TP53 mutations in primary breast carcinomas from white and African-Brazilian patients

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Abstract. We have attempted to determine the incidence, nature and clinical significance of *TP53* mutation in a group of white (242 cases) and African-Brazilian (52 cases) patients with breast cancer. The interethnic admixture as estimated by STR markers showed that white subjects displayed 67.9±0.4%, 25.0±1.7% and 7.0%±1.6% and the black populations had 34.4±1.9%, 56.2±1.9 and 9.4±2.2% respectively of European, African and Amerindian genes. Clinical parameters such as age, lymph node status and steroid receptors were similar in both groups. African-Brazilian patients presented more advanced lesions. Mutation screening was performed using polymerase chain reaction-single strand conformation analysis followed by sequencing. Compared to whites (13.6%), a relatively high frequency of *TP53* mutation was found in blacks (32.7%) (p=0.001). African-Brazilian women have a larger proportion of mutations in exons 5 and 7, whereas white women have more mutations in exon 8. Mutations within exon 4 were found only in tumors of white patients. The spectra of *TP53* mutations show that A:T→G:C nucleotide transversion and G:C→C:G transition were more common in African-Brazilian women whereas G:C→T:A transversion occurs very frequently in whites. A high prevalence of G:C→A:T nucleotide transitions and deletions was detected in both groups. No association was found between p53 gene mutation and tumor or clinical parameters independently of the ethnic group. With a median follow-up of 35.6 months for whites and 43.4 months for the blacks, no differences in overall survival were found. If white patients were stratified according to the type and location of *TP53* mutations, patients with mutations affecting amino acids directly involved in DNA or Zn binding displayed a poor prognosis. The pattern of mutations found in our population seems to reflect a base line pattern observed in populations with similar ethnic profile with some modifications, which might be derived from specific etiological factors.

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Introduction

The tumor suppressor gene *TP53* is a transcription factor that is implicated in the regulation of several biological pathways, such as cell growth, gene transcription, apoptosis, senescence and genomic stability (1). The biological activities of p53, especially in the regulation of DNA repair and apoptosis make it a potential prognostic and predictive marker. In addition, it is a useful end point marker in human cancer epidemiology. The patterns of *TP53* mutations might reflect the effects of chronic carcinogen exposure in populations with different geographical and ethnic backgrounds (2,3).

TP53 mutations have been associated with the tumorigenic process in the majority of human cancers (4). Of the *TP53* mutations 80-90% involve exons 5 to 8 spanning the evolutionary conserved region of the protein (domains II to V). However, the nature, type and site of these mutations vary among different tumor types and depend on different carcinogen exposure (5).

Breast cancer is the most frequent fatal cancer in women, presenting a variable and unpredictable course of disease. Several groups have investigated the occurrence of *TP53* mutations in breast cancer. The frequency of these *TP53* mutations varies considerably ranging from 12 to 60% (6). An increasing number of studies have attempted to analyse the value of p53 mutational status in prognostic evaluation (7-10).

Patterns of somatic *TP53* mutations in sporadic breast cancer seem to vary according to race and geographical location. Differences in *TP53* mutational spectra between women from different American or European regions as well as between breast tumors from patients from Northern and Southern Japan have been documented (reviewed in ref. 11). The spectrum of *TP53* mutations in breast cancer appears to vary between black and white women in USA and may contribute to the described racial disparity in breast cancer survival (12-14).

The Brazilian population is highly heterogeneous as a consequence of European colonizers, African slaves and autochthonous Amerindians (15,16). The Portuguese colonists settled in the country since the beginning of the 16th century. Between the 16th and 19th centuries about 2.5 to 4.0 million of African blacks were brought as slaves (17) and between 1819-1947, Brazil received almost 5 million immigrants who settled mainly in to South and Southeast (18). Therefore, the southeast population is formed mainly by descendents of European immigrants (Portuguese, Spanish, Italian and German). Recent studies of allele distribution indicated that the degree of intermixing of the South and Southeast white population is low (19). On the other hand, the intermixing between whites and blacks in the North-eastern states is higher (20).

