Study of p53 codon 72 polymorphism in patients with breast cancer

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Summary

Breast cancer is a common disease in Western societies, with an incidence of 46.31/100,000 women/year in Brazil. The tumor suppressor gene TP53 is one of the most studied genes regarding the presence of mutations. Indeed, 50% of all tumors are known to exhibit changes in the TP53 nucleotide sequence due to carcinogenic processes. As to the presence of polymorphism, the TP53 gene is polymorphic at the nucleotide residue 347 (codon 72).

In the current study, we examine if this polymorphism is associated with the clinicopathological parameters of breast cancer patients in a Brazilian population. One hundred and thirteen patients with breast cancer were included. The polymorphic region of the TP53 gene was PCR-amplified from genomic DNA obtained from buccal cells. Specific primers for the Pro and Arg allele were used.

Correlations of polymorphism with age, staging, nuclear grade, lymph node status, estrogen receptor status and lymphatic and/or blood vessel invasion were evaluated. Statistical analysis was performed using the Fisher's exact test The frequency of p53 Arg/Arg was 57% and of the heterozygous allele Arg/Pro it was 39%.

There was no correlation between polymorphism and clinicopathological parameters. According to our results, the TP53 polymorphism, at the 347 residue, is not associated with any clinicopathological findings of patients with breast cancer.

Key words: p53 polymorphism; Breast cancer; Prognosis.

Introduction

Breast cancer is a highly prevalent disease in Western populations, with higher incidence in North America and Western Europe [1]. In Brazil, it is estimated there were approximately 49 new cases per 100,000 women in 2005 [2]. Since this is such a prevalent and severe disease among women, advances in studies that make an attempt to detect new genes related to breast cancer, or even its interaction with environmental factors involved in mammary carcinogenesis, could result in improvement in prevention and treatment of this neoplasm.

Genetic factors are responsible for only 5% of cases, 85% of cases being sporadic and approximately 10% familial [1]. Studies on inherited susceptibility to breast cancer suggested that the predominant susceptibility genes are transmitted in an autosomal dominant mode [1]. Polymorphisms are among other genetic factors of lower penetrance that are responsible for familial cases.

This genetic variation is responsible for the remaining familial risks not attributed to high penetrance genes. Polymorphisms in genes that codify enzymes, receptors or other proteins which act in metabolic pathways and are potentially relevant in breast cancer, could influence the function of these proteins and create differences among individuals in their metabolic activity. It is not known if polymorphism per se produces a modest risk or if interaction with other carcinogenic factors is needed.

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The TP53 gene is associated with breast cancer when mutated. This is a tumor suppressor gene located in the short arm of chromosome 17 and composed of only 11 exons. It codifies a 393- amino acid nuclear phosphoprotein called p53, with 53 kDa [3, 4]. It acts as the cell molecular guardian, monitoring the genome integrity through the control of the cell cycle, repair of DNA and apoptosis [5, 6]. The TP53 mutations are considered the most common genetic alterations in human cancer and are present in approximately 50% of all cancers [7-9]. In breast cancer, TP53 is mutated in 20-30% of tumors [10].

The prevalence of TP53 mutations in the germ lineage of women with breast cancer and aged under 40 years has been estimated in approximately 1% [1].

Polymorphism is defined as a variation sequence in a gene that occurs in more than 1% of alleles [1]. Polymorphism in TP53 is considered a risk factor for malignant diseases including breast cancer [7, 11], due to the crucial relation of this gene in genome maintenance.

Only codon 72 polymorphism seems to be related to breast cancer [12]. The polymorphic TP53 in codon 72 of the protein it codifies means it has variants in the same p53 protein, which do not characterize a mutation. In the amino acid structure, guanine or cytosine in residual nucleotide 347 results in an arginine (CGC) or proline (CCC) codon [11] for the amino acid.

The p53 Pro 72 is different from p53 Arg72 and this is reflected by different electrophoretic mobility. The Arg72 migrates faster in agarose gel [11, 13]. Tumors of patients with Pro 72 are smaller and grow less [11, 13]. The p53 Pro presents higher transcriptional activity than p53 Arg, which could be related to stronger affinity for transcrip-

tional factors TAFII32 and TAFII70 [5]. On the other hand, p53 Arg seems to induce apoptosis faster than p53 *Pro in vitro* [5, 14].

