# Review

# Shaping Genetic Alterations in Human Cancer: The p53 Mutation Paradigm

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p53 mutations are found in 50% of human cancers. Molecular epidemiology has shown strong correlations between the spectrum of p53 mutations and exposure to exogenous carcinogens. This spectrum is influenced quantitatively and qualitatively by various upstream genetic filters that modulate carcinogen activation, detoxification, and/or DNA repair. In this review, we will discuss how other factors such as tissue specificity, SNP of genes associated with the p53 pathway, other genetic alterations, or p53 mutant heterogeneity can act as a second set of downstream filters that also have a profound impact on the spectrum of p53 mutations.

## Introduction

Somatic p53 missense mutations are found in approximately 50% of human cancers (Soussi et al., 2006), but it is generally assumed that the p53 pathway is also inactivated in wild-type (WT) p53-carrying tumors via indirect mechanisms such as MDM2/MDMX amplification leading to p53 destabilization (Marine et al., 2007; Brooks and Gu, 2006). An increasing number of studies indicate that a subset of p53 mutant proteins are oncogenic and actively participate in neoplastic transformation (Weisz et al., 2007; Peart and Prives, 2006). These observations have several implications, including a possible heterogeneous clinical phenotype depending on whether p53 itself is mutated and on the site of mutations or whether p53 function is modified indirectly (Prives and Manfredi, 2005).

Analysis of the spectrum of p53 mutations in human cancer demonstrates a link between exposure to various types of carcinogens and the development of specific cancers (Hussain et al., 2000). In skin tumors, a high frequency of p53 mutations is observed at dipyrimidine sites, a molecular signature of mutagenesis by solar UV rays (Ziegler et al., 1994). The relationships between G → T transversion and lung cancer in smokers or mutation of codon 249 observed in aflatoxin B1-induced liver cancers are also very demonstrative (Staib et al., 2003). This spectrum related to carcinogen-induced adducts can be modulated qualitatively and quantitatively by various factors that act as positive or negative filters (Figure 1). These factors include genetic heterogeneity of the activation process that transforms an unreactive carcinogen into a form that binds DNA or heterogeneity of the detoxification process that eliminates the activated molecule. Similarly, SNP leading to variations in the activity of DNA repair genes associated with base excision repair (BER) or nucleotide excision repair (NER) have been detected in a normal population and can also shape the pattern of mutations (Hung et al., 2005). Many

studies have focused on the analysis of these various upstream filters that control the transformation of a DNA adduct into a stable mutation. Although mutations can occur at multiple positions in the genome, only those that lead to a selective advantage for the cell will be identified on analysis of tumors.

So far, analyses of the spectrum of p53 mutations and inactivation have not taken into account another set of selection events that can be referred to as downstream filters. They include tissue specificity, functional polymorphisms in genes associated with the p53 pathway, or other oncogenic modifications occurring in the tumor. In this review, we will discuss how these filters can quantitatively and qualitatively shape p53 alterations; how they can act by selecting specific mutant p53 whose properties, either loss or gain of function, are associated with a transforming phenotype in a given genetic and physiologic environment; and how these observations can be translated into clinical practice.

## p53 Mutant Heterogeneity

The main activity of p53 is to act as a transcription factor that binds DNA via a domain localized in the core region of the protein (CR; residues 100 to 300). The DNA sequence found in the various p53 response elements (p53 RE) is markedly degenerated, and the affinity of p53 for these various binding sites is highly heterogeneous, an important feature in the regulation of the p53 response, as it imposes a hierarchy in the occupancy of the various p53 response elements (Qian et al., 2002; Inga et al., 2002). Several thousand genes have been shown to be directly regulated by p53, but as all these studies were performed in cell lines and only limited data are available about tissue specificity, the spatiotemporal response of p53 in vivo and the way in which promoter heterogeneity regulates p53 response remain unclear. Furthermore, specific cofactors, some of which have tissue-specific expression, can bind to various regions of
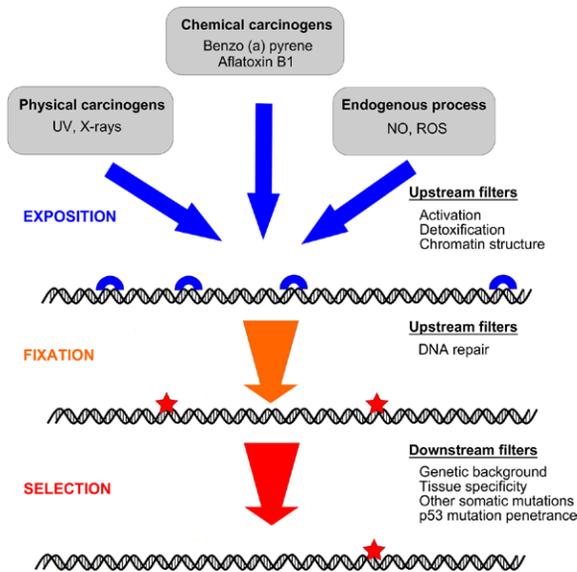
**Figure 1. Shaping p53 Mutations in Human Cancer**
In the exposure step, exogenous or endogenous carcinogens lead to the generation of a wide range of DNA adducts (blue half-ring shapes). These adducts are influenced qualitatively and quantitatively by several factors, including the efficiency of the activation and detoxification process, the affinity of the carcinogen for a given nucleotide, the chromatin structure, and any other factors that can modify the interaction of an active carcinogen with DNA. In a second step, most of the lesions are eliminated, but the cellular contexts (phases of the cell cycle, cell type) have an impact on the efficiency of DNA repair. In the fixation step, nonrepaired lesions are transformed into stable mutations (red stars) that are transmitted to daughter cells after cell division, resulting in three possible outcomes. First, if the mutation leads to a lethal phenotype, the cell dies and this counterselection prevents propagation of that specific alteration. A second outcome, which is the most common, is that the mutation does not result in any particular phenotype. This is the case for the majority of intergenic or intronic alterations, but also for certain intragenic mutations that target nonessential amino acid residues. Mutations targeting the third base of nucleotide codons also fall into this category, although it should be kept in mind that they can lead to defects in RNA stability or splicing. These "neutral mutations" are not involved in any selection process and are expanded at the rate of normal cell division of the original tissue. Nevertheless, if this particular cell is the target of a second alteration that leads to its clonal expansion, the first mutation will be coselected despite the absence of associated phenotype. Such mutations, called "passenger mutations," as opposed to the "driving mutations" that lead to clonal expansion, are quite common, and their frequency is related to the efficiency of the mechanism controlling genetic stability. The presence of passenger mutations is a real problem for molecular diagnosis, as the increased throughput and sensitivity of genetic analysis will reveal a wide variety of passenger mutations that will be difficult to distinguish from true driving mutations in the absence of functional assays. The third possible outcome for stable somatic mutations is a functional modification that results in a new phenotype that contributes to the neoplastic process. These true driving mutations are fortunately rare, but a selection process can be modulated by factors such as tissue specificity, the presence of other somatic genetic modifications, or the genetic background of the individual, as discussed in the text.

