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p53 Codon 72 Polymorphism as a Risk Factor in the Development of Breast Cancer

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The p53 gene is polymorphic at amino acid 72 of the protein that it encodes. It has been reported that patients with the arginine form have a higher risk of developing other forms of cancer than those with the proline form. The purpose of this study was to examine whether p53 Arg at the polymorphic position 72 could represent a risk factor for women with breast lesions. The study population included 56 biopsies from patients with breast lesions. Also, 61 normal blood samples were used as controls. There was a difference in the distribution of p53 genotypes between breast cancer lesions and the normal samples. The allele frequency of p53 Arg/Arg was much higher than that of the normal samples (61% versus 20%). Based on the findings of this study, it is suggested that p53 Arg homozygosity could represent a risk factor for the tumorigenesis of the breast. © 2000 Academic Press

***Key Words: p53* gene; codon 72; breast cancer.**

Breast cancer incidence is almost 1 in 9 women and current therapies for the disease are inadequate once it has metastasized. The disease is characterized by high morbidity and mortality. Normal as well as malignant growth is regulated by endocrine hormones and by local tissue factors, such as polypeptide growth factors. Breast cancer seem to progress as hyperplastic ductal or lobular epithelial growth, acquiring progressive genetic changes (including those of oncogenes and tumor suppressor genes) leading to clonal outgrowths of progressively malignant cells (1).

The p53 tumor suppressor gene is commonly altered in human cancer and the spectrum of p53 mutations in these cancers provide clues to the etiology and molecular pathogenesis of neoplasia (2).

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In human populations, the p53 gene is polymorphic at amino acid 72 of the protein that it encodes (3–5). In the reading frame used, the G or C at the nucleotide residue 347 resulted in an arginine codon (CGC) or proline (CCC) for the amino acid residue. Matlashewski *et al.* (3), concluded from their observations that p53 with Pro-72 is structurally different from p53 with Arg-72, and this is reflected by its altered electrophoretic mobility. p53 with Arg-72 migrated more rapidly on gels than did p53 with Pro-72 (3). It was also noted that the tumors produced by the Pro-72 p53 containing cells, appeared more slowly and were smaller in each case than the Arg-72 p53 tumors and that both forms of human p53 can increase the tumorigenicity but the Arg-72 form of human p53 is more oncogenic in this respect than the Pro-72 form of human p53.

In the current study, we examine whether p53 Arg at the polymorphic position 72 could represent a risk factor for patients with malignant breast lesions, in comparison with a normal control group. Breast lesions were found to carry the Arg/Arg polymorphism in p53 codon 12 in 62% whereas this genotype appeared only in 20% in the normal blood samples. Therefore, it is suggested that p53 Arg homozygosity could possibly represent a potential risk factor for the tumorigenesis of the breast. No statistical correlation was observed between p53 status and the clinicopathological parameters.

MATERIALS AND METHODS

Subjects and Blood Samples

The study population included 56 patients with breast cancer from Greece. Directly after dissection the specimens were stored at −70°C until DNA extraction. Peripheral blood was obtained from 61 healthy normal people aged ≥55 years with no known breast lesions, because no normal breast tissue could be obtained in order to be used as control.

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DNA Extraction from Breast Tissue and Blood Samples

DNA extraction was performed under a standard protocol using organic detergents (6).

PCR Amplification of p53 Polymorphic Sequences

The polymorphic region of the p53 gene was PCR-amplified from the genomic DNA of both breast tissues and blood samples for the amplification of the Pro allele using primer pairs p53Pro+/p53− and p53+/Arg− for the amplification of the Arg allele (7). In every PCR reaction two blank samples were employed as negative controls to ensure that no contaminants were introduced. The mixture was heated for 1 min at 95°C and samples were subjected to 30 cycles of amplification at 94°C for 40 s, 60°C for 40 s, and 72°C for 30 s (p53+/Arg−), at 94°C for 40 s, 54°C for 40 s, and 72°C for 30 s (p53Pro+/p53−). Elongation was at 72°C for 5 min.

PCR products were analyzed on a 2% agarose gel and photographed on a UV light transilluminator.

Statistical Analysis

The presence of p53 codon 12 polymorphism was analyzed for correlation with age of the patient, clinical stage, histological grade, estrogen and progesterone receptor status. Statistical analysis of the results was performed with the package SPSS 6.0 (for Windows). Statistical significance was set at $P \leq 0.05$.

RESULTS

Histological data of the specimens

The presence of amplifiable DNA, using primers for a fragment of β-globin gene, was confirmed in all the 56 breast cancer specimens and the 61 blood samples examined (data not shown).

p53 Codon 72 Polymorphism

To analyze the codon-72 polymorphism, we used a PCR-based assay that specifically detects either the p53 Pro or p53 Arg allele. The primer pair p53+/Arg− gives a PCR product of 141 bp of the Arg allele (Fig. 1), whereas the Pro+/p53− primer pair gives a PCR product of 177-bp fragment of the proline allele (Fig. 2).

