REVIEW

The rebel angel: mutant p53 as the driving oncogene in breast cancer

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Breast cancer is the most frequent invasive tumor diagnosed in women, causing over 400,000 deaths yearly worldwide. Like other tumors, it is a disease with a complex, heterogeneous genetic and biochemical background. No single genomic or metabolic condition can be regarded as decisive for its formation and progression. However, a few key players can be pointed out and among them is the TP53 tumor suppressor gene, commonly mutated in breast cancer. In particular, TP53 mutations are exceptionally frequent and apparently among the key driving factors in triple negative breast cancer—the most aggressive breast cancer sub-group—whose management still represents a clinical challenge.

The majority of TP53 mutations result in the substitution of single aminoacids in the central region of the p53 protein, generating a spectrum of variants ('mutant p53s', for short). These mutants lose the normal p53 oncosuppressive functions to various extents but can also acquire oncogenic properties by gain-of-function mechanisms. This review discusses the molecular processes translating gene mutations to the pathologic consequences of mutant p53 tumorigenic activity, reconciling cell and animal models with clinical outcomes in breast cancer. Existing and speculative therapeutic methods targeting mutant p53 are also discussed, taking into account the overlap of mutant and wild-type p53 regulatory mechanisms and the crosstalk between mutant p53 and other oncogenic pathways in breast cancer. The studies described here concern breast cancer models and patients—unless it is indicated otherwise and justified by the importance of data obtained in other models.

Significance of TP53 mutations in breast cancer

TP53 gene and its mutations in spontaneous breast cancer

P53 protein, encoded by the TP53 tumor suppressor gene, is one of the main molecular decision makers of stress response in human cells (1). Embedded within a complex signaling pathway, p53 senses a plethora of stress signals originating from deregulated expression of oncopgenes, DNA damage, metabolic deprivation or telomere erosion. Depending on the cellular context and on the type of stress, p53 elicits apoptosis, DNA repair, transient or permanent cell cycle arrest and, lately found as surprisingly crucial, metabolic homeostasis maintenance (2). P53 activation—inactivation upon stress depends on a repertoire of posttranslational modifications (PTMs) and interactions with proteins that induce p53 stabilization and subcellular relocation, allowing it to induce appropriate sets of genes. The oncosuppressive functions of p53 may be inhibited by several mechanisms, but TP53 has come to researchers’ attention primarily due to its exceptional mutation frequency—higher than in any other tumor suppressor gene in humans overall. On average, TP53 is mutated in 31% of all tumors included in the Catalog of Somatic Mutations in Cancer (COSMIC) database (3), and is mutated in ~23% of breast cancer samples, where it is the second most frequently mutated gene after the PIK3CA protooncogene (26% in COSMIC). Mutations in TP53 occur more frequently in other types of tumors, in particular ovarian (50% of cases in COSMIC), large intestine (43%) and lung (36%) cancers. Although these sporadic cancers depend more heavily on TP53 mutations than breast cancer, the presence of mutated TP53 is still one of the main molecular characteristics of this type of tumor.

According to the current release of the International Agency for Research on Cancer (IARC) TP53 database (http://www-p53.iarc.fr/), included in COSMIC, ~70% of the breast cancer alterations in TP53 are missense mutations (4). This proportion, as well as the spectrum of mutated codons in the gene (the hotspots), reflect the p53 mutational pattern of other tumors (Figure 1 and Table 1). A noteworthy difference is codon 220, which is the fourth most frequent missense mutation in breast cancer (3.6%), whereas it ranks seventh in other cancers (2%). Another such overrepresentation is codon 163 (2% in breast cancer, 1% in other cancers) (5). Although no explanation of these differences has been provided, geographic or ethnical characteristics have been suggested to influence the occurrence of specific mutations, possibly due to the link to environmental mutagens (6, 7). Associations of TP53 mutation with breast-cancer-predisposing BRCA1/2 germline mutations have been also found, probably favored by a bias in the dysfunctional DNA repair mechanisms (8, 9). In sporadic breast cancers, high TP53 mutation frequencies have been significantly associated with two polymorphisms: the homozygous Arginine at codon 72 of p53 (10); and the presence of the highly active allelic variant G of glutathione-S-transferases (GSTs) (11). Importantly, differences have been found in the specific TP53 mutation occurrence in breast cancer types and grades, as well as in the survival of patients bearing particular hotspot mutations (discussed in part IV).

Hereditary TP53 mutations and breast cancer

The significance of TP53 gene alterations in breast cancer is supported by the frequent occurrence of this cancer type in the Li–Fraumeni syndrome, a hereditary tumor-predisposing disorder associated with germline TP53 mutations (12). Taking into account the tissue and organ specificity of tumors, breast cancer is the single most frequent event in Li–Fraumeni syndrome, accounting for ~25% of all tumors in affected families (13). The mutational spectrum of TP53 in Li–Fraumeni breast cancer resembles that in spontaneous breast cancer, with ~65% missense mutations, but differs in the hotspot distribution (Figure 1). This is mainly due to the unusually high frequency (up to 16%) of codon 337 mutation in Li–Fraumeni patients, which is 11% in syndrome-related breast cancers (IARC TP53 database). This bias probably reflects a founder effect in the Southern Brazilian population (14), due to the genetic background dependence of the mutation’s penetrance (15). The tumor spectrum associated with germline alterations at codon 337 is particular, with 67% of diagnosed adrenal tumors and breast cancers down to 11.6% (IARC TP53 database). This underlines that specific kinds of TP53 alterations may have a different impact on breast cancer development.

The fact that most inborn TP53 mutations preferentially induce breast cancer may imply that p53 alterations are the early events in spontaneous mammary tumors also. The fact that missense mutations in TP53 are more frequent in high-grade spontaneous breast carcinomas (16, 17) suggests that early p53 mutations might be one of the decisive events in the development of breast cancers on the high-grade-like rather than ‘low-grade-like’ molecular pathway,
taking into account their proposed early divergence (18,19). The rise of new approaches for monitoring genomes and global expression profiles of single cells (20) or tumor mass fragments (21) should allow to draw a more accurate picture of the timing and 'topology' of TP53 mutations in breast cancer.

