p53 polymorphisms: cancer implications

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Abstract | The normal functioning of p53 is a potent barrier to cancer. Tumour-associated mutations in *TP53*, typically single nucleotide substitutions in the coding sequence, are a hallmark of most human cancers and cause dramatic defects in p53 function. By contrast, only a small fraction, if any, of the >200 naturally occurring sequence variations (single nucleotide polymorphisms, SNPs) of *TP53* in human populations are expected to cause measurable perturbation of p53 function. Polymorphisms in the *TP53* locus that might have cancer-related phenotypical manifestations are the subject of this Review. Polymorphic variants of other genes in the p53 pathway, such as *MDM2*, which might have biological consequences either individually or in combination with p53 variants are also discussed.

Polymorphism
A germline variation within a gene (often at a single nucleotide, known as single nucleotide polymorphisms, SNPs) that exists at a frequency of at least 1% in the general population.

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The tumour suppressor p53 is a key player in stress responses that preserve genomic stability, responding to a variety of insults including DNA damage, hypoxia, metabolic stress and oncogene activation[1,2]. The most well-documented mechanism by which p53 exercises its protective roles is as a transcription factor. By binding to specific response elements in DNA, p53 modulates the transcription of genes that govern the major defences against tumour growth, which include cell cycle arrest, apoptosis, maintenance of genetic integrity, inhibition of angiogenesis and cellular senescence[3]. p53 also interacts with numerous cellular proteins, including several that control programmed cell death, and these molecular interactions might contribute to the inhibitory role of p53 in tumorigenesis[4–9].

Malfunction of the p53 pathway is an almost universal hallmark of human tumours[1,2]. Somatic mutation of *TP53* that results in the absence or dysfunction of p53 is one of the most common mechanisms by which the p53 pathway is damaged during tumorigenesis. The direct loss of properly functioning p53 is also associated with an unfavourable prognosis in some types of cancer[10]. In this Review, three distinct sets of sequence alterations at the *TP53* locus will be referred to: tumour-associated mutations, germline Li–Fraumeni mutations and germline p53 polymorphisms.

The tumour-associated mutations in sporadic cancers arise in somatic cells, both spontaneously and as a consequence of DNA damage. The mutations selected for in tumorigenesis are usually single base substitutions that result in amino acid substitutions in the DNA-binding domain (DBD) of p53 (missense mutations). Typically, the mutant tumour-associated p53 proteins have lost most or all of the normal p53 functions. Although mutation in *TP53* is a frequent mechanism by which p53 function is lost in tumorigenesis, in some types of cancer other routes prevail, such as gene amplification of key negative regulators of p53, *MDM2* and *MDM4*.

Germline *TP53* mutations are found in individuals with Li–Fraumeni syndrome, which confers an increased risk of developing various cancers, including sarcomas, breast and brain cancers, and adrenocortical tumours, at an early age of onset[11]. As is the case for somatic tumour-associated mutations, Li–Fraumeni mutations are most often missense base substitutions in the DBD, and encode defective proteins.

Germline *TP53* polymorphisms are the subject of this Review. Unlike mutations associated with Li–Fraumeni syndrome or somatic tumour-associated mutations, most polymorphisms are expected to be phenotypically silent, with an occasional variant that might affect cancer risk by compromising the normal activities of p53, although the effects of these variants are probably more subtle than those of p53 mutations associated with cancer or Li–Fraumeni syndrome. To avoid confusion, single nucleotide polymorphisms (SNPs) are not usually referred to as mutations, which is a term generally reserved for p53 sequence changes that arise during tumorigenesis or are found in patients with Li–Fraumeni syndrome.

TP53 is unique among tumour suppressor genes because so many different missense mutations can occur within it that generate a range of mutant p53 proteins with varying levels of residual activity.

At a glance

- *TP53*, which encodes p53, is a tumour suppressor gene that is frequently mutated in sporadic cancers. The mutations are usually single base substitutions that disrupt function, and some confer new oncogenic (gain-of-function) properties. Over 200 single nucleotide polymorphisms (SNPs; germline variants) in *TP53* have been identified; in contrast to tumour-associated mutations, most of these *TP53* SNPs are unlikely to have biological effects. Owing to the importance of p53 in tumour suppression, the polymorphisms that alter p53 function might affect cancer risk, progression and/or response to treatment.
- p53 lies at the hub of a vast signalling network. Polymorphisms in upstream activators, repressors or downstream effectors of p53 might individually modulate cancer risk or interact with polymorphisms or mutations in *TP53*.
- Because the effects of a polymorphism can be subtle and can vary according to genetic background, there are rigorous methodological challenges associated with determining the effect of a polymorphism on cancer risk. Even for the most studied SNP in p53 at codon 72, R72P, the results have been inconsistent, particularly those from population studies that have investigated associations with cancer risk.
- Population studies require large sample sizes (in the thousands). High-throughput sequencing and the development of genome-wide SNP maps are allowing larger and more comprehensive studies of polymorphisms to be carried out. To date, no study of a sufficient size has reported a significant association between SNPs at the *TP53* locus and altered cancer risk.
- Molecular studies examining the effects of p53 polymorphisms have been based principally on *in vitro* models with transfected cell lines. The biological effects of p53 pathway variants at the molecular level in primary cells or *in vivo* still need to be determined. The design of genetically engineered mice using knock-in and knockout technology to study human polymorphisms is currently underway.

Penetrance
A measure of the proportion of genetically similar individuals that show any phenotypical manifestation of a mutation that they have in common.

These diverse tumour-associated mutations have been compiled in databases, together with details of the frequency of the mutations and a summary of molecular studies that have characterized the activities of each mutant (the IARC *TP53* mutation database, the p53 Knowledgebase, the *TP53* Web Site and the Database of germline p53 mutations). Depending on the mutation, different elements of normal p53-mediated responses can be lost and some mutants can gain new non-wild-type functions. The oncogenic properties of gain-of-function mutant p53 proteins are currently under intensive investigation because an in-depth understanding of the molecular mechanisms might ultimately have translational benefits in the clinic[12]. There is evidence to suggest that gain-of-function mutants can interact with other transcription factors, such as nuclear transcription factor Y (NF-Y) or p73, to transcriptionally activate or repress a unique subset of genes, leading to disruption of cell cycle regulation and apoptosis[13]. Mutant p53 interactions with multiprotein complexes that are involved in recombination and DNA double-strand break repair might also contribute to the deregulation of homologous recombination, interchromosomal translocations and aneuploidy, which are observed in neoplastic cells that express gain-of-function p53 mutants[14–16].

A major lesson from the human tumour-associated p53 mutation databases is that *TP53* is unusually vulnerable to a large range of single nucleotide alterations that compromise the function of the wild-type protein and can even confer new oncogenic activities. This raises the possibility that at least some of the germline polymorphisms of *TP53* in healthy populations might also impinge on p53 function. This is despite the fact that only a small fraction of the many polymorphic sequence variations at gene loci in the human genome (most of which are intronic) are likely to have any cancer-associated phenotypical manifestations. Features that suggest a potential phenotypical consequence include polymorphisms in the coding sequence that alter amino acid sequence, or variants that affect expression levels; for example, polymorphisms in promoters, splice sites, untranslated regions (UTRs) or protein-binding elements. Unlike the high-penetrance germline mutations that underlie syndromes such as Li–Fraumeni and ataxia–telangiectasia (discussed later), polymorphisms are generally expected to have more modest effects, such as causing an earlier age of disease onset or leading to a small increase in the risk of cancer. This presents methodological challenges for establishing and verifying the biological effects of polymorphisms. Identifying polymorphisms that have a small effect on cancer risk requires a multidisciplinary approach, including molecular studies and population-based research using high-throughput sequencing and high-resolution SNP mapping.

