Exclusive Intronic p53 Point Mutations Detected in Heterozygous Invasive Ductal Breast Carcinoma Samples

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Introduction

Breast cancer is the most common cancer in women, with a 33% increase in incidence rate in 10 years[1], and is the leading cause of mortality in women. One approach that may advance the understanding of the increased incidence of breast cancer is to study the p53 tumour suppressor gene, which orchestrates cell cycle control, DNA repair, apoptosis and control of angiogenesis.[2-3] A high frequency of mutations of this tumour suppressor gene in human cancers (mutations found in over 50% of human cancers) reflects the importance of its involvement in maintaining the stability of the genome during cellular stress. This reason has made the gene to be best suited for mutational spectrum analysis besides its modest size (11exons, 393 amino acids) which allows for the study of the entire coding region. Thus, the presence of p53 mutations in human cancers might indicate the value of the gene as a molecular marker for early prognosis especially in breast cancer since mutations in p53 result in poor prognosis, weak response to therapy and decreased overall survival time[4].

Most common p53 mutations are found in both familial and sporadic breast cancers[5-6]. However, the majority of mutations in human cancers are somatic rather than germline. Approximately 80% of the p53 mutations are missense point mutations which mostly concentrate within the central DNA binding region (codons 110-307)[7]. In breast cancer cases, many investigations have concluded that the mutations are more common in invasive ductal breast carcinoma than in lobular cancers where exons 5-8 being the region for mutation hotspots. However, mutation lying outside this region or in intronic sequences may be significant and have been previously underestimated. In addition, studies on mutations of p53 have focused on immunohistochermical analysis of p53 expression in paraffin embedded tissues[8]. In the present study, a molecular screening technique was applied and the p53 gene was screened for potential mutations in intron 4 through exon 9 using a non-isotopic RNAse cleavage assay (NIRCA) analysis.

Materials and Methods

Samples: Breast tissues processed as paraffin-embedded samples were obtained in duplicate sets and were randomly selected from patients with invasive ductal breast carcinoma aged 18 to 62 years. A total of thirty-one tissue samples were stained with Hematoxylin and Eosin (H&E) for identification of the cancerous regions, which was confirmed by a pathologist, and subjected to DNA extraction. Tissue samples from non-cancerous regions were used as controls.

DNA Extraction and Amplification: Based on the identification of the cancerous regions on the H&E stained slides, DNA extraction was carried out from the duplicate paraffin-embedded slides using a Pin-Point DNA extraction kit (Zymo Research) according to the manufacturer's protocols. The DNA obtained was then subjected to two rounds of polymerase chain reaction (PCR) to amplify exons 5-9. The T7 and T7 promoter-incorporated primers (Ambion) were used to amplify the target regions (exons 5-6, 7 and 8-9). Standard PCR conditions were optimized and performed using a Mastercycler Gradient Thermocycler (Eppendorf).

Non-Isotopic RNase Cleavage Assay: Transcription of RNA was carried out by adding 50-150ng of the PCR product into 4ul of transcription assay according to the protocol suggested by the manufacturer (Ambion). Hybridization solution was added into the reaction mixture prior to denaturation of the RNA duplexes at 97^0C for 7 minutes. The hybridization of the denatured RNA transcripts was then carried out at room temperature for 5 minutes followed by cleavage of RNA mismatches by RNase digestion at 37^0C for 35 minutes. The digestion reaction was stopped by the addition of the stop-dye. The digested fragments were then analyzed on a 2.5% agarose gel in 1X Tris-Borate EDTA buffer at 130 V for two hours.

DNA Sequencing Analysis: Fragments having alterations, and one of each fragment without alteration of the p53 gene as detected by NIRCA were sent for automated sequencing (IBS Genome Center, UPM) to assess the reliability of the methods used and also to determine the exact locations of the mutations.

Results and Discussion

The methods used in the study were successfully optimized and found reliable to be used in establishing a p53 gene mutation screening procedure. All of the 31 cases of breast cancer samples screened showed potential mutation spots by NIRCA and when sequenced, were confirmed to be mutations. No alteration was detected when samples from non-cancerous regions were used. The results obtained were categorized into four groups based on the pattern and size of the bands produced after RNase digestion. Sequence analysis also revealed the mutations to be exclusively located in intron 7. Presently, there are no confirmed prognostic variables yet established for early diagnosis of breast cancer. Current diagnosis of breast cancer is based on combination of traditional clinical staging (such as local extension, size, and the presence or absence of nodal or distant metastases), standard histological analysis of the tumour specimen, and expression of hormone receptors in the tumour[9].

Thus, most cases presented are normally patients with late stage of the tumour. Recent studies on the genetic composition of the cancer cell and molecular biological probes in detecting variations in oncogene and other related gene expression have revealed several putative prognostic factors in breast cancer. Based on the the present study, two mutation hot spots have been identified within intron 7, a transversion of T to G at nucleotide 2702 (55% occurrence) and a transversion of T to C at nucleotide 2682 (84 % occurrence). It is unlikely that these are intronic polymorphisms since they were not detected in DNA samples from non-cancerous regions. The mutations detected are believed to be located at the splice sites which may cause aberrant splicing of the p53 mRNAs and hence the production of a truncated proteins.

Conclusions

To our knowledge, this is the first report of a breast cancer mutation pattern of the p53 gene in the Malaysian population. However, a larger sample size would be needed to enable additional information such as cancer predisposition, response to therapy and disease progression to be obtained. Nevertheless, the mutation detection strategy used in this study was found to be rapid and provided a high-throughput method for screening purposes.

Benefits from the study

The results would be useful in identifying the location of the mutations specifically in the Malaysian population and a comparison to a global mutational database for breast cancer can be obtained. Analysis of the mutated regions would give significant information in our understanding of the functions of the p53 gene and perhaps contribute towards the development of a therapy against breast cancer.

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