In Brazil, breast cancer is the main cause of morbidity and mortality among women (www.inca.org.br). The incidence of breast cancer in São Paulo, the largest Brazilian city located in the Southeast region, is similar to that reported for other western populations.

The aim of the present study was to determine the incidence and pattern of *TP53* genetic alterations in primary breast carcinomas from a group of white (mostly of Western and Southern European ancestry) and African-Brazilian patients. We further investigated whether there was a relationship between *TP53* genetic alterations and clinicopathological characteristics and survival of the patients.

Materials and methods

Patients. Two hundred and ninety-four samples of primary breast carcinomas were obtained from patients with primary breast cancer (242 whites and 52 African-Brazilian). All white patients were diagnosed at the A.C. Camargo Hospital, in São Paulo (a Southern Brazilian city in the state of São Paulo). The majority of African-Brazilian patients came from Hospital Aristides Maltez, Salvador (a Northeastern city in the state of Bahia). We obtained surgical pathology report, demographic information and follow-up for each patient from the respective hospital records. The ethnic/race sub group classification was based on morphological criteria and family history. 'Mixed type' patients have been left out of the study. The largest diameter of the tumors was recorded. The number of lymph node metastases was determined by microscopic examination of an average of 24 lymph nodes per patient. All cases were submitted to histopathological review of tumor slides in order to confirm diagnosis. All tumors were classified according to the WHO Histological Typing of Breast Tumors. Clinical stage of the patients was determined according to the UICC TNM staging system. Immunohistochemical expression of estrogen and progesterone receptors was also evaluated with the following antibodies: ID5 (Dako A/S, Glostrup, Denmark) and 1.A6 (Novocastra, Newcastle, UK), respectively. A case was considered positive if >10% of the tumor cells were stained.

DNA extraction. Tumor samples were dissected to remove residual normal tissue before freezing and storage in liquid nitrogen. Tissue was ground to a powder using a Frozen Tissue Pulverizer (Termovac). The powder was ressuspended in 1 ml of lysis buffer (10 mM Tris-HCl, pH 7.6, 1 mM EDTA and 0.6% SDS) and 100 μg/ml proteinase K, and incubated at 37˚C overnight. High molecular weight DNA was extracted with phenol-chloroform and precipitated with ethanol.

Polymerase chain reaction-single stranded conformation polymorphism (PCR-SSCP) analysis. Six sets of oligonucleotide primers were used to amplify exons 4 to 9 of the *TP53* gene. The primers used were the same described by Murakami *et al* (22). PCR reactions were performed in 25 μl volumes using 50-100 ng of genomic DNA template, 1 μM of each primer, 1.5 mM $MgCl_2$, 200 μM of each deoxynucleotide triphosphate, 0.1 μCi of [α-^{32}P]-dCTP (Amersham, specific activity, 3,000 Ci/mmol), 50 mM KCl, 10 mM Tris-HCl pH 8.0, and 0.5 unit of *Taq* DNA polymerase (Pharmacia, NJ, USA). Samples were overlaid with mineral oil and amplified for 35 cycles of denaturation, annealing and extension optimized for each primer set. The reactions were performed with an automated Thermal Cycler - Perkin Elmer 480. Amplification products (1 μl) were diluted 10-fold in a buffer containing 95% formamide, 20 mM EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol, heated at 83˚C for 5 min and applied (3 μl/lane) on two 6% polyacrylamide non-denaturing gels, one containing 5% and the other 10% glycerol. Electrophoresis was performed at 6 W for 14-16 h at room temperature with two cooling fans. Band shift mobility was detected by autoradiography of dried gels using Kodak X-Omat XAR film with an intensifying screen for 12 to 48 h at -70˚C.

Direct DNA sequencing. Exons with suspected *TP53* mutations as judged by SSCP gels were amplified. The PCR products obtained were purified using Wizard PCR Preps kit (Promega Corporation, Madison, USA) according to the manufacturer's procedure. Three to 5 μl out of the purified DNA was subjected to a dideoxy chain termination reaction using a double stranded DNA cycle sequencing kit (Life Biotechnology) for both sense and antisense primers. Sequencing reaction products were denatured and resolved on 6% denaturing urea/polyacrylamide gels. Gels were fixed for 15 min in a 10% methanol/10% acetic acid solution, dried and exposed to X-ray film overnight. In some cases the DNA sequences were determined by BigDye Dye Terminator Cycle Sequencing kit and analyzed on an semi-automated sequencer ABI377 (Perkin-Elmer, Cetus).