There are studies in the literature that consider the Arg/Arg genotype in codon 72 as a risk factor for breast cancer when compared to a control population. This is related to the fact that the prevalence of Arg/Arg 72 is 20% in the general population and 62% in breast cancer patients [11].

Several authors studied this association, but the results are still inconclusive [11, 15]. Polymorphism in p53 codon 72 varies according to the ethnic group and to geographic latitude [5, 11, 16]. The frequency of Arg 72 genotype increases with latitude, whereas that of Pro 72 decreases with latitude. There is also an association of this polymorphism with larynx, colorectal, cervical, vesical and lung cancers.

In breast cancer patients a higher number of mutations were found in homozygous individuals for Arg; and even in those heterozygous - Arg/Pro - the frequency of mutations located in the arginine allele is higher. Therefore, the p53 Arg 72 polymorphism is already considered a risk factor in certain populations and could also be a prognostic factor. Its presence could be related to a higher number of lymph nodes affected and higher histological grade, among other factors.

It would be relevant to conduct this investigation in the Brazilian population. The objective of this study was to verify the association between the TP53 codon 72 polymorphism and clinical-pathological parameters of breast cancer patients, in order to verify the eventual prognostic value of this polymorphism.

Materials and Methods

This study was conducted in the Discipline of Mastology, Department of Gynecology at the *Universidade Federal de Sao Paulo* (UNIFESP), and patients who underwent surgery for breast cancer in the period from 1999 to 2004 were enrolled. The patients were chosen when they were admitted for postoperative follow-up visits at outpatient clinics.

The research project was approved by the Institution Research Ethics Committee. After signing an informed consent, all patients were submitted to buccal cell collection using a cytobrush. According to the protocol, data were gathered from patients' medical charts, such as age at onset of disease, past history, staging, recurrences and metastases. Tumor immunohistochemical and histopathological data were obtained from the postoperative pathological record.

DNA extraction and genotyping: Cytological samples obtained were preserved at -80°C until further genomic DNA extraction. DNA was extracted according to the Kit GFX® protocol (Amersham-Pharmacia) for buccal cells. Analysis of the amount of DNA obtained in these extractions was made by spectrophotometry with 260 nm wave length (Spectronic model Genesys 5). The p53 genotyping was performed according to Brenna *et al*. Briefly, for the arginine allele reactions, 200 ng of the genomic DNA were used in a final reaction volume of 25 µl containing: 10 pmol/µl of each primer (sense 5'- TCC CCC TTG CCG TCC CAA -3'; anti-sense 5'- CTG GTG CAG GGG CCA CGC-3'). For the proline allele reactions, 200 ng of the genomic DNA were used in a final reaction volume of 25 µl containing: 10 pmol/µl of each primer (sense 5'-GCC AGA GGC TGC TCC CCC–3'; anti-sense 5'-CGT GCA AGT CAC AGA CTT-3'). Electrophoresis was conducted in a 2% agarose gel containing ethidium bromide staining and the patterns obtained in this reaction have been described elsewhere [17].

Statistical analysis was made by Fisher's exact test and the differences between allele status and clinicopathological parameters were considered significant when $p < 0.05$.

Results

One hundred and thirteen patients participated in the study. In the genetic analysis of the TP53 codon 72 polymorphism in this Brazilian population we obtained 61 (61/113) homozygous cases for arginine (Arg/Arg), 48 heterozygous cases (Arg/Pro) and four homozygous cases for proline (Pro/Pro).

The age varied from 25 to 84 years, with a mean of 53.88 years and median of 56 years. Among all patients, 87.6% (99/113) were aged 40 years or older, and they presented a similar distribution in both heterozygous (Arg/Pro) (87.5%) and homozygous Arg/Arg (86.2%), and Pro/Pro (100%) cases. There was a family history of breast cancer in 8.8% of the cases. Among homozygous arg/arg patients, 11.5% presented a positive family history, whereas only 6.2% of the heterozygous cases expressed this variable (Table 1).

