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High incidence of protein-truncating TP53 mutations in BRCA1-related breast cancer

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Abstract

Approximately half of all hereditary breast cancers are compromised in their DNA repair mechanisms due to loss of BRCA1 or BRCA2 function. Previous research has found a strong correlation between BRCA mutation and TP53 mutation. However, TP53 mutation status is often indirectly assessed by immunohistochemical staining of accumulated p53 protein. We sequenced TP53 exons 2 to 9 in 21 BRCA1-related breast cancers and 37 sporadic breast tumors. Strikingly, all BRCA1-related breast tumors contained TP53 mutations, whereas only half of these tumors stained positive for p53 accumulation. Positive p53 staining correlates with the presence of TP53 hotspot mutations in both BRCA1-related and sporadic breast tumors. However, whereas the majority of sporadic breast tumors that stained negative for p53 accumulation had wild-type TP53, the majority of BRCA1-associated breast tumors that stained negative for p53 accumulation had protein-truncating TP53 mutations (nonsense, frameshift, and splice mutations). Therefore, the strong selection for p53 loss in BRCA1-related tumors is achieved by an increase of protein-truncating TP53 mutations rather than hotspot mutations. Hence, immunohistochemical detection of TP53 mutation could lead to misdiagnosis in approximately half of all BRCA1-related tumors. The presence of deleterious TP53 mutations in most, if not all, BRCA1-related breast cancers suggests that p53 loss of function is essential for BRCA1-associated tumorigenesis. BRCA1-related tumors may therefore be treated not only with drugs that target BRCA1 deficiency (e.g., poly(ADP-ribose) polymerase inhibitors) but also with drugs that selectively target p53-deficient cells. This raises interesting possibilities for combination therapies against BRCA1-deficient breast cancers and BRCA1-like tumors with homologous recombination deficiency. (Cancer Res 2009;69(8):OF1–9)
Introduction

Currently, 1 in 8 women in Western countries is estimated to develop breast cancer in her lifetime, which makes it the most common cancer in these women (1). Approximately 5-10% of all breast cancers are hereditary, and 30%-80% of these are related to BRCA1 or BRCA2 loss (2). BRCA1- or BRCA2-deficient cells display genomic instability due to defective double strand break (DSB) repair (3). A defective cell-cycle checkpoint is necessary to facilitate propagation of cells with genomic damage mutation (4). As ‘guardian of the genome’ (5), p53 (also known as Transformation Related Protein or Tumor Protein 53, TP53) is a key factor in the cellular response to DNA damage, and p53 loss may, therefore, be a prerequisite for development of BRCA1 and BRCA2-associated breast tumors (6). Indeed, mouse studies have shown that Brca1-/- and Brca2-/- mice are embryonic lethal due to growth arrest associated with p53 dependent up-regulation of p21\textsuperscript{Waf1} and that concomitant Trp53 knock-out in Brca1-/- and Brca2-/- mice partially rescues the embryonic lethality to a later developmental stage (7-9). Also, development of mouse mammary tumors in conditional Brca1 and Brca2 knock-out mice was greatly accelerated in a (conditional) Trp53 knockout background (10-13). Summarizing, there is ample evidence that homozygous BRCA loss induces cellular lethality by activating a p53 dependent checkpoint in mouse embryos or in mammary epithelial cells and that impairment of this checkpoint by p53 loss alleviates the cell-lethal effects of BRCA loss.

Previous studies found that breast tumors from human BRCA-mutation carriers have an increased frequency of TP53 mutations with distinct properties compared to those found in tumors from non-BRCA1/2 carriers (14-17). However, TP53 mutation is usually scored indirectly by immunohistochemical detection of p53\textsuperscript{IHC+} cells with accumulation of dominant-negative mutant p53 protein (18-20). Cells that stain negative for p53 (p53\textsuperscript{IHC-}) have either wild type p53 or TP53 mutations that do not give rise to accumulation of mutant p53 (21). Consequently, p53-immunohistochemistry only detects a fraction of TP53 mutations (22-25). Indeed, tumors with no p53 expression have been shown to have a higher frequency of protein-truncating TP53 mutations compared to tumors with p53 expression (37% compared to 7.5%) (26). Several studies have analyzed TP53 mutations and p53
immunohistochemistry status in BRCA1 tumors (17,27-29), however tumor numbers were small and different methods were used for scoring for immunohistochemical positivity and for TP53 mutation detection. Moreover, they did not investigate whether p53 immunohistochemistry- and mutation status are differentially distributed in BRCA1-related breast tumors compared to sporadic breast tumors. To investigate the frequency of TP53 mutation in BRCA1-related tumors and sporadic breast cancers and to assess the correlation of TP53 mutation frequency with p53-immunohistochemistry status we sequenced the TP53 exons 2-9 in 21 BRCA1-related breast cancers and 37 sporadic breast tumors and correlated TP53 mutation properties with p53IHC+ or p53IHC- status. Our results show that most, if not all BRCA1-related breast tumors harbor deleterious TP53 mutations suggesting that p53 inactivation is a prerequisite for the development of BRCA1-related breast tumors. About half of all BRCA1 tumors have mutations leading to accumulated p53 protein, which can be detected with immunohistochemistry. The other half of the BRCA1 tumors has a protein-truncating TP53 mutation, which does not result in accumulated p53 protein. Hence, indirect detection of TP53 mutation status by immunohistochemistry could result in misdiagnosis of half of all BRCA1-related breast tumors.

