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Structural and functional characterization of BAP1 human protein

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ABSTRACT

Cancer is a deadly disease in humans and arises from uncontrolled cell division. Cancer are broadly therefore, categorized into two types: primary cancers (in the organ or tissue where cancer originated) and secondary cancers (metastatic tumor). In 1990, the first breast cancer susceptibility gene BRCA1 was identified on chromosome 17q12-21 by linkage analysis of multiple families affected by early onset breast and ovarian cancer. BRCA1 is large spread over 80 kb genomic DNA composed of 24 exons, 22 codings and 2 non-coding exons that are transcribed into a 7.8 kb mRNA encodes a protein containing 1863 amino acids. The approximate molecular mass of the BRAC1 protein is 220 kDa. The BRCA1 gene bears no homology with other genes, with the exception of a RING finger motif at the amino-terminal end. In other proteins such a motif has been shown to interact with nucleic acids and to from protein complexes, suggesting a role of BRCA1 in transcription. Nuclear localization sequence in exon 11, and a conserved acidic carboxy terminus, the BRCT (BRCA1 carboxyl terminal) domain. Till date, more than 800 different mutations mostly frame shift mutations in the BRCA1 gene have been reported. BRCA1 gene contains N-terminal RING finger domain and two C-terminal BRCT (BRCA1-C-Terminal) domain, both involved in protein-protein interactions. Exon 11 of BRCA1 gene contains over 60% of protein and encodes two putative localization signals, also contain a domain that interacts with RAD51, a homology of *E. Coli* rec. a involved in DNA damage repair. BRACA1 was associated and co-purified with RNA polymerase complex, and interacts with RNA helicase in a transcription Germline mutation in BRCA1 has been detected in approximately 80-90% of familial breast/ovarian cancer and about 45-50% of familial breast cancer alone.

Keywords: Heteroresistance, Multidrug resistant tuberculosis, Extensively drug resistant tuberculosis, rpoB gene

INTRODUCTION

Cancer is a deadly disease in humans and arises from uncontrolled cell division. Cancer are broadly therefore, categorized into two types: primary cancers (in the organ or tissue where cancer originated) and secondary cancers (metastatic tumor). Depending upon what types of normal cells are

converted into cancerous cells, cancers may also be classified into the following different types:

a. Carcinoma: cancer that begins in the skin cell or in the outer lining of visceral organs such as the liver or Kidney
b. Sarcoma: cancer that arises from altered cells of mesenchymal origin
c. Leukemia: cancer of the blood or bone marrow
d. Lymphoma: cancer of the lymphocytes
e. Myeloma: cancer of plasma cells from the bone marrow
f. Mixed types: include the tumours developed from different tissues.

Cancer can be in the form of either a solid tumour or a liquid/ "soft" tumour that arises from alteration in specific regions of genes. The genetic alterations may be due to external factors such as radiation, carcinogens, or due to internal factors such as deficiencies in the DNA damage repair mechanisms within the cells (Arver *et al*., 2000). The genes which are altered or mutated in cancer are described as cancer-susceptibility genes. The cancer genes can be divide into three major categories: gatekeeper genes, caretaker genes and landscaper genes (Schwartz and Torre, 1995). Gatekeeper genes are responsible for regulation of cell growth and differentiation, and these include tumor suppressor genes and oncogenes (Li *et al*., 2000). Caretaker genes maintain the genomic integrity of the cell, and when affected, they are indirectly responsible for cancer progression (Welcsh and King, 2001). Landscaper genes indirectly affect cancer progression by controlling cellular environment around the tumour.

Breast cancer (BC) is the third largest public health problem worldwide and incidence of breast cancer in India is around (17.2%) of all the cancers (Welcsh and King, 2001). In developed countries, one in ten women is diagnosed with BC, while in developing countries, the rates are slightly lower, but the incidences are increasing day by day (Atlas *et al*., 2000). The most prominent and well established risk factors for BC are early menarche (beginning of menstrual function) and late age at first childbirth.

Breast cancer (BC) can be divided into two categories, sporadic BC and hereditary BC. The genetic changes in sporadic BC are due to external factors while they are inherited in hereditary BC. Hall *et al* (1990) studied and identify linkage analysis of the breast cancer susceptibility gene. The gene was found on the long arm of human chromosome 17 (Ruffner *et al*., 1999); this gene was subsequently named BRCA1 (Ruffner *et al*., 1999); BRCA1 is known to act as a tumour suppressor gene because in more than 90% of breast cancer cases, this gene is found to be mutated (Xu *et al*.,1999).

