p53 REVIEW ARTICLE

TP53 and Breast Cancer

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For the p53 Special Issue

The TP53 gene (p53) is found altered in breast carcinomas in approximately 20–40% of all cases depending on tumor size and stage of the disease. It seems to be an early event in breast tumorigenesis. Several polymorphisms in the TP53 gene have been detected and their possible roles in breast cancer risk and association to type of cancer developed are discussed. The different mutation spectra seen in geographical and ethnic populations may be used to identify environmental exposure contributing to breast cancer development. The role of TP53 mutation as a prognostic marker is reviewed as well as its role as a predictor for therapy response. All data available on TP53 mutation analyses of human breast carcinomas, as well data from transgenic animal studies and experimental cell studies, support an important role for TP53 in mammary carcinogenesis. Hum Mutat 21:292–300, 2003. © 2003 Wiley-Liss, Inc.

KEYWORDS: breast cancer; cancer; tumor; p53; TP53; loss of heterozygosity; LOH; Li-Fraumeni syndrome; LFS

DATABASES:
TP53 – OMIM: 191170, 151623 (LFS); GenBank: NM_000546 (mRNA)
http://p53.curie.fr/ (p53 Web Site at Institut Curie)
www.iarc.fr/P53 (IARC p53 Mutation Database)

INTRODUCTION

Breast cancer is the most common malignancy among females worldwide and more than 1,000,000 new cases are diagnosed every year [Ferlay et al., 2001]. The incidence and the mortality rate vary between different ethnically and geographically distinct populations by at least fourfold with the lowest incidence among Asians and the highest among North Americans. Although the incidence has increased over the last 20 years, the prognosis has improved, partly because of early diagnosis and as a result of more active treatment against systemic spread. The use of adjuvant hormone therapy and chemotherapy, as well as radiotherapy, have improved the survival rate, but the success of adjuvant systemic treatment depends on identification of patients at risk for developing disseminated disease. It is therefore important to identify markers that can predict tumor aggressiveness and predict the response to the selected therapy.

Breast cancer is associated with different types of somatic genetic alterations such as mutations in oncogenes and tumor suppressor genes. To date, the most frequent sites of gene mutations are in the TP53 gene (MIM# 191170) with approximately 30% of the tumors having a mutation, often accompanied by loss of the wild-type allele (LOH). Overview of reported mutations is found in various databases [Beroud and Soussi, 1998; Soussi et al., 2000; Olivier et al., 2002; Beroud and Soussi, 2003].

A large number of studies have assessed the prognostic and predictive role of TP53 alterations in breast cancer yielding conflicting results. Two different methodologies have been used to assess TP53 alterations: DNA sequencing and immunohistochemistry (IHC). Most TP53 alterations found in breast carcinomas are point mutations leading to the synthesis of a stable, mal-functional, and non-degradable protein that accumulates in tumor cells, and thus can be detected by IHC. The correlation between TP53 protein accumulation measured by IHC and TP53 mutation detected by sequencing is, however, less than 75% in breast carcinomas [Norberg et al.,

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1998; Geisler et al., 2001). The reason for this is that not all mutations yield a stable protein and some mutations result in protein truncation and are thus not detected by IHC. Non-mutated TP53 protein may also accumulate in some cells as a result of a response to DNA damage or by binding to other cellular proteins, giving a positive IHC result. Studies that have used sequencing to detect mutations all showed a strong association to survival whereas most studies using IHC failed to detect such an association [reviewed by Soussi and Beroud, 2001]. For this reason the present review mainly focuses on studies where mutation analyses have been performed.

AN EARLY EVENT IN BREAST TUMORIGENESIS

That TP53 was involved in breast cancer development was first recognized when germline mutation in this gene was found to be responsible for Li Fraumeni Syndrome (LFS; MIM# 151623) [Malkin et al., 1990]. Individuals with LFS confer an increased risk of several cancers with the most frequent being breast cancer. This implies an important role for TP53 inactivation early in the development of breast cancer, although not a presupposition. In studies of breast carcinomas from LFS patients the other allele is inactivated by LOH in approximately only half of the tumors [Varley et al., 1997] and in sporadic tumors harboring a somatic TP53 mutation LOH is seen in approximately 70%, indicating that a haplo-insufficiency mechanism may exist, or that gain of function mutations or dominant negative mutations occur.

