HUMAN MUTATION 21:292–300 (2003)

p53 REVIEW ARTICLE

TP53 and Breast Cancer

Anne-Lise Børresen-Dale*

Department of Genetics, Institute for Cancer Research, The University Hospital, The Norwegian Radium Hospital, Montebello, Oslo, Norway

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.**

KEY WORDS: 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.,

*Correspondence to: Anne-Lise Børresen-Dale, Department of Genetics, Institute for Cancer Research, The University Hospital The Norwegian Radium Hospital, Montebello, 0310 Oslo, Norway. E-mail: alb@radium.uio.no

DOI 10.1002/humu.10174
Published online in Wiley InterScience (www.interscience.wiley.com).

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 Børresen, 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, $p = 0.0003$). 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%) [Langerød 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 1. Results of TP53 Mutation Testing and Survival Analyses for Individual Studies (Updated from Pharoah et al., 1999)

Study	Mutation detection method	Case selection	No. of cases	TP53 alterations %	Sequencing results					Relative hazard
					MS(%)	NS(%)	F(%)	IF(%)	Other (%)	R=relapse/ D=death
Andersen et al. [1993]	CDGE exons 5–8	Unselected	163	22	77	6	17	0	0	2.3/2.9
Elledge et al. [1993]	SSCP exons 5–9	Node-negative	200	14[a]	NA	NA	NA	NA	NA	2.2/NA
Riou et al. [1993]	Sequencing exons 2–11	Inflammatory breast cancer	24	38[a]	NA	NA	NA	NA	NA	NA/8.6
Thorlacius et al. [1993, 1995]	CDGE exons 5,7,8	Unselected	106	19	70	5	25	0	0	NA/3.3
Caleffi et al. [1994]	CDGE exons 5–9	Unselected	192	22	85	10	0	0	5	NA/ns
Saitoh et al. [1994]	ddF exons 2–11	Unselected	52	39	NA	NA	NA	NA	NA	NA/NA
Bergh et al. [1995]	Sequencing cDNA	Unselected	312	22	65	10	16	9	0	NA/2.0
		Node-positive	97	30						NA/2.4
		Node-negative	201	18						NA/1.1
Børresen et al. [1995]	CDGE exons 5–8	Unselected	600	20	77	12	9	2	0	NA/NA
Shiao et al. [1995]	SSCP exons 5–8	Unselected	92	20	56	11	11	0	22	NA/NA
		White American	47	19	78	0	11	0	11	NA/5.6
		Black American	45	20	33	22	12	0	33	NA/0.8
de Witte [1996]	SSCP exons 5–8	Unselected	142	34	NA	NA	NA	NA	NA	2.4/3.0
Gretarsdottir [1996]	CDGE exons 5–8	Unselected	186	16	70	6	12	0	12	1.0/1.0
Kovach et al. [1996]	ddF exons 4–10	Unselected	44	30	57	0	14	22	7	4.7/23.2
Seshadri et al. [1996]	SSCP exons 5–6	Unselected	727	8	NA	NA	NA	NA	NA	2.3/2.4
		Node-negative	424							1.9/2.0
		Node-positive	303							2.5/2.7
Soong et al. [1997]	SSCP exons 4–10	Unselected	375	19	67	10	23	0	0	NA/2.5
Valgardsdottir [1997]	CDGE exons 5–8	Unselected	87	17	84	8	0	8	0	NA/6.6
Berns et al. [1998]	SSCP exons 5–8	Metastatic	222	35	81	2	6	0	11	1.6/1.5
Falette et al. [1998]	Sequencing exons 2–11	Node-negative	113	16	100	0	0	0	0	NA/1.8
Iacopetta et al. [1998]	SSCP exons 4–8	Node-negative	422	18	NA	NA	NA	NA	NA	1.6/2.1
Tsuda [1998]	SSCP exons 4–8	Node-positive	150	25	NA	NA	NA	NA	NA	1.9/2.7
Alsner et al. [2000]; Overgård et al. [2000]	DGGE exons 2–11	Unselected	315	23	66	24	10	0	0	2.5/4.0
Blaszyk et al. [2000]	ddF exons 4–10	Unselected	90	36	56	44[b]	0	0	0	1.8/1.9
Berns et al. [2000]	Sequencing exons 2–11	Metastatic	243	37	69	12	13	0.5	0	2.6/2.0
Kandioler-Eckersberger et al. [2000]	Sequencing exons 2–11	Locally advanced	67	19	54	46	0	0	0	NA/NA
Geisler et al. [2001]	CDGE exons 2–11	Locally advanced	91	29	61	15	20	4	0	2.8/3.2
Powell et al. [2001]	SSCP exons 4–8	Unselected	908	19	NA	NA	NA	NA	NA	NA/2.1
Simao et al. [2002]	SSCP exons 5–8	Unselected	120	20	58	8	13	0	21	NA/NA
Conway et al. [2002]	SSCP exons 4–8	Unselected	456	24	57	10	23	3	7	NA/NA