Calculation of ethnic admixture. Ethnic admixture was calculated on the basis of the allele frequencies of 5 short tandem repeats (STR) (D1S80, APOB, D4S43, F13A1, located in intron 1 of the A chain of coagulation factor 13, and vW-I, which occurs within intron 40 of the von Willebrand gene) using the gene identity method (23,24), using the ADMIX 3

Table I. Distribution of patients and tumor characteristics by ethnic group.

Characteristics	White pts. frequency (%)	African-Brazilian pts. frequency (%)	p-value
Age (years)			
≤50	100 (41.3)	26 (50.0)	0.251
>50	142 (58.7)	26 (50.0)	
Clinical stage			
I	36 (15.1)	1 (2.0)	0.053
II	114 (47.7)	24 (47.1)	
III	73 (30.5)	21 (41.2)	
IV	16 (6.7)	5 (9.8)	
Tumor size (cm)			
≤1.9	43 (18.2)	3 (6.4)	0.050
2-4.9	122 (51.7)	23 (48.9)	
>4.9	71 (30.1)	21 (44.7)	
Lymph nodes			
≤0	88 (38.3)	17 (34.7)	0.379
1-3	62 (27.0)	10 (20.4)	
>3	80 (34.8)	22 (44.9)	
Recurrence			
No	164 (67.8)	35 (67.3)	0.949
Yes	78 (32.2)	17 (32.7)	
Status			
Alive, well	127 (52.5)	28 (53.8)	0.661
Alive with recurrence	14 (5.8)	4 (7.7)	
Death due to cancer	57 (23.5)	8 (15.4)	
Death due to other cause	2 (0.8)	1 (1.9)	
Lost of follow-up	42 (17.4)	11 (21.2)	
ER			
Negative	105 (44.7)	13 (44.8)	0.988
Positive	130 (55.3)	16 (55.2)	
PR			
Negative	129 (57.1)	18 (62.1)	0.609
Positive	97 (42.9)	11 (37.9)	
p53 mutation			
No	209 (86.4)	35 (67.3)	0.001
Yes	33 (13.6)	17 (32.7)	
Mutation type[a]			
1	12 (5.0)	5 (9.6)	0.135
2	8 (3.3)	4 (7.7)	
3	222 (91.7)	43 (82.7)	

[a]Mutation type: 1, patients with tumors with missense mutations affecting one of the amino acids directly involved in DNA or zinc binding; 2, patients with tumors with null mutations or missense mutations inside the structural/conserved domains, but not affecting any of the amino acids directly involved in DNA or zinc binding; 3, patients with tumors with missense mutations outside structural/conserved domains including wild-type (without *p53* mutation). p-value obtained from Chi-square test.

program, kindly made available to us by Dr R. Chakraborty. Details of the laboratory methods of STR analysis were described (19). The parenteral frequencies used for those evaluations were obtained from African, Amerindian and European populations (25).

Statistical analyses. The Chi-square test and Fisher's exact test for frequency data in contingency tables were performed to determine the associations between the p53 genetic alterations, ethnic groups and the clinicopathological characteristics of the patients. The overall survival time was defined as the interval between the date of the beginning of treatment or surgery and the date of last follow-up appointment (censored observation) or date of death (uncensored observation). Overall survival probabilities were calculated based on the Kaplan-Meier product limit technique and the log-rank test was assessed to compare survival curves. For all statistical tests the significance level assumed was $p<0.05$. The data analyses were performed using the STATA software pV 7.0.