Mutant TP53 in mouse models: importance of an oncogenic p53 gain-of-function

Although the idea that missense mutations can confer oncogenic properties to p53, in contrast to a mere loss-of-function, has been around for many years(22–24), only specific mouse models have proved that mutant p53 gain-of-function (GOF)—defined as the ability of p53 missense mutants to actively contribute to tumor progression and aggressiveness—indeed affects tumorigenesis in vivo.

The most accurate mouse models of Li–Fraumeni TP53 missense mutations were generated by gene knockin (Table 1). The mutant TP53 alleles used in crucial studies encoded R172H and R270H p53 variants, the murine counterparts of human R175H and R273H hotspot mutants (25–27). Tumor spectra in these mice differed, and metastasis frequency was increased, when compared with mice having TP53 +/– and –/– genotypes; there were also differences between R172H and R270H variants (26).

In contrast to Li–Fraumeni patients with the corresponding R175H or R273H mutations, these mice displayed low frequency of mammary carcinomas (26,27), similar to TP53 knockout mice (Table 1) (28,29). This apparent inconsistency may find an explanation in their particular genetic backgrounds, as well as in the fact that early appearance of other tumors in these animals would mask the formation of mammary carcinomas. Indeed, when a single TP53 R270H allele was expressed specifically in mammary epithelium, increased formation of breast cancer was observed (30).

Mouse models generated so far provided a great deal of important data (Table 1), but as illustrated by the Li–Fraumeni reconstruction attempts, possess limitations in simulating the phenotypic effects of human TP53 mutations. Nonetheless, after inclusion of more TP53 mutations, combined with alterations in other oncogenic pathways, careful dissection of the impact of genetic background and extensive use of cancer xenografts, mouse models may yet provide a tremendous contribution to the understanding of the role of p53 in breast cancer.

Molecular mechanism of mutant p53 action in breast cancer cells

Transcriptional activity of mutant p53

The role of wild-type (wt) TP53 in tumor suppression is strongly linked to the molecular properties of the p53 protein. Even though p53 has important transcription-independent roles (31), the protein works primarily as a tetrameric transcription factor (32). Solving the crystal structure of the DNA-binding domain (DBD) of wt p53, the largest and most structured of its domains, in complex with the target DNA sequence (Figure 1B) was crucial for understanding how oncogenic hotspot mutations affect p53 activity (33). The most commonly changed residues in breast cancer—R248Q and R273H—affect contact between p53 and DNA and hence have been dubbed ‘contact mutants’. In contrast, R175H and Y220C substitutions generate p53 ‘structural mutants’, with distorted DBD structure under physiological conditions. Careful biophysical studies in vitro uncovered a gradient in the extent of p53 DBD destabilization by the specific hotspot TP53 mutations (34), suggesting that different mutants may be functionally different
Table I. Oncogenic properties of most frequent TP53 missense mutations in breast cancer

| Codon | Frequency of missense mutations in breast cancer (# most frequent in breast cancer); in other tumors (# most frequent) | Ten-year mortality rate (/1000) in breast cancer patients, based on (17) | Human breast cancer cell lines with endogenous mutation, based on Handbook of p53 Mutations In Cell Lines, v. 1.0 (http://p53.free.fr/) | Human mammary epithelial cell characteristics associated with the presence of mutant p53 | Knockin mouse models with mutant TP53 expressed in mammary epithelium | Mammary carcinomas associated with mutant p55 in mouse models |
|-------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| 248   | 10.1% (1); 8.8% (2) | 78.65 (R248Q: 69.06; R248W: 108.84) | H-31 (R248Q) HCC1143 (R248Q) HCC2157 (R248W) HCC70 (R248Q) | Altered growth and cell polarity in 3D cultures, EMT induction (MCF10A cells with introduced p53 R248W mutant) (104,121) | Mammary gland-specific expression (R245W) (166) | No increase in mammary carcinomas compared with parental strain (166,167) |
| 273   | 7.5% (2); 8.9% (1) | 68.29 (R273H: 67.29; R273C: 64.86) | HCC38 (R273L) MDA-MB-468 (R273H) R30T (R273C) SUM229PE (R273C) | Disordered growth in 3D cultures, induction of mevalonate pathway genes; induction of migration-related mutant p53 signature genes; inhibition of apoptosis (MDA-MB-468) (48,90,104); Altered growth and cell polarity in 3D cultures, EMT induction (MCF10A cells with introduced p53 R273H mutant) (104,121); Immortalization of normal mammary epithelial cells (95) | Mammary gland-specific expression (R270H) (26) | Low incidence of mammary carcinoma; altered tumor spectrum compared with p53-/- and +/- (26) |
| 175   | 7.0% (3); 6.5% (3) | 68.29 | HCC1395 (R175H) SK-BR-3 (R175H) | Increased growth rate, tumorigenic potential, chemoresistance; increased expression of pro-angiogenic genes, NF-Y and NF-kB targets; inhibition of p73; inhibition of apoptosis mediated by the vit. D receptor (SK-BR-3) (44,47,89,91,168,169); Altered growth and cell polarity in 3D cultures, EMT induction (MCF10A cells with introduced p53 R273H mutant) (104,121) | Germline knockin (R172H) (26,27,57) | No mammary carcinomas; altered tumor spectrum compared with p53-/- and +/- (26); Increased mutant p53 stability and tumorigenesis in mdm2-/- or p16-/- background (57) |
| 220   | 3.6% (4); 2.0% (7) | 45.63 | HCC1419 (Y220C) MDA-MB-330 (Y220C) | Unknown | None | Unknown |
| 245   | 3.4% (5); 4.1% (4) | 37.68 | None | Altered growth and cell polarity in 3D cultures, weak GOF (MCF10A cells with introduced p53 G245S mutant) (104,121) | None | Unknown |
| 280a   | 1.3% (16); 1.3% (13) | Unknown | CAMA-1 (R280T) MDA-MB-231 (R280K) | Disordered growth in 3D cultures, induction of mevalonate pathway genes; induction of migration-related mutant p53 signature genes and migratory phenotype; inhibition of p63 downstream transcriptional program and TGFβ-induced metastasis; inhibition of apoptosis mediated by the vit. D receptor, induction of chemokines and other inflammatory mediators (MDA-MB-231) (48,75,91,104,108) | None | Unknown |

