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Somatic *TP53* Mutations in the Era of Genome Sequencing

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Amid the complexity of genetic alterations in human cancer, *TP53* mutation appears as an almost invariant component, representing by far the most frequent genetic alteration overall. Compared with previous targeted sequencing studies, recent integrated genomics studies offer a less biased view of *TP53* mutation patterns, revealing that >20% of mutations occur outside the DNA-binding domain. Among the 12 mutations representing each at least 1% of all mutations, five occur at residues directly involved in specific DNA binding, four affect the tertiary fold of the DNA-binding domain, and three are nonsense mutations, two of them in the carboxyl terminus. Significant mutations also occur in introns, affecting alternative splicing events or generating rearrangements (e.g., in intron 1 in sporadic osteosarcoma). In aggressive cancers, mutation is so common that it may not have prognostic value (all these cancers have impaired p53 function caused by mutation or by other mechanisms). In several other cancers, however, mutation makes a clear difference for prognostication, as, for example, in *HER2*-enriched breast cancers and in lung adenocarcinoma with *EGFR* mutations. Thus, the clinical significance of *TP53* mutation is dependent on tumor subtype and context. Understanding the clinical impact of mutation will require integrating mutation-specific information (type, frequency, and predicted impact) with data on haplotypes and on loss of heterozygosity.

In 1989, 10 years after the discovery of the p53 protein, studies by Baker et al. (1989), Nigro et al. (1989), and Takahashi et al. (1989) revealed that the *TP53* gene was frequently mutated in many forms of human cancer. More than 25 years later, in the era of genome-wide sequencing of cancer DNA, *TP53* is confirmed as the most frequently mutated gene associated with cancer in general. This finding is one of the most surprising lessons from cancer genome sequencing. There is no a priori reason why candidate gene-centered sequencing approaches would have correctly identified one unique gene among 20,000 genes as the most frequently mutated one. Indeed, a comparison of significantly mutated genes (SMGs) detected by whole-exome analysis in 15 types of solid tumors shows that *TP53* is the most frequently mutated gene in 11 of them (glioblastoma, clear cell renal cell carcinoma, head and

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neck squamous cell cancer, serous ovarian carcinoma, non-small-cell lung cancer, small-cell lung cancer, gastric cancer, hepatocellular carcinoma [HCC], cholangiocarcinoma, breast cancer [BC], prostate cancer) (average: 25%–60%), whereas it ranks second to *KRAS* in pancreatic and colorectal cancer, and third to *BRAF* and *NRAF* in melanoma (Watson et al. 2013). In hematopoietic malignancies, *TP53* mutations are less frequent (10%–15%) but are among the 10 most frequent SMGs in five of six documented malignancies (the exception is pediatric acute lymphoblastic leukemia [Zhang et al. 2012]).

The first studies on *TP53* mutations focused on tumors with allelic deletions at the *TP53* locus (17p13.1) and showed that mutations resulting in single amino acid substitutions were frequent on the allele that was retained. This observation was consistent with the theoretical hallmark of a tumor-suppressor gene and Knudsen's two-hit hypothesis. Soon, however, it was noted that tumors that retained both parental alleles also frequently carried point mutations on one allele, suggesting a form of dominant effect of the mutation. Indeed, independent of the allelic context, nuclear accumulation of mutant p53 protein was detected in a wide spectrum of primary and metastatic lesions, suggesting a selective retention of mutant p53 during tumor development and dissemination (Bartek et al. 1990). In 1991, distinct patterns of somatic *TP53* mutations were revealed, respectively, in UV-induced nonmelanoma skin cancers (Brash et al. 1991) and in aflatoxin-related HCC (Bressac et al. 1991). These mutation patterns were consistent with the ones induced by these mutagens in experimental assays, confirming the idea that "carcinogens [can] leave fingerprints" in *TP53* sequence as evidence of their etiological role in carcinogenesis (Vogelstein and Kinzler 1992).

In subsequent years, the results literally sparked an industry of sequencing *TP53* exons using the Sanger technique of chain-terminating inhibitors in cancer tissues. From a few hundred at the turn of the 1990s, the number of somatic *TP53* mutations identified in cancer grew steadily to more than 10,000 by the end of the millennium (Hainaut and Hollstein 2000). Soon, computational resources were developed to compile, retrieve, and compare the already identified somatic mutations. In 1994, two databases collecting and annotating mutations detected by sequencing and published in the peer-reviewed literature were established, and they have been since maintained continuously: the *TP53* database at the International Agency for Research on Cancer (IARC), initiated by Monica Hollstein and Curtis Harris (p53.iarc.fr) (Olivier et al. 2002), and the UMD-p53 database, initiated by Thierry Soussi (p53.fr/index.html) (Beroud and Soussi 1998). These databases currently contain more than 30,000 annotated mutations and provide a number of flexible tools to analyze the distribution of mutations across cancers. They have been the basis of several comprehensive reviews discussing the variability and heterogeneity of *TP53* mutation patterns with respect to mutagenic processes, cancer etiology, or potential impacts on cancer biology (Greenblatt et al. 1994; Hainaut and Hollstein 2000; Petitjean et al. 2007; Soussi 2014).

In recent years, the development of second-generation sequencing technologies (also called next-generation sequencing [NGS]) has enabled the systematic analysis of cancer exomes and genomes, expanding our understanding of mutation patterns far beyond the knowledge derived from single genes such as *TP53*. However, despite numerous studies reporting an association between somatic *TP53* mutations and poor prognosis and unfavorable treatment outcomes (Olivier et al. 2010; Robles and Harris 2010), the only accepted recommendation for clinical testing to date is for chronic lymphocytic leukemia (CLL), in which somatic *TP53* mutation is predictive of poor response to conventional therapies (Stilgenbauer et al. 2014). Thus, the clinical significance of somatic *TP53* mutations remains elusive in most forms of cancer. In this review, we revisit our understanding of *TP53* mutation patterns in the light of recent data generated by next-generation sequencing, and we discuss how somatic *TP53* mutation testing may contribute to inform clinical practice.

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TOWARD AN UNBIASED SOMATIC *TP53* MUTATION SPECTRUM

TP53 occupies ~19.14 kb of genomic DNA on chromosome 17p13.1 and is oriented on the minus strand, antisense to other genes in the neighborhood. It comprises 14 exons, including 10 exons constituting the coding sequence of the canonical, full-length p53 protein of 393 amino acids, one noncoding exon 1, and three alternative exons, exon 2/3, exon 9β, and exon 9γ (exon 9α being the regularly spliced form of exon 9) involved in the synthesis of alternatively spliced transcripts encoding p53 protein isoforms (Fig. 1A). The noncoding exon 1 is separated from the rest of the coding sequence by a highly conserved intron that covers more than half of the gene (10,738 bp). Another gene, *WRAP53*, is located upstream of the *TP53* coding sequence on the opposite (plus) strand. It overlaps with exon 1 and the proximal part of intron 1 and encodes antisense transcripts that are complementary to exon 1 transcripts (Mahmoudi et al. 2009). Two main promoter domains, P1 and P2, have been identified. P1 is the main promoter and is located upstream of exon 1. P2 is located within intron 1 at about 1000 bp of the 3′ end of exon 1 and regulates the expression of an intronic polyadenylated transcript of 1125 b, Hp53int1 (D17S2179E, GenBank: U58658.1) (Reisman et al. 1996). The p53 gene has a complex expression pattern with a single major transcript (encoding the full-length p53 protein) and more than 12 documented transcripts of variable abundance generated by alternative splicing and/or internal promoter usage (a weak promoter has been identified in a region encompassing exons 2–4 [Marcel et al. 2010]). These transcripts encode protein isoforms that differ from canonical p53 by the lack of amino-terminal domains (Δ40p53, lacking residues 1–39; Δ133p53, lacking residues 1–132) or by alternative carboxyl-terminal domains (p53β and p53γ) (Courtois et al. 2004; Khoury and Bourdon 2010; Marcel et al. 2011). Although the precise functions of these isoforms is still poorly understood, there is strong experimental evidence that amino-terminally truncated p53 variants may counteract the suppressor functions of full-length p53 protein (reviewed in Marcel et al. 2011; see also Joruiz and Bourdon 2016).

