Investigation of Single Strand Conformational Alterations of the TP53 Gene in Epithelial Hyperplasias of the Breast

Investigação das Alterações Conformacionais de Fita Simples do Gene TP53 em Hiperplasias Epiteliais da Mama

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Abstract

Generally, benign breast lesions behave as innocuous and limited proliferations, but sometimes they can represent pre-cancerous diseases. The practical importance of epithelial hyperplasias studies is related to their potential for malignant transformation. The TP53 tumor supressor gene suffers the greatest number of mutations in human cancer Using single strand conformational polymorphism, we did a mutation screening in exons 5 to 8 of the TP53 gene in the tumoral tissues of five patients with epithelial hyperplasias of the breast. The obtained results do not show any polymorphism that indicates mutation. The lack of mutation indicates that this gene is not involved in the intial process of malignization, strengthening the hypothesis that mutations on TP53 gene are a late event in the breast carcinogenesis.

Key words: epithelial hyperplasia of breast; single strand conformational polymorphism (SSCP); TP53 gene.

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Resumo

As lesões benignas da mama geralmente comportam-se como proliferações inócuas e limitadas, mas algumas vezes podem representar patologias pré-cancerosas. A importância prática do estudo das hiperplasias epiteliais (HE) está relacionada com o seu potencial de transformação maligna. O gene supressor de tumor TP53 é o que sofre o maior número de mutações em câncer humano. Usando a técnica do polimorfismo conformacional de fita simples, triamos mutações nos exons 5 ao 8 do gene TP53 em hiperplasias epiteliais da mama. Os resultados obtidos não revelaram qualquer polimorfismo indicativo de mutação. A ausência de mutação é um indicativo de que este gene não está envolvido no processo de iniciação da malignização, reforçando a hipótese de que as mutações no gene TP53 são um evento tardio na carcinogênese mamária.

Palavras-chave: hiperplasias epiteliais da mama, polimorfismo conformacional de fita simples (SSCP), gene TP53.

Introduction

Atypical epithelial hyperplasias (EHs) are mammary lesions treated and classified as benign proliferation disorders, and are considered to be clinical risk markers, given their association with a four or fivefold increase in the risk of developing breast cancer[1]. While the development and progression of human breast neoplasia is dependent on the accumulation of several somatic alterations, it is not clear whether mutations usually occur in noninvasive lesions before invasion[2].

The study of benign proliferations of the breast may reveal a possible relationship between genetic alterations and the condition of the tissue. Only malignant EH homologues have been studied to date, and there are few published descriptions of genetic analyses of mammary hyperplasia[2-7]. The comparison of the molecular alterations in these tissues to their malignant counterparts may contribute to the understanding of the genes that both act in cellular proliferation and lead to malignant transformations and invasions of the cell[8].

The TP53 gene suffers the greatest number of mutations in human cancer[9]. In sporadical breast tumours as well as in germ line of family members with breast cancer history, most of the mutations in TP53 gene are found on exons 5 to 8[10-12]. The frequency of mutations of gene TP53 in breast cancer is approximately 25%[13, 14].

The identification of mutations in the early stage of mammary neoplasias would provide a correlation between the occurrence of these mutations and the stage of the disease,15 as well as identifying a subgroup of patients with a greater risk of developing mammary carcinomas[6].

In the present study, five cases of breast EHs (one atypical and four moderate) were analysed in order to identify conformational alterations of the TP53 gene.

Material and Method

Sample

In the present study, five samples of EHs were studied on a molecular level. The samples' histological types are summarized in Table 1. The patients from whom the samples were taken had not been previously submitted to either radiation and/or chemotherapy. Two samples was used as control: Control 1 – a sample of healthy tissue taken from a young patient with no histological alteration of the breast; Control 2 – a sample of blood taken from a young women with no familial history of the breast cancer.

Extraction of DNA from tumor tissue

To obtain DNA of high molecular weight, the tissue samples were pulverized in liquid nitrogen, homogeneity in lise buffer (Tris-HCl 10 mM, pH 8,0; EDTA 5 mM; SDS 0,6%),

Table 1. Histological diagnosis of the benign mammary lesions analyed in the present study

Case(Inicials)	Histopathological Diagnosis	Age (years)	Sex	Racial group	Breast affected
1(LMB)	Epithelial hyperplasia with fibrocystic alterations and areas of adenosis.	40	F	White	No data
2(OAA)	Diffuse mammary fibroepithelial hyperplasia.	58	F	Non white	No data
3(DSP)	Fibroepithelial hyperplasia of the breast with foci of typical epitheliosis and cystic transformation.	27	F	Non white	Right
4(CSS)	Fibroepithelial hyperplasia of the breast with foci of typical epitheliosis and apocryne metaplasia.	24	F	White	Right
5(MCQ)	Atypical ductal epithelial hyperplasia.	29	F	Non white	Left

and digested with K proteinase (100mg/ml) at 37°C for 16-18 hours. Following digestion, samples were extracted using phenol/chloroform and precipitated with sodium-ethanol acetate.

