

RESEARCH ARTICLE

Absence of P53 Gene Mutations in Exons 5 - 7 Among Breast Cancer Patients of Bengalee Hindu Caste Females, West Bengal, India

Abhishikta Ghosh Roy^{1,4}, BN Sarkar^{1*}, Rakesh Roy², VR Rao³, AR Bandyopadhyay⁴

Abstract

Background: The high incidence and relatively good prognosis of breast cancer has made it the most prevalent cancer in the world today. A large number of distinct mutations and polymorphisms in the p53 gene have been reported worldwide, but there is no report regarding the role of this inherited susceptibility gene in breast cancer risk among the Bengalee Hindu Caste females of West Bengal, India. **Aim of the Study:** We investigated the distribution and the nature of p53 gene mutations and polymorphisms in exons 5-7 in a cohort of 110 Bengalee Hindu breast cancer patients and 127 age, sex and caste matched controls by direct sequencing. **Results:** We did not observe any mutations and polymorphisms in our studied individuals. **Conclusion:** We therefore conclude that mutations in exons 5-7 of p53 gene are rare causes of breast cancer among Bengalee Hindu caste females, and therefore of little help for genetic counseling and diagnostic purposes.

Keywords: p53 - exons 5, 6 and 7 - Bengalee Hindu caste females - breast cancer - India

Asian Pacific J Cancer Prev, 13 (9), 4477-4479

Introduction

Carcinoma of the breast is one of the most common malignancies that affects and is the leading cause of female death worldwide (Ferlay et al., 2008). Breast cancer, like other malignancies, is a genetic disease with multiple genetic events leading to the malignant phenotype. Epidemiological studies suggest several risk factors that include family history, genetic, environmental agents, fat diet, nulliparity, early age at menarche, late age at menopause (Zubo et al., 2009). It has been implicated in the pathogenesis of breast carcinoma, amongst which p53 tumor suppressor gene is one of the most commonly mutated gene. The p53 is a 20kb gene located on the short arm of chromosome 17 at 17p13. It contains 11 exons, first of which is non-coding and some 10kb away from the rest (Dhingra and Hortobagy, 1996). The p53 tumor suppressor gene is the most involved genetic factor for breast cancer (Mabrouk et al., 2003).

It is now accepted that inactivation of p53 gene, as a result of mutation, is a key step in neoplastic transformation and progression (Shojaie and Tirgari, 2008). It is estimated that about 50% of all human cancers contain a mutation in the p53 gene (Gasco et al., 2002). However, it is not yet completely clear which of the many properties of p53 are particularly important in oncogenesis. The wild type p53 protein was believed to be "guardian of the genome" (Gretarsdottir et al., 1996; Smardova et al., 2001). The p53

gene is mutated in 25-40% of the breast carcinoma cases. This suggests an etiological role of exogenous chemical carcinogenesis in sporadic breast carcinoma (Levine et al., 1991). Mutations in p53 are often but not always accompanied by allele loss at 17p13. Studies have directly correlated p53 mutations with breast cancer prognosis and it has recently been demonstrated that p53 gene mutations (exons 4-10) are the single most predictive indicator for recurrence and death in breast cancer. Direct detection of p53 mutations has substantially greater prognostic value than immuno histochemical detection.

In the present study, our aim was to detect mutations in exons 5, 6 and 7 of the p53 tumor suppressor gene in breast cancer patients of Bengali Hindu caste population by Polymerase Chain Reaction amplification of the Target DNA followed by direct sequencing and understand their possible role as a prognostic indicator. To the best of our knowledge, this is the first report on screening mutations of p53 gene among the Bengalee Hindu females of West Bengal.

Materials and Methods

Collection of samples

The subjects included in this study are 110 patients with histo pathologically confirmed Breast Carcinoma visiting the Cancer Centre Welfare Home and Research Institute, Kolkata, India, from April, 2010 to December,

¹Anthropological Survey of India, ²Department of Medical Oncology, Cancer Centre Welfare Home and Research Institute, ⁴Department of Anthropology, University of Calcutta, Kolkata, West-Bengal, ³Department of Anthropology, Delhi University, Delhi, India *For correspondence: breres.anthro@gmail.com

2011. Clinical data and detailed family history of each patient were collected with the help of collaborating clinicians after clinical examinations. Ethical approval of the research project using human subjects was obtained from the Institutional Ethical Committee of Anthropological Survey of India, Ministry of Culture, Government of India. Patient samples were mainly collected from The Cancer Centre Welfare Home and Research Institute, Kolkata and National medical College and Hospital is the main referral centre for cases related to Breast Carcinoma. The demographic data (name, age, address, origin, occupation, family history, educational status, etc) were collected using schedule method.

