

# TP53 Mutations in Human Cancers: Origins, Consequences, and Clinical Use

Magali Olivier<sup>1</sup>, Monica Hollstein<sup>2</sup>, and Pierre Hainaut<sup>1</sup>

<sup>1</sup>Group of Molecular Carcinogenesis, International Agency for Research on Cancer, 150 Cours Albert Thomas, 69372 Lyon Cedex 08 France

<sup>2</sup>LIGHT Laboratories, University of Leeds, United Kingdom

Correspondence: [hainaut@iarc.fr](mailto:hainaut@iarc.fr)

Somatic mutations in the TP53 gene are one of the most frequent alterations in human cancers, and germline mutations are the underlying cause of Li-Fraumeni syndrome, which predisposes to a wide spectrum of early-onset cancers. Most mutations are single-base substitutions distributed throughout the coding sequence. Their diverse types and positions may inform on the nature of mutagenic mechanisms involved in cancer etiology. TP53 mutations are also potential prognostic and predictive markers, as well as targets for pharmacological intervention. All mutations found in human cancers are compiled in the IARC TP53 Database (<http://www-p53.iarc.fr/>). A human TP53 knockin mouse model (Hupki mouse) provides an experimental model to study mutagenesis in the context of a human TP53 sequence. Here, we summarize current knowledge on TP53 gene variations observed in human cancers and populations, and current clinical applications derived from this knowledge.

Genetic variations in the tumor suppressor gene *TP53* (OMIM #191117) contribute to human cancers in different ways. First, somatic mutations are frequent in most cancers (Hollstein et al. 1991). The antiproliferative role of p53 protein in response to various stresses and during physiological processes such as senescence makes it a primary target for inactivation in cancer (Levine 1997). The main modes of *TP53* inactivation are single-base substitution and loss of alleles, with inactivation by viral or cellular proteins playing a major role in specific cancers (Tommasino et al. 2003). Second, inheritance of a *TP53* mutation causes predisposition to early-onset cancers including breast

carcinomas, sarcomas, brain tumors, and adrenal cortical carcinomas, defining the Li-Fraumeni (LFS) and Li-Fraumeni-like (LFL) syndromes (Li et al. 1988; Olivier et al. 2003). Third, *TP53* is highly polymorphic in coding and noncoding regions and some of these polymorphisms have been shown to increase cancer susceptibility and to modify cancer phenotypes in *TP53* mutation carriers (Whibley et al. 2009).

Whereas tumor suppressors are commonly inactivated by frameshift or nonsense mutations, most *TP53* mutations are missense and cause single amino-acid changes at many different positions. Mutations are thus diverse in their type, sequence context, position, and structural

---

Editors: Arnold J. Levine and David Lane  
Additional Perspectives on The p53 Family available at [www.cshperspectives.org](http://www.cshperspectives.org)

Copyright © 2010 Cold Spring Harbor Laboratory Press; all rights reserved; doi: 10.1101/cshperspect.a001008  
Cite this article as *Cold Spring Harb Perspect Biol* 2010;2:a001008

M. Olivier, M. Hollstein, and P. Hainaut

impact, making it possible to identify mutation patterns in relation with cancer type and etiology. The occurrence of special mutation patterns may inform on the nature of the mutagens that have caused them, making TP53 an interesting gene to analyze in the realm of molecular epidemiology.

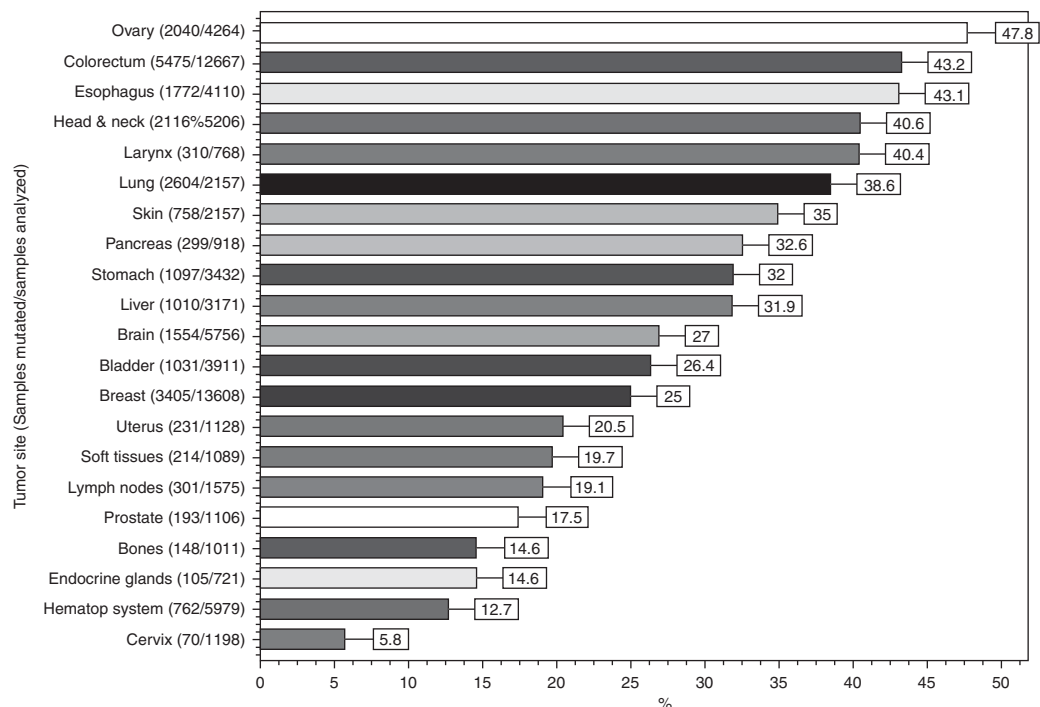
Data on mutation prevalence in human cancer can be conveniently accessed through the IARC TP53 database (<http://www-p53.iarc.fr/>), a resource that compiles all TP53 gene variations reported in human cancers with annotations on tumor phenotype, patient characteristics, and structural and functional impact of mutations (Petitjean et al. 2007b). Recently, it has become possible to confront these observations with experimental data generated in a novel mouse model, the HupKi mouse, that contains a human TP53 sequence at the mouse TP53 locus and recapitulates the effects of environmental mutagens in a human sequence context (Luo et al. 2001). In this article, we review the current knowledge on the origin,

causes, and consequences of TP53 variations and mutations in cancer and we discuss their significance as biomarkers in epidemiology and in the clinics.

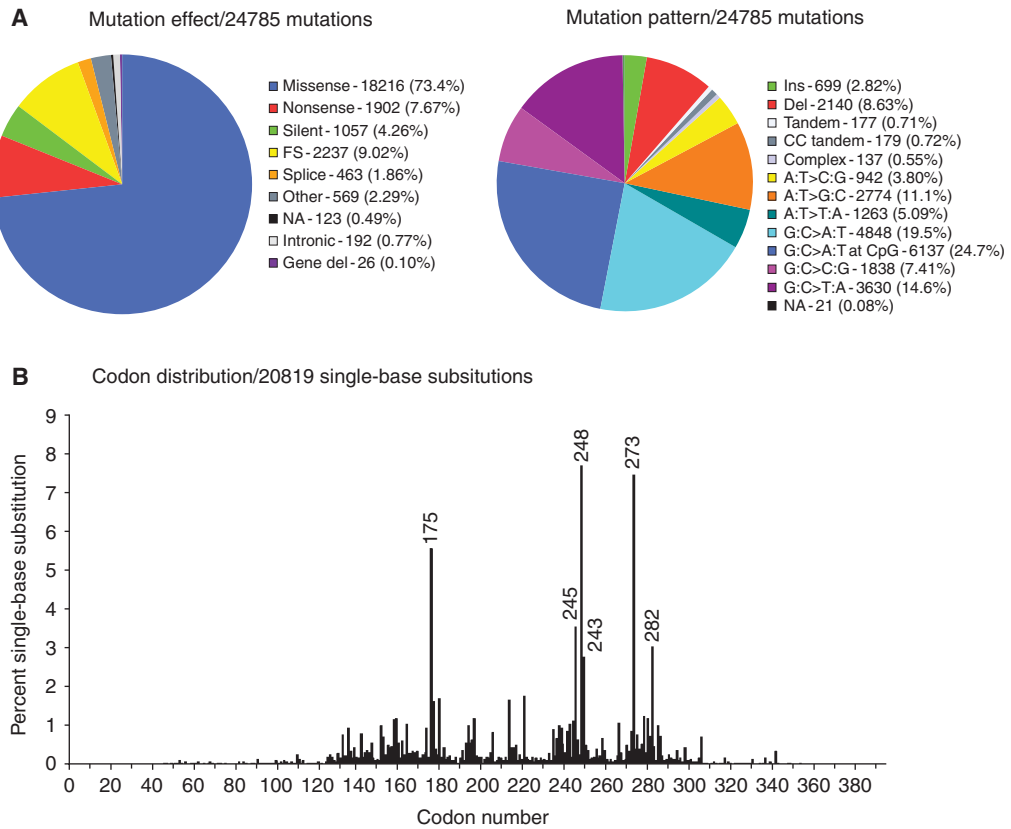
## TP53 VARIATION LANDSCAPES IN HUMAN CANCERS AND POPULATIONS

### Somatic Mutations

Somatic TP53 mutations occur in almost every type of cancer at rates from 38%–50% in ovarian, esophageal, colorectal, head and neck, larynx, and lung cancers to about 5% in primary leukemia, sarcoma, testicular cancer, malignant melanoma, and cervical cancer (Fig. 1). Mutations are more frequent in advanced stage or in cancer subtypes with aggressive behavior (such as triple negative or HER2-amplified breast cancers) (Wang et al. 2004a; Wang et al. 2004b; Langerod et al. 2007). In cancers with low mutation rates, p53 is often inactivated by alternative mechanisms. This is



**Figure 1.** TP53 mutations prevalence in sporadic cancers. The proportion of tumors with somatic TP53 mutations is indicated. Data from IARC TP53 Database (R13, November 2008)(Petitjean et al. 2007b).



