

Clinical Outcomes and Correlates of TP53 Mutations and Cancer

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The initial observation that p53 accumulation might serve as a surrogate biomarker for TP53 mutation has been the cornerstone for vast translational efforts aimed at validating its clinical use for the diagnosis, prognosis, and treatment of cancer. Early on, it was realized that accurate evaluation of p53 status and function could not be achieved through protein-expression analysis only. As our understanding of the p53 pathway has evolved and more sophisticated methods for assessment of p53 functional integrity have become available, the clinical and molecular epidemiological implications of p53 abnormalities in cancers are being revealed. They include diagnostic testing for germline p53 mutations, and the assessment of selected p53 mutations as biomarkers of carcinogen exposure and cancer risk and prognosis. Here, we describe the strengths and limitations of the most frequently used techniques for determination of p53 status in tumors, as well as the most remarkable latest findings relating to its clinical and epidemiological value.

GERMLINE TP53 MUTATIONS AND INHERITED CANCER PREDISPOSITION

Germline mutations in the TP53 tumor suppressor gene are associated with the Li-Fraumeni multicancer predisposition syndrome (LFS; OMIM #151623). This autosomal dominant syndrome is characterized by a high incidence of early-onset cancers comprising mostly breast cancer, sarcoma, adrenocortical carcinoma (ACC), leukemia, and brain tumors. The similarity between cancer types developed in patients with clinical features of LFS, and those with the highest incidence of somatic mutations in TP53 were instrumental to the discovery of inherited TP53 mutations as the basis for

the syndrome (Malkin et al. 1990). Diagnostic testing for germline TP53 mutations in families with suspected LFS through direct DNA sequencing of exons 2–11 has now been validated in a large clinical cohort (Gonzalez et al. 2009). From that study, it has been proposed that childhood ACC and the presence of a family member with one of the “core” cancers, sarcoma, brain tumor, breast cancer, or ACC, are the most likely indicators of a germline TP53 mutation that should prompt a request for diagnostic testing by molecular screening. A detailed analysis of TP53 mutation types in a large cohort of French LFS families has established that missense mutations are the most frequent and are

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associated with earlier onset of cancer as compared with loss-of-function mutations (Bougeard et al. 2008). Summary results from this study are depicted in Figure 1A. Although such missense mutations were determined to be transcriptionally inactive based on yeast functional assays, their association with earlier onset of cancers argues in favor of an additional gain-of-function oncogenic effect (Sigal and Rotter 2000; Brosh and Rotter 2009). In terms of the codon distribution along the p53 molecule, germline mutations follow a similar pattern to somatic mutations in sporadic cancers, with hot-spot mutations being associated mainly with the DNA binding domain (Fig. 1B). One notable exception is the high frequency of codon 337 mutations in LFS families. Single base-pair substitutions at Arg337 have been described in 72 families and 233 individuals with LFS (<http://www-p53.iarc.fr>), but are exceedingly rare in sporadic cancers (0.1% single base-pair substitutions). The R337H mutation, predominantly found in LFS families from southern Brazil, is a risk allele for pediatric ACC and is a unique example of tissue-specific predisposing mutation (Figueiredo et al. 2006). In all, much continues to be learned that is allowing better recognition and management of this complex disease.

COMMON GENETIC VARIANTS OF TP53 AS BIOMARKERS OF CANCER RISK AND OUTCOME

A common polymorphism at codon 72 of TP53 (rs1042522) results in either proline (p53pro) or arginine (p53arg). This nonconservative amino-acid change is found in the proline-rich domain and affects certain p53 biochemical properties. p53pro is a strong transcriptional activator, but a poorer inducer of apoptosis than p53arg (Thomas et al. 1999). In addition, p53arg is more susceptible to degradation by the human papillomavirus (HPV) E6 protein (Storey et al. 1998), leading to a controversial hypothesis that the arginine variant could be associated with risk and/or progression of cervical cancer (Ojeda et al. 2003), a finding disputed by additional meta-analysis (Koushik et al. 2004) and a recent large population study

(Koshiol et al. 2009). Several epidemiological studies have reported an association between the presence of a pro/pro genotype and the risk for lung adenocarcinoma development (Weston et al. 1992; Fan et al. 2000; Liu et al. 2001), which has been confirmed by meta-analysis (Matakidou et al. 2003). In a case-only study of lung cancer, p53pro was also associated with an increased frequency of somatic mutations in TP53 (Mechanic et al. 2005). Homozygous Pro/Pro is more prevalent in African Americans, and is associated in this population, either by itself or within its haplotype block, with worse survival for lung cancer (Mechanic et al. 2007) and colorectal cancer (Katkooori et al. 2009).

