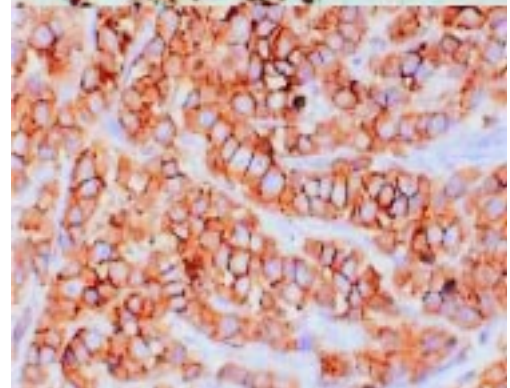


Breast Cancer

BREAST cancer is emerging as a leading women cancer in metropolitan cities like Delhi and Mumbai. In India, cancer of breast is second most common cancer in women after the cervical cancer with annual incidence exceeding 80,000. A number of well recognized exogenous and endogenous risk factors have been associated with the genesis of breast cancer. The exogenous factors include high fat diet, lack of physical activity, alcohol abuse, cigarette smoking, socio-economic status, environmental exposures to pollutants, pesticides, electromagnetic field and ionizing radiations. Among the endogenous factors, the duration of exposure to steroid hormones play a vital role. This in turn depends on several factors such as early age of menarche, late menopause, late pregnancy, nulliparity, and obesity. Presence of strong family history of breast and/or ovarian cancer is also one of the major endogenous risk factor for breast cancer. Both endogenous and/or exogenous factors ultimately cause structural or functional alterations in cellular genes resulting in development of breast cancer. Recently, two major dominantly inherited genes have been identified are BRCA1 and BRCA2 predisposing to breast cancer. BRCA1/BRCA2 genes have been linked to almost 45% of sporadic breast cancers and about 90% breast and ovarian cancer families. However, the role of these genes in sporadic breast cancer is still unclear. In addition, tumor suppressor gene p53 has been observed mutated in about 25–30% of sporadic cancers. In view of the paucity of comprehensive study on BRCA1, BRCA2 and p53 genes in Indian breast cancer patients, present studies have been designed to detect and characterize *BRCA1*, *BRCA2* and *p53* gene mutations, their expression profile and regulation of expression by transcription factors, NF- κ B and AP-1, in sporadic and breast cancer patients and these findings are being correlated with the clinico-pathological variable of the disease.

In addition, ICPO is working out to develop the use of Fine needle aspiration cytology and automated image cytometry to measure various morphometric parameters on cytology smears to differentiate benign lesions from malignant lesions of the breast.

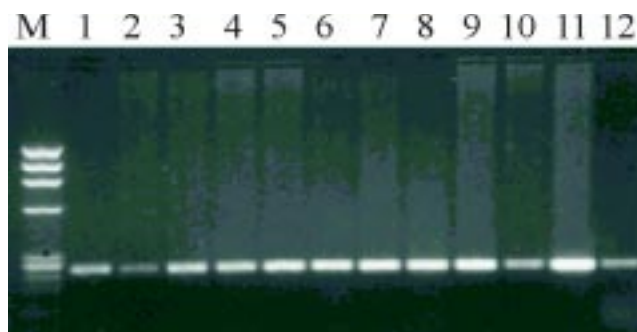


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Study of *BRCA1*, *BRCA2* and *p53* Gene Mutations and Expression in Human Breast Cancer Cells

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<i>Co-Investigators</i>	: Suresh Bhambani Suresh Hedau Alok C. Bharti
<i>Students & Staff</i>	: Neeraj Jain Rakesh Kumar Rashmi Sapru
<i>Collaborators</i>	: Ravi Kant , MAMC & LNJP Hospital, New Delhi Syad Akhtar Husain , Jamia Milia Islamia, New Delhi S.K. Kochar , Pawan Gupta , Dharamshila Cancer Hospital, New Delhi