*280a280 codon is included as in Figure 1; a Increased mortality from total codon 273 changes is due to other 273 variants, albeit very few in number (17)
proteins. Transactivation assays in yeast or human cultured cells have been designed to assess the activity of mutant p53s (35–37). In a study concerning TP53 mutants frequently expressed in breast cancer, many mutant p53 variants were shown to possess an altered promoter activation spectrum (48,75). For instance, mutation Y220C, for instance, transactivates the most sensitive wt p53 response element (from the promoter of the p21 gene—WAF1), whereas other response elements are not activated (38). The shift, rather than a full displacement in the transcriptional specificity of wt p53, is an element of mutant p53 GOF. Transcriptional analysis of the activity of six different TP53 hotspot mutants in a p53−/− background of H1299 lung carcinoma cells indicated that mutant p53s regulate predominantly a specific subset of genes whose promoters are also bound by wt p53 (39). Nevertheless, an accumulating body of evidence in different models, including breast cancer cells, suggests that mutant p53 also acquires distinct DNA-binding and transactivation properties because many loci lacking p53-responsive elements are direct transcriptional targets of hotspot p53 mutants (40–44). Mutant p53 can also directly activate transcription of specific micro-RNA (45) and attenuate micro-RNA processing, presumably affecting their general levels in cells (46). Using breast cancer cell lines, Blandino and colleagues found 40 promoters bound de novo by R175H p53 (47), whereas our group identified 10 novel genes controlled and bound by R280K p53 (48).

Despite increasing interest, no consensus target sequence of mutant p53 has been defined so far. However, an overrepresentation of nuclear factor-kappaB (NF-kB) target sites has been observed in promoters bound by the p53 R175H mutant (47), and a p63-binding consensus emerged as the main feature of sites bound by mutant p53s in H1299 lung carcinoma cells and HaCaT keratinocytes (39,49), suggesting that mutant p53 cooperation with different transcription factors may be an important route to execute its GOF activity. Because p53 is a tetrameric transcription factor, hetero-oligomerization of wt and mutant monomers results in a dominant negative effect. Even though this dominant negative effect of mutant p53 has been demonstrated (50,51), its importance in tumorigenesis is controversial. In H1299 cells, mutations in the DBD of p53 have been shown to be relatively ineffective in the functional inactivation of heterotetramers of wt p53 (52), and the influence of heterozygous TP53 missense mutations in cell lines and tumor samples (including breast cancer) has been questioned (53,54). Taking into account different observations, it is conceivable that many mutant p53 variants can interfere with wt p53 to facilitate tumor formation by lowering the tumor suppressor barrier, but mutant p53 GOF provides an additional boost, such as increased invasiveness and chemoresistance, for tumorigenesis; this effect could be potentiated when the wt allele is lost (27,55). The cell/tumor type context is also an important variable to consider in further research—because the mutant p53’s GOF penetration may be dependent on the particular molecular background.

**Stability and PTMs of mutant p53**

Complementing the altered transcriptional abilities, the oncogenic properties of most frequent p53 mutants also benefit from an increased p53 level. In normal cells, p53 is destabilized mainly by the action of Mdm2, an E3 ubiquitin ligase that is a direct p53 transcriptional target (56). In tumor cells, this negative feedback is frequently abrogated, resulting in increased p53 level. The importance of mutant p53 accumulation is underscored by the observation that Li–Fraumeni mouse models with R172H p53 knockin, despite different tumor spectra and metastasis incidence, have survival curves similar to p53−/− mice (26,27). However, when they are crossed with MDM2−/− mice, resulting in increased mutant p53 stability, survival of the animals is drastically shortened (57), indicating that R175H p53 GOF is strongly dependent on p53 stability. Even though mutant p53 variants can be ubiquitinated by MDM2 as well as other E3 ligases and degraded (58), several mechanisms may counteract this process in mammmary tumor cells: for instance, p16INK4a upregulation (59) or Hsp90-mediated stabilization (60).

Mutant p53 stabilization and activity can depend also on PTMs other than ubiquitination, although not many studies have addressed their role (61,62). In breast cancer cell lines and tumor samples, p53 phosphorylation is detected regardless of TP53 mutational status (63), indicating that oncogenic stress can modify mutant p53 on the same regulatory sites as it does on wt p53 (48). Expression of a non-phosphorylatable variant (S392A) enhances the oncogenic potential of p53 R175H in cultured cells, suggesting that Ser 392 phosphorylation might negatively affect the GOF. Accordingly, in breast cancer samples with high levels of mutant p53, the phosphorylation at S392 was reduced (64).

Despite the massive amount of knowledge on wt p53 PTMs, the same aspect of mutant p53 still holds many unknowns. In particular, next to nothing is known about acetylation, methylation or sumoylation specific to mutant p53 in breast cancer, whereas acetylation by p300/CPB-associated factor (PCAF) was found to reactivate selected p53 mutants in H1299 cells (65). This field of research deserves greater efforts, because pharmacological intervention on enzymes that apply regulatory PTMs on mutant p53 might become an approach of antitumor treatment.

