Mutational Analysis of *BRCA 2* gene for SNP detection Responsible for Breast Cancer

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Abstract

The aim of this research was put a SNP of BRAC 2 gene that suffer from mutation to analysis by P.C.R and see how that effect to cause breast cancer in 22 women and masseur the amount of DNA by nano drop device and give the accurate shapes of bands under gel-electrophoresis for that mutant gen .

1. Introduction

Cancer is due to failures of the mechanisms that usually control the growth and proliferation of cells. During normal development and throughout adult life, intricate genetic control systems regulate the balance between cell birth and death in response to growth signals, growth-inhibiting signals, and death signals. Cancer occurs when the mechanisms that maintain normal growth rates malfunction to cause excess cell division (Renkema, 2004). The losses of cellular regulation that give rise to most or all cases of cancer are due to genetic damage that is often accompanied by influences of tumor-promoting chemicals, hormones, and sometimes viruses (Silverman et al. 2001). Mutations in two broad classes of genes have been implicated in the onset of cancer: proto-oncogenes and tumor-suppressor genes. Proto-oncogenes normally promote cell growth; mutations change them into oncogenes whose products are excessively active in growth promotion. Oncogenic mutations usually result in either increased gene expression or production of a hyperactive product. Tumor suppressor genes normally restrain growth, so mutations that inactivate them allow inappropriate cell division (Wechsberg, 2001). A third, more specialized class of genes called caretaker genes is also often linked to cancer. Caretaker genes normally protect the integrity of the genome; when they are inactivated, cells acquire additional mutations at increased rare-including mutations that cause the deregulation of cell growth and proliferation and lead to cancer. Many of the genes in these three classes encode proteins that help regulate cell proliferation (i.e., entry into and progression through the cell cycle) or cell death by apoptosis; others encode proteins that participate in repairing damaged DNA.
Breast cancer is one of the most common malignant tumors contributing to the high mortality of females worldwide (MacDonald et al. 2005: 372;

Norman & Brain 2005).The etiology of breast cancer is a complex combination of both environmental and genetic factors, so the determination of genetic polymorphism provided a new way to investigate the etiology of such complex genetic disease. Breast cancer begins in breast tissue. Most of the tumors that develop in breast tissue are benign (not cancerous). Some breast tumors are cancerous, but have not yet spread to other parts of the body. This type of breast cancer is called "in situ," and it can almost always be cured with treatment. The most serious type of breast cancer is invasive, meaning that the cancerous tumors have spread to other parts of the body (Hailey et al., 2000).

Breast cancer is the second most common cancer among women (after skin cancer). According to the current statistics of the Centers for Disease Control and Prevention, breast cancer is the most common cancer in women in the United States (excluding skin cancer) accounting for 32 percent of all female cancers (Williams et al., 2002). The good news is that the rate of death from breast cancer has declined over the last few years. This is probably because more tumors have been found early, when treatment can help the most. The National Cancer Institute estimates that about 1 in 8 women in the United States (approximately 13.3 percent) will develop breast cancer during her lifetime. This estimate is based on cancer rates from 1997 through 1999. Regular screening mammograms and breast exams (both self-exams and exams by a doctor) can help find breast cancers early (Umeh & Rogan-Gibson, 2001). In many of these cases, a person has inherited a gene from his or her parents that has mutated (changed from its normal form). This mutated gene makes it more likely for a person to get breast cancer. Breast cancer is a disease in which certain cells in the breast become abnormal and multiply without control or order to form a tumor. The most common form of breast cancer begins in cells lining the ducts that carry milk to the nipple (ductal cancer). Other

forms of breast cancer begin in the glands that produce milk (lobular cancer) or in other parts of the breast (Skinner et al., 1998).

Early breast cancer usually does not cause pain and may exhibit no noticeable symptoms. As the cancer progresses, signs and symptoms can include a lump or thickening in or near the breast; a change in the size or shape of the breast; nipple discharge, tenderness, or retraction (turning inward); and skin irritation, dimpling, or scaliness (Iglehart et al., 1998). These changes can occur as part of many different conditions, however. Having one or more of these symptoms does not mean that a person definitely has breast cancer. In some cases, cancerous tumors can invade surrounding tissue and spread to other parts of the body. If breast cancer spreads, cancerous cells most often appear in the bones, liver, lungs, or brain (Lipkus et al., 1999). Tumors that begin at one site and then spread to other areas of the body are called metastatic cancers.