the p53 protein and modulate its activity. Biochemical and structural studies have shown that the CR of p53 is highly flexible and cycles rapidly between folded and unfolded states (Joerger and Fersht, 2007). This fragility explains the high concentration of missense muta-

tions in the 600 bp that codes for this domain. Each residue in this region has been found to be mutated in human tumors with frequencies ranging from twice for infrequent mutants to more than 1000 times for hot spot mutants (UMD p53 database 2007_R1; http://p53.free.fr/). Biochemical and functional studies have clearly demonstrated that different p53 mutants in the CR have a marked heterogeneity in terms of loss of structure and function (Soussi and Lozano, 2005). Using a library of 2500 different p53 mutants, Kato et al. showed that, while hot spot mutants found in human cancers display total loss of transactivating capacity, other mutants retain a partial transactivation activity for a subset of target genes, leading to a wide range of possible mutant activities (Figure 2) (Kato et al., 2003). This observation is of importance, as hot spot mutants at codons 175, 248, and 273 represent only 10% of all somatic p53 mutations found in human tumors. It is also noteworthy that the fragility of the p53 core domain and susceptibility of p53 to inactivating point mutations have apparently not been selected against during the course of evolution, presumably because loss of p53 function and development of cancer usually occur long after reproduction and the raising of offspring. p53 CR is also critical for interactions with various cofactors. Cellular proteins such as Bcl-Xl or 53BP2/ASPP2 interact with WT p53, but each protein requires a specific set of partially overlapping p53 residues of the CR (Samuels-Lev et al., 2001; Mihara et al., 2003). p53 interaction with Bcl-Xl is specifically associated with transcription-independent p53-induced apoptosis, whereas 53BP2/ASPP binding specifically enhances transactivation of proapoptotic genes such as *BAX* and *PIG 3* but has no effect on *WAF1* (Samuels-Lev et al., 2001; Mihara et al., 2003). This intricate promiscuity of various functions in the CR of p53 raises a number of questions concerning the interplay between the loss of these functions and p53 point mutations (Soussi, 2007). Some mutants, such as R175H, are totally defective for transactivation and do not bind to 53BP2/ASPP2, whereas other mutants such as R273H retain a normal conformation and/or the capacity to bind 53BP2/ASPP2 despite loss of their transactivation activity (Tidow et al., 2006). Whether loss of the transcriptional transactivation function of p53 via impaired DNA binding is the only consequence of p53 mutations selected during tumor evolution remains an open question (Soussi, 2007).

The impact of the mutation on the various biological functions of p53 is therefore an important factor in the selection process that will act together with the other filters discussed below such as tissue specificity or genetic background. Mouse models support these observations on the heterogeneity of p53 mutations. Knockin mice expressing two hot spot p53 mutations (R172H and R270H corresponding to human R175H and R273H, respectively) have a different phenotype from *p53⁻/⁻* mice (Olive et al., 2004; Lang et al., 2004). They develop a different spectrum of tumors with 50% of carcinomas with a high metastatic potential, while both of

these features are absent in *p53*^{-/-} mouse. Furthermore, the tumor spectra are different in mice expressing the two p53 mutants in the same genetic background. More relevant to the human population, expression of the same mutant p53 (R175H) in two different genetic backgrounds results in a different tumor spectrum. How tissue specificity and genetic background can contribute to the heterogeneity of p53 mutations both quantitatively (frequency of mutations) and qualitatively (diversity of p53 mutations) is discussed below.

## Tissue Specificity
### The p53 Family Network and Tissue Specificity

After discovering the p53 family members p63 and p73, it has become clear that all three proteins are intertwined in a complex crossregulation network (Yang and McKeon, 2000; Deyoung and Ellisen, 2007). Both p73 and p63 are expressed as several protein isoforms that either contain a p53-like transactivation domain (TA isoforms) or that lack this domain (ΔN isoforms). Each p53, p63, and 73 isoform possesses a homologous DNA-binding domain and shares transcriptional targets but with opposite effects, as the binding of DN isoforms can block transactivation (Stiewe, 2007). Recent reports indicate that p53 is also expressed as multiple isoforms (Rohaly et al., 2005; Bourdon et al., 2005). Although p53 expression is relatively ubiquitous, p73 expression and p63 expression are more restricted to specific tissue with various ratios of TA and ΔN isoforms. p63 is essential for the proliferation and differentiation cascade in stratified epithelia, and the ΔN isoforms play a major role in this function (Perez and Pietenpol, 2007). TAp73 has a strong proapoptotic activity after DNA damage induced by drugs used in chemotherapy such as cisplatin or adriamycin (Yuan et al., 1999; Gong et al., 1999; Agami et al., 1999). It is therefore not surprising that deregulation of p73 or p63 will depend on tissue type and the ratio of the various isoforms. The patterns of deregulation of this p63/73 network in human cancer have been recently discussed in several reviews (Deyoung and Ellisen, 2007; Ratovitski et al., 2006). In the present review, we will focus on how p53 mutations can contribute to deregulation of this network and their relationship to tissue specificity. WT p53 does not oligomerize with p73, but some mutant p53 proteins bind to p73 and inhibit its apoptotic activity (Strano et al., 2000; Di Como et al., 1999). This interaction occurs specifically via the CR of both proteins and is restricted to structural mutant p53 that display a change of conformation such as R249S or R175H (Gaiddon et al., 2001). Patients with head and neck SCC presenting these mutant p53 have a poor response to therapy associated with a lack of p73-associated apoptosis (Bergamaschi et al., 2003). More recently, Leong et al. reported that patients with the triple-negative subset of breast cancer (Basal-like, negative for estrogen and progesterone receptor and negative for *ERBB2* amplification) display a high frequency of p53 inactivation, overexpression of ΔNp63 associated with inhibition of p73 apoptotic activity and loss of chemosensitivity (Leong et al., 2007).
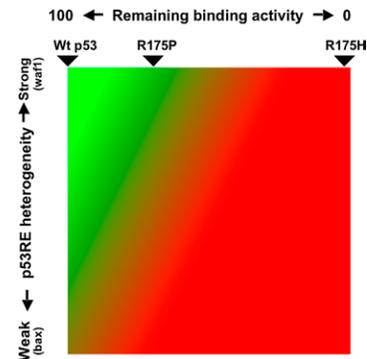


**Figure 2. Heterogeneity of the DNA-Binding Activity of Mutant p53**

Due to the marked degeneracy of the p53 DNA response element, there is a wide variation in the affinity of wild-type p53 to DNA, with two extremes represented by p21/WAF1 (strong binding) and bax (weak binding) (vertical axis). p53 mutation generates a large number of p53 variants with unique properties depending on the remaining affinity for DNA that will dictate transcription of a subset of p53-responsive genes (horizontal axis). Highly penetrant mutants such as R175H show a total loss of DNA binding with no transcriptional activity. However, the R175P mutant displays only a partial loss of activity with no binding to the bax promoter but normal binding to the p21/WAF promoter. This mutant is deficient for induction of apoptosis but retains wild-type activity with respect to induction of growth arrest. The observation that a subset of p53 mutants has also lost the ability to bind coactivators such as ASSP2 adds another level of complexity. Red and green represent wild-type and mutant p53 activity, respectively.