BAP1 is a member of the UCH family which possesses a conserved catalytic domain containing histidine, cysteine, and aspartic acid catalytic triad (Kauraniemi *et al*., 2000). The gene BRCA1 is about 100 kb long and comprises of 24 exons, of which exon one is non-coding while exon four has Alu repeats (transposable element) (Scully *et al*., 1997). The coding region begins in exon 2. Exon 11 constitutes more than 60% of the coding region, while most of the other exons are relatively small (Miki *et al*., 1994). BRCA1 encodes a 7.8 kb mRNA that codes for a BRCA1 protein having 1,863 amino acids a nuclear phosphoprotein of 220 kDa (Chen *et al*., 1996a). BRCA1 has two distinct domains, N-terminal RING finger domain and C-terminal BRCT domain (Saurin *et al*., 1996). In addition, BRCA1 has two nuclear localization signals in exon 11 (Chen *et al*., 1995). These signals help the protein to cross the nuclear membrane easily (Callebaut and Mornon, 1997). Carboxyl terminal domain of BRCA1 is transactivation domain of much transcription factors (Abel *et al*., 1995). And contains two domains, which are situated in tandem consisting of amino acids 1646-1736 and 1760-1855. BRCA1 is conserved in mammals but not in lower animals (Wang *et al*., 2000). On analyzing BRCT domain of BRCA1 it acts as a phosphopeptide recognition sequence. The basic function of the BRCT domain is to act as a protein-protein interaction. In breast and ovarian cancer most of the mutations are found in the BRCT and RING finger domains of BRCA1. Khurshid *et al* (2005) reported that efficient differences in the model and the X-ray structure were situated at some of the important sites. Further, they analyzed the active centre of the enzyme and other sites that are involved in DNA repair. They explained that amino acid bind the bases located in the major/minor grooves of DNA. An insertion of Arg 176 in the Major groove and Met270 in the minor groove caps the DNA bound enzymes's active site, stabilizing the extra helical AP site conformation and effectively locking the protein onto the AP-DNA. Three BAP1 mutants were also modeled and analyzed as regards the changes in the structure. Substitution of Arg 176--> Ala leads to the loss of DNA binding whereas mutation of Asp282--> Ala and His308--> Asn leads to a decreases in the enzymatic activity (Khurshid *et al*., 2005).

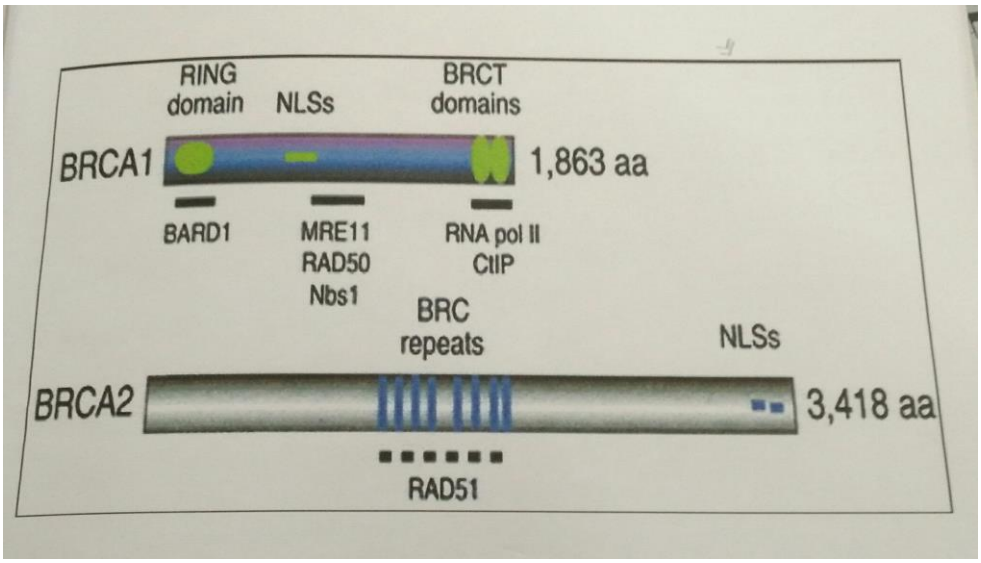

Figure 1: Structure analysis of the human BRCA1 protein. (Venkitaraman, 2002).