LIKE other cancers, breast cancer has a strong genetic basic and recently two breast cancer susceptibility genes, *BRCA1* and *BRCA2* have strongly been implicated in the development of breast and ovarian cancer. It has been suggested that *BRCA1* gene is altered in the structural level of hereditary breast cancer and at transcriptional level in sporadic breast cancer. Breast cancer is also known to be influenced by steroid hormones, which induce transcription factors such as AP-1 that modulate genes responsible for cellular growth and differentiation. Genetic alteration in breast cancer genes, *BRCA1* and *BRCA2*, cause 5 to 10% inherited predisposition to breast cancer, but they may be responsible for 80–90% of multiple case of breast cancers. The life-time risk of developing breast cancer approaches to 90% in those women who carry mutations in *BRCA1* and *BRCA2* genes. But, in India, no information is available on the status of breast genes *BRCA1* and *BRCA2* and their incidence, type of mutation, expression and



PCR amplification of exon 2 of *BRCA1* gene showing amplicons of 258bp; Lane M– Hae III digested λ X 174 molecular weight marker; Lane 1-12: Breast cancer tumor samples

Sequence variations detected in the *BRCA1*, *BRCA2* and *p53* tumor suppressor gene in sporadic breast cancer

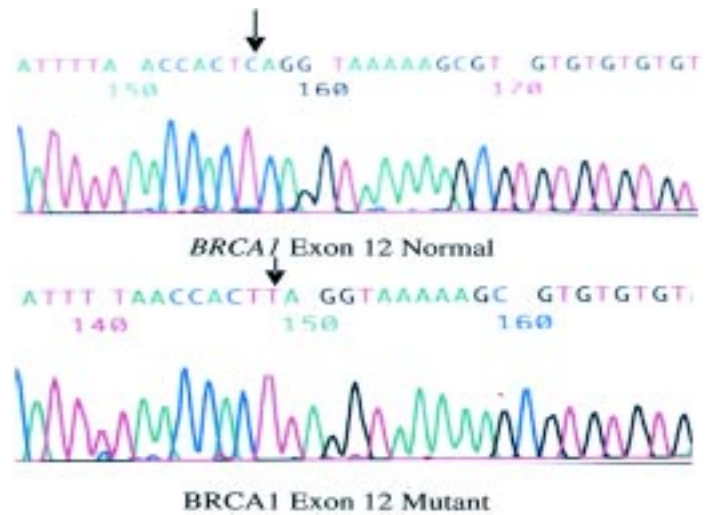
Genes	Exon	Codon	Nucleotide change	Amino acid change	Type of mutation
<i>BRCA1</i>	2	3	A128G	Leu→ Ser	Transversion silent
	2	4	T131G	Leu→ Ser	Transition silent
<i>BRCA2</i>	2		G292C	Ala→ Pro	Transversion missense
	2		C282G	Arg→ Arg	Transversion silent
<i>p53</i>	5	175	G775A	Arg→ His	Transition missense
	5	175	G775A	Arg→ His	Transition missense
	5	157	G720T	Val→ Phe	Transversion missense

Sequence variations detected in the *BRCA1* and *p53* tumor suppressor gene in familial breast cancer

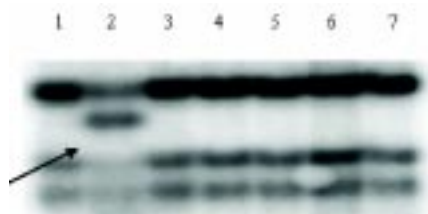
Genes	Exon	Codon	Nucleotide change	Amino acid change	Type of mutation
<i>BRCA1</i>	7	110	A448C	Lys→ Thr	Transversion missense
	7	114	T459C	Ser→ Pro	Transition missense
	12	1395	C4302T	Glu→ Stop codon	Transition nonsense
	16	1621	4956insG	TGA at codon 1621	Frameshift
<i>p53</i>	4	72	G540A	Val→ Ile	Transition missense



