. *Clin. Cancer Res.* **14**, 89–96 (2008).
123. Ma, X. *et al.* TP53 and KRAS mutations as markers of outcome of adjuvant cisplatin-based chemotherapy in completely resected non-small-cell lung cancer (NSCL): the International Adjuvant Lung Cancer Trial (IALT) biological program. *Ann. Oncol. Abstr.* **19**, viii61–62, (2008).
124. Poeta, M. L. *et al.* TP53 mutations and survival in squamous-cell carcinoma of the head and neck. *N. Engl. J. Med.* **357**, 2552–2561 (2007).
125. Monti, P. *et al.* Transcriptional functionality of germ line p53 mutants influences cancer phenotype. *Clin. Cancer Res.* **13**, 3789–3795 (2007).
126. Sakuragi, N. *et al.* Functional analysis of p53 gene and the prognostic impact of dominant-negative p53 mutation in endometrial cancer. *Int. J. Cancer* **116**, 514–519 (2005).
127. Salinas-Sanchez, A. S. *et al.* Implications of p53 gene mutations on patient survival in transitional cell carcinoma of the bladder: a long-term study. *Urol. Oncol.* **26**, 620–626 (2008).
128. Ecke, T. H., Sachs, M. D., Lenk, S. V., Loening, S. A. & Schlechte, H. H. TP53 gene mutations as an independent marker for urinary bladder cancer progression. *Int. J. Mol. Med.* **21**, 655–661 (2008).
129. Olivier, M. *et al.* The clinical value of somatic TP53 gene mutations in **1**, 794 patients with breast cancer. *Clin. Cancer Res.* **12**, 1157–1167 (2006).
A large-scale analysis of the prognostic association of *TP53* mutations in breast cancer, including comparison of the clinical effects of different mutation categories.
130. Young, K. H. *et al.* Mutations in the DNA-binding codons of TP53, which are associated with decreased expression of TRAILreceptor-2, predict for poor survival in diffuse large B-cell lymphoma. *Blood* **110**, 4396–4405 (2007).
131. Young, K. H. *et al.* Structural profiles of TP53 gene mutations predict clinical outcome in diffuse large B-cell lymphoma: an international collaborative study. *Blood* **112**, 3088–3098 (2008).
132. Wang, Y., Kristensen, G. B., Borresen-Dale, A. L. & Helland, A. TP53 mutations and codon 72 genotype--impact on survival among ovarian cancer patients. *Ann. Oncol.* **18**, 964–966 (2007).
133. Batchelor, T. T. *et al.* Age-dependent prognostic effects of genetic alterations in glioblastoma. *Clin. Cancer Res.* **10**, 228–233 (2004).
134. Chang, S. C. *et al.* Relationship between genetic alterations and prognosis in sporadic colorectal cancer. *Int. J. Cancer* **118**, 1721–1727 (2006).
135. Ruano, Y. *et al.* Worse outcome in primary glioblastoma multiforme with concurrent epidermal growth factor receptor and p53 alteration. *Am. J. Clin. Pathol.* **131**, 257–263 (2009).
136. Yang, Y. C. *et al.* Joint association of polymorphism of the FGFR4 gene and mutation TP53 gene with bladder cancer prognosis. *Br. J. Cancer* **95**, 1455–1458 (2006).
137. Boersma, B. J. *et al.* Association of breast cancer outcome with status of p53 and MDM2 SNP309. *J. Natl Cancer Inst.* **98**, 911–919 (2006).
138. Burke, L. *et al.* Prognostic implications of molecular and immunohistochemical profiles of the Rb and p53 cell cycle regulatory pathways in primary non-small cell lung carcinoma. *Clin. Cancer Res.* **11**, 232–241 (2005).
139. Bull., S. B. *et al.* The combination of p53 mutation and neu/erbB-2 amplification is associated with poor survival in node-negative breast cancer. *J. Clin. Oncol.* **22**, 86–96 (2004).
140. Dearth, L. R. *et al.* Inactive full-length p53 mutants lacking dominant wild-type p53 inhibition highlight loss of heterozygosity as an important aspect of p53 status in human cancers. *Carcinogenesis* **28**, 289–298 (2007).
141. Ahrendt, S. A. *et al.* Rapid p53 sequence analysis in primary lung cancer using an oligonucleotide probe array. *Proc. Natl Acad. Sci. USA* **96**, 7382–7387 (1999).

