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REVIEW

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

Dawid Walerych[1], Marco Napoli[1,2], Licio Collavin[1,2] and Giannino Del Sal[1,2,*]

[1] Laboratorio Nazionale CIB (LNCIB), Area Science Park, 34149 Trieste, Italy and [2] Dipartimento di Scienze della Vita, Università degli Studi di Trieste, 34127 Trieste, Italy

*To whom correspondence should be addressed. Email: giannino.delsal@lncib.it Tel +39-040-3756801, Fax +39-040-398990

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 subgroup—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 oncogenes, 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 relocalization, 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 PI3KCA 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,

Abbreviations: COSMIC, Catalog of Somatic Mutations in Cancer; DBD, DNA-binding domain; ER, estrogen receptor; GOF, gain-of-function; HER2, human epidermal growth factor receptor 2; IARC, International Agency for Research on Cancer; NF-κB, nuclear factor-kappaB; PR, progesterone receptor; PTM, posttranslational modification; TNBC, triple-negative breast cancer; TopBP1, topoisomerase IIβ-binding protein.

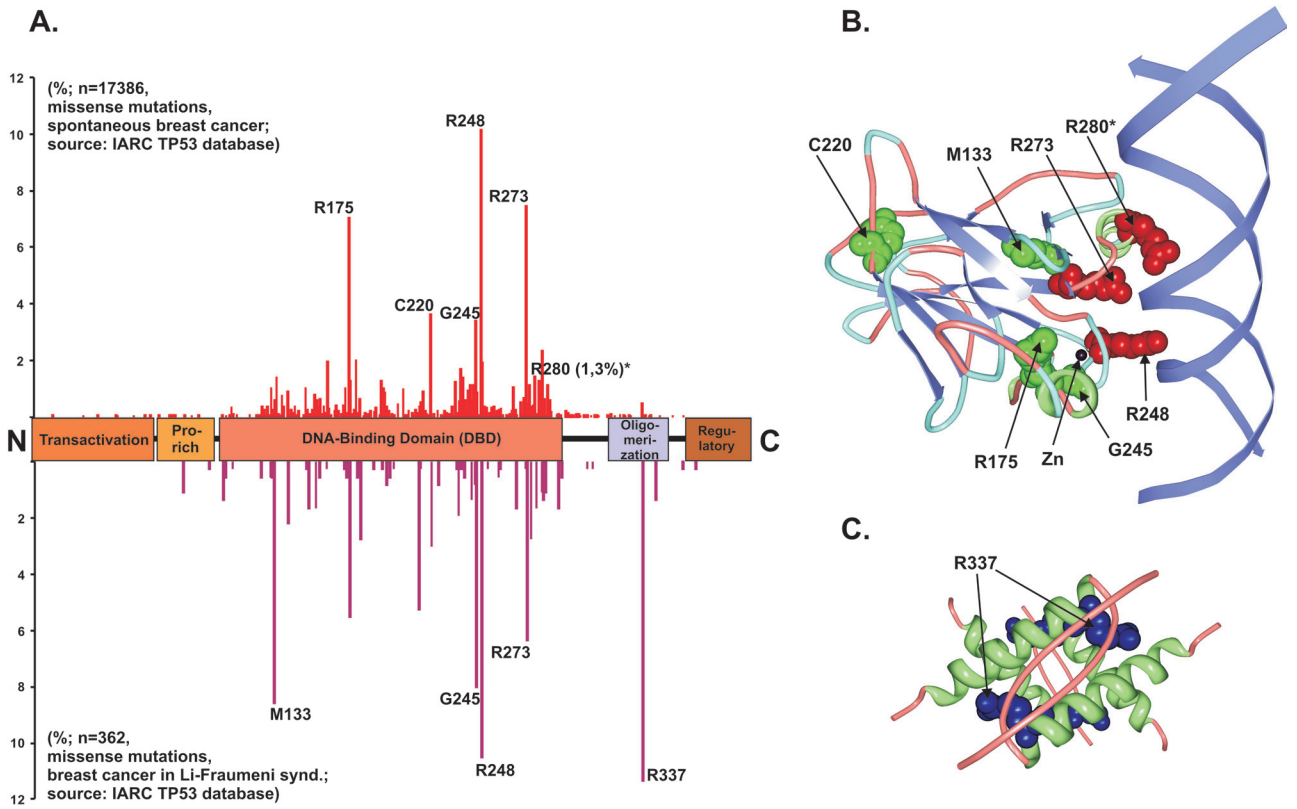

Fig. 1. Frequency and structure of p53 missense alterations in breast cancer. (**A**) Human p53 domain structure with indicated frequency (percent bars) of missense changes in TP53 found in spontaneous (above) or Li–Fraumeni-associated (below) breast cancer. The five most frequently changed codons are indicated by numbers and residue names. The codon 280 marked with (*) is included due to the widespread use of the MDA-MB-231 cell line, bearing R280K p53, as a model for invasive breast cancer (see Table I). (**B**) Structural position of residues affected by most frequent missense-mutation-related changes in the DNA-binding domain (DBD) of human p53. DNA-contacting Arginine side chains are colored red (R280 is marked with * as in A), residues whose change affects DBD folding in p53 'structural mutants' are colored green. The position of the DBD-stabilizing Zinc (Zn) atom is also indicated. Derived from Protein Data Bank (PDB) ID: 1TUP (33). (**C**) Structural position of the residues most frequently altered in Li–Fraumeni breast cancer—Arginine 337 (side chains colored blue)—in the oligomerization domains of human p53 tetramer. Derived from PDB ID: 1C26 (163).

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 p53 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 R248 mutant) (104,121)	Mammary gland-specific expression (R245W) (166)	No increase in mammary carcinomas compared with parental strain (166,167)
					HupKI – exons 4–9 of murine p53 replaced by the human sequence (R248W) (84)	No mammary carcinomas; increased genomic instability and altered tumor spectrum compared with p53 –/– (84)
273	7.5% (2); 8.9% (1)	68.29 (R273H: 67.29; R273C: 64.86) [b]	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)	Germline knockin (R270H) (26)	Low incidence of mammary carcinoma; altered tumor spectrum compared with p53 –/– and +–- (26)
					Mammary gland-specific expression (R270H) (30,51,167)	High frequency of mammary tumors; DN but not GOF of mutant p53 observed (30,51); Higher tumor grade compared with wt p53 in the presence of T antigen (167)
					HupKI – exons 4–9 of murine p53 replaced by the human sequence (R273H) (84)	No mammary carcinomas; Altered tumor spectrum compared with p53–/– (84)