Results

A total of 294 primary breast tumors, 52 from African-Brazilian and 242 from white Brazilian patients were examined for *TP53* mutations using PCR-SSCP and direct DNA sequencing. The interethnic admixture calculated for the two populations defined by the morphological and family history data showed significant different contributions of blacks and whites, as estimated by STR markers. The white subjects from São Paulo showed 67.9±0.4%, 25.0±1.7% and 7.0±1.6% of genes of European, African and Amerindian origins, respectively, whereas the black populations from São Paulo and Salvador had 34.4±1.9%, 56.2±1.9 and 9.4±2.2% of European, African and Amerindian genes.

Distribution of patient and tumor characteristics by ethnic groups was listed in Table I. The age of the white patients at the time of surgery ranged from 25-93 years (median 53 years). For patients classified as African-Brazilian the median age was 55 years (range 28-88). Tumor metastases at lymph nodes were detected in 175 patients.

The patients were followed up from 1988 to July 2002. The median follow-up time and mean ± standard deviation obtained for white patients were respectively 35.6 months and 47.2±33.0, and for African-Brazilians were 43.4 months and 48.7±35.2. During the follow-up period, it was observed that around 17% of white and 21% of African-Brazilian patients were lost to follow-up. No statistically significant differences in various parameters were observed from blacks and whites excepting that patients of the African-Brazilian group had more advanced lesions than those of the white group.

Fifty out of 294 (17%) tumors examined showed *TP53* mutations. Mutations were observed among five exons (4 in exon 4; 8 in exon 5; 7 in exon 6; 16 in exon 7; 16 in exon 8). In addition, 26 cases showed a neutral polymorphism for codon 213 of exon 6 (CGA→CGG; Arg→Arg), 4 cases showed polymorphisms for exon 4 in codon 47 (2 cases; CCG→TCG, Pro→Ser), in codon 72 (2 cases; CGC→CCC, Arg→Pro) and in codon 262 (1 case, GGT→GGC, Gly→Gly). Compared to whites (13.6%), a relatively high frequency of

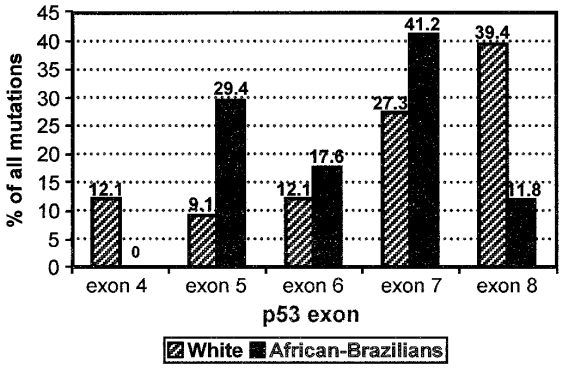

Figure 1. Exon distribution of *TP53* mutations in primary breast carcinomas.

TP53 mutations was found in blacks (32.7%) (p=0.01). The frequency of the neutral polymorphism in codon 213 of the *TP53*, was 10.5% among whites and 3.8% among blacks. Relative distribution of mutations within the different exons and the frequencies of specific mutations among black and white patients were also compared (Fig. 1). African-Brazilian women have a larger proportion of mutations in exons 5 (29.4%) and 7 (41.2%), whereas white women have more mutations in exons 7 (27.3%) and 8 (39.4%). Fig. 2 shows the pattern and codon distribution of p53 mutations in breast cancer. Sequencing results are summarized in Tables II and III. White patients displayed 20% of deletions, 9% of insertions and 6% of intronic mutations, while 29% of deletions and 6% of insertions were observed in African-Brazilian patients. Base substitutions comprising 65% of the mutations were observed in both white and black patients. However, the spectra of *TP53* mutations show that A:T→G:C transitions and G:C→C:G transversions were more common in African-Brazilian compared to white women (27.3% vs 9.5% and 27.3% vs 4.8% respectively). White patients were found to have a higher frequency of G:C→T:A transversion than their black counterparts (23.8% vs 9%). There was a prevalence of G:C→A:T nucleotide transitions in both groups. Seventy-two percent of these transitions occurred at CpG dinucleotide sites. Transversions A:T→C:G and A:T→T:A were found only in the white population.

