Implication of *BRCA1* gene in breast cancer

S K Kachhap* & S N Ghosh

Cell Biology Division, Cancer Research Institute, Tata Memorial Centre, Parel, Mumbai-400012, India.

Breast cancer susceptibility gene (*BRCA1*) is known to be responsible for hereditary breast and ovarian cancer. This gene is highly penetrant conferring a risk for 0.92 by the age of 70. Germline mutation in this gene leads to susceptibility to breast and ovarian cancer, with a genotype phenotype correlation. Frequency of mutations of this gene in normal population of breast cancer is low suggesting that the effort of primary screening for *BRCA1* gene should be restricted to only familial cases with a strong history of breast and ovarian cancer. Recent studies indicate that *BRCA1* is a tumor suppressor gene responsible for both normal development and carcinogenesis of the breast. Normal function elucidated so far, reveal *BRCA1* to be a multifunctional protein involved in DNA repair, cell cycle regulation and transcription. There is circumstantial evidence that gene interacts with p53, a protein involved in cell cycle control, DNA repair and apoptosis.

Cancer, the characteristic of uncontrolled growth is always accompanied by alterations in normal pathways of differentiation and development. Reproductive endocrine factors such as menarche, menopause and age at first full term pregnancy which control breast development also influence breast cancer risk, supports the hypothesis that mammary gland development and mammary gland carcinogenesis are fundamentally related.

Epidemiological data indicate that incidence of breast cancer differs markedly in different countries. It is high in North America and North Europe and low in Japan. This indicates that breast cancers arise due to complex interaction between environmental factors and genetic factors. Among the environmental factors both endogenous and exogenous hormones and fat intake play a significant role. While among genetic factors susceptibility genes either common with less penetrant and rare with highly penetrant genes are found to be associated.

A variety of epidemiological and animal studies suggest that hormones play a critical role in both mammary gland development and mammary carcinogenesis. An increased risk of breast cancer is found among women with early onset of menarche and late menopause. It is reported that environmental estrogen or xenoestrogen have the ability to interfere with several physiological process that are normally estrogen regulated which could result in alteration in reproduction and susceptibility to breast cancer. Several investigators have reported that an excess risk of breast cancer is associated with oral contraceptive use in young women with family history. Colditz has summarized the different sources of data concerning the relationship of both endogenous and exogenous hormones to the etiology of breast cancer and concluded that evidence is now sufficient to infer that estrogen is a cause of breast cancer. However, a mechanism by which estrogen affects proliferation of certain target cells is not fully understood. Several evidences indicate that they can induce expression of cell cycle regulatory genes, that directly suggests a primary role of estrogen receptor (ER) in regulating cell cycle progression. Higher androgen levels have been reported in Caucasian women (high risk group) who showed familial clustering. Recent studies also indicate that serum concentration of bioavailable estradiol and free testosterone and plasma prolactin are associated with future risk of breast cancer. Factor for increase of insulin concentration of insulin in plasma is also found to be associated with increase in risk of premenopausal but not postmenopausal breast cancer.

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*Present address: John Hopkins Oncology Centre, John Hopkins School of Medicine, Bunting-Blanstein, Cancer Research Building, 1650 Orleans St./RM132, Baltimore, MD 21231-1006, USA.*
Dietary intake of fats in particular ω-6 polyunsaturated fatty acids have been shown to increase breast tumor incidence in rodents\textsuperscript{10,11}. On the contrary an intake of ω-3 polyunsaturated fatty acids have been shown to decrease tumor incidence\textsuperscript{12}. Thus the type of fat intake can decide the outcome of the disease and a ratio of ω-6/ω-3 fatty acids might be a better indicator of cancer risk than the amount of fats\textsuperscript{13}. Hilakivi-Clarke et al.\textsuperscript{14} have reported that consumption of diet high in fat (primarily in the form of n-6 polyunsaturated fatty acid, linoleic acid) during pregnancy increased risk of developing carcinogen-induced mammary tumors, possibly by increasing the level of circulating estrogen.