**Effect of TP53 mutations on p53 paralogs and isoforms in breast cancer**

Human p53 possesses two main paralogs—p63 and p73—expressed as multiple isoforms sharing a significant similarity to p53 (66,67). Though not being classical tumor suppressors, both possess antitumorogenic functions (68,69). p63 does not hetero-oligomerize with its paralogs (70), but p73 and p63 were found to bind mutant p53 via its DBD (71,72). As a consequence, p73 and p63 can be drawn into mutant p53 aggregates in osteosarcoma cells, presumably blocking their normal function (73). Such interactions are involved in the direct negative effect of mutant p53 on the antitumor activity of its paralogs in different tumor models (26,27,70,74). In breast cancer cells, p53 mutant variants were shown to repress p73- and p63-driven transcriptions of target genes (71,72). In response to transforming growth factor (TGF)-beta signaling, mutant p53 binds p63 in a complex with Smad2, preventing transcription of Sharp-1 and Cyclin G2, two crucial p63 target genes that suppress metastatic behavior of breast cancer cells (48,75). Similarly, mutant p53 variants introduced into epithelial lung H1299 and mammary MCF-10A cells were found to promote an invasive phenotype by inhibiting p63-dependent regulation of integrin and epidermal growth factor receptor (EGFR) recycling (76). Finally, high-throughput microarray and ChIP-seq data from lung and skin cancer cells (39,49) revealed that p63-promoter-binding sites are frequently targeted by mutant p53, indicating that direct influence on p63 is one of the distinctive mechanisms of mutant p53 GOF.

The last 10 years have brought significant advances in our knowledge of p53 isoforms (77). Indeed, p53 can be expressed in various alternative N-terminal and C-terminal isoforms. Notably, they were reported to be differentially expressed between normal and breast cancer tissues (78). In normal breast tissue, the C-terminally truncated and modified β and γ isoforms were both present, whereas p53β was only detected in 33% of tested tumors, and p53γ in none. In contrast, although N-terminally truncated Δ133p53 was not detectable in healthy controls, it was found in 80% of all tested breast tumors. Successive studies demonstrated that expression of the p53γ isoform significantly improves the outcome of breast cancer patients bearing mutant p53 (79). The mutations were present in both full-length p53 and p53γ, leading to a conclusion that oncogenic properties induced in TP53 are not simply transferred to a shorter p53 isoform. It will be interesting to address in further research whether there is an influence of specific hotspot mutations on the function of p53 isoforms, and whether this function extends beyond the influence on full-length p53.

**Mutant p53 involvement in mechanisms of breast cancer development**

**Mutant p53 in early tumorigenesis of breast cancer: DNA damage response, genomic instability and apoptosis avoidance**

Mutant p53 influence in breast cancer may stretch from early events, raising the probability of tumor development (as in Li–Fraumeni
Mutant p53 in breast cancer

A large fraction of breast cancers expresses estrogen (ER) and progesterone receptors (PR), and their proliferation depends on hormonal stimulation. Despite prognostic associations discussed in the following sections, there is little clarity in the functional interactions between ER and PR and mutant p53. Block of estrogen-dependent signaling was shown to reduce wt and mutant p53 protein levels in ER-positive mammary tumor cells (98,99). ER-alpha and mutant p53 may also potentially cooperate to transcribe selected target genes by cooperative docking to non-canonical promoter-binding sites (100). In theory, such a complex can form also with some p53 mutants, and therefore ER-alpha may potentially influence the transcriptional landscape of cells expressing mutant p53. Additional studies are urgently needed to clarify the functional interaction between ER and mutant p53 in breast cancer, especially in view of the clinical use of anti-estrogenic drugs.

One of the earliest discoveries regarding the pro-oncogenic activities of mutant p53 was its ability to sustain tumor growth of fibroblast cells injected subcutaneously in immunocompromised mice with poor patient survival (94). Fourth, mutant p53 may affect cellular senescence. In this respect, specific studies are needed to understand the contribution of mutant p53 variants to overcoming cellular senescence in breast cancer, because this is an effect already observed in mammary epithelial cells (95) and in other cancers (96,97).

Mutant p53 in breast cancer growth: effect on metabolism, inflammation and angiogenesis

A large fraction of breast cancers expresses estrogen (ER) and progesterone receptors (PR), and their proliferation depends on hormonal stimulation. Despite prognostic associations discussed in the following sections, there is little clarity in the functional interactions between ER and PR and mutant p53. Block of estrogen-dependent signaling was shown to reduce wt and mutant p53 protein levels in ER-positive mammary tumor cells (98,99). ER-alpha and mutant p53 may also potentially cooperate to transcribe selected target genes by cooperative docking to non-canonical promoter-binding sites (100). In theory, such a complex can form also with some p53 mutants, and therefore ER-alpha may potentially influence the transcriptional landscape of cells expressing mutant p53. Additional studies are urgently needed to clarify the functional interaction between ER and mutant p53 in breast cancer, especially in view of the clinical use of anti-estrogenic drugs.
Currently, specific transcriptional programs or cell phenotypes cannot be correlated with selected missense p53 mutations or even with the broad distinction between contact and structural mutants (39) (Table 1). However, we suggest that future research on breast tumor progression and its clinical applications will have to take into account the properties of the specific mutants. This will require extensive application of DNA sequencing to biological samples along with the analysis of p53 expression levels. TP53 mutations were found to be more frequent in high-grade, large-size, node-positive cases and in estrogen- and progesterone-receptor-negative (ER-, PR-) tumors (17). Considering division of breast tumors according to the current molecular subtype classification (128,129), TP53 mutation numbers are usually low in well-differentiated and hormone–receptor-positive luminal subtype A and significantly higher in human epidermal growth factor receptor 2 (HER2) positive and basal-like tumors. In a recent study involving a detailed, genome-scale analysis of nearly 2000 breast cancer cases, TP53 mutations were found in 34% basal-like, 22% HER2, 13% luminal B and 5% luminal A molecular subtypes (130). Taking into account the hormone receptors, TP53 mutations were detected in 8% cases of ER+ and/or PR+ tumors and in 29% of hormone-receptor-negative ones (130). Interestingly, in ER+/PR+ tumors, despite the reduced frequency, the prognostic value of TP53 mutation remains strong (17). TP53 alterations correlate with poor clinical outcome also in HER2-positive cancers (131,132), TNBCs (ER-, PR- and HER2-), which mainly belong to the molecular basal-like subtype (129), are more likely to be grade 3 tumors (16). A recent study conducted with high-throughput genomic approaches confirmed that in TNBCs, mutations in TP53 are more frequent than in any other oncogene or tumor suppressor, reaching 54% of samples (133). The increase in TP53 mutations in hormone-receptor-negative tumors and their exceptionally frequent occurrence in TNBC suggests that in the absence of hormone-related stimulatory signaling characteristic to the mammary epithelium, mutant p53 may become increasingly critical for breast cancer progression. Moreover, once established, mutant p53 dependence seems to provide a more severe pro-oncogenic activity compared with hormone dependence. This is supported by the fact that taking into account all the heterogeneity of breast cancer, whenever present, TP53 mutations are more frequent in high-grade, large-size, node-positive cases, thus possessing a prognosis-worsening, driving role (17,130).