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; Vähäkangas, 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; Blaszyk 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 Alsner 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). Berns and collaborators [Berns et al., 2000] studied whether *TP53* mutations can predict response in patients with advanced disease to either first-line tamoxifen or up-front chemotherapy. A total of 243 patients were included in the study. Patients with *TP53* mutations in codons that directly affected DNA binding or mutations within the zinc binding domain L3 showed the lowest response to tamoxifen. In the group of patients receiving chemotherapy the *TP53* mutation carriers also had a poor response, although not significant. In another smaller study of advanced breast cancer patients treated with either FEC (fluorouracil, epirubicin, cyclophosphamide) (35 patients) or paclitaxel (32 patients) in a neoadjuvant setting, a strong correlation between lack of response and presence of a *TP53* mutation was observed in the FEC-treated group but not in the paclitaxel-treated group [Kandioler-Eckersberger et al., 2000]. The study was too small to evaluate the effect of different mutations, although all the seven mutations in the nonresponders to FEC resided in the zinc binding domains or in areas coding for amino acids involved in DNA binding.

All these studies point to a significant clinical implication for specific *TP53* mutations, and show that *TP53* analysis of the primary tumor is helpful in predicting the response to DNA damaging drugs like doxorubicin, FUMI, FEC, and tamoxifen in patients with metastatic disease. The type of mutation and its biological function should be considered in the analyses of the predictive value of *TP53*, and it is justified that *TP53* mutation analyses should be included in prospective studies where a large number of cases, matched for tumor size and nodal status and therapy, are evaluated for response to different treatment regimes. Only such studies will allow definite clarification of the added value of the *TP53* mutational status in prognostication and in introducing tailored therapy.

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 Sørlie 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 [Sørlie 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.

ACKNOWLEDGMENTS

I thank Hilde Johnsen for critically reviewing all the reported mutations and grouping them into categories.

REFERENCES

Aas T, Borresen AL, Geisler S, Smith-Sorensen B, Johnsen H, Varhaug JE, Akslen LA, Lonning PE. 1996. Specific P53 mutations are associated with de novo resistance to doxorubicin in breast cancer patients. Nat Med 2:811–814.

Alsner J, Yilmaz M, Guldberg P, Hansen LL, Overgaard J. 2000. Heterogeneity in the clinical phenotype of TP53 mutations in breast cancer patients. Clin Cancer Res 6:3923–3931.

Andersen TI, Holm R, Nesland JM, Heimdal KR, Ottestad L, Borresen AL. 1993. Prognostic significance of TP53 alterations in breast carcinoma. Br J Cancer 68:540–548.

Andersen TI, Børresen AL. 1995. Alterations of the TP53 gene as a potential prognostic marker in breast carcinomas. Advantages of using constant denaturant gel electrophoresis in mutation detection. Diagn Mol Pathol 4:203–211.

Barbareschi M. 2002. Prognostic value of the immunohistochemical expression of p53 in breast carcinomas: a review of the literature involving over 9,000 patients. Appl Immunohistochem 4:106–116.

Bergh J, Norberg T, Sjogren S, Lindgren A, Holmberg L. 1995. Complete sequencing of the p53 gene provides prognostic information in breast cancer patients, particularly in relation to adjuvant systemic therapy and radiotherapy. Nat Med 1:1029–1034.