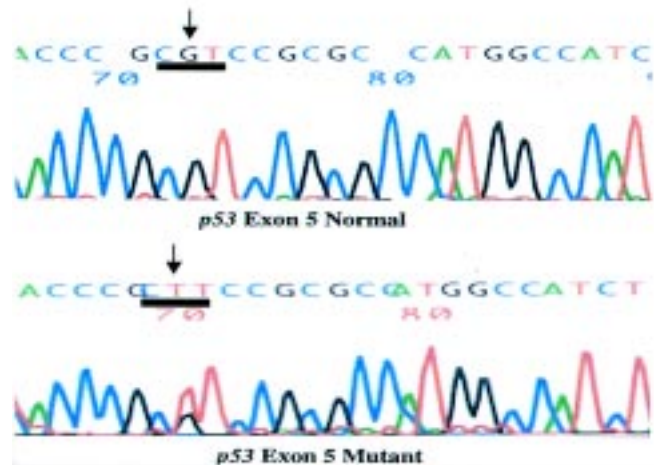
SSCP analysis of exon 2 of *BRCA1* tumour suppressor gene in breast tumors. Arrow showing altered band mobility indicating of presence of a mutation



Automated DNA sequencing showing normal and mutant sequence of exons 12 of *BRCA1* gene revealed CAG→TAG mutations respectively

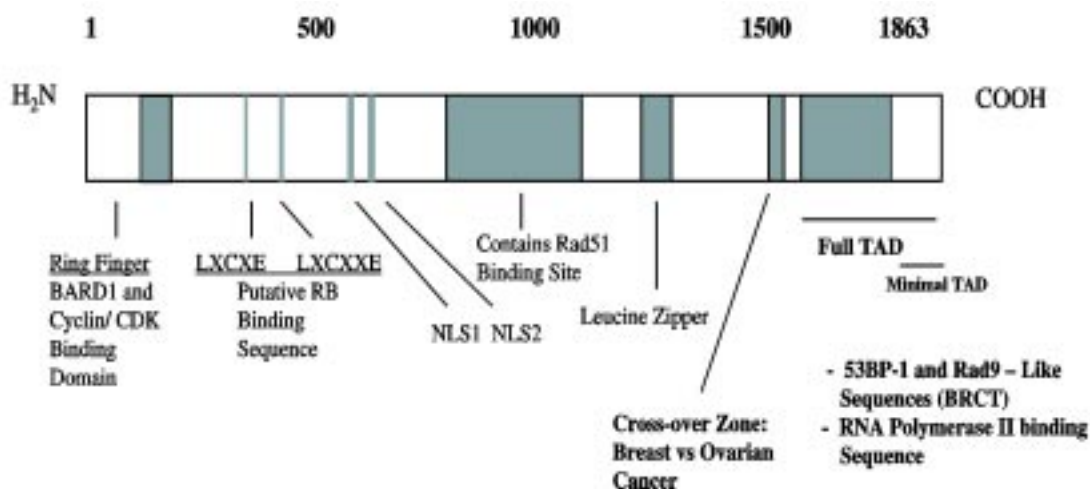


Representative PCR-SSCP of the *p53* exon 4 in familial breast cancer specimens showing altered band mobility at lanes 2 marked by arrows that indicates presence of *p53* gene mutation

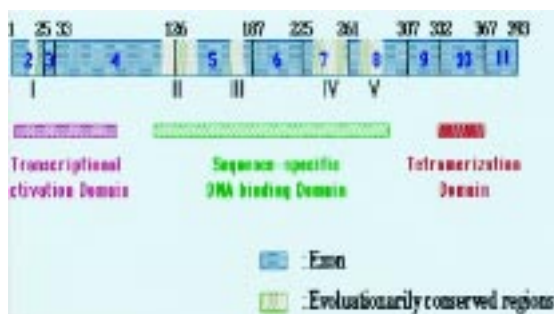
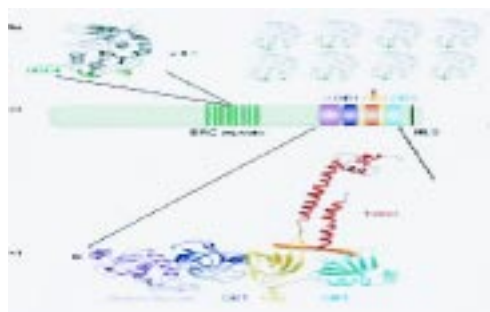


DNA Sequencing of Exon 5 *p53* genes

regulation. It is most important to look for these breast cancer genes in women from India where different ethnic populations and varied incidence of breast cancer exist. Neither expression nor the regulatory role of the *BRCA1* and *BRCA2* gene on malignant transformation and growth has been analyzed. Therefore, the present study has been designed to analyze *BRCA1* and *BRCA2* gene mutation, expression and their comparison with the status of *p53* tumor suppressor gene in different grades of both sporadic and familial breast cancer in women.