For clinical applications, it would be important to improve the reliability of p53 mutation as the prognostic marker. Different studies...
Fig. 3. Mutant p53 as the hub of oncogenic pathways in breast cancer. The activity of mutant p53 is regulated through upstream signal transducers as well as regulators of transcription, stability and structure. Its biological effects are mediated by direct transcriptional activity and through association with downstream protein effectors. These mechanisms are interconnected (small arrows), especially downstream of mutant p53, as most proteins bound directly affect the tumorigenic features of breast cancer models or patients and target genes found to be directly regulated by mutant p53. *PTMs—Posttranslational Modifications may be affected by upstream factors and may influence downstream effects of mutant p53. †TopBP1 is implicated as a coordinator protein of mutant p53 GOF exerted via NF-Y, p63 and p73.
have shown that this can be achieved by combining the analysis of TP53 mutational status with the assessment of other factors relevant for either mutant p53 stability or pro-oncogenic functions (Figure 3). Examples of these are the Polo-like kinase 1 and the phosphorylation-dependent prolyl isomerase Pin1 (48,134). In particular, combined analysis of Pin1 protein levels and TP53 missense mutations has been shown to outperform the mere determination of TP53 mutational status as the predictor of clinical outcome (48). Further research is needed to identify other molecular parameters to improve the prognostic value of TP53 mutational status.

**Predictive value of mutant p53 in breast cancer**

Despite increasing evidence supporting the prognostic significance of TP53 mutations, few studies have been performed in order to assess its predictive value in patient response to therapy. Among the most commonly used chemotherapeutic regimens, the clearest available results concern treatment with anthracyclines, as several preclinical and clinical reports have indicated that mutant TP53-bearing breast cancer cells and tissues are more resistant to these drugs, compared with wt TP53-bearing ones (37,135,136). A more complex scenario arises when taxane-based chemotherapy is considered. Despite promising early results (137), a large phase-3 clinical trial has recently demonstrated that mutant TP53-harboring breast carcinomas have similar sensitivity to taxanes as those with wt TP53, and TP53 status seems not to be decisive in selecting patients for such chemotherapy (138).

As suggested for the prognostic value of TP53 status, the combination of p53 assessment with the analysis of additional pathways or molecular markers—primarily those controlling mutant p53 stability or activity—should be considered in order to strengthen its predictive properties. Furthermore, it would be important to verify whether the combination of chemotherapeutic drugs with molecules directly or indirectly impinging on mutant p53 activity could be useful as a therapeutic strategy. A striking example is provided by the recent results obtained by treating TNBC tumors implanted in mice with a combination of Chk 1 inhibitors and the DNA-damaging drug irinotecan (139). Under these conditions, tumors with mutant p53 responded to a significantly better extent, indicating that it may be a key determinant influencing tumor response to therapy. Another recent intriguing example has been provided by the observation that Wnt-induced mouse mammary tumors expressing mutant p53 show a superior clinical response to doxorubicin treatment when compared to similar tumors with wild-type p53 (140).

**Mutant p53 as the drug target in breast cancer**

**Direct targeting of mutant p53**

Mutant p53 has been considered a potential direct target of therapy and structural information on p53 has been used to rationally identify molecules reactivating wt transcriptional function of mutant p53 or restoring its native structure (141,142). Peptides designed to interfere with p53/p73 binding were able to sensitize breast cancer cells to chemotherapeutic drugs (143). Another noteworthy study concerns a rational design of small compounds predicted to bind and stabilize in silico the structure of one mutant p53 variant distinctive in breast cancer—Y220C (144). Although these are elegant examples of how the detailed structural information on p53 could be potentially translated to clinics, all these above-mentioned compounds have not yet been tested to confirm their structural effects in vivo.

Instead, a random screening approach allowed the identification of short peptide aptamers that bind specifically to mutant p53 and, importantly, trigger cell death in breast cancer cells expressing mutant, but not wt, p53 (145). Other interesting molecules of potential therapeutic impact identified by screening approaches are CP-31398 (146), P53R3 (147), RETRA (148) and PRIMA-1 (149). This last compound was one of the first examples of drugs capable of restoring wt p53 conformation, thereby allowing sequence-specific DNA binding and induction of p53 target genes (149). In particular, PRIMA-1 induces apoptosis in tumor cells (other than breast cancer) and inhibits human xenograft tumor growth in SCID mice (149). PRIMA-1 derivatives have been produced to increase its efficacy and the most powerful one is its methylated form, PRIMA-IMET/APR-246 (150), which is currently in a clinical trial (151).

**Indirect targeting of mutant p53—challenging mechanisms common to wt and mutant p53**

The alternative to targeting mutant p53 itself is to block its upstream activators. Results obtained in several models have suggested candidates, such as the signaling pathway components EGFR (76), transforming growth factor TGBβR1 (75), Ras-activated kinases like p38, JNK1-2, MEK/extracellular signal-regulated kinase ERK and CK1ε/δ (48,75), and the Polo-like kinase family members 1 (151) and 2 (152), for pharmacological inhibition in breast cancer. Other potential targets include proteins affecting mutant p53 levels and structure, such as prolyl isomerase Pin1 (48), Hsp90 (153) or the histone deacetylases (154,155).