Berns EM, van Staveren IL, Look MP, Smid M, Klijn JG, Foekens JA. 1998. Mutations in residues of TP53 that directly contact DNA predict poor outcome in human primary breast cancer. Br J Cancer 77:1130–1136.

Berns EM, Foekens JA, Vossen R, Look MP, Devilee P, Henzen-Logmans SC, van Staveren IL, van Putten WL, Inganas M, Meijer-van Gelder ME, Cornelisse C, Claassen CJ, Portengen H, Bakker B, Klijn JG. 2000. Complete sequencing of TP53 predicts poor response to systemic therapy of advanced breast cancer. Cancer Res 60:2155–2162.

Beroud C, Soussi T. 1998. p53 gene mutation: software and database. Nucleic Acids Res 26:200–204.

Beroud C, Soussi T. 2003. The UMD-p53 database: new mutations and analysis tools. Hum Mutat 21:176–181.

Blaszyk H, Hartmann A, Cunningham JM, Schaid D, Wold LE, Kovach JS, Sommer SS. 2000. A prospective trial of midwest breast cancer patients: a p53 gene mutation is the most important predictor of adverse outcome. Int J Cancer 89:32–38.

Borresen AL, Andersen TI, Eyfjord JE, Cornelis RS, Thorlacius S, Borg A, Johansson U, Theillet C, Scherneck S, Hartman S. 1995. TP53 mutations and breast cancer prognosis: particularly poor survival rates for cases with mutations in the zinc-binding domains. Genes Chromosomes Cancer 14:71–75.

Caleffi M, Teague MW, Jensen RA, Vnencak-Jones CL, Dupont WD, Parl FF. 1994. p53 gene mutations and steroid receptor status in breast cancer. Clinicopathologic correlations and prognostic assessment. Cancer 73:2147–2156.

Conway K, Edmiston SN, Cui L, Drouin SS, Pang J, He M, Tse CK, Geradts J, Dressler L, Liu ET, Millikan R, Newman B. 2002. Prevalence and spectrum of p53 mutations associated with smoking in breast cancer. Cancer Res 62:1987–1995.

de Witte HH, Foekens JA, Lennerstrand J, Smid M, Look MP, Klijn JG, Benraad TJ, Berns EM. 1996. Prognostic significance of TP53 accumulation in human primary breast cancer: comparison between a rapid quantitative immunoassay and SSCP analysis. Int J Cancer 69:125–130.

Done SJ, Eskandarian S, Bull S, Redston M, Andrulis IL. 2001a. p53 missense mutations in microdissected high-grade ductal carcinoma in situ of the breast. J Natl Cancer Inst. 93:700–704.

Done SJ, Arneson CR, Ozcelik H, Redston M, Andrulis IL. 2001b. P53 protein accumulation in non-invasive lesions surrounding p53 mutation positive invasive breast cancers. Breast Cancer Res Treat 65:111–118.

Dunning AM, Healey CS, Pharoah PD, Teare MD, Ponder BA, Easton DF. 1999. A systematic review of genetic polymorphisms and breast cancer risk. Cancer Epidemiol Biomarkers Prev 8:843–854.

Elledge RM, Fuqua SA, Clark GM, Pujol P, Allred DC, William L. 1993. McGuire memorial symposium. The role and prognostic significance of p53 gene alterations in breast cancer. Breast Cancer Res Treat 27:95–102.

Falette N, Paperin MP, Treilleux I, Gratadour AC, Peloux N, Mignotte H, Tooke N, Lofman E, Inganas M, Bremond A, Ozturk M, Puisieux A. 1998. Prognostic value of P53 gene mutations in a large series of node-negative breast cancer patients. Cancer Res 58:1451–1455.

Ferlay J, Bray F, Pisane P Parkin DM. 2001. Cancer incidence, mortality and prevalence worldwide. Globocan 2000 (CD-ROM). IARC Press.

Gasco M, Yulug IG, Crook T. 2003. TP53 mutations in familial breast cancer: functional aspects. Hum Mutat 21:301–306.