The p53 pathway

Regulation and activation of p53. The levels of p53 are key to its activity and are tightly controlled in the cell, largely by covalent modifications[17]. Numerous stress sensors that converge at p53 result in the phosphorylation, acetylation, ubiquitylation and methylation of specific p53 residues[17–20]. This range of modifications elicits downstream p53 responses that counteract the deleterious consequences of DNA damage, hypoxia, metabolic stress or oncogene activation (FIG. 1). Polymorphisms at loci that alter the activity of any single upstream event that activates p53 should not entirely abrogate the p53 response, owing to the high level of redundancy in stress responses, but cellular responses could be attenuated by altering one or more of the triggers for p53 activation.

Under normal, unstressed conditions, a key negative regulator of p53 is MDM2, which binds to the transactivation domain of p53 and ubiquitylates the protein, targeting it for degradation[21]. Because p53 transcriptionally activates MDM2, the expression levels of p53 and MDM2 are balanced through a negative feedback loop, which is altered by an increase in p53 levels following stresses such as DNA damage[22]. The related protein MDM4 (also known as MDMX) also modulates p53 activity[23], and the interplay between p53, MDM2 and MDM4 at the molecular level is complex. For example, MDM2 binds to *TP53* mRNA, controlling the rate of translation[24], and MDM2 regulates the levels of itself, MDM4 and p53 (REFS 23,25,26). The pivotal role of MDM2 and MDM4 in the control of p53 function argues that polymorphisms at these loci should be scrutinized for potential modulation of p53 function.

The range of post-translational modifications and p53 upregulation elicited by stress are well-studied features of the p53 network. Less is known about the physiological

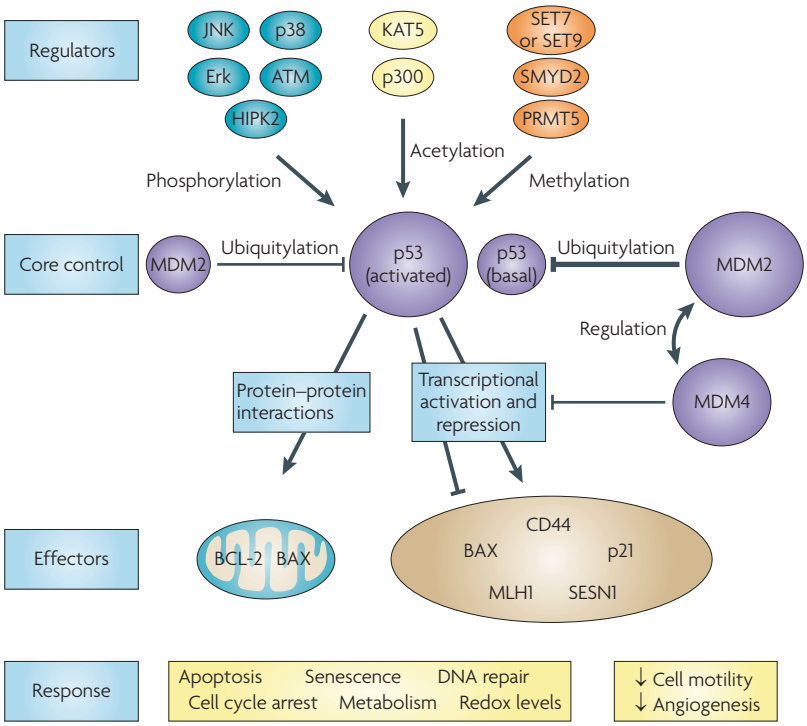


Figure 1 | **The p53 pathway.** The p53 pathway is complex. At least 50 different enzymes can covalently modify p53 to alter its stability, cellular location or activity[17]. Under normal cellular conditions, MDM2 represses p53 by binding and sequestering p53, and by ubiquitylating p53, targeting it for degradation. DNA damage, oxidative stress and oncogene activation are among the processes that can activate p53 by a range of regulators. Basal levels of p53 and p53 in cells that are undergoing low levels of stress can also affect cell physiology. Under high levels of stress, the interactions between MDM2, MDM4 and p53 are disrupted by post-translational modifications of these proteins. This allows activated p53 to act as a transcription factor, activating or repressing genes involved in apoptosis, cell cycle arrest and senescence. p53 can also move to the mitochondria, where it physically interacts with members of the Bcl-2 family to form pores in the mitochondrial membrane, leading to the release of cytochrome *c* and subsequent apoptosis. Some of the more intensively studied activators, regulators and effectors of p53 are shown in the figure. ATM, ataxia–telangiectasia mutated; BAX, BCL-2-associated X; HIPK2, homeodomain-interacting protein kinase 2; JNK, Jun N-terminal kinase; KAT5, K (lysine) acetyltransferase 5; MLH1, MutL protein homologue 1; PRMT5, protein arginine methyltransferase 5; SESN1, sestrin 1; SMYD2, SET and MYND domain-containing 2.

roles of p53 that are not necessarily linked to tumour suppression and that can be executed by low levels of p53 and in the absence of a severe insult or stress (TABLE 1). These homeostatic activities might also be affected by polymorphisms in p53, although there are limited data on this subject (not discussed in this Review).

Events following p53 activation. p53 controls multiple cellular functions by inducing or repressing target genes with p53 response elements[27] (FIG. 2). Several hundred p53-responsive genes have been identified, more than 100 of which have been verified by several different methods to be directly regulated by p53 (REF. 27). These include genes involved in cell cycle arrest, such as *CDKN1A*, which encodes p21; genes involved in apoptosis, such as *BBC3* (BCL-2-binding component 3, also known as PUMA), *BAX* (BCL-2-associated X) and *PERP* (p53 apoptosis effector related to PMP22); genes involved in the inhibition of angiogenesis, such as *THBS1* (thrombospondin 1); and genes involved in cellular senescence, such as *CDKN1A* and *PML* (promyelocytic leukaemia). p53 binding to response elements is regulated by the structure and conformation of p53, post-translational modifications of p53, and the structure and sequence of the response element[28].

The p53 response element has been extensively characterized and is one of the key factors that determines the pleiotropy of the p53 stress response. Slight differences in the sequence of response elements can dramatically alter the inducibility of the target gene by p53 (REFS 27,29). The numerous splice variant isoforms, and amino-terminal shortened forms of *TP53* and p53 family members (*TP63* and *TP73*) add another layer of complexity to p53 transcriptional activity and the expression of p53 downstream targets[30–33]. The ability of p53 to repress the transcription of specific genes is also important for its tumour suppressor activity, as shown in a recent report on p53 repression of CD44 expression[34].