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-κB 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 +/– (26,27);Increased mutant p53 stability and tumorigenesis in mdm2 –/– or p16 –/– background (57)
					Mammary gland-specific expression (R172H) (170)	Low level of spontaneous tumorigenesis, predisposition to the development of mammary tumors when treated with the chemical carcinogen DMBA; increased genomic instability (170)
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
280[a]	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

[a]280 codon is included as in Figure 1; [b] 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. Y220C, for instance, still 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. Transcriptomic 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-κB) 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 penetrance 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 mammary tumor cells: for instance, $p16^{INK4}$ 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/CBP-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 antitumorigenic functions (68,69). P53 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 transcription 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 by a mutation 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

patients), to processes characteristic of advanced stages of cancer (Figure 2). The loss of p53 function as the 'guardian of the genome' has been classically associated with a deregulation of its downstream targets and impairment of DNA repair, cell cycle arrest or apoptosis (80,81). However, the presence of p53 mutant variants introduces additional oncogenic layers to the cell stress response.

First, signaling pathways and mechanisms normally controlling wt p53 in response to genotoxic stress may similarly activate and stabilize mutant p53 (82,83). Second, one of the characteristics of mutant p53 GOF in various models, involving transcription-independent mechanisms, is the contribution to genomic instability (84,85). In breast cancer samples, mutations in TP53 have been associated with increased incidence of chromosomal abnormalities in patients (86,87). In mouse mammary epithelial cells, introduction of R172H p53 induced centrosome amplification and increased the frequency of aberrant mitoses, leading to altered chromosome numbers (88). Third, mutant p53 reduces sensitivity to both spontaneous and DNA-damage-induced apoptosis (88). This effect was present in different breast carcinoma cell lines and was found to depend on mutant p53, as opposed to the loss of p53 (89,90). Several studies have explored the mechanism of these anti-apoptotic effects: mutant p53 variants were found to convert Vitamin D3 from a pro- to an anti-apoptotic factor, shifting the transactivation spectrum of Vitamin D receptor toward a prosurvival profile (91). Positive association in breast cancer has been found between mutant p53 and expression of the anti-apoptotic splice variant of Survivin (92). Mutant p53s have been also found to increase the levels of Bcl-2 (93). Recently, Bcl-2 has been described to be frequently overproduced in triple-negative breast cancers (TNBCs) and, in contrast to other mammary tumor types, to correlate 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). Despite this potential cooperation, ER-alpha was shown to inhibit wt p53-dependent transcription in breast tumor cell lines via direct binding and recruitment of transcriptional repressors (101–103). 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