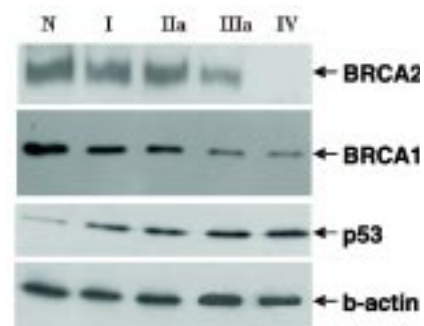


Localization of functional domain within the full-length BRCA1 protein



Structural organization of p53 protein

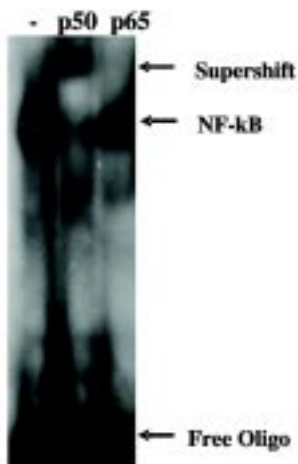
Out of 100 sporadic breast cancer cases analyzed, only exon 2 of both *BRCA1* and *BRCA2* in 2 different patients, 2 (2%) in *BRCA1* and 2 (2%) in *BRCA2* and only exon 5 of *p53* gene in three cases (3%) showed alteration in band mobility in SSCP assay. Of four patients with *BRCA1* gene mutations observed out of 16 familial breast cancer cases showed a novel frameshift 4956insG leading to TGA at codon 1621 in exon 16.



A western blot showing BRCA2, BRCA1 and p53 protein expression in normal and different clinical stages of breast cancer specimens

Transcription Factors AP-1 and NF- κ B in Breast Carcinomas

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Collaborator	: Ravi Kant , MAMC & LNJP Hospital, New Delhi Syad Akhtar Husain , Jamia Milia Islamia, New Delhi S.K. Kochar, Pawan Gupta , Dharamshila Cancer Hospital, New Delhi



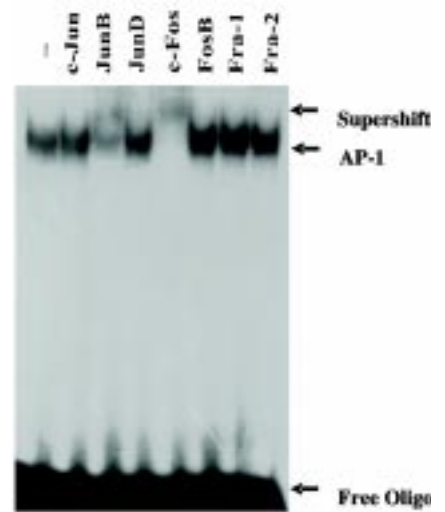
Constitutive activation of NF- κ B and its composition in breast cancer tissue

TRANSSCRIPTION Factors, such as NF- κ B and AP-1, play a very important role in the breast carcinogenesis. Activating protein-1 (AP-1) transcription factors consist of homodimers and heterodimers of Jun (c-Jun, v-Jun, JunB, JunD) and Fos (c-Fos, v-Fos, FosB, Fra1, and Fra2) subfamilies. The AP-1 transcriptional factors are expressed in most cells and are activated by specific kinases, which are in turn activated by diverse signals such as growth factors stimulation, exposure to UV light, oxidative stress, tumor promoters or oncogene over-expression or activation, mitogenes and heat shock. AP-1 is essential for mitogenic signal transduction induced by many different growth factors (EGF, TGF α , heregulin, bFGF, IGF-1 and estrogen). While these growth factors also activate other signaling pathways such as Rho or Akt-dependent pathways, blockade at the level of AP-1 is sufficient to prevent growth despite the stimulation of other AP-1 independent pathways.