After initial studies reported that somatic mutations were clustered over ~1200 bases in conserved regions of exons 5–8, most studies have sequenced only these regions. Conventional sequencing methods are optimized for the detection of small sequence alterations (single-base mutations, short indels). Larger indels or rearrangements are not readily scored by these techniques. These biases have caused an underrepresentation of somatic mutations occurring outside exons 5–8, many of which are indels. Whole-genome sequencing (WGS) and whole-exome sequencing (WES) mutation data generated by NGS may be, in principle, less biased toward an a priori selection of "hotspot" mutation areas. The COSMIC database maintained at the Sanger Institute (Hinxton, United Kingdom) is the largest public database designed to store and display somatic mutation information relating to cancer. The Whole Genomes Resource (v73) of this database (cancer.sanger.ac.uk/wgs) compiles a total of ~5000 *TP53* mutations detected in WGS/WES projects, corresponding to 1206 different mutation events (as of May 1, 2015). Of those, only 12 mutations are highly recurrent, representing each at least 1% of the entire mutation data set (Table 1). These mutations represent mutational DNA "hotspots." Of note, 11 of these hotspots occur at CpG sites and constitute a molecular signature of the random deamination of unstable 5-methylcytosine. Altogether, the 12 DNA hotspots represent ~25%–30% of the total number of mutations. In contrast, 773 mutations are rare events, each occurring only once or twice in the data set, representing altogether ~20% of the total number of mutations. Because several DNA hotspot mutations fall within the same codons (e.g., 245, 248, or 273), only six "major hotspot" codons comprise each at least 2% of all mutations (175, 213, 245, 248, 273, and 282). Another 13 codons represent "mini hotspots," comprising each between 1% and 2% of all mutations (158, 176, 179, 193, 195, 196, 220, 249, 266, 278, 306, 337, and 342). Figure 1B shows the distribution of WGS/WES mutations along the p53 coding sequence and

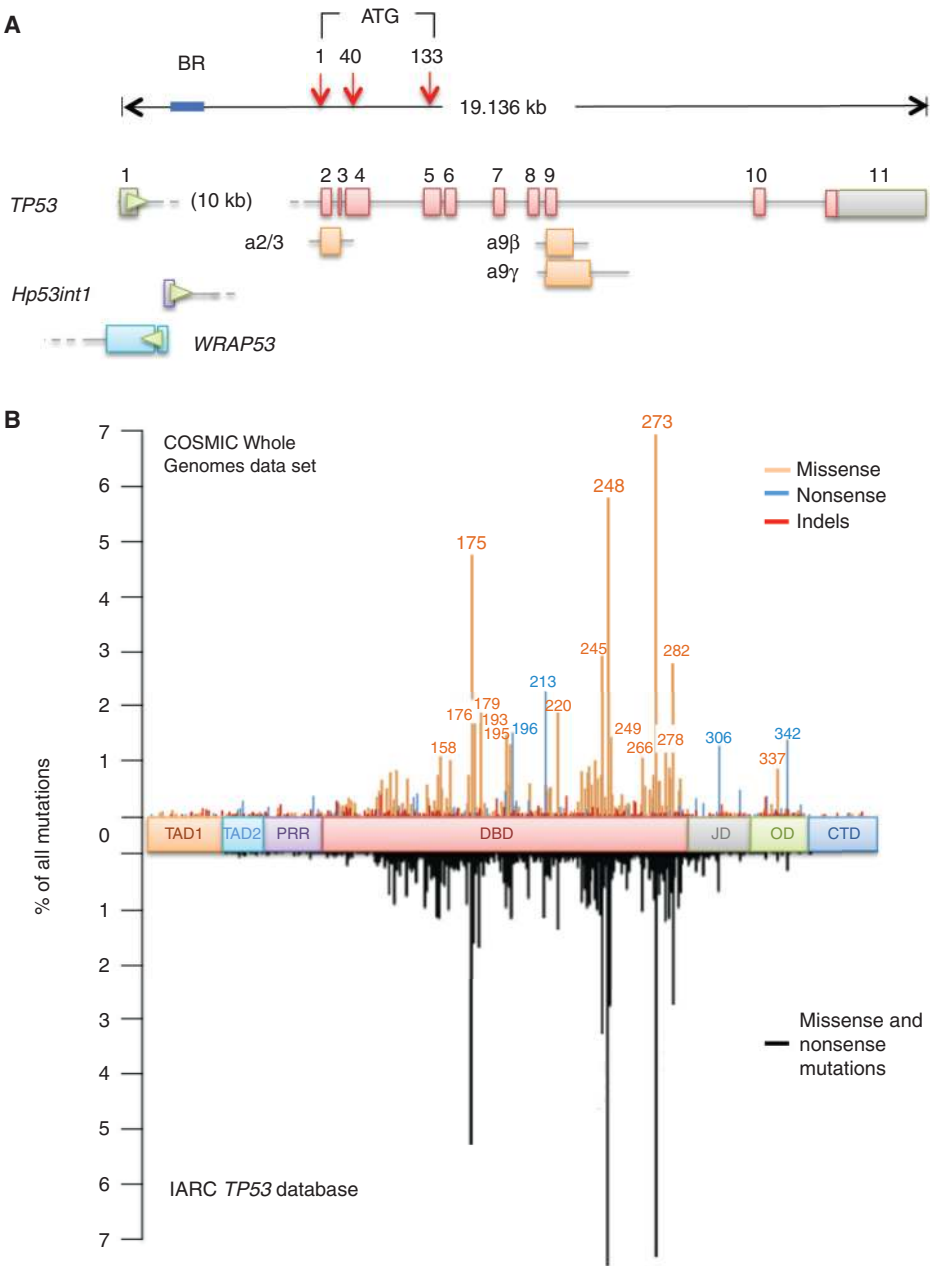

Figure 1. *TP53* mutation spectrum in human cancer. (*A*) *TP53* locus on chromosome 17p13.1, showing main intron/exon structure (coding exons, red; noncoding exon 1 and 3′ UTR in exon 11, gray) and alternative exons 2/3, 9α, and 9β (orange). The position of Hp53int1 in intron 1 is shown, as well as the region of exon1/intron 1 overlapping with alternative exons 1 of *WRAP53*. All introns and exons are represented to scale, except intron 1. Blue bar, location of rearrangement breakpoints in osteosarcoma. Red arrows, position of the main (+1) and alternative (+40, +133) protein initiation sites. Green arrowheads, orientation of the coding sequences (*TP53* is located on the minus strand of DNA, *WRAP53* on the plus strand). (*Legend continues on following page.*)

Table 1. *TP53* mutation "hotspots": 12 mutations representing each of at least 1% of all mutations in the COSMIC Whole Genome Dataset (cancer.sanger.ac.uk/wgs)

Position (codon)	cDNA/base change	Protein/amino acid change	Type of mutation	%	CpG site	Mutation effect
175	c.524G>A	p.R175H	Substitution/missense	4.0	Yes	Structural
196	c.586C>T	p.R196*	Substitution/nonsense	1.4	Yes	Structural
213	c.637C>T	p.R213*	Substitution/nonsense	2.1	Yes	Null
220	c.659A>G	p.Y220C	Substitution/missense	1.5	No	Structural
245	c.733G>A	p.G245S	Substitution/missense	1.3	Yes	Structural
248	c.742C>T	p.R248W	Substitution/missense	2.1	Yes	DNA-binding
248	c.743G>A	p.R248Q	Substitution/missense	2.6	Yes	DNA-binding
273	c.818G>A	p.R273H	Substitution/missense	2.6	Yes	DNA-binding
273	c.817C>T	p.R273C	Substitution/missense	2.9	Yes	DNA-binding
282	c.844C>T	p.R282W	Substitution/missense	2.2	Yes	DNA-binding
306	c.916C>T	p.R306*	Substitution/nonsense	1.1	Yes	Carboxy-terminal truncation
342	c.1024C>T	p.R342*	Substitution/nonsense	1.3	Yes	Carboxy-terminal truncation

Structural, mutation affecting the folding of the DNA-binding domain; null, mutation predicted to impair protein synthesis; DNA-binding, mutation replacing an amino acid making direct and specific contact with DNA; carboxy-terminal truncation, mutation predicted as generating a protein lacking the entire (p.R306*) or part of (p.R342*) carboxy-terminal oligomerization domain.

compares it with the distribution compiled from the IARC mutation data set in 2010, before the systematic usage of NGS (of note, the latest release of the IARC database [p53.iarc.fr] has included 866 mutations identified by WGS/WES [3% of the data set]). Although these distributions look very similar, the WGS/WES pattern shows that mutations outside exons 5–8 represent 22.5% of the total, almost double that estimated by Sanger sequencing (10%–15%). The higher number of silent mutations detected by NGS explains only 1.5% of this difference. Within the DNA-binding domain of the p53 protein, NGS data show that mutations at codon 213 (80% nonsense, p.R213*) and codon 220 (90% missense, p.Y220C) are hotspots almost as high as the well-defined hotspot codons 245 and 282. NGS data also show a frequently mutated area in the oligomerization domain (residues 326–355, 5% of all mutations). Within this domain, codon 342 (91% nonsense, p.R342*) constitutes a minor hotspot codon. Thus, the Sanger data set appears to be biased toward underrepresentation of mutations outside exons 5–8 and overrepresentation of the mutations known as major hotspots (codons 175, 245, 248, 273, and 282), perhaps because of a tendency of investigators to not fully analyze exon 6 (which is GC-rich and hard to sequence) and to preferentially report mutations detected at codons in exons 5, 7, and 8. Interestingly, the newly scored hotspot codons 213 and 342 contain CpG dinucleotide sequences, as do the standard hotspot codons 175, 245, 248, 273, and 282.