The effects of p53 on cellular functions are also mediated by protein–protein interactions, which constitute a second axis in the suppression of tumorigenesis by p53. Numerous proteins involved in cell cycle control, DNA repair, signalling or gene transcription can bind to wild-type p53 (REF. 35). Some of these interactions modulate the participation of p53 in the mitochondrial apoptotic programme[4,7,8]. The p53 protein binds to members of the Bcl-2 family, increasing mitochondrial permeability and the release of mitochondrial intermembrane molecules that trigger downstream apoptotic events[36,37]. Tumour-associated mutations in the p53 DBD are unable to activate this pathway[38], consistent with the importance of these interactions in inhibiting tumorigenesis.

Polymorphisms of the *TP53* locus

As is true of the human genome as a whole (in which over 3.1 million sequence variations have been mapped, which represent 25–35% of the total estimated SNPs[39,40]), numerous SNPs and other sequence variations are present at the *TP53* locus (FIG. 3) (see also the International HapMap Project and the NCBI SNP database). Most of these variations are intronic and can be presumed to have no cancer-related biological consequences. Few of the many p53 polymorphisms have been assessed for altered biochemical and/or biological function, or for their effects on cancer risk in population studies. It is an ongoing challenge to find appropriate criteria for selecting variants that are worthy of assessment in laboratory-based studies or to devise methods to screen unselected sets for functional activity.

Polymorphisms in non-coding sequences. More than 90% of the polymorphisms in *TP53* occur in the non-coding sequences. The most well-characterized intronic *TP53* polymorphism is a 16 base pair insertion in intron 3 (REF. 41). This is the only intronic polymorphism that has been associated with an increase in the risk of several types of cancer[42–45]; however, the close proximity of this polymorphism to the codon 72 polymorphism in

p53 response element
A short sequence of DNA in or near a gene that can bind p53, which then regulates the transcriptional activity of that gene.

p53 family
This family includes p53, p63 and p73, which have a significant degree of sequence homology particularly in the sequence-specific DNA-binding domain.

Table 1 | **Additional roles for the p53 tumour suppressor**

Physiological roles of p53	Examples of proposed mechanisms	Refs
Reproduction	Transcription of LIF is controlled by p53, with enhancement by the p53-R72 variant Levels of LIF affect implantation success	65,66
Prolonged organismal life span	p53 controls stem cell self-renewal In carriers of the p53-P72 variant, apoptosis can be attenuated, with a reduced age-associated attrition of stem cell populations	85, 161–163
Antioxidant functions	Transcriptional activation of genes encoding enzymes that reduce the cellular levels of reactive oxygen species, including GPX1, SOD2 and sestrins	164
Autophagy	Transcriptional activation of DRAM, a lysosomal membrane protein required for autophagy Inhibition of mTOR, a negative regulator of autophagy	165,166
Glucose metabolism	Transcriptional activation of TIGAR, which blocks glycolysis and directs the pathway to the pentose phosphate shunt	167,168
Mitochondrial respiration	Transcriptional activation of SOCS2, a cytokine-induced negative regulator of cytokine signalling	169
Embryonic stem cell pools	Suppression of NANOG by p53 causes embryonic stem cell differentiation	170

DRAM, damage-regulated autophagy mediator; GPX1, glutathione peroxidase 1; IGF1R, insulin-like growth factor 1 receptor; LIF, leukaemia-inhibitory factor; SOCS2, suppressor of cytokine signalling 2; SOD2, superoxide dismutase [Mn], mitochondrial; TIGAR, TP53-induced glycolysis and apoptosis regulator.

exon 4 (discussed below) might partly explain the proposed association of this allele with cancer. One study showed that this allele was associated with lower levels of *TP53* transcripts, suggesting that the polymorphism causes an alteration in mRNA processing, providing a possible molecular basis for the associated increase in risk of developing cancer[45].

Synonymous polymorphisms in TP53 coding sequences. Of the 19 exonic polymorphisms that have been reported in *TP53*, eight are synonymous. Although these polymorphisms do not change the amino acid sequence or structure of the protein, in theory, changes in base sequence and codon use could modify protein expression, folding and function, or provoke new splicing events[24,46,47]. The finding that MDM2 binds to and facilitates the translation of *TP53* mRNA, and that this interaction is abrogated by silent mutations in the N-terminal region suggests another mechanism by which synonymous polymorphisms could affect p53 function[24]. A silent mutation at codon 36 (CCG to CCT) was shown to reduce the ability of p53 to activate apoptosis by lowering the affinity of the *TP53* mRNA for MDM2, consequently reducing p53 levels[24]. Three synonymous polymorphisms — D21D (GAC to GAT), P34P (CCC to CCA) and P36P (CCG to CCA) — are located in the region that is crucial for *TP53* mRNA binding to MDM2 and their roles await functional analysis. The recent finding that microRNAs (miRNAs) can inhibit translation by targeting the coding sequences of genes invites speculation that there are as yet undiscovered miRNAs that can target p53 in a similar fashion, which might explain how silent *TP53* polymorphisms in the coding region of the gene could influence p53 activity[48–50].

Non-synonymous polymorphisms in TP53 coding sequences. The remaining 11 polymorphisms in *TP53* are non-synonymous, resulting in an amino acid change in the protein. Only four of these polymorphisms have been validated by multiple submissions of the polymorphism to p53 databases, reports on the frequency of the polymorphism, or inclusion of the polymorphism in the HapMap database. In addition, they have not been reported as somatic mutations in tumours (see the IARC *TP53* mutation database).

Changes in the amino acid sequence can alter the ability of p53 to bind to response elements of target genes (as shown by tumour-associated p53 mutations[29]), alter recognition motifs for post-translational modifications, or alter the protein stability and interactions with other proteins[32,51]. For two of the polymorphisms, there is sufficient molecular evidence to suggest that the polymorphisms cause a functional change in the p53 pathway (P47S[52] and R72P[53]; discussed below). The remaining two validated non-synonymous polymorphisms have not been associated with an altered cancer risk to date (V217M and G360A).

Codon 47 (P47S)

P47S, a rare polymorphism in the N-terminal transactivation domain of p53, results from a C>T base substitution at position 1 of codon 47. It has only been reported in populations of African origin, in which it is found at an allele frequency of approximately 5% [REF. 52].

Phosphorylation of the N-terminal domain of p53 has been shown to regulate its transactivation properties[17,54,55]. p38 and homeodomain-interacting protein kinase 2 (HIPK2) phosphorylate S46, which enhances the transcription of apoptosis-related genes and hence promotes p53-mediated apoptosis[17]. These two kinases are directed to phosphorylation sites by a proline residue adjacent to S46. Thus, replacement of P47, as occurs with the P47S polymorphism, would be expected to decrease phosphorylation at S46, decrease transactivation of pro-apoptotic target genes and thus potentially increase cancer risk[56,57].