Nuclear factor—kappa B (NF- κ B) is an inducible transcription factor that is constitutively activated in most of the cancers including breast cancer. It plays a central role in the integrating intra- and extra-cellular signals and assuring appropriate gene expression. The members of the family include p50, p52, p65, c-Rel, and RelB. The best-characterized members of NF- κ B family are p50/p65 heterodimer and p50/p50 homodimer complexes. In most cells, NF- κ B is present in the cytoplasm in an inactive form bound to inhibitor protein (I κ B). A wide variety of endogenous and exogenous factors such as cytokines, growth factors, viruses and environmental hazards can facilitate dissociation of I κ B/NF- κ B complex resulting in to release and translocation of NF- κ B in to the nucleus. Downstream targets of NF- κ B include anti-apoptotic genes which allow cells to evade apoptosis and proliferate.

Aim of this investigation is to study the DNA binding activity and expression patterns of AP-1 and NF- κ B in order to unravel the transcriptional regulation during breast carcinogenesis. In vitro studies will be carried out using breast cancer cell lines to investigate the regulatory effects of certain cytokines (TNF α), steroid hormones including taxol, the plant derived drug recently being used for breast cancer genes during breast carcinogenesis, to develop the molecular approaches to control transcription of breast cancer genes during progression of breast cancer in humans.

Preliminary studies carried out in breast tumor specimens in comparison to that of normal controls indicate high binding activity of AP-1 and NF- κ B in malignant cases. It is also shows selectively high expression of c-fos and down-regulation of fra-1 in breast cancer. This is reconfirmed again in immunoblotting of AP-1 components using specific antibodies raised against each of AP-1 members. The expression patterns of p50 showed very high expression in cancer cases while nil or negligible in normal tissue. However, the p65 expression was found to be very low in malignant as well as in normal tissue samples.



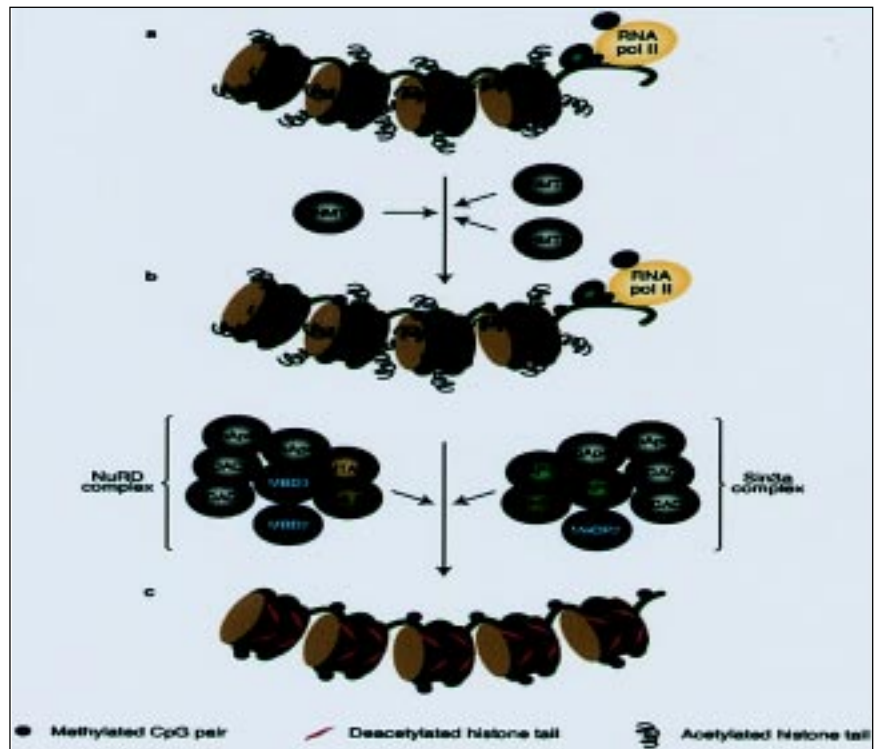
Constitutive activation of AP-1 and its composition in breast cancer tissues

Role of Promoter Methylation in the Expression of *BRCA1* Gene in Breast Cancer

Team Leader : **Bhudev C. Das**
Co-Investigator : **Suresh Hedau**
Alok C. Bharti