**Figure 2.** Type of somatic *TP53* mutations in human cancers. (A) Pie charts showing the proportion of the different types of *TP53* somatic mutations found in all human cancers. (B) Histogram displaying the position of somatic point mutations in the coding sequence of the *TP53* gene. Data from the IARC *TP53* Database (R13, November 2008)(Petitjean et al. 2007b).

the case for cervical cancer in which p53 is targeted for degradation by HPV E6 (Tommasino et al. 2003) or for sarcoma that overexpress amplified HDM2.

Based on studies that examined the whole coding sequence, 86% of mutations cluster between codons 125 and 300, corresponding mainly to the DNA binding domain (Fig. 2). Most mutations in this region are missense (87.9%). In contrast, outside this region, missense mutations represent only about 40%, the majority of mutations being nonsense or frameshift. Among single-base substitutions, about 25% are C:G>T:A substitutions at CpG sites. CpG dinucleotides mutate at a rate 10 times higher than other nucleotides, generating transitions (Jones et al. 1992). About 3%–5%

of cytosines in the human genome are methylated at position 5' by a postreplicative mechanism that is restricted to CpG dinucleotides and is catalyzed by DNA methyltransferases. The 5' methylcytosine (5mC) is less stable than cytosine and undergoes spontaneous deamination into thymine at a rate five times higher than the unmethylated base. This process is enhanced by oxygen and nitrogen radicals, leading to a higher load of CpG transitions in cancers arising from inflammatory precursors such as Barrett's mucosa or ulcerative colitis (Schmutte et al. 1996; Ambs et al. 1999; Vaninetti et al. 2008). Among the 22 CpG of the DNA-binding domain (DBD), three hotspot codons (175, 248, and 273) represent 60% of CpG mutations and another five residues

M. Olivier, M. Hollstein, and P. Hainaut

(196, 213, 245, 282, and 306) account for 26% of these mutations. The lack of mutations at other CpG sites reflects the fact that substitution at these residues does not generate a dysfunctional protein. Although the same CpG hotspot mutations occur in many cancer types, other types of mutations tend to show differences from one cancer to the other. Some of these differences have been linked to the effect of specific mutagens. Geographic differences have also been reported in relation with environmental exposures. These aspects have been extensively discussed in other reviews (Hainaut et al. 2000; Olivier et al. 2004) and some examples are briefly discussed later in this article.

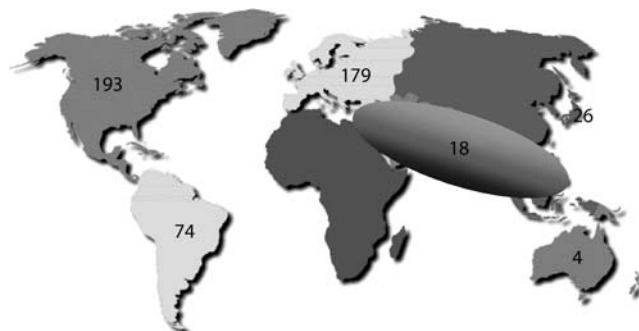
### Germline Mutations: Li-Fraumeni Syndrome

*TP53* germline mutations are the underlying cause of LFS, a familial clustering of early onset tumors including sarcomas, breast cancers, brain tumors, and adrenal cortical carcinomas (Li et al. 1988; Malkin et al. 1990). Over the past 20 years, *TP53* germline mutations have been detected in about 500 families or individuals with complete or partial LFS features (the latter defined as Li-Fraumeni-like, LFL) (Olivier et al. 2003). LFS/LFL has been generally considered as a rare syndrome (Eeles 1995). However, screening for *TP53* germline mutation in patients with early onset breast cancer and unselected for familial history has shown *TP53* mutations in 2%–3% of the cases (Laloo et al. 2006), whereas screening of 525 patients with any kind of

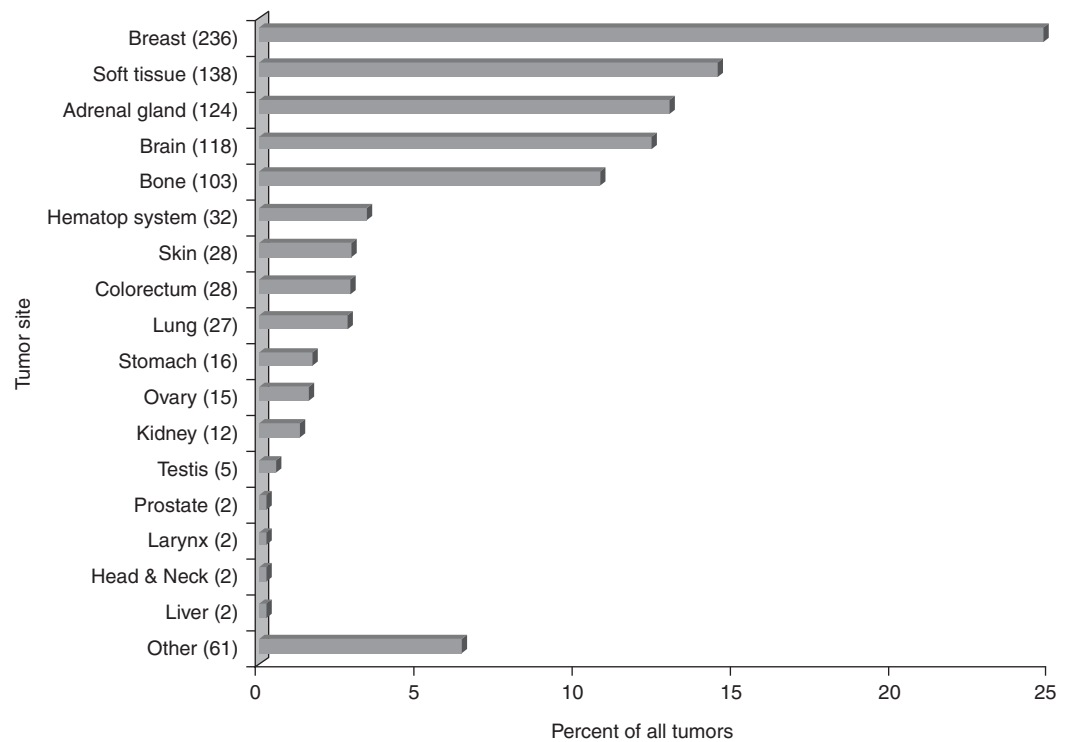
cancer family history has identified 91 (17.3%) *TP53* mutations (Gonzalez et al. 2009). Based on these results, *TP53* mutation may contribute to up to 17% of all familial cancer cases. Studies in southern Brazil have identified many families with a founder mutation (R337H) (Fig. 3). Thus, *TP53* germline mutations may be more common than previously recognized, occurring in about 1 in 5,000 to 1 in 20,000 births (Laloo et al. 2006) (Gonzalez et al. 2009).

Breast cancer and soft tissue and bone sarcoma account for over 50% of tumors in *TP53* mutation carriers, followed by adrenocortical carcinomas and brain tumors (Fig. 4). Other cancers include hematological malignancies, gastric, colorectal, and ovarian cancers, occurring at earlier ages than in the general population (Olivier et al. 2003). Rarer cancers associated with *TP53* germline mutation are choroid plexus carcinoma or papilloma before the age of 15, Wilms' tumor, and malignant phyllodes tumors (Birch et al. 2001; Gonzalez et al. 2009).

The distribution of germline mutations is similar to somatic mutations, with mostly missense mutations (77%) located at the same hotspots. The proportion of CpG mutations (54% vs. 25% in somatic mutations) may reflect the spontaneous nature of germline mutations. Genotype–phenotype correlations suggest that the most significant defect is loss of function because large deletions encompassing the whole *TP53* gene have been found in LFS families with aggressive features (Bougeard et al. 2003).



**Figure 3.** Geographic distribution of germline *TP53* mutations. Number of *TP53* germline mutation carrier families in each world region. Data from the IARC *TP53* Database (R13, November 2008) (Petitjean et al. 2007b).



**Figure 4.** Tumor spectrum in individuals with a germline *TP53* mutation. The proportion of specific tumor types among all tumors reported in confirmed *TP53* germline mutation carriers is indicated. Data from IARC *TP53* Germline Database (R13, November 2008, <http://www-p53.iarc.fr/Germline.html>).

### TP53 Polymorphisms

Over 80 *TP53* polymorphisms have been identified and validated in human populations (IARC *TP53* Database, R13). The majority (90%) are located in introns, outside splice sites, or in noncoding exons. Few of them have been tested in functional assays or studied for effects on cancer risk. Among 18 exonic SNPs (Table 1), five are silent and seven are located after the stop codon in exon 11. Four exonic polymorphisms alter the protein sequence and have only subtle effects on transactivation capacity as measured in yeast-based assays (Kato et al. 2003).

V217M is the only nonsilent polymorphism in the DBD. In yeast functional assays, the rare variant shows increased transactivation of some p53 response elements (CDKN1A, BAX, and PMAIP1) and may thus be protective against cancer. G360A, located next to the tetramerization

domain (TET) showed slightly reduced activity that may result in an increased cancer risk. However, the impact of these SNP has not been investigated in clinical or epidemiological studies.