A small percentage of all breast cancers cluster in families. Hereditary cancers are those associated with inherited gene mutations. Hereditary breast cancers tend to occur earlier in life than non-inherited (sporadic) cases and are more likely to involve both breasts(Silva & Zurrida 2003: 16). Researchers estimate that more than 178,000 new cases of invasive breast cancer will be diagnosed in U.S. women in 2007. Most breast cancers occur in women, but they can also develop in men. Scientists estimate that more than 2,000 new cases of breast cancer will be diagnosed in men in 2007. In addition to specific genetic changes, researchers have identified many personal and environmental factors that may influence a person's risk of developing breast cancer. These factors include gender, age, ethnic background, a history of previous breast cancer, certain changes in breast tissue, and hormonal factors. A history of breast cancer in closely related family members is also an important risk

factor, particularly if the cancer occurred at an early age (Tittle et al. 2002). Some breast cancers that cluster in families are associated with inherited mutations in particular genes, such as BRCA1 or BRCA2 where it is estimated that 5 percent to 10 percent of all breast cancers are hereditary.

The genetic determinants of breast cancer are under intensive study (Fry et al. 2005). Some women with a strong family history of breast cancer inherit BRCA1 or BRCA2 mutations, which have a variable penetrance for breast cancer, between 40 to 66%, suggesting that additional factors contribute to cancer risk among BRCA1 and BRCA2 carriers, studies of high risk populations generally help uncover the molecular mechanisms of a disease and provide guidance and direction for studies of sporadic disease (Foxcroft et al. 2004). While BRCA1and BRCA2 mutations are highly penetrant, resulting in higher risk for breast cancer, both of these genes are also highly polymorphic. Moreover, several of their variants result in amino acid changes which could ultimately change the structure and function of the genes (Ganz et al. 2003). It is therefore plausible that the combination of genetic changes in these genes or in genes in their pathway may at least contribute to the disease or the mechanisms associated with the disease in the general population.

2. Genes are related to breast cancer

Variations of the BRCA1, BRCA2, CDH1, STK11, and TP53 genes increase the risk of developing breast cancer. The AR, ATM, BARD1, BRIP1, CHEK2, DIRAS3, ERBB2, NBN, PALB2, RAD50, and RAD51 genes are associated with breast cancer. After the BRCA1 gene was discovered in 1994 and BRCA2 in 1995 (Miki et al., 1994; Wooster et al., 1995) genetic testing for breast cancer susceptibility was introduced into clinical practice in North America and in Europe. Over the past decade,

testing has also been initiated in Asia and in developing countries to a much lesser extent (Chalmers & Thomson 1996; Hopwood 2000).

3. Function of BRCA1 and BRCA2:

BRCA1 and BRCA2 are tumor suppressors which are essential for the faithful repair of double-strand DNA breaks by homologous recombination (Narod and Foulkes, 2004). However BRCA1 also participates in several other cellular functions which are important in maintaining genomic integrity, including the assembly of the mitotic spindle, centrosome duplication, cell-cycle control, and chromatin remodeling at sites of double-strand DNA breaks(Frankel & Devers 2000: 113). The role of BRCA2 appears primarily to regulate RAD51 filament formation — this is a critical step in catalyzing strand invasion and initiating homologous recombination. Cells lacking BRCA1 or BRCA2 are unable to repair double strand breaks by homologous recombination and therefore repair proceeds through error-prone pathways, such as non-homologous end-joining(Green & Thorogood 2004: 104). These cells may incur mutations during strand repair and often accumulate chromosomal rearrangements during successive rounds of cell division (Narod and Foulkes, 2004). While the majority of these rearrangements result in cell death, in some cases the mutant daughter cells are stable and these lead to the emergence of a dominant cell lineage that acquires the capabilities of autonomous cell division and of metastatic potential, two of the hallmarks of cancer. BRCA1 has also been shown to be required for the activation of both S- and G2/M-phase cell-cycle arrest after DNA damage, the latter being dependent on prior phosphorylation of BRCA1 by the master checkpoint kinase ATM [ataxia telangiectasia mutated (Xu et al., 2001)]. BRCA1 has been shown to interact with multiple DNA repair/recombination proteins, including Rad51, the Rad50/MRE11/Nibrin complex, Bloom’s helicase, and the Fanconi D2 protein (reviewed in Yun and Hiom, 2009).

Furthermore, the roles for BRCA1 in transcriptional regulation and proliferation are mediated through associations with CTIP, ZBRK, p300, estrogen receptor (ER), HDAC, Rb, p53, RNA polymerase II holoenzyme, cyclin D1, c-myc, and at least one member of the Swi/Snf complex (Narod and Foulkes, 2004).