The DNA was dissolved in TE buffer (Tris-HCl 10 mM, pH 8,0; EDTA 1 mM) and incubated with RNAse at 37°C for 30 min. to eliminate contaminating RNA. Following this treatment, the DNA was once again extracted with phenol/chloroform and then precipitated with ethanol[16].

Polymerase Chain Reaction (PCR)

Primers 17 were used to amplify exons 5 to 8. Approximately 200 ng of genomic DNA were mixed with 50 pmol of each primer and *Taq* polymerase buffer (Cenbiot, RS, BR), with a final concentration of Tris-HCl 10 mM; pH 8.4; KCl 50 mM and $MgCl_2$ 1.5 mM; 50 mM of each triphosphated desoxinucleotide and 1.25 units of *Taq* polymerase.

Amplifications were carried out using a Perkin Elmer/Cetus, USA, thermal cycler, under the following conditions:

a) 1 cycle at 95°C for 5 minutes;
b) 35 cycles at 95°C for 1 minute, 60°C for 1 minute, 72°C for 1 minute;
c) 1 cycle at 72°C for 7 minutes.

Analysis of Single-Strand Conformational Polymorphisms (SSCP)

For the analysis of SSCP, the DNA was amplified by PCR using the specific p53 gene primers. Following reaction, the products were diluted in 100ml of a solution of SDS 0.1%, EDTA 10 mM. Three ml of the diluted sample were mixed with 4 ml of formamide stain, denatured for 10 min. at 95°C and kept refrigerated prior to the application of the gel. Electrophoresis was carried out in a 40 cm 5-10% polyacrilamid gel containing 10% glicerol for 16-18 hours at 6 W and room temperature.[18] The single-strand DNA was stained with silver nitrate. The migration patterns of single-stranded DNA were then examined for differences.

Results

Detection of Specific TP53 Gene Mutations.

We checked whether the conformational alterations in TP53 exons 5, 6 and 8 detected in the PCR-SSCP test were initial events in the genesis of these benign proliferative disorders. No alterations were found in our samples.

Discussion

Breast cancer occurs most frequently after menopause, but it seems likely that the first steps in the development of the mammary carcinoma occur prior to the menopause, given the long period – ten to thirty years – that precedes the clinical manifestation of this neoplasia[19].

Epidemiological studies suggest that there is a moderate increase in the chances of developing cancer in the case of moderate proliferative epithelial lesions, and a substantial increase in that of atypical proliferative lesions. The atypical EH is an intermediate stage in relation to the carcinoma[20]. This type of mammary lesion can be defined as a proliferation similar to a normal hyperplasia, but may also exhibit concomitant *in situ* carcinomas[21].

The practical importance of EH studies is related to their potential for malignant transformation. The deactivation of tumor-suppressing genes is a common genetic mechanism in breast cancer, and is of considerable significance for the pathogenesis of human cancer[22]. Given this, we examined tissue from the tumors of the five patients with EH studied here (four moderate and one atypical EHs) for the presence of alterations of the exons 5 to 8 of tumor-suppressing gene TP53, given that this gene suffers the greatest number of mutations in human cancer. Alterations of gene TP53 are found in half of all tumours[23]. In breast cancer, the presence of mutations of this gene implies an unfavorable prognosis[24]. Mutations of this gene are detected frequently in breast carcinomas[8]. However, there have been few studies of this gene in benign mammary lesions. The presence or absence of these alterations in the EHs would indicate the participation of gene p53 in the early or late stages, respectively, of mammary transformation.

SCHMITT *et al.*[5] and MOMMERS *et al.*[7] studied the expression of p53 protein in intraductal EHs using immunocytochemical techniques. Expression was observed in 4.5% and 8% of the samples respectively, suggesting that this phenomenon is already present in intraductal proliferations of the breast. Although Umekita *et al*.[4] observed no expression in an investigation of p53 protein expression in sixteen atypical ductal hyperplasias and thirty-nine benign epithelial hyperplasias.

The analyses of EHs presented here did not reveal the presence of polymorphisms indicating exon mutations. Millikan *et al.*[3] found a low percentage of TP53 point mutations in an analysis of sixty benign breast biopsies. Done *et al*.[2] analyzed foci of epithelial hyperplasia adjacent to invasive carcinomas with known TP53 mutations and found no such mutations, including an atypical case, but the same mutation of invasive carcinoma was present when the adjacent lesion was *in situ* ductal carcinoma. The absence of alterations indicates that this gene may not be involved in the initial stages of proliferation, which reinforces the hypothesis that the mutations of the TP53 gene are relatively late events in mammary carcinogenesis. An evidence that supports this hypothesis is the absence of alterations in the chromosome 17p13, the region containing p53,[25, 26] in literature descriptions of Ehs[27-29]. In this case, mutations of this gene in breast cancer may play the same role as their homologues in adenocarcinomas of the colon[15], where mutations of the TP53 gene occur during the transition from adenoma to carcinoma[30]. Further research will be necessary to evaluate the usefulness of these markers as tools for the evaluation of the malignant potential of benign breast tissue.

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