About 2% of all mutations occur at intron/exon boundaries and affect splicing donor or acceptor sites. Genome-wide splicing mutation analysis has identified *TP53* as the gene most commonly affected by splicing mutation in BC

Figure 1. (*continued*) (*B*, *top*) Codon distribution of mutations (missense, nonsense, and indels) in the coding sequence of *TP53*, based on mutation data derived from integrated genomic studies compiled in the Whole Genomes Resource (v73) of the COSMIC mutation database (cancer.sanger.ac.uk/cosmic/signatures), showing the position of major hotspots (>2% of all mutations) and mini hotspots (1%–2% of all mutations). (*Bottom*) Codon distribution of missense and nonsense mutations based on studies using conventional targeted sequencing approaches and compiled from the International Agency for Research on Cancer (IARC) *TP53* mutation database (version R.13, 2008 [Olivier et al. 2010]). The IARC distribution is displayed as a mirror image of the COSMIC distribution. TAD1, TAD2, Transcriptional activation domains 1 and 2; PRR, proline-rich region; DBD, DNA-binding domain; JD, junctional domain; OD, oligomerization domain; CTD, carboxy-terminal domain.

(Dorman et al. 2014). However, significant somatic variations occur in *TP53* introns at sites other than those implicated in splice junctions, which are not covered in many conventional or exome-sequencing programs and thus are not reported in mutation databases. In BC, mutations in intron 9 colocalizing with alternative exons 9α and 9β have been detected using yeast functional assays (Iggo et al. 2013). These mutations alter the balance between fully spliced and alternatively spliced p53 transcripts, leading to the preferential synthesis of a protein that lacks part of the oligomerization domain. In osteosarcoma, somatic rearrangements in intron 1 have been detected by WGS in ~20% of the cases (Ribi et al. 2015). The rearrangement breakpoints are located across the entire sequence of intron 1, but most of them cluster in a region of 1.7 kb located immediately upstream of the locus encoding the intronic Hp53int1 transcript, suggesting that breakpoint may be facilitated by open chromatin conformation at this locus (Fig. 1A) (Chen et al. 2014; Ribi et al. 2015). So far, intron 1 rearrangements have not been observed in other sporadic cancers and the reason for the narrow tissue specificity is unknown. The impact of these rearrangements on p53 function remains to be analyzed, but it is noteworthy that an intron 1 rearrangement (455-kb inversion) cosegregates with inherited risk of several cancers in a Li–Fraumeni family. The tumors of these patients show impaired *TP53* transcription and loss of heterozygosity (LOH) affecting the wild-type allele (Ribi et al. 2015). Osteosarcoma has been considered as a type of cancer with low rate of *TP53* mutations in exons 2–11 (20%), partly balanced by frequent functional inactivation of p53 protein through amplification of *MDM2* (Ladanyi et al. 1993). The finding of intron 1 rearrangements in about half of sporadic osteosarcomas leads to a call for reconsidering them as a cancer with a high rate of somatic *TP53* mutations.

BEYOND MUTATIONS: IMPACT OF *TP53* HAPLOTYPES

TP53 contains more than 100 validated naturally occurring single-nucleotide polymorphisms (SNPs; see p53.iarc.fr/TP53GeneVariations.aspx) but only a few of them have been studied for their effect on p53 functions. The best characterized exonic SNP is a nonsilent substitution at codon 72 in exon 4 (rs1042522, g.7520197G>C, p.R72P), which is present at minor allele (P) frequency between 10% (Caucasians, Northern Europe) and 50% (Africans, Yoruba), depending on population (Whibley et al. 2009). Several studies have shown that this SNP specifies protein variants with different functional properties in vitro and in experimental in vivo models. The form of p53 with R (arginine) at codon 72 (p53R72) more effectively induces p53-mediated apoptosis than p53P72 (proline), partially through targeting p53 to the mitochondria (Dumont et al. 2003). Meanwhile, p53P72, when compared with p53R72, more efficiently induces cell-cycle arrest and DNA repair (Pim and Banks 2004; Siddique and Sabapathy 2006). The ability of mutant p53 to bind p73, neutralize p73-induced apoptosis, and experimentally transform cells in cooperation with EJ-Ras is enhanced when the mutant protein carries an arginine at position 72 (Marin et al. 2000). In addition, R72 alleles appear to be preferentially mutated and retained in squamous cell carcinomas arising in patients who are R72P germline heterozygotes. Thus, this intragenic polymorphism may act as a modifier of mutant *TP53* effect, suggesting that mutations and /or LOH may occur at different rates on different *TP53* haplotypes. This hypothesis is supported by results from Mechanic et al. (2007) who analyzed 14 intragenic *TP53* SNPs in a case-control study of lung cancer and uncovered an association between several combinations of SNPs and the risk of somatic mutation. The SNPs associated with mutation were located between intron 2 and intron 7, including R72P/rs1042522 (exon 4), rs9895829 (intron 4, c.376-125T>C), rs1625895 (intron 6, c.672+62A>G), and rs12951053 (intron 7, c.782+92T>G), defining the haplotype containing R-T-A-G as more likely to carry a mutation. The reason why this haplotype is preferentially mutated is unknown. In HCC carrying the aflatoxin-induced p.R249S mutation, analysis of 19 SNPs spanning the

entire *TP53* locus has shown a strong association between mutation and haplotypes that carry a combination of two SNPs (rs17882227 and rs8064946) defining a linkage disequilibrium block extending from upstream of noncoding exon 1 to the first half of intron 1. This domain contains two coding sequences overlapping with *TP53* (*WRAP53* and Hp53int1) (Ortiz-Cuaran et al. 2013). It is possible that these SNPs may modulate the p53 antisense activity of these transcripts, thus having an impact on p53 expression levels and therefore on the rate at which specific mutant alleles occur and are retained during tumor development. A simple way to rationalize this notion is to consider that different haplotypes express p53 at different levels, defining a continuum of functional p53 responses from "weak" to "strong" *TP53* alleles, which may differ for different biological p53 effects (e.g., cell-cycle arrest, apoptosis, control of metabolism, or DNA repair) (Fig. 2). Mutations on a "weak" haplotype may be more likely to require the loss of the "strong" wild-type allele to exert a significant effect. In contrast, mutation on a "strong" haplotype may have disrupting, dominant effects even if a weaker wild-type allele is present.

MUTATION PATTERNS AS SIGNATURES OF MUTAGENIC PROCESSES

TP53 mutations have been extensively used as clues for the etiology of endogenous and exogenous mutagenic processes that operate during carcinogenesis in humans. Multiple independent studies have been conducted by Sanger sequencing of *TP53* in tumors selected for their suspected association with a mutagen or mutagenic processes. The spectra produced by the aggregation of these somatic mutations were compared with the mutations experimentally generated by the suspected mutagens in vitro or in vivo systems (Hollstein et al. 1999; Pfeifer 2015). Table 2 summarizes the "molecular signatures" identified in *TP53*, caused by specific agents or mutagenic processes identified in *TP53*. This information has been extensively discussed in previous reviews (Greenblatt et al. 1994; Hainaut and Hollstein 2000; Olivier et al. 2004, 2010; Robles and Harris 2010; Pfeifer 2015). Nonmelanoma skin cancer (basal and squamous cell carcinoma) and melanoma show a predominance of G:C>A:T transitions at dipyrimidine sites (mutated base underlined), including about 10% of CC>TT tandem mutations, a type of mutation resulting from UV-light-induced covalent coupling of C=C double bonds at adjacent pyrimidines. These mutations are not caused by other known carcinogens and are almost never observed in tumors of internal organs (Luo et al. 2001). In HCC, a unique transversion at codon 249 (p.R249S; G:C>T:A) is highly prevalent in geographic areas in which the mycotoxin aflatoxin is a widespread contaminant of the food (parts of Africa, Eastern Asia, South America) (reviewed in Gouas et al. 2009). In lung cancer, ~30% of *TP53* mutations are G:C to T:A transversions in smokers, but not in never-smokers. This class of mutation is the main mutagenic effect of polycyclic aromatic hydrocarbons (PAHs) from the tar fraction of cigarette smoke. Exposure of human bronchial cells to the diol epoxide of the PAH benzo[*a*]pyrene causes DNA damage at the same G:C pairs that are frequently mutated into T:A in lung cancers from smokers (Denissenko et al. 1996; Tretyakova et al. 2002). Recently, a mutational fingerprint of aristolochic acid has been reported in urothelial carcinomas of subjects with a history of exposure to herbal remedies and food products containing seeds of *Aristolochia clematitis* (Hollstein et al. 2013; Poon et al. 2013). Last, all cancer types harbor at least 20% of G:C>A:T mutations at hypermutable CpG dinucleotides, attributed to the normal cellular event of deamination of 5-methylcytosine to thymine. This process is enhanced by inflammation, resulting in a high prevalence of these mutations in cancers associated with chronic inflammation (Ambs et al. 1999; Cooks et al. 2014). CpG mutations are exceptionally common in a few specific types of cancer including colorectal, gastric, and esophageal cancers (frequently occurring from precursor inflammatory lesions) as well as in brain cancers.