### The p53 Response Is Tissue Specific

Analysis of p53 response in cell lines after various types of stress has led to the general belief that stabilization and activation of p53 always occur regardless of the cell type. However, this universal p53 response does not apply to whole animals (Toledo and Wahl, 2006; Soussi, 2007). As early as 1995, Midgley et al. demonstrated a marked tissue-specific restriction of the p53 response after gamma irradiation (Midgley et al., 1995). Accumulation of p53 protein following whole-body irradiation was associated with a strong apoptotic response in the spleen and thymus, while no response was observed in hepatocytes. Using in situ hybridization with probes corresponding to various p53 target genes, Fei et al. extended these observations and also showed strong tissue specificity with distinct regulation of various p53 target genes in different tissues (Fei et al., 2002). In liver cells, only the *Waf1* gene involved in growth arrest was induced, whereas none of the proapoptotic p53 target genes were upregulated. This observation is the inverse of what occurs in the spleen with specific induction of the proapoptotic gene *Puma*, whereas *Waf1* or other apoptotic genes such as *Noxa* or *Dr5* were barely detectable. Another study based on transgenic mice expressing a *lacZ* reporter gene fused to the *Mdm2* promoter showed that the pattern of p53 transcriptional response is fairly homogeneous in the early embryo but becomes more restricted with increasing embryo age and with differentiation of the tissues (Gottlieb et al., 1997). A predominant p53 response after DNA damage was observed in

the spleen, thymus, and small intestine. Recently, Ringshausen et al., using an inducible p53 mouse model, showed that p53 activation leads to specific apoptosis of radiosensitive tissue such as bone marrow, thymus, or spleen, whereas radioresistant tissues only displayed marked growth arrest (Ringshausen et al., 2006). All these observations reveal that the phenotype of a cell line has only a limited relation to the original tissue from which it is derived. Although this is not unexpected, as p53 is a pleiotropic stress sensor and in vitro cell culture in plastic dishes is quite unnatural, there is a general tendency to overinterpret in vitro studies and transfection experiments (Toledo and Wahl, 2006).

The observed tissue-dependent p53 responses also raise some questions concerning whether tissue origin could specify the selection of particular p53 mutations. This issue is complex, as several confounding factors such as the tissue specificity of several carcinogens or the presence of other genetic alterations (see below) could influence the outcome. Nevertheless, in hepatocellular carcinoma, several findings support the notion that the R249S mutation is specifically selected in liver cells. Epidemiologic studies unambiguously show that the p53 mutation at codon 249 in liver cancer is linked to aflatoxin B1 (AFB1) exposure (Staib et al., 2003). In vitro studies exposing human liver cells to AFB1 also revealed the same R249S hot spot (Puisieux et al., 1991). Two nonexclusive explanations can account for the high prevalence of this particular mutation in liver cancer. First, codon 249 may be highly sensitive to the mutagenic effect of AFB1, as indicated by several studies. Second, it is possible that this mutant provides a special growth advantage to liver cells. The second hypothesis is supported by several findings. (1) Although AFB1 binds specifically to codon 249 in vitro, it is not the major site of AFB1 adducts, and stronger binding has also been observed at other codons, such as 245 and 248, at which mutations are found in other cancer types (Puisieux et al., 1991; Denissenko et al., 1998). The fact that these mutants are not found in liver cancer indicates a preferential selection for R249S. (2) Mutant R249S properties such as dominant-negative activity or increased survival effects are more potent in liver cells than those of other mutants (Ponchel et al., 1994). There is no explanation for this liver specificity of the R249S mutant, but a better understanding of the various gain-of-function activities of mutant p53 and identification of the tissue specificity of p53 isoforms could uncover some novel functions for this mutant. Another association between tissue specificity and a particular p53 mutant has been found in adrenal cortical carcinoma (ACC). In south Brazil, a germline mutation at codon 337 (R337H) has been specifically associated with pediatric ACC, with several cases in one family (Ribeiro et al., 2001). This mutation is localized in the oligomerization domain but analysis of classical functions such as cell cycle arrest or apoptosis failed to reveal any defect of this mutant despite its strong association with pediatric

ACC. This paradox has been resolved by structural analysis demonstrating a very high sensitivity of the R337H mutant to pH-induced denaturation as compared to WT p53 (DiGiammarino et al., 2002). As the adrenal gland is known to undergo extensive apoptosis during pre- and postnatal development, it has been postulated that an increased intracellular pH may lead to p53 inactivation and impair apoptosis specifically in these cells.

Several cancers display a very low frequency of p53 mutation, indicating that the upstream or downstream p53 pathways could be impaired. Indeed, amplification of MDM2 is observed in sarcoma (Michael and Oren, 2003), and Laurie et al. recently demonstrated that amplification of MDMX disrupts the p53 pathway and suppresses the apoptotic response in retinoblastoma (Laurie et al., 2006). In neuroblastoma, inactivation of p53 can often occur through nuclear exclusion via amplification of the Parc protein (Nikolaev et al., 2003; Moll et al., 1995). Whether this high frequency of indirect p53 inactivation, predominantly observed in nonepithelial tumors, has any biological significance remains to be analyzed.

The importance of the tissue specificity of p53 alteration was recently highlighted by analysis of p53 restoration in animal models (Martins et al., 2006; Ventura et al., 2007; Xue et al., 2007). Although these three studies show that restoring WT p53 activity in a p53-deficient tumor leads to p53-dependent regression of the tumor, the outcome differs according to tumor type. In solid tumors, p53 induced marked growth arrest, whereas in lymphoma, p53 induced intense and rapid apoptosis (Martins et al., 2006; Ventura et al., 2007; Xue et al., 2007).

### p53 Mutations and Genetic Background
#### Exonic p53 SNP

Thirty-seven SNP have been identified in the p53 gene, 18 of which have a frequency higher than 5% in the SNP500 cancer populations. The first exonic SNP discovered results in a proline-to-arginine substitution at codon 72 (Harris et al., 1986). A striking bias in the distribution of this SNP in the human population was noted (Beckman et al., 1994). The frequency of the Pro/Pro haplotype is 16% in Scandinavian populations and 63% in the Nigerian population. The reason for this North/South gradient is unknown at the present time. Residue 72 is localized in the proline-rich domain (PRD) of the protein that has been associated with a regulatory function of p53 apoptosis. Transfection studies have shown that deletion of the PRD motif in both human and mouse p53 leads to specific abrogation of the apoptotic activity of p53, maintaining an intact growth arrest function (Walker and Levine, 1996; Sakamuro et al., 1997; Venot et al., 1998). These observations were not reproduced in MEF from mouse lacking the PRD that are unable to induce a cell cycle arrest but display a weak apoptotic activity (Toledo et al., 2006). The Arg72 p53 variant has a more potent proapoptotic capacity compared to the Pro72 variant (Dumont et al., 2003; Bergamaschi et al., 2006). Several nonexclusive explanations have been

proposed to interpret this observation. Dumont et al. showed that a stronger interaction of the R72 variant with the nuclear-export protein CRM1 leads to enhanced nuclear export and greater accumulation in mitochondria (Dumont et al., 2003). This differential interaction is specific to human cells and absent in mice, indicating that an appropriate environment is important to display the specificity of each human p53 variant (Phang and Sabapathy, 2007). Another explanation has been proposed by Bergamaschi et al., who showed that iASPP, a specific cellular inhibitor of p53, binds to the PRD of p53 and interacts more strongly with the P72 variant, leading to a more pronounced inhibitory effect on its apoptotic activity (Bergamaschi et al., 2006). Epidemiologic studies have been performed to determine whether this SNP is associated with an increased risk of cancer. Results of these studies are very contradictory and have not demonstrated any highly significant correlations, suggesting that, if such an association exists, it may not be very strong or may be due to other as yet unidentified genetic factors (Pietsch et al., 2006). Similarly, analysis of the prognostic value of codon 72 SNP in various types of cancer has not been fully conclusive. A possible explanation is that p53 mutant gain of function differs for the two p53 variants. As mentioned in a previous section, some structural p53 mutants bind and inactivate the apoptotic activity of p73. The binding of mutant p53 for p73 is stronger for the R72 variants compared to mutants with the Pro72 variants and generates mutant p53 with a strong gain of function (Marin et al., 2000; Bergamaschi et al., 2003). Patients with head and neck cancer who display these structural p53 mutants associated with Arg72 variants have a very poor outcome. Therefore, two confounding effects should be considered: the lower apoptotic activity of the P72 variant and the enhanced gain of function of mutant p53 carrying the R72 polymorphism, both of which can be associated with a poor response to therapy or poor outcome (Figure 3). In tumors with a high frequency of TP53 mutation, the R72 variant can be associated with a poor prognosis, whereas in tumors with a low frequency of p53 mutation, the low apoptotic variant P72 could be associated with a poorer outcome. It is unclear at the present time whether this model can be generalized to other settings.