Since 10-15% of breast cancer is familial, susceptibility to the disease is inherited. Lynch et al.\textsuperscript{15} have estimated that among breast cancer patients about 15-20% patients have a positive history of breast cancer in an immediate family member, mother, sister or daughter. These cases are characterized by early onset and bilateral disease. Daughters, who have a mother with bilateral\textsuperscript{16,17} or early onset of breast cancer have more risk of breast cancer than do daughters of mother with unilateral or late onset of breast cancer. Common environmental exposure may also lead to familial clustering of the disease\textsuperscript{17,18}. This complicates the recognition of families in which clustering is due to common predisposing genes with low penetrance. Number of breast cancer cases caused by low penetrance genes is however considered to be much higher than the number of hereditary cases caused by mutation in higher penetrance genes. A number of low and high penetrance genes have been discovered in breast cancer. Polymorphism in CYP1A1, NAT1 and CAG repeat in AR gene are among the low penetrance gene responsible for breast cancer, while \( p^3 \), BRCA1, BRCA2, ATR and ATM are high penetrance gene that account for majority of hereditary breast cancer\textsuperscript{19}.

In 1992, Mary Claire King and her co-workers have identified a 8M region in 17q12-q21 by linkage analysis in 23 breast cancer families using 11 polymorphic marker\textsuperscript{20}. This region is linked to breast cancer families that shows an early onset of the disease. The gene has been named as breast cancer susceptibility gene (\( BRCA1 \)) and found to be responsible for breast cancer in about half of all families with a dominant predisposition of the disease and between 80 and 90% of families in which multiple cases of both breast and ovarian cancer occurs. These mutations are highly penetrant, conferring a risk of about 90% of either breast or ovarian cancer by the age of 70 years\textsuperscript{21}. Later, Miki et al.\textsuperscript{22} have identified a strong candidate for \( BRCA1 \) gene by positional cloning. Predisposing mutations have been identified in 5 of 8 kindreds in family linked to \( BRCA1 \). The gene has been found to be expressed in various tissues including breast and ovaries with maximum expression in thymus and testis. It encodes a predicted protein of 1863 amino acids. The protein contained a zinc finger domain in its amino terminal, indicating its role as a transcription factor. \( BRCA1 \) is encoded by a gene which has 24 exons that spans a 81-kb genomic length.

Since in the breast connection between carcinogenesis and development is illustrated by the existence of endocrine risk factors for breast cancer that are related to timing of normal development events such as menarche, menopause and age at full \( 1 \)st term pregnancy, and if \( BRCA1 \), the susceptible gene for breast cancer is involved in breast cancer, then function of this gene should alter during all these developmental phases. This review illustrates hormonal regulation of \( BRCA1 \) gene and its function at the time of development, growth and differentiation of mammary gland and how in the absence, mutations or down regulation of expression of this gene promotes breast carcinogenesis.

**Germline mutations of \( BRCA1 \)—**The proportion of families attributable to \( BRCA1 \) mutation/s vary widely among different populations\textsuperscript{23}. \( BRCA1 \) gene does not seem to reveal a hotspot. Mutational studies in various populations differing ethnically and geographically have demonstrated \( BRCA1 \) mutations to be scattered throughout the length of the gene. However, majority of the mutations lie on exon 11 which is expected as this exon codes for 60% of the \( BRCA1 \) protein. Using various techniques to identify \( BRCA1 \) mutations which include the protein truncation test, single strand conformation polymorphism, heteroduplex analysis and direct sequencing, various researchers have identified mutations throughout the length of the gene. Of all the mutations detected in the gene, about 90% result in truncation. \( BRCA1 \) mutation seems to be common in Russian breast and/or ovarian cancer families (70%). Two alleles (5382insC and 4153delA) are the most common in this population. Of these, 5382insC is also common among European population while 4153delA is unique to the Russian. The second population that shows the highest \( BRCA1 \) mutations are the Ashkenazi Jewish families of Israel. One of the most common alleles present in this population is 185delAG, a founder mutation in majority of the
Ashkenazi Jews. Almost every mutation analyzed in Italian breast cancer families seems to be unique and does not have a founder effect. Studying the proportion of 185delAG in various populations gives an insight about its origin and propagation along with the migration of population. The age of 185delAG mutation was estimated at 46 generations or 1000-1500 years. However the Iraqi, Iranian and Ashkenazi Jewish families share the same haplotype indicating that mutation predates the separation of the communities and is ≥ 2000 years old. Many European mutations have been found in United States or Canada indicating the migration of this population to these areas. BRCA1 is estimated to be responsible for 20 to 25% of high risk families in Britain, France, Scandinavia and Hungary and <20% of high risk families in Holland, Belgium, Germany and Norway.