There are, however, risks associated with inhibiting some of these factors because of the numerous regulatory mechanisms shared by wt and mutant p53. This includes p53 degradation, which in the case of both wt and mutant depends at least partially on Mdm2 and p16INK4 (57,58). Wt p53 folding, stability and activity are supported by Hsp70–Hsp90 chaperone machinery (156) and prolyl isomerase Pin1 (157), whereas the same proteins are found to support mutant p53’s oncogenic potential (48,158). Therefore, an important prerequisite to their clinical applications is to know the status of TP53 sequence and expression in the tumor; Mdm2 and Hsp90 inhibitors, such as Nutlin and 17AAG, respectively, are being considered candidate drugs for breast cancer (159,171). A more appealing possibility is to target specific inducers of mutant p53. One example is topoisomerase IIβ-binding protein (TopBP1), which is aberrantly expressed in breast cancer (160), where it is associated with high tumor grade and shorter patient survival (161). TopBP1 in breast cancer cells inhibits wt p53 function, whereas it promotes GOF of mutant p53 variants (161,162). This makes TopBP1 a very interesting potential drug target in breast cancers bearing either wt or mutant p53.

**Conclusions**

The TP53 gene is mutated less frequently in breast cancer than in other tumors, yet it is the second most frequent genetic alteration in this type of cancer. TP53 mutation frequency and its prognostic value differ between breast cancer subtypes, where, like in other tumors, p53 has to be considered within a web of highly interconnected tumorigenic pathways (Figure 3). In some cases, like TNBC, mutant p53 probably acts as a balance-shifting ‘driver’, whereas in others, it may just be a contingent ‘passenger’. However, in overall majority of cases, missense mutant p53 seems associated with an aggressive tumor phenotype, suggesting that mutant p53 is intrinsically predisposed to overtake the driver role.

The tumor-driving potential of mutant p53 is exerted at multiple levels: dampening oncosuppressive pathways (p63 and other growth suppressors); enhancing multiple oncogenic pathways relevant in breast cancer (such as PI3K/AKT, Ras/mitogen-activated protein kinase (MAPK) and NF-xB) both directly and indirectly; and altering the cellular physiology at posttranscriptional level via non-coding RNAs. Additional functional intersections of mutant p53 with cellular pathways will certainly emerge in the future due to new technologies and high-throughput approaches. Following validation in breast cancer models, definition of these cross talks will provide essential conceptual tools for basic and clinical research, granting a better understanding of breast cancer biology and allowing a more accurate stratification of patients for personalized therapy. This knowledge will be critical not only for the development of novel anticancer approaches, but also—and perhaps more importantly—to allow more efficient use of currently available drugs.
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References

1. Horn,H.F. et al. (2007) Coping with stress: multiple ways to activate p53. Oncogene, 26, 1306–1316.
2. Li,T. et al. (2012) Tumor Suppression in the Absence of p53-Mediated Cell-Cycle Arrest, Apoptosis, and Senescence. Cell, 149, 1269–1283.
3. Forbes,S.A. et al. (2011) COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer. Nucleic Acids Res., 39, D945–D950.
4. Petitjean,A. et al. (2007) Impact of mutant p53 functional properties on TP53 mutation patterns and tumor phenotype: lessons from recent developments in the IARC TP53 database. Hum. Mutat., 28, 622–629.
5. Feki,A. et al. (2004) Mutational spectrum of p53 mutations in primary breast and ovarian tumors. Crit. Rev. Oncol. Hematol., 52, 103–116.
6. Olivier,M. et al. (2001) TP53 mutation patterns in breast cancers: searching for clues of environmental carcinogenesis. Semin. Cancer Biol., 11, 353–360.
7. Hill,K.A. et al. (2002) p53 as a mutagen test in breast cancer. Environ. Mol. Mutagen., 39, 216–227.
8. Greenblatt,M.S. et al. (2001) TP53 mutations in breast cancer associated with BRCA1 or BRCA2 germline mutations: distinctive spectrum and structural distribution. Cancer Res., 61, 4092–4097.
9. Mani,E. et al. (2009) High frequency of TP53 mutation in BRCA1 and sporadic basal-like carcinomas but not in BRCA1 luminal breast tumors. Cancer Res., 69, 663–671.
10. Langerød,A. et al. (2002) The TP53 codon 72 polymorphism may affect the function of TP53 mutations in breast carcinomas but not in colorectal carcinomas. Cancer Epidemiol, Biomarkers Prev., 11, 1684–1688.
11. Nedelcheva Kristensen,V. et al. (1998) Single tube multiplex polymerase chain reaction genotype analysis of GSTM1, GSTT1 and GSTP1: relation of genotypes to TP53 tumor status and clinicopathological variables in breast cancer patients. Pharmacogenetics, 8, 441–447.
12. Malkin,D. (2011) Li-fraumeni syndrome. Cell, 149, 1245–1256.
13. Kato,S. et al. (2003) Understanding the function-structure and function-mutation relationships of p53 tumor suppressor protein by high-resolution missense mutation analysis. Proc. Natl. Acad. Sci. U.S.A., 100, 8424–8429.
14. Deissler,H. et al. (2004) Spectrum of p53 mutations in biopsies from breast cancer patients selected for preoperative chemotherapy analysed by the functional yeast assay to predict therapeutic response. Oncol. Rep., 11, 1281–1286.
15. Berthau,P. et al. (2007) Exquisite sensitivity of TP53 mutant and basal breast cancers to a dose-dense epirubicin-cyclophosphamide regimen. PLoS Med., 4, e90.
16. Jordan,J.J. et al. (2010) Altered-function p53 missense mutations identified in breast cancers can have subtle effects on transactivation. Mol. Cancer Res., 8, 701–716.
17. Neilsen,P.M. et al. (2011) Mutant p53 uses p63 as a molecular chaperone to alter gene expression and induce a pro-invasive secretome. Oncotarget., 2, 1203–1217.
18. Chicas,A. et al. (2000) Mutant p53 forms a complex with Sp1 on HIV-LTR DNA. Biochem. Biophys. Res. Commun., 279, 383–390.
19. Scian,M.J. et al. (2004) Tumor-derived p53 mutants induce oncogenesis by transactivating growth-promoting genes. Oncogene, 23, 4430–4443.
20. Weiss,L. et al. (2004) Transactivation of the EGR1 gene contributes to mutant p53 gain of function. Cancer Res., 64, 8318–8327.
21. Yan,W. et al. (2009) Identification of GR01 as a critical determinant for mutant p53 gain of function. J. Biol. Chem., 284, 12178–12187.
22. Bonnemagi,G. et al. (2009) The execution of the transcriptional axis mutant p53, E2F1 and ID4 promotes tumor neo-angiogenesis. Nat. Struct. Mol. Biol., 16, 1086–1093.
23. Donzelli,S. et al. (2011) MicroRNA-128-2 targets the transcriptional repressor E2F5 enhancing mutant p53 gain of function. Cell Death Differ., 18, 1038–1048.
24. Suzuki,H.I. et al. (2009) Modulation of microRNA processing by p53. Nature, 460, 529–533.
25. Dell’Orso,S. et al. (2011) ChiP-on-chip analysis of in vivo mutant p53 binding to selected gene promoters. OMICS, 15, 305–312.
26. Girardin,I.E. et al. (2011) A Pin1/mutant p53 axis promotes aggressiveness in breast cancer. Cancer Cell., 20, 79–91.
27. Martynova,E. et al. (2012) Gain-of-function p53 mutants have widespread genomic locations partially overlapping with p63. Oncotarget, 3, 132–143.
28. Chène,P. (1998) In vitro analysis of the dominant negative effect of p53 mutants. J. Mol. Biol., 281, 205–209.
29. Wijnhoven,S.W. et al. (2007) Dominant-negative but not gain-of-function effects of a p53.R270H mutation in the p53 tumor suppressor gene mimic human breast cancer development. Cancer Res., 67, 4648–4656.
30. Chan,W.M. et al. (2004) How many mutant p53 molecules are needed to inactivate a tetramer? Mol. Cell. Biol., 24, 3536–3551.
31. Monti,P. et al. (2011) Dominant-negative features of mutant TP53 in germline carriers have limited impact on cancer outcomes. Mol. Cancer Res., 9, 271–279.
32. Stoczyńska-Fidelus,E. et al. (2011) Limited importance of the dominant-negative effect of TP53 missense mutations. BMC Cancer, 11, 243.
33. Junk,D.J. et al. (2008) Different mutant/wild-type p53 combinations cause a spectrum of increased invasive potential in nonmalignant immortalized human mammary epithelial cells. Neoplasia, 10, 450–461.
relative of p53 not only in structure but also in anti-cancer approach? et al., 18 spokes in tumor suppression.

