Full Length Research Paper

Expression profiling of \textit{brca1} gene in familial breast cancer in India

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The incidence of breast cancer in India is on the rise. This was indicated by the recent report from Indian Council of Medical Research wherein it was reported that 1 in 22 women in India was likely to suffer from breast cancer in their life time. About 40 to 50\% of hereditary breast cancers and most of hereditary breast and ovarian syndromes are thought to be due to mutation in the breast cancer susceptibility gene (BRCA1).

**Key words:** Familial Breast Cancer, BRCA1, Q-PCR, India.

**INTRODUCTION**

Inheritance of a mutant \textit{BRCA1} or \textit{BRCA2} gene (numbers 113705 and 600185, respectively in Online Mendelian Inheritance in man, (a catalogue of inherited diseases) confers a lifetime risk of breast cancer of 50 to 85\% and a lifetime risk of ovarian cancer of 15 to 45\% (Miki et al., 1994; Wooster et al., 1995; Ford et al., 1994, 1998; Easton et al., 1995; Thorlacius et al., 1998). These germ-line mutations account for a substantial proportion of inherited breast and ovarian cancers (Szabo et al., 1997), but it is likely that additional susceptibility genes maybe discovered (Kainu et al., 2000; Seitz et al., 1997). Certain pathological features can help to distinguish breast tumors with \textit{BRCA1} mutations from those with \textit{BRCA2} mutations. Tumors with \textit{BRCA1} mutations are high-grade cancers with a high mitotic index, "pushing" tumor margins (that is to say, non-infiltrating, smooth edges), and a lymphocytic infiltrate, whereas tumors with \textit{BRCA2} mutations are heterogeneous, and often with relatively high grade, and display substantially less tubule formation. The proportion of the perimeter with continuous pushing margins can distinguish both types of tumors from sporadic cases of breast cancer (Lakhani et al., 1998). Tumors with \textit{BRCA1} mutations are generally negative for both estrogen and progesterone receptors, whereas most tumors with \textit{BRCA2} mutations are positive for these hormone receptors (Loman et al., 1998; Johannsson et al., 1997; Karp et al., 1997; Verhoog et al., 1998). These differences imply that the mutant \textit{BRCA1} and \textit{BRCA2} genes induce the formation of breast tumors through separate pathways.

The \textit{BRCA1} and \textit{BRCA2} proteins participate in DNA
repair and homologous recombination and probably other cellular processes (Welsh et al., 2000). A cell with a mutant BRCA1 or BRCA2 gene which therefore lacks functional BRCA1 or BRCA2 protein has a decreased ability to repair damaged DNA. In animal models, this defect causes genomic instability (Yu et al., 2000). In humans, breast tumors in carriers of mutant BRCA1 or BRCA2 genes are characterized by a large number of chromosomal changes, some of which differ depending on the genotype (Tirkkonen et al., 1997). To explore the expression patterns of BRCA1 gene in our population, we examined gene expression of BRCA1 in peripheral blood samples from patients with familial breast cancer (n = 25) and their first degree relatives (n = 33), in 20 families.

**Materials and Methods**

Here in, we reported the results obtained from our real-time PCR based expression profiling of peripheral blood cells for BRCA1 gene in 20 families of familial breast cancer patients within the age group of 35 to 65 years and their unaffected first degree female relatives irrespective of the age which were studied for BRCA1 gene expression profiling. From peripheral blood cells, a total of mRNA was isolated, quantified and expression patterns were analysed using real-time polymerase chain reaction (PCR). GAPDH was used as a house keeping gene. The gene expression levels were quantified using the delta-delta Ct method.

**Selection of Patients**

Data of 1039 breast cancer patients from year 2001 to 2009 was collected from Karnataka Cancer Therapy and Research Institute (KCTRI). The patient’s data were analyzed; familial breast cancer patients were identified based on the condition that each of them had at least one first degree relative affected with breast cancer (Phipps and Perry 1988). The criteria for the selection of familial breast cancer patients were based on earlier studies (Kumar et al., 2002). Out of these, 196 were identified as familial breast cancer patients. The frequency of familial breast cancer in this population was 18.86%. This is quite high when compared with the incidence of familial breast cancer globally. Convenience sampling was done and 20 familial breast cancer patients and their unaffected first degree relatives who were comfortable with the stated study have been included in this study.

**Clinical Sample (Blood) Collection**

Institutional ethical clearance was obtained from KCTRI. Consent was obtained from the subjects who were enrolled in the study. Peripheral blood samples were collected in PAXgene Blood RNA Tube (BD, Cat. No. 762165) and stored at -70°C.