Several studies have sought to identify the stage of breast tumorigenesis at which a somatic TP53 mutation occurs. Careful studies of microdissected tumor material have shown that TP53 mutations can occur in ductal carcinoma in situ (DCIS) before the development of invasive breast cancer, and that the frequency increases from around zero in low-grade DCIS to 30–40% in high-grade DCIS [Ho et al., 2000; Done et al., 2001a,b]. These results point to an important role of TP53 alterations early in the carcinogenic process of the breast.

FREQUENCY OF MUTATIONS IN DIFFERENT PATIENT COHORTS

The frequency of TP53 mutations reported in breast tumors ranges from 15 to 71% (Table 1) [reviewed by Andersen and Borresen, 1995; Pharoah et al., 1999; Hill and Sommer, 2002]. Significant differences are seen among populations, although the different distribution of various stages as well as whether the whole gene was screened or only the conserved region from exons 5–8 may have influenced the frequency found in the various studies. The frequencies in node-negative patients are on the whole considerably lower (15–18%) than in node-positive patients, and large tumors and tumors from patients with advanced disease have a higher frequency of mutations than small tumors. An increased frequency during tumor progression has also been observed. In a study by Norberg et al. [2001] of 30 breast cancer patients with recurring tumors, the prevalence of TP53 mutations was higher in the recurrent tumors than in the primaries.

Most studies investigating all coding exons (2–11) have found that approximately 10% of alterations reside outside the conserved region (exons 5–8). Younger patients seem to have a higher frequency of TP53 mutations in their tumors [Berns et al., 2000], possibly reflecting a higher proportion of BRCA1/2 carriers in the younger cohort. Several recent studies [reviewed by Greenblatt et al., 2001] investigating TP53 mutations in tumors from BRCA1 or BRCA2 germ-line mutation carriers have found that the frequency of somatic alterations in TP53 are more common than in sporadic cancers (OR 2.8, p = 0.003). When the spectrum of the TP53 mutations in the BRCA1/2 carriers was compared to that of sporadic breast cancer reported in the IARC TP53 mutation database [Olivier et al., 2002] it also differed significantly. Mutations at A:T base pairs were more common as well as strand bias suggesting an influence of DNA repair abnormalities. These results suggest that BRCA1/BRCA2 function influences the type and distribution of TP53 mutations seen in breast cancer [see also Gasco et al., 2003].

In a study by Nedelcheva et al. [1998] investigating the influence of the genotype of the different glutathione-S-transferases (GSTs) on the frequency of TP53 mutations in breast tumors, a higher frequency of mutations was found among carriers of a high activity G-allele of the GSTP1 (38% vs. 21%).

In a study by Gudmundsdottir et al. [2001], patients homozygous for the GSTT1 null allele had a higher frequency of TP53 mutations in their tumors than those with an active allele (26.4% vs. 12.4%). They also found a suggestive trend for higher TP53 mutation frequency in tumors from patients with the GG genotype of the GSTP1 gene confirming the results by Nedelcheva et al. [1998]. These results indicate that polymorphisms in genes involved in detoxification of mutagens play a role in the tumorigenesis of the breast by increasing the frequency of mutations in the TP53 gene.

Recently, the frequency of TP53 mutations was shown to differ in breast tumors from individuals carrying different alleles of the codon 72 polymorphism of the TP53 gene itself. Patients homozygous for the Arg allele had a significantly higher frequency of TP53 mutations in their tumors than those homozygous for the Pro allele (28.5% vs. 3.8%) [Langeröd et al., 2002]. No difference in the TP53 mutation frequency was seen between the two different homozygotes in a series of colon cancer patients who
<table>
<thead>
<tr>
<th>Study</th>
<th>Mutation detection method</th>
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<th>Relative hazard</th>
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<td></td>
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<td></td>
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<td>MS(%)</td>
<td>NS(%)</td>
<td>F(%)</td>
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<td>456</td>
<td>24</td>
<td>57</td>
<td>10</td>
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</table>

MS, missense; NS, nonsense; F, frameshift; IF, in frame insertion/deletion; CDGE, constant denaturant gel electrophoresis; SSCP, single-strand conformation polymorphism; ddF, dideoxy fingerprinting; DGGE, denaturing gradient gel electrophoresis; NA, not available; ns, not significant.