DNA methylation is a universal reversible mechanism, which regulates gene expression, chromatin structure and genomic stability. Methylation of the DNA occurs most frequently on the 5' cytosine residues within 5'-CpG-3' di-nucleotides, which often cluster together in CpG islands that can stretch for several kilobases. In actively transcribed genes,

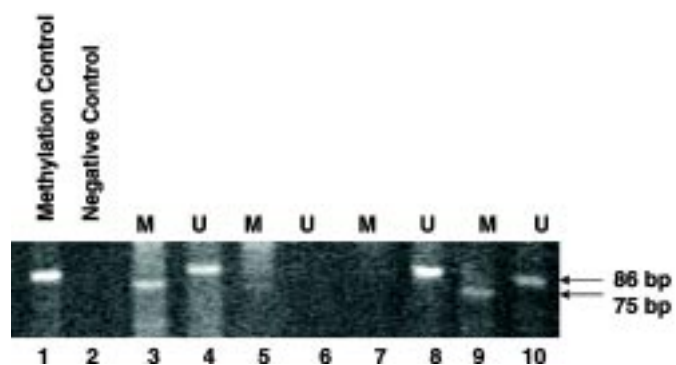
Proposed Mechanism by which DNA methylation leads to transcriptional repression



CpG islands within regulatory regions are often unmethylated. In contrast, methylation at these sites represses transcription by altering chromatin structure such that the transcriptional machinery does not have proper access to functionally important regions of the promoter. Methylation of promoter region is frequently used during normal development to epigenetically down-regulate gene expression in a tissue-specific manner.

Highly reduced expression of BRCA1 has been observed by us and other in sporadic as well as familial breast cancers although the actual mechanism(s) remains unclear. Aberrant cytosine methylation of the BRCA1 promoter is associated with decreased BRCA1 expression in human breast cancer. The role of abnormal methylation leading to loss of expression during carcinogenesis had already been established for several genes such as Rb, VHL, p15, p16, APC, E-cadherin, MGMT, GSTP1 etc. Hypermethylation of the BRCA1 promoter region has been also strongly correlated with lack of estrogen and progesterone receptor expression. It suggests that epigenetic silencing may be one of the mechanisms of transcriptional inactivation of BRCA1 in sporadic breast cancer.

Present study has been carried out to see the status of methylation within the BRCA1 promoter region in a large spectrum of sporadic as well as familial breast cancer. We analyzed 50 tumor biopsies from sporadic breast cancer and 10 normal blood samples for the methylation of the BRCA1 gene promoter region. Only 15 cases (30%) showed hypermethylation of the BRCA1 gene. Further study is in progress.



Methylation Analysis of the BRCA1 promoter region in tumour samples in sporadic breast cancer. Lane 1 - Methylated control of BRCA1; Lane 2 - Negative control; Lane 3 and lane 9 shows hypermethylated BRCA1 promoter

Morphological Parameters and Image Cytometry of the Fine Needle Aspirates of Histo-pathologically Confirmed Benign and Malignant Breast and Other Lesions

Team Leader : **Suresh Bhambhani**
Investigator : **Veena Kashyap**
Collaborator : **S. Rao**, Northern Railway Hospital, New Delhi



Physician taking the FNAC samples from Breast and other lesions

FINE needle aspiration cytology continues to gain clinical acceptance as a diagnostic technique for collecting cells from palpable and non-palpable breast lesions. Automated image cytometry is relatively newer technique, which can rapidly and accurately measure the various morphometric parameters on cytology smears which can be used to differentiate benign lesions from malignant lesions of the breast. The nuclear morphometry reinforced by image cytometry may separate breast carcinoma into low and high nuclear grades. The image cytometry may pick up some of the nuclear features, which are sub-visual and overlooked by human eye. The measurements of various nuclear parameters by image cytometry would be helpful in future applications of automated diagnosis and grading of breast carcinomas from cytologic materials.

Under this study histologically confirmed fifty cases of fibroadenoma (Benign breast disease) and fifty cases of breast carcinoma were selected to delineate cytomorphological parameters such as cellularity, cell uniformity, cell dissociation, nuclear granules etc as well as for image cytometry of nuclear area, perimeter and diameter.