RRRCW →	WGYYY ←	(spacer)	RRRCW →	WGYYY ←	Consensus motif
GGGCA	GGCCC	-	GGGCT	TGTCG	*BAX*
AGACA	TGTCC	ac	AGACT	TGTCT	*TIGAR* (C12orf5)
CTGCA	AGTCC	-	TGACT	TGTCC	*BBC3* (PUMA)
GAACA	TGTCT	-	AAGCA	TGCTG	*GADD45*
GAAGA	AGACT	-	GGGCA	TGTCT	*CDKN1A* (p21)
AGTTA	AGTCC	-	TGACT	TGTCT	*MDM2* response element 1
GGTCA	AGTTC	-	AGACA	CGTTC	*MDM2* response element 2
AGGCA	AGCTC	-	CAGCT	TGTTC	*PERP*
GCGCT	GGCCT	ggagccag	GGGCA	TGTCC	*PML*
GGACA	AGTCT	-	CCACA	AGTCA	*SESN1* (sestrin)
TGACA	TGCCC	-	AGGCA	TGTCT	*p53R2*

Figure 2 | **Sequences of p53 response elements.** The canonical p53 response element is displayed in the box. This comprises two palindromic 10 base pair sequences separated by a spacer varying between 0 and 13 base pairs[27]. The p53 response elements of several p53 target genes are listed beneath the consensus sequence, with bases matching the consensus sequence listed in the appropriate colour, non-matching bases are not coloured. Several p53 responsive genes contain multiple p53 response elements; the two MDM2 response elements are shown as an example. BAX, BCL-2-associated X; BBC3, BCL-2-binding component 3; PERP, p53 apoptosis effector related to PMP22; PML, promyelocytic leukaemia; R, purine; SESN1, sestrin 1; TIGAR, TP53-induced glycolysis and apoptosis regulator; W, adenine or thymine; Y, pyrimidine.

Li *et al.* observed reduced phosphorylation at S46, decreased induction of the pro-apoptotic genes *P53AIP1* (p53-regulated apoptosis-inducing protein 1) and *BBC3*, and decreased apoptosis in human cell lines transfected with the p53-S47 variant[58]. An earlier study concluded that the ability of p53 to suppress cell growth is not affected by the p53-S47 variant (REF. 52), but the induction of apoptosis was not examined. Surprisingly, in a yeast-based screening assay, the p53-S47 variant generated a 'super-transactivation' p53, which could activate five of the panel of eight p53 target genes (including *P53AIP1*) at >150% of the levels induced by wild-type p53; only the induction of *MDM2* was attenuated (FIG. 4; the IARC *TP53* mutation database)[59]. The incongruent results could be due to the different test systems used (transactivation activity of p53 in a yeast assay, compared with p53 activity in mammalian cells). The occurrence of unexpected findings might also indicate that the link between p53 transactivation of pro-apoptotic genes and induction of apoptosis is not as simple as was originally thought, as proposed in a comprehensive study that tested a library of constructs encoding p53 mutants in yeast and p53-null human cells[60]. Finally, it is noteworthy that blocking single regulatory modifications of p53, such as phosphorylation at S46 in mouse models, has unexpectedly modest phenotypical consequences in contrast to the predictions from *in vitro* studies of p53 (REFS 26,56). This is only one example of many from p53 research that showed that the expectations derived from cell culture transfection studies were not met when put to the test in appropriate mouse models[26].

Codon 72 (R72P)

In human populations, codon 72 of p53 has either the sequence CCC, which encodes proline, or CGC, which encodes arginine. The variants are hereafter abbreviated p53-P72 and p53-R72. Comparative sequence analyses in non-human primates suggest that p53-P72 is the ancestral form, although p53-R72 occurs at a high frequency (>50%) in some populations[61]. A latitude gradient in variant frequency (an increasing frequency of the p53–72 variant towards the equator[62,63]) invited early speculation that p53-P72 might protect against adverse consequences of sunlight or other environmental cancer risk factors. Interestingly, p53 influences the tanning response to sunlight by inducing the expression of pro-opiomelanocortin (POMC)[64], which leads to the conjecture that p53 polymorphisms influence *POMC* transactivation. The contribution of p53-mediated control of leukaemia-inhibitory factor expression, which is crucial for blastocyst implantation, has recently been considered in the discussion of evolutionary selection of specific alleles in the p53 pathway[65,66].

Codon 72 is in exon 4 in the segment of *TP53* that encodes the polyproline domain, which lies between the N-terminal transactivating domain and the DBD, in which most tumour-associated mutations are found. The precise contribution of the polyproline domain to p53 regulation and function is still unclear. Unlike the DBD, the polyproline domain is less well-conserved across species than the DBD and is not a common location of tumour-associated mutations. Deletion studies in cells and mice support the view that the polyproline domain is essential for p53 to mount a full apoptotic response to stress and inhibit tumorigenesis[67–70]. However, there are differing interpretations of whether specific PXXP or other protein-binding motifs in the domain are crucial to p53 stability and function and, if so, which motifs are important[69,71,72].

The expression of p53 pro-apoptotic target genes and the mitochondrial apoptotic response in p53-null cell lines stably transfected with constructs that encode p53-R72 is higher than in cell lines expressing the p53-P72 variant, supporting the current view[7] that p53-R72 is a more potent inducer of apoptosis than p53-P72. p53-P72 binds more weakly than p53-R72 to the positive regulatory protein PIN1, a prolyl isomerase[73], yet interacts more readily with the inhibitory protein iASPP (inhibitor of ASPP)[74]. These attributes also predict that p53-P72 has a weaker apoptotic potential than p53-R72.

The current consensus is that p53-R72 is more effective at inducing apoptosis and protecting stressed cells from neoplastic development than p53-P72. However, it is not yet understood how universal these functional differences between p53-P72 and p53-R72 might be in different cell types or whether they are relevant *in vivo*. There are insufficient experimental data to establish consistent differences in biological activities between p53-R72 and p53-P72 in normal primary human cells or in tumours. However, studies using various cell culture techniques, such as transfection experiments in tumour cell lines, have reported differences in mitochondrial localization, apoptosis, cell cycle progression, DNA