P47S has been reported in African populations only, at a frequency of approximately 5% (Felleo-Bosco et al. 1993). This polymorphism may affect phosphorylation at S46, which enhances p53-mediated apoptosis (Pistritto et al. 2007; Oda et al. 2000). Phosphorylation of S46 by p38 and homeodomain-interacting protein kinase 2 (HIPK2) requires a proline adjacent to S46. Presence of a serine may thus alter kinase recognition. However, functional data in different experimental systems have proved inconsistent. In mice, blocking S46 phosphorylation has only modest phenotypical consequences (Feng et al. 2006; Toledo et al. 2006). It is possible that this SNP may impact on p53 function only under specific stress conditions. Its

M. Olivier, M. Hollstein, and P. Hainaut

**Table 1.** Selected polymorphisms in TP53 gene

Exon/Intron	g_Description	c_Description	AvgHet	SNPlink	Effect
4-exon	g.11333C>A	c.102C>A	-	11575998	silent
4-exon	g.11339G>A	c.108G>A	0.012738	1800370	silent
4-exon	g.11370C>T	c.139C>T (P47S)	0.029329	1800371	missense
4-exon	g.11446C>G	c.215C>G (R72P)	0.492248	1042522	missense
6-exon	g.12708A>G	c.639A>G	0.023526	1800372	silent
6-exon	g.12718G>A	c.649G>A (V217M)	0.0098	35163653	missense
10-exon	g.16970G>C	c.1079G>C (G360A)	0.009892	35993958	missense
11-exon	g.18096C>T	c.1182+105C>T	0.019761	35919705	silent
11-exon	g.18196G>A	c.1182+205G>A	0.024239	16956880	silent
11-exon	g.18305G>A	c.1182+314G>A	0.019569	34486624	noncoding
11-exon	g.18319G>A	c.1182+328G>A	0.010246	17881366	noncoding
11-exon	g.18476G>A	c.1182+485G>A	0.091723	4968187	noncoding
11-exon	g.18560_18561del2	c.1182+569_1182+570del2	0.436246	17886358	noncoding
11-exon	g.18604C>A	c.1182+613C>A	0.023872	17879353	noncoding
11-exon	g.18817G>A	c.1182+826G>A	0.136576	17884306	noncoding
11-exon	g.18877G>A	c.1182+886G>A	0.009892	35659787	noncoding
11-exon	g.19166A>C	c.1182+1175A>C	0.00555	-	intronic
3-intron	g.11259_11274del16	c.96+41_96+56del16	0.5	17878362	intronic
2-intron	g.11117C>G	c.74+38C>G	0.468059	1642785	Intronic
9-intron	g.16143G>A	c.994-742G>A	0.401235	1641549	Intronic
9-intron	g.14496C>T	c.993+431C>T	0.3432	1642791	Intronic
7-intron	g.13511T>G	c.782+92T>G	0.310766	12951053	Intronic
9-intron	g.15185T>C	c.993+1120T>C	0.256705	12949655	Intronic
7-intron	g.13491C>T	c.782+72C>T	0.246362	12947788	Intronic
4-intron	g.12273G>A	c.376-91G>A	0.212093	2909430	Intronic
6-intron	g.12803A>G	c.672+62A>G	0.210458	1625895	Intronic
4-intron	g.12239T>C	c.376-125T>C	0.157451	9895829	Intronic
3-intron	g.11299C>A	c.97-29C>A	0.15648	17883323	intronic

Complete list available at <http://www-p53.iarc.fr/PolymorphismView.asp>.

Missense polymorphisms are highlighted (amino-acid substitution and codon into brackets).

impact on cancer susceptibility remains to be elucidated.

The P72R polymorphism is the most extensively studied both in experimental and population studies. Sharp ethnic differences have been observed, the P72 allele showing a north–south gradient from 0.17 in Swedish Saamis to 0.63 in Africans (Yorubas). A recent report has shown an association of this SNP with latitude within Asian populations (Shi et al. 2009) and has proposed that allele frequency may be an adaptation to differences in winter temperature. R72 is more active than P72 in transactivating the expression of leukaemia-inhibitory factor (Kang et al. 2009), which is critical for blastocyst implantation, leading to the conjecture that R72 could thus provide a selective advantage by reducing the risk of implantation failure in colder climates.

Codon 72 is located within a proline-rich region and may affect a putative SH3-binding domain. The current consensus from a large number of studies is that R72 is more effective in inducing apoptosis than P72. However, the relevance of these observations for cancer susceptibility is not understood. An early suggestion that individuals homozygous for R72 may be at greatly higher risk for HPV-related cervical cancers (Storey et al. 1998) has not been substantiated by extensive population-based studies (Klug et al. 2001). Many studies have reported associations between the R72P SNP and risk of different cancers, with inconsistent results. Meta-analyses for breast (Schmidt et al. 2007), lung (Matakidou et al. 2003), and other cancers do not support a significant role for this polymorphism in susceptibility.



The P72R SNP has been studied for its impact on prognosis or response to treatment. Many of these studies must be interpreted cautiously as they lack power and rigorous design. There is, however, evidence of a modifier effect on *TP53* mutations, with a bias toward expression of mutant R72 allele in tumors of heterozygous patients with LOH affecting wild-type P72 (Langerod et al. 2002; Bergamaschi et al. 2003; Bonafe et al. 2003; Vikhanskaya et al. 2005; Nelson et al. 2005; Zawlik et al. 2009). Furthermore, experimental studies showed that R72 mutants were more potent in cooperating with EJ-Ras in transforming primary cells and in neutralizing p73 activity (Marin et al. 2000; Bergamaschi et al. 2003). A modifier effect of the R72P SNP has also been reported in germline *TP53* mutation carriers, in whom R72 was associated with earlier age at first diagnosis of cancer (Bougeard et al. 2006).

P72R is in linkage disequilibrium with a polymorphism in intron 3 (PIN3), consisting of a duplication of a 16 bp, GC rich sequence (allele frequency: 0.2 in Caucasians). PIN3 has been associated with increased risk of several cancers (Wang-Gohrke et al. 1998; Wang-Gohrke et al. 1999; Gemignani et al. 2004). The duplicated PIN3 allele is associated with reduced expression of *TP53* mRNA in lymphoblastoid cell lines (Gemignani et al. 2004). Recently, *TP53* germline mutation carriers with duplicated PIN3 were shown to develop cancer, on average, 20 years later than carriers with nonduplicated alleles (Marcel et al. 2009).

Given the complex polymorphic structure of *TP53*, haplotypes may provide more relevant information than individual polymorphisms. In addition, polymorphisms in gene regulating p53 have a critical impact, as for example MDM2 SNP309 polymorphism (See Grochola et al. 2010).

## STRUCTURAL AND FUNCTIONAL IMPACT OF MUTATIONS

### Impact on Protein Structure

The p53 DBD is made of an immunoglobulin-like  $\beta$ -sandwich of two antiparallel  $\beta$ -sheets,

providing a scaffold for a flexible DNA-binding surface (Cho et al. 1994). This surface is formed by two large loops (L2 and L3) stabilized by a zinc atom and a loop-sheet-helix motif (loop L1). Zinc binding (coordinated by H179, C176, C238, and C242) is critical for correct folding, and requires reduction of thiol groups on cysteines. Hotspot mutations are at residues involved either in making contacts with DNA or in supporting the structure of the DNA-binding surface. Mutant proteins have thus been classified as “contact” (e.g., R248 and R273) or “structural” (e.g., R175, G245, R249, and R282) (Joerger et al. 2007). The structure of several common mutants has been elucidated by NMR spectroscopy or X-ray crystallography (Joerger et al. 2005; Joerger et al. 2006). DNA-contact mutants retain the overall architecture of the DBD with loss of a critical DNA contact. They may actively prevent DNA binding if a large hydrophobic side chain is introduced (e.g., S241E, R248W, and C277F). Zinc-binding mutants affect the zinc coordination sphere (e.g., C176F, H179R, and C242F). This category includes R175H, the most frequent hotspot mutant, because introduction of histidine residue causes distortions that directly interfere with zinc binding. Substitutions introducing smaller residues at this position have been shown to be less destabilizing with partial retention of function (Bullock et al. 2000). Structural mutants cause distortions that create internal cavities or surface crevices in the protein scaffold, inducing conformational changes in the DNA binding surface. Overall, these studies highlight the structural heterogeneity of mutant proteins, with possible consequences on their biochemical and biological properties.

### Impact on Transcriptional Activities

p53 regulates transcription through specific binding to response elements in the promoters or introns of target genes (Riley et al. 2008). p53REs are variations of a consensus constituted by two decamers (PuPuPuC(A/T)(T/A)GPyPyPy) separated by a spacer of variable length (El-Deiry et al. 1992). These sequence variations affect p53 binding affinity and may contribute to shape the repertoire of genes

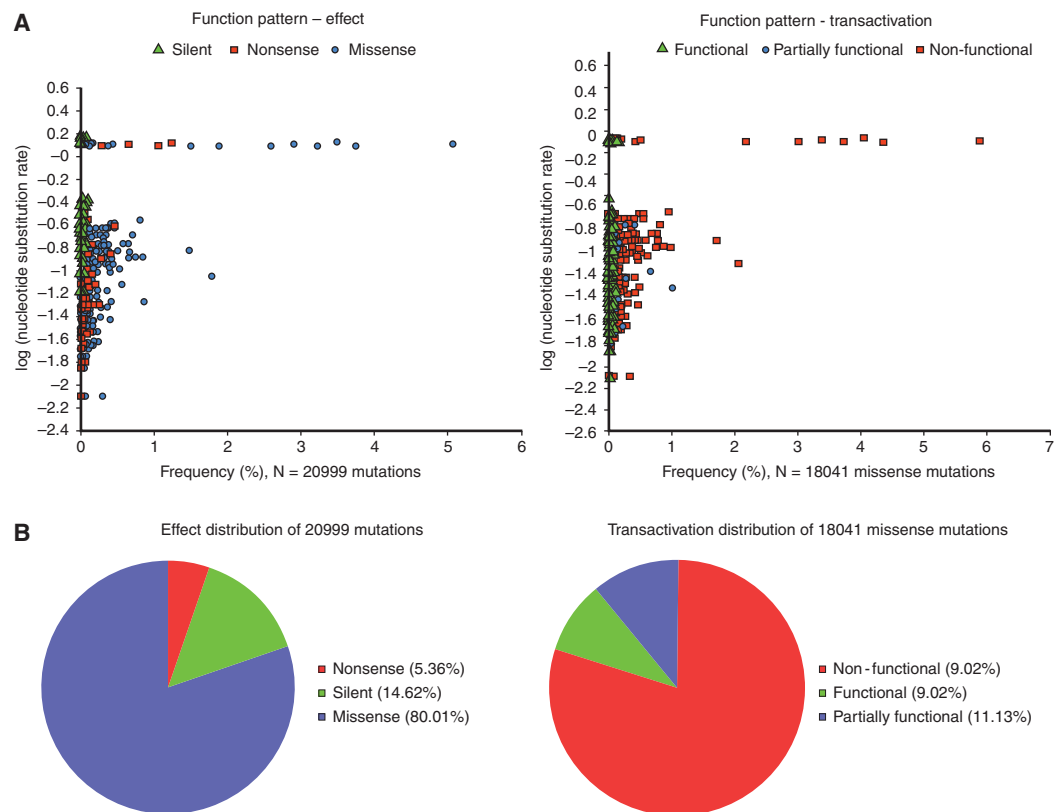
M. Olivier, M. Hollstein, and P. Hainaut