SOMATIC TP53 MUTATIONS

TP53 is frequently mutated in human cancer and the mutational spectrum can be a molecular link to etiological causes of cancer (Hollstein et al. 1991; Levine et al. 1991; Petitjean et al. 2007). The cancer-associated mutations in TP53 are primarily missense substitutions nonrandomly distributed along the molecule, particularly the central DNA-binding-domain (<http://www-p53.iarc.fr>). These single amino-acid changes affect p53's transcriptional activity to various degrees. Not all mutations have equal deleterious effect on p53 function and some missense mutants may even acquire new functions (Weisz et al. 2007; Brosh and Rotter 2009). The TP53 mutational spectrum is characterized by the presence of mutations at six discrete hot-spot codons within the DNA binding domain of the molecule: Codons 175, 245, 248, 249, 273, and 282 (Fig. 1C). The TP53 mutational pattern has proved to be a clinically relevant "molecular sensor" of genotoxic exposure to environmental carcinogens and endogenous mutagens (Hussain and Harris 1999).

METHODOLOGIES FOR ASSESSMENT OF P53 STATUS IN CLINICAL AND EPIDEMIOLOGICAL STUDIES

The need for standardized methods for assessment of p53 mutation status in tumors has

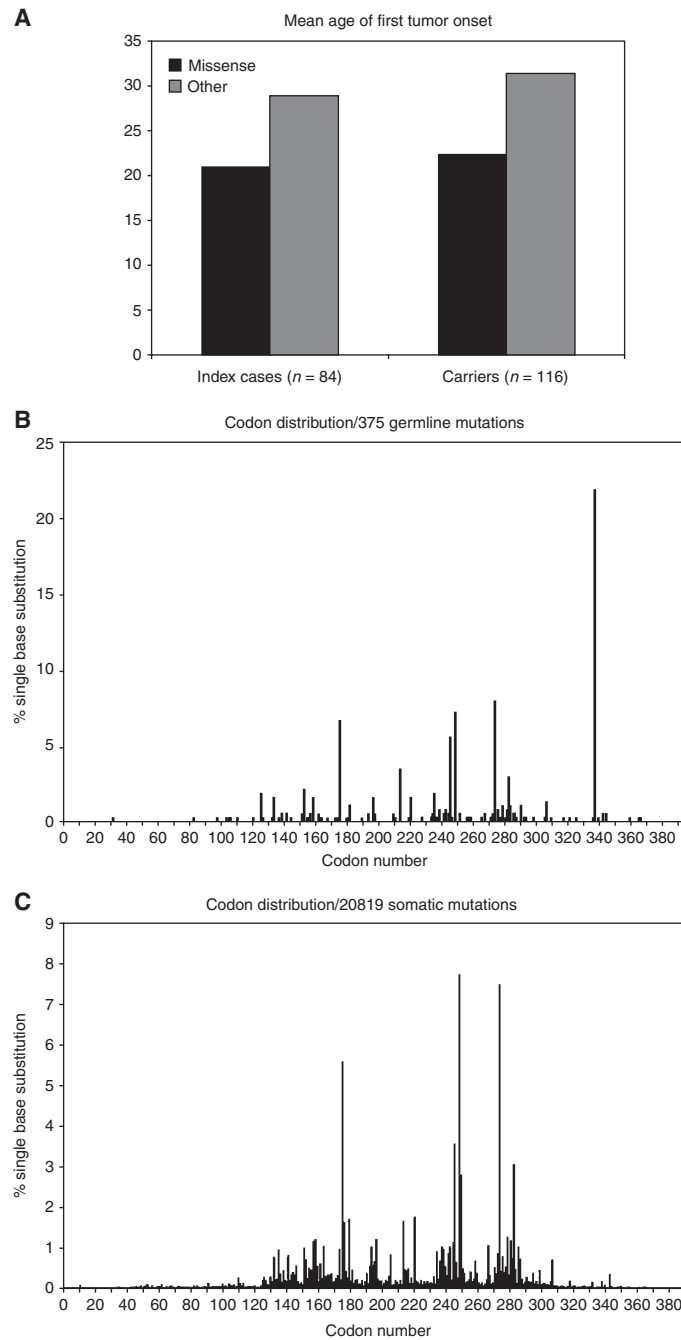


Figure 1. The TP53 mutational spectrum in familial and sporadic cancer. (A) Germline TP53 missense mutations result in earlier onset of cancers in LFS ($p = .017$ for index cases, and $p = .004$ for carriers). Mutation spectrum of (B) germline and (C) somatic TP53 mutations. Reproduced from the IARC p53 database (<http://www-p53.iarc.fr>) using the provided bioinformatics tools (Petitjean et al. 2007). (A, Adapted, with permission, from Bougeard et al. 2008 [© BMJ Group].)

been recognized and guidelines were established as a starting point to develop p53 as a biomarker (Soussi and Beroud 2001).