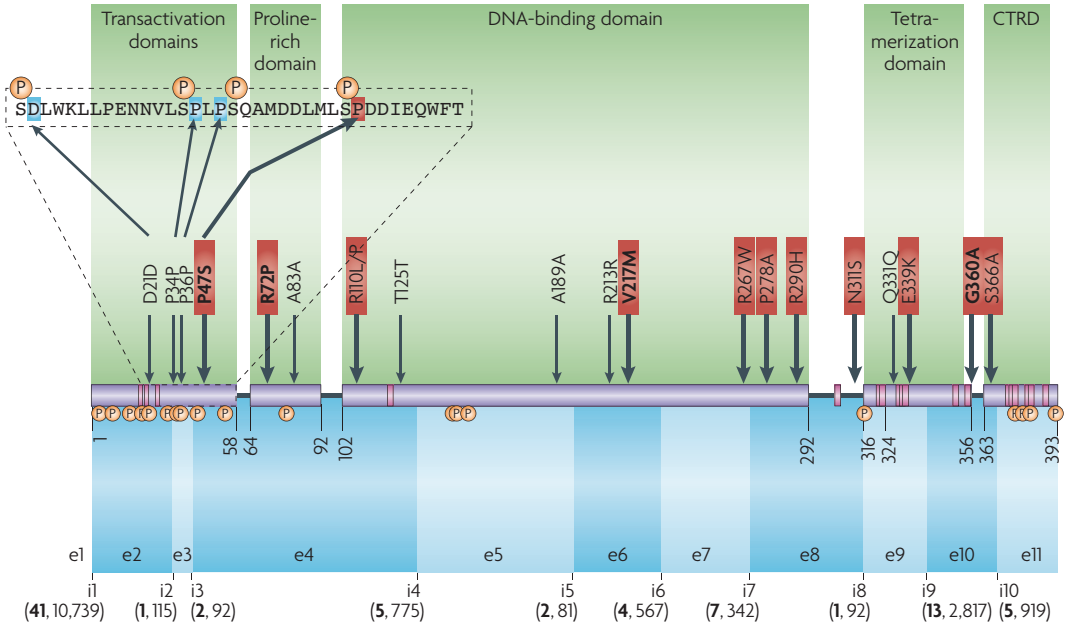
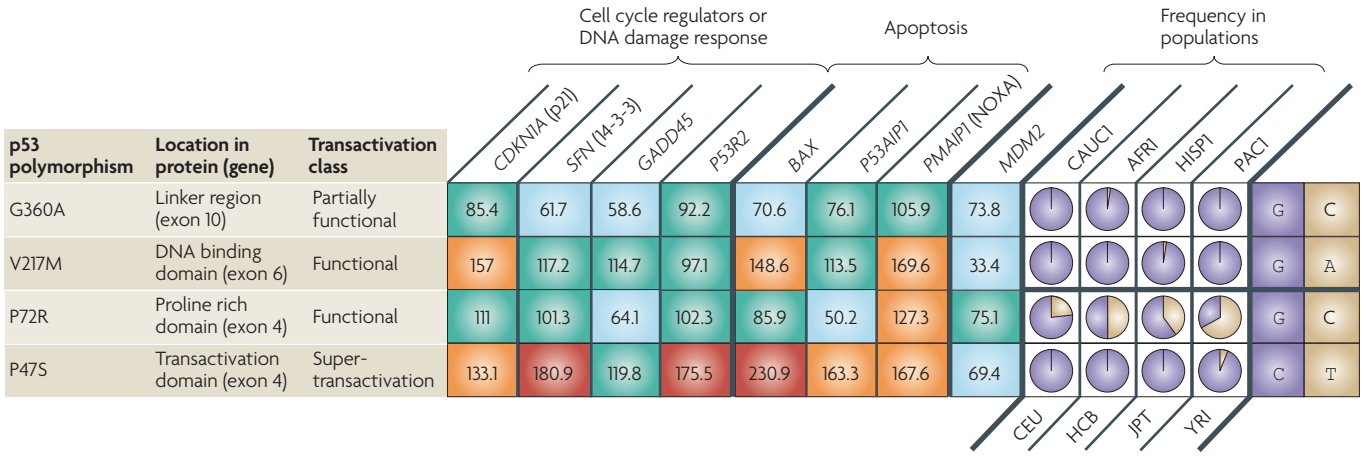


Figure 3 | **p53 single nucleotide polymorphisms: locations in the p53 protein and DNA sequences.** The p53 protein comprises three major domains: an amino-terminal transactivation domain; a central DNA-binding domain, which is crucial in transcriptional transactivation by p53 and the region that is most frequently mutated in cancers; and a carboxy-terminal tetramerization domain. The C-terminal and N-terminal domains contain many post-translational modification sites that alter the stability, activity and protein–protein interactions of p53. The yellow circles (P) indicate sites at which phosphorylation occurs and the pink rectangles indicate a number of other post-translational modification sites, including acetylation, sumoylation, methylation and neddylation. Other important structural features of p53 are the proline-rich domain, the C-terminal regulatory domain (CTRD) and several nuclear localization and nuclear export signals. Exonic polymorphisms are indicated on the protein structure, with non-synonymous polymorphisms in red. The polymorphisms discussed in the text are shown in bold. The inset region shows the amino acid sequence encompassing the proline-directed phosphorylation site adjacent to the non-synonymous polymorphism P47S. The intronic regions are shown below the protein structure, with the number of validated polymorphisms identified in each intron in bold, and the size of the intron (i) in base pairs; e, exon.

repair, growth arrest and transcriptional transactivation[7,73–80]. As yet, few of the >100 known, well-validated p53 target genes[27] have been examined in any detail with respect to their differential induction by the codon 72 p53 variants.

Cancer susceptibility and the codon 72 polymorphism. A decade ago, a dramatic effect of the p53 codon 72 polymorphism on the risk of cervical cancer was reported. This effect was explained by the finding that the E6 oncoprotein from high-risk mucosal human papilloma viruses (HPVs) causes more efficient degradation of p53-R72 than p53-P72, reducing cellular levels of p53 and increasing the risk of HPV-associated cancers in p53-R72 homozygotes[79]. This sparked an intensive investigation — that is still continuing — into the potential effect of the p53 codon 72 polymorphism on susceptibility to various cancers or cancer-related phenotypes. The NIH genetic association database, which is not comprehensive, has records on over 230 studies evaluating the effect of the codon 72 polymorphism on susceptibility to a wide variety of cancers. Many of these studies have reported 'statistically significant' associations. However, interpretation of these studies is beset by many problems: the major issues are a combination of small sample size, publication bias and inadequately stringent levels for statistical significance. These problems were common to many of the early association studies[81].

The best evidence is provided by large, carefully designed case–control studies of thousands of samples. A type I error rate (p-value threshold for significance) of 10^{-7} is generally regarded as genome-wide significance and no single study has reported such a highly statistically significant association between the codon 72 polymorphism and any cancer. Several formal meta-analyses combining data from multiple studies have been published on breast[82], gastric[83] and lung[84] cancer, and these do not support a role for this polymorphism in the risk of developing these cancers. A screen of all publications up to 2005, which assessed a total of 61 populations and combined all types of cancer, estimated a pooled weak cancer risk in p53-P72 homozygotes of questionable significance ($p < 0.05$)[85]. A meta-analysis of studies investigating the R72P polymorphism in cervical cancer reported some evidence of association. The authors found that women who carry two copies of the arginine allele were at increased risk compared with those carrying one copy, but this association was not highly statistically significant ($p = 0.001$)[86]. Furthermore, there was no difference in the risk between women who carry two



p53 polymorphism	Location in protein (gene)	Transactivation class	CDKN1A (p21)	SFN (14-3-3)	GADD45	P53R2	BAX	P53AIP1	PMAIP1 (NOXA)	MDM2	CAUC1	AFRI	HISP1	PACI
G360A	Linker region (exon 10)	Partially functional	85.4	61.7	58.6	92.2	70.6	76.1	105.9	73.8	G	C		
V217M	DNA binding domain (exon 6)	Functional	157	117.2	114.7	97.1	148.6	113.5	169.6	33.4	G	A		
P72R	Proline rich domain (exon 4)	Functional	111	101.3	64.1	102.3	85.9	50.2	127.3	75.1	G	C		
P47S	Transactivation domain (exon 4)	Super-transactivation	133.1	180.9	119.8	175.5	230.9	163.3	167.6	69.4	C	T		