*a*Only a small number of samples sequenced.

*b*Includes both NS and F.
have a different spectrum of TP53 mutations in their tumors. These results suggest a selective growth advantage for cells carrying a type of TP53 mutation seen in breast carcinomas when the mutation resided on an Arg allele.

**POLYMORPHISMS IN THE TP53 GENE AND BREAST CANCER RISK**

At least 14 different polymorphisms have been described in the TP53 gene [see the databases described in Olivier et al., 2002; www.iarc.fr/P53] of which five are in exons with the most common the Arg/Pro polymorphism, in codon 72 described above. There are more than 120 studies that have sought an association between TP53 polymorphisms and risk of cancer in general, several focusing on breast cancer, with divergent and inconclusive results. In a review by Dunning et al. [1999] performing meta-analyses, a statistically significant difference in genotype frequencies was found for the Arg72Pro polymorphism with Pro carriers having an OR of 1.27 (95% CI 1.02–1.59). Some studies have found that a haplotype of three polymorphisms (codon 72, intron 3, and intron 6) conveyed a breast cancer risk of almost 2 (95% CI 1.14–3.4) [Weston et al., 1998; Keshava et al., 2002]. Several groups have also reported interesting associations between various polymorphisms and phenotypic features of breast cancer. In the study by Powell et al. [2002], the 16-bp insertion polymorphism in intron 3 was strongly associated with poor histological grade independently of other pathological features. Whether these findings reflect an association to TP53 mutations, as seen in the study by Langerød et al. [2002], remains to be seen.

**TYPE OF MUTATION AS A MUTAGEN TEST?**

Sommer and colleagues have suggested that the TP53 gene can be used as a “mutagen test” also for breast cancer [Hartmann et al., 1997; Hill and Sommer, 2002]. The TP53 mutation spectrum has been used as a signature for exposure in cancers like lung cancer, known to be induced by exogenous mutagens [Toyooka et al., 2003; Vähåkangas, 2003]. Despite intensive studies, the origin of sporadic breast cancer is largely unknown, and studies examining the role of specific putative carcinogen exposure in breast cancer have found either inconsistent or weak associations. A comparison of the TP53 mutation spectrum in breast tumors from 15 geographically and ethnically diverse populations showed a significantly distinct pattern [Hill and Sommer, 2002]. Pairwise analyses of the mutation pattern confirmed an excess of differences among the populations, and interestingly the authors observed that low-risk populations from southern Japan showed an intermediate pattern of mutations, such that there was no significant difference from any of the high-risk populations. The authors hypothesized that the mutation pattern in the low-risk populations predominantly reflects a baseline endogenous pattern, while mutagens present in the high-risk populations might skew that endogenous pattern in different directions. This will generate mutation patterns in these high-risk populations that are more different from each other than from the endogenous mutation pattern. These diverse TP53 mutation patterns seen in breast tumors from patients belonging to different populations are consistent with a significant contribution by a diversity of exogenous mutagens, although they remain to be identified. Data from the IARC database also support the hypothesis that a fraction of breast cancer mutations occur as a consequence of environmental exposure [Olivier and Hainaut, 2001].

**PROGNOSTIC SIGNIFICANCE**

Initial studies trying to elucidate the role of a mutated TP53 gene in breast cancer prognosis were based on detecting TP53 accumulation using IHC. In a meta-analysis of more than 9,000 breast cancer patients, the prognostic and predictive value of the TP53 overexpression appeared weak [Barbareschi, 2002]. However, strong prognostic significance of TP53 mutations using sequencing has been reported in more than 25 studies to date involving over 6,000 patients (see Table 1) [reviewed by Hartmann et al., 1997; Pharoah et al., 1999; Blasyk et al., 2000]. A comprehensive meta-analysis of 16 of these studies including over 3,500 patients was performed by Pharoah et al. [1999]. They found that the RR of dying of breast cancer for unselected patients with a TP53 mutation in their tumor was 2.0 (95% CI 1.7–2.5). For node-negative patients the RR was 1.7 (95% CI 1.2–2.3), and for node-positive patients the RR was 2.6 (95% CI 1.7–3.9). This and later studies have confirmed that mutations in the TP53 gene confer a worse overall and disease-free survival in breast cancer cases, and this effect is independent of other risk factors. In several of the studies the presence of a TP53 mutation was the single most adverse prognostic indicator for both recurrence and death.