Gene Sequencing

DNA sequencing is the gold standard for identification of TP53 mutations. Gel-based mutation screening assays, such as SSCP or PCR-RFLP, are routinely used before sequencing. In this technique, TP53 is amplified and resulting PCR fragments are subjected to enzymatic restriction using an enzyme for which a site is predicted to be created or destroyed by the presence of mutation. The resulting gel profile is used as an indicator for the presence of mutation, and sequencing of that area is undertaken using direct sequencing methods. For small samples, whole-genome amplification can precede exon-specific PCR and sequencing (Lim et al. 2009). Most TP53 mutations identified in tumors are circumscribed to the area encompassing exons 5–8 and therefore many translational studies have limited their mutational analysis to this portion of the gene. This has caused bias in the TP53 mutation literature because mutations outside exons 5–8 may have been missed. The problem is likely to be solved with more sophisticated targeted high-throughput DNA sequencing strategies that may in the future be used to gather nucleotide-level information about TP53 and other critical genes in clinical samples (Ding et al. 2008). Still, the presence of mutation does not unequivocally indicate that p53 is fully inactive, nor does the absence of it indicate that p53 is functionally proficient. Thus, assessing functional activity of p53 mutants is essential for an accurate indication of clinical relevance.

Immunohistochemistry (IHC)

The overexpression and accumulation of p53 protein has been widely used as surrogate for detection of p53 abnormalities in tumors. The principle behind this assay is that mutant p53 is unable to transcriptionally activate MDM2, thus resulting in loss of negative-feedback loop

and accumulation of p53 protein. Surprisingly, p53 protein is not stabilized in nontumor tissues of patients with LFS or mice bearing a TP53 mutation through homologous recombination (gene knockin). So, overexpression of p53 is context-dependent and intimately associated with tumor development. In addition, positive IHC will not recognize null mutations, which comprise nonsense mutations, deletions, insertions, and splicing junction mutations, and account for up to 30% of somatic TP53 mutations. So, as could be expected, IHC is not a good readout for TP53 mutation when the frequency of null mutations is high (Alsner et al. 2008). Importantly, it has been shown that null mutations can be more predictive of outcome than positive IHC (Hashimoto et al. 1999), further casting doubt on the role of IHC in the clinical setting. Moreover, the interpretation of positive p53 immunostaining in early lesions should be done with caution, because it may be a sign of wild-type protein accumulation in response to an inflammatory microenvironment (Hofseth et al. 2003), or even senescence (Collado et al. 2005). The obvious advantage of IHC is that it is a straightforward technique that can be applied on tissue preserved in a similar manner as required for routine histopathological assessment and scored in a routine pathology laboratory. It does not require a priori knowledge or assumptions about the specific mutational event and can be easily incorporated to existing panels of biomarkers for specific subtypes of cancer. The evaluation of MDM2 as well as p53 staining in tumors provides readout for transcriptional activity as well as of functional inactivation in the absence of mutation. This strategy was reported to accurately identify the TP53 status of tumors (Nenutil et al. 2005). When used as part of a panel of biomarkers, p53 IHC may provide important clinical information. For example, p53 IHC in combination with hormone receptor, ERBB2, BCL2 staining, and MYC status can yield a robust global profile that predicts risk of relapse for breast cancer patients undergoing adjuvant therapy and can help inform clinical decisions of the appropriate level of postoperative adjuvant therapy (Bremer et al. 2009).



TP53 Mutational Load Assay

This methodology relies on the existence of specific enzyme restriction sites in the wild-type TP53 sequence that are destroyed by the mutation that is being measured. Upon elimination of the TP53 wild-type sequence through enzymatic digestion, the mutated sequence is enriched by PCR, cloned, and hybridized to oligonucleotides complementary to wild type or mutant sequences in the presence of standards and internal controls to determine mutation frequency. Initially developed to detect codon 249 mutations in cell culture (Aguilar et al. 1993), this method has been adapted for detection and measurement of codon 249 mutations in nontumor tissue of Wilson's disease patients (Hussain et al. 2000b), codon 247 and 248 mutations in noncancerous colon tissue of ulcerative colitis patients (Hussain et al. 2000a), and codon 157 G-to-T transversions in nontumorous lungs of lung cancer patients (Hussain et al. 2001).

Mass Spectrometry

Short oligonucleotide mass analysis (SOMA) has been developed for analysis of codon 249 TP53 mutations that are associated with dietary exposure to Aflatoxin B1 (Qian et al. 2002). The high sensitivity of this technique allows detection of specific TP53 mutations present in plasma DNA at low frequency. It is also quantitative, providing a means to correlate cumulative exposures with cancer risk. It requires prior knowledge of the mutation being detected so that recognition patterns of mass spectrometry and standards can be developed.