activated by p53 in response to specific signals (Inga et al. 2002). Recent genome-wide analyses of p53 binding sites suggest that hundreds of genes may be up- or down-regulated by p53 (Smeenk et al. 2008). Kato et al. (2003) have used a yeast-based functional assay to analyze the transactivation of 2314 different missense mutants on eight p53REs. All hotspot mutants displayed loss of function on all tested p53REs (Fig. 5). However, several rare mutants retained partial activity on all REs or even full activity on some REs. A small category of mutants even showed increased activity compared with wild-type p53 (so-called “supertrans mutants,” often occurring in loop L1, which is not a frequent

target for cancer-related mutations). Using a different type of yeast based assay, Inga et al. (2002) further showed that differential transactivation by wild-type and mutant proteins depends on both protein levels and target sequence.

### Dominant–Negative and Gain-of-Function Effects

Mutant p53 proteins often accumulate in the nucleus of in situ and metastatic cancer cells, suggesting an oncogenic effect in addition to loss of wild-type suppressor function. Experimentally, several hotspot mutants have been



**Figure 5.** Functional impact of somatic *TP53* mutations in human cancers. (A) Scatter plots showing the frequency (x-axis) of single amino-acid substitutions in relation to their effect (left panel) or functional impacts on transactivation (right panel), and expected substitution rates (y-axis in log). Each point represents a single amino-acid substitution that is shaped and colored according to the mutation effect or functional impact. (B) Pie charts displaying the proportion of all somatic single amino-acid substitutions according to their effect (left panel) or functional impacts on transactivation (right panel). Data from the IARC *TP53* Database (R13, November 2008)(Petitjean et al. 2007b).





shown to cooperate with oncogenes for cellular transformation (Hinds et al. 1990), setting the concept of gain-of-function (GOF). Alternatively, mutant p53 proteins may exert dominant-negative effects (DNE) over wild-type p53. Indeed, transcriptional activity relies on the formation of p53 tetramers (dimers of dimers) and incorporation of mutant p53 in these structures may preclude their activity.

DNE has been experimentally studied in yeast and human cell assays, generating data on the DNE effects for over 200 mutants (Brachmann et al. 1996). Analysis of the IARC TP53 database showed that mutants with DNE toward p53-RE from WAF-1 and RGC promoters were over-represented in cancer (Petitjean et al. 2007b), suggesting that DNE plays a role in selecting for mutations during carcinogenesis.

Only a few mutants have been studied for GOF activities and there is no consensus on the molecular definition of such activities (Petitjean et al. 2007b). Knockin mice with R175H and R273H mutants (Lang et al. 2004; Olive et al. 2004) showed increased metastases and different cancer distribution compared with p53 knockout mice, providing in vivo evidence of GOF. Various molecular mechanisms have been proposed as the basis of GOF, such as interference with the p53-family proteins p63 and p73, interference with the ATM DNA repair pathway (Song et al. 2007), activation of genes normally unaffected or repressed by wild-type p53, interference with other transcription factors, and resistance to drugs (see Oren and Rotter 2010). However, no systematic study has been performed to distinguish mutants with GOF properties. Thus, there is so far no definite evidence that GOF contribute to mutant p53 selection in human cancers.

It should be noted that, when compared with expected numbers, if all mutations were equal, missense mutations in p53 DBD are only slightly overrepresented in cancer (87.1% observed vs. 72.9% expected), whereas nonsense mutations are overrepresented by a factor of two (8% vs. 3.6%) and silent mutations underrepresented by a factor of four (4.8% vs. 22.9%) (Table 2). These observations further support that loss of function is the critical

factor for the selection of mutations in cancer. This is particularly striking when considering mutations at hypermutable CpG sites. Of 34 possible missense mutations at CpG sites in the DBD, only seven are frequently observed in cancers. These seven mutants show complete loss of transactivation activities, whereas the 27 rare mutants retain significant transactivational activity on one or more p53RE.

## MUTAGEN-INDUCED MUTATION SPECTRA

### Human Data

In several types of cancers, mutation patterns bear the hallmarks of chemical damage induced by particular mutagens, leading to the concept of “mutagen fingerprints” (Table 3). These fingerprints are defined by the relative frequencies, types of base change, strand orientation, and location of base substitutions. The factors shaping mutation patterns have been studied in detail in various organisms and experimental models. They include (1) the nature of the environmental agent that chemically modifies a base, (2) the DNA sequence itself (base context), (3) epigenetic modifications at the target sequence (e.g., base methylation), (4) DNA repair and replication processes, (5) transcription activity at the locus, and (6) biological selection (as discussed previously).

Although in many cancers transitions at CpG sites predominate, a complex picture emerges when concentrating on rarer types of mutations. The patterns of these mutations can vary dramatically between two types of cancers, or two patient populations with a given cancer type. In some instances, the mutations reveal

**Table 2.** Observed and expected frequency of single-nucleotide substitutions within exons 5–8 of *TP53* gene

Mutation type	Expected mutations	Observed mutations*
Missense	1150 (73.4%)	17,191 (87.9%)
Nonsense	58 (3.7%)	1435 (7.3%)
Silent	359 (22.9%)	932 (4.8%)
<b>Total</b>	<b>1567</b>	<b>19,558</b>

\*Somatic mutations reported in the IARC TP53 Database (R13, November 2008).

M. Olivier, M. Hollstein, and P. Hainaut

**Table 3.** Human carcinogen *TP53* signature mutations

Carcinogen/ (exposure)	Target organ	Metabolic activation	TP53 Signature		
			Base substitution		Distinctive
			IARC DB	HUF assay	Hotspots (codons)
PAH (B[a]P) (smoking)	Lung	Epoxidation	G to T with strand bias	G to T with strand bias	157, 158, 273
AA (dietary contam.)	Urothelium	Nitroreduction	A to T with strand bias	A to T with strand bias	131, 209, (280)
Aflatoxin B1 (dietary contam.)	Liver	Epoxidation	G to T	n.i.	249 (3rd base)
UV radiation (sunlight)	Skin	n.a.	CC to TT	CC to TT	248, 278
3-NBA (diesel)	Lung	Nitroreduction	n.i.	G to T	n.i.
Vinyl chloride	Liver	Epoxidation	A:T to T:A with strand bias	n.i.	

characteristics expected from the effects of environmental mutagens on DNA. There are four well-documented examples of associations between an etiologic agent and a tumor mutation fingerprint. Three of these have been reviewed in detail: exposure to sunlight and the presence of tandem CC to TT transitions in nonmelanoma skin cancers (Giglia-Mari et al. 2003), tobacco smoking and PAH-induced G to T transversions at specific G:C base pairs oriented with the (premutated) guanine on the nontranscribed strand (codons 157, 158, 245, 248, and 273) (Pfeifer et al. 2002; Pfeifer et al. 2003), and exposure to dietary aflatoxinB1 and G to T transversions at the third base of codon 249 (AGG to AGT) in tumors from HBV carriers (Hussain et al. 2007). The fourth and most recent example links TP53 A to T transversions with crops contaminated with seeds of *Aristolochia sp.*, and is worth mentioning even though the estimated number of cancer patients exposed worldwide to aristolochic acids (AA), the carcinogenic agent, is expected to be small in comparison to the three exposures just cited (Arlt et al. 2007). Pre-mutagenic AA-specific DNA adducts have been detected in individuals suffering from Balkan endemic nephropathy (BEN), a form of nephropathy associated with high risk of urothelial cancer that is historically common in some parts of southeastern Europe. *TP53*

mutations in urothelial tumors of these patients are predominantly (74%) A to T transversions (Grollman et al. 2007), a form of mutation that is rare in urothelial tumors from non-BEN patients (4%) as well as in human tumors overall (5%). Moreover, BEN tumor mutations show a strong strand bias, with the premutated adenine base residing on the nontranscribed strand in 93% of the instances reported so far. This bias suggests preferential (transcription-coupled) repair of bulky lesions on the DNA strand that serves as template for mRNA synthesis. Reminiscent of the first discoveries of AFB1-associated codon 249 G to T transversions in two small sets of liver tumors (Hsu et al. 1991; Bressac et al. 1991), the AA-associated mutations suggest a mutation fingerprint even though only 19 mutations in this cohort of BEN patients have been reported thus far.

Recent large-scale sequencing studies are having a profound impact on our understanding of mutation load in human cancers (Ding et al. 2008; Stratton et al. 2009). In keeping with the smokers' lung tumor TP53 mutation data, a screen of 623 genes in 188 lung adenocarcinomas confirmed the higher mutation load and higher fraction of strand-biased G to T transversions in lung tumor of smokers when compared with nonsmokers (Ding et al. 2008). Genome-wide studies highlighted an unexpected and statistically significant elevation in



G:C to C:G transversions in breast cancers (28% of all substitutions) compared with colorectal, pancreatic, and brain cancers (7–10%) (Stephens et al. 2005; Sjoblom et al. 2006; Greenman et al. 2007; Jones et al. 2008). Oddly, this is not seen in breast cancer TP53 mutations (9% of which are G:C to C:G transversions) (see Pfeifer and Besaratinia 2009 for discussion of G:C to C:G mutations). Next generation DNA sequencing technologies will offer new opportunities for studying factors that govern mutation load in normal and pre-neoplastic cell populations, including stem cells, which may be especially pertinent to elucidating origins of critical cancer-causing mutations.