**RNA Isolation and Quantification**

PAXgene blood RNA Kit (Qiagen Cat. No 762174) was used for isolation of total cellular RNA from human blood. Glassware was treated before use to ensure that it is RNase-free. Glassware used for RNA work was cleaned with detergent, thoroughly rinsed and oven baked at 180°C for >4 h (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases, but oven-baking will. Alternatively, glassware can be treated with diethyl pyrocarbonate (DEPC). Glassware was filled with 0.1% DEPC (0.1% in water) and allowed to stand overnight (12 h) at 37°C and then autoclaved or heated to 100°C for 15 min to denature residual DEPC. The isolated total RNA was quantified on a micro-volume UV and UV-Visible spectrophotometer (Q3000, Quawell)

**RT-PCR**

The high capacity cDNA Reverse Transcription Kit (Part no: 4368814, Applied Biosystems) was used for the quantitative conversion of total RNA in a single 20 μL reaction to single stranded cDNA. The kit includes Random Primers, optimized RT Buffer, dNTP’s and MultiScribe™ MuLV reverse transcriptase. First, 10 μL of 2 X RT master mixes was prepared by mixing 10X RT Buffer 2 μL, 25 X dNTP mixes (100 mM) 0.8 μL, 10 X RT Random Primers 2 μL, MultiScribe™ reverse transcriptase 1 μL, RNase Inhibitor 1 μL, Nuclease-free water 3.2 μL. To this mixture, 10 μL of RNA sample was added. The PCR was programmed to the thermal cycles of one step each at 25°C for 10min, 37°C for 120 min, 85°C for 5 min, and finally 4°C hold.

**qPCR**

Power SYBR® Green PCR master mix (Part no: 4367659, Applied Biosystems) was used for real-time PCR analysis using SYBR® Green 1 Dye. The 20 μL reaction mixture contains Power SYBR Green PCR master mix (2X) 10 μL, forward and reverse primers (Table 1), 1 μL (100 pmol), tamplet 1 μL (50 ng), and Nuclease-free water 7 μL. The PCR cycles included 1 step of initial denaturation for 95°C for 2 min, 40 cycles of 95°C for 15 s, Tm (53.7°C for BRCA1 primers and 58°C for GAPDH primers) for 45 s and 72°C for 30 s, followed by final elongation at 72°C for 5 min.

**Analysis**

The qPCR based BRCA1 gene expression results were analyzed for fold variation based on Ct values using the following formula:

\[ \text{Fold Variation} = 2^{(\Delta\Delta Ct)} \]

Where, ΔΔCt is the Ct (GOI) – avg. (Ct (HKG)); GOI is the gene of interest, and HKG is the housekeeping gene.

**Results and Discussion**

In this study, 20 Indian families with hereditary breast cancer were studied for BRCA1 gene expression profiling along with GAPDH as the house keeping gene. The qPCR based BRCA1 gene expression was examined in 25 familial breast cancer patients and 33 unaffected first degree female relatives. The fold variation changes showed that BRCA1 gene is down regulated in all the samples; affected familial breast cancer patients and their unaffected female first degree relatives (Table 2). This study suggested that BRCA1 down regulation plays a role in the development of familial breast cancer among the Indian women.Q-PCR based technology is a versatile technique that can be used in a rich diversity of approaches to help understand cancer development, improve patient treatment and management, and also to
identify those predisposed to developed cancer. The power of Q-PCR technology is that it allows global analysis of simultaneous gene expression. This can identify novel gene-gene interactions or enable the entire control pathways to be followed. Gene expression patterns could be integrated on one hand with clinical data to identify new markers to predict biological behavior of tumors, and on the other hand with data bases of drug sensitivity to unravel the molecular basis of drug action.

In this study, we analyzed 20 families with familial breast cancer background within the age group of 35 to 65 years. All the samples show down regulation of BRCA1 gene. According to Table 2, our results showed a similar gene expression pattern of down regulation of BRCA1 in affected familial breast cancer patients and their first degree relatives. In order to find out the prevalence of any specific biomarker, a large number of families need to be investigated. However, this study with 20 families suggested that BRCA1 down regulation does play a role in the development of familial breast cancer among the Indian women.

Along with BRCA1, other tumor suppressor genes like BRCA2, p53, ATM, CHK2, PTEN, Rb, p27, APC, p16, and oncogenes like HER-2, Ras, P13K, AKT, c-myc, c-fos, Cyclin D1, Cyclin E, may be analyzed to identify the exact role of BRCA1 in the development of familial breast cancer.

**Conclusions**

Our study demonstrated that BRCA1 gene is down regulated in all the familial breast cancer patients and their first degree female relatives. Down regulation of BRCA1 gene in first degree female relatives of the patients at the early stage of their life suggested that they
might be at high risk of development of breast cancer in the nearest future. This makes it necessary to screen a large number of families, perhaps within each group, in order to get a true picture of the contribution of BRCA1 gene expression to familial breast cancer and attempts for this are on in our laboratory.

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