Materials and Methods

Breast tumors
We used DNA isolated from archival material from 22 verified pathogenic BRCA1 germline mutation carriers and 39 patients with sporadic breast cancer without family breast cancer history. Clinical data, tumor characteristics and DNA isolation methods were described previously (30).

p53 immunohistochemistry and sequencing
p53 immunohistochemistry (p53IHC) status was determined by staining formalin-fixed, paraffin-embedded (FFPE) tumor tissues (30) with mouse-anti-human p53 monoclonal antibody (Dako M 7001, clone DO-7 Glostrup Denmark) that recognizes the N-terminus (aa19-21) of p53 (31). If more than 50% of the cells stained positive, the tumor was designated p53IHC+ otherwise, p53IHC-. Direct sequencing for exons 2-9
of the TP53 gene was performed on the tumor DNA of each tumor. Each exon was amplified individually using 25 ng of genomic DNA, followed by re-amplification using at least one nested primer (HotStarTaq Master Mix Kit, QIAGEN). ~5 ng of purified PCR product (QIAquick PCR purification kit, QIAGEN) was directly sequenced using the BigDye Terminator Reaction kit, version 3.1 and an ABI 3730 DNA sequencer (Applied Biosystems, Foster City, CA). Sequence data was analyzed with SEQUENCHERTM software, version 4.5 (Gene Codes Corp., Ann Arbor, MI). For primer sequences, see Supplementary Table 6 online. We were able to obtain sequence data from 21 BRCA1 and 37 sporadic tumors.

**TP53 mutation analysis**

To discriminate between homozygous and heterozygous TP53 mutations present in the tumor DNA, the abundance of the aberrant base was estimated from the

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<table>
<thead>
<tr>
<th>Tumor sample</th>
<th>Deleterious mutations</th>
<th>Neutral missense mutations</th>
<th>Silent mutations</th>
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NOTE: p53 sequence was compared with a consensus sequence as given by the IARC TP53 database. Mutations are classified by their (predicted) effect on p53 function. Deleterious p53 mutations include truncating mutations (red), in-frame deletions (green), and common hotspot p53 mutations according to Walker and colleagues (blue). Other missense mutations are classified by the effect predicted by the SIFT algorithm to be deleterious or neutral (black). Codons H214, P177, H214, and V216 were also identified as hotspots by Walker and colleagues, but these did not occur as frequently as the common hotspots. All mutations are shown in bold print, except when the abundance of the aberrant base within the p53 mutation was estimated <25%. These mutations were not taken along in data set comparisons.

*Known SNP.
**V218I: valine (GTG) > isoleucine (ATA): two homozygous mutations in one codon. p53IHC: percentage of tumor cells that stain positive with immunohistochemistry.
sequence chromatogram from both the forward and reverse sequencing runs. When comparing mutations types found in the tumor groups, we minimized the influence of tumor heterogeneity by only including \textit{TP53} mutations that had an estimated abundance of more than 25% in the tumor DNA (shown in bold print in Table 1 and Table 2). For all \textit{TP53} mutations, we looked up several properties in the IARC \textit{TP53} database Release 12 \textsuperscript{2} (32) (Supplementary Table 2), and used this data for our comparative analyses. The \textit{TP53} mutations were divided into three categories: deleterious mutations, neutral missense mutations and silent mutations.