### Experimental Systems

Experimental mutagenesis studies in lower organisms and mammalian cells have provided evidence linking the four human *TP53* tumor mutation signatures discussed above to the suspected carcinogens (Hainaut et al. 2000; vom Brocke J. et al. 2006). However, this approach is difficult to extrapolate to more complex situations involving multiple suspected carcinogens and where etiologic clues are needed. Classical experimental systems to generate mutation spectra are based on mutations in reporter genes that are not cancer related, but that allow efficient selection and recovery of mutants (e.g., the microbial *lacI* and *LacZ* genes, or the mammalian *TK* and *HPRT* genes). However, a reporter gene will differ from *TP53* or any other genes of interest in base sequence context, transcriptional level (thus transcription-coupled repair), and biological selection of mutants, three key factors that shape human *TP53* tumor mutation patterns. Until recently, the human *TP53* gene was considered a “nonselectable” gene in an experimental setting, which would preclude its use as a reporter of experimentally induced mutations. However, innovative approaches have been developed to map experimental DNA damage along the human *TP53* sequence as a first approximation of the sites vulnerable to accumulation of mutations. This strategy was used successfully to show the correlation between human tumor *TP53*

mutations and the positions of the main DNA photoproducts induced by UV light or those of adducts formed by metabolites of PAH (Denissenko et al. 1996). Another powerful approach was devised to score mutations in human *TP53* sequences cloned into a vector by assessing loss of p53 transcriptional transactivation function when the plasmid is then introduced into specially designed yeast strains (Frebourg et al. 1992; Ishioka et al. 1993).

A direct method to induce and select *TP53* mutations in mammalian cells would be to conduct a rodent cancer assay and sequence the murine *TP53* gene in the carcinogen-induced tumors (Zielinski et al. 2002). Although this approach has been used successfully to examine UV-induced mutations in murine skin tumors, it has limited applicability considering the unexplained paucity of *TP53* mutations in most mouse tumor types other than skin. Furthermore, murine and human *TP53* genes, although highly homologous, differ in DNA sequence at critical hotspot codons, posttranslational modification sites, and intragenic suppressor sites (Hergenhahn et al. 2004). An in vivo carcinogenesis assay in mouse harboring human *TP53* gene sequences would bypass this latter difficulty. However, its applicability may still be limited to studies of skin lesions induced by topical application of suspected agents. The HUF Assay (Hupki fibroblast assay) is a novel mutagenesis test in primary cells from “humanized” mice that was designed to circumvent the difficulties discussed above (Liu et al. 2004). HupKi (Human p53-Knockin) mice harbor human *TP53* sequences encoding both the polyproline and the DNA binding domain, where most human tumor mutations arise (Luo et al. 2001; Reinbold et al. 2008), replacing the homologous murine gene segments within the mouse *TP53* locus. Hupki p53 is functional and regulated normally in mice. The “domain-swap” strategy is necessary, rather than replacement of the entire murine p53 by its human counterpart, because in a complete swap the *TP53* gene loses wild-type function in mice, due to aberrant human p53 N-terminal interaction with the murine major negative regulator Mdm2 (Dudgeon et al. 2006).

M. Olivier, M. Hollstein, and P. Hainaut



The HUF assay takes advantage of the fact that p53 inactivation is the critical rate-limiting event in escape of replicative senescence and spontaneous immortalization of mouse embryo fibroblasts (MEF) in culture (Hahn et al. 2002). Fibroblasts are explanted from Hupki embryos, exposed to carcinogens, and cultured until immortalization has occurred. *TP53* gene is then sequenced in MEF cell lines in search of mutations induced by carcinogens in a human sequence context. Several human carcinogens have been tested in the HUF assay and shown to induce *TP53* mutations that are consistent with signatures identified in human tumors (Pfeifer and Besaratinia 2009; vom Brocke et al. 2006; Reinbold et al. 2008). Of note, aristolochic acid-induced A to T mutations in HUF cells correspond remarkably well with the *TP53* tumor mutations in cancer patients with BEN (Nedelko et al. 2009). The HupKi mouse concept is now being extended to a whole family of mice carrying humanized *TP53* sequence variants (recapitulating human *TP53* polymorphisms, hotspot *TP53* mutations, or mutations abrogating critical post-translational modification sites). By crossing Hupki mice with other genetically modified mouse strains (e.g., mice with humanized metabolic or DNA repair enzymes, or knockout strains with deficiencies in specific defense systems), many outstanding questions on the mechanisms of mutagenesis pertinent to human tumorigenesis could be addressed.

### EPIDEMIOLOGICAL, DIAGNOSTIC, AND THERAPEUTIC UTILITY OF *TP53* MUTATIONS

#### Biomarkers in Molecular Epidemiology

As discussed above, specific *TP53* mutations are observed in some types of cancer that are likely to have arisen from exposure to known carcinogens. The existence of important variations in mutation patterns between different groups of patients with the same cancer suggests that further mutation fingerprints related to environmental exposures are still to be discovered. This is particularly the case in squamous cell carcinomas of the head and neck and of the

esophagus, where variations have been described in relation with geographic origin of the patients, environmental risk factors, and lifestyle. However, in the natural history of human cancers, there is often no evidence for high exposure to genotoxic substances and most mutations may arise through spontaneous replication errors, endogenous pro-mutagenic states (induced by reactive oxygen species), spontaneous deamination, or other mechanisms contributing to the spontaneous decay of DNA (Lindahl 1993). Furthermore, the association between characteristic mutation type and carcinogen needs support from experimental systems and other lines of evidence to gain plausibility. Moreover, any characteristic single-base “fingerprint” mutation induced by an exogenous risk factor may also arise spontaneously, i.e., in the absence of exposure (albeit perhaps rarely), calling for corroborative studies to substantiate links between exposures and cancer mutations. In the realm of molecular epidemiology, where large numbers, control groups, and robust statistics are mandatory, such studies are facilitated by technical advances allowing the identification of mutations in DNA extracted from surrogate biological samples such as plasma, urine, sputum, or exfoliated cells from bronchus, bladder, oral cavity, and esophagus (Kirk et al. 2000). The use of such surrogate materials is compatible with the development of studies that follow consistent epidemiological designs, e.g., case-control and prospective cohort studies.

#### Biomarkers in the Clinics

Mutations in *TP53* are useful markers of tumor clonality to compare, in individual patients, separate clusters of tumor cells from the same lesion, or multiple lesions arising in the same tissue (Dix et al. 1995; Ponten et al. 1997; Franklin et al. 1997). Mutations are also useful for the follow-up of minimal residual disease, for comparison between primary and recurrent tumors and for tracing the origin of distant metastases (Franklin et al. 1997). However, the finding of different *TP53* mutations in separate clusters of a tumor does not exclude a clonal

origin with occurrence of *TP53* mutations at a late stage. Detection of *TP53* mutations may also help to identify early lesions at a high risk of evolution. For example, in esophageal and endometrial cancers, detection of a mutation in low-grade dysplasia should be considered as an indicator of high risk of malignant evolution (Montesano et al. 1998; Jia et al. 2008).

*TP53* mutations, but not p53 positive immunohistochemistry (IHC), have been consistently associated with poor prognosis in cancers such as breast, colorectal, head and neck, and leukemia (Petitjean et al. 2007a). In breast cancers, there is now clear evidence that *TP53* mutation is an independent marker of poor prognosis, in particular in hormone receptor-positive cases. A large number of studies that have used IHC as a surrogate marker for mutation status have failed to provide such consistent results. IHC leads to an unacceptable number of misclassified cases (false-positive and false-negative) and to a large interstudy variability. *TP53* gene status has also been associated with response to specific treatment regimens in breast cancer (Bergh et al. 1995; Aas et al. 1996; Olivier et al. 2006). However, it should be noted that the prognostic and predictive significance of *TP53* mutations is extremely variable according to tumor type and/or treatment (Bertheau et al. 2008), and there is therefore no simple, universal clinical message that can be delivered by *TP53* mutation analysis.

Mutations may also serve as biomarkers for targeted therapy. Several strategies have been developed to reactivate normal p53 functions in p53 mutated tumors. They include molecules that target broad classes of mutants to reactivate suppressive functions in tumor cells (PRIMA, RITA, scFv) (Caron de Fromentel et al. 1999; Issaeva et al. 2004; Bykov et al. 2005), or compounds that specifically target particular missense mutants to restore wild-type-like structure (Phikan059 targeting R220C) (Boeckler et al. 2008). Interestingly, several of these small pharmacological compounds appear to share a common chemical activity as Michael acceptors and can modify thiols in p53, suggesting that they affect p53 folding through redox regulation (Lambert et al. 2009). Peptides have also been

generated that interact specifically with several p53 mutants and block their nonspecific transactivation capacities, inhibiting GOF properties. These approaches complement other approaches such as gene therapy that target tumors that do not express p53 mutants (Senzer et al. 2009).

## CONCLUDING REMARKS

The *TP53* gene has been the most extensively sequenced tumor gene before the era of large scale sequencing, generating a large amount of data to identify links between carcinogens, mutation fingerprints, and tumorigenesis. Recent data produced by genome-wide sequencing have confirmed that overall mutation patterns in cancer genomes are quite similar to those in *TP53* itself. The existence of several in vitro and in vivo mutagenesis assays such as the HUF assay provides tools to experimentally corroborate observations on mutation patterns in human cancers. Thus, studies on *TP53* mutations will further contribute to the understanding of gene-environment interactions in cancer, in particular when comparing variations in *TP53* mutation patterns in relation to different cohorts of patients.