In order to investigate whether *TP53* mutations were associated with the development and progression of primary breast tumor the clinicopathological characteristics of the patients with tumors showing *TP53* mutations were compared with those patients with tumors without *TP53* genetic alterations. We could not find a statistically significant correlation between the presence of *TP53* mutations and any clinicopathological characteristics, such as, age, clinical stage, tumor size, steroid hormone receptors or lymph node status independently of the ethnic group (Tables IV and V).

For white patients the 5- and 10-year overall survival probability was 71.1 and 62.9% respectively. No correlation was detected between *p53* abnormalities and overall survival. Survival estimates were not presented for the African-Brazilians patients in this study, because of the high percentage of loss from follow-up status for this group of patients, which could introduce a bias in the survival estimates.

We further investigated the prognostic impact of *TP53* mutations at specific sites, which may produce different effects on the p53 function. Breast cancer patients were stratified according to the type of *TP53* mutations as defined by Alsner *et al* (26): type 1, patients with tumors showing missense mutations affecting one of the amino acids directly involved

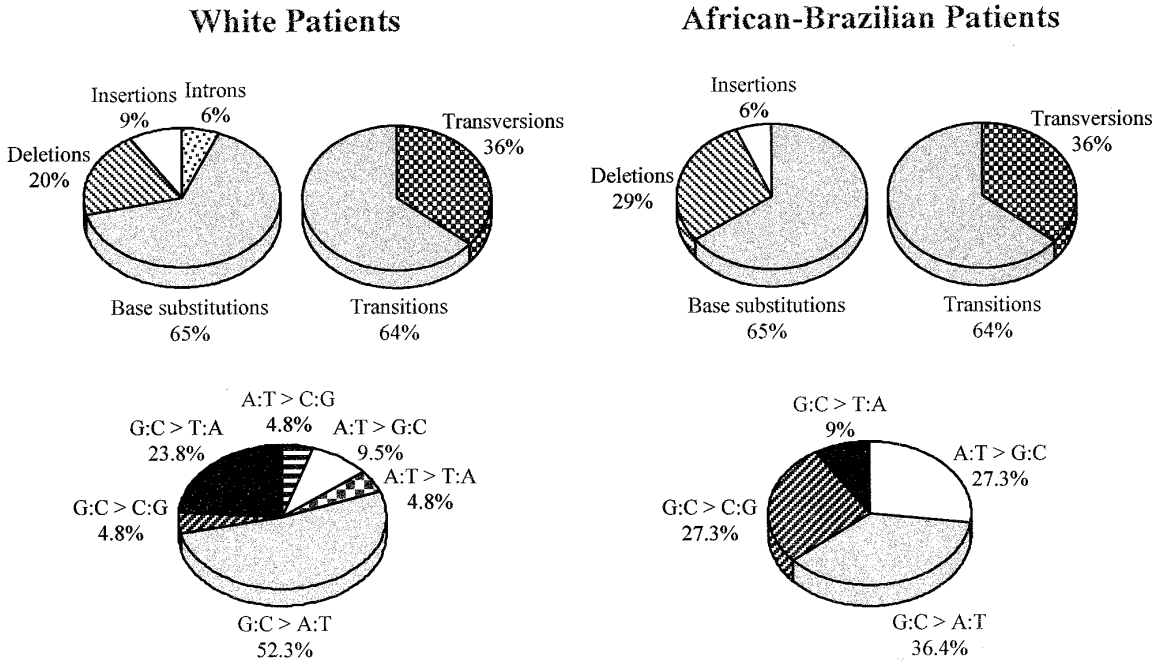

Figure 2. *TP53* mutational spectrum in primary breast carcinomas. Mutation patterns are given as pie charters showing the proportions of the different type of mutations.

Table II. Type of *TP53* mutations in tumors from white breast cancer patients.

