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CLONING AND DNA FINGERPRINTING OF BREAST CANCER GENE BY ARBITRARILY PRIMED POLYMERASE CHAIN REACTION (AP-PCR) – A CASE REPORT

Clinical Research

King Solomon Ebenezer* Bioscience Research Foundation, Chennai – 602002, Tamil Nadu, India *Corresponding Author

Rajesh Kannan Velu Department Microbiology, Bharathidasan University, Tiruchirapalli-620024, Tamil Nadu, India

ABSTRACT

Globally the breast cancer is the most common cancer in women than other cancers. Recently, molecular studies urge for these familial syndromes to dig out the etiology, but several defective tumor suppressor genes been associated with familial breast cancer. Mainly, the BRCA-I gene at 17q21 as tumor suppressor gene, since a single mutation in BRCA-1 dramatically increases a woman's risk for breast cancer. Here we analyze the genetic instability in breast cancer tissue compared with normal tissues from the same individual through cloning and AP-PCR (Arbitrary primed polymerase chain reaction) using 4 different primers. Among these OPC4 showed presence of and variation in the genetic set up of cancer tissue and further it purified and cloned in to a T- tailed vector viz. pTZ57R/T. Presence of amplified DNA fragments was indicating homozygous insertion of 1270 bp observed in breast cancer plasmid.

KEYWORDS

Arbitrary primed polymerase chain reaction, vector, primer, genes and Breast cancer.

INTRODUCTION

Breast Cancer is common cancer among women characterized by uncontrolled growth of cells and may cause both internal and external factors which leads to death. Mainly, genetic cause plays a vital role to begin, alteration and development of normal cells in to cancer cells [1]. The couple of genes are important for formation of cancer cells such as tumor suppressor genes and oncogenes. But, genetically in breast cancer over activation of proto-oncogenes and inactivate of tumor suppressor genes [2]. A trouble-free process which is discrete from conventional PCR process to detect mutations called arbitrarily primed PCR (AP PCR). Based on the use of known gene sequences as oligonucleotide primers for amplification of genomic DNA to detect genetic polymorphism and used to build genetic maps [3]. In this case study, genetic alterations in the breast cancer tissue detected using AP-PCR technique and further confirmed through cloning process.

CASE STUDY

DNA extracted from breast cancer tissue which collected from vital diagnostic clinic, Bangalore. by Phenol-Chloroform-Isoamyl alcohol method [34] and Quantification was also done AP-PCR performed using procedures [5]. amplifications done for breast cancer gene using four different primers. Each 25-µl reaction contained 75 ng of DNA in 2.5µl of 10xassay buffer, 2.5mMgcl^2, 100M of each dNTPs, 100nM random tenmer primer, 1.5 unit of Taq DNA polymerase (Bangalore Genei, India). Primers used to amplify were OPA 04 - CCGCCCAAAC, OPC 15 - TGTCTGGGTG, OPK 03 - CCAGCTTAGG, OPC 04 - CCGCATCTAC. Reaction mixture incubated for 45 cycles:1st cycle (3.5min at 92° C,1min at 34°C,2min at 72°C) Subsequently, PCR for next 44 cycles (1min at 92°C,1min at 34°C,2minat72°C) followed by a final Extension cycle of 15min at 72°C.PCR of thermal cycles were performed in a Corbett thermal cycler (Germany). Each thermal cycle consisted of denaturation, annealing and early extension, and Final extension. PCR products purified by using SIGMA- Gene elute PCR clean-up kit and resolved by submarine 1.5% agarose gel electrophoresis [6]. After electrophoresis process the purified PCR product was ligated into T tailed vector (pTZ57R/T) by using Ins T/A clone PCR product cloning kit (MBI Fermentas). The ligated products transformed in to E. coli DH5 and transformants screened on LB agar plates containing 100g/ml ampicillin .Finally it confirmed by agarose gel electrophoresis of the isolated plasmid from the transformed colonies.

The Breast cancer gene of the *Homo sapiens* was amplified by PCR method using arbitrary primers. The primers were able to amplify breast cancer genes. The size of the amplicon determined based on comparison with the standard molecular marker run along with it. The PCR product matched with the expected size of 1.2 Kb. Four random ten-mers were used to analyze instability in the genome of breast cancer tissue using AP-PCR DNA Fingerprinting .Of these 4 primers, 3 primers OPA-04, OPC-15, OPK-03 produced similar amplification patterns in normal and cancerous cell DNA sample. The remaining primer OPC-04 detected genetic alteration in the cancerous cell giving an amplicon of size 1270 bp (Fig.1)

Fig. 1: Gel picture showing the amplified Breast cancer genes using OPC-04 primer: LANE 1 Ladder1 kb: LANE 2 Amplified Breast Cancer Genomic DNA (OPCO4): LANE 3 Amplified Normal Genomic DNA (OPCO4).

The cloned amplified products in the Plasmid p TZ57R were identified by plasmids isolation. Totally four transformants identified, among these one of them was recombinant based on the sizes (molecular weight) of the plasmid DNA. The plasmids with insert differed in their mobility in the agarose gel when compared to the control plasmid. The study revealed that AP-PCR fingerprinting was very useful in the identification and characterization of the regions of the genome of breast cancer tissues that have undergone alterations in comparison to their corresponding uninvolved breast tissues samples. Changes in the genome that were observed in this study include homozygous insertions, indicated by the presence of amplified DNA fragments in breast cancer DNA compared to the control normal DNA.

CONCLUSION

The most important finding that emerged from the present study that an insertion of a 1270bp amplified fragment was observed in cancer sample using primer OPC-04 that was absent in control normal DNA. The insertion of the locus in the genome of breast cancer was most likely the result of mutation commonly associated with breast cancer and, presumably specific for the breast cancer occurring at the OPC-04 primer binding sites.

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FINALASSISTANCE

None

CONFLICT OF INTEREST

None declared

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