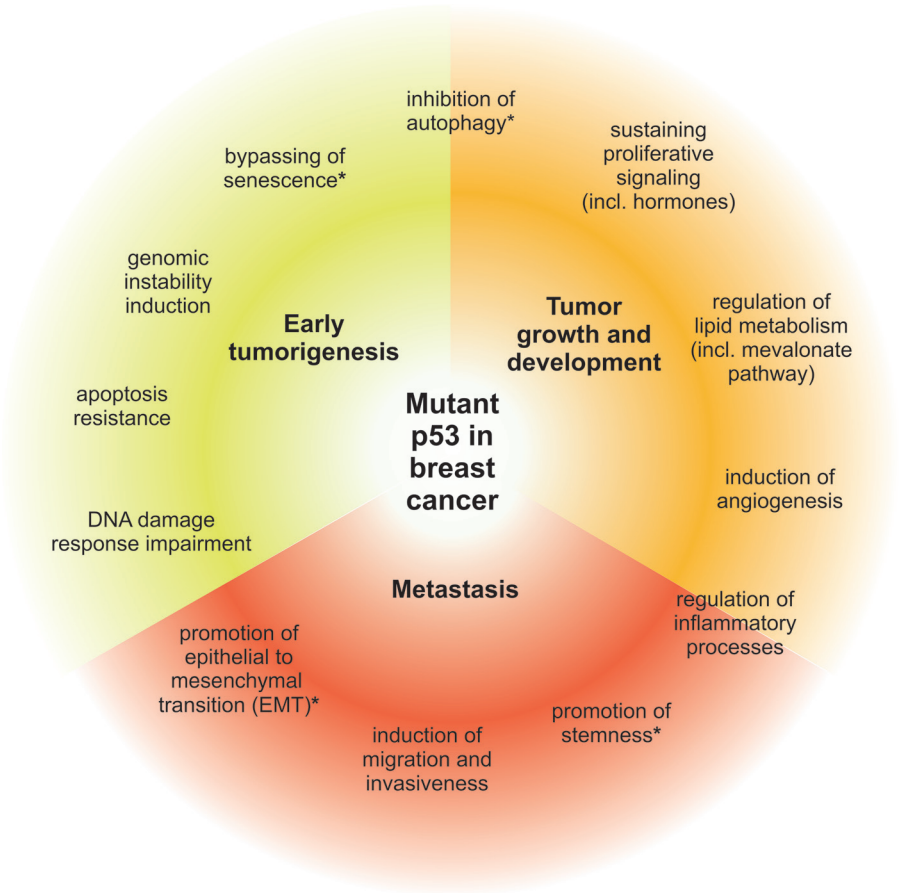

Fig. 2. Mutant p53 involvement in processes associated with breast cancer development. Mutant p53 is known to affect multiple oncogenic processes (164,165). Although different oncogenic mechanisms overlap during tumorigenesis, here they are arbitrarily divided into mechanisms indispensable for early tumorigenesis at the level of single-cell biochemistry (green), mechanisms supporting multicellular tumor mass growth (orange), and features necessary for metastasis to secondary sites (red). The asterisks (*) indicate oncogenic mechanisms known to be important for breast cancer, linked to p53 gain-of-function in other tumors, but not yet directly tested for mutant p53 dependence in mammary carcinoma cells or mouse models. See text for detailed information and references.

(24). Recently, mutant p53s were shown to promote breast cell proliferation in both two- (2D) and three-dimensional (3D) cultures by inducing the expression of genes involved in the mevalonate pathway (104). Indeed, either mutant p53 depletion or treatment with inhibitors of this pathway could reduce the growth and tumorigenic potential of mutant p53-harboring cells (104). The involvement of mutant p53 in tumor-associated inflammation may also be relevant, because cases of inflammatory breast cancer display increased NF-κB signaling and frequency of p53 mutation (105,106). Tissue culture experiments indicate that mutant p53 promotes NF-κB activity in response to tumor necrosis factor (TNF)-alpha (107) and sustains expression of inflammatory cytokines and receptors in breast cancer cell lines (108).