ID no.	Exon	Codon	Nucleotide change	Amino acid change
T108	4	54	1 bp deletion	1bp
M121	4	Intron	Insertion	
M158	4	44	1 bp deletion	Frameshift
M257	4	109/110	6 bp insertion	Frameshift
TL59	5	134	TTT→TTA	Phe→Leu
M165	5	144/145	3 bp deletion	Frameshift
CM29	5	141/142	2 bp insertion	Frameshift
CM302	6	217	GTG→GCG	Val→Ala
TSG32	6	195	ATC→ACC	Iil→Thr
M117	6	198	GAA→TAA	Glu→stop
M319	6	192	CAG→TAG	Gln→stop
M133	7	230	1 bp deletion	Frameshift
M149	7	248	CGG→CAG	Arg→Gln
M113	7	245	GGC→AGC	Gly→Ser
M127	7	258	GAA→TAA	Glu→stop
M187	7	245	GGC→AGC	Gly→Ser
M208	7	237	ATG→ATA	Met→Ile
M212	7	245	GGC→GAC	Gly→Asp
M291	7	235	3 bp deletion	Frameshift
CM73	7	248	CGG→CAG	Arg→Gln
TL44	8	Intron	48 bp deletion	Frameshift
TL101	8	273	CGT→CAT	Arg→His
TE141	8	Intron	T→G	
TE154	8	Intron	T→G	
M74	8	272	GTG→ATG	Val→Met
M182	8	275	TGT→TTT	Cys→Phe
TSG1	8	273	CGT→TGT	Arg→Cys
TSG19	8	275	TGT→TTT	Cys→Phe
TSG26	8	270	TTT→TGT	Phe→Ser
CM50	8	262	GGT→GGC	Gly→Gly
TL96	8	269-285	47 bp deletion	Frameshift
TL112	8	282	CGG→TGG	Arg→Trp
CM49	8	282	CGG→GGG	Arg→Gly
CM159	8	298	GAG→TAG	Glu→Stop

Table III. Type of *TP53* mutations in tumors from African-Brazilian patients.

ID no.	Exon	Codon	Nucleotide change	Amino acid change
S08	5	179	CAT→CGT	His→Arg
S10	5	156	CGC→GGC	Arg→Gly
S13	5	148	GGT→GAT	Gly→Asp
S16	5	176	TGC→TGG	Cys→Trp
TER153	5	159	6 bp insertion	Frameshift
S06	6	192	CAG→TAG	Gln→Stop
S09	6	193	CAT→CGT	His→Arg
M289	6		27 bp deletion	Frameshift
S03	7	237	1 bp deletion	Frameshift
S18	7	237	1 bp deletion	Frameshift
TL61	7	244	3 bp deletion	Frameshift
TL67	7	241/242	3 bp deletion	Frameshift
M152	7	245	GGC→GAC	Gly→Asp
M171	7	248	CGG→CAG	Arg→Gln
M232	7	238	TGT→TAT	Cys→His
CM48	8	282	CGG→GGG	Arg→Gly
TL74	8	285	GAG→TAG	Glu→Stop

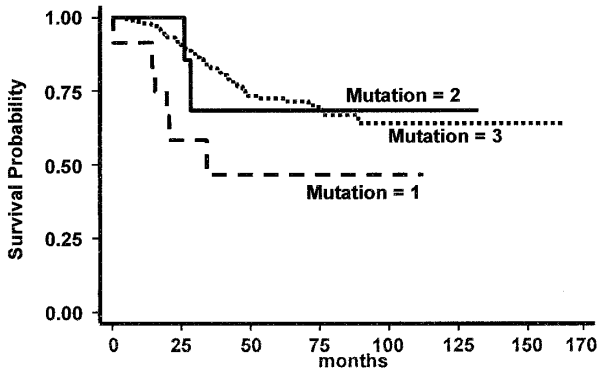

Figure 3. Kaplan-Meier estimates of overall survival in breast cancer patients stratified according to the *TP53* mutations. 1, patients with tumors with missense mutations affecting one of the amino acids directly involved in DNA or zinc binding; 2, patients with tumors with null mutations or missense mutations inside the structural/conserved domains, but not affecting any of the amino acids directly involved in the DNA contact or zinc binding and 3, wild-type together with missense mutations outside structural/conserved domains.

in DNA or zinc binding; type 2, patients with tumors showing null mutations and missense mutations inside the structural/conserved domains, but not affecting any of the amino acids directly involved in the DNA contact or zinc binding; and type 3, patients with tumors showing missense mutations outside structural/conserved domains. The number of patients in each category is presented in Table I.