BRCA1 mutations are by far rare in Japanese population with every mutation being unique to that country. Further studies on Asian population should throw a light of the population specific mutations among this group. From a study comprising 33 breast and breast-ovarian familial cancer cases of Indian origin, we have observed a frequency of 12% germ line mutations in Indian women. The mutations are unique to our population and have not been reported elsewhere.

Earlier studies on BRCA1 mutation prevalence suggest that about half of breast cancer families are attributable to BRCA1 mutations. However, recent analysis has revealed that actual number of BRCA1 mutations in high risk families might be as low as 12.8 to 16% (Ref. 27, 28). Our findings are in accordance with other ethnic population. The reduced prevalence may be explained as early studies have involved families with large numbers of breast and ovarian cancer cases in comparison to more representative mixture of families with breast cancer alone and with both breast and ovarian cancer as reported in recent studies. The studies conclude that widespread screening of BRCA1 is unwarranted and the efforts should be concentrated on high risk families with breast and breast ovarian cancer where BRCA1 mutation is more prevalent.

Somatic mutations of BRCA1—Somatic mutations of BRCA1 in sporadic breast tumors are yet to be detected. Although loss of heterozygosity (LOH) at BRCA1 locus has been reported with varying frequency with the loss of one allele and the presence of mutation in the other allele has not been detected, few somatic alterations of BRCA1 have been reported in sporadic ovarian tumors (S/29) selected for LOH on 17q (Ref.32). One reason may be that in sporadic breast cancer BRCA1 mutation could be present in a regulatory region other than the coding sequence. Another possibility is that BRCA1 expression is temporally regulated by hormones and may function during puberty and this could possibly explain why germline mutation in the gene predisposes one for early onset breast cancer. Another reason could be that BRCA1 may be regulated at the transcriptional level in sporadic tumors. This hypothesis is supported by the findings of Thompson et al. who have reported lower levels of BRCA1 mRNA in sporadic tumors in comparison to normal tissue.

Genotype and phenotype correlation and factors affecting BRCA1—Mutation analysis of BRCA1 seems to indicate a phenotypic correlation. In a study of 60 families with a history of breast and/or ovarian cancer twenty-two different germ-line mutations have been detected. A significant correlation between location of mutation in the gene and the ratio of breast to ovarian cancer incidence within each family has been observed. Mutations at 3' end of the gene have been shown to be associated with a lower proportion of ovarian cancer. Their findings indicate that this region of the mutant BRCA1 may have domain that might retain an active function in ovarian cell but not in the breast epithelium.