A second exonic polymorphism at codon 47 changing a proline to serine has also been described (Felley-Bosco et al., 1993). The frequency is very low, ranging from 0.5% to 5% in various studies. This polymorphism is close to serine 46, phosphorylation of which is a key event for the apoptotic function of p53. In vitro studies have shown that the S47 variant is a poorer substrate for S46 phosphorylation and has an impaired proapoptotic ability (Li et al., 2005). The clinical significance of this p53 variant is not known.

### Functional SNP in the p53 Pathways
As discussed in a previous section, the p53 RE recognized by WT p53 is highly degenerated. The consensus sequence is composed of two copies of the
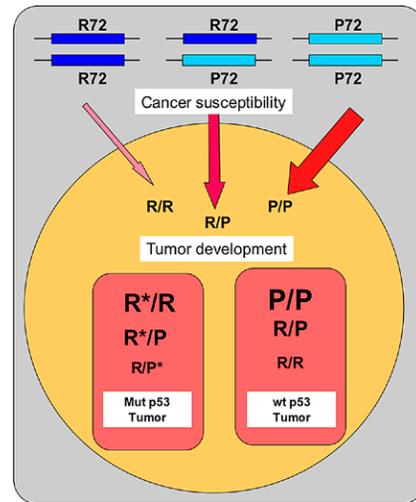


**Figure 3. Complexity of p53 R72P Polymorphism**
In normal cells (gray shade), p53 72R has a more potent apoptotic activity compared to p53 P72, suggesting that individuals with this SNP will have a higher cancer susceptibility compared to R72 individuals, as symbolized by the thickness of the arrows. In tumors (orange shade) the situation is more complex. In tumors with a low frequency of p53 mutations (red shade, right), p53 polymorphism P72 is more frequent, and tumors are less sensitive to apoptosis-inducing treatment. On the other hand, in tumors with a high frequency of p53 mutations (red shade, left, mutation symbolized by asterisk), mutant p53 encoded by the R72 allele may bind to p73 and inhibit its apoptotic activity, and are therefore preferentially selected during tumor development. This is supported by the observation that head and neck tumors bearing p53 mutations with the R72 allele are more resistant to chemotherapy, and these patients have a shorter survival.

5′PuPuPuC(A/T)(T/A)GPyPyPy-3′ motif separated by a spacer of 0 to 13 bases (Funk et al., 1992; el-Deiry et al., 1992). It has been clearly established that variation in this sequence can drastically affect the efficiency of transactivation (Qian et al., 2002). Several studies have been performed to determine whether certain natural SNP are present in functional p53 RE. Although a difficult challenge, using p53 as a paradigm for these studies is facilitated by the large number of genes regulated by p53 (more than a thousand), the length and degeneracy of the p53 RE, and our good knowledge of the p53 network. Two types of analysis were used for these studies, a candidate gene approach performed by Bonds et al. and a global unbiased screening of the whole genome performed by Tomso et al. (Bond et al., 2004; Tomso et al., 2005). The first type of analysis led to the detection of SNP309 in the *MDM2* gene (Bond et al., 2004). This polymorphism changes a T to G at nucleotide 309 of intron 1 close to the p53 RE and creates a higher-affinity DNA-binding site for Sp1 that leads to increased levels of *MDM2* RNA and protein in cells. Cell lines homozygous for the G allele express high levels of *MDM2* and have an impaired p53 response after DNA damage with poor induction of p53 and weak p53-induced apoptosis (Bond et al., 2004). The frequency of individuals with the G/G genotype is around 1%, which is sufficiently high
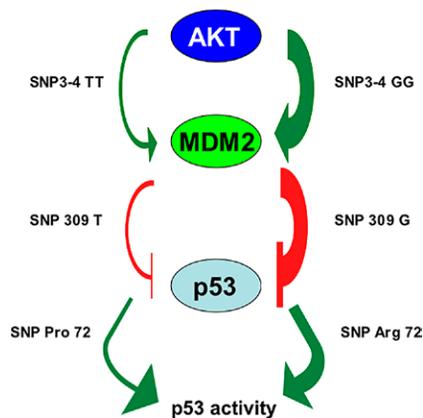
**Figure 4. Heterogeneity of the p53 Pathway**
AKT kinase, the level of which is controlled by SNP 3-4, phosphorylates MDM2 protein and enhances MDM2-mediated ubiquitination and degradation of p53. The level of *MDM2* expression is modulated by SNP309 SNP. p53 activity is also believed to be controlled by the Arg-Pro polymorphism at codon 72. In the worst case scenario, an individual with strong AKT activity (SNP 3-4 GG), high MDM2 expression (SNP 309 GG), and a p53 SNP Pro 72 with low apoptotic activity will be at high risk for cancer and will experience a very low response to DNA damage induced by chemotherapeutic agents. On the other hand, individuals with the opposite genotype will experience the best response. Between these two possibilities, multiple genotypes in these three genes and other unknown modifier genes will lead to a marked heterogeneity of p53 responses. Whether this heterogeneity contributes to cancer incidence or clinical status and response to treatment remains to be analyzed.

for population studies. Indeed, individuals with the Li-Fraumeni syndrome carrying the G allele (either in the heterozygous or homozygous state) have an earlier age of tumor onset. SNP309 is found in a region of the *MDM2* promoter that is regulated by hormonal signaling pathways, and recent studies have shown that the clinical penetrance of this SNP is stronger in women than in man. The onset of cancer was found to be significantly earlier in patients with sarcoma, breast cancer, colorectal cancer, or lymphoma (Bond et al., 2006; Bond and Levine, 2007). A second SNP (SNP 354) in exon 12 of the *MDM2* gene has been recently associated with an increased breast cancer incidence, but its biological significance is unknown (Boersma et al., 2006).

A specific combination of SNP in the *AKT* gene has been associated with an increased amount of AKT protein in cells. A large body of data indicate that the IGF-1-AKT survival pathway is strongly integrated in the p53 network (Levine et al., 2006). AKT protein phosphorylates and stabilizes MDM2 and downregulates p53 (Zhou et al., 2001; Mayo and Donner, 2001). AKT is negatively regulated by the phosphatase PTEN, a p53 response gene that is inactivated in a wide variety of cancers. AKT also phosphorylates and inactivates TSC2, which is encoded by a p53 target gene, that negatively regulates the mTOR pathway after glucose starvation. AKT is therefore able to impair p53 response at multiple points in various pathways. Several SNP associated with variable expression of AKT have been

identified. Analysis of p53-dependent apoptosis after irradiation in 113 lymphoblastoid cell lines showed that individuals expressing high levels of AKT displayed a reduced apoptosis compared to individuals with lower AKT expression (Harris et al., 2005). Whether or not this population with high AKT activity will be less responsive to radiotherapy or more prone to cancer has yet to be determined.