Interestingly, Livingston and colleagues have proposed that BRCA1 protein is implicated in genetic silencing of the X-chromosome. They propose that direct binding of BRCA1 with XIST (X-inactive specific transcript) is critical to keep the X-chromosome silent (Ganesan et al., 2002). This relationship between the BRCA1 and XIST, the main mediator of X-chromosome inactivation, remains controversial (Vincent-Salomon et al., 2007). Some have speculated that this effect of BRCA1 could be part of a broader effect on heterochromatin maintenance and/or gene regulation (Pageau et al., 2007). In support of this hypothesis, Verma and colleagues argue that no unifying framework can link all of the reported biochemical activities of BRCA1 to its tumor suppressor function and they propose that many of the tumor suppressor functions of BRCA1 are due to its maintenance of global heterochromatin integrity (Zhu et al., 2011). Although there is no consensus yet on mechanism, loss of BRCA1 or BRCA2 functions in cell lineages is a critical step in breast and ovarian carcinogenesis among women with a mutation.

4. Disease characteristics:

A germline mutation in *BRCA1* or *BRCA2* predisposes to breast and ovarian cancer as well as other cancers. The risk of developing cancer that is associated with a germline *BRCA1* or *BRCA2* mutation, which has been derived from families with multiple affected individuals, families with few affected individuals, and from population-based studies, appears to be variable within families(Grbich 1999b: 70). Prognosis for breast and ovarian cancer depends on the stage at which the cancer is diagnosed;

however, studies on survival have revealed conflicting results for individuals with germline *BRCA1* or *BRCA2* mutations when compared to controls (Llewellyn et al. 1999: 214; Tuckett 2004: 49).

5. Risks Associated with a Mutation:

The lifetime risk of breast cancer in women with a BRCA1 or BRCA2 mutation is approximately 75%. For BRCA1, there is little evidence that the risk varies for different mutations; however, this is not the case for BRCA2 (McKenzie et al. 2005: 121). The risk of breast cancer for women with the common Ashkenazi founder mutation BRCA2 6714delT is approximately half that of women with other BRCA2 mutations. There is also a common truncating variant in the terminal exon 27 of BRCA2 that does not appear to increase the risk of breast cancer (Mazoyer et al., 1996), although it may increase the risk of pancreatic cancer (Martin et al., 2005) and esophageal cancer (Akbari et al., 2008).

There have been many studies of potential modifiers of risk of BRCA1 and BRCA2 published over the last decade. Penetrance may vary by geographic region; perhaps this reflects the underlying cancer rates in the general population. For example, the risk of breast cancer for women in Poland with a BRCA1 mutation appears to be much lower than that for women in North America (Lubinski et al., 2010). The difference could not be explained by mutation type, by reproductive factors, exogenous hormones, body-mass index (BMI), or by screening intensity. In this study, the difference was attributable to different risks early in life. In North America, the annual risk of breast cancer for women ages 25-39 with a BRCA1 mutation was 3.8% and in Poland it was 1.4% ($p < 0.001$). It has also been suggested that the risk of breast cancer in BRCA1 carriers is increasing with recent year of birth (Litton et al., 2011).

6. Methods and materials

1-After taking samples from 10 females have history in their families and also suffer from breast cancer after using crashing technique for smash the cells and extract DNA from cells and

2- identified the BARC 2 gen by P.C.R technique

BRCA2 primer sequences

Forward primer: 5'CATCTGTTTTGATAGGTCTTAG 3'

Reverse primer: 5'CAGCGTTTGCTTCATGGAAA 3'