The introduction of NGS has provided a stepping-stone for a giant leap in our under-

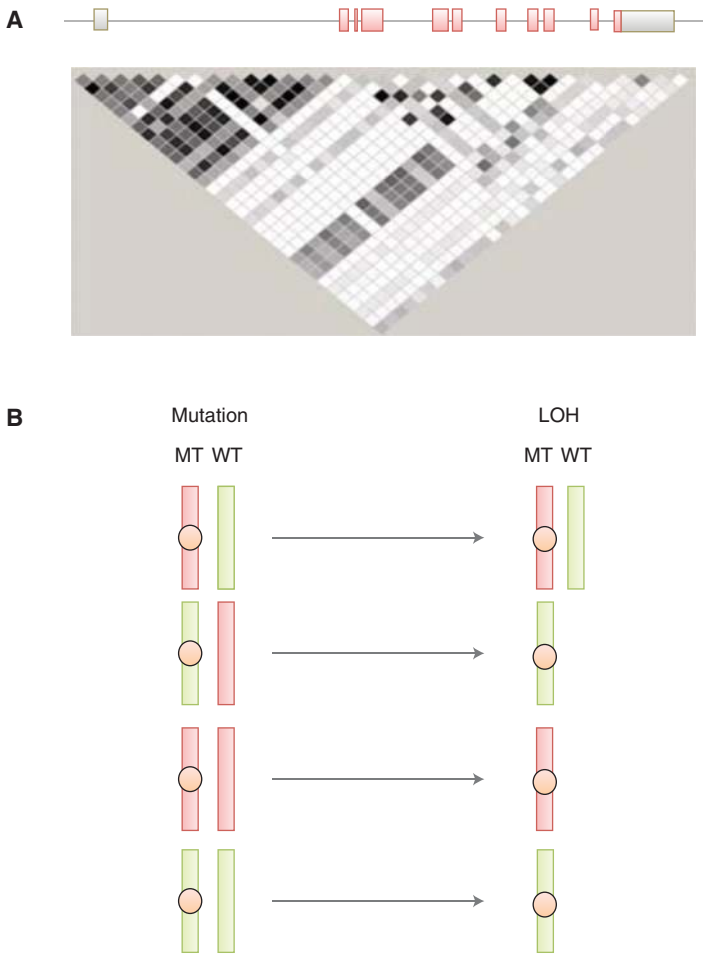

Figure 2. Variability and potential impact of *TP53* haplotypes. (*A*) Linkage disequilibrium (LD) plot in the HapMap Panel for Caucasians, using 29 tag SNPs (tSNPs) for typing *TP53* haplotypes (Garritano et al. 2010). The degree of LD is indicated by shades from black (strong LD) to white (no LD). This figure shows that most tSNPs in intron 1 and the *TP53* promoter are in strong LD, forming a conserved haplotype block. (*B*) Model for loss of heterozygosity (LOH) at the wild-type (WT) allele depending on the interplay between "strong" (red) and "weak" (green) mutant haplotypes. (*Top* row) If the mutation (MT) occurs on a "strong" haplotype, its effects may dominate the residual WT "weak" haplotype, making LOH not compulsory. In all other haplotype combinations, LOH is required to eliminate a WT haplotype stronger than or equally strong as the one carrying the mutation.

standing of the signatures of mutational processes in human cancer. In addition to access to large data sets (between a few hundred and >10,000 mutations per tumor genome), NGS data have supported the development of a computational framework that allows deconstructing distinct but overlapping patterns from a set of cancer samples (Alexandrov et al. 2013a,b). Somatic mutations in the genome of any cancer are the result of cumulative mutagenic processes encompassing endogenous and exogenous mechanisms, each operating with different temporal patterns and with different strengths. Often, the cumulative pattern in cancer does not match any of the known single-operative mutational processes. By using algorithms that decipher the minimal set of mutagenic signatures that optimally explain the proportion of each mutation type in each cancer, it is now possible to estimate the contri-

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Table 2. *TP53* mutation patterns as a signature of mutational processes

Base change (underlined)	Type	Mechanism/ agent	Promutagenic lesion	Typical *TP53* mutations	Cancers	References
CC>TT tandem	Transition	Solar UV	Dipyrimidine dimers	p.S127F, p.Q136*, p.R158C, p.H179Y, p.R196*, p.R248W, p.R278F, p.R282W	Nonmelanoma skin	Brash et al. 1991
G:C>T:A	Transversion	Aflatoxin (AFB1)	AFB1-N7-guanine adducts	p.R249S	Liver (hepatocellular carcinoma)	Bressac et al. 1991
G:C>T:A	Transversion	Tobacco smoke	PAH-N2-guanine adducts	p.V157F, p.R158L, p.G245V, p.G245C, p.R248L, p.R249M, p.R273L, p.D281E, p.E298*	Bronchus and lung, head and neck, esophagus	Denissenko et al. 1996; Tretyakova et al. 2002
A:T>T:A	Transversion	Aristolochic acid	Aristolactam-DNA adducts	p.Q104L, p.N131Y, p.K139*, p.K164*, p.R209*, p.R280S, p.K291*	Bladder/urothelium	Hollstein et al. 2013
G:C>A:T at CpG	Transition	Random deamination	5- methylcytosine	p.R175S, p.R213*, p.R248Q, p.R248W, p.R282W, p.R306*, p.R342*	All cancers, in particular, colon, stomach, brain	Ambs et al. 1999

bution of different signatures to each cancer mutation spectrum.

The first NGS studies focusing on mutation patterns were published in *Nature* in 2010 and reported the patterns of somatic mutations in malignant melanoma (Pleasance et al. 2010a) and small-cell lung carcinoma (Pleasance et al. 2010b). These studies confirmed the widespread impact of UV light and tobacco carcinogens, respectively, in these cancers. Since then, many international consortia have undertaken the sequencing of large numbers of cancer samples, and mutational signature genomic patterns have been identified for many of them (Alexandrov et al. 2013a; Roberts and Gordenin 2014). The COSMIC database website provides a resource documenting up to 30 mutational signatures based on pooled NGS data from 10,952 exomes and 1048 whole genomes across 40 different types of cancers (accessible at cancer.sanger.ac.uk/cosmic/signatures). Several of these signatures confirm and refine mutational processes already described in *TP53* (COSMIC signature 1, spontaneous deamination of 5meC; signature 4, tobacco smoke; signature 7, UV light; signature 22, aristolochic acid; and signature 24, aflatoxin).

Surprisingly, there are only a few new mutational signatures informing on exogenous carcinogen exposure in addition to those already detected in *TP53*. One example is signature 11, which corresponds to a distinct C>T mutation pattern caused by the alkylating agent temozolomide in recurrent glioblastoma multiforme of patients that have undergone this type of chemotherapy. On the other hand, NGS mutational signatures provide new insights into endogenous mutation processes, including mutational signatures caused by the error-prone polymerase η (signature 9, detected in chronic lymphocytic leukemia and malignant B-cell lymphomas), by proofreading defects attributed to polymerase ε (signature 10, in subsets of colorectal and uterine cancers), by defective mismatch repair (signatures 6, 15, 30, and 26, in gastric, colorectal, and endometrial cancer), and by defective repair of DNA strand breaks associated with germline and somatic BRCA1 and BRCA2 mutations (signature 3, breast, pancreatic, and ovarian cancers). Several new mutation signatures are still to be assigned to a specific mechanism (signatures 5, 8, 12, 14, 17–19, 21, 23, 25, 28, and 30). For example, esophageal adenocarcinomas carry a very unusual type of mutation, A-to-C mutations at 5′ AA dinucleotides (Pfeifer 2015). There are no known mutational processes, either endogenous or exogenous, that have been shown experimentally to result in these types of mutations. Because these events are highly specific for a tissue traversed by ingested materials, it is plausible that these mutations are caused by exposure to an exogenous mutagen. Furthermore, in addition to the aforementioned exposures to aflatoxins, liver cancer genomes, unlike other cancer genomes, have very diverse patterns of mutations targeting A/T base pairs. Because the liver is the primary organ for metabolism of xenobiotics, it would not be surprising if these A/T-targeted mutations were caused by metabolites of environmental carcinogens.