It has been shown that tumors from BRCA1 mutant carriers are often histologically aggressive, steroid receptor negative, DNA aneuploid, highly proliferative as well as TP positive and ERBB2 negative by immunostaining. Eisinger et al. have reported that there is an existence of two subgroups of BRCA1 associated breast cancer families. The first group (78%) is composed of cases with high proliferation rate and the second group (22%) is composed of cases with low proliferating breast cancer. To test for the existence of two types of BRCA1 alleles, differently affecting breast tumor growth, Sobol et al. have analyzed the distribution of mitotic index, grade of tumor, and matching them with the location of germline mutation on breast cancer cases from 20 families. They have observed a prevalence of highly proliferative tumors when the mutation occurs in two terminal domains (amino and carboxyl terminals) of BRCA1 protein. This result therefore suggests that based on the site of germline mutation one might predict the development and behaviour of the tumor. Serry et al. have identified an association between low levels of BRCA1 expression and acquisition of distant metastas-
sis in sporadic disease and suggested that a suppression of BRCA1 has a role to play in the progression of significant fraction of sporadic cancer and thus it can be used as a prognostic marker for the disease. However, Marcus et al. have reported that compared with other hereditary breast cancer (HBC), there is lower rate of recurrence in BRCA1 related HBC group which seems to be more paradoxical because their study as well as other studies have shown that BRCA1 related cancers are more likely to be aneuploid, estrogen negative and highly proliferative, a feature associated with poor prognosis. According to these authors, these paradoxical phenomena can be explained based on the sensitivity of these tumor cells and suggest that perhaps BRCA1 linked tumors are more chemosensitive or radiosensitive and therefore have higher cured fraction as has been reported for other highly proliferative breast cancer.

It is evident that despite of high penetrance of BRCA1, the development of the disease in carriers are not always expressed. Development is now known to be influenced by several other low risk genetic and environmental factors. Substantial variation exists in the age at which breast cancer is diagnosed in BRCA1 mutation carriers. This suggests that genes other than BRCA1 may modify BRCA1 associated age specific risk for breast cancer. Association studies using polymorphisms found in glutathione-S-transferase suggest that genetic modifiers of BRCA1 penetrance do exist. Recently, Rebbeck et al. have studied the effect of a CAG repeat length polymorphism found in exon 1 of androgen receptor gene (AR-CAG) on age at diagnosis of breast cancer in known mutation carriers. The androgen receptor gene functions as a ligand dependent transcriptional activator in response to androgens and is thought to be involved in breast tumor growth and progression. The authors have compared AR-CAG repeat lengths in affected and unaffected women with germline BRCA1 mutation. They have shown that carrier women who have at least one BRCA1 allele with 28 or more CAG repeats have an increased risk of cancer. The data suggest that women who carry larger repeat units are more prone to develop the disease than women who have shorter repeat lengths. We have compared free androgen levels in familial breast cancer individuals screened for BRCA1 mutation with sporadic and control individuals and found it to be higher in familial cancer cases and significantly higher in individuals carrying BRCA1 mutations suggesting an increase in free androgen level and familial clustering. Besides, other factors do exist that can lower BRCA1 expression in sporadic tumors. Several reports have indicated that unsaturated fatty acid like linoleic acid can regulate the expression of p53 gene and H-ras oncogene and estradiol that can modulate BRCA1 gene expression thereby breast carcinogenesis. We have observed increased proliferation and anchorage independent growth with concomitant down regulation of BRCA1 expression in MCF-7 cells treated with linoleic acid and estradiol compared to untreated controls. Our results suggest that BRCA1 expression is down regulated during breast carcinogenesis. Furthermore, it has been reported that such as hypermethylation of BRCA1 gene promoter could also be responsible for lower expression of BRCA1 gene. Methylation is the main epigenetic modification in humans, and changes in patterns of methylation play an important role in the tumorigenesis. In particular, hypermethylation of normally unmethylated CpG islands located in the promoter regions of many tumor suppressor and DNA repair genes, such as p16, p15, Rb, VHL, E-cadherin, GSTP1, and MLH1, is associated with its loss of expression in cancer cell lines and primary tumours. Several reports suggest that aberrant methylation of BRCA1 could occur in breast carcinoma. Esteller et al. have reported that BRCA1 promoter methylation shows an unusual distribution among histopathologic types of breast carcinoma. BRCA1 promoter hypermethylation has been seen to be more frequent in medullary and mucinous subtypes of breast cancer.