Figure 4 | **Transcriptional activity and frequency of the p53 polymorphic variants discussed in the text.** Frequency data were extracted from two polymorphism frequency databases and the activity data from Kato *et al.*, who expressed each of the missense variants of p53 in a yeast reporter system, together with promoter–luciferase constructs for each of the promoters of the listed genes. Activity data are colour-coded, and expressed as percentage of wild-type p53 induction at each promoter in the same system[59]. The colours are grouped accordingly: 0–75% blue, 75–125% green, 125–175% orange, >175% red. The allelic frequencies of each polymorphism in various human populations (AFR1, individuals of self-described African or African–American heritage; CAUC1, individuals of self-described Caucasian heritage; CEU, CEPH Utah residents with ancestry from northern and western Europe; HCB, Han Chinese in Beijing, China; HISP1, individuals of self-described Hispanic heritage; JPT, Japanese in Tokyo, Japan; PAC1, individuals of self-described Pacific Rim heritage; YRI, Yoruba in Ibadan, Nigeria) in which they have been studied are shown as pie-charts, with the corresponding base changes displayed in the final columns: G360A, GGG or GCG; V217M, GTG or ATG; P72R, CCC or CGC; P47S, CCG or TCG. (Note that with respect to the codon 72 polymorphism, most reports have referred to the p53-P72 (CCC) allele as the minor variant). The frequencies of codon 360 and codon 217 variants are reported from the NCBI SNP database, and the codon 72 and codon 47 variant frequencies are reported from the HapMap database. BAX, BCL-2-activated X; GADD45, growth arrest and DNA damage-inducible 45; P53AIP1, p53-regulated apoptosis-inducing protein 1; P53R2, p53-inducible ribonucleotide reductase small subunit 2-like; SFN, stratifin.

arginine alleles and those who carry two proline alleles. This pattern of risk, combined with the observation that none of the studies on which this analysis was based included more than 200 cases, suggests that the result should be interpreted with some caution. Much larger studies will be required to confirm this observation.

The past 12 months have seen the publication of empirical, genome-wide association studies reporting common susceptibility loci for breast cancer[87–89], colorectal cancer[90,91], lung cancer[92,93], malignant melanoma[94] and prostate cancer[95–97]. None of the loci reported in these studies have been close to *TP53*.

The other cancer-related phenotype that has been studied in relation to the codon 72 polymorphisms is prognosis or response to treatment. An earlier median age of onset of squamous cell carcinoma of the head and neck, hereditary non-polyposis colorectal cancer and oral cancer has been reported in patients homozygous for p53-P72 (REFS 98,99). Patients with breast, lung or head and neck cancer with the p53-R72 genotype were reported to have higher response rates and survival after receiving chemotherapy and radiotherapy[80,100–102]. However, an extensive assessment of overall survival in a group of 619 lung cancer patients found no evidence for an effect of the codon 72 polymorphism on prognosis[103]. In general, evaluation of survival studies is affected by problems similar to those described for cancer risk association studies and, as yet, no large studies with statistically robust results have reported significant associations.

It remains plausible that the codon 72 polymorphism is a modifier of cancer risk or age at onset when considered in the context of other genetic variations (such as the MDM2-309 polymorphism, discussed below) and cancer-associated mutations in other genes. The phenotypical effect of the codon 72 variants in conjunction with deleterious mutations in the p53 DBD has been investigated by several research groups. The molecular rationale for examining the combined effect on cancer risk or prognosis is that the codon 72 polymorphism in *cis* with dysfunctional missense mutations might affect properties of the mutant p53 protein. Tumour-associated mutants that alter the conformation of p53 can bind to the pro-apoptotic transcription factor p73α, inhibiting its activity. This acquired deleterious property is enhanced when the somatic mutation is in *cis* with R72, increasing the disruption of p73α apoptotic activity[51,104]. Families with Li–Fraumeni syndrome are a naturally occurring informative cohort to assess the codon 72 polymorphism in relation to DBD mutant p53 and some studies have reported that the age of onset and/or survival are affected by the residue at position 72, but there are inconsistencies as to which variant is beneficial[105–107].

Codons 217 and 360 (V217M and G360A)

V217M (resulting from a G>A transition) is the only validated coding polymorphism that is located in the DBD of p53 (FIGS 3,5); thus, in principle, it could dramatically affect the activity of p53. Functional studies

have been limited to transactivation assays in yeast[59], which indicate that this polymorphism results in little loss of activity (FIG. 4). The genes that show the most variation in activation are *CDKN1A*, *BAX* and *PMAIP1* (also known as NOXA), but the p53-M217 variant leads to greater expression of these genes than the more common p53-V217 variant. Extrapolating from this result, one can speculate that the V217M polymorphism might be protective against cancer. However, as seen with P47S, results in one experimental system might not accurately predict results *in vivo* and more extensive functional studies in a mammalian system would be required before proposing this as a protective or even significant variant that would be worthy of further study in a clinical setting.

G360A is located in the linker region adjacent to the tetramerization domain of p53 (FIGS 3,5). Again, the functional data for this polymorphic variant have been provided by transactivation studies in yeast[59], which showed a slight decrease in the transactivation of *BAX*, *MDM2* and *P53AIP*, and a more marked decrease in stratifin (*SFN*, also known as 14-3-3 sigma) and *GADD45* (growth arrest and DNA damage-inducible 45) (FIG. 4). These results might indicate an attenuated DNA damage response but, again, confirmation in a mammalian system is required.

Polymorphisms in p53 pathway genes

Many cellular proteins interact with or are under the control of p53 (FIG. 1). Polymorphisms in any of these proteins might influence cancer risk or synergize with p53 polymorphisms or mutations to modify cancer risk. Several p53 pathway genes have been found to contain polymorphisms of potential clinical interest (TABLE 2). These include both well-characterized p53 pathway genes, such as *MDM2,* and genes whose links to the p53 pathway have only recently been elucidated such as *NQO1* (NADPH dehydrogenase quinone 1)[108]. The most well-characterized polymorphisms are discussed below.

MDM2 SNP309. MDM2 is overexpressed in approximately 10% of cancers, and mouse models that overexpress MDM2 are more susceptible to cancer development[109]. Although a complete lack of MDM2 in *Mdm2*$^{-/-}$ mice is embryonic lethal[110,111], this can be overcome by concomitant knockout of *Trp53*, and modest reductions in MDM2 can be protective against cancer[112]. Thus, a polymorphism with even a small effect on MDM2 expression levels could have a measurable effect on the cancer phenotype.

Although several polymorphisms have been identified in *MDM2*, a T>G SNP at nucleotide 309 in the first intron has been the most intensively characterized polymorphism[113]. An assessment of the growing literature on MDM2 SNP309 (see Supplementary Information S1 (table)) is beyond the scope of this Review; however, a few salient points are mentioned here. The T allele is the ancestral allele, but the high frequency of the G allele in many populations indicates that selective pressure favours the G variant, thus fixing it in the population[114], despite this allele being a potential risk variant for cancer.