The patients were classified as early stage (64.6%) and advanced stage (35.4%) (Table 1). Approximately 69%

Table 1. — *Correlation between P53 codon 72 polymorphism and clinical-pathological parameters*.

	Arg/Pro (%)	Arg/Arg	Pro/Pro	p*
Family history				
Negative	45 (93.8)	54(88.5)	4 (100)	0.662
Positive	3(6.2)			
Clinical stage				
I and II	32 (66.7)	39 (63.9)	2 (50)	0.774
III and IV	16 (33.3)	22 (36.1)	2 (50)	
ER status				
Negative	13 (27.1)	20 (32.8)	2 (50)	0.581
Positive	35 (72.9)	41 (67.2)	2 (50)	
Lymphatic and blood vessel invasion				
Negative	28 (58.3)	31 (50.8)	1 (25.0)	0.406
Positive	20 (41.7)	30 (49.2)	3 (75.0)	
Nuclear grade				
Low	8 (17.8)	10 (17.9)	1 (33.3)	0.623
High	37 (82.2)	46 (82.1)	2 (66.7)	
Capsular invasion				
Negative	11 (39.3)	13 (34.2)	1 (50.0)	0.904
Positive	17 (60.7)	25 (65.8)	1 (50.0)	
Local recurrence				
Negative	46 (95.8)	56 (91.8)	4 (100)	0.585
Positive	2 (4.2)	5 (8.2)	0	
Metastasis				
Negative	4.0 (85.1)	53 (86.8)	3 (75.0)	0.608
Positive	7 (14.9)	8 (13.1)	1 (25.0)	
Death				
No	47 (97.9)	54 (91.5)	4 (100)	0.378
Yes	1 (2.1)	5 (8.5)	0	

presented positive hormone receptors, and the percentage of negative hormone receptors was higher in homozygous (32.8%) than in heterozygous patients (27.1%). In the analysis of histopathological parameters, there was angiolymphatic invasion in 49.2% of homozygous cases compared to only 41.7% of heterozygous cases (p = 0.406). The analysis of nuclear grade showed the cases with low nuclear grades (nuclear grade 1) corresponded to 18.3%, while 81.7% were high nuclear grade (nuclear grade 2 and 3).

In the analysis of polymorphisms there was similarity regarding the presence of high-grade tumors in both homozygous (82.1%) and heterozygous cases (82.2%) (Table 1). Out of 68 patients with nodal involvement, 65.8% presented capsular invasion among the homozygous and 60.7% among the heterozygous cases (Table 1). Only 6.2% of the women had local recurrence during the follow-up which varied from six months to five years.

The development of metastasis during follow-up occurred in 14.2% of patients, with similar percentages in both homozygous and heterozygous cases (Table 1). Among six cases of deaths, five were homozygous for arginine.

Discussion

The TP53 gene is polymorphic at position 72, containing a residue of proline or arginine in this position. Two decades ago it was initially studied as a change in the p53 mobility in SDS-polyacrylamide gel [18].

This alteration in mobility suggested that change in the amino acid could modify the protein structure and function, and produce functionally distinct proteins. This leads to the hypothesis that there could be individual variations in apoptosis induced by p53 due to natural population variations at the codon 72 of the p53 protein and therefore several authors have examined arginine homozygosity as a possible risk factor for skin and HPV-associated cervical tumorigenesis [11, 15, 19]. This occurs because despite Arg 72 pro-apoptotic activity, it seems to be more susceptible to a HPV 18 association and, consequently, to degradation of p53 function.

Papadakis *et al*. [11] found a higher prevalence (62%) of Arg/Arg genotype among breast cancer patients compared to the control group with no disease (20%). In our population of patients we also observed a higher incidence of Arg/Arg genotype (54%).

In Papadakis *et al's* control group, 67% of cases were heterozygous (Arg/Pro) and only 20% homozygous (Arg/Arg). This shows that even though a case-control study was not conducted, our sample is very similar to the genotypic situation of breast cancer patients in Greece. Only four of our cases were homozygous for proline (Pro/Pro) compared to 21% of Papadakis *et al's* cases, thus corroborating the hypothesis that the frequency of p53 codon-72 genotypes varies according to the ethnic group and to geographic location.