Cold Spring. et al. (2010) Restoration of DNA-binding and growth-suppressive function of mutant p53. Cancer Res., 60, 901–911.

Gaiddon,C. et al. (2010) p53-family proteins and their regulators: hubs and Harb. Perspect. Biol. 2, a004887.

Hanel,W. et al. (2010) Physical interaction with human tumor-derived p53. J. Cell. Biochem., 101, 29503–29512.

Morton, J.P. et al. (2010) Mutant p53 drives metastasis and overcomes growth arrest/senescence in pancreatic cancer. Proc. Natl. Acad. Sci. U.S.A., 107, 246–251.

Skinner,H.D. et al. (2012) TP53 disruptive mutations lead to head and neck cancer treatment failure through inhibition of radiation-induced senescence. Clin. Cancer Res., 18, 290–300.

Liu,G. et al. (2000) Estrogen receptor protects p53 from deactivation by human double minute-2. Cancer Res., 60, 1810–1814.

Fernández-Cuesta, L. et al. (2011) Estrogen levels act as a rheostat on p53 levels and modulate p53-dependent responses in breast cancer cell lines. Mol. Cell. Biol., 21, 35–42.

Menendez,D. et al. (2010) Estrogen receptor acting in cis enhances WT and mutant p53 transactivation at canonical and noncanonical p53 target sequences. Proc. Natl. Acad. Sci. U.S.A., 107, 1500–1505.

Konduri,B.D. et al. (2010) Mechanisms of estrogen receptor antagonism toward p53 and its implications in breast cancer therapeutic response and stem cell regulation. Proc. Natl. Acad. Sci. U.S.A., 107, 15081–15086.

Liu,W. et al. (2006) Estrogen receptor-alpha binds p53 tumor suppressor protein directly and represses its function. J. Biol. Chem., 281, 9837–9840.

Sayeed,A. et al. (2007) Estrogen receptor alpha binds p53-mediated transcriptional repression: implications for the regulation of apoptosis. Cancer Res., 67, 7746–7755.

Freed-Pastor,W.A. et al. (2012) Mutant p53 disrupts mammalian tumour architecture via the mevalonate pathway. Cell, 148, 244–258.

Azziz,S.A. et al. (2001) Case control study of prognostic markers and disease outcome in inflammatory carcinoma breast: a unique clinical experience. Breast J., 7, 398–404.

Dawood,S. et al. (2011) Inflammatory breast cancer: what progress have we made? Oncology (Williston Park, N.Y.), 25, 264–70, 273.

Weisz,L. et al. (2007) Mutant p53 enhances nuclear factor kappab activation by tumor necrosis factor alpha in cancer cells. Cancer Res., 67, 2396–2401.

Yeudall,W.A. et al. (2012) Gain-of-function mutant p53 upregulates CXC chemokines and enhances cell migration. Carcinogenesis, 33, 442–451.

Kellavakar,U.P. et al. (1999) Effects of mutant p53 expression on human 15-lipoxygenase-promoter activity and murine 12/15-lipoxygenase gene expression: evidence that 15-lipoxygenase is a mutator gene. Proc. Natl. Acad. Sci. U.S.A., 96, 4378–4383.

Reddy,N. et al. (1997) Characterization of a 15-lipoxygenase in human breast carcinoma BT-20 cells: stimulation of 13-HODE formation by TGF alpha/EGF. Biochem. Biophys. Res. Commun., 231, 111–116.

Werner,H. et al. (1996) Wild-type and mutant p53 differentially regulate transcription of the insulin-like growth factor I receptor gene. Proc. Natl. Acad. Sci. U.S.A., 93, 8318–8323.