\textsuperscript{2}http://ww-p53.iarc.fr
Deleterious mutations render a nonfunctional or nontranscribed p53 protein and can be frameshift-, nonsense-, splice-, inframe- and deleterious missense mutations. Frameshift, nonsense, and splice mutations are protein-truncating mutations that lead to premature translational stops and frequently to non-sense mediated mRNA decay (NMD) (33). The effect of in-frame insertions or deletions is not always known, but they can alter p53 function when they occur in crucial domains of the protein. All missense mutations found were classified according to their predicted impact on p53 function as determined by the SIFT algorithm\(^3\) (Sorting Intolerant From Tolerant) (34) in Table 1 and Table 2. Since no matched normal/germline DNA was available, some benign germline variants may have been identified as deleterious somatic mutations by SIFT.

Neutral missense mutations are point mutations that cause an amino acid change that is predicted to have no or low impact on p53 function. Silent \(TP53\) mutations are point mutations that do not lead to an amino acid change, and therefore presumably do not alter p53 functionality. However, p53 missense mutations often render a dominant-negative p53 protein species that attenuate the function of wild type p53 protein encoded by the non-mutated allele, thereby abrogating complete p53 function. These mutants can be contact mutants with disrupted p53-DNA binding, or conformational mutants that disrupt the secondary structure of p53, and thus affect p53 oligomerization. These missense \(TP53\) mutations are predicted to have a deleterious effect on p53 function and some of these are frequently found in multiple tumor types and are hence referred to as \(TP53\) hotspot mutations. Walker \textit{et al.} (35) identified 73 significant hotspot mutations in \(TP53\), 29 of these mutations were most common (\(p<0.001\)) and we refer to these mutations as “common hotspot mutations”, K132, C135, P151, V157, R158, Y163, V173, R175, C176, H179, H193, Y205, Y220, Y234, M237, C238, S241, C242, G245, M246, R248, R249, G266, R273, P278, R280, D281, R282 and E285.

The three groups of deleterious \(TP53\) mutations (protein-truncating mutations, in-frame deletions or insertions, and deleterious missense mutations) are shown in red, green and blue respectively in Tables 1 and 2.

\(^3\)http://sift.jcvi.org/
TP53 mutations in BRCA1-associated and sporadic breast tumors

We analyzed TP53 mutation status in a previously published cohort of 22 tumors from confirmed pathogenic BRCA1 germ-line mutation carriers (30). For BRCA1-mutations, see Supplementary Table 1. Positive p53 immunohistochemical staining (p53IHC+) was observed in 50% (11/22) of the BRCA1-associated breast cancers. All p53IHC- tumors had a staining percentage of ≤ 1%, except one sporadic tumor, which had a staining percentage of 20%. All p53IHC- tumors had a staining percentage of ≥ 50%. To compare TP53 mutations found in p53IHC+ and p53IHC- cases of BRCA1-related and sporadic breast cancers, we selected a group of 39 age-matched, sporadic breast tumors.
tumors with a similar proportion (48.7%, 19/39) of p53IHC+ tumors as the BRCA1-related tumor group. We performed direct sequencing of TP53 exons 2–9 on genomic DNA from 22 BRCA1 tumors and 39 sporadic tumors. We were able to obtain sequence data from 21 BRCA1 tumors (Table 1) and 37 sporadic tumors (Table 2). For 58 tumors, all 8 exons were sequenced twice (forward and reverse) except exon 3 in 24 cases. Using the TP53 mutation data summarized in Table 1 and Table 2, we analyzed whether there were any properties that could distinguish TP53 mutations found in p53IHC+ or p53IHC− BRCA1-related breast tumors and sporadic breast cancers. The comparisons are shown in Table 3.
We found 51 *TP53* mutations in the 21 BRCA1 tumors, and 67 *TP53* mutations in 37 sporadic tumors, excluding SNPs and mutations with an abundance of less than 25%, as estimated from the sequence chromatogram. In many tumors, multiple co-occurring *TP53* mutations were found, and these were categorized according to their predicted effect on p53 function (see Materials and Methods).