Microarray Analysis

Several versions of spotted oligonucleotide arrays have been developed to increase the sensitivity and specificity of mutation detection, as well as maximize gene coverage (Ahrendt et al. 1999; Fouquet et al. 2004). Among them, the p53 GeneChip (Ahrendt et al. 1999) is designed to detect single base-pair substitutions and single base-pair deletions in the TP53 coding sequence (Grollman et al. 2007). After one round

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amplification of TP53 exons 2–11 followed by DNase I-mediated fragmentation, labeled, fragmented DNA amplicons are hybridized to oligonucleotide probes representing one wild-type, three possible base-pair mutations, and one single base-pair deletion for each nucleotide on the full TP53 coding sequence. The re-engineered Amplichip p53 (Olivier et al. 2009) is now under commercial development.

Functional Analysis

Although more than 80% of mutations found in tumors map to the DNA binding domain of the protein and are likely to affect its transcriptional activity, not all TP53 mutations have similarly deleterious effects. Only a fraction of TP53 mutations found in tumors are completely devoid of transcriptional activity and others are partially able to transactivate canonical p53 targets. Besides staining for downstream target genes (Nenutil et al. 2005), the effect of tumor-derived mutants on transcriptional activity can also be determined in yeast (Kato et al. 2003). For example, TP53 mutations were classified as active or inactive based on yeast functional assay and their prognostic and predictive values were evaluated in a large cohort of colorectal carcinoma cases (Iacopetta et al. 2006). Inactive p53 mutants were associated with poor survival only in late-stage tumors. The functional class of mutant p53 did not predict benefit from adjuvant chemotherapy.

Mutations in TP53 as Biomarkers of Exposure to Environmental Carcinogens

Aflatoxin B1 and 249ser Mutation

One of the first pieces of evidence that the mutational pattern of TP53 could be used as a molecular fingerprint associating exposure with cancer etiology arose from the finding that hepatocellular carcinomas (HCC) from areas with aflatoxin-contaminated crops bear a specific nucleotide substitution at the third base of codon 249 (AGG to AGT) (Bressac et al. 1991; Hsu et al. 1991). Consistently, the same



mutation was found after exposure of human hepatocytes to aflatoxin B₁ in vitro (Aguilar et al. 1993). A comprehensive review of the TP53 mutation spectra in hepatocellular carcinomas from different regions of the world showed that in areas with low aflatoxin exposure, the codon 249 mutation was less prevalent (Montesano et al. 1997). Codon 249 mutation proved to be not only a surrogate end-point marker for aflatoxin exposure, but a dynamic dose-response sensor that reflects the degree of aflatoxin exposure (Stern et al. 2001). This mutation is a biomarker for early cancer detection, because it can be measured in plasma before HCC diagnosis (Jackson et al. 2003), as well as a biomarker of exposure and risk (Kirk et al. 2005), that can synergistically predict the risk for HCC together with hepatitis B virus antigen in serum (Table 1) (Kirk et al. 2005; Hussain et al. 2007). In areas of West Africa with lower aflatoxin exposure, 249ser mutation can still be detected in plasma in HCC patients, and is under development as an early diagnostic marker in the first intervention study of its kind (Szymanska et al. 2004). Lately, the presence of 249ser mutation as a biomarker of exposure to aflatoxin has uncovered a link between aflatoxin exposure and cirrhosis (Kuniholm et al. 2008).

Sunlight and Pyrimidine Dimers

Exposure to UV-radiation from sunlight is an etiological factor for nonmelanoma skin cancers. In vitro, UV-radiation causes a characteristic

pattern of CC to TT tandem mutations that are also frequently found in the mutational spectrum of TP53 in skin cancers but not internal cancers (Brash et al. 1991). It has been proposed that presence of this mutation can be used as a biomarker of photoprotection against skin cancer (Benjamin et al. 2008).

Tobacco and G-to-T Transversions

The dose-dependent association between cigarette smoking and G-to-T transversions in TP53 in lung cancer subjects has long been recognized (Suzuki et al. 1992). A G-to-T transversion at codon 157 of TP53 (GTC to TTC) is frequent in lung cancers from smokers, but not in other types of cancer, including lung cancers from never-smokers (Hainaut and Pfeifer 2001; Vahakangas et al. 2001). The presence of TP53 mutations in plasma DNA is a biomarker of tobacco exposure (Hagiwara et al. 2006).

SOMATIC TP53 MUTATIONS AS BIOMARKERS OF CANCER RISK IN PREDISPOSING CONDITIONS

The overrepresentation of C to T transitions in the tumor spectra of TP53 mutations has prompted the proposal that endogenous reactive molecules, such as nitric oxide, may contribute to cancer etiology (Ambs et al. 1997). Nitric oxide is overproduced in chronic inflammatory diseases, such as ulcerative colitis, that are cancer prone and have high TP53 mutational load in nontumor tissues (Hussain et al. 2000a). The interplay of nitric oxide production and TP53 mutation may lead to clonal selection and tumor growth in sporadic colon cancer as well (Ambs et al. 1998; Goodman et al. 2004). Other chronic inflammatory diseases that predispose to cancer, such as hemochromatosis and Wilson disease (Ambs et al. 1999), are also associated with detectable TP53 mutations in nontumor tissue (Hussain et al. 2000b). There would be clear clinical benefit from the availability of sensitive assays that would identify mutations in individuals at risk for cancer before overt tumor development (Olivier et al. 2004). Chronic inflammation and cancer risk