142. Tonisson, N. *et al.* Evaluating the arrayed primer extension resequencing assay of TP53 tumor suppressor gene. *Proc. Natl Acad. Sci. USA* **99**, 5503–5508 (2002).
143. Kringen, P. *et al.* Evaluation of arrayed primer extension for TP53 mutation detection in breast and ovarian carcinomas. *Biotechniques* **39**, 755–761 (2005).
144. Dicker, F. *et al.* The detection of TP53 mutations in chronic lymphocytic leukemia independently predicts rapid disease progression and is highly correlated with a complex aberrant karyotype. *Leukemia* **23**, 117–124 (2009).
145. Balogh, G. A. *et al.* Mutant p53 protein in serum could be used as a molecular marker in human breast cancer. *Int. J. Oncol.* **28**, 995–1002 (2006).
146. Chang, S. C., Lin, J. K., Lin, T. C. & Liang, W. Y. Genetic alteration of p53, but not overexpression of intratumoral p53 protein, or serum p53 antibody is a prognostic factor in sporadic colorectal adenocarcinoma. *Int. J. Oncol.* **26**, 65–75 (2005).
147. Soussi, T. p53 Antibodies in the sera of patients with various types of cancer: a review. *Cancer Res.* **60**, 1777–1788 (2000).
148. Kumar, S., Mohan, A. & Guleria, R. Prognostic implications of circulating anti-p53 antibodies in lung cancer--a review. *Eur. J. Cancer Care (Engl.)* **18**, 248–254 (2009).
149. Usui, H. & Otani, T. Detection of K-ras and p53 gene mutations in pancreatic juice for the diagnosis of intraductal papillary mucinous tumors. *Pancreas* **22**, 108–109 (2001).
150. Dong, S. M. *et al.* Detecting colorectal cancer in stool with the use of multiple genetic targets. *J. Natl Cancer Inst.* **93**, 858–865 (2001).
151. Done, S. J., Eskandarian, S., Bull., S., Redston, M. & Andrulis, I. L. p53 missense mutations in microdissected high-grade ductal carcinoma *in situ* of the breast. J. *Natl Cancer Inst.* **93**, 700–704 (2001).
152. Keohavong, P., Gao, W. M., Mady, H. H., Kanbour-Shakir, A. & Melhem, M. F. Analysis of p53 mutations in cells taken from paraffin-embedded tissue sections of ductal carcinoma *in situ* and atypical ductal hyperplasia of the breast. *Cancer Lett.* **212**, 121–130 (2004).
153. Lazarus, P. *et al.* A low incidence of p53 mutations in pre-malignant lesions of the oral cavity from non-tobacco users. *Int. J. Cancer* **60**, 458–463(1995).
154. Qin, G. Z., Park, J. Y., Chen, S. Y. & Lazarus, P. A high prevalence of p53 mutations in pre-malignant oral erythroplakia. *Int. J. Cancer* **80**, 345–348 (1999).
155. Bougeard, G. *et al.* Molecular basis of the Li-Fraumeni syndrome: an update from the French LFS families. *J. Med. Genet.* **45**, 535–538 (2008).
156. Sigal, A., Matas, D., Almog, N., Goldfinger, N. & Rotter, V. The C-terminus of mutant p53 is necessary for its ability to interfere with growth arrest or apoptosis. *Oncogene* **20**, 4891–4898 (2001).
157. Ventura, A. & Jacks, T. MicroRNAs and cancer: short RNAs go a long way. *Cell* **136**, 586–591 (2009).
158. Wang, W. & El-Deiry, W. S. Restoration of p53 to limit tumor growth. *Curr. Opin. Oncol.* **20**, 90–96 (2008).
159. Joerger, A. C. & Fersht, A. R. Structure-function-rescue: the diverse nature of common p53 cancer mutants. *Oncogene* **26**, 2226–2242 (2007).
160. Suad, O. *et al.* Structural basis of restoring sequence-specific DNA binding and transactivation to mutant p53 by suppressor mutations. *J. Mol. Biol.* **385**, 249–265 (2009).