The pro72 allele is selected for individuals who live in high levels of ultra-violet radiation (low latitudes), since this polymorphism characteristically varies according to latitude. The lower the latitude, the more Pro 72 and the higher, the more Arg 72. The latitude of Sao Paulo is 23 degrees, which leads to a higher frequency of Arg and lower frequency of Pro/Pro, as shown in the study. In Bologna, 6.35% of breast cancer were Pro/Pro, close to our incidence (3.5%); 43.3% were heterozygous (Arg/Pro) and 47.8% homozygous for arginine [20].

In our study initial stages were predominant, but there was a higher percentage of homozygous for arginine in advanced stages, similar to the results obtained by Papadakis *et al*., in which only seven cases were Stage III and six of these were homozygous for Arg/Arg.

As for hormone receptors, 69% of our cases were positive for hormone receptors, like in Papadakis *et al's* study mentioned above [11]. However, our homozygous cases presented a higher number of negative hormone receptors, in disagreement with their analysis in which positive and negative hormone receptors are equally distributed among heterozygous and homozygous cases for arginine. As in the Greek study, our patients also had nuclear grade 2 and 3.

The attempt to find differences between the clinical-pathological characteristics of patients is based on studies reporting that variants in codon 72 polymorphism are not biochemically equivalent; they differ in the capability of linking to the transcriptional machinery and in apoptosis modulating a variety of experimental systems [18, 20]. Therefore, individuals presenting these variants could behave differently during manifestation of the condition, elucidating a variant that would modulate higher susceptibility to aggressive disease.

Bonafé *et al*. [20] found that the presence of arginine in one of the codon 72 alleles was associated with lower overall survival (OS) and disease-free survival (DFS), regardless of other established prognostic factors such as nodal involvement. This study also demonstrated that patients who maintained arginine in tumor tissue usually had positive estrogen receptors (p = 0.06).

Hence, those authors concluded that the presence of arginine in the polymorphism variant at the moment of diagnosis does not mean a poorer prognosis, but interferes in long-term OS and DFS. Like in our cases, this study did not show statistical significance between the clinical-pathological factors analyzed (nuclear grade, tumor size, nodal involvement, age, hormone receptor status and Ki67) as compared to variants of p53 codon 72. However, it shows similarity in expression of receptors.

In a study conducted on individuals with transitional cell carcinoma in the urinary tract [21], it was observed that patients with mutating tumors containing Arg in tumor tissue had larger lesions. There was no statistically significant correlation between the other parameters, such as age, tumor grade and gender. This makes us consider that the presence of arginine homozygosity, which could be correlated with poorer prognostic factors in breast cancer, would be observed if we had selected only patients presenting a mutant p53; later, a comparative study of prognostic factors and variants will be performed.

Arginine allele preferential retention in neoplastic tissue has been described in many carcinomas, such as head and neck, vulvar, esophageal, urinary and lung. It is speculated that this polymorphism may affect the mutation functions of p53, resulting in growth advantage for tumors in which the mutation resides in the arginine allele. The reason is when the mutation occurs at this site it is capable of inactivating p73. Yamamoto *et al*. [22] demonstrated p73 apoptotic activity, an increase in mitotic activity and a decrease of apoptosis in patients with blocked p73 activity. In that study, patients with a functional deficit of this protein had higher tumor proliferation and higher number of metastases, suggesting that p73 would be a prognostic factor. Despite all efforts and the increasing number of studies, the biopathological meaning of these variants is not yet clear.

Polymorphism acts as an intragenic modifier of mutant p53 behavior and has an effect in p53 biological activity. Currently, the meaning of p53 codon 72 polymorphism is still obscure in terms of cancer biopathology and epidemiology. Further studies using a spectrum of different carcinoma tissue samples are required to understand the association of polymorphism and human carcinogenesis. In conclusion according to our findings, different alleles of p53 codon 72 polymorphism have not shown any association with breast cancer clinical-pathological factors.

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