When regarding all p53IHC+ and p53IHC- tumors as one group, we found significantly
more TP53 mutations in BRCA1-related tumors (100%; 21/21) compared with sporadic tumors, (73%; 27/37, p=0.009 Fisher’s exact test, Supplementary Table 3). All TP53 mutations were looked up in the IARC TP53 database (32) and those predicted to impair p53 function by the SIFT algorithm (34) were defined as “deleterious”. We found that significantly more BRCA1-related tumors (90.5%; 19/21) had deleterious TP53 mutations compared with sporadic tumors (59.5%; 22/37, p=0.016 Fisher’s exact test) (Figure 1A, Supplementary Table 3). Next, we analyzed whether BRCA1-associated tumors had different types of TP53 mutations compared with sporadic tumors (Figure 1B, Table 3A). Most types of TP53 mutations occurred in very similar proportions in BRCA1 tumors or sporadic tumors; however, we found a significantly larger proportion of protein-truncating frameshift, nonsense and splice mutations in the BRCA1 tumor group (21.6%; 11/51) compared with the sporadic tumor group (3.0%; 2/67, P=0.0061, Table 3A). Hence, within our tumor groups, BRCA1 tumors have a more than 7-fold increase in protein-truncating TP53 mutations.

**Correlation of TP53 mutation types with p53-immunohistochemistry**

To investigate the correlation between p53-immunohistochemistry and distinct TP53 mutations, we compared types of TP53 mutations in p53IHC+ and p53IHC- BRCA1-associated and sporadic breast tumors. All BRCA1 tumors had one or more TP53 mutation(s) and the total number of tumors with one or more TP53 mutation(s) is very similar in the p53IHC+ and p53IHC- BRCA1-associated tumor group (Figure 1C, left, Supplementary Table 3). Both p53IHC+ and p53IHC- sporadic tumor groups included cases with wild type TP53, and the p53IHC+ group had a greater percentage of tumors with only one TP53 mutation (44.4%) compared with the p53IHC- sporadic tumors (5.3%).

Overall, the mean number of deleterious TP53 mutations was higher in the BRCA1-related breast tumors (1.43 mutations per tumor) compared to the sporadic tumors (0.84 mutations per tumor) (Figure 1C, right, Supplementary Table 3). This could be a reflection of the high selection pressure for loss of p53 activity in these homologous recombination deficient (HRD) tumors. This selection pressure seems to be identical for all BRCA1-related tumors, as there was no difference in the amount of deleterious mutations per tumor between the p53IHC+ and the p53IHC- BRCA1-related tumors. In contrast, sporadic p53IHC+ tumors had more deleterious
TP53 mutations (1 mutation per tumor) than p53\textsuperscript{HIC-} tumors (0.68 mutations per tumor) (Supplementary Table 3). Interestingly, the difference in numbers of TP53 mutations found in p53\textsuperscript{HIC-} BRCA1-related and sporadic tumors was due to an increase in protein-truncating nonsense, frameshift and splice mutations (Figure 1D, Figure 2, Supplementary Table 3). Of the p53\textsuperscript{HIC-} BRCA1 tumors, 72.7% (8/11) had protein-truncating mutations compared to 10.5% (2/19) of the p53\textsuperscript{HIC-} sporadic tumors. Furthermore, 60% (6/10) of p53\textsuperscript{HIC-} BRCA1 tumors and 66.7% (12/18) of p53\textsuperscript{HIC-} sporadic tumors had common hotspot mutations compared with 9.1% (1/11) and 5.3% (1/19) of p53\textsuperscript{HIC-} BRCA1 and p53\textsuperscript{HIC-} sporadic tumors respectively (Supplementary Table 3). Indeed, logistic regression analysis (Table 3B) showed that p53\textsuperscript{HIC-} tumors are significantly more likely to have protein-truncating TP53 mutations than p53\textsuperscript{HIC+} tumors, regardless of their BRCA1 status (O.R =27.1, p=0.0067). Similarly, BRCA1-related breast tumors were also significantly more likely to have a protein-truncating TP53 mutation compared to sporadic tumors (OR=24.5, p=0.0012). Therefore, p53\textsuperscript{HIC-} and BRCA1 status are independent tumor characteristics that correlate positively with the likelihood of having a protein-truncating TP53 mutation. Conversely, p53\textsuperscript{HIC+} tumors were significantly more likely to have a common TP53 hotspot mutation compared with p53\textsuperscript{HIC-} tumors (O.R. = 25.2, p=0.0001), independent of BRCA1 status. In sum, BRCA1 tumors have significantly more mutations than sporadic tumors and this difference is found specifically in the p53\textsuperscript{HIC-} tumor groups, where 72.7% (8/11) of the p53\textsuperscript{HIC-} BRCA1 tumors had protein-truncating TP53 mutations compared to only 10.5% (2/19) of the p53\textsuperscript{HIC-} sporadic tumors. The distribution of the TP53 mutations over the TP53 coding region shown in Figure 2, with most mutations mapping to the DNA binding domain.