Geisler S, Lonning PE, Aas T, Johnsen H, Fluge O, Haugen DF, Lillehaug JR, Akslen LA, Borresen-Dale AL. 2001. Influence of TP53 gene alterations and c-erbB-2 expression on the response to treatment with doxorubicin in locally advanced breast cancer. Cancer Res 61:2505–2512.

Greenblatt MS, Chappuis PO, Bond JP, Hamel N, Foulkes WD. 2001. TP53 mutations in breast cancer associated with BRCA1 or BRCA2 germ-line mutations: distinctive spectrum and structural distribution. Cancer Res 61:4092–4097.

Gretarsdottir S, Tryggvadottir L, Jonasson JG, Sigurdsson H, Olafsdottir K, Agnarsson BA, Ogmundsdottir H, Eyfjord JE. 1996. TP53 mutation analyses on breast carcinomas: a study of paraffin-embedded archival material. Br J Cancer 74:555–561.

Gudmundsdottir K, Tryggvadottir L, Eyfjord JE. 2001. GSTM1, GSTT1, and GSTP1 genotypes in relation to breast cancer risk and frequency of mutations in the p53 gene. Cancer Epidemiol Biomarkers Prev 10:1169–1173.

Hartmann A, Blaszyk H, Kovach JS, Sommer SS. 1997. The molecular epidemiology of p53 gene mutations in human breast cancer. Trends Genet 13:27–33.

Hill KA, Sommer SS. 2002. p53 as a mutagen test in breast cancer. Environ Mol Mutagen 39:216–227.

Ho GH, Calvano JE, Bisogna M, Borgen PI, Rosen PP, Tan LK, Van Zee KJ. 2000. In microdissected ductal carcinoma in situ, HER-2/neu amplification, but not p53 mutation, is associated with high nuclear grade and comedo histology. Cancer 89:2153–2160.

Iacopetta B, Grieu F, Powell B, Soong R, McCaul K, Seshadri R. 1998. Analysis of p53 gene mutation by polymerase chain reaction-single strand conformation polymorphism provides

independent prognostic information in node-negative breast cancer. Clin Cancer Res 4:1597–1602.

Jain AN, Chin K, Borresen-Dale AL, Erikstein BK, Eynstein LP, Kaaresen R, Gray JW. 2001. Quantitative analysis of chromosomal CGH in human breast tumors associates copy number abnormalities with p53 status and patient survival. Proc Natl Acad Sci 98:7952–7957.

Jansson T, Inganas M, Sjogren S, Norberg T, Lindgren A, Holmberg L, Bergh J. 1995. p53 status predicts survival in breast cancer patients treated with or without postoperative radiotherapy: a novel hypothesis based on clinical findings. J Clin Oncol 13:2745–2751.

Kandioler-Eckersberger D, Ludwig C, Rudas M, Kappel S, Janschek E, Wenzel C, Schlagbauer-Wadl H, Mittlbock M, Gnant M, Steger G, Jakesz R. 2000. TP53 mutation and p53 overexpression for prediction of response to neoadjuvant treatment in breast cancer patients. Clin Cancer Res 6: 50–56.

Keshava C, Frye BL, Wolff MS, McCanlies EC, Weston A. 2002. Waf-1 (p21) and p53 polymorphisms in breast cancer. Cancer Epidemiol Biomarkers Prev 11:127–130.

Kovach JS, Hartmann A, Blaszyk H, Cunningham J, Schaid D, Sommer SS. 1996. Mutation detection by highly sensitive methods indicates that p53 gene mutations in breast cancer can have important prognostic value. Proc Natl Acad Sci 93:1093–1096.

Kucera E, Speiser P, Gnant M, Szabo L, Samonigg H, Hausmaninger H, Mittlbock M, Fridrik M, Seifert M, Kubista E, Reiner A, Zeillinger R, Jakesz R. 1999. Prognostic significance of mutations in the p53 gene, particularly in the zinc-binding domains, in lymph node- and steroid receptor positive breast cancer patients. Austrian breast cancer study group. Eur J Cancer 35:398–405.