Table 1. Biomarkers of Aflatoxin B₁ (AFB₁), TP53 mutation, and Hepatitis B Virus (HBV) are synergistic in predicting risk of hepatocellular carcinoma (HCC)

Biomarker	HCC RR (95% CI) ^a	
	Urinary AFB ₁ ^c	Serum TP53 mutant ^d
Minus/Minus	1	1
Plus/Minus	7 (2–24)	10 (5–20)
Minus/Plus	3 (1–10)	13 (5–35)
Plus/Plus	59 (17–212)	399 (49–3272)

^aRR: relative risk; CI: confidence interval.

^bHBVSag.

^cUrinary AFB₁ biomarker: AFB₁, AFP₁, AFM₁, and AFB₁-N⁷-Gua.

^dTP53 Codon 249^{ser} mutant in serum DNA.

is an exciting field for research that may yield early diagnostic and therapeutic opportunities (Hussain and Harris 2007).

Thus, the presence of TP53 mutations with a specific pattern or representing a plausible biological mechanism can be used clinically to study the etiology of cancers as well as screening tools for cancer predisposition and risk.

TP53 Abnormalities as Biomarkers of Cancer Prognosis

A multitude of retrospective studies have associated abnormal p53 protein expression as well as somatic mutation with poor survival or lack of response to therapy. Nonetheless, the clinical significance of p53 status for patient outcome has been (Roth 1999) and continues to be one of the most controversial areas of p53 research (Hoff 2005; Munro et al. 2005). The problem largely stems from the inherent complexity of the p53 pathway, the abundance of mutations in different tumor types and clinical stages, and their disparate effects, as well as lack of randomized prospective studies compounded by heterogeneity in experimental designs, sample types, and techniques. In addition, it would perhaps be naïve to expect that a ubiquitously mutated gene would have the same clinical value regardless of context. That being said, there have been encouraging findings regarding survival prediction by TP53 mutations. This is illustrated in Figure 2 using data extracted from the IARC TP53 database and updated with the addition of 20 sequencing-based studies published between 2007 and 2009. For cancers of the breast, head and neck, liver, hematopoietic, and lymphoid systems, a majority of studies show an association of TP53 mutation with worsened survival (Fig. 2). For cancers of the bladder, brain, lung, colon, esophagus, and ovary, about as many studies found an association as did not. For cancers of the pancreas, prostate gland, rectum, and stomach, the number as well as the cumulative number of patients in the respective cohorts reported by each publication were small. The largest patient cohorts have been reported for breast and colorectal carcinomas.

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Colorectal Cancer

Two large meta-analysis reports on the predictive value of p53 abnormalities on outcome of colon cancer show contradictory results regarding response to chemotherapy, but an essentially similar lack of significant effect on survival prediction (Munro et al. 2005; Russo et al. 2005). In contrast, a sequencing-based, prospective study on primary operable colorectal cancer patients undergoing resective surgery showed that specific TP53 mutations were associated with worse prognosis (Bazan et al. 2005). Similarly, a subset of functionally inactive mutations predicted poor survival in late stage colorectal cancers (Iacopetta et al. 2006).

Breast Cancer

Breast cancer seems to show the clearest association of TP53 mutation with poor survival in large, prospective, sequence-based studies. The presence of TP53 mutation was an independent predictor of cancer-specific death in a large cohort of women with primary breast cancer and associated with poorest prognosis when combined with the absence of progesterone receptor (Olivier et al. 2006). The overall picture that is emerging from breast cancer indicates that missense mutations that affect DNA binding are particularly deleterious and are associated with the worse survival, whereas nonmissense mutations in the DNA-binding motifs or null mutations are associated with intermediate reduction of survival compared with no mutations (Olivier et al. 2006; Alsner et al. 2008). Kaplan-Meier survival curves from the paper by Olivier et al. (2006) illustrating this finding are shown in Figure 3. In another large prospective study, TP53 mutation was found to be associated with poor survival as well as “basal-like” and “ERBB2+” gene expression subgroups, which themselves had very high mortality (Langerod et al. 2007). Missense TP53 mutations also conferred higher risk of recurrence and death compared with wild-type TP53 in women with node-negative breast cancer (Ozcelik et al. 2007). In contrast to the analysis of TP53 mutations, the overall clinical