Properties of BRCA1-related TP53 mutations

It has been reported previously that TP53 mutations in BRCA-related breast tumors have specific properties when compared to TP53 mutations in sporadic breast tumors (14-17). Previous authors have suggested TP53 mutations in BRCA1-associated tumors comprise fewer recurrent hotspot mutations and more non-hotspot missense mutations than sporadic tumors. Moreover, these rarely recurring non-hotspot mutations localized to p53 protein regions not normally mutated in sporadic tumors.
At the base pair level, BRCA-specific TP53 mutations had a prevalence of A:T base pair changes. To verify these observations, we analyzed these properties of the TP53 mutations found in our BRCA1 and sporadic tumor groups. In contrast to earlier findings, we found that all these properties of TP53 mutations occurred with similar frequencies in the BRCA1-related and sporadic breast tumors (Figure 3; for details of the analysis, see Supplementary Results and Supplementary Tables 3 and 4 online). Hence, our data do not support the notion of previous reports on the specificity of TP53 mutations in BRCA-related breast tumors.

**Discussion**

The rapid induction of p53-mediated cell cycle arrest by DNA DSB damage implies a strong requirement for TP53 mutation in BRCA1-related tumors with defective DSB repair. Previous studies have found that 60–77% of BRCA1 tumors stain positive for p53 (17, 27, 29, 36). Studies that also sequenced the TP53 gene found that 30–68% of BRCA1-related tumors have TP53 mutations at the DNA level (17, 27, 29). However, the correlation between p53-immunohistochemistry status and TP53 mutation status in BRCA1-related breast cancers has never been investigated.

In this study, we sequenced TP53 exons 2-9 in 21 BRCA1-related breast tumors and in 37 sporadic breast cancers, and compared the properties of TP53 mutations between these two tumor groups. We find that TP53 mutations occur in all BRCA1-related breast tumors, suggesting a general requirement of p53 loss in these tumors. Since TP53 mutation status is often scored by p53 immunohistochemistry, we also investigated the correlation of TP53 mutations status with p53-immunohistochemistry data in BRCA1-related and sporadic tumors.

Half of the BRCA1-related tumors consisted of tumors that stained positive for mutant p53 protein (p53IHC+), and we compared these tumors with p53IHC- sporadic tumors. The remaining p53IHC- BRCA1-related tumors were compared to p53IHC- sporadic tumors. Independent of BRCA1 status, p53IHC- status correlated significantly with the presence of common hotspot mutations, which are the most common TP53 mutations found in all tumors. Conversely, p53IHC- status correlated significantly with protein-truncating p53 mutations. Strikingly, BRCA1 status also correlated significantly with p53-truncation status. Indeed, the increased frequency
of deleterious p53 mutations in BRCA1-related tumors is caused by an increase in protein-truncating (i.e. nonsense, frameshift and splice) TP53 mutations in p53$^{\text{BRCA1}}$-BRCA1 tumors compared to sporadic tumors (42.9% vs. 5.4%), rather than by an increase in TP53 hotspot mutations. Consequently, scoring for p53 mutations in BRCA1-related breast tumors by immunohistochemistry could lead to misdiagnosis in approximately half of all BRCA1-related tumors.

The selective increase in protein-truncating TP53 mutations in BRCA1-related breast cancers suggests that non-dominant-negative TP53 mutations may be more effectively homozygozed during BRCA1-associated tumorigenesis than during sporadic tumor formation. This could be due to several reasons: i. Genomic instability induced by BRCA1 loss might facilitate mutation of the remaining wild-type TP53 allele in BRCA1-deficient cells with a heterozygous protein-truncating TP53 mutation. (ii) Alternatively, since BRCA1 is also involved in the G2-M and spindle assembly checkpoints (37), LOH at the TP53 locus might occur more efficiently in BRCA1-deficient cells. (iii) Since TP53 and BRCA1 are both located