Using a global genome analysis, Menendez et al. identified a SNP in a putative p53 response element on the promoter of the *FLT-1* gene (Fms-like tyrosine kinase 1). The *FLT-1* gene encodes one of the receptors activated via binding of VEGF, a molecule essential for angiogenesis and associated with metastasis. In vitro analysis showed that only one of the two alleles is induced by p53, whereas the other is unaffected (Menendez et al., 2006). Although no clinical data are yet available, this finding shows that the p53 response in a normal individual could be highly heterogeneous. As discussed in a previous section, animal models also indicate that genetic background has a strong impact on tumor heterogeneity, as knockin mouse models expressing mutant p53 R172H (R175H in human) in different genetic backgrounds develop different types of tumors (Iwakuma and Lozano, 2007).

Taken together, these data indicate that the p53 network is genetically heterogeneous, a feature that can lead to a wide variation in the p53 response (Figure 4). Furthermore, data presented here are certainly the tip of the iceberg, as only a small component of the p53 pathway has been analyzed. A multitude of SNP affecting the p53 response will undoubtedly be identified in the near future. Understanding how all these SNP will collectively define tumor risk or predict tumor behavior, prognosis, or response to treatment will constitute an important challenge.

### p53 Mutations and Other Tumor-Associated Genetic Alterations

Although p53 mutations are fairly ubiquitous and can be found in more than 50% of human tumors, it is now well known that most types of cancer harbor specific genetic defects such as mutations in *APC* in colon cancer, *BRCA1* and *BRCA2* in breast cancer, and *B-RAF* in melanoma (Futreal et al., 2004). As the p53 network is closely linked to many other cellular pathways, it is likely that defects in any of these pathways, either inherited or acquired somatically, could influence p53 function qualitatively or quantitatively. We have previously discussed how PTEN and p53 mutations are mutually exclusive in breast cancer.

Another example is the relationship between the BRCA1 and p53 pathways. BRCA1 acts both as a checkpoint and a DNA damage repair gene that ensures genome integrity (Venkitaraman, 2002). *BRCA1* germline mutations are associated with an increased risk of developing breast and ovarian carcinoma. *Brca1* null mouse embryos die early during development, but this phenotype can be partially rescued by p53 defi-

ciency. Furthermore, mice carrying a partial deletion of the carboxy terminus of Brca1 (*Brca1Δ11*) have a milder developmental defect that is totally rescued in a *p53*⁺/⁻ background, but the resulting mice are prone to cancer (Cao et al., 2006). In breast cancer families, p53 mutations arise in a BRCA1-deficient background associated with genetic instability and DNA repair deficiency, and the high frequency of p53 mutations in this tumor is therefore not surprising (Crook et al., 1997). Furthermore, the spectrum of these mutations is different compared to matched sporadic breast tumors of the same grade. Only a few mutations are localized at hot spot codons, and several new p53 mutations have been identified in BRCA1-deficient tumors (Smith et al., 1999). Functional analysis of these BRCA1-associated p53 mutants shows that several of them have a partial defect of biological activities such as growth arrest or apoptosis. Other mutants behave in a manner undistinguishable from WT p53 except for a weak transforming activity when cotransfected with *H-RAS* into rat cells (Smith et al., 1999). As animal models suggest that p53 inactivation is a key step for survival of *Brca1*-deficient cells, this increased frequency of p53 mutation in BRCA families is not unexpected, and we can exclude the hypothesis that these mutations are passenger events randomly coselected in an unstable genetic background. The observation that many of the BRCA1-associated p53 mutants retain WT function to a large extent is interesting. It is possible that they target a specific functional activity of p53 that has not yet been identified, such as specific gene transactivation in mammary glands or cooperation with the BRCA network in DNA repair, as several proteins such as Rad51 can bind to both p53 and BRCA1. Finally, the possibility that these mutations may affect p53's transcription-independent apoptosis function or affect functions of newly identified p53 isoforms should be considered (Bourdon et al., 2005; Mihara et al., 2003).

Several genetic pathways leading to cell transformation have been identified in sporadic colorectal carcinomas. In tumors associated with microsatellite instability (MSI+), the frequency of p53 mutation is low (less than 10%) compared to other sporadic colorectal carcinomas (around 40%). This observation can be explained by the propensity of MSI+ tumors to use a pathway for tumor development that requires mutations in genes containing a repeated polynucleotide tract such as *BAX*, *TGFβR*, or *IGFR* (Konishi et al., 1996). It is still unclear whether the spectrum of p53 mutations is the same in MSI+ and MSI− tumors. In inflammatory breast cancer and neuroblastoma, the frequency of p53 mutation is low, but immunohistochemical and molecular analysis indicates accumulation of WT p53 in the cytoplasm of tumor cells. This abnormal localization of p53 disrupts its function after DNA damage (Zaika et al., 1999). Whether this particular mechanism for p53 inactivation is associated with a specific cell type or with other genetic defects remains to be determined.

## Prospects

This review puts into perspective several fields of investigation that have never been previously reviewed together. It should provide us with a novel working framework to determine how p53 inactivation in human cancer should be analyzed to allow a better understanding of p53 pathways but also to improve our strategy for the clinical analysis of p53 alterations. The value of p53 mutations as a clinical biomarker in various human tumors has been the subject of intense investigation (Soussi, 2005). Despite these efforts, no consensus has been reached, and p53 mutation analysis is not yet used in clinical practice. p53 alterations are a very complex issue, and this review describes how p53 mutations could be shaped by several factors, including genetic background, other tumor-associated genetic alterations, and tissue-specific factors. Other factors that have not been discussed here, including gender, ethnic background, and age, which could also have a major impact on p53 mutant selection, must also be considered. Furthermore, p53 is only one component of a giant surveillance network whose efficiency is modulated by many other elements including the other members of the p53 family and several other signaling pathways. The essential question concerns the strategy used to assess p53 status in human tumors, as a mutation is only one of the multiple ways to impair the p53 pathway. Whether or not these different pathways are associated with the same tumor phenotype is of importance. *MDM2* amplification observed in a high frequency of sarcomas is certainly associated with the p53-independent oncogenic function of the MDM2 protein. As the main function of p53 protein is to act as a transcription factor with a very broad spectrum of target genes, comparing expression profiles of tumors with different p53 status could be very useful to identify a specific p53 signature, as several analyses have shown that p53 mutations are associated with a specific expression signature in breast cancer (Sorlie et al., 2001; Miller et al., 2005). It has yet to be determined whether this expression signature is similar in other tissues or associated with indirect p53 inactivation.

The recent demonstration that therapy based on p53 reactivation in p53-deficient tumors can lead either to potent apoptosis or cellular senescence, depending on the type of tumor, should encourage us to undertake more global analysis of the p53 status in tumor cells (Martins et al., 2006; Ventura et al., 2007; Xue et al., 2007). Several novel strategies are already underway, including small molecules that activate WT p53 or target mutant p53 (Wiman, 2006; Vassilev, 2007). The effect of mutant p53 rescue by compounds such as CP-31398 and PRIMA-1 will depend on the specific type of p53 mutation, as certain mutants appear less amenable to reactivation. Many other factors, including p53 and *MDM2* polymorphisms and the expression of p53 family isoforms and other p53-regulating proteins, e.g., iASPP, can also affect therapeutic efficacy.

Activation of WT p53 via inhibition of p53-MDM2 binding by low-molecular-weight compounds such as Nutlin 3 or RITA is applicable to tumors that retain WT p53 but also to tumors with mutant p53, and the efficiency of this type of therapy could be modulated by *MDM2* SNP309 (Issaeva et al., 2004; Tovar et al., 2006).