Some of the most interesting new data provided by NGS is the identification of an endogenous pathway involving cytidine deaminases of the AID/APOBEC family (signatures 2 and 13). Abnormal targeting of cytidine deaminases to random chromosomal locations appears to cause frequent C>T mutations at the cytosine of 5′TpC dinucleotides. The human genome encodes eight APOBEC enzymes (seven of them located at the APOBEC3 gene cluster) that normally serve to restrict viral infection and retrotransposon mobility by deaminating cytosines during the ssDNA stage of their replication stages. These enzymes target TCA/TCT trinucleotides in ssDNA in which they deaminate C to U, which is either directly copied to T or excised by uracil DNA glycosylase, followed by copying of the resulting abasic site into C>T or C>G mutations. Because of the processivity of APOBEC enzymes, these mutations often occur in clusters. AID/APOBEC signatures are present in many forms of cancer, and a germline deletion polymorphism affecting APOBEC3A and APOBEC3B is associated with large numbers of them (Nik-Zainal et al. 2014).

It is now possible to revisit *TP53* mutation patterns in the light of these extended and re-

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fined mutational signatures. Recent studies have identified APOBEC mutation patterns in *TP53* in BC (Lindley 2013) and lung adenocarcinoma (Waters et al. 2015). Although these studies are useful to better understand the sequence and codon context of mutation formation in *TP53*, the amount of etiological information they reveal is limited compared with multigene studies using NGS.

FUNCTIONAL DIVERSITY OF *TP53* MUTATIONS

The spectrum of *TP53* mutation in cancer cells and tissues is the result of combined processes acting as filters that select cancer mutations among a broad range of possible mutations. Sequence-context-dependent mutagenesis and DNA repair represent two of these filters. The biological selection of functionally "meaningful" mutations represents a third filter. However, what is a "meaningful" human cancer mutation is still largely unclear.

The lowest common denominator of cancer mutations is loss of transcriptional function (LOF) altering the p53-dependent transcriptional repertoire. Not all mutants are equal in this respect. The use of standardized yeast-based assays to test for LOF at total of 2314 mutants representing all possible substitutions throughout the protein has shown that LOF is virtually complete for frequently occurring mutants (including hotspots) but that some of the rarely occurring mutants retain quasi-wild-type functionality for transactivation (Kato et al. 2003). Most of these rare functional mutants and occur only once or twice in the COSMIC WGS/WES data set (representing $<3\%$ of all mutations). They may simply represent passenger mutations with no functional significance, detected only because of their accidental presence in an expanded clone of transformed cells. An interesting parallel exists between these mutants and those occasionally detected in noncancer tissues such as rheumatoid arthritis (RA), a noncancer precursor chronic inflammatory disease characterized by oligoclonal proliferation of nontransformed synoviocytes. RA tissues frequently contain clusters of cell clones with atypical *TP53* mutations that include a high proportion of silent mutations (14%), mutations retaining wild-type transactivation capacity (60%), and mutations rarely found in any cancer (70% of them are either absent or reported only once in the WGS/WES COSMIC data set) (Firestein et al. 1997; Yamanishi et al. 2002). Such mutants are unlikely to be selected during carcinogenesis but are carried over as passengers in expanding subclones of RA synoviocytes.

There is compelling experimental evidence that *TP53* mutations induce functional changes well beyond the scope of LOF. Many missense p53 mutants are expressed as stable proteins that exert dominant negative (DN) effects by interfering with the product of the remaining wild-type allele. A "prion-like" effect of some mutant p53 over wild type has been shown in vitro, in which the mutant enforces wild-type protein to adopt a denatured, mutant-like conformation (Milner and Medcalf 1991). DN effects may also result from a "sponge" effect of stable mutant p53 protein, absorbing the low-abundance wild-type proteins into oligomers dominated by mutant forms. Aside from DN effects, gain-of-function (GOF) effects have been demonstrated through which mutant p53 exerts a number of prooncogenic biochemical activities. GOF effects have been extensively studied in experimental cell systems and in genetically modified mouse models (Brosh and Rotter 2009; Muller and Vousden 2014). A number of mechanisms have been proposed for mutant p53 GOF, including direct binding to DNA changing (rather than abolishing) gene expression, binding to a large panel of transcription factors enhancing or impairing their function (e.g., binding to the p53 family members p63 and p73), binding to a number of proteins not directly involved in transcription (e.g., binding to the MRE11-RAD50-NSB1 complex, disrupting its function), and regulating microRNA networks (e.g., miR130b, miR155, and miR205) (Brosh and Rotter 2009; Muller and Vousden 2014). Recently, several p53 mutant proteins have been shown to up-regulate a number of chromatin regulatory genes, including the methyltransferases *MLL1/KMTA2A* and *MLL2 (KMT2D)* and the acetyltransferase *MOZ/KAT6A*,

resulting in a global increase in histone methylation and acetylation and suggesting that mutant p53 may induce GOF effects through sweeping changes in the epigenetic control of chromatin dynamics (Zhu et al. 2015). The functional consequences of these GOF effects encompass the entire spectrum of the "hallmarks of cancer" (Hanahan and Weinberg 2011; Solomon et al. 2011). Thus, in contrast to LOF, there is no simple standard assay for scoring GOF effects. Furthermore, there is no clear understanding of the structural properties of mutant p53 that support GOF effects. Given the multiplicity of GOF mechanisms, it is unlikely that all effects depend on a single structural feature. In addition, mutants with the most perturbed protein structure are not necessarily those with the strongest GOF effects. In contrast, these effects are expected to be subtle and context-dependent, because they require interactions between mutant p53 and proteins that are themselves expressed in a tissue- and context-dependent manner. The subtlety of GOF effects is illustrated by p.R249S, the mutant caused by aflatoxin-induced mutagenesis in HCC arising in a context of chronic hepatitis B (HB) infection. This mutant is a clear LOF mutant, but is not commonly considered as GOF in most studies. However, its mechanism of action involves the binding of the HB antigen HBx, activating it as a viral oncogene in liver cells (Jiang et al. 2010; Gouas et al. 2012). This effect is a typical context-dependent GOF effect. The binding specificity of p.R249S to HBx may explain the surprisingly narrow spectrum of aflatoxin-induced *TP53* mutations in HCC, because other mutants potentially induced by aflatoxin may not bind HBx with the same efficacy as p.R249S, preventing their selection during HB-dependent hepatocarcinogenesis (Denissenko et al. 1998).

CLASSIFYING *TP53* MUTATIONS FOR CLINICAL USE

Although a multitude of experimental studies have shown different effects of various mutants on proliferation, apoptosis, or responses to cytotoxic drugs, there is surprisingly little clinical evidence for association between different types of mutations and clinicopathological variables, prognostics, or prediction of therapeutic responses. This does not imply that such associations do not exist. Perhaps the best evidence for clinically relevant differences between different mutations is found in subjects who carry germline *TP53* mutations. In a recent report on 322 *TP53* mutation carriers from French Li–Fraumeni families, the mean age of tumor onset was statistically different between carriers of missense mutations (23.8 years) and those carrying nonsense mutations or genomic rearrangements (28.5 years). The difference was even larger when comparing hotspot mutations, such as p.R175H or p.R248W (mean age of tumor onset, 15–20 yr) with complete deletion of the *TP53* gene (38.5 yr) (Bougeard et al. 2015). Assuming that complete deletion of *TP53* represents a "straight" LOF effect, these observations suggest that the more severe clinical phenotype of hotspot mutants is caused by their capacity to impair the wild-type allele (DN effects) and/or by specific GOF effects. It is likely that similar genotype–phenotype correlations occur for somatic mutations.