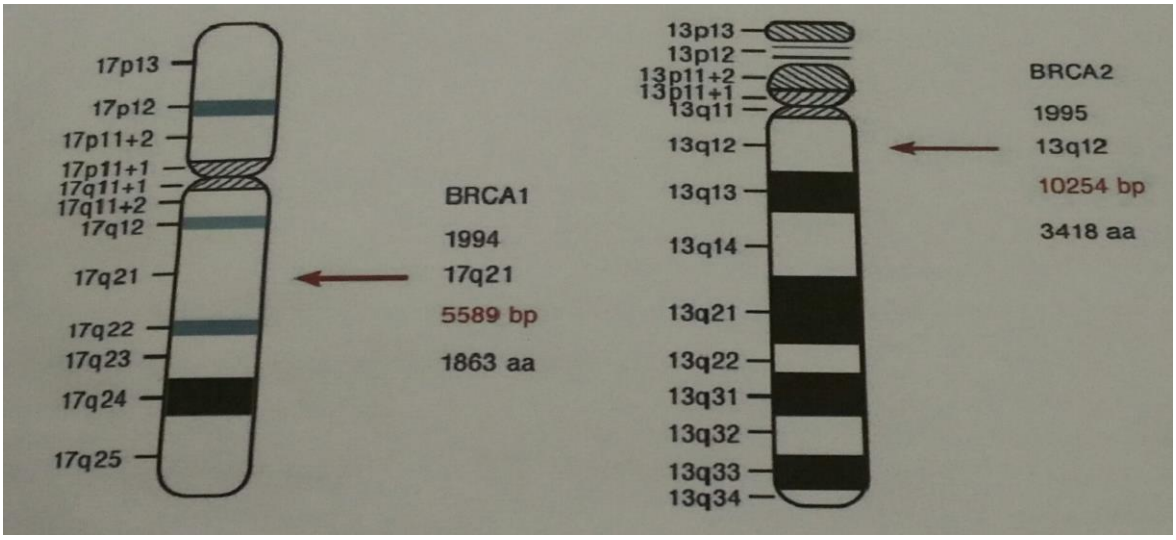

Figure 2: Tumour suppressor genes BRCA1 and BRCA2

(BRCA1 contains an N-terminal RING domain, nuclear localization signals (NLSs), and two C-terminal BRCT domains of ~110 residues also found in several proteins with functions in DNA repair or cell cycle control.

BRCA2 contains eight repeats of the ~40 residue BRC motifs. Six of the eight motifs in human BRCA2 can bind directly to RAD51 when expressed in vitro).

Mechanism of BRCA-mediated breast cancer formation

In 1990, the first breast cancer susceptibility gene BRCA1 was identified on chromosome 17q12-21 by linkage analysis of multiple families affected by early onset breast and ovarian cancer. BRCA1 is large spread over 80 kb genomic DNA composed of 24 exons, 22 codings and 2 non-coding exons that are transcribed into a 7.8 kb mRNA encodes a protein containing 1863 amino acids (Wooster *et al*., 1994). The approximate molecular mass of the BRAC1 protein is 220 kDa (Gudmundsson *et al*., 1995). The BRCA1 gene bears no homology with other genes, with the exception of a RING finger motif at the amino-terminal end. In other proteins such a motif has been shown to interact with nucleic acids and to from protein complexes, suggesting a role of BRCA1 in transcription. Nuclear localization sequence in exon 11, and a conserved acidic carboxy terminus, the BRCT (BRCA1 carboxyl terminal) domain. Till date, more than 800 different mutations mostly frame shift mutations in the BRCA1 gene have been

reported. BRCA1 gene contains N-terminal RING finger domain and two C-terminal BRCT (BRCA1-C-Terminal) domain, both involved in protein-protein interactions. Exon 11 of BRCA1 gene contains over 60% of protein and encodes two putative localization signals, also contain a domain that interacts with RAD51, a homology of *E. Coli* rec. a involved in DNA damage repair. BRACA1 was associated and co-purified with RNA polymerase complex, and interacts with RNA helicase in a transcription (Futreal *et al*., 1994) Germline mutation in BRCA1 has been detected in approximately 80-90% of familial breast/ovarian cancer and about 45-50% of familial breast cancer alone (Futreal *et al*., 1994).

BRCA2 was the second gene identified as breast and/ovarian cancer susceptibility gene to be discovered (Knudson, 1971). BRCA2 contains eight repeats of the ~40 residue BRC motifs. Six of the eight motifs in human BRCA2 can bind directly to RAD51 when expressed in vitro. BRCA2 plays an important role in the error-free repair of DNA double strand breaks as well as transcriptional regulation (Beger *et al*., 2001). In normal cells, BRCA2 ensures the stability of the DNA and helps to prevent uncontrolled cell growth. BRCA2 mutations have been characterized in different populations worldwide, with significant variation of the relative contribution of these genes to hereditary cancer between populations. (Beger *et al*., 2001) Various population-based studies have shown population specific BRCA2 founder mutations and also variable number of novel mutations in different populations, and thus have defined high and low risk subsets for developing breast cancer based on ethnic origin (Esteller *et al*., 2000).