In terms of clinical applications, *TP53* mutations have proven to be extremely complex biomarkers. Despite impressive progress in mechanistic understanding of p53 structure and function, p53 research has not yet generated applications of wide impact on cancer management and therapy. Somehow, the complexity of the p53 field may have acted as a deterrent for clinical applications. The fact that mutations in *TP53* are diverse in their biological effects and that they may occur at many different stages before or during tumor development makes it impossible to derive simple messages uniformly applicable to all clinical contexts. Translating *TP53* mutation into the clinics will require large, structured clinical trials in which patients with defined p53 status are recruited on the basis of specific inclusion criteria, randomized for treatment according to determined regimens, and followed up for long-term therapeutic and clinical end points. Databases such as the current *TP53* mutation databases will

M. Olivier, M. Hollstein, and P. Hainaut

have a critical role in collecting, structuring, and annotating these data, allowing for the interpretation of *TP53* mutation and their educated use in standard molecular pathology practice.

## REFERENCES

- Aas T, Borresen AL, Geisler S, Smith-Sorensen B, Johnsen H, Varhaug JE, Akslen LA, Lonning PE. 1996. Specific P53 mutations are associated with de novo resistance to doxorubicin in breast cancer patients. *Nat Med* **2**: 811–814.
- Ambs S, Hussain SP, Marrogi AJ, Harris CC. 1999. Cancer-prone oxyradical overload disease. *IARC Sci Publ* **150**: 295–302.
- Arlt VM, Stiborova M, vom BJ, Simoes ML, Lord GM, Nortier JL, Hollstein M, Phillips DH, Schmeiser HH. 2007. Aristolochic acid mutagenesis: Molecular clues to the aetiology of Balkan endemic nephropathy-associated urothelial cancer. *Carcinogenesis* **28**: 2253–2261.
- Barker N, Ridgway RA, van Es JH, van de Wetering M, Begthel H, van den Born M, Danenberg E, Clarke AR, Sansom OJ, Clevers H. 2009. Crypt stem cells as the cells-of-origin of intestinal cancer. *Nature* **457**: 608–611.
- Bergamaschi D, Gasco M, Hiller L, Sullivan A, Syed N, Trigiante G, Yulug I, Merlano M, Numico G, Comino A, et al. 2003. p53 polymorphism influences response in cancer chemotherapy via modulation of p73-dependent apoptosis. *Cancer Cell* **3**: 387–402.
- Bergh J, Norberg T, Sjogren S, Lindgren A, Holmberg L. 1995. Complete sequencing of the p53 gene provides prognostic information in breast cancer patients, particularly in relation to adjuvant systemic therapy and radiotherapy. *Nat Med* **1**: 1029–1034.
- Bertheau P, Espie M, Turpin E, Lehmann J, Plassa LF, Varna M, Janin A, de TH. 2008. TP53 status and response to chemotherapy in breast cancer. *Pathobiology* **75**: 132–139.
- Birch JM, Alston RD, McNally RJ, Evans DG, Kelsey AM, Harris M, Eden OB, Varley JM. 2001. Relative frequency and morphology of cancers in carriers of germline TP53 mutations. *Oncogene* **20**: 4621–4628.
- Boeckler FM, Joerger AC, Jaggi G, Rutherford TJ, Veprentsev DB, Fersht AR. 2008. Targeted rescue of a destabilized mutant of p53 by an in silico screened drug. *Proc Natl Acad Sci* **105**: 10360–10365.
- Bonafe M, Ceccarelli C, Farabegoli F, Santini D, Taffurelli M, Barbi C, Marzi E, Trapassi C, Storci G, Olivieri F, et al. 2003. Retention of the p53 codon 72 arginine allele is associated with a reduction of disease-free and overall survival in arginine/proline heterozygous breast cancer patients. *Clin Cancer Res* **9**: 4860–4864.
- Bougeard G, Baert-Desurmont S, Tournier I, Vasseur S, Martin C, Brugieres L, Chompert A, Bressac-de Paillerets B, Stoppa-Lyonnet D, Bonaiti-Pellie C, et al. 2006. Impact of the MDM2 SNP309 and p53 Arg72Pro polymorphism on age of tumour onset in Li-Fraumeni syndrome. *J Med Genet* **43**: 531–533.
- Bougeard G, Brugieres L, Chompert A, Gesta P, Charbonnier F, Valent A, Martin C, Raux G, Feunteun J, Bressac-de Paillerets B, Frebourg T. 2003. Screening for TP53 rearrangements in families with the Li-Fraumeni syndrome reveals a complete deletion of the TP53 gene. *Oncogene* **22**: 840–846.
- Brachmann RK, Vidal M, Boeke JD. 1996. Dominant-negative p53 mutations selected in yeast hit cancer hot spots. *Proc Natl Acad Sci* **93**: 4091–4095.
- Bressac B, Kew M, Wands J, Ozturk M. 1991. Selective G to T mutations of p53 gene in hepatocellular carcinoma from southern Africa. *Nature* **350**: 429–431.
- Bullock AN, Henckel J, Fersht AR. 2000. Quantitative analysis of residual folding and DNA binding in mutant p53 core domain: Definition of mutant states for rescue in cancer therapy. *Oncogene* **19**: 1245–1256.
- Bykov VJ, Issaeva N, Zache N, Shilov A, Hultcrantz M, Bergman J, Selivanova G, Wiman KG. 2005. Reactivation of mutant p53 and induction of apoptosis in human tumor cells by maleimide analogs. *J Biol Chem* **280**: 30384–30391.
- Caron de Fromentel C, Gruel N, Venot C, Debussche L, Conseiller E, Dureuil C, Teillaud JL, Tocque B, Bracco L. 1999. Restoration of transcriptional activity of p53 mutants in human tumour cells by intracellular expression of anti-p53 single chain Fv fragments. *Oncogene* **18**: 551–557.
- Cho Y, Gorina S, Jeffrey PD, Pavletich NP. 1994. Crystal structure of a p53 tumor suppressor-DNA complex: Understanding tumorigenic mutations. *Science* **265**: 346–355.
- Denissenko ME, Pao A, Tang M, Pfeifer GP. 1996. Preferential formation of benzo[a]pyrene adducts at lung cancer mutational hotspots in P53. *Science* **274**: 430–432.
- Ding L, Getz G, Wheeler DA, Mardis ER, McLellan MD, Cibulskis K, Sougnez C, Greulich H, Muzny DM, Morgan MB, et al. 2008. Somatic mutations affect key pathways in lung adenocarcinoma. *Nature* **455**: 1069–1075.
- Dix BR, Robbins PD, Spagnolo DV, Padovan GL, House AK, Iacopetta BJ. 1995. Clonal analysis of colorectal tumors using K-ras and p53 gene mutations as markers. *Diagn Mol Pathol* **4**: 261–265.
- Dudgeon C, Kek C, Demidov ON, Saito S, Fernandes K, Diot A, Bourdon JC, Lane DP, Appella E, Fornace AJ Jr, et al. 2006. Tumor susceptibility and apoptosis defect in a mouse strain expressing a human p53 transgene. *Cancer Res* **66**: 2928–2936.
- Eeles RA. 1995. Germline mutations in the TP53 gene. *Cancer Surv* **25**: 101–124.
- El-Deiry WS, Kern SE, Pietenpol JA, Kinzler KW, Vogelstein B. 1992. Definition of a consensus binding site for p53. *Nat Genet* **1**: 45–49.
- Felley-Bosco E, Weston A, Cawley HM, Bennett WP, Harris CC. 1993. Functional studies of a germ-line polymorphism at codon 47 within the p53 gene. *Am J Hum Genet* **53**: 752–759.
- Feng L, Hollstein M, Xu Y. 2006. Ser46 phosphorylation regulates p53-dependent apoptosis and replicative senescence. *Cell Cycle* **5**: 2812–2819.
- Franklin WA, Gazdar AF, Haney J, Wistuba II, La Rosa FG, Kennedy T, Ritchey DM, Miller YE. 1997. Widely dispersed p53 mutation in respiratory epithelium. A novel