Many clinical studies have tried to subdivide somatic mutations into severity classes based on localization and predicted structural effects. Small clinical cohorts, suboptimal methods used to assess *TP53* mutations status, and lack of consistency in how the mutations are classified have hindered a robust correlation with clinical features. Computational approaches using sequence similarities such as SIFT (sorting intolerant from tolerant) generate relatively accurate prediction for loss of transactivation function in vitro (specificity, 90%; sensitivity, 70% [Mathe et al. 2006]) but their clinical usefulness is limited to the identification of rare passenger mutations. Two simple classification algorithms have been used with some success (Fig. 3). The Poeta algorithm classifies mutations into two a priori classes, disruptive and nondisruptive mutations. Disruptive mutations combine predicted-null mutations (nonsense, frameshift, and splice site) and chemically deleterious substitutions in loops forming the DNA-binding surface of the protein (Poeta et al. 2007). Nondisruptive mutations include

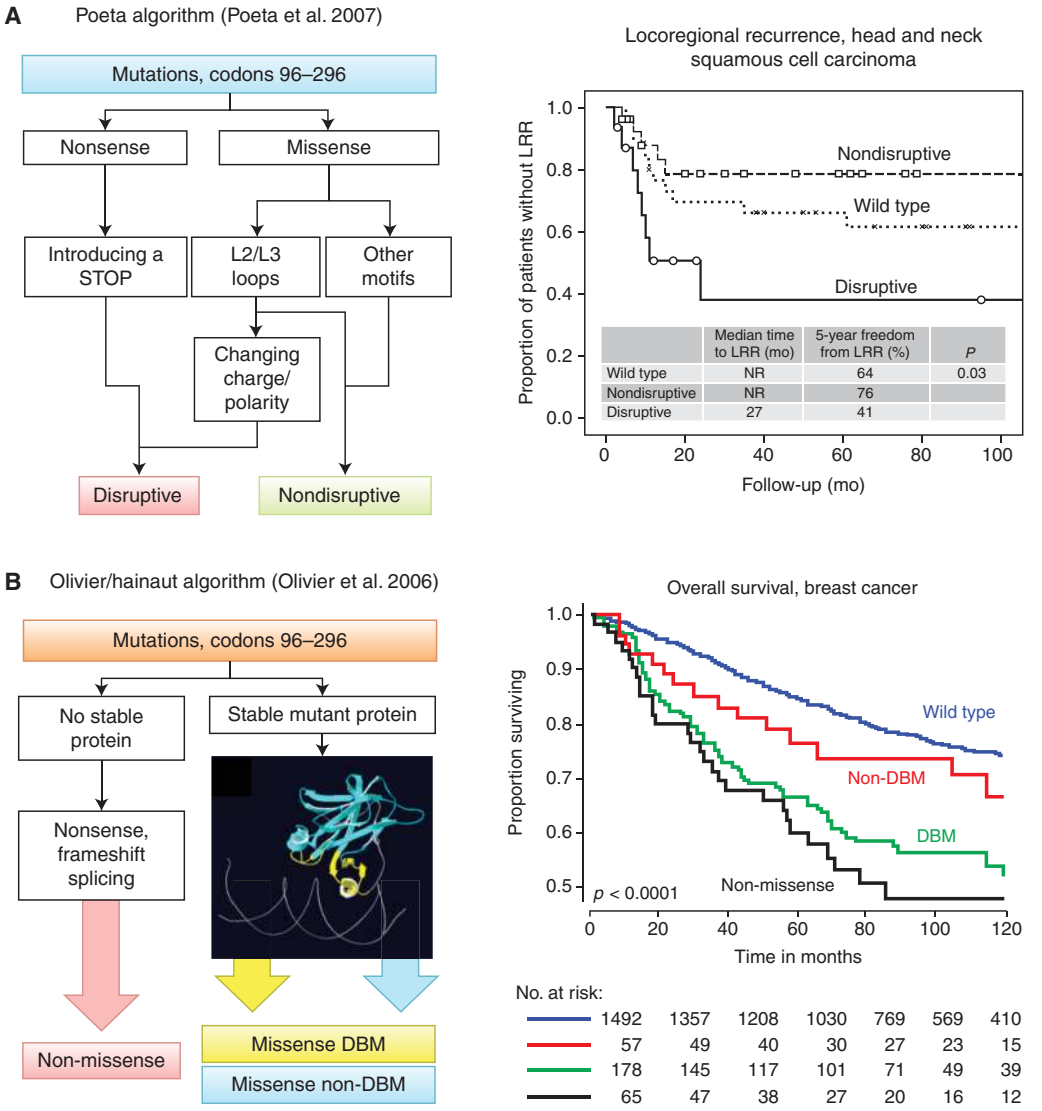

Figure 3. *TP53* mutation classification algorithms. (*A*) The Poeta algorithm (*left*) (Poeta et al. 2007) classifies mutations occurring in the DNA-binding domain in two groups, disruptive (D) and nondisruptive (ND) mutations. D mutations either preclude the synthesis of p53 (nonsense mutations) or cause a structurally important amino acid change in the L2/L3 loops of the DNA-binding domain. Other missense mutations are classified as ND. (*Right*) D and ND mutations have different prognostic value for locoregional recurrence (LRR) in a cohort of 74 patients with squamous head and neck cancer (Skinner et al. 2012). (*B*) The Olivier/Hainaut algorithm (*left*) classifies mutations in three groups: mutations predicting a null p53 protein (non-missense), missense mutations in DNA-binding motifs (missense DBM), and missense mutations outside the DNA-binding motif (missense non-DBM). (*Right*) These three categories show different prognostic values for overall survival in a cohort of 1794 patients with breast cancer (Olivier et al. 2006).

any other missense mutation. In head and neck squamous cell cancer (HNSqCC), disruptive mutations are associated with decreased overall survival, whereas there is no significant association with nondisruptive mutations. Moreover, disruptive mutations predict locoregional recurrence and HNSqCC cell lines with disruptive mutations are significantly more radioresistant than those expressing nondisruptive mutations (Skinner et al. 2012). The Olivier/Hainaut algorithm specifies three classes of mutations, predicted-null, missense mutation in the DNA-binding motif (DBM), and missense mutation in non-DNA-binding motif (non-DBM) (Olivier et al. 2006). The distinction between DBM and non-DBM mutations is purely topological, based on the observation that interspecies conservation of amino acids in the loops of the DBM is higher than in β-strands and short intervening loops of the non-DBM. The non-DBM is thus predicted to be more tolerant to mutations than the DBM (Mathe et al. 2006). In a large series of 1791 BCs from European patients, the algorithm identified predicted-null mutations as having the strongest association with poor survival, followed by DBM, whereas non-DBM were just marginally worse than wild-type *TP53* (Olivier et al. 2006). In the METABRIC BC cohort (1420 patients), no statistical difference was found between the three mutation classes, although DBM mutations appear to have a marginally more severe effect (Silwal-Pandit et al. 2014). Incidentally, the fact that the two different classes of missense mutations appear to have the same (or less) effects as predicted-null mutations argues against DN or GOF effects of missense mutations in BC. When applied to non-small-cell lung cancer (NSCLC), the algorithm does not identify any prognostic value associated with either mutation class. However, in a randomized trial of platinium-based therapy after surgery (525 patients, 221 with mutation; 42%), non-DBM mutations were associated with poor overall survival in the group that received chemotherapy but not in the group treated by surgery only (Ma et al. 2014). This effect was not seen with predicted null or DBM mutations. This observation suggests that non-DBM may exert DN and/or GOF effects that confer resistance to adjuvant therapy. It is interesting to note that non-DBM mutations are frequent in NSCLC of smokers, because of the frequent targeting by tobacco carcinogens of codons encoding residues located in the hydrophobic core of the protein (Le Calvez et al. 2005). The selection of these mutations may contribute to a form of drug resistance in tumor cells.

Overall, these results indicate that the clinical severity of mutations is tumor- and context-dependent. Thus, the same mutations do not carry the same clinical consequences in different cancers. Although it can be assumed that the LOF effects of mutations might be roughly equivalent in different tissue contexts, DN effects are dependent on the retention, expression, and activity of the wild-type allele, and GOF effects may depend on factors that are expressed in a tissue- and context-dependent manner.

TP53 IN THE GENOMIC LANDSCAPE OF CANCER: SEEING THE NEEDLE WITHIN THE HAYSTACK

Until recently, *TP53* mutations have been mostly approached as stand-alone events in studies of limited size and statistical power. The development of integrative genomics combining WGS/WES with DNA copy number alterations and studies on mRNA and protein expression provides an unprecedented view of the general genomic contexts in which *TP53* mutations occur (Kristensen et al. 2014). In recent months, large consortium studies have combined multidimensional molecular approaches to characterize the landscape of genomic alterations in human cancers. These studies are generating a wealth of information that remains to be fully explored using *TP53* mutation as a magnifying lens. Here, we briefly highlight some of the new knowledge that integrative genomics is bringing to our understanding of *TP53* mutations in breast and non-small-cell lung cancer.