MATERIALS AND METHODS

The strategies used in this study were as follows:

1. Transformation of clone in to *E. Coli* (BL21) strain

Full-length BRCA1 gene (cDNA) inserted into PGZ1KT vector containing ampicillin resistant marker and TEV endonucleases restriction sites were provided by ACTREC Mumbai. There are 500 µ1 of BL21 (*E. Coli*) glycerol stock was inoculated into 5ml of LB medium and cells were grown at 37^0C, shaking at 200 rpm to an optical density (OD) of 0.4-0.6 at 590 nm. 1.5ml of culture was centrifuged atv13000 rpm for 1min at 4^0C and suspended in 500µ1 of ice cold 50 mM $CaCl_2$. After 30 min incubation on ice, the cells were centrifuged and the pellet was suspended in 100 µ1 of ice cold 50mM $CaCl_2$. (1µ1 (BAP1 gene cloned in PGX 1KT vectors)-(1 mg/µ1) was added to the cells and incubated on ice for 30min). The cells were heat shocked at 42^0C for 90 seconds and then incubated on ice for 2min. 1ml of LB medium was added onto the cells and incubated at 37^0C for 1h to allow the expression of antibiotic resistance gene before plating the cells. After incubation, the transformation mixture was plated on LB agar plates containing 100µg/ml ampicillin and IPTG. Plates were incubated overnight at 37^0C to select the recombinant clones carrying the gene of interest. (Collins,1995). The white colonies were picked up and grown in LB medium containing 100µg/ml ampicillin. The bacterial culture was directly used as for DNA extraction.

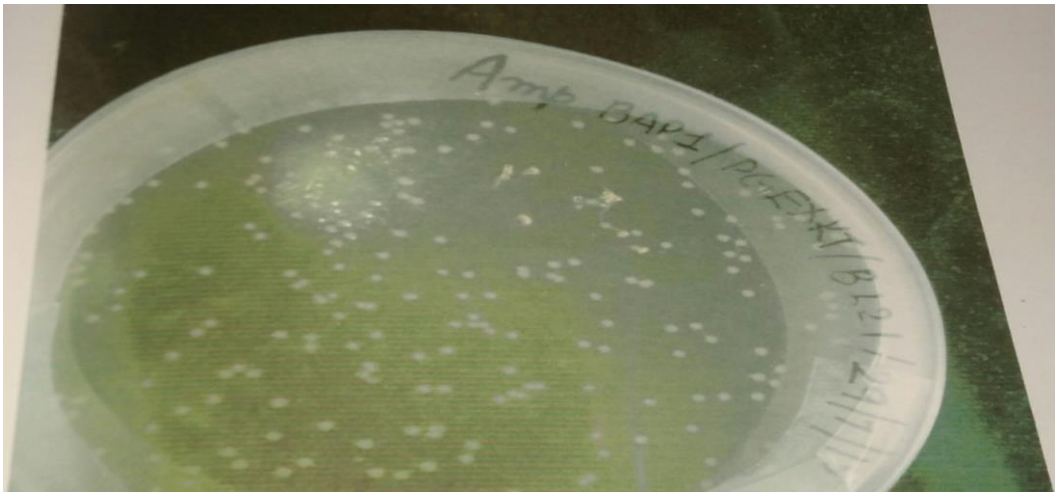

Figure: 3 - Amp BAP1/PGEXKT

2. Identification of clone:

The plasmid DNA from recombinant bacterial clone was isolated using protocol based on the alkaline Lysis method (Birnboim and Doly, 1979) with minor modifications. Restriction enzyme digestions were performed in 15-50 reaction volumes containing 1-10µg plasmid DNA. Reactions were carried out with the appropriate reaction buffer, according to the manufacturer's recommendations. DNA fragments were fractionated by horizontal gel electrophoresis by using 1XTAE buffer and 5x gel loading buffer. On digesting the plasmid with restriction enzymes $(TEV)_2$ run the digested material on 1% agarose gel visualized under trans-illuminator.

There are two different bands were observed one is 5kb (vectors) and other is 1kb (insert) as shown in the figure.

3. Sequencing of clone:

For further confirmation of sequence of gene of interest DNA fragments were extracted from TAE agarose gels by using the QIAEX II gel extraction kit according to the manufacturer's instructions. Gel purification with the QIAEX II kit yields 60-70% recovery of DNA fragments between 1.0 kb to 5.0 kb in 10-20µ1 volumes. Extracted DNA was sequenced using next generation sequencer and analyzed with the Genbank/EMBL and Expressed sequence Tag (EST) databases. Homology search of the sequenced gene that came out from this study was mainly performed by comparing the data with gene bank. This result was satisfactory since construct was cloned correctly according to the sequencing results.