Univariate survival analysis was carried out for the group of white patients. For each p53 mutation type the overall survival estimates for 5 and 10 years respectively to type 1 were 46.4% and 46.4%; to type 2 were 69.2% and 69.2% and to type 3 were 72.5% and 63.6%. The *p53* mutation type 1 showed the lowest survival probability rates, and a significant difference among survival curves by log-rank test was observed with p-0.0357 (Fig. 3).

Discussion

The spectrum of *TP53* somatic mutations in specific types of tumors offers the possibility of detecting differences among populations that may be attributed to genetic susceptibility and environmental exposures. In the present study we performed a detailed analysis of *TP53* mutation in breast carcinomas from Brazilian patients with two ethnic backgrounds. Although a degree of miscegenation was demonstrated for both the white and black populations, a significant

Table IV. Associations of p53 gene mutation with clinico-pathological characteristics of white breast cancer patients.

Characteristics	p53 mutation status		p-value
	Negative frequency (%)	Positive frequency (%)	
Age (years)			
≤50	87 (41.6)	13 (39.4)	0.809
>50	122 (58.4)	20 (60.6)	
Clinical stage			
I	31 (15.1)	5 (15.1)	0.669
II	101 (49.0)	13 (39.4)	
III	60 (29.1)	13 (39.4)	
IV	14 (6.8)	2 (6.1)	
Tumor size (cm)			
≤1.9	38 (18.7)	5 (15.1)	0.678
2-4.9	106 (52.2)	16 (48.5)	
>4.9	59 (29.1)	12 (36.4)	
Lymph nodes			
≤0	75 (37.3)	13 (44.8)	0.739
1-3	55 (27.4)	7 (24.1)	
>3	71 (35.3)	9 (31.0)	
Recurrence			
No	145 (69.4)	19 (57.6)	0.178
Yes	64 (30.6)	14 (42.4)	
Censored status			
Death	50 (23.9)	9 (27.3)	0.667
Alive or lost of follow-up	159 (76.1)	24 (72.7)	
ER			
Negative	89 (43.8)	16 (50.0)	0.515
Positive	114 (56.2)	16 (50.0)	
PR			
Negative	113 (58.2)	16 (50.0)	0.382
Positive	81 (41.8)	16 (50.0)	

p-value obtained from Chi-square test.

Table V. Associations of p53 gene mutation with clinico-pathological characteristics of African-Brazilian breast cancer patients.

Characteristics	p53 mutation status		p-value
	Negative frequency (%)	Positive frequency (%)	
Age (years)			
≤50	19 (54.3)	7 (41.2)	0.555
>50	16 (45.7)	10 (58.8)	
Clinical stage			
I	1 (2.9)	0 (0.0)	
II	20 (58.8)	4 (23.5)	
III	11 (32.4)	10 (58.8)	
IV	2 (5.9)	3 (17.7)	
Tumor size (cm)			
≤1.9	3 (9.4)	0 (0.0)	
2-4.9	16 (50.0)	7 (46.7)	
>4.9	13 (40.6)	8 (53.3)	
Lymph nodes			
≤0	13 (39.4)	4 (25.0)	0.258
1-3	8 (24.2)	2 (12.5)	
>3	12 (36.4)	10 (62.5)	
Recurrence			
No	24 (68.6)	11 (64.7)	0.780
Yes	11 (31.4)	6 (35.3)	
Censored status			
Death	7 (20.0)	2 (11.8)	0.700
Alive or lost of follow-up	28 (80.0)	15 (88.2)	
ER			
Negative	10 (47.6)	3 (37.5)	0.624
Positive	11 (52.4)	5 (62.5)	
PR			
Negative	12 (57.1)	6 (75.0)	0.671
Positive	9 (42.9)	2 (25.0)	

p-value obtained from Chi-square test.

difference was observed between them, with a predominance of genes of African origin in the group of black patients and a predominance of genes of European origin in the sample of white individuals. A certain degree of miscegenation is expected when dealing with ethnic groups living within a multiracial population, which tends to reduce differences between the populations, thus strengthening the findings of the present study.