Langerød A, Bukholm IRK, Bregård A, Lønning PE, Andersen TI, Rognum TO, Meling GI, Lothe RA, Børresen-Dale A-L. 2002. The TP53 codon 72 polymorphism may affect the function of TP53 mutations in breast carcinomas but not in colorectal carcinomas. Cancer Epid Biomarker and Prev 11:1684–1688.

Malkin D, Li FP, Strong LC, Fraumeni JF Jr, Nelson CE, Kim DH, Kassel J, Gryka MA, Bischoff FZ, Tainsky MA. 1990. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science 3 250:1233–1238.

Nedelcheva KV, Andersen TI, Erikstein B, Geitvik G, Skovlund E, Nesland JM, Borresen-Dale AL. 1998. Single tube multiplex polymerase chain reaction genotype analysis of GSTM1, GSTT1 and GSTP1: relation of genotypes to TP53 tumor status and clinicopathological variables in breast cancer patients. Pharmacogenetics 8:441–447.

Norberg T, Lennerstrand J, Inganas M, Bergh J. 1998. Comparison between p53 protein measurements using the luminometric immunoassay and immunohistochemistry with detection of p53 gene mutations using cDNA sequencing in human breast tumors. Int J Cancer 79:376–383.

Norberg T, Klaar S, Karf G, Nordgren H, Holmberg L, Bergh J. 2001. Increased p53 mutation frequency during tumor progression–results from a breast cancer cohort. Cancer Res 61:8317–8321.

Olivier M, Hainaut P. 2001. TP53 mutation patterns in breast cancers: searching for clues of environmental carcinogenesis. Semin Cancer Biol 11:353–360.

Olivier M, Eeles R, Hollstein M, Khan MA, Harris CC, Hainaut P. 2002. The IARC TP53 database: new online mutation analysis and recommendations to users. Hum Mutat 19:607–614.

Overgaard J, Yilmaz M, Guldberg P, Hansen LL, Alsner J. 2000. TP53 mutation is an independent prognostic marker for poor outcome in both node-negative and node-positive breast cancer. Acta Oncol 39:327–333.

Perou CM, Sorlie T, Eisen MB, van de RM, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lonning PE, Borresen-Dale AL, Brown PO, Botstein D. 2000. Molecular portraits of human breast tumours. Nature 406:747–752.

Pharoah PD, Day NE, Caldas C. 1999. Somatic mutations in the p53 gene and prognosis in breast cancer: a meta-analysis. Br J Cancer 80:1968–1973.

Pollack JR, Sørlie T, Perou CM, Res C, Eisen M, Jeffrey S, Johnsen H, Lønning PE, Botstein D, Børresen-Dale AL, Brown PA. 2002. Microarray analysis reveals a major direct role of DNA copy number alteration in the transcriptional program of human breast tumors. PNAS 99:12963–12968.

Powell B, Soong R, Iacopetta B, Seshadri R, Smith DR. 2000. Prognostic significance of mutations to different structural and functional regions of the p53 gene in breast cancer. Clin Cancer Res 6:443–451.

Powell BL, Bydder S, Grieu F, Gnanasampanthan G, Elsaleh H, Seshadri R, Berns EM, Iacopetta B. 2001. Prognostic value of TP53 gene mutation in adjuvant treated breast cancer patients. Breast Cancer Res Treat 69:65–68.

Powell BL, van Staveren IL, Roosken P, Grieu F, Berns EM, Iacopetta B. 2002. Associations between common polymorphisms in TP53 and p21WAF1/Cip1 and phenotypic features of breast cancer. Carcinogenesis 23:311–315.

Riou G, Le MG, Travagli JP, Levine AJ, Moll UM. 1993. Poor prognosis of p53 gene mutation and nuclear overexpression of p53 protein in inflammatory breast carcinoma. J Natl Cancer Inst 85:1765–1767.

Saitoh S, Cunningham J, De Vries EM, McGovern RM, Schroeder JJ, Hartmann A, Blaszyk H, Wold LE, Schaid D, Sommer SS. 1994. p53 gene mutations in breast cancers in midwestern US women: null as well as missense-type mutations are associated with poor prognosis. Oncogene 9:2869–2875.