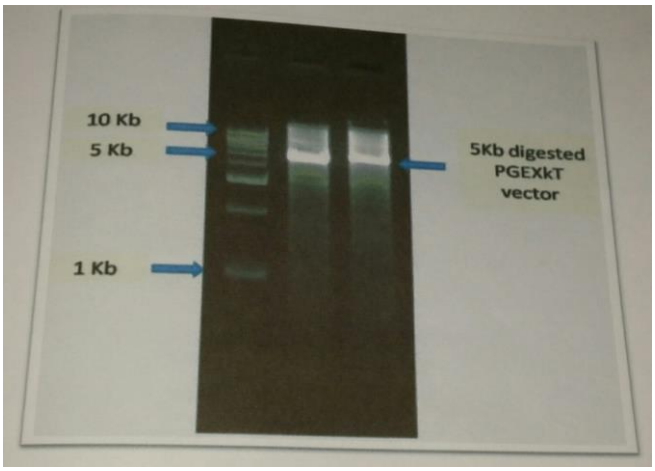

Figure: 4 - Digestion of clone using TEV enzymes

Purification of expressed proteins:

After conformation of correct clone using DNA sequencing, transformed bacterial cells were lysed using lysis buffer (50mM Tris (pH-7.5) 300mM NaCl containing 1mM PMSF and 1x protease inhibitor to the solution volume), 20ml/ lit culture medium. Cells were sonicated at 50-60 pulse for 1min for 5-7 times. After sonication. The cells were centrifuged at 18,000 rpm for 45 min at 4^0C to remove the insoluble proteins and cell debris. Supernatant were taken and 20µ1 of samples were loaded on GST resin calibrated with distilled water followed by lysis buffer. Expressed proteins were trapped inside the column with GST beads. To obtain the desired proteins TEV enzyme was added to the GST beads and incubates for 2-3 hrs. Eluted fraction were collected and analyzed by SDS-PAGE.