It is therefore reasonable to anticipate that analysis of the p53 status in human cancer will comprise a combination of SNP analysis to evaluate the patient's individual "p53 network genotype" and precise analysis of the tumor status at the DNA level (p53 mutation) and/or at the RNA level (expression signature). Only such studies will be able to determine whether or not p53 will keep all of its promises in clinical oncology.

### REFERENCES

Agami, R., Blandino, G., Oren, M., and Shaul, Y. (1999). Interaction of c-Abl and p73alpha and their collaboration to induce apoptosis. Nature *399*, 809–813.

Beckman, G., Birgander, R., Sjalander, A., Saha, N., Holmberg, P.A., Kivela, A., and Beckman, L. (1994). Is p53 polymorphism maintained by natural selection? Hum. Hered. *44*, 266–270.

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.

Bergamaschi, D., Samuels, Y., Sullivan, A., Zvelebil, M., Breyssens, H., Bisso, A., Del Sal, G., Syed, N., Smith, P., Gasco, M., et al. (2006). iASPP preferentially binds p53 proline-rich region and modulates apoptotic function of codon 72-polymorphic p53. Nat. Genet. *38*, 1133–1141.

Boersma, B.J., Howe, T.M., Goodman, J.E., Yfantis, H.G., Lee, D.H., Chanock, S.J., and Ambs, S. (2006). Association of breast cancer outcome with status of p53 and MDM2 SNP309. J. Natl. Cancer Inst. *98*, 911–919.

Bond, G.L., Hu, W., Bond, E.E., Robins, H., Lutzker, S.G., Arva, N.C., Bargonetti, J., Bartel, F., Taubert, H., Wuerl, P., et al. (2004). A single nucleotide polymorphism in the MDM2 promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. Cell *119*, 591–602.

Bond, G.L., Hirshfield, K.M., Kirchhoff, T., Alexe, G., Bond, E.E., Robins, H., Bartel, F., Taubert, H., Wuerl, P., Hait, W., et al. (2006). MDM2 SNP309 accelerates tumor formation in a gender-specific and hormone-dependent manner. Cancer Res. *66*, 5104–5110.

Bond, G.L., and Levine, A.J. (2007). A single nucleotide polymorphism in the p53 pathway interacts with gender, environmental stresses and tumor genetics to influence cancer in humans. Oncogene *26*, 1317–1323.

Bourdon, J.C., Fernandes, K., Murray-Zmijewski, F., Liu, G., Diot, A., Xirodimas, D.P., Saville, M.K., and Lane, D.P. (2005). p53 isoforms can regulate p53 transcriptional activity. Genes Dev. *19*, 2122–2137.

Brooks, C.L., and Gu, W. (2006). p53 ubiquitination: Mdm2 and beyond. Mol. Cell *21*, 307–315.

Cao, L., Kim, S., Xiao, C., Wang, R.H., Coumoul, X., Wang, X., Li, W.M., Xu, X.L., De Soto, J.A., Takai, H., et al. (2006). ATM-Chk2-p53 activation prevents tumorigenesis at an expense of organ homeostasis upon Brca1 deficiency. EMBO J. *25*, 2167–2177.

Crook, T., Crossland, S., Crompton, M.R., Osin, P., and Gusterson, B.A. (1997). p53 mutations in BRCA1-associated familial breast cancer. Lancet *350*, 638–639.

Denissenko, M.F., Koudriakova, T.B., Smith, L., O'Connor, T.R., Riggs, A.D., and Pfeifer, G.P. (1998). The p53 codon 249 mutational hotspot in hepatocellular carcinoma is not related to selective formation or persistence of aflatoxin B1 adducts. Oncogene *17*, 3007–3014.

Deyoung, M.P., and Ellisen, L.W. (2007). p63 and p73 in human cancer: Defining the network. Oncogene *26*, 5169–5183.

Di Como, C.J., Gaiddon, C., and Prives, C. (1999). p73 function is inhibited by tumor-derived p53 mutants in mammalian cells. Mol. Cell. Biol. *19*, 1438–1449.

DiGiammarino, E.L., Lee, A.S., Cadwell, C., Zhang, W., Bothner, B., Ribeiro, R.C., Zambetti, G., and Kriwacki, R.W. (2002). A novel mechanism of tumorigenesis involving pH-dependent destabilization of a mutant p53 tetramer. Nat. Struct. Biol. *9*, 12–16.

Dumont, P., Leu, J.I., Della Pietra, A.C., III, George, D.L., and Murphy, M. (2003). The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. Nat. Genet. *33*, 357–365.

el-Deiry, W.S., Kern, S.E., Pietenpol, J.A., Kinzler, K.W., and Vogelstein, B. (1992). Definition of a consensus binding site for p53. Nat. Genet. *1*, 45–49.

Fei, P., Bernhard, E.J., and El-Deiry, W.S. (2002). Tissue-specific induction of p53 targets in vivo. Cancer Res. *62*, 7316–7327.

Felley-Bosco, E., Weston, A., Cawley, H.M., Bennett, W.P., and Harris, C.C. (1993). Functional studies of a germ-line polymorphism at codon 47 within the p53 gene. Am. J. Hum. Genet. *53*, 752–759.

Funk, W.D., Pak, D.T., Karas, R.H., Wright, W.E., and Shay, J.W. (1992). A transcriptionally active DNA-binding site for human p53 protein complexes. Mol. Cell. Biol. *12*, 2866–2871.

Futreal, P.A., Coin, L., Marshall, M., Down, T., Hubbard, T., Wooster, R., Rahman, N., and Stratton, M.R. (2004). A census of human cancer genes. Nat. Rev. Cancer *4*, 177–183.

Gaiddon, C., Lokshin, M., Ahn, J., Zhang, T., and Prives, C. (2001). A subset of tumor-derived mutant forms of p53 down-regulate p63 and p73 through a direct interaction with the p53 core domain. Mol. Cell. Biol. *21*, 1874–1887.

Gong, J.G., Costanzo, A., Yang, H.Q., Melino, G., Kaelin, W.G.J., Levrero, M., and Wang, J.Y. (1999). The tyrosine kinase c-Abl regulates p73 in apoptotic response to cisplatin-induced DNA damage. Nature *399*, 806–809.

Gottlieb, E., Haffner, R., King, A., Asher, G., Gruss, P., Lonai, P., and Oren, M. (1997). Transgenic mouse model for studying the transcriptional activity of the p53 protein: Age- and tissue-dependent changes in radiation-induced activation during embryogenesis. EMBO J. *16*, 1381–1390.

Harris, N., Brill, E., Shohat, O., Prokocimer, M., Wolf, D., Arai, N., and Rotter, V. (1986). Molecular basis for heterogeneity of the human p53 protein. Mol. Cell. Biol. *6*, 4650–4656.

Harris, S.L., Gil, G., Robins, H., Hu, W., Hirshfield, K., Bond, E., Bond, G., and Levine, A.J. (2005). Detection of functional single-nucleotide polymorphisms that affect apoptosis. Proc. Natl. Acad. Sci. USA *102*, 16297–16302.

Hung, R.J., Hall, J., Brennan, P., and Boffetta, P. (2005). Genetic polymorphisms in the base excision repair pathway and cancer risk: A HuGE review. Am. J. Epidemiol. *162*, 925–942.

Hussain, S.P., Hollstein, M.H., and Harris, C.C. (2000). p53 tumor suppressor gene: At the crossroads of molecular carcinogenesis, molecular epidemiology, and human risk assessment. Ann. N Y Acad. Sci. *919*, 79–85.

Inga, A., Storici, F., Darden, T.A., and Resnick, M.A. (2002). Differential transactivation by the p53 transcription factor is highly dependent on p53 level and promoter target sequence. Mol. Cell. Biol. *22*, 8612–8625.