3-QUALITATIVE ESTIMATION OF DNA BY NANODROP

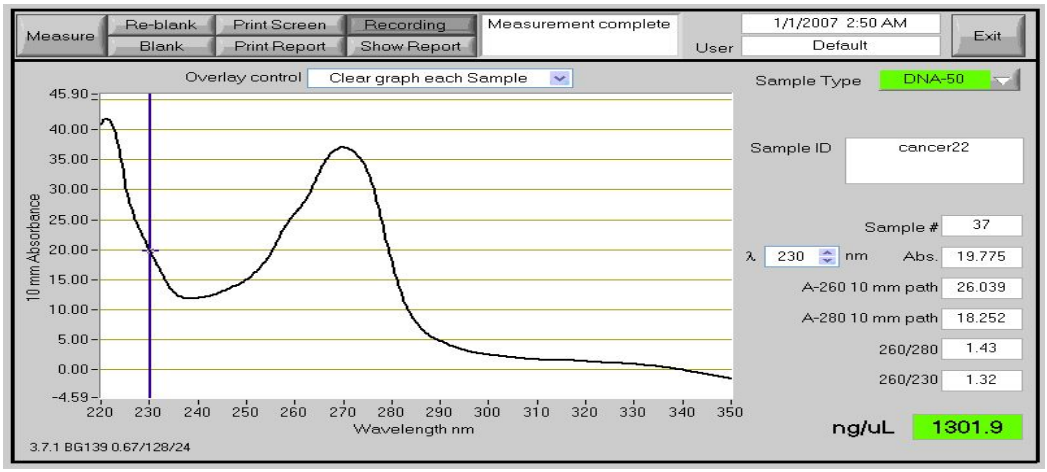

Figure1. Nanodrop Quantification of sample 22

4- QUALITATIVE ANALYSIS OF DNA AGAROSE GEL ELECTROPHORESIS

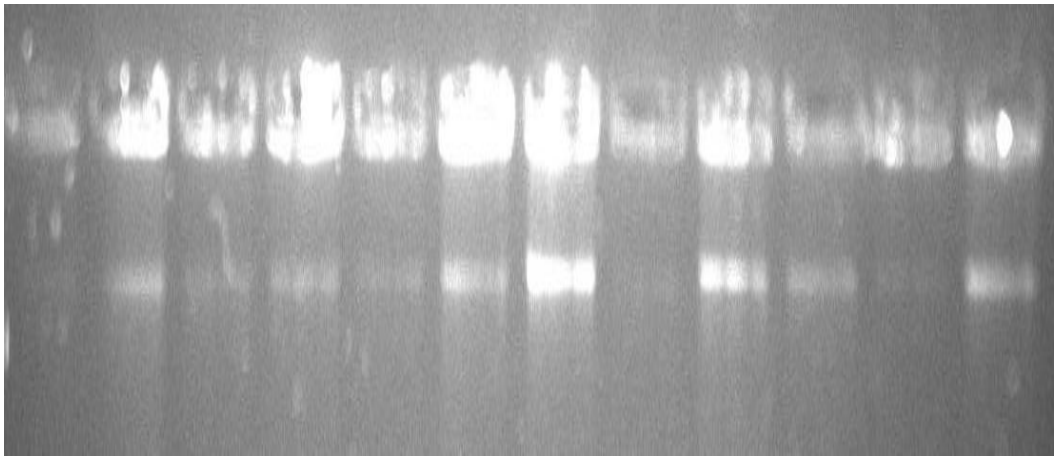

Figure 2 Analysis of DNA Agarose Gel Electrophoresis.

7. DISCUSSION

The present study deals with the isolation and detection of single nucleotide polymorphism from clinical human breast cancer samples. The clinical sample was collected from hospitals in Bangalore, India. The gene screened for SNP was BRCA2. To identify genetic defects of the BRCA2 gene that contribute to the development of breast cancer, we detected a previously reported PCR and RFLP.

Genomic DNA was extracted using Phenol-choroform extraction method and quantified at 260 nm in a Thermo Scientific Nanodrop 1000 spectrophotometer. The quality of the DNA was assessed in 0.8% agarose gel. The normal ratio of absorbance and wavelength for DNA should be 1.6 to 2.0, which indicates that the DNA is pure. If the ratio is above 2.0, it means the sample has RNA contamination, which can be removed by treating it with RNAse enzyme. If the ratio is below 1.6, it means the sample has protein contamination, which can be removed by treating it with ProteinaseK enzyme. Different concentrations of the DNA was found in different samples as displayed.

BRCA2 gene was amplified using BRCA2 forward primer (5’-CATCTGTTTTGATAGGTCTTAG -3’) and BRCA2 reverse primer (5’-CAGCGTTTGCTTCATGGAAA-3’) the amplified samples were incubated with specific restriction enzyme (HINF 1). Restricted samples were run on 2% agarose gel. The results were analyses and from the band patterns it was revealed that out of 22 samples, one sample was cut by restriction enzyme, indicating the mutation at specific site, hence among the Bangalore breast cancer patients; one patient was detected with SNP carrying exon-8 genotype. BRCA2 was found to be significantly associated breast cancer. SNPs can also be used to track the inheritance of disease genes within families. This study can be continued further for the

discovery of drugs for this mutation. SNPs have application in Genome Wide Association Studies, like as a high resolution marker in gene mapping related to diseases and normal traits. This study can be continued further for the discovery of drugs for this mutation.

8. Conclusions

1- Increased chance of being infected person who has a history of his family with the disease
2- We can diagnosis of his willingness to infection by detecting the gene even before the tumor be

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