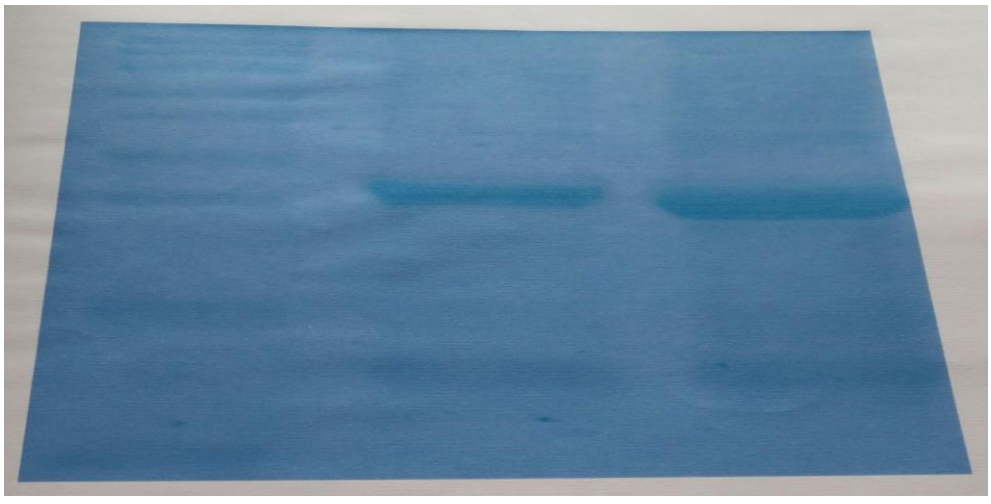

Figure: 5 SDS-PAGE of purified protein

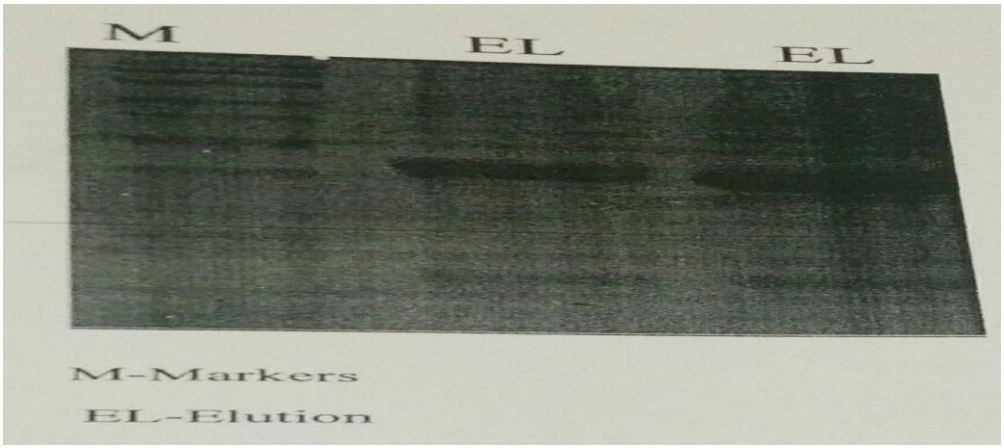

Figure: 6 SDS-PAGE of purified proteins

Analysis of protein bands using SDS-PAGE

Proteins profiling of samples was performed using SDS-polyacrylamide gels as described by Laemmli, (1970). One hundred fifty micrograms (150µg) of proteins obtained from purification along with protein molecular weight marker (SM0671, Fermentas) were loaded into 12% gels. Electrophoresis was performed at constant voltage (100 volts). Staining of gels was done in 0.025% coomassie Brilliant blue 250 containing 40% methanol and 7% acetic acid, while distaining was done in the same solution without dye.

On analyzing the SDS-PAGE results multiple bands of proteins are observed showed the variation in the molecular weight of the polypeptides. The major components of all the polypeptides were in the molecular weight range of 95 to 11 kDa, with the variation in relative mobility values.

MATERIALS:

General reagents

The general laboratory chemicals were supplied from Sigma chemical Co.(st. Louis, U.S.A)

Nucleic Acids and proteins

There are 100 bp DNA Ladder used in this study were supplied from MBI fermentas and protein markers were from Bio-Rad (U.S.A.)

Bacterial strains

The bacterial host used in this study was *E. Coli* (BL21). Bacterial strains were stored at-70^{o}C in LB medium containing 50% (v/v) glycerol for long-term storage. Recombinant clones were stored under the same conditions in media supplemented with appropriate antibiotics. Strains were maintained as isolated colonies on LB agar plated at 4^{o}C for short-term storage.

Plasmids

PGX1KT BRCA1clone was constructed by cloning the full-length BRCA1 (cDNA) insert into PGX1KT (provided by ACTREC Mumbai).

Kits

Gel extraction Kits were purchased from Qiagen (Germany). Restriction endonucleases were supplied by fermentas.

Solutions and Media:

General solutions

1XTAE: 40 mM Tris-acetate, 2mM EDTA (pH 8.0)

TE: 10mM Tris, 1 mM EDTA (pH 8.0)

Ethidium bromide: 10mg/ml in water (stock solution), 30 mg/ml (working solution)

5x agarose gel loading buffer: 0.25% bromophenol blue, 0.25% xylene cyanol, 50% glycerol, 1 mM EDTA.

Solutions for plasmid DNA isolation:

Solution I: 50 mM glucose, 25 mM Tris pH: 8.0, 10 mM EDTA

Solution II: 0.2 N NaOH, 1% SDS

Solution III: 3M potassium acetate, pH 4.8

Microbiological media and antibiotics

LB broth: 1% tryptone, 0.5% yeast extract, 1% NaCl

LB agar plates: LB+2% agar

IPTG: 0.5 M solution in water, filter sterilized and stored at-20^{0}C in the dark (working solution 2.5 mM) X-Gal: 250 mg/ml solution in DMF (stock solution) 6.5 mg/ml (working solution) Ampicillin: 100 mg/ml solution in water sterilized by filtration and stored at-20^{0C}.(working solution 100 µg/ml)

Protein Separation:

Acrylamide-Bisacrylamide solution: 29 gram acrylamide,1gram bisacrylamide were mixed make up the final volume up to 100 ml with ddH_2O and stored in dark bottle. 10% APS: 0.1 gram APS dissolved in 1ml ddH_2O.