Whether the prognostic significance of all types of mutations is the same, is still under debate. Børresen et al. [1995] reported that patients with mutations effecting or disrupting the zinc binding domains L2 and L3 (codons 163–195 and 236–251) have worse prognosis than patients with mutations elsewhere. Berns et al. [1998] found that mutations affecting amino acids directly involved in DNA binding, many of these residing in the zinc binding domain, were related with the poorest prognosis. These findings were confirmed in a study by Alsnr et al. [2000], where patients with missense mutations affecting DNA binding or zinc binding displayed a very
aggressive phenotype with a short survival. Kucera et al. [1999] found that patients with mutations residing in the zinc binding areas only gave a marginally significant difference for overall survival in multivariate analyses, but not in univariate analyses.

Powell et al. [2000] found that mutations causing denaturation of the protein structure were associated with poor survival, but they could not confirm that mutations in the DNA contact region including the L2/L3 domains had a worse survival. However, in the two latter studies only missense mutations residing within these domains were considered and not all mutations that also disrupted these domains like the truncating mutation. Bergh et al. [1995] reported that the prognosis for mutations in the conserved regions II and V was worse than for mutations in the conserved regions II and IV and non-conserved regions. Further work is required to determine the role of the different mutations in breast cancer prognosis. The poor prognosis for patients with specific TP53 mutations could reflect that these types of mutations have a gain-of-function effect or a particularly strong dominant-negative phenotype. Integrated mutation data has to be incorporated in structural analyses, and experimental systems have to be developed to evaluate the biochemical and biological effect of the different mutations associated with poor prognosis.

To evaluate whether the poor prognosis associated with these mutations reflects an aggressive tumor more likely to metastasize, or whether it predicts response to specific types of adjuvant therapy, carefully designed studies have to be performed. Patients with similar clinical phenotype and treated similarly should be evaluated for the response to the specific treatment given before these questions can be answered reliably. Several studies of this kind have been performed but no clear conclusion can so far be drawn (see below).

Recent reports have described the detection of tumor-specific DNA circulating in plasma from breast cancer patients. TP53 mutations were detected in 30 out of 40 breast cancer cases with a mutation in their primary tumor [Shao et al., 2001]. The presence of a TP53 mutation in plasma strongly correlated with various clinicopathological parameters, and was shown to be a significant prognostic marker.

**PREDICTOR OF THERAPY RESPONSE**

Since TP53 is involved in control of the cell cycle, in repair after DNA damage, and in apoptosis, there is a strong biological rationale for investigating whether mutations are predictors of response to DNA-damaging agents. Several studies have assessed this question in relation to different chemotherapy and radiotherapy regimes. One study suggested that locoregional radiotherapy improves survival in cancer cases with TP53 mutations but not for those with wild-type TP53 [Jansson et al., 1995]. However, in another study from the same group they found that adjuvant systemic therapy, especially with tamoxifen, along with radiotherapy was of less value for patients with TP53-mutated tumors [Bergh et al., 1995]. In a study by Aas et al. [1996] on 63 locally advanced breast cancers, treated with doxorubicin in a neoadjuvant setting, there was strong evidence that specific mutations disrupting the zinc binding domains correlate with primary resistance to the drug, and that presence of such mutations was predictive of an early relapse. These findings were further supported in an updated study from the same group including 90 patients [Geisler et al., 2001]. Similar findings have also been seen in a group of 35 locally advanced breast cancers treated with FUMI (5-fluorouracil and mitomycin C) in a neoadjuvant setting (unpublished).