Breast Cancer (BC)

With mutation in 20%–25% of all cases, *TP53* ranks first in a series of seven genes that are

mutated in >10% of BCs (including *PIK3CA*, *ERBB2*, *MYC*, *FGFR1/ZNF703*, *GATA3*, and *CCND1*) (Stephens et al. 2012). A global study of the genomic and transcriptomic architecture of breast cancers has identified up to 10 molecular subtypes (integrative clusters) in which *TP53* mutations occur at significantly different frequencies (Curtis et al. 2012). A detailed analysis of the *TP53* mutation spectrum in the 1420 BCs of the METABRIC cohort has identified mutations in 28.3% of the cases (402 patients), with significant variations across BC subtypes (Silwal-Pandit et al. 2014). Mutations are frequent in basal-like and *HER2*-enriched tumors (65% and 53.4%, respectively), rare in luminal A and normal-like tumors (9.3% and 11%, respectively), and occur at an intermediate rate in luminal B tumors (24.8%). LOH at the *TP53* locus is observed in 80.9% of the mutated tumors, independently of molecular subtype. However, in *TP53* wild-type cases, the frequency of LOH varies across subtypes (from 24% in basal-like to 52% in luminal B). The distribution of mutations show striking differences between subtypes, with hotspot mutations dominating the spectrum of basal-like tumors, whereas a more uniform mutation distribution is seen in other subtypes.

When examining the associations between mutation and prognosis, *TP53* mutation appears to be strongly correlated with poor overall survival in ER-positive patients but not in ER-negative patients. In fact, the presence of a mutation seems to wipe out the prognostic beneficial effect of ER positivity (Olivier et al. 2006; Silwal-Pandit et al. 2014). This observation suggests that p53 may function as a rate-limiting factor for ER signaling in BC, a conjecture supported by experimental data implicating p53 in estrogen responses in breast cancer cells (Fernandez-Cuesta et al. 2011a,b). Patients with mutant *TP53* may thus not respond as well to endocrine breast cancer therapy as patients with wild-type *TP53*. In a recent study, p53 protein accumulation has been identified as a strong predictor of recurrence in ER-positive BC patients treated by aromatase inhibitor. It remains to be shown whether this accumulation is associated with mutant p53 (Yamamoto et al. 2014).

The association between *TP53* mutation and BC prognosis is not uniform across subtypes. Mutations are significantly associated with poor survival in *HER2*-enriched, luminal B, and normal-like but not in basal-like or luminal A, suggesting a diverse role and impact for *TP53* mutations (Fig. 4) (Silwal-Pandit et al. 2014). In basal-like cancers, disruption of the p53 pathway may be an obligate mechanism in virtually all tumors, either by mutation or by other mechanisms. Presence of wild-type *TP53* may therefore not imply an intact p53 pathway, explaining the lack of prognostic value of *TP53* mutations. In *HER2*-enriched cancers, mutation may disable the capacity of p53 to operate a safeguard mechanism against oncogene-driven cell proliferation induced by activated *HER2*. In luminal B and normal-like cancers, disruption of *TP53* function is an optional mechanism and the occurrence of a mutation and/or LOH makes a significant difference for tumor growth and progression, thus entailing a clear negative prognostic value. In contrast, in luminal A cancers, mutation is a rare event that may be relatively unimportant. The fact that luminal A mutations rarely occur at hotspots suggest that many of them might be passenger mutations. There is no significant difference in prognostic value between different classes of mutations in any of the subtypes.

With respect to predictive value of mutations, it has long been suggested that docetaxel may confer a greater therapeutic advantage over anthracyclines in BC with mutated compared with wild-type *TP53*. This hypothesis has not been substantiated in two large clinical trials, which have confirmed the prognostic value of mutant *TP53* but have not provided evidence for a predictive effect of mutation on response to therapy (Bonnefoi et al. 2011; Fernandez-Cuesta et al. 2012).

Non-Small-Cell Lung Cancer (NSCLC)

NSCLC comprises two major histological entities, squamous cell carcinoma (SqCC) and adenocarcinoma (AdC), and several minor histological subtypes including large-cell carcinomas (LCCs). WES reveals *TP53* mutations in 81% of SqCC, a frequency at the higher end of

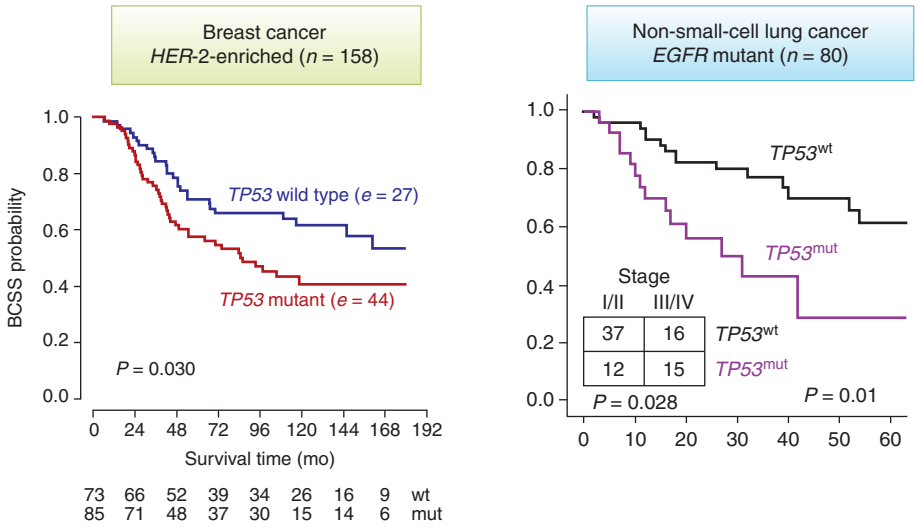

Figure 4. Prognostic value of *TP53* mutation in breast and lung cancer driven by oncogenes of the EGF receptor family. (*Left*) Prognostic value of *TP53* mutations in patients with *HER2*-enriched breast cancer subtype (Silwal-Pandit et al. 2014). (*Right*) Prognostic value of *TP53* mutations in patients with adenocarcinoma of the lung with mutant *EGFR* (Clinical Lung Cancer Genome Project 2013).

those reported in studies using Sanger sequencing (range, 50%–80%), in which *TP53* mutation load in SqCC appeared to correlate with smoking history (Le Calvez et al. 2005). *TP53* is by far the most frequently mutated gene in SqCC, ahead of *MLL2* (20%), *PI3KCA* (16%), *CDKN2A* (15%), *NFE2L2* (15%), and *KEAP1* (12%) (Clinical Lung Cancer Genome Project 2013). There is no obvious difference in the patterns of mutated genes between *TP53*-mutated and wild-type cases, suggesting that disruption of the p53 pathway is an obligate mechanism in the pathogenesis of SqCC. However, the high frequency precludes using *TP53* mutations as a classifier for identifying molecular subtypes or for investigating prognostic and predictive value. In AdC, mutations occur in 46% of the cases, ahead of *KRAS* (33%), *KEAP1* (17%), *STK11* (17%), *EGFR* (14%), *NF1* (11%), and *BRAF* (10%). It is striking to note that *TP53* is the only gene that is commonly mutated in both major histological types of NSCLC. In both AdC and SqCC, the pattern of *TP53* mutations is characterized by a high rate of transversions occurring at G bases on the nontranscribed strand, a typical signature of mutations induced by tobacco smoke. This pattern is seen in all histological subtypes but less marked in AdC than SqCC. In AdC, this pattern is not detected in *EGFR*-mutated cases, which mostly develop in never smokers, and in which *TP53* mutations occur at a lower frequency than in smoking-related *KRAS*-mutated cases (30% vs. 60%, respectively). Independent of this association with smoke-related mutation signatures, *TP53* mutation does not appear to be associated with any specific molecular feature of AdC.

A multitude of cohort studies has failed to identify a prognostic effect for *TP53* mutations on NSCLC survival, even in AdC and after separating *TP53* into different classes (see, e.g., Scoccianti et al. 2012; Ma et al. 2014). However, in a comprehensive analysis of 1255 clinically annotated lung cancers by the Clinical Lung Cancer Genome Project (CLCGP), *TP53* mutation was found to be associated with a significant negative prognostic value in AdC with mutated *EGFR* (Fig. 4) (Clinical Lung Cancer Genome Project 2013). In fact, AdC with mutated *EGFR* seems to have a more favorable prognosis than AdC with other driver oncogene mutations, including *KRAS*. Although *TP53* mutation does not appear to have an effect on the prognosis of AdC with mutated *KRAS*, it

cancels the apparent beneficial effect of *EGFR* mutation. It should be noted that this beneficial effect is not only attributable to the response of these patients to targeted *EGFR* therapies. It is also observed in a retrospective series of AdC patients who have been treated with conventional therapies. These observations suggest that wild-type p53 functions as a limiting factor in AdC driven by oncogenic activation of *EGFR*. There is a striking parallelism between the effect of *TP53* mutation in *EGFR*-mutated lung AdC and in *HER2*-enriched breast cancer (Fig. 4).