Figure 3. TP53 mutation properties in BRCA1-related and sporadic breast tumors. Several TP53 mutation properties, which were previously reported to be BRCA-specific (15, 16, 18, 19), occurred with similar frequency in our series of BRCA1-related and sporadic breast tumors. A. Missense mutations: For each tumor group, missense mutations were subdivided in hotspot mutations (orange), non-hotspot mutations predicted to be deleterious by the SIFT algorithm used by the IARC TP53 database (35) (blue), and non-hotspot mutations predicted to be neutral (gray). Hotspot TP53 mutations were those according to Walker et al., 1999 (38). B. Rarity in breast cancer: All mutations were subdivided into mutations previously reported in breast cancer (orange), and those new to breast cancer (blue), as reported in the IARC TP53 database (35). C. Rarity in cancer in general: All mutations were subdivided into mutations previously reported in cancer (orange) and those new to cancer in general (blue) as reported in the IARC TP53 database (35). D. Domain function: Indicated are the fraction of TP53 mutations found in SH3/Pro Rich domain (orange), the DNA Binding domain (blue) or other domains (gray). E. Basepair changes: Indicated are basepair changes within the TP53 coding region, occurring at A:T sites (orange) or at G:C sites (blue) of the coding strand. Other mutations types (gray) include basepair deletions and insertions.
on chromosome 17, simultaneous LOH at TP53 and BRCA1 via missegregation of chromosome 17 might take place in case protein-truncating TP53 mutations occur in cis with the BRCA1 germline mutation.

Previous studies have not shown an increased frequency of protein-truncating TP53 mutations in p53HIC\(^{-}\) BRCA1-related tumors. This could be due to the lower proportion of p53HIC\(^{-}\) tumors or the lower number of protein-truncating TP53 mutations found in these studies. First, some studies did not include p53HIC data (28), and others used different methods to determine p53HIC\(^{-}\) staining: a quick score method (17) or a cutoff of 10% positive staining cells (27, 29). We used a more stringent cutoff of 50% positive staining cells. Second, all previous studies have used pre-screening of TP53 amplicons by single strand conformation polymorphism (SSCP) analysis and/or direct sequencing of only selected TP53 exons. This could result in recovery of lower numbers of TP53 mutations, including protein-truncating mutations. Crook et al (17) sequenced 12 independent plasmid clones for each exon for each tumor and identified only one protein-truncating TP53 mutation in a panel of 70 BRCA1, BRCA2 and sporadic tumors. Armes et al (27) detected mutations in TP53 exons 5–10 by direct sequencing, SSCP or sub-cloning. Mutations that were detected at least twice from different PCR reactions were designated TP53 mutation-positive. Armes et al found only seven TP53 mutations in 40 BRCA1, BRCA2 and sporadic tumors of which one was a TP53 frameshift mutation. Using direct sequencing of TP53 exons 5–9, Foulkes et al (29) found 8 TP53 mutations in 13 BRCA1 tumors, one of which was a frameshift mutation. Phillips et al (28) analyzed TP53 exons 4–10 in 46 breast tumors by SSCP, and sequenced only those fragments that showed aberrant migration patterns. This way they found 20 TP53 mutations, of which 13 were protein-truncating.

The above-mentioned studies find very different proportions of (protein-truncating) TP53 mutations, suggesting that the TP53 mutation detection methods used in these studies give rise to an incomplete TP53 mutation spectrum. Misdetection of TP53 mutations may be minimized by direct sequencing of most – ideally all – protein coding TP53 exons from PCR amplified tumor DNA without pre-screening of TP53 amplicons by SSCP. This strategy may also minimize possible bias for or against protein-truncating TP53 mutations and permit detection of multiple TP53 mutations within each tumor.
TP53 mutation is strongly associated with high grade, hormone receptor negative, basal like breast tumors and with increased global genomic instability (38-42) which is a fitting description of BRCA1-related breast tumors (27, 43). Furthermore, protein-truncating TP53 mutations have been found to have a prognostic value similar to TP53 hotspot mutations (44) and they have recently been linked to poor prognosis in breast cancer (44) and squamous head and neck cancer patients (45).

The strong requirement for TP53 mutation in BRCA1-related breast tumors could be an explanation for their high tumor grade and high proliferation. This high frequency of p53 mutations might not be limited to BRCA1-related breast tumors, but might be characteristic for other types of HRD tumors. The intimate link between BRCA1 mutation and TP53 mutation suggests that BRCA1-related and BRCA1-like tumors might be most effectively treated with combinations of HRD targeting therapeutics (such as DNA damaging drugs or PARP inhibitors (46) and therapeutics that target p53 deficiency, such as Chek1 inhibitors (47). Conversely, HRD may occur more frequently in p53 deficient tumors, therefore, HRD-targeting drugs might be more active against p53-deficient tumors compared with p53 wild type tumors. In line with this, p53-mutated breast tumors showed increased sensitivity to high-dose chemotherapy or dose-dense epirubicin-cyclophosphamide (48-50).

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**References**

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