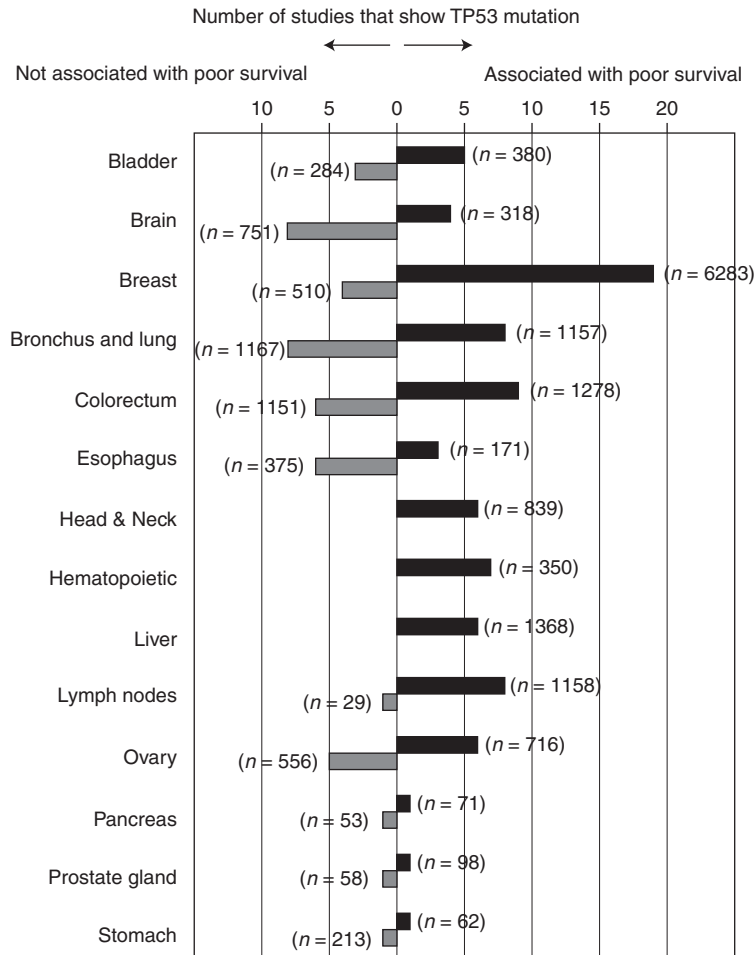


Figure 2. Graphical representation of the number of studies that have shown an association or lack of association of TP53 mutation with poor survival. The cumulative number of patients in all cohorts reported in those studies is indicated as n for each cancer type. Data were extracted from the IARC p53 database R13 release (<http://www-p53.iarc.fr>) and were updated with 20 new studies appearing in 2006–2009.

value of p53 immunostaining in breast cancer is poor with respect to other clinical parameters, including Her-2 positivity (Soerjomataram et al. 2008). Thus, the routine use of p53 immunostaining is not recommended in clinical practice (Harris et al. 2007).

Head and Neck Cancers

A prospective, multicenter trial analyzed TP53 mutation status in squamous-cell carcinoma of the head and neck through p53 GeneChip and found that presence of any TP53 mutation had an adverse effect on survival, but those mutations

that resulted in disrupted DNA binding led to an even worse prognosis (Poeta et al. 2007). In agreement, TP53 mutations in direct DNA contact areas resulted in accelerated tumor progression and reduced therapeutic responsiveness in head and neck squamous cell carcinoma (Erber et al. 1998; Temam et al. 2000).

Lung Cancer

Among patients with stage I NSCLC, survival was higher in those with wild-type p53 than in those with mutant p53 (Ahrendt et al. 2003), but TP53 mutations were not associated with

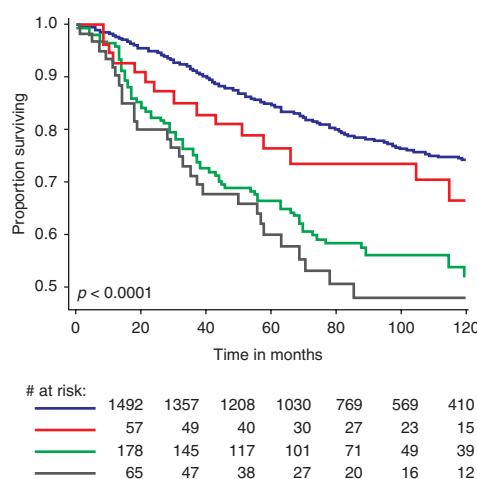


Figure 3. Kaplan-Meier survival curves of patients with breast cancer stratified by the type of TP53 gene mutation found in their tumor. Survival of patients without mutation or with a silent mutation within exons 5 to 8 (blue line), with a missense mutation within exons 5 to 8 but outside the DNA binding domains (red line), with a missense mutation in the DNA binding domains (green line), and with a mutation other than missense within exons 5 to 8 (black line). (Reprinted, with permission, from Olivier et al. 2006 [© AACR].)

the very limited survival of cases with advanced-stage NSCLC (Lim et al. 2009). TP53 mutations were, however, found to be significantly associated with recurrence of NSCLC (Ludovini et al. 2008).