Bond *et al.* demonstrated that the 309G variant is bound more efficiently by the transcription factor SP1 (a co-activator for many hormone receptors) than the 309T variant[113]. The oestrogen receptor also binds the *MDM2* promoter in the region of SNP309. Hu and colleagues determined that, in oestrogen-responsive cells, oestrogen preferentially induced the transcription of MDM2 from the SNP309 promoter and that the levels of MDM2 in SNP309G/G cells were higher than in heterozygous SNP309G/T or SNP309T/T cells[22]. As a result, individuals carrying the SNP309G variant might have higher levels of MDM2 than individuals with the SNP309T variant when oestrogen levels are higher. This increase in MDM2 would lead to p53 suppression and might lead to a higher risk of hormone-related cancers.

This hypothesis is supported by several studies that show an earlier age of onset or an increased susceptibility to breast cancer in premenopausal women with the MDM2 SNP309G variant[113,115,116]. However, some studies have shown an association between this polymorphism and increased cancer risk, or treatment outcome in both males and females, whereas other studies show no association of this polymorphism with cancer

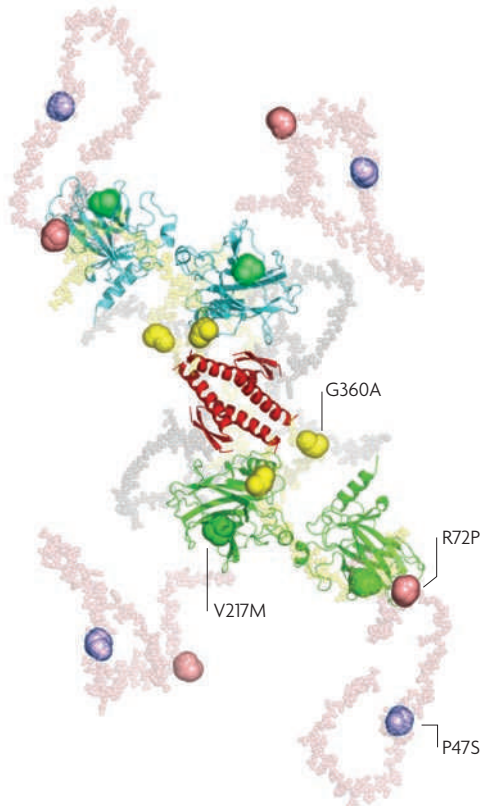


Figure 5 | **Three-dimensional structure of p53 in its tetrameric form.** The four coding sequence polymorphisms discussed in the text are indicated. Glycine 360 (polymorphic variant is alanine) is displayed in yellow; valine 217 (polymorphic variant is methionine) is displayed in green; arginine 72 (polymorphic variant is proline) is displayed in pink; proline 47 (polymorphic variant is serine) is shown in purple. Figure is modified, with permission, from REF. 160 © (2007) National Academy of Sciences, USA. Figure courtesy of A. Fersht, University of Cambridge, UK.

Table 2 | **Polymorphisms in p53 pathway genes**

Gene	Relation to p53	Cellular function	Polymorphisms potentially associated with cancer	Disease association	Potential or associated functional change	Refs
TP53	Encodes p53	Tumour suppressor: induces cell cycle arrest and apoptosis and senescence in response to stress	R72P P47S Intron 3 16 bp insertion	p53 often mutated in cancer Germline *TP53* mutations in Li–Fraumeni syndrome Knockout mice are susceptible to tumours	See text	1–3
MDM2	Regulator of p53 protein Induced by p53	Core control of p53 levels, activity and localization within the cell	SNP309 (promoter region)	Homozygous knockout in mice is embryonic lethal, but can be rescued by simultaneous knockout of *Trp53*	Enhanced SP1 binding site (G variant) increases transcription by ER Higher MDM2 levels suppress p53, reducing tumour suppression	113
CDKN1A	Induced by p53	Regulator of cell cycle progression	S31R R84Q	Knockout mice develop normally, but develop tumours early	Reduced G1 arrest in response to DNA damage, which could alter responses to therapy	131, 141, 142
ATM	Phosphorylates (and activates) p53 in response to DNA damage	Recognizes DNA damage and activates p53 and DNA damage response pathways	D1853N	Homozygous mutant *ATM* results in AT, which confers a 100-fold increased cancer risk	Decreased activation of p53	122, 171
BAX	Induced by p53 Interacts with other apoptotic proteins at the mitochondria	Forms a heterodimer with BCL-2 , activating MOMP and the mitochondrial apoptotic pathway	g>a (125 bp upstream of start site)	Lower levels of BAX in multiple tumour types predict poor response to chemotherapy		148, 172
TP53I3	Induced by p53	Oxidoreductase-like protein involved in apoptosis	Promoter microsatellite		Increased induction by p53 with increased microsatellite length improves apoptotic response	145
AKT1	Activates MDM2, which inhibits p53	A Ser–Thr kinase induced by growth factors that inhibits apoptosis by activating MDM2	The haplotype (five SNPs in *AKT1*) in Caucasian populations results in higher levels of AKT1 than in African populations	Some polymorphisms are associated with schizophrenia Knockout mice are smaller (*Akt1*$^{-/-}$)	Lower levels of AKT1 sensitize cells to apoptotic signals	173, 174
TP73	Induces p53 Induced by p53 Physically interacts with p53	p53-like protein, shares many p53 functions, including induction of apoptosis and cell cycle arrest	Two linked SNPs in the non-coding region of exon 2		In combination with MDM2 SNP309 increases cancer risk	175, 176
NQO1	Stabilizes p53 by an MDM2-independent mechanism	Protects against oxidative stress and carcinogenesis	P187S	NQO1 variant associated with susceptibility to various cancers Altered expression in many tumours and Alzheimer's disease	P187S causes loss of NQO1 activity, and its tumour- suppressive and antioxidative effects, affecting cancer risk and response to therapy	108, 177, 178

AT, ataxia–telangiectasia; *ATM*, ataxia–telangiectasia mutated; bp, base pair; *CDKN1A*, cyclin-dependent kinase inhibitor 1; ER, oestrogen receptor; MOMP, mitochondrial outer membrane permeabilization; NQO1, NADPH dehydrogenase quinone 1; SNP, single nucleotide polymorphism; TP53I3, tumour protein p53 inducible protein 3.

risk, even in premenopausal women (Supplementary Information S1 (table)). Clearly, additional factors such as the effect of p53 mutations add complexity to this model[105]. The first report on the functional significance of the MDM2 SNP309G polymorphism showed an association between this variant and accelerated tumour formation in patients with Li–Fraumeni syndrome[113], a finding that has been repeated in subsequent studies[106,117–119]. Modifications in p53 could be masked by altered expression of MDM2; for example, in a study by Boersma *et al.*, mutant p53 predicted poor survival in patients who were homozygous for the SNP309T allele, but not for patients who carried the SNP309G allele[120].

ATM (ataxia–telangiectasia mutated). ATM is a protein kinase that plays an important part in activating the cellular response to DNA damage, particularly DNA double-strand breaks[121]. ATM phosphorylates a range of proteins, including p53 (at S15 and T18)[17,122] and CHK2, which in turn phosphorylates p53 at S20. These modifications activate p53 by preventing its interaction with MDM2 (REFS 123,124). ATM also phosphorylates MDM2 and MDM4, inhibiting their interactions with p53 (REFS 125,126).