Issaeva, N., Bozko, P., Enge, M., Protopopova, M., Verhoef, L.G., Masucci, M., Pramanik, A., and 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.

Iwakuma, T., and Lozano, G. (2007). Crippling p53 activities via knock-in mutations in mouse models. Oncogene *26*, 2177–2184.

Joerger, A.C., and Fersht, A.R. (2007). Structure-function-rescue: The diverse nature of common p53 cancer mutants. Oncogene *26*, 2226–2242.

Kato, S., Han, S.Y., Liu, W., Otsuka, K., Shibata, H., Kanamaru, R., and 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. USA *100*, 8424–8429.

Konishi, M., Kikuchi-Yanoshita, R., Tanaka, K., Muraoka, M., Onda, A., Okumura, Y., Kishi, N., Iwama, T., Mori, T., Koike, M., et al. (1996). Molecular nature of colon tumors in hereditary nonpolyposis colon cancer, familial polyposis, and sporadic colon cancer. Gastroenterology *111*, 307–317.

Lang, G.A., Iwakuma, T., Suh, Y.A., Liu, G., Rao, V.A., Parant, J.M., Valentin-Vega, Y.A., Terzian, T., Caldwell, L.C., Strong, L.C., et al. (2004). Gain of function of a p53 hot spot mutation in a mouse model of Li-Fraumeni syndrome. Cell *119*, 861–872.

Laurie, N.A., Donovan, S.L., Shih, C.S., Zhang, J., Mills, N., Fuller, C., Teunisse, A., Lam, S., Ramos, Y., Mohan, A., et al. (2006). Inactivation of the p53 pathway in retinoblastoma. Nature *444*, 61–66.

Leong, C. O., Vidnovic, N., Deyoung, M. P., Sgroi, D., and Ellisen, L. W. (2007). The p63/p73 network mediates chemosensitivity to cisplatin in a biologically defined subset of primary breast cancers. J. Clin. Invest. *117*, 1370–1380.

Levine, A.J., Feng, Z., Mak, T.W., You, H., and Jin, S. (2006). Coordination and communication between the p53 and IGF-1-AKT-TOR signal transduction pathways. Genes Dev. *20*, 267–275.

Li, X., Dumont, P., Della Pietra, A., Shetler, C., and Murphy, M.E. (2005). The codon 47 polymorphism in p53 is functionally significant. J. Biol. Chem. *280*, 24245–24251.

Marin, M.C., Jost, C.A., Brooks, L.A., Irwin, M.S., O'Nions, J., Tidy, J.A., James, N., McGregor, J.M., Harwood, C.A., Yulug, I.G., et al. (2000). A common polymorphism acts as an intragenic modifier of mutant p53 behaviour. Nat. Genet. *25*, 47–54.

Marine, J.C., Dyer, M.A., and Jochemsen, A.G. (2007). MDMX: From bench to bedside. J. Cell Sci. *120*, 371–378.

Martins, C.P., Brown-Swigart, L., and Evan, G.I. (2006). Modeling the therapeutic efficacy of p53 restoration in tumors. Cell *127*, 1323–1334.

Mayo, L.D., and Donner, D.B. (2001). A phosphatidylinositol 3-kinase/Akt pathway promotes translocation of Mdm2 from the cytoplasm to the nucleus. Proc. Natl. Acad. Sci. USA *98*, 11598–11603.

Menendez, D., Krysiak, O., Inga, A., Krysiak, B., Resnick, M.A., and Schonfelder, G. (2006). A SNP in the flt-1 promoter integrates the VEGF system into the p53 transcriptional network. Proc. Natl. Acad. Sci. USA *103*, 1406–1411.

Michael, D., and Oren, M. (2003). The p53-Mdm2 module and the ubiquitin system. Semin. Cancer Biol. *13*, 49–58.

Midgley, C.A., Owens, B., Briscoe, C.V., Thomas, D.B., Lane, D.P., and Hall, P.A. (1995). Coupling between gamma irradiation, p53 induction and the apoptotic response depends upon cell type in vivo. J. Cell Sci. *108*, 1843–1848.

Mihara, M., Erster, S., Zaika, A., Petrenko, O., Chittenden, T., Pancoska, P., and Moll, U.M. (2003). p53 has a direct apoptogenic role at the mitochondria. Mol. Cell *11*, 577–590.

Miller, L.D., Smeds, J., George, J., Vega, V.B., Vergara, L., Ploner, A., Pawitan, Y., Hall, P., Klaar, S., Liu, E.T., and Bergh, J. (2005). An expression signature for p53 status in human breast cancer predicts mutation status, transcriptional effects, and patient survival. Proc. Natl. Acad. Sci. USA *102*, 13550–13555.

Moll, U.M., LaQuaglia, M., Benard, J., and Riou, G. (1995). Wild-type p53 protein undergoes cytoplasmic sequestration in undifferentiated neuroblastomas but not in differentiated tumors. Proc. Natl. Acad. Sci. USA *92*, 4407–4411.

Nikolaev, A.Y., Li, M., Puskas, N., Qin, J., and Gu, W. (2003). Parc: A cytoplasmic anchor for p53. Cell *112*, 29–40.

Olive, K.P., Tuveson, D.A., Ruhe, Z.C., Yin, B., Willis, N.A., Bronson, R.T., Crowley, D., and Jacks, T. (2004). Mutant p53 gain of function in two mouse models of Li-Fraumeni syndrome. Cell *119*, 847–860.

Peart, M.J., and Prives, C. (2006). Mutant p53 gain of function: The NF-Y connection. Cancer Cell *10*, 173–174.

Perez, C.A., and Pietenpol, J.A. (2007). Transcriptional programs regulated by p63 in normal epithelium and tumors. Cell Cycle *6*, 246–254.

Phang, B. H., and Sabapathy, K. (2007). The codon 72 polymorphism-specific effects of human p53 are absent in mouse cells: Implications on generation of mouse models. Oncogene *26*, 2964–2974. Published online November 20, 2006. 10.1038/sj.onc.1210112.

Pietsch, E.C., Humbey, O., and Murphy, M.E. (2006). Polymorphisms in the p53 pathway. Oncogene *25*, 1602–1611.

Ponchel, F., Puisieux, A., Tabone, E., Michot, J.P., Froschl, G., Morel, A.P., Frebourg, T., Fontaniere, B., Oberhammer, F., and Ozturk, M. (1994). Hepatocarcinoma-specific mutant p53–249ser induces mitotic activity but has no effect on transforming growth factor beta 1-mediated apoptosis. Cancer Res. *54*, 2064–2068.

Prives, C., and Manfredi, J.J. (2005). The continuing saga of p53—More sleepless nights ahead. Mol. Cell *19*, 719–721.

Puisieux, A., Lim, S., Groopman, J., and Ozturk, M. (1991). Selective targeting of p53 gene mutational hotspots in human cancers by etiologically defined carcinogens. Cancer Res. *51*, 6185–6189.

Qian, H., Wang, T., Naumovski, L., Lopez, C.D., and Brachmann, R.K. (2002). Groups of p53 target genes involved in specific p53 downstream effects cluster into different classes of DNA binding sites. Oncogene *21*, 7901–7911.

Ratovitski, E., Trink, B., and Sidransky, D. (2006). p63 and p73: Teammates or adversaries? Cancer Cell *9*, 1–2.