TP53 mutations have been reported as having a predictive effect on the survival of patients treated with adjuvant platinum-based therapy, a standard treatment regimen for stage II–III completely resected NSCLC (Ma et al. 2014). This effect predicts a marginal benefit of adjuvant therapy in patients with wild-type *TP53* and marginal detrimental effect in patients with mutated *TP53* mutation. There is no effect of *TP53* mutation in patients who are treated by surgery alone. As discussed above, this effect is significant only for non-DBM mutations, a class of mutations occurring in regions encoding the hydrophobic core structure of the p53 DNA-binding domain. Mutations in the hydrophobic core domain are frequent in lung cancers of smokers, because of the targeting of codons in this region by carcinogens from tobacco smoke. These mutations may carry GOF effect that, although having no impact on natural tumor development (no prognostic value), accelerates tumor progression or relapse under adjuvant treatment (negative predictive value). This particular situation is a rare illustration of a possible GOF effect of mutant *TP53* in a large randomized clinical trial. Presence of a non-DBM mutation appears to be associated with an adverse effect of adjuvant therapy, suggesting that these treatments should be restricted to wild-type *TP53* cases. The molecular basis of this effect remains to be identified.

CONCLUDING REMARKS: SOLVING THE *TP53* MUTATION EQUATION

The biological and clinical significance of *TP53* mutation is the result of a complex equation that combines structural and functional terms. The main structural terms are the mutation itself (position, type, and effect on p53 protein structure), the retention (or not) of a functional wild-type allele (LOH at *TP53* locus), and the haplotype structure of both wild-type and mutant alleles, which may be critical in the fine balancing act by which one allele may dominate over the other. Current sequencing strategies are tailored to assess only the first of these terms, and only in an incomplete manner because most studies remain focused on mutations occurring in exons. A recent initiative of researchers in the *TP53* mutation field, led by Thierry Soussi, is recommending that the entire sequence of the *TP53* gene should be taken into consideration when developing mutational analysis strategies to assess *TP53* status. As a next step, it will be important to design algorithms that enable the assignment of haplotypes and LOH from sequencing data, thus providing the technical basis for an assay capable of scoring all the structural terms of the mutant *TP53* equation.

The functional terms of the equation are even more complex. Although the major generic term is LOF, DN and GOF represent additional variables that may be extremely context-dependent. There is currently no simple and robust assay to score DN and GOF functions in a clinical setting. This complexity is compounded by the fact that, in many cancers, the p53 pathway may be disrupted by other mechanisms than structural alteration of the *TP53* gene, so that tumors that apparently retain intact alleles actually do not have an intact p53 pathway. Many studies have analyzed the transcriptomic patterns of *TP53* dysfunction in cancer cells and tissues but so far no robust consensus signature has emerged. Although a number of confirmed p53 target genes exist, their aberrant expression in cancer tissue may not be detectable without an inducer or treatment, or the expression of these genes in cancer tissue may be altered by p53-independent mechanisms. Efforts should be continued to identify surrogate biomarkers of a disrupted p53 pathway. A promising approach is to investigate p53-regulated long and short noncoding RNA networks (Donzelli et al. 2014).

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Integrative genomics studies are confirming that somatic *TP53* mutation lies at the very center of the patterns of alterations that characterize the genomic landscape of cancer. They also confirm that the biological and clinical significance of *TP53* mutation is very context-dependent, with large differences among histological subtypes of the same cancers. We propose to distinguish three patterns of association between *TP53* and genomic landscapes, highlighting their contrasting clinical messages.

High Mutation Frequency in Tumors with Profoundly Rearranged Genomes

In many subtypes of cancer (in particular in solid tumors), the mutation frequency is so high that it can be assumed that deregulation of the p53 pathway is an obligate mechanism for carcinogenesis. These cancers often show very disorganized genomes with high overall mutation load, often associated with intense exposure to endogenous or exogenous mutagens. They are poorly responsive to either conventional or pathway-targeted therapies. This is the situation observed in basal-like breast cancer or in SqCC of the lung. The overarching role of *TP53* mutation in these cancers might be to promote and maintain metabolic and/or epigenetic programs essential for the expansion of aggressive cancer cells with stem-like phenotypes. In turn, loss of p53 function may promote genomic instability by favoring the occurrence of massive catastrophic genomic rearrangements such as chromothripsis (Rausch et al. 2012). In these tumors, *TP53* mutation in itself may not be of much clinical significance for subclassifying cases or stratifying patients. However, a deeper evaluation of all structural terms of the *TP53* mutation equation may uncover significant differences in the clinical behavior of cancers with different mutants. Despite having an overall poor prognosis, these tumors with frequent *TP53* mutation show a range of clinical behaviors that may depend on factors that modulate the penetrance of *TP53* mutation, such as LOH, haplotypes, and alteration of regulators of p53 function. In breast cancer, combining *TP53* mutation, LOH, and *MDM2* overexpression reveals an additive negative prognostic effect, suggesting that several mechanisms of p53 inactivation may cooperate within the same tumor (Silwal-Pandit et al. 2014).

Intermediate Mutation Frequency in Oncogene-Driven Cancers

Knowledge of *TP53* mutation status is proving of more direct relevance in tumors with intermediate *TP53* mutation frequencies, such as *EGFR*-mutated lung AdC and *HER2*-enriched BC. In these oncogene-driven cancers, *TP53* mutation occurs at frequencies between 30% and 50% and is a clear indicator of poor prognosis. In both cases, the driving event is the oncogenic activation of a cell-surface tyrosine kinase receptor of the EGF superfamily and it is plausible that *TP53* mutation has similar consequences in both cancers. An interesting hypothesis is that this effect is caused by the involvement of p53 in regulating cell responses to oncogenic signaling through the p14arf–Mdm2 pathway. Inactivation of p53 by mutation may switch off this pathway, thus eliminating a natural safeguard against excessive cell proliferation (Lomazzi et al. 2002). In support of this hypothesis, we have reported that p14arf expression is often down-regulated in lung AdC with mutated *EGFR* and wild-type *TP53*, suggesting that this pathway is under strong negative selection pressure in *EGFR*-mutated lung cancers (Mounawar et al. 2007; Cortot et al. 2014). However, why this effect is seen with *EGFR* mutation or *HER2* enrichment and not with *KRAS* mutation is not clear. Manipulating the p53 pathway to increase and stabilize wild-type responses may prove a valuable companion approach in the treatment of these cancers, in particular with targeted therapies.

Low Mutation Frequencies in Tumors with Actionable Wild-Type *TP53*

A broad range of cancers has rare *TP53* mutations, in particular at early stages. These cancers include lesions of overall good prognosis such as adenoma of the colon or luminal A breast cancers, in which rare *TP53* mutations might

just be occasional passengers. Most of these lesions are adequately curated by surgery. However, *TP53* mutations are also rare in several cancer types requiring more aggressive treatment, such as melanoma (6%) or osteosarcoma (10%–12%). In these cases, a reassessment of *TP53* mutation status is warranted. It is possible that significant structural alterations in *TP53* have been missed in conventional studies because they occur in regions of the gene that are not routinely screened. A dramatic example of such a situation is observed in osteosarcoma, in which frameshift alterations/translocations are detected in intron 1 in ~50% of the cases with no mutation in the coding sequence (Chen et al. 2014; Ribi et al. 2015). Another potential intragenic mechanism of inactivation is overexpression of dominant-negative p53 isoforms, either caused by deregulation of transcription and/or splicing, or by mutations in introns that activate the expression of naturally occurring isoforms (Marcel et al. 2011). For melanoma, the frequent deletion or mutation of the *CDKN2A* locus, which includes alterations of p14/ARF, may be a contributing factor for the lower mutation prevalence in the *TP53* gene itself (Hodis et al. 2012). The fact that many of these tumors have in common the retention of a potentially functional p53 pathway suggests that it may be possible to pharmacologically "resuscitate" a suppressor function. A proof of principle has been given in cutaneous melanoma, in which overexpression of *MDM4* promotes the survival of human metastatic melanoma by antagonizing p53 proapoptotic function (Gembarska et al. 2012). Inhibition of the *MDM4*–p53 interaction restores p53 function in melanoma cells, resulting in increased sensitivity to cytotoxic chemotherapy and to inhibitors of the *BRAF* (V600E) oncogene, thus identifying *MDM4* as a promising target for therapy in melanoma.

In the coming years, *TP53* mutation data will continue to grow at an unprecedented, exponential pace because at least the frequently mutated exons of *TP53* are included in most current next-generation sequencing panels. During the weeks of redaction of this article (May–July 2015), the COSMIC WGS/WES *TP53* mutation data set grew by more than 1000 units. A large concerted effort is needed to mine and annotate this enormous mass of information and to turn it into a source of detailed knowledge for the biologist, the clinician and, ultimately, for the benefit of the patients.

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