ATM was identified as the gene mutated in the inherited, recessive neurodegenerative disease ataxia–telangiectasia, which is characterized by ataxia and a marked increase in cancer incidence, especially lymphomas and leukaemia[122]. There is disagreement as to whether the heterozygous carriers of the mutant gene have an increased risk of cancer, especially breast cancer: evidence suggests that the type of ATM mutation might influence the degree of risk experienced by heterozygous carriers because some mutants have dominant-negative properties[127].

An ATM polymorphism that has been reported to influence cancer risk, disease progression, severity and disease-free survival is D1853N (rs1801516), a common variant present in 15–18% of the population[128]. This polymorphism might be a risk factor for hereditary non-polyposis colorectal cancer[129], with a reported alteration in splicing attributed to this variant[130], but these results remain to be confirmed.

p21. p21 is a cyclin-dependent kinase inhibitor that prevents cell cycle progression at G1. It is induced by wild-type p53 following DNA damage[131], and p21-null cells are deficient in DNA damage-induced, p53-mediated G1 arrest[132]. Although p21 plays a key part in cell cycle regulation[131,132], somatic mutations in *CDKN1A* are not associated with human cancer, although p21-null mice have been shown to precociously develop tumours[133]. Thus, p21 polymorphisms might have little effect in modulating the risk of developing cancer. A deficiency in DNA damage-induced cell cycle arrest might alter the susceptibility of tumour cells to radiotherapeutic and chemotherapeutic agents because these agents target cancer cells by causing DNA damage.

Several polymorphisms in p21 have been described and the S>R polymorphism at codon 31 (REF. 134) in the CDK inhibitor domain is currently receiving the most attention. The frequency of this polymorphism varies widely in different population groups and, as is the case for the p53 polymorphisms, there are conflicting reports on the association of this polymorphism with cancer risk[135–139]. It seems likely that the discrepancies are due in part to factors associated with experimental design (for example, inadequate sample sizes and poorly defined populations) and possibly to variation in the effects of p21 on cancer development in different tissues and under different kinds of carcinogenic stress. The functional consequences of the S31R polymorphism were studied experimentally, but the kinase inhibition and growth suppression activities of the variants did not differ[134,140]. One study in peripheral leukocytes from donors reported an association of this polymorphism with reduced levels of *CDKN1A* mRNA that was amplified when combined with p53-P72 (REF. 141). Other polymorphisms at the *CDKN1A* locus have been described, but these do not directly alter the functional domains of p21 (REF. 142).

p53 response element polymorphisms. Polymorphisms have been reported in the promoters of p53 target genes with both canonical and non-canonical response elements. Slight sequence variations in response elements can alter the responsiveness of genes to p53 by up to 1,000-fold[143]. Tomso *et al.* recently identified more than 200 SNPs in putative p53 response elements using a bioinformatics approach and high-resolution SNP maps[144]. Eight of these SNPs were selected for functional evaluation, on the basis of their proximity to genes involved in classical p53 response pathways. In all eight cases, the polymorphic variants altered the levels of transactivation by p53 in yeast reporter assays and six of the genes were inducible by endogenous p53 in cell culture assays. Although none of these genes had previously been identified as p53 target genes, four are known to have a potential role in tumorigenesis[144].

The promoter of the p53 target gene, *TP53I3* (tumour protein 53 inducible 3; also known as *PIG3*), contains a non-canonical p53 response element containing a polymorphic pentanucleotide microsatellite region. The number of pentanucleotide repeats is directly proportional to the inducibility of *TP53I3* (REF. 145). The protein encoded by *TP53I3* is an oxidoreductase that plays a part in p53-mediated apoptosis. Increased inducibility of *TP53I3* could be protective against cancer because cells might more readily undergo apoptosis after stress. Although one study has shown no association with a low number of the *TP53I3* pentanucleotide repeats in breast or lung cancer[146], another study reported a correlation between higher-grade bladder cancer and shorter pentanucleotide repeats[147].

Polymorphisms have been identified in the promoter regions (although not in the p53 response elements) of *BCL2* and *BAX*[148], that either decrease (in the case of *BCL2*) or increase (in the case of *BAX*) the risk for squamous cell carcinoma of the head and neck.

Concluding remarks

Elucidating the effect of p53 pathway polymorphisms on cancer risk is a challenge. Traditional studies that investigated individual *TP53* polymorphisms in case–control studies of limited sizes have not given definitive answers and new approaches are required. High-throughput methodologies and consortium studies investigating large numbers of individuals will provide the power these investigations require and a more unbiased approach. Recent publications verify that, when large populations are examined, polymorphisms that have a low but significant effect on cancer risk can be identified[149].

Investigating gene polymorphisms of potential interest with genetically engineered knock-in mouse models might offer a useful approach to the difficulties

that arise from genetic diversity in human populations. It allows testing of *in vitro* hypotheses in an *in vivo* context, with the caveat that some components of the p53 network will differ in mice and men, so the design of the models has to be based on knowledge of the underlying molecular biology[26,150–152]. Knock-in mouse strains to investigate the p53 codon 72 polymorphism and a strain harbouring a *CHEK2* variant linked to breast cancer susceptibility are available[153,154], and undoubtedly other mouse models, for example, strains to investigate the MDM2 SNP309, will be forthcoming. Animal models have an increasing potential as strategies for exploring the functional effect of selected, potentially important SNPs in human disease, particularly because more sophisticated methods have been developed for genetic engineering in mice and the models have become more relevant to human disease processes[152,155,156].

Increasingly, the combined effects of a polymorphism and environmental exposures such as tobacco smoke, and the effect of several polymorphisms that act together on cancer risk or clinical course, are under study. A conspicuous example is the reported effect of MDM2 SNP309 in conjunction with p53 variants and smoking[157,158]. Laboratory-based research to elucidate the molecular basis of phenotypes associated with polymorphisms would profit from independent unbiased validation of interesting new findings; from a reassessment of early observations, using multiple models, including primary cells; and from corroboration by different methodologies. Biological evaluation of p53 variants in a well-designed mammalian cell-based assay that encompasses comprehensive functional analyses, akin to the pioneering approach developed to assess *BRCA2* mutations in mouse embryonic stem cells, could be of benefit[159].

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DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
BAX | *BBC3* | *CDKN1A* | *GADD45* | *MDM2* | *MDM4* | *NQO1* | *PERP* | *PMAIP1* | *PML* | *SFN* | *THBS1* | *TP53* | *TP53I3* | *TP63*
OMIM: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
ataxia–telangiectasia | Li–Fraumeni
UniProtKB: http://www.uniprot.org
ATM | HIPK2 | iASPP | NF-Y | p53 | P53AIP | p73 | PIN1

FURTHER INFORMATION

M. Hollstein's homepage: http://www.leeds.ac.uk/light/staff/hollstein_m.html
Database of germline p53 mutations: http://www.lf2.cuni.cz/projects/germline_mut_p53.htm
IARC *TP53* mutation database: http://www-p53.iarc.fr/
International HapMap Project: http://www.hapmap.org/
NCBI SNP database: http://www.ncbi.nlm.nih.gov/projects/SNP/
NIH genetic association database: http://geneticassociationdb.nih.gov/cgi-bin/index.cgi
p53 Knowledgebase: http://p53.bii.a-star.edu.sg/index.php
***TP53* Web Site:** http://p53.free.fr/

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