Gallagher,E.I. et al. (2011) Minireview: IGF, Insulin, and Cancer. Endocrinology, 152, 2546–2551.

Morselli,E. et al. (2008) Mutant p53 protein localized in the cytoplasm inhibits autophagy. Cell Cycle, 7, 3056–3061.

Debnath,J. (2011) The multifaceted roles of autophagy in tumors-implications for breast cancer. J. Mammary Gland Biol. Neoplasia, 16, 173–187.

Kieser,A. et al. (1994) Mutant p53 potentiates protein kinase C induction of vascular endothelial growth factor expression. Oncogene, 9, 963–969.

Linderholm,B.K. et al. (2001) The expression of vascular endothelial growth factor correlates with mutant p53 and poor prognosis in human breast cancer. Cancer Res., 61, 2256–2260.
117. Kalluri,R. et al. (2009) The basics of epithelial-mesenchymal transition. *J. Clin. Invest.*, **119**, 1420–1428.

118. Rogers,L. et al. (2010) Gain of oncogenic function of p53 mutant regulates E-cadherin expression uncoupled from cell invasion in colon cancer cells. *J. Cell Sci.*, **123**(Pt 8), 1295–1305.

119. Kogan-Sakin,L. et al. (2011) Mutant p53(R175H) upregulates Twist1 expression and promotes epithelial-mesenchymal transition in immortalized prostate cells. *Cell Death Differ.*, **18**, 271–281.

120. Ohashi,S. et al. (2010) Epidermal growth factor receptor and mutant p53 expand an esophageal cellular subpopulation capable of epithelial-to-mesenchymal transition through ZEB transcription factors. *Cancer Res.*, **70**, 4174–4184.

121. Zhang,Y. et al. (2011) Mutant p53 disrupts MCF-10A cell polarity in three-dimensional culture via epithelial-to-mesenchymal transitions. *J. Biol. Chem.*, **286**, 16218–16228.

122. Mani,S.A. et al. (2008) The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell*, **133**, 704–715.

123. Cicalese,A. et al. (2009) The tumor suppressor p53 regulates polarity of self-renewing divisions in mammary stem cells. *Cell*, **138**, 1083–1095.

124. Mizuno,H. (2003) A mutant TP53 gene status is associated with a poor prognosis and anthracycline-resistance in breast cancer patients. *Eur. J. Cancer*, **39**, 701–713.

125. Perou,C.M. et al. (2000) Molecular portraits of human breast tumours. *Nature*, **460**, 47–52.

126. Sorlie,T. et al. (2001) Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc. Natl. Acad. Sci. U.S.A.*, **98**, 10869–74.

127. Curtis,C. et al. (2012) The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. *Nature*, **486**, 346–352.

128. Rahko,E. et al. (2003) A mutant TP53 gene status is associated with a poor prognosis and anthracycline-resistance in breast cancer patients. *Eur. J. Cancer*, **39**, 447–453.

129. Yamashita,H. et al. (2004) Coexistence of HER2 over-expression and p53 mutation to Taxol by increasing G2/M arrest and apoptosis. *Proc. Natl. Acad. Sci. U.S.A.*, **101**, 4330–4340.

130. Wang,Z. et al. (2005) PRIMA-1(MET) synergizes with cisplatin to induce tumor cell apoptosis. *Cancer Cell*, **18**, 2507–2510.

131. Modis,S. et al. (2011) Mutant p53 oncogenic functions are sustained by PI3 kinase through an autoregulatory feedback loop. *Cell Cycle*, **10**, 6290–6307.

132. Bykov,V. et al. (2002) Restoration of the tumor suppressor function to mutant p53 by a low-molecular-weight compound. *Nat. Med.*, **8**, 282–288.

133. Guida,E. et al. (2008) Peptide aptamers targeting mutant p53 induce apoptosis in tumor cells. *Cancer Res.*, **68**, 6550–6558.

134. Foster,B.A. et al. (1999) Pharmacological rescue of mutant p53 conformation and function. *Science*, **266**, 2507–2510.

135. Fornace,A.J. et al. (2012) The clonal and mutational evolution spectrum of breast cancer. *Oncogene*, **31**, 4864–4874.

136. Kalluri,R. et al. (2009) The basics of epithelial-mesenchymal transition. *J. Cell Sci.*, **122**, 1541–1552.

137. Jackson,J.G. et al. (2012) p53-Mediated Senescence Impairs the Apoptotic Response to Chemotherapy and Clinical Outcome in Breast Cancer. *Cancer Cell*, **21**, 793–806.

138. Selivanova,G. et al. (1997) Restoration of the growth suppression function of mutant p53 by a synthetic peptide derived from the p53 C-terminal domain. *Nat. Med.*, **3**, 632–638.

139. Friedler,A. et al. (2002) A peptide that binds and stabilizes p53 core domain: chaperone strategy for rescue of oncogenic mutants. *Proc. Natl. Acad. Sci. U.S.A.*, **99**, 9377–942.

140. Martynova,E. et al. (2012) Gain-of-function p53 mutants have widespread genomic locations partially overlapping with p63. *Oncotarget*, **3**, 132–143.

141. Boeckler,F.M. et al. (2008) Targeted rescue of a destabilized mutant of p53 by an si silo screened drug. *Proc. Natl. Acad. Sci. U.S.A.*, **105**, 10533–10538.

142. Kalluri,R. et al. (2009) The basics of epithelial-mesenchymal transition. *J. Clin. Invest.*, **119**, 1420–1428.

143. Rogers,L. et al. (2010) Gain of oncogenic function of p53 mutant regulates E-cadherin expression uncoupled from cell invasion in colon cancer cells. *J. Cell Sci.*, **123**(Pt 8), 1295–1305.

144. Kogan-Sakin,L. et al. (2011) Mutant p53(R175H) upregulates Twist1 expression and promotes epithelial-mesenchymal transition in immortalized prostate cells. *Cell Death Differ.*, **18**, 271–281.