Ribeiro, R.C., Sandrini, F., Figueiredo, B., Zambetti, G.P., Michalkiewicz, E., Lafferty, A.R., DeLacerda, L., Rabin, M., Cadwell, C., Sampaio, G., et al. (2001). An inherited p53 mutation that contributes in a tissue-specific manner to pediatric adrenal cortical carcinoma. Proc. Natl. Acad. Sci. USA *98*, 9330–9335.

Ringshausen, I., O'Shea, C.C., Finch, A.J., Swigart, L.B., and Evan, G.I. (2006). Mdm2 is critically and continuously required to suppress lethal p53 activity in vivo. Cancer Cell *10*, 501–514.

Rohaly, G., Chemnitz, J., Dehde, S., Nunez, A.M., Heukeshoven, J., Deppert, W., and Dornreiter, I. (2005). A novel human p53 isoform is an essential element of the ATR-intra-S phase checkpoint. Cell *122*, 21–32.

Sakamuro, D., Sabbatini, P., White, E., and Prendergast, G.C. (1997).

The polyproline region of p53 is required to activate apoptosis but not growth arrest. Oncogene 15, 887–898.

Samuels-Lev, Y., O'Connor, D.J., Bergamaschi, D., Trigiante, G., Hsieh, J.K., Zhong, S., Campargue, I., Naumovski, L., Crook, T., and Lu, X. (2001). ASPP proteins specifically stimulate the apoptotic function of p53. Mol. Cell 8, 781–794.

Smith, P.D., Crossland, S., Parker, G., Osin, P., Brooks, L., Waller, J., Philp, E., Crompton, M.R., Gusterson, B.A., Allday, M.J., and Crook, T. (1999). Novel p53 mutants selected in BRCA-associated tumours which dissociate transformation suppression from other wild-type p53 functions. Oncogene 18, 2451–2459.

Sorlie, T., Perou, C.M., Tibshirani, R., Aas, T., Geisler, S., Johnsen, H., Hastie, T., Eisen, M.B., van de Rijn, M., Jeffrey, S.S., et al. (2001). Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc. Natl. Acad. Sci. USA 98, 10869–10874.

Soussi, T. (2005). The p53 pathway and human cancer. Br. J. Surg. 92, 1331–1332.

Soussi, T. (2007). p53 alterations in human cancer: More questions than answers. Oncogene 26, 2145–2156.

Soussi, T., and Lozano, G. (2005). p53 mutation heterogeneity in cancer. Biochem. Biophys. Res. Commun. 331, 834–842.

Soussi, T., Ishioka, C., Claustres, M., and Beroud, C. (2006). Locus-specific mutation databases: Pitfalls and good practice based on the p53 experience. Nat. Rev. Cancer 6, 83–90.

Staib, F., Hussain, S.P., Hofseth, L.J., Wang, X.W., and Harris, C.C. (2003). TP53 and liver carcinogenesis. Hum. Mutat. 21, 201–216.

Stiewe, T. (2007). The p53 family in differentiation and tumorigenesis. Nat. Rev. Cancer 7, 165–168.

Strano, S., Munarriz, E., Rossi, M., Cristofanelli, B., Shaul, Y., Castagnoli, L., Levine, A.J., Sacchi, A., Cesareni, G., Oren, M., and Blandino, G. (2000). Physical and functional interaction between p53 mutants and different isoforms of p73. J. Biol. Chem. 275, 29503–29512.

Tidow, H., Veprintsev, D.B., Freund, S.M., and Fersht, A.R. (2006). Effects of oncogenic mutations and DNA response elements on the binding of p53 to p53-binding protein 2 (53BP2). J. Biol. Chem. 281, 32526–32533.

Toledo, F., and Wahl, G.M. (2006). Regulating the p53 pathway: In vitro hypotheses, in vivo veritas. Nat. Rev. Cancer 6, 909–923.

Toledo, F., Krummel, K.A., Lee, C.J., Liu, C.W., Rodewald, L.W., Tang, M., and Wahl, G.M. (2006). A mouse p53 mutant lacking the proline-rich domain rescues Mdm4 deficiency and provides insight into the Mdm2-Mdm4-p53 regulatory network. Cancer Cell 9, 273–285.

Tomso, D.J., Inga, A., Menendez, D., Pittman, G.S., Campbell, M.R., Storici, F., Bell, D.A., and Resnick, M.A. (2005). Functionally distinct polymorphic sequences in the human genome that are targets for p53 transactivation. Proc. Natl. Acad. Sci. USA 102, 6431–6436.

Tovar, C., Rosinski, J., Filipovic, Z., Higgins, B., Kolinsky, K., Hilton, H., Zhao, X., Vu, B.T., Qing, W., Packman, K., et al. (2006). Small-molecule MDM2 antagonists reveal aberrant p53 signaling in cancer: Implications for therapy. Proc. Natl. Acad. Sci. USA 103, 1888–1893.

Vassilev, L.T. (2007). MDM2 inhibitors for cancer therapy. Trends Mol. Med. 13, 23–31. Published online November 28, 2006.

Venkitaraman, A.R. (2002). Cancer susceptibility and the functions of BRCA1 and BRCA2. Cell 108, 171–182.

Venot, C., Maratrat, M., Dureuil, C., Conseiller, E., Bracco, L., and Debussche, L. (1998). The requirement for the p53 proline-rich functional domain for mediation of apoptosis is correlated with specific PIG3 gene transactivation and with transcriptional repression. EMBO J. 17, 4668–4679.

Ventura, A., Kirsch, D.G., McLaughlin, M.E., Tuveson, D.A., Grimm, J., Lintault, L., Newman, J., Reczek, E.E., Weissleder, R., and Jacks, T. (2007). Restoration of p53 function leads to tumour regression in vivo. Nature 445, 661–665.

Walker, K.K., and Levine, A.J. (1996). Identification of a novel p53 functional domain that is necessary for efficient growth suppression. Proc. Natl. Acad. Sci. USA 93, 15335–15340.

Weisz, L., Oren, M., and Rotter, V. (2007). Transcription regulation by mutant p53. Oncogene 26, 2202–2211.

Wiman, K.G. (2006). Strategies for therapeutic targeting of the p53 pathway in cancer. Cell Death Differ. 13, 921–926.

Xue, W., Zender, L., Miething, C., Dickins, R.A., Hernando, E., Krizhanovsky, V., Cordon-Cardo, C., and Lowe, S.W. (2007). Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. Nature 445, 656–660.

Yang, A., and McKeon, F. (2000). P63 and P73: P53 mimics, menaces and more. Nat. Rev. Mol. Cell Biol. 1, 199–207.

Yuan, Z.M., Shioya, H., Ishiko, T., Sun, X., Gu, J., Huang, Y.Y., Lu, H., Kharbanda, S., Weichselbaum, R., and Kufe, D. (1999). p73 is regulated by tyrosine kinase c-Abl in the apoptotic response to DNA damage. Nature 399, 814–817.

Zaika, A., Marchenko, N., and Moll, U.M. (1999). Cytoplasmically "sequestered" wild type p53 protein is resistant to Mdm2-mediated degradation. J. Biol. Chem. 274, 27474–27480.

Zhou, B.P., Liao, Y., Xia, W., Zou, Y., Spohn, B., and Hung, M.C. (2001). HER-2/neu induces p53 ubiquitination via Akt-mediated MDM2 phosphorylation. Nat. Cell Biol. 3, 973–982.

Ziegler, A., Jonason, A.S., Leffell, D.J., Simon, J.A., Sharma, H.W., Kimmelman, J., Remington, L., Jacks, T., and Brash, D.E. (1994). Sunburn and p53 in the onset of skin cancer. Nature 372, 773–776.