The mutation rate detected in this white population (13.6%) was lower than the 18% established by Pharoach *et al* (7) in a meta-analysis of breast cancer studies, being in the lowest side of the range reported for various American and European populations examined in 1,425 breast tumor samples worldwide (27). The more prevalent mutation was the nucleotide transition G:C→A:T (52.3%) which is similar to the frequency reported in the IARC data base of *p53* gene mutations (http://www.iarc.fr/P53/index.html) (28). This transition is a frequent event in the European patients (29-34). The frequency of G:C→T:A (23.8%) and A:T→T:A (4.8%) transversions and A:T→G:C transition (9.5%) matched those reported by most other studies of American and European populations (11). The relative distribution of mutations within exons 6 and 7 was similar to that reported in the IARC data base (28) excepting that a lower proportion of mutations was observed in exon 5 and a higher proportion in exons 4 and 8 in the current study.

Analysis of the profile of *p53* mutation in African-Brazilian women indicated that the frequency of G:C→A:T transition

(36.4%) resembles that one of the American black population of Detroit, being considerable lower from that reported for blacks from the New Orleans area (12,13). Frequency of A:T→G:C (27.3%) transition was also similar to that reported for the Detroit population although this transition has not been detected in the New Orleans population. On the other hand, our black population had a prevalence of G:C→C:G and showed abundance of micro-deletions and insertions as compared to the African American profile. The different mutation spectrum of *p53* between this study and the American reports may in part reflect differences in the origin of the slave trade from Africa to North and South America. Brazil received slaves mainly from regions of Bantu populations (68%) and the remainder from the Bight of Benin (19). Studies based on the haplotypes associated with the sickle-cell gene indicated that the Bantu haplotype predominated all over the country due to the homogenizing effect introduced by internal slave trade (36,37), thus differing from the equivalent populations in the USA originated mainly from a Benin population.

Analysis of frequency and mutation types in the current study indicated significant intergroup differences on the pattern of *p53* mutations. The major differences lie in the prevalence of G:C→C:G transversions and A:T→G:C transitions in the black population whereas white patients were found to have a higher frequency of G:C→T:A transversion. It has been suggested that an excess of A:T→G:C in black populations might result from an increased exposure to exogenous or endogenous nitric oxide and/or defective repair of deaminated adducts induced by nitric oxide (11). Differences in inherited characteristics of the population, such as polymorphism for several enzymes involved in the activation and detoxification of carcinogens might also contribute to differences in the *TP53* mutation spectrum between black and white populations. Moreover the frequency of *p53* mutations in the black population was significantly greater than that of the white population. Although no statistically significant differences in several tumor and clinical parameters were observed between our two ethnic populations, patients of the African-Brazilian group appear to have more advanced lesions than those of the white group. As it is generally believed that *p53* mutations occur more frequently in tumors with more aggressive features (37), this difference may in part explain the difference in the prevalence of *p53* mutations in both groups.

Our data on global rate of *p53* mutation and high frequency of G:C→A:T transitions is also in accordance with that reported in a recent study of 120 patients living in Rio de Janeiro, a Brazilian southeastern city. However, the cohort described there seems to be ethnically heterogeneous (38).

Several studies showed an association of *p53* mutations with conventional clinicopathological features and survival but our study failed to show such a correlation (7-10,39,40). Reasons for discrepancies between the current results and those of other studies cited above may be related to small tumor numbers or reflect population characteristics.

The prognostic significance of mutations in different locations and functional domains of the p53 gene has been investigated (8,26 and therein). In the present study, in an attempt to identify any correlation between the outcome of white breast cancer patients with the type and location of the *TP53* mutations, we adopted the criteria used by Alsner *et al* (26) to stratify the patients. Our data support the concept that breast cancer carrying *p53* mutations in the zinc binding regions or affecting the DNA contact domain was associated with reduction in patient survival. This analysis was not carried out in black patients due to limited sample size of this specific cohort, as the frequency of the black population is presently small in our country (6.1%) (41).

The findings suggest that the racial differences in p53 mutations seems to reflect a base line pattern observed in populations with similar ethnic profile with some modifications, which might be derived from specific etiological factors.

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