Another 127 controls were collected; those were age sex and ethnic group matched. Controls didn't have any family history of breast cancer.

DNA Isolation

Approximately 5mL peripheral blood samples were collected in BD Vacutainer K2 EDTA (6 mL) with written and informed consent from patients with Breast Carcinoma, and from normal individuals as controls, without any history of breast carcinoma. Genomic DNA was prepared from fresh whole blood by using the conventional phenol-chloroform method (Sambrook and Russel, 2001). Genomic DNA was dissolved in TE (10mM Tris-HCl and 0.1mM EDTA, pH 8.0).

PCR Amplification

Polymerase chain reaction (PCR) was carried out to amplify exons and adjacent flanking region in a total volume of 10.0 µL containing 40-100 ng genomic DNA, 0.4 mM of each primer, 0.2 mM of each dNTP, 0.5-1.5 mM of MgCl₂ (as appropriate), and 0.2 unit of Taq polymerase (Invitrogen, Carlsbad, CA) in a Thermocycler (GeneAmp-9700; PE Applied Biosystems, Foster City, CA). Annealing temperature is calculated based on T_m of the primer pairs. The p53 exons (5-7) were amplified using the designed primers (Table 1). PCR amplified DNA fragments were analyzed on 2% agarose gel and then visualized by ethidium bromide staining.

Mutation and Polymorphism Detection

The PCR products free of contaminating bands due to non specific amplification were directly sequenced in forward and reverse direction in DNA Analyzer 3730 (Applied Biosystems, USA). Nucleotide changes were detected by comparing sequence obtained in chromatogram with the normal p53 gene sequence using pair-wise BLAST (Tatusova et al., 1999) and SeqScape software v2.5

Results

A total of 237 study subjects were included in the present study. The p53 mutations were screened for 110 patients and 127 controls.

The p53 mutations were screened for a total of 110 patients with histopathologically confirmed breast carcinoma (mean±SD, age at diagnosis in years, 52±10.9; age range in years, 27-76) and 127 controls having no

Table 1. Primer List for p53 Gene, Exons 5-7

Exon	Length	Sequence
5	248	F: AACTCTGTCTCCTTCCTCTT R: AACCAGCCCTGTCGTCTCTC
6	181	F: GCCTCTGATTCCTCACTGAT R: TTAACCCCTCCTCCCAGAGA
7	238	F: AGGTCTCCCAAGGCGCACT R: CAGGGTGGCAAGTGCTCCT

Table 2. Distribution of Age in the Studied Sample Population

	N (Total Number)	Mean±SD (in years)	Range (in years)
Patients	110	54.0370±10.38324	30-78
Controls	127	53.9685±8.55323	32-72

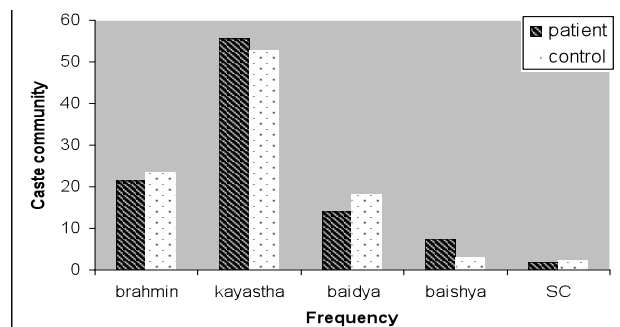


Figure 1. Distribution of Patients and Controls According to the Castes.

family history of cancer (Table 2). The age distribution of the patients and controls were similar. The patients and controls were classified into five caste groups namely Brahmin, Kayastha, Baidya, Baishya and Scheduled Castes (Figure 1). Most of the patients underwent chemotherapy and had a family history of breast cancer (66.7%).

All of them received treatment from the referral centres and the clinical data were collected with the help of collaborating clinicians. Socio-demographic data like age, occupation, marital status, nationality, origin, diet, etc., were collected using pre-tested schedule method. Genetic analysis of the exons (5-7) of the p53 gene revealed none of the commonly reported mutations in 237 subjects studied.