Prostate Cancer

Abnormal p53 expression in prostate cancer was related to increased risk of disease-specific death as well as the development of distant metastasis at 5 years (Che et al. 2007). Most importantly, this study provided support for using p53 as a marker to stratify patients undergoing androgen deprivation and radiotherapy, by showing that long-term androgen deprivation may significantly improve the cause-specific survival for those with abnormal p53 (Che et al. 2007).

Ovarian Cancer

A meta-analysis of 62 studies addressing the prognostic significance of p53 abnormalities using IHC or mutational analysis found that overall p53 aberrant status confers poor

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survival, but the effect was modest (de Graeff et al. 2009).

Hematopoietic Cancers

Although the prevalence of TP53 mutations in chronic lymphocytic leukemia (CLL) is low (<10%), TP53 disruption is frequently observed in CLL through del17p13, and both these abnormalities are predictive of poor survival (Zenz et al. 2008; Rossi et al. 2009). Also, most of TP53 mutations found in CLL cluster within the DNA binding domain of p53.

Lymphoid Cancers

An international collaborative study on 477 patients with diffuse large B-cell lymphoma (DLBCL) showed that p53 DNA-binding mutants have a worse effect on overall survival in DLBCL than WT p53 or mutants in other domains (Young et al. 2008).

Sequencing-based randomized prospective studies have started to uncover the prognostic value of TP53 mutational analysis. Overall, the finding that null mutations have a somewhat intermediate effect on survival gives support to “gain-of-function” properties that may translate into newly acquired transcriptional functions (Weisz et al. 2007).

TP53 ABNORMALITIES AS BIOMARKERS OF THERAPEUTIC RESPONSE

The complexity of the data resulting from studies examining various patient cohorts, treatment modalities, and indices of response to therapy is overwhelming. There is no possible generalization of the role of TP53 mutation as a predictor of therapeutic response, perhaps because there is no single answer. There are, however, recent studies that evaluate it in well-defined patient cohorts and treatment modalities. It is under these conditions that evaluation of p53 status, either by IHC or sequencing, yields the most promising results. For example, TP53 mutation is a biomarker of responsiveness to dose-dense epirubicin-cyclophosphamide regimen in basal breast cancer (Bertheau et al. 2007),

and to carboplatin/gemcitabine treatment in NSCLC (Cortinovis et al. 2008). These results are encouraging in that they point to specific therapeutic regimens for patients with the worse prognosis. There is no clear picture of the role of TP53 mutation in the response to first-line chemotherapy in colorectal cancer patients (Hoff 2005; Munro et al. 2005; Russo et al. 2005; Iacopetta et al. 2006). However, mutant TP53 is a predictor of better clinical outcome in patients with chemotherapy-refractory metastatic colorectal cancer treated with the anti-EGFR antibody cetuximab (Oden-Gangloff et al. 2009). Interestingly, this association was only found in the subset of patients who lack KRAS mutations, because mutant KRAS is a strong predictor of cetuximab resistance in metastatic colorectal cancer (Lievre et al. 2008). This finding suggests that status of p53 may modulate the response of cancers to molecularly targeted drugs, even if the drugs are not designed to target the p53 pathway directly. Thus, TP53 genotyping may be useful for stratification and selection of patients that will most benefit from certain molecularly targeted therapies.

CONCLUDING REMARKS

The lack of clear predictive value of TP53 mutations in colorectal cancer compared with breast cancer starts to make sense when looking at the genomic landscapes of those two cancer types (Wood et al. 2007). Although the single most frequently mutated gene found in breast cancer is TP53, there are at least two other mutant genes that are just as prevalent in colon cancer, KRAS, and APC (Fig. 4). The interplay of several driver mutations is likely to have an impact in chemotherapeutic response, in such a way that no single mutational event may be clinically meaningful, but rather an understanding of the compounded effects of several driver mutations may be needed to inform clinical course.

Technological advances have allowed the unbiased sequencing of the whole genome in individual cancers. As a result, we now have an overall view of the mutational profile of

selected human tumors. This bird's eye view of the genomic landscape of cancers has in part served to reinforce what we already knew, that TP53 is the most frequently mutated gene in human tumors (Wood et al. 2007; Ding et al. 2008). Thus, the tantalizing proposition that evaluation of the p53 status may have prognostic value warrants further analysis.