- mechanism for field carcinogenesis. *J Clin Invest* **100**: 2133–2137.
- Frebourg T, Barbier N, Kassel J, Ng YS, Romero P, Friend SH. 1992. A functional screen for germ line p53 mutations based on transcriptional activation. *Cancer Res* **52**: 6976–6978.
- Gemignani F, Moreno V, Landi S, Moullan N, Chabrier A, Gutierrez-Enriquez S, Hall J, Guino E, Peinado MA, Capella G, et al. 2004. ATP53 polymorphism is associated with increased risk of colorectal cancer and with reduced levels of TP53 mRNA. *Oncogene* **23**: 1954–1956.
- Giglia-Mari G, Sarasin A. 2003. TP53 mutations in human skin cancers. *Hum Mutat* **21**: 217–228.
- Gonzalez KD, Noltner KA, Buzin CH, Gu D, Wen-Fong CY, Nguyen VQ, Han JH, Lowstuter K, Longmate J, Sommer SS, et al. 2009. Beyond Li Fraumeni Syndrome: clinical characteristics of families with p53 germline mutations. *J Clin Oncol* **27**: 1250–1256.
- Greenman C, Stephens P, Smith R, Dalgleish GL, Hunter C, Bignell G, Davies H, Teague J, Butler A, Stevens C, et al. 2007. Patterns of somatic mutation in human cancer genomes. *Nature* **446**: 153–158.
- Grochola LF, Zeron-Medina J, Mériaux S, Bond GL. 2010. Single nucleotide polymorphisms in the p53 signaling pathway. *Cold Spring Harb Perspect Biol* **2**: a001032.
- Grollman AP, Shibutani S, Moriya M, Miller F, Wu L, Moll U, Suzuki N, Fernandes A, Rosenquist T, Medverec Z, et al. 2007. Aristolochic acid and the etiology of endemic (Balkan) nephropathy. *Proc Natl Acad Sci* **104**: 12129–12134.
- Hahn WC, Weinberg RA. 2002. Modelling the molecular circuitry of cancer. *Nat Rev Cancer* **2**: 331–341.
- Hainaut P, Hollstein M. 2000. p53 and human cancer: The first ten thousand mutations. *Adv Cancer Res* **77**: 81–137.
- Hergenbahn M, Luo JL, Hollstein M. 2004. p53 designer genes for the modern mouse. *Cell Cycle* **3**: 738–741.
- Hinds PW, Finlay CA, Quartin RS, Baker SJ, Fearon ER, Vogelstein B, Levine AJ. 1990. Mutant p53 DNA clones from human colon carcinomas cooperate with ras in transforming primary rat cells: A comparison of the “hot spot” mutant phenotypes. *Cell Growth Differ* **1**: 571–580.
- Hollstein M, Sidransky D, Vogelstein B, Harris CC. 1991. p53 mutations in human cancers. *Science* **253**: 49–53.
- Hsu IC, Metcalf RA, Sun T, Welsh JA, Wang NJ, Harris CC. 1991. Mutational hotspot in the p53 gene in human hepatocellular carcinomas. *Nature* **350**: 427–428.
- Hussain SP, Schwank J, Staib F, Wang XW, Harris CC. 2007. TP53 mutations and hepatocellular carcinoma: Insights into the etiology and pathogenesis of liver cancer. *Oncogene* **26**: 2166–2176.
- Inga A, Storic F, Darden TA, Resnick MA. 2002. Differential transactivation by the p53 transcription factor is highly dependent on p53 level and promoter target sequence. *Mol Cell Biol* **22**: 8612–8625.
- Ishioka C, Frebourg T, Yan YX, Vidal M, Friend SH, Schmidt S, Iggo R. 1993. Screening patients for heterozygous p53 mutations using a functional assay in yeast. *Nat Genet* **5**: 124–129.
- Issaeva N, Bozko P, Enge M, Protopopova M, Verhoef LG, Masucci M, Pramanik A, Selivanova G. 2004. Small molecule RITA binds to p53, blocks p53-HDM-2 interaction and activates p53 function in tumors. *Nat Med* **10**: 1321–1328.
- Jia L, Liu Y, Yi X, Miron A, Crum CP, Kong B, Zheng W. 2008. Endometrial glandular dysplasia with frequent p53 gene mutation: A genetic evidence supporting its precancer nature for endometrial serous carcinoma. *Clin Cancer Res* **14**: 2263–2269.
- Joerger AC, Fersht AR. 2007. Structure-function-rescue: The diverse nature of common p53 cancer mutants. *Oncogene* **26**: 2226–2242.
- Joerger AC, Ang HC, Fersht AR. 2006. Structural basis for understanding oncogenic p53 mutations and designing rescue drugs 6. *Proc Natl Acad Sci* **103**: 15056–15061.
- Joerger AC, Ang HC, Veprintsev DB, Blair CM, Fersht AR. 2005. Structures of p53 cancer mutants and mechanism of rescue by second-site suppressor mutations 8. *J Biol Chem* **280**: 16030–16037.
- Jones PA, Rideout WM, Shen JC, Spruck CH, Tsai YC. 1992. Methylation, mutation and cancer. *Bioessays* **14**: 33–36.
- Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Kamiyama H, Jimeno A, et al. 2008. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science* **321**: 1801–1806.
- Kang HJ, Feng Z, Sun Y, Atwal G, Murphy ME, Rebbeck TR, Rosenwaks Z, Levine AJ, Hu W. 2009. Single-nucleotide polymorphisms in the p53 pathway regulate fertility in humans. *Proc Natl Acad Sci* **106**: 9761–9766.
- Kato S, Han SY, Liu W, Otsuka K, Shibata H, Kanamaru R, Ishioka C. 2003. Understanding the function-structure and function-mutation relationships of p53 tumor suppressor protein by high-resolution missense mutation analysis. *Proc Natl Acad Sci* **100**: 8424–8429.
- Kirk GD, Camus-Randon AM, Mendy M, Goedert JJ, Merle P, Trepo C, Brechot C, Hainaut P, Montesano R. 2000. Ser-249 p53 mutations in plasma DNA of patients with hepatocellular carcinoma from The Gambia. *J Natl Cancer Inst* **92**: 148–153.
- Klug SJ, Wilmotte R, Santos C, Almonte M, Herrero R, Guerrero I, Caceres E, Peixoto-Guimaraes D, Lenoir G, Hainaut P, et al. 2001. TP53 polymorphism, HPV infection, and risk of cervical cancer. **10**: 1009–1012.
- Lalloo F, Varley J, Moran A, Ellis D, O’ dair L, Pharoah P, Antoniou A, Hartley R, Shenton A, Seal S, et al. 2006. BRCA1, BRCA2 and TP53 mutations in very early-onset breast cancer with associated risks to relatives 1. *Eur J Cancer* **42**: 1143–1150.
- Lang GA, Iwakuma T, Suh YA, Liu G, Rao VA, Parant JM, Valentin-Vega YA, Terzian T, Caldwell LC, Strong LC, et al. 2004. Gain of function of a p53 hot spot mutation in a mouse model of Li-Fraumeni syndrome. *Cell* **119**: 861–872.
- Langerod A, Bukholm IR, Bregard A, Lonning PE, Andersen TI, Rognum TO, Meling GI, Lothe RA, Borresen-Dale AL. 2002. The TP53 codon 72 polymorphism may affect the function of TP53 mutations in breast carcinomas but not in colorectal carcinomas. *Cancer Epidemiol Biomarkers Prev* **11**: 1684–1688.
- Langerod A, Zhao H, Borgan O, Nesland JM, Bukholm IR, Ikdhahl T, Karesen R, Borresen-Dale AL, Jeffrey SS. 2007. TP53 mutation status and gene expression profiles are

M. Olivier, M. Hollstein, and P. Hainaut

- powerful prognostic markers of breast cancer. *Breast Cancer Res* **9**: R30.
- Levine AJ. 1997. p53, the cellular gatekeeper for growth and division. *Cell* **88**: 323–331.
- Li FP, Fraumeni JFJ, Mulvihill JJ, Blattner WA, Dreyfus MG, Tucker MA, Miller RW. 1988. A cancer family syndrome in twenty-four kindreds. *Cancer Res* **48**: 5358–5362.
- Lindahl T. 1993. Instability and decay of the primary structure of DNA. *Nature* **362**: 709–715.
- Liu Z, Hergenhahn M, Schmeiser HH, Wogan GN, Hong A, Hollstein M. 2004. Human tumor p53 mutations are selected for in mouse embryonic fibroblasts harboring a humanized p53 gene. *Proc Natl Acad Sci* **101**: 2963–2968.
- Luo JL, Yang Q, Tong WM, Hergenhahn M, Wang ZQ, Hollstein M. 2001. Knock-in mice with a chimeric human/murine p53 gene develop normally and show wild-type p53 responses to DNA damaging agents: A new biomedical research tool 9. *Oncogene* **20**: 320–328.
- Malkin D, Li FP, Strong LC, Fraumeni JFJ, Nelson CE, Kim DH, Kassel J, Gryka MA, Bischoff FZ, Tainsky MA. 1990. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science* **250**: 1233–1238.
- Marcel V, Palmero EI, Falagan-Lotsch P, Martel-Planche G, Ashton-Prolla P, Olivier M, Brentani RR, Hainaut P, Achatz MI. 2009. TP53PIN3 and MDM2 SNP309 polymorphisms as genetic modifiers in the Li-Fraumeni syndrome: Impact on age at first diagnosis. *J Med Genet*. (in press)
- Marin MC, Jost CA, Brooks LA, Irwin MS, O’Nions J, Tidy JA, James N, McGregor JM, Harwood CA, Yulug IG, et al. 2000. A common polymorphism acts as an intragenic modifier of mutant p53 behaviour. *Nat Genet* **25**: 47–54.
- Matakidou A, Eisen T, Houlston RS. 2003. TP53 polymorphisms and lung cancer risk: A systematic review and meta-analysis. *Mutagenesis* **18**: 377–385.
- Montesano R, Hainaut P. 1998. Molecular precursor lesions in oesophageal cancer. *Cancer Surv* **32**: 53–68.
- Nedelko T, Arlt VM, Phillips DH, Hollstein M. 2009. TP53 mutation signature supports involvement of aristolochic acid in the aetiology of endemic nephropathy-associated tumours. *Int J Cancer* **124**: 987–990.
- Nelson HH, Wilkojmen M, Marsit CJ, Kelsey KT. 2005. TP53 mutation, allelism and survival in non-small cell lung cancer 1. *Carcinogenesis* **26**: 1770–1773.
- Oda K, Arakawa H, Tanaka T, Matsuda K, Tanikawa C, Mori T, Nishimori H, Tamai K, Tokino T, Nakamura Y, Taya Y. 2000. p53AIP1, a potential mediator of p53-dependent apoptosis, and its regulation by Ser-46-phosphorylated p53. *Cell* **102**: 849–862.
- Olive KP, Tuveson DA, Ruhe ZC, Yin B, Willis NA, Bronson RT, Crowley D, Jacks T. 2004. Mutant p53 gain of function in two mouse models of Li-Fraumeni syndrome. *Cell* **119**: 847–860.
- Olivier M, Goldgar DE, Sodha N, Ohgaki H, Kleihues P, Hainaut P, Eeles RA. 2003. Li-Fraumeni and related syndromes: Correlation between tumor type, family structure, and TP53 genotype. *Cancer Res* **63**: 6643–6650.
- Olivier M, Hussain SB, Caron de Fromental C, Hainaut P, Harris CC. 2004. TP53 mutation spectra and load: A tool for generating hypotheses on the etiology of cancer. *IARC Sci Publ* **157**: 247–270.
- Olivier M, Langerod A, Carrieri P, Bergh J, Klaar S, Eyfjord J, Theillet C, Rodriguez C, Lidereau R, Bieche I, et al. 2006. The clinical value of somatic TP53 gene mutations in 1,794 patients with breast cancer. *Clin Cancer Res* **12**: 1157–1167.
- Oren M, Rotter V. 2010. Mutant p53 gain of function in cancer. *Cold Spring Harb Perspect Biol* **2**: a001107.
- Petitjean A, Achatz MI, Borresen-Dale AL, Hainaut P, Olivier M. 2007a. TP53 mutations in human cancers: Functional selection and impact on cancer prognosis and outcomes. *Oncogene* **26**: 2157–2165.
- Petitjean A, Mathe E, Kato S, Ishioka C, Tavtigian SV, Hainaut P, Olivier M. 2007b. 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.
- Pfeifer GP, Besaratinia A. 2009. Mutational spectra of human cancer. *Hum Genet* **125**: 493–506.
- Pfeifer GP, Hainaut P. 2003. On the origin of G → T transversions in lung cancer. *Mutat Res* **526**: 39–43.
- Pfeifer GP, Denissenko ME, Olivier M, Tretyakova N, Hecht SS, Hainaut P. 2002. Tobacco smoke carcinogens, DNA damage and p53 mutations in smoking-associated cancers. *Oncogene* **21**: 7435–7451.
- Pistrutto G, Puca R, Nardinocchi L, Sacchi A, D’Orazi G. 2007. HIPK2-induced p53Ser46 phosphorylation activates the KILLER/DR5-mediated caspase-8 extrinsic apoptotic pathway. *Cell Death Differ* **14**: 1837–1839.
- Ponten F, Berg C, Ahmadian A, Ren ZP, Nister M, Lundberg J, Uhlen M, Ponten J. 1997. Molecular pathology in basal cell cancer with p53 as a genetic marker. *Oncogene* **15**: 1059–1067.
- Reinbold M, Luo JL, Nedelko T, Jerchow B, Murphy ME, Whibley C, Wei Q, Hollstein M. 2008. Common tumour p53 mutations in immortalized cells from Hupki mice heterozygous at codon 72. *Oncogene* **27**: 2788–2794.
- Riley T, Sontag E, Chen P, Levine A. 2008. Transcriptional control of human p53-regulated genes. *Nat Rev Mol Cell Biol* **9**: 402–412.
- Schmidt MK, Reincke S, Broeks A, Braaf LM, Hogervorst FB, Tollenaar RA, Johnson N, Fletcher O, Peto J, Tammiska J, et al. 2007. Do MDM2 SNP309 and TP53 R72P interact in breast cancer susceptibility? A large pooled series from the breast cancer association consortium. *Cancer Res* **67**: 9584–9590.
- Schmutte C, Yang AS, Nguyen TT, Beart RW, Jones PA. 1996. Mechanisms for the involvement of DNA methylation in colon carcinogenesis. *Cancer Res* **56**: 2375–2381.
- Senzer N, Nemunaitis J. 2009. A review of contusugene ladenev (Advexin) p53 therapy. *Curr Opin Mol Ther* **11**: 54–61.
- Shi H, Tan SJ, Zhong H, Hu W, Levine A, Xiao CJ, Peng Y, Qi XB, Shou WH, Ma RL, et al. 2009. Winter temperature and UV are tightly linked to genetic changes in the p53 tumor suppressor pathway in Eastern Asia. *Am J Hum Genet* **84**: 534–541.
- Sjoberg T, Jones S, Wood LD, Parsons DW, Lin J, Barber TD, Mandelker D, Leary RJ, Ptak J, Silliman N, et al. 2006. The