Discussion

The association between p53 gene mutations and breast carcinoma has been attempted by several studies, however, the issue is still controversial. The high incidences of breast cancer have prompted many researchers to understand its risk factors at the genomic level. We did not observe any mutations, after screening a cohort of 110 patients and 127 unrelated matched controls from the Bengalee Hindu caste females of West Bengal for the presence of common p53 mutations in exons 5, 6 and 7.

Our study has some limitations. The studied cohort is relatively small. Although the common mutations are absent in our study, their contribution to breast carcinoma cannot be completely excluded, as p53 is a large gene and mutations, other rearrangements, or upstream region

polymorphisms may exist in this population. Thus, mutation screening of the entire coding region including 11 exons of p53 is important to determine the contribution of this gene to breast cancer among the Bengalee Hindu caste females. Based on our results, routine testing for these mutations from diagnostic purpose and genetic counseling may not be cost effective, at least in the studied population.

Although, p53 mutations are reported at various frequencies in different populations, this study indicates that these mutations do not significantly contribute to Breast Cancer among the cases cutting across all the castes taken in our consideration; thereby, they are of little relevance for their pathogenic role in this disease inheritance and cannot be recommended for the diagnostic screening.

Acknowledgements

This research work is supported by the grant from Anthropological Survey of India, Ministry of Culture, Government of India. The authors are thankful to the patients with Breast Carcinoma and control subjects for voluntarily taking part in this research work and donating their blood samples.

References

- American Cancer Society (1999-2000). Breast Cancer Facts and Figures BRCAFF99
- Andersen TI, Holm R, Nesland JM, et al (1993). Prognostic significance of TP53 alterations in breast carcinoma. *Br J Cancer*, **69**, 540-8.
- Davidoff AM, Kerns BJM (1991). Maintenance of p53 alterations throughout breast cancer progression. *Cancer Res*, **51**, 2605-10.
- Dhingra K, Hortobagyi GN (1996). TITLE?????. *Semin Oncol*, **23**, 436-45.
- Gretarsdottir S, Tryggvadottir L, Jonasson JC, et al (1996). TP53 mutation analyses on breast carcinomas. A study of paraffin-embedded archival material. *Br J Cancer*, **74**, 555-61.
- Harris CC (1996). P53 tumor suppressor gene: At the crossroads of molecular carcinogenesis, molecular epidemiology, and cancer risk assessment. *Environ Health Perspect*, **104**, 435-9.
- Hollstein M, Sidransky D, Vogelstein B, et al (1991). p53 mutations in human cancers. *Science*, **253**, 49-53.
- Levine AJ, Momand J, Finley CA (1991). The p53 tumour suppressor gene. *Nature*, **351**, 453-6.
- Mabrouk I, Baccouche S, El-Abed R, et al (2003). No evidence of correlation between p 53 codon 72 polymorphism and risk of bladder or breast carcinoma in Tunisian patients. *Ann NY Acad Sci*, **1010**, 764-70.
- Madrigal L (1994). Twinning rates in admixed Costa Rican populations. *Am J Hum Biol*, **6**, 215-8.
- Sambrook J, Russel DW (2000). Molecular cloning: a laboratory manual in: Irwin N (ed) Cold Spring Harbor Press, NY. Science
- Smardova J, Nemažerova A, Trbusek M, et al (2001). Rare somatic p53 mutation identification in breast cancer - A case report. *Tumor Biology*, **22**, 59-66.
- Thorlacius S, Borrensens AL, Eyfjord JE (1993). Somatic p53 mutations in human breast carcinomas in an Icelandic population- a prognostic factor. *Cancer Res*, **53**, 1637-41.
- Mancuso T (1995). Mutation frequencies at codon 248 of the p53

tumor suppressor gene are not increased in colon cancer cell lines with the RER+ phenotype. PLEASE CHECK!!! *Oxford university press*, ?, 3643-8.

Varley JM, Brammer WJ (1991). Loss of chromosome 17 p13 sequences and mutation of p53 in human breast carcinomas. *Oncogene*, **6**, 413-21.

Zubo P, Stanclova A, Kajo K, et al (2009). The p53 codon 72 exon 4 BstUI polymorphism and endometrial cancer in Caucasian women. *Clin Trans Res*, **76**, 173-83.