161. Kurose, K. *et al.* Frequent somatic mutations in PTEN and TP53 are mutually exclusive in the stroma of breast carcinomas. *Nature Genet.* **32**, 355–357 (2002).
162. Patocs, A. *et al.* Breast-cancer stromal cells with TP53 mutations and nodal metastases. *N. Engl. J. Med.* **357**, 2543–2551 (2007).
163. Campbell, I. G., Qiu, W., Polyak, K. & Haviv, I. Breast-cancer stromal cells with TP53 mutations. *N. Engl. J. Med.* **358**, 1634–1635 (2008).
164. Zander, C. S. & Soussi, T. Breast-cancer stromal cells with TP53 mutations. *N. Engl. J. Med.* **358**, 1635 (2008).
165. Haupt, S. *et al.* Promyelocytic leukemia protein is required for gain of function by mutant p53. *Cancer Res.* **69**, 4818–4826 (2009).
166. Harris, N. *et al.* Molecular basis for heterogeneity of the human p53 protein. *Mol. Cell Biol.* **6**, 4650–4656 (1986).
167. Whibley, C., Pharoah, P. D. & Hollstein, M. p53 polymorphisms: cancer implications. *Nature Rev. Cancer* **9**, 95–107 (2009).
168. Li, F. P. & Fraumeni, J. F. Jr Rhabdomyosarcoma in children: epidemiologic study and identification of a familial cancer syndrome. *J. Natl Cancer Inst.* **43**, 1365–1373 (1969).
169. Li, F. P. & Fraumeni, J. F. Jr Soft-tissue sarcomas, breast cancer, and other neoplasms. A familial syndrome? *Ann. Intern. Med.* **71**, 747–752 (1969).
170. Varley, J. M. Germline TP53 mutations and Li-Fraumeni syndrome. *Hum. Mutat.* **21**, 313–320 (2003).
171. Birch, J. M. *et al.* Cancer phenotype correlates with constitutional TP53 genotype in families with the Li-Fraumeni syndrome. *Oncogene* **17**, 1061–1068 (1998).
172. Iwanaga, Y. & Jeang, K. T. Expression of mitotic spindle checkpoint protein hsMAD1 correlates with cellular proliferation and is activated by a gain-of-function p53 mutant. *Cancer Res.* **62**, 2618–2624 (2002).
173. Preuss, U., Kreutzfeld, R. & Scheidtmann, K. H. Tumor-derived p53 mutant C174Y is a gain-of-function mutant which activates the fos promoter and enhances colony formation. *Int. J. Cancer* **88**, 162–171 (2000).
174. Deb, S., Jackson, C. T., Subler, M. A. & Martin, D. W. Modulation of cellular and viral promoters by mutant human p53 proteins found in tumor cells. *J. Virol.* **66**, 6164–6170 (1992).
175. Mizuarai, S., Yamanaka, K. & Kotani, H. Mutant p53 induces the GEF-H1 oncogene, a guanine nucleotide exchange factor-H1 for RhoA, resulting in accelerated cell proliferation in tumor cells. *Cancer Res.* **66**, 6319–6326 (2006).
176. Yan, W., Liu, G., Scoumanne, A. & Chen, X. Suppression of inhibitor of differentiation 2, a target of mutant p53, is required for gain-of-function mutations. *Cancer Res.* **68**, 6789–6796 (2008).
177. Werner, H., Karnieli, E., Rauscher, F. J. & LeRoith, D. Wild-type and mutant p53 differentially regulate transcription of the insulin-like growth factor I receptor gene. *Proc. Natl Acad. Sci. USA* **93**, 8318–8323 (1996).
178. Yan, W. & Chen, X. Identification of GRO1 as a critical determinant for mutant p53 gain of function. *J. Biol. Chem.*, **284**, 12178–12187 (2009).
179. Weisz, L. *et al.* Transactivation of the *EGR1* gene contributes to mutant p53 gain of function. *Cancer Res.* **64**, 8318–8327 (2004).