- consensus coding sequences of human breast and colorectal cancers. *Science* **314**: 268–274.
- Smeenk L, van Heeringen SJ, Koeppl M, van Driel MA, Bartels SJ, Akkers RC, Denisov S, Stunnenberg HG, Lohrum M. 2008. Characterization of genome-wide p53-binding sites upon stress response. *Nucleic Acids Res* **36**: 3639–3654.
- Stephens P, Edkins S, Davies H, Greenman C, Cox C, Hunter C, Bignell G, Teague J, Smith R, Stevens C, et al. 2005. A screen of the complete protein kinase gene family identifies diverse patterns of somatic mutations in human breast cancer. *Nat Genet* **37**: 590–592.
- Song H, Hollstein M, Xu Y. 2007. p53 gain-of-function cancer mutants induce genetic instability by inactivating ATM. *Nat Cell Biol* **9**: 573–580.
- Storey A, Thomas M, Kalita A, Harwood C, Gardiol D, Mantovani F, Breuer J, Leigh IM, Matlashewski G, Banks L. 1998. Role of a p53 polymorphism in the development of human papillomavirus-associated cancer. *Nature* **393**: 229–234.
- Stratton MR, Campbell PJ, Futreal PA. 2009. The cancer genome. *Nature* **458**: 719–724.
- Toledo F, Wahl GM. 2006. Regulating the p53 pathway: In vitro hypotheses, in vivo veritas. *Nat Rev Cancer* **6**: 909–923.
- Tommasino M, Accardi R, Caldeira S, Dong W, Malanchi I, Smet A, Zehbe I. 2003. The role of TP53 in Cervical carcinogenesis. *Hum Mutat* **21**: 307–312.
- Vaninetti NM, Geldenhuys L, Porter GA, Risch H, Hainaut P, Guernsey DL, Casson AG. 2008. Inducible nitric oxide synthase, nitrotyrosine and p53 mutations in the molecular pathogenesis of Barrett's esophagus and esophageal adenocarcinoma. *Mol Carcinog* **47**: 275–285.
- Vikhanskaya F, Siddique MM, Kei LM, Brogini M, Sabapathy K. 2005. Evaluation of the combined effect of p53 codon 72 polymorphism and hotspot mutations in response to anticancer drugs. *Clin Cancer Res* **11**: 4348–4356.
- vom Brocke J, Schmeiser HH, Reinbold M, Hollstein M. 2006. MEF immortalization to investigate the ins and outs of mutagenesis. *Carcinogenesis* **27**: 2141–2147.
- Wang Y, Helland A, Holm R, Skomedal H, Abeler VM, Danielsen HE, Trope CG, Borresen-Dale AL, Kristensen GB. 2004a. TP53 mutations in early-stage ovarian carcinoma, relation to long-term survival. *Br J Cancer* **90**: 678–685.
- Wang Y, Kringen P, Kristensen GB, Holm R, Baekelandt MM, Olivier M, Skomedal H, Hainaut P, Trope CG, Abeler VM, et al. 2004b. Effect of the codon 72 polymorphism (c.215G>C, p.Arg72Pro) in combination with somatic sequence variants in the TP53 gene on survival in patients with advanced ovarian carcinoma. *Hum Mutat* **24**: 21–34.
- Wang-Gohrke S, Rebbeck TR, Besenfelder W, Kreienberg R, Runnebaum IB. 1998. p53 germline polymorphisms are associated with an increased risk for breast cancer in German women. *Anticancer Res* **18**: 2095–2099.
- Wang-Gohrke S, Weikel W, Risch H, Vesprini D, Abrahamson J, Lerman C, Godwin A, Moslehi R, Olipade O, Brunet JS, et al. 1999. Intron variants of the p53 gene are associated with increased risk for ovarian cancer but not in carriers of BRCA1 or BRCA2 germline mutations. *Br J Cancer* **81**: 179–183.
- Whibley C, Pharoah PD, Hollstein M. 2009. p53 polymorphisms: cancer implications. *Nat Rev Cancer* **9**: 95–107.
- Zawlik I, Kita D, Vaccarella S, Mittelbronn M, Franceschi S, Ohgaki H. 2009. Common polymorphisms in the MDM2 and TP53 genes and the relationship between TP53 mutations and patient outcomes in glioblastomas. *Brain Pathol* **19**: 188–194.
- Zielinski B, Liu Z, Hollstein M, Hergenbahn M, Luo JL. 2002. Mouse models for generating P53 gene mutation spectra. *Toxicol Lett* **134**: 31–37.



## TP53 Mutations in Human Cancers: Origins, Consequences, and Clinical Use

Magali Olivier, Monica Hollstein and Pierre Hainaut

*Cold Spring Harb Perspect Biol* 2010; doi: 10.1101/cshperspect.a001008 originally published online November 11, 2009

---

Subject Collection [The p53 Family](#)

---

**The Origins and Evolution of the p53 Family of Genes**

*Vladimir A. Belyi, Prashanth Ak, Elke Markert, et al.*

**Mouse Models of p53 Functions**

*Guillermina Lozano*

**TP53 Mutations in Human Cancers: Origins, Consequences, and Clinical Use**

*Magali Olivier, Monica Hollstein and Pierre Hainaut*

**p53 Research: The Past Thirty Years and the Next Thirty Years**

*David Lane and Arnold Levine*

**Transcriptional Regulation by P53**

*Rachel Beckerman and Carol Prives*

**p53-based Cancer Therapy**

*David P. Lane, Chit Fang Cheok and Sonia Lain*

**Phylogeny and Function of the Invertebrate p53 Superfamily**

*Rachael Rutkowski, Kay Hofmann and Anton Gartner*

**Tied Up in Loops: Positive and Negative Autoregulation of p53**

*Xin Lu*

**The Tumor Suppressor p53: From Structures to Drug Discovery**

*Andreas C. Joerger and Alan R. Fersht*

**p53 Regulation of Metabolic Pathways**

*Eyal Gottlieb and Karen H. Vousden*

**The Regulation of the p53-mediated Stress Response by MDM2 and MDM4**

*Mary Ellen Perry*

**Zebrafish Models of p53 Functions**

*Narie Y. Storer and Leonard I. Zon*

**p63 and p73, the Ancestors of p53**

*V. Dötsch, F. Bernassola, D. Coutandin, et al.*

**Pathologies Associated with the p53 Response**

*Andrei V. Gudkov and Elena A. Komarova*

**Single-nucleotide Polymorphisms in the p53 Signaling Pathway**

*Lukasz F. Grochola, Jorge Zeron-Medina, Sophie Mériaux, et al.*

**Clinical Outcomes and Correlates of TP53 Mutations and Cancer**

*Ana I. Robles and Curtis C. Harris*

For additional articles in this collection, see <http://cshperspectives.cshlp.org/cgi/collection/>

Gene Link™ All Modifications and Oligo Types Synthesized  
Long Oligos • Fluorescent • Chimeric • DNA • RNA • Antisense

*Oligo Modifications?*  
Your wish is our command.