Functions of BRCA1—Since cloning of BRCA1 gene, an attempt has been made to describe its possible functions. Another aspect that is intriguing is that though BRCA1 is ubiquitously expressed in various tissues with the maximum expression in thymus and testes, why is mutation in the gene does not have a pleiotropic effect? Why should it only predispose to breast, ovarian and prostate cancer? Even though the expression is maximum in the testis and thymus none of the reported cases ever shows a thymic lymphoma or a testicular carcinoma. The answers to such questions can only be elucidated by knowing the function of BRCA1 and understanding various factors that govern the expression of the gene.

Once the role of BRCA1 as a tumor suppressor gene is established in familial tumors by LOH and mutation analysis and in sporadic tumors by transcriptional down regulation efforts have been focused on the discerning the function of BRCA1. Earlier studies using knock out mice reveal that homoz-
gous mice lacking BRCA1 gene die within 10 and 13 days of embryonic development, suffering from a variety of neuro-epithelial defects. Mice having homozygous BRCA1 gene do not show any evidence of cancer. This indicates that the gene is essential for early embryonic proliferation and development. Subsequently, BRCA1 has been shown to be widely expressed in developing embryos and has a preference for replicating cells as it is associated with terminal differentiation of the ectoderm and mesoderm derived tissues. Expression of BRCA1 in testis is restricted to meiotic germ cell and in mammary tissue to alveolar and ductal epithelial cells. Since carriers of BRCA1 mutant alleles exhibit an increased susceptibility to carcinoma of the breast, as well as ovary, colon and prostate, it is likely that these genes play an important role in regulation of growth and differentiation of epithelial cells, particularly those which are hormone responsive. BRCA1 expression is enhanced considerably during pregnancy and lactation and declines after parturition, indicating that expression is hormonally induced. Indeed steroid hormones like estrogen and progesterone as well as peptide hormones are shown to enhance BRCA1 gene expression.

BRCA1 mRNA and proteins are highly expressed during late G1-early S phase of the cell cycle. Cloning of BRCA1 reveals a RING finger near the amino terminal (amino acids 1-112), the only sequence homologous to a group of proteins that binds DNA. RING fingers are zinc binding domains that mediate protein-protein or protein-DNA interactions. Many proteins that form a macromolecular complex have such RING finger. They are also present on gene that facilitate ubiquitination, a common mechanism that results in degradation of proteins. Thus, BRCA1 may function as one of the proteins that mediate ubiquitination and a loss of RING finger as a result of mutation may cause certain proteins to accumulate thereby bringing about a disregulation of proliferation.

Other clues to the function of BRCA1 came from a combination of different techniques like yeast two-hybrid system, immunoprecipitation and immunohistochemical localization that identified proteins physically linked to BRCA1. The RING finger domain of BRCA1 binds to the protein BARD1 (BRCA1 associated RING domain) which is a de-ubiquitinating enzyme. This association may probably regulate the ubiquitination pathway of BRCA1. BRCA1 C-terminal (BRCA1 C-terminal) a motif that is shown to be present in p53 binding protein like 53BP1 and other proteins involved in DNA repair or metabolism. BRCA1 immunostaining reveals discrete, nuclear foci during S phase of cell cycle. These foci have been shown to contain BARD1 and RAD51. Eukaryotic RAD51 proteins are homologous of bacterial RecA gene and are required for recombination during mitosis and meiosis. Rad 51 mediates double strand exchanges and brings about recombination. BRCA1 exon 11 encodes sequence(s) (residues 758-1064) that interact with Rad51. BRCA1-Rad may serve as complexes that participate in double strand break repair and recombination. BRCA1 has also been found to co-localize with Rad50 complexes. Rad50 forms complexes with Mre11 and p95-nibrin upon irradiation. It functions as homologous recombination, non-homologous end joining, meiotic recombination, DNA damage response and telomere maintenance. Formation of irradiation-induced foci positive for Rad50, Mre11, BRCA1 is dramatically reduced in HCC1937 breast cancer cells carrying homozygous mutation for BRCA1 and is restored upon transfection with wild type BRCA1. These irradiation induced foci are discrete and different from Rad 50-BRCA1 complexes, indicating that cells appear to have two distinct BRCA1 foci. One fraction co-localizes with Rad50 and other co-localizes with Rad51 complexes. These two foci are mutually exclusive as cells with both Rad50 and Rad51 complexes are rarely seen. Thus, it appears that BRCA1 may have different roles in two foci observed. Rad 50 complex participates in non-homologous end joining or homologous recombination in DNA double strand breaks. In homologous recombination, it is postulated that Rad 50 complex brings about end joining and Rad 51 complex is involved in strand exchange during a subsequent step. BRCA1 may be involved in coupling the two steps.