5x loading buffer: 62.5 mM Tris-HCl pH 6.8, 5% mercaptoethanol, 2% SDS, 15% glycerol, and 0.001% bromophenol blue

5x running buffer: 125 mM Tris, 1.25 M glycine, 0.5% SDS; pH 8.3

General methods

Transformation of E.coli

500µ1 of BL21 glycerol stock was inoculated into 5ml of LB medium and cells were grown at 37^0C, shaking at 200 rpm to an optical density (OD) of 0.4-0.6 at 590nm. 1.5ml of culture was centrifuge at 13000 rpm for 1min at 4^0C and suspended in 500µl of ice cold 50mM $CaCl_2$. After 30 min incubation on ice, the cells were centrifuged and the pellet was suspended in 100µ1 of ice cold 50mM $CaCl_2$. 1µ1 of plasmid DNA (1mg/µ1) was added to the cells and incubated on ice for 30 min. The cells were heat shocked at 42^0C for 90 seconds and then incubated on ice for 2min. 1ml of LB medium was added onto the cells and incubated at 37^0C for 1h to allow the expression of antibiotic resistance gene before plating the cells. After incubation, the transformation mixture was plated on LB agar plates containing 100µg/ml ampicillin. Plates were incubated overnight at 37^0C to select the recombinant clones carrying the plasmid of interest. (Sambrook *et al*., 1989).

Table 1: Total Reaction Volume

DNA	7µl
10X FD Buffer	2µl
TEV enzyme	1µl
Total reaction volume	10µl

Digestion of insert and vectors:

Separated DNA fragments were digested with TEV enzyme and again separated using 1% agarose. On analyzing the bands we get two fragment 1 kb and 5kb. One kb fragment is gene of interest and 5 kb fragment is vector. Again 1kb bands were extracted using gel extraction kit and subjected for sequencing.

A typical Restriction digestion reaction of insert +vector The reaction was set up and incubated at 37^0C for 1.5 hr. After incubation the contents were loaded on1% agarose.

Automated DNA sequencing:

Gene of interest (1kb) was eluted from agarose gel producing consistent banding patterns eluted using a gel extraction kit procured from Qiagen, Germany. The eluted DNA was cloned into pGEM®-T easy vector promega, USA following the manufacturer's instruction. Sequencing were carried out with Next Generation Sequencer. Nucleotide sequences were analyzed with the Genbank/EMBL and Expressed sequence Tag (EST) databases. Homology search of the sequenced clones that came out from this study was mainly performed by BLAST search, available for public use at http://www.ncbi.nlm.nih.gov/Blast.

Calibrated with distilled water followed by lysis buffer. Expressed proteins were trapped inside the column with GST beads. To obtain the desired proteins TEV enzyme was added to the GST beads and incubates for 2-3hrs. Eluted fraction were collected analyzed by SDS-PAGE.

PROTEIN PROFILING USING SDS-PAGE:

PERFORMING SDS-PAGE

Table 2: Preparation of 12% separating gel (10ml) was as follows

H_2O	3.2 ml
30% Acrylamide mix	4.0 ml
1.5 M Tris, pH: 8.8	2.6 ml
10% SDS	0.1 ml
10%APS	0.1 ml
TEMED	10ml

Table 3: Preparation of 5% stacking gel (10ml) was as follow.

H_2O	6.8 ml
30% Acrylamide mix	1.7 ml
1.5 M Tris, pH: 8.8	1.25 ml
10% SDS	0.1 ml
10%APS	0.1 ml
TEMED	10ml

RESULTS

Cancer is an unpredictable and uncertain disease that can create heightened vulnerability for many individuals. Breast cancer has emerged as the most common cancer in women and the most common cause of cancer death in women. The life-time risk of developing breast cancer is found to be 1:30 in urban India and 1:65 in rural India as compared to 1 in 8 in the USA (Mello *et al*., 2002). The data obtained from Tata Memorial Hospital, Cancer hospital in Mumbai of the undergoing treatment for cancer revealed that about 60% of them have early breast cancer, 35% with advanced stage and 5% have the disease spread to other organs (Monteiro *et al*., 1996).

However, the survival rate of breast cancer has increased in recent time due to the advanced technology available for early detection, effective treatment and medical care delivery. It enables the victims to live longer and enhances their quality of life.

BRCA1 and BRCA2 are genes known to be associated with breast cancer. Women who Inherite a faulty BRCA1 or BRCA2 gene have an increased risk of breast cancer. Carrying a faulty gene does not necessarily mean that the woman will get breast cancer; a mutation of the gene in the breast is what causes to develop the breast cancer. In the general population it is estimated that 1 in 800 women carries faulty BRCA1 gene. Nishikawa *et al* (2009) reported that BAP1 interacts with BARD1 to inhibit the E3 ligase activity of BRCA1/BARD1. Domain comprised by residues 182-365 of BAP1 interact with the ring finger domain of BARD1. They performed surface plasmon resonance spectroscopy (BIA core) and the result showed that BAP1 interferes with the BRCA1/BARD1 association. The perturbation resulted in inhibition of BRCA1 auto ubiquitination and NPM1/B23 ubiquitination by BRCA1/BARD1. Significantly, inhibition of BAP1 expression by short hairpin RNA resulted in hypersensitivity of the cells to ionizing irradiation and in retardation of S-phase progression. On the basis of result obtained they have suggested, BAP1 and BRCA1/BARD1 co coordinately regulate ubiquitination during the DNA damage response and the cell cycle