Two recent discoveries related to p53 may have significant clinical implications. MicroRNAs are small, evolutionarily conserved non-coding RNAs that exert diverse regulatory activities on mRNA stability and translation (Ambros 2001). They are frequently deleted and/or dysregulated in cancer (Calin and Croce 2006) and can be biomarkers of cancer diagnosis, prognosis, and therapeutic outcome (Bartels and Tsongalis 2009). Certain microRNAs, e.g., mir-34a, b, and c, are transcriptionally transactivated by p53 (Chang et al. 2007; He et al. 2007; Raver-Shapira et al. 2007; Tarasov et al. 2007), whereas others, e.g., mir-125b, negatively regulate its expression (Le et al. 2009). So, it is becoming apparent that not only do TP53 mutations affect its downstream functions, such as apoptosis and senescence, but also epigenetic or mutational changes in related microRNAs need to be considered, because abnormalities in microRNAs, such as methylation of mir-34 (Lujambio et al. 2008) and/or amplification of mir-125b (Bousquet et al. 2008) may affect downstream p53 functions (Fig. 5). In addition, the discovery of p53 isoform variants arising from alternative splicing and promoter usage may also have clinical implications (Bourdon et al. 2005). These variants can interact with wild-type p53 through either dominant-negative or cotransactivating functional modulation. Most importantly, p53 isoforms are expressed in human cancers (Boldrup et al. 2007; Fujita et al. 2009) so that certain isoforms may alter the function of wild-type p53 and complicate the interpretation of nonmutant TP53 and immunohistochemical results in prediction of cancer risk, prognosis, and therapeutic response.

When we first discussed the clinical consequences of TP53 mutations (Harris and Hollstein 1993), it seemed that widespread translational

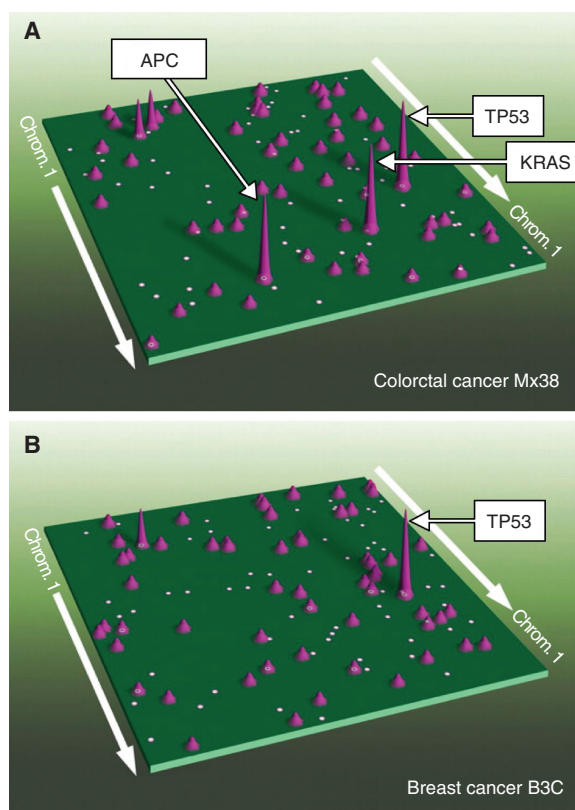


Figure 4. Cancer genome landscapes of typical (A) colorectal or (B) breast tumors. Nonsilent somatic mutations are plotted in two-dimensional space representing chromosomal positions of RefSeq genes. Peaks indicate individual genes mutated at high frequency, with peak height reflecting mutation prevalence. The dots represent genes that were somatically mutated in the individual colorectal (Mx38) or breast (B3C) tumor displayed. The mountain on the right of both landscapes represents TP53 (chromosome 17), and is shared by both breast and colorectal cancers. (Reprinted, with permission, from Wood et al. 2007 [© AAAS].)

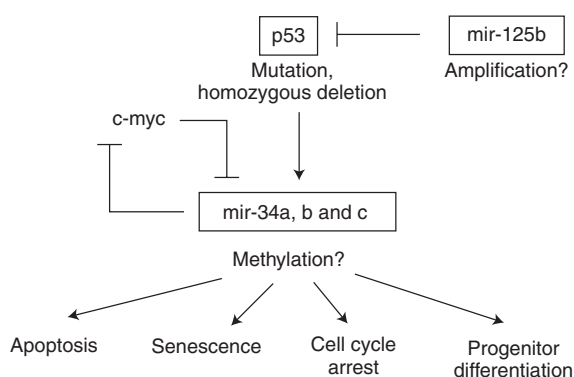


Figure 5. MicroRNAs are integral to the p53 network and their abnormalities may have clinical implications. MicroRNAs mir-34a, b, and c, are transcriptionally transactivated by p53 and are downstream effectors of p53 function. MicroRNA mir-125b negatively regulates p53 expression. In addition to TP53 mutations and deletions, abnormalities in microRNAs, such as methylation of mir-34 (Lujambio et al. 2008) and/or amplification of mir-125b (Bousquet et al. 2008) may affect downstream p53 functions.

applications for p53 functional analysis and genotyping were within reach. We now know that reliable assessment of the integrity of p53's function in sporadic cancers will require more than immunostaining, and even more than sequencing. It will require a systems biology approach that takes into account the genomic and epigenomic landscapes of cancer.

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