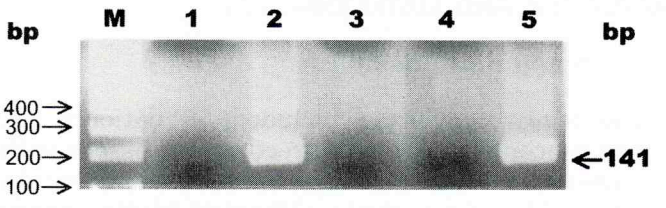

FIG. 1. p53-72 Arg allele amplification products (141 bp) employing PCR. PCR products were electrophoresed through a 2% agarose gel. Lanes 2 and 5: positive samples; lanes 1, 3, and 4: negative samples; lane M: 100 bp molecular weight marker.

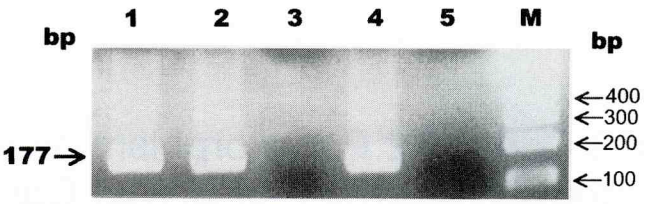

FIG. 2. p53-72 Pro allele amplification products (177 bp) employing PCR. PCR products were electophoresed through a 2% agarose gel. Lanes 1, 2, and 4: positive samples; lanes 3 and 5: negative samples; lane M: 100 bp molecular weight marker.

The results of the p53 polymorphism distribution of the 56 breast cancer lesions and also the distribution of the 61 normal blood samples used as controls are summarized in Table I. There was a difference in the distribution of p53 genotypes between breast cancer lesions and that of normal samples. The allele frequency of p53 Arg/Arg was much higher (61%) than the normal samples (20%). The Arg/Pro heterozygosity frequency was 18% in breast cancer lesions compared to 67% in blood samples. The Pro/Pro frequency was low both in breast lesions (21%) and in the control group (10%). The results of the p53 polymorphism distribution of the 56 breast cancer lesions were correlated to the age of the patient, clinical stage, histological grade, estrogen and progesterone receptor status as summarized in Table II.

A statistically significant correlation was not observed between p53 status and the clinicopathological parameters.

DISCUSSION

Breast cancer accounts for the most common cancer in women in Europe (8). A subset of molecular alterations have been associated with the development of the disease.

p53 is polymorphic at amino acid 72 of the protein that it encodes, thus p53 may contain either a proline or an arginine residue at this position. We (9, 10) and others (11–13) have examined whether p53 arginine homozygosity may represent a possible risk factor for HPV-associated cervix and skin tumorigenesis. However, the results are still inconclusive.

In the present study, we analyzed the p53 genotype of breast lesions and control samples using a PCR-based assay. Our results confirm the difference in the Arg/Arg genotype between breast lesions and our control (62% versus 20%). In our control group the frequency of p53 Arg/Pro heterozygosity is 67% whereas

TABLE I

Frequencies of Codon 72 Polymorphism

Histological diagnosis	Number of samples	Arginine	Arginine/proline	Proline	Other
Blood samples	61	12 (26)	41 (67)	6 (10)	2 (3)
Breast cancer lesions					
Total (%)	56	34 (61)	10 (18)	12 (21)	—

there seems to be some difference in the prevalence of p53Pro and p53 Arg homozygosity (10 and 20%, respectively). It has been postulated that the frequencies of p53 codon-72 genotypes vary according to the ethnic group. The frequency of p53 Arg homozygosity in our control group is lower than that found in a Japanese study (14) and in a Norwegian study (15).

However, in our breast cancer lesions there is a significant over-representation of p53 Arg homozygosity (62%) compared to the p53 Pro homozygosity (21%). The frequency of p53 Arg/Pro heterozygotes is 17%.

Our results indicate that p53 Arg homozygosity may represent a possible risk factor for breast tumorigenesis. In the breast tumors studied, there was an over-representation of homozygous p53 Arg compared with heterozygous or homozygous Pro alleles.

A small number of control samples where found to carry no Arg or Pro in the amino acid residue 72 of p53. This can be due to a deletion of that coding sequence of the p53 gene or as Matlashewski *et al.* (3) have stated, further alleles, such as Cys, may exist at position 72 of the p53 protein.

In conclusion, our study indicates that p53 Arg homozygosity is associated with breast cancer and could possibly represent a potential risk factor for tumorigenesis of the breast. Further investigation is needed in different ethnic populations to determine the influence of this p53 polymorphism on carcinogenesis.

TABLE II

p53 Polymorphism of Breast Cancer Lesions Correlated to the Age of the Patient, Clinical Stage, Histological Grade, and Estrogen and Progesterone Receptor Status

	Pro/Pro	Pro/Arg	Arg/Arg
Age			
≤40		1	1
40–50		2	10
>50	12	7	23
Stage			
I	6	3	15
II	5	6	14
III		1	6
Grade			
I	4		4
II	6	7	29
III	1	3	2
Estrogen receptor			
Positive	6	6	18
Negative	5	4	17
Progesterone receptor			
Positive	6	7	18
Negative	5	3	17

Note. ER− or PgR− represents ER or PgR receptor level below 5 fmol mg^{-1} protein; ER+ or PgR+ represents ER or PgR receptor level above 5 fmol mg^{-1} protein.

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