(Nishikawa *et al*., 2009). Machiada *et al* (2009) reported that growth inhibitory activity is possessed by the deubiquitinating enzyme BRCA1 associated protein 1(BAP1). It functions as a tumour suppressor. They also reported that BAP1 play significant roles in cell proliferation. BAP1 depletion by RNAi inhibits cell proliferation and dose over expression of a dominant negative mutant of BAP1. They performed mass spectrometry and analysis of co purified protein revealed that BAP1 is associated with factors involved in chromatin modulation and transcriptional regulation. They showed the interaction with host cell factor-1, a cell-cycles regulator composed of host cell factor-1N and host cell factor-1C, is critical for the BAP1 mediated growth regulation. The significance of the BAP1 hot cell factor-1 interaction is underscored by the fact that growth suppression by the dominant negative BAP1 mutant is entirely dependent on the host cell factor-1binding motif. These results suggest that BAP1 regulates cell proliferation by deubiquitinating host cell factor-1(Machiada *et al*., 2009). Cabone *et al* (2013) have characterized the germ line BAP1 mutations cause a novel cancer syndrome, at least in the affected families which have been studied so far, by the onset at an early age of benign melanocytic skin tumors with mutated BAP1, and later in life by a high incidence of mesothelioma, uveal melanoma, cutaneous melanoma and possibly other cancers.(Carbon *et al*., 2013). So study and characterization of these genes are necessary for cancer detection. In this study the cloning of BRCA1 paved the way for the start of a large number of large number of large number of studies aimed at defining the nature of the gene and its interactions. First, molecular biology studies were undertaken to characterize the BRCA1 protein and the pathway(s) in which it functioned.

As previously described, obtaining this information is a priority because defining the pathway of breast cancer carcinogenesis could potentially allow for the development of specially-targeted preventative or therapeutic measures. Second, genetic epidemiological studies were undertaken to better define the frequency of BRCA1 mutations in the population and to determine the risk of cancer faced by carriers of such mutations. This information is useful, in part, because it can allow accurate genetic counselling for at-risk individuals. Third, studies were undertaken to evaluate the efficacy of possible treatment aimed at preventing cancer development in mutation carriers. Finally BRCA1 accounted for only a percentage of hereditary breast cancer cases, it was necessary to continue the search of other genes for breast cancer. So in this study we attempted to characterized the BRC1 gene and function.

DISCUSSION AND CONCLUSION

Among cancers, breast cancer has become an important cancer with significant psycho-social impacts. Though advanced diagnostic procedures and treatment breast cancer can be cured. Socio-cultured background and stigma associated with cancer further influence the women's harrowing experiences. Financial strain of treatment cost either delay the treatment of breast cancer affects the continuity. On the other hand, effect of treatment procedures cause physical disability, body image disturbance and psychological distress. Role-changes and economic burden caused by the disease put major stress on the patient's family and affect family relationship. Family may avoid its social network because of the fear of disclosure of cancer to others and the subsequent alteration of body image. Women from poor economic strata have to spend a major portion of their earning and saving for surgery and continuous medical treatment; hence, breast reconstructive surgery or prosthesis is a distant dream for them. Being chronic cancer patients, they depend on their families for long-time psycho-social support and help. Therefore, a study of this nature is not only necessary to dwell at length the biological, psychological and sociological situations of breast cancer survivors but also important to suggest suitable steps for reducing and relieving stress so as to enhance the coping strategies among the patients and their families. BAP1 first showed as a tumor suppressor in cultured cells, where deubiquitinase domain and nuclear localization sequences were required for BAP1 to suppress cell growth. (Ventii *et al*., 2008). It possesses growth inhibitory activity and functions as tumor suppressor. It plays an important role in cell proliferation. BAP1 mutation were identified in small number in breast and lunges cancer cell lines.

In this study we attempted to characterize the expression of BARC1 gene for further analysis of structure and function. Cloned, were provided by ACTREC Mumbai. On expression and analyzing the protein bands we found a multiple bands of proteins, not the single bands. Our interest of protein was also present in the gel it is 33kD so it needs more purification or either due to the GST tag problem or any other practical error. On the other hands our transformations result are accurate and size of insert is same as BRC1 gene. So it further need to be analyzed using more appropriate techniques like separation using nickel chrome which is also mentioned in literature.

Competing interests:

None of the authors have an association that poses any conflict of interest. The funders had no part in the decision to publish the manuscript.

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