180. Buganim, Y. *et al.* Mutant p53 protects cells from 12-O-tetradecanoylphorbol-13-acetate-induced death by attenuating activating transcription factor 3 induction. *Cancer Res.* **66**, 10750–10759, (2006).
181. Lavra, L. *et al.* Gal-3 is stimulated by gain-of-function p53 mutations and modulates chemoresistance in anaplastic thyroid carcinomas. *J. Pathol.* **218**, 66–75 (2008).
182. Zalcenstein, A. *et al.* Mutant p53 gain of function: repression of CD95(Fas/APO-1) gene expression by tumor-associated p53 mutants. *Oncogene* **22**, 5667–5676 (2003).
183. Zalcenstein, A. *et al.* Repression of the MSP/MST-1 gene contributes to the antiapoptotic gain of function of mutant p53. *Oncogene* **25**, 359–369 (2006).
184. Lee, Y. I., Lee, S., Das, G. C., Park, U. S. & Park, S. M. Activation of the insulin-like growth factor II transcription by aflatoxin B1 induced p53 mutant 249 is caused by activation of transcription complexes; implications for a gain-of-function during the formation of hepatocellular carcinoma. *Oncogene* **19**, 3717–3726 (2000).
185. Yang, X., Pater, A. & Tang, S. C. Cloning and characterization of the human BAG-1 gene promoter: upregulation by tumor-derived p53 mutants. *Oncogene* **18**, 4546–4553 (1999).
186. Pugacheva, E. N. *et al.* Novel gain of function activity of p53 mutants: activation of the dUTPase gene expression leading to resistance to 5-fluorouracil. *Oncogene* **21**, 4595–4600 (2002).
187. Kalo, E. *et al.* Mutant p53 attenuates the SMAD-dependent transforming growth factor β1 (TGF-β1) signaling pathway by repressing the expression of TGF-β receptor type II. *Mol. Cell Biol.* **27**, 8228–8242 (2007).
188. Kelavkar, U. P. & Badr, K. F. Effects of mutant p53 expression on human 15-lipoxygenase-promoter activity and murine 12/15-lipoxygenase gene expression: evidence that 15-lipoxygenase is a mutator gene. *Proc. Natl Acad. Sci. USA* **96**, 4378–4383 (1999).
189. Loging, W. T. & Reisman, D. Elevated expression of ribosomal protein genes L37, RPP-1, and S2 in the presence of mutant p53. *Cancer Epidemiol. Biomarkers Prev.* **8**, 1011–1016 (1999).
190. Dhar, G. *et al.* Gain of oncogenic function of p53 mutants induces invasive phenotypes in human breast cancer cells by silencing CCN5/WISP-2. *Cancer Res.* **68**, 4580–4587 (2008).
191. Khromova, N. V., Kopnin, P. B., Stepanova, E. V., Agapova, L. S. & Kopnin, B. P. p53 hot-spot mutants increase tumor vascularization via ROS-mediated activation of the HIF1/VEGF-A pathway. *Cancer Lett.* **276**, 143–151 (2008).

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DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
Cdkn1a | *CDKN2A* | *HRAS* | *MDM2* | *MDR1* | *MYC* | *TP53* | *Trp53*
National Cancer Institute drug dictionary: http://www.cancer.gov/DRUGDICTIONARY
doxorubicin | vitamin D
OMIM: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
Li–Fraumeni Syndrome
UniProtKB: http://www.uniprot.org
E2F1 | ETS1 | ERBB2 | GADD45α | HDAC1 | HSP90 | INK4A | MRE11 | NF-κB | NF-Y | p53 | p63 | p73 | PTEN | SP1 | STUB1 | TNFα | VDR

FURTHER INFORMATION

Varda Rotter's homepage: http://www.weizmann.ac.il/mcb/Varda/
IARC TP53 Mutation Database: http://www-p53.iarc.fr/

SUPPLEMENTARY INFORMATION

See online article: S1 (table)

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