Seshadri R, Leong AS, McCaul K, Firgaira FA, Setlur V, Horsfall DJ. 1996. Relationship between p53 gene abnormalities and other tumour characteristics in breast-cancer prognosis. Int J Cancer 69:135–141.

Shao ZM, Wu J, Shen ZZ, Nguyen M. 2001. p53 mutation in plasma DNA and its prognostic value in breast cancer patients. Clin Cancer Res 7:2222–2227.

Shiao YH, Chen VW, Scheer WD, Wu XC, Correa P. 1995. Racial disparity in the association of p53 gene alterations with breast cancer survival. Cancer Res 55:1485–1490.

Simao TA, Ribeiro FS, Amorim LMF, Albano RM, Andrea-Serpa MJ, Cardoso LEB, Mendonca GAS, deMoura-Gallo CV. 2002. TP53 mutations in breast cancer tumors of

patients from Rio De Janeiro, Brazil: association with risk factors and tumor characteristics. Int J Cancer 101:69–73.

Sivaraman L, Conneely OM, Medina D, O'Malley BW. 2001. p53 is a potential mediator of pregnancy and hormone-induced resistance to mammary carcinogenesis. PNAS 98:12379–12384.

Soong R, Iacopetta BJ, Harvey JM, Sterrett GF, Dawkins HJ, Hahnel R, Robbins PD. 1997. Detection of p53 gene mutation by rapid PCR-SSCP and its association with poor survival in breast cancer. Int J Cancer 74:642–647.

Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de RM, Jeffrey SS, Thorsen T, Quist H, Matese JC, Brown PO, Botstein D, Eystein LP, Borresen-Dale AL. 2001. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. PNAS 98:10869–10874.

Sørlie T, Børresen-Dale A-L, Botstein D. 2003. Expression profiling of breast cancer: from molecular portraits to clinical utility. Brennan C, Dugan DJ, Editors. In: Oncogenomics: molecular approaches to cancer. Wiley & Sons. In press.

Soussi T, Dehouche K, Beroud C. 2000. p53 website and analysis of p53 gene mutations in human cancer: forging a link between epidemiology and carcinogenesis. Hum Mutat 15:105–113.

Soussi T, Beroud C. 2001. Assessing TP53 status in human tumours to evaluate clinical outcome. Nat Rev Cancer 1:233–240.

Thorlacius S, Borresen AL, Eyfjord JE. 1993. Somatic p53 mutations in human breast carcinomas in an Icelandic population: a prognostic factor. Cancer Res 53:1637–1641.

Thorlacius S, Thorgilsson B, Bjornsson J, Tryggvadottir L, Borresen AL, Ogmundsdottir HM, Eyfjord JE. 1995. TP53 mutations and abnormal p53 protein staining in breast carcinomas related to prognosis. Eur J Cancer 31A:1856–1861.

Toyooka S, Tsuda T, Gazdar AF. 2003. The *TP53* gene, tobacco exposure and lung cancer. Hum Mutat 21:229–239.

Tsuda H, Sakamaki C, Tsugane S, Fukutomi T, Hirohashi S. 1998. A prospective study of the significance of gene and chromosome alterations as prognostic indicators of breast cancer patients with lymph node metastases. Breast Cancer Res Treat 48:21–32.

Vähäkangas K. 2003. TP53 mutations in workers exposed to occupational carcinogens. Hum Mutat 21:240–251.

Valgardsdottir R, Tryggvadottir L, Steinarsdottir M, Olafsdottir K, Jonasdottir S, Jonasson JG, Ogmundsdottir HM, Eyfjord JE. 1997. Genomic instability and poor prognosis associated with abnormal TP53 in breast carcinomas. Molecular and immunohistochemical analysis 105:121–130.

Varley JM, Thorncroft M, McGown G, Appleby J, Kelsey AM, Tricker KJ, Evans DG, Birch JM. 1997. A detailed study of loss of heterozygosity on chromosome 17 in tumours from Li-Fraumeni patients carrying a mutation to the TP53 gene. Oncogene 14:865–871.

Weston A, Wolff MS, Morabia A. 1998. True extended haplotypes of p53: indicators of breast cancer risk. Cancer Genet Cytogenet 102:153–154.