In different experimental systems, mutant p53 was also reported to affect numerous other proliferative signals and metabolic pathways potentially important for development of breast cancer, although no specific experiments with mammary cells were performed (see also Figure 2). Mutant p53 has been demonstrated to promote the expression levels of 15-lypoxygenase (109), an enzyme that was found to be associated with mammary tumor progression and survival of breast cancer cells (110). Mutant p53 was also shown to induce the expression levels of the insulin-like growth factor I receptor gene (IGF1R (111)), which once activated is able to trigger both phosphatidylinositol 3-kinase (PI3K)/AKT and Ras/mitogen-activated protein kinase (MAPK) prosurvival pathways, often deregulated in breast cancer (112). Mutant p53 was also demonstrated to inhibit autophagy (113), a process shown to modulate breast tumor formation and development (114).

In order to sustain faster growth and match the increased requirement of oxygen and nutrients, the tumor mass needs to be vascularized. Mutant p53 was reported to promote vascular endothelial growth factor (VEGF) expression in NIH 3T3 cells (115) and to trigger E2F1-dependent induction of ID4, which in turn contributes to stabilization of the mRNAs of the pro-angiogenic factors IL8 and GRO-α in breast cancer cells (44). In accordance, increased levels of mutant p53 and VEGF have been described to be correlated with poor clinical outcome of breast carcinoma patients (116).

Mutant p53 in metastasis, epithelial to mesenchymal transition and stemness

The majority of deaths in breast cancer patients are associated with metastasis. Mutant p53 has been recently reported to boost the metastatic potential of breast cancer cells. As discussed earlier, this process is in part linked to mutant p53-dependent inhibition of its paralog p63. However, mutant p53 upon phosphorylation-dependent prolyl isomerization by Pin1 can promote tumor cell migration and invasion by directly inducing a transcriptional program sustaining the aggressive potential of breast cancer (48).

Metastasis formation is a complex process intimately connected to cell plasticity and epithelial to mesenchymal transition within the primary tumor (117). In breast cancer cells, mutant p53 is able to both repress epithelial markers such as E-cadherin (118) and induce some crucial transcription factors regulating mesenchymal phenotypes such as Twist (119), ZEB-1 and ZEB-2 (120). Accordingly, MCF-10A-immortalized mammary epithelial cells ectopically expressing mutant p53 undergo epithelial to mesenchymal transition, losing cell polarity and the capability to grow as spheroids in 3D cultures, an effect that can be only partially achieved by the endogenous wt p53 knockdown (121).

Enhanced plasticity due to epithelial to mesenchymal transition can also generate cancer stem-like cells, ultimately responsible of tumor growth and aggressiveness (122). In this regard, wt p53 has recently been shown to act as a crucial safeguard of stem cell self-renewal. Its loss promotes symmetric cell division, favoring expansion of cancer stem cells, breast tumor growth and dissemination (123). Taking into account these observations, it is conceivable to hypothesize that mutant p53 could affect many aspects of cancer stem cell formation and expansion. Supporting this idea, the presence of mutant p53 in primary breast tumors correlates with stem cell transcriptional signatures (124), whereas poorly differentiated and grade 3 breast carcinomas displaying the highest stem cell content (125) are characterized by high frequency of TP53 alterations (17). Recently, mutant p53 was shown to facilitate somatic cell reprogramming, thus increasing the malignant phenotype of such cells (126). This evidence further underlines the multifaceted role of mutant p53 in controlling stem cell phenotypes and tumor aggressiveness.

Diagnostic implications of mutant p53 in breast cancer

Mutant p53 variants in breast cancer prognosis and heterogeneity

Increasing evidence regarding the oncogenic activities of mutant p53 prompted many groups to analyze TP53 mutations as a prognostic or predictive marker (127). In an important study analyzing 1794 breast cancer patients, the stratification of different kinds of TP53 mutations showed that all loss-of-function p53 mutants correlated with poor prognosis compared with wt p53 cases (17). However, mutant p53 GOF and dominant negative indications were also found: among the most frequent missense mutations, codon 248 alterations were linked to reduced survival, whereas mutations at codon 220 and 245 were associated with a better prognosis compared with any other missense variants (Table 1) (17). Interestingly, a difference was found between R248Q and R248W variants, where the latter, though less frequent, correlated with significantly higher patient mortality. This indicates that the two mutant proteins might be functionally different; this observation, together with the mouse model studies (26), underlines that distinguishing the tumorigenic properties of specific p53 hotspot mutants is a potentially critical and still open problem in oncology. 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). P53 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