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MOLECULAR ALTERATIONS ASSOCIATED WITH MUTATED TP53

The currently accepted model for the function of wild-type TP53 protein is a multifunctional transcription factor involved in the control of cell cycle progression, DNA maintenance and genome integrity, repair after DNA damage, and apoptosis. Loss of TP53 function eliminates the growth arrest response to DNA damage and may allow replication of damaged template DNA. As a consequence of this, tumors with TP53 mutations would be expected to have a higher frequency of mutations in other genes and an increased frequency of gene amplification and gene deletion. An altered expression of the TP53 transcriptional target genes is also expected.

Recurrent findings in most studies are that TP53 mutated breast tumors simultaneously have amplification of the ERBB2 amplicon pointing to an interdependent role for these two genes, and that the TP53 mutated tumors are more likely to be estrogen receptor negative. Quantitative analysis of chromosomal CGH to detect copy-number abnormalities in 52 selected breast tumors with and without TP53 mutation showed a significantly higher frequency of gain and losses in the TP53-mutated tumors [Jain et al., 2001]. Interestingly the gain and losses were not random but were restricted to certain loci, gain at 8q24 and loss at 5q15-5q21. This correlation may therefore point to a selective advantage for cells having a TP53 mutation and an alteration in any of these loci. In another study of breast carcinomas using array CGH and expression arrays with 9,000 clones, samples with TP53 mutations had a significantly higher frequency of amplification and overexpression of genes residing on 8q24 confirming the previous results [Pollack et al., 2002].

Molecular expression profiling of breast tumors using cDNA arrays has been used to distinguish tumor subclasses with different underlying biology [Perou et al., 2000]. In a study by Sorlie et al. [2001], at least five different subclasses were seen. TP53 analyses of the tumors included in this study showed that the frequency of mutated tumors differed significantly among the subclasses. As the TP53 gene was not included in the genelist used to create the subclasses, the distribution of TP53 mutations among the different tumor groups nevertheless points to a significant role for this gene in determining the gene expression patterns in the various tumor subtypes. In survival analyses the patients belonging to the different tumor subclasses showed significantly different outcomes, with the poorest survival for those groups with the highest frequency of TP53 mutations.

Taking advantage of the microarray data, the effect of TP53 mutations on the genome-wide expression patterns across tumors was analyzed [Sorlie et al., 2002]. In a search for genes whose expression was consistently different between TP53-mutated and TP53 wild-type tumors (using Wilcoxon rank sum test) many genes could be identified. Several of the highly expressed genes in the TP53-mutated tumors were cell-cycle regulated genes like BUB1, CDC25B, and S100A8. Among the genes highly expressed in the tumors with a wild-type TP53 gene were ER, and ER-regulated genes including LIV-1, and genes that normally cluster together with the ER such as GATA-3 and HNF3A. Further examination is needed to determine which of the genes are direct targets of TP53 and which are only associated with a particular expression phenotype.

The recent finding that TP53 is hormonally responsive, and that exposure to pregnancy levels of estrogen and progesterone in mice can induce and sustain chronic nuclear TP53 expression in a way that inhibits carcinogen-induced mammary tumors [Sivaraman et al., 2001] is exciting, and may open up possibilities for interventions.

CONCLUSIONS

All data available on TP53 mutation analyses of human breast carcinomas, as well data from transgenic animal studies and experimental cell studies, support an important role for TP53 in mammary carcinogenesis. Although only a fraction of breast tumors harbor a TP53 mutation, accumulating data over the past years points to an inactivation of the TP53 activity by alterations of either upstream or downstream targets in the TP53 pathway in a large proportion of breast tumors. Several other mechanisms for inactivation of TP53 itself have been described including amplification of one of the many TP53 binding proteins such as MDM2, alterations in genes coding for proteins responsible for the phosphorylation, acetylation and ribosylation of the TP53 protein like ATM and CHK2, and in genes coding for transcription factors of the TP53 gene itself, like HoxA5. These discoveries will provide a foundation when performing high-throughput genome analyses allowing the analyses of all genes in the TP53 pathway simultaneously, and will certainly provide new insight into its role in breast tumorigenesis. Molecular pathological analyses of specific components in the TP53 pathway are likely to give a great impact on the diagnosis, prognostication, and selection of the right treatment for individual breast cancer patients.

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REFERENCES