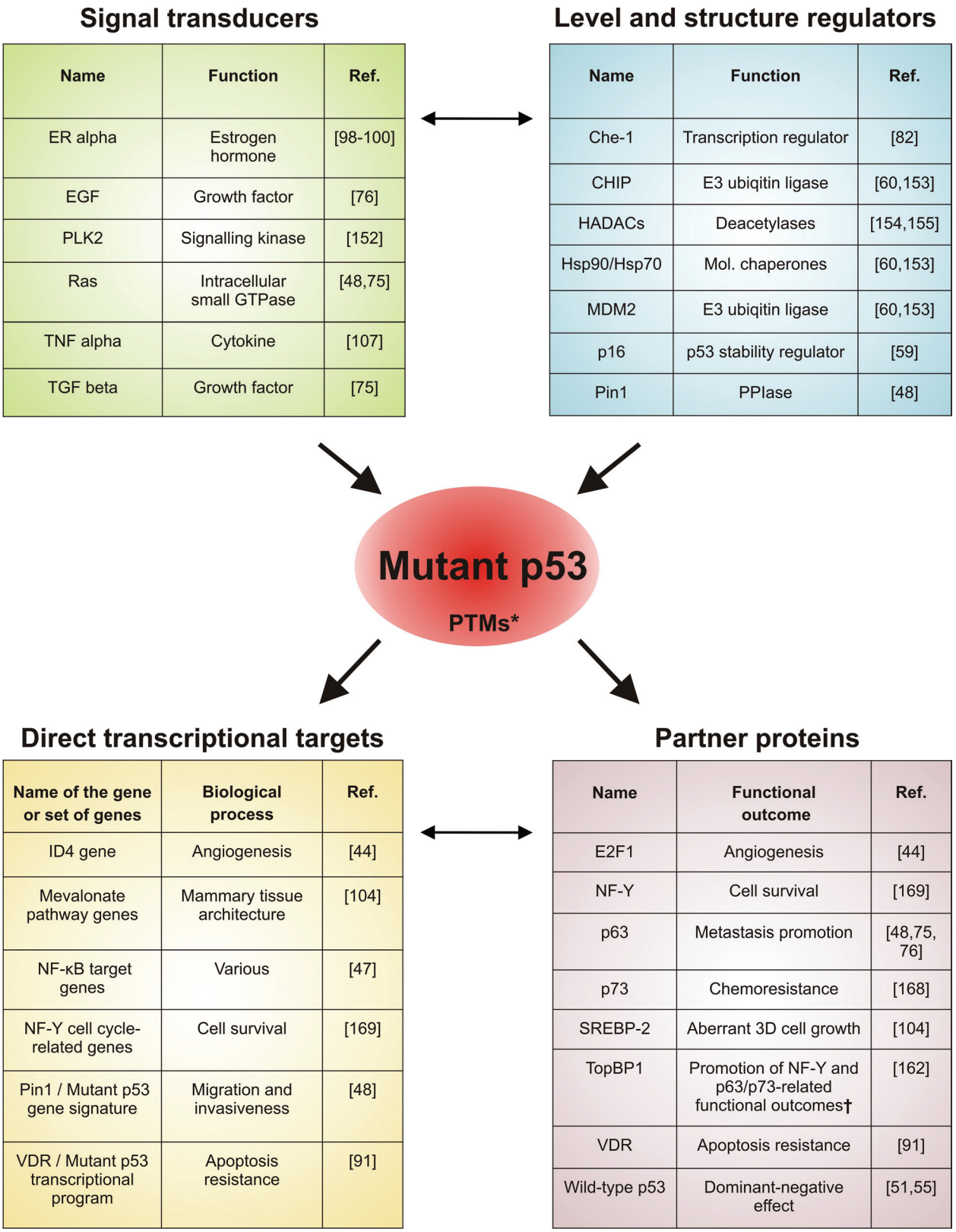

Fig. 3. Mutant p53 as the hub of oncogenic pathways in breast cancer. The activity of mutant p53 is regulated through upstream signal transducers as well as regulators of transcription, stability and structure. Its biological effects are mediated by direct transcriptional activity and through association with downstream protein effectors. These mechanisms are interconnected (small arrows), especially downstream of mutant p53, as most proteins bound directly are transcription regulators. The figure includes only the factors/processes that were found to 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 phoshorylation-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 transferred to clinics, all these above-mentioned compounds have not yet been tested to confirm their 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-1MET/APR-246 (150), which is currently a promising drug candidate and is being tested in a clinical phase I/II trial, though not in breast cancer (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 TGFBR1 (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 $p16^{INK4}$ (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-κB) 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 near 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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