Experiments based on response to various DNA damaging agents reveal distinct changes in BRCA1 protein. Following treatment of MCF7 cells with hydroxy urea and a low dose UV treatment BRCA1 has been found to have relocated onto subnuclear sites of active DNA replication as evident by co-localization with PCNA, (a marker of replication). BRCA1 undergoes specific phosphorylation at S phase which is different from known phosphorylation that occurs during G1-S transition. This phosphorylation seems to be dependent on mutation of Ataxia Telangiectasia (ATM) (Ref.71). Thus, BRCA1 is engaged in double
stranded break repair upon damage by various types of DNA damaging agents. Efficient DNA repair is important because unrepaired DNA leads to chromosomal breaks, translocation and genomic instability. This is further supported by the fact that BRCA1 deficient cell lines show an increased genetic instability. Xu et al. have used murine embryonic fibroblasts derived from mice with a targeted deletion of BRCA1 exon 11 and have shown that the cells have a defective G2-M checkpoint accompanied by chromosomal abnormalities, multiple functional centrosomes, unequal chromosomal segregation, and abnormal nuclear division, leading to aneuploidy. Hsu and White have found that BRCA1 is associated with gamma tubulin, a component of centrosome, during mitosis. The observation that BRCA1 co-localizes to centrosome suggests that it may act in a manner similar to other proteins that regulate cell cycle and co-localize with centrosome such as cyclin A, cyclin B, cdc 2, and 14-3-3. It is also known that p53 associates with centrosomes and that p53 nullizygous mouse embryonic fibroblasts have a high frequency of amplified centrosome and abnormal mitosis. Retinoblastoma protein co-localizes with the centrosome, but retinoblastoma protein does not affect centrosome amplification. It is proposed that mutations in BRCA1 might disrupt centrosome duplication and hence chromosome segregation leading to abnormal chromosomes. The fact that BRCA1 deficient cells and embryos both show a high level of abnormal chromosomes further strengthens the hypothesis that BRCA1 gene has a major role in genomic integrity. Our results using clonal cell culture of MCF-7 cell line, that has a low expression of BRCA1, revealed that these cells have higher number of chromosomal breaks and dicentrics in comparison to HeLa cells that have a normal level of BRCA1 gene expression. Further, these cells are more sensitive to gamma rays in comparison to T-47D or HeLa cells, suggesting a poor repair capacity in these cells. These results indicate that lower expression of BRCA1 could also bring about genome wide chromosomal instability and such cells are more radiosensitive.

Harkin et al. have reported the involvement of BRCA1 in apoptosis. By using oligonucleotide arrays and functional assays, this group has shown expression of BRCA1 induced GADD45 and c-Jun N-terminal kinase/stress activated protein kinase (JNK/SAPK)-dependent apoptosis. Using a cell line engineered for inducible expression of BRCA1, they have shown an increase programmed cell death. This induction is a result of increase in BRCA1 expression through activation of JNK/SAPK, thereby leading to apoptosis. This observation let them to suggest that BRCA1 has a role in induction of apoptosis.

There is also increasing amount of evidence that BRCA1 may serve as a transcription factor and regulates gene expression. BRCT repeats located at the C-terminus of BRCA1 may serve as interacting domains with p53 protein. However, interaction of BRCA1 with p53 is yet to be established. Nevertheless, full length BRCA1 when co-transfected with p53 is shown to induce transcription of p21 and MDM2 genes both of which are activated by p53. BRCA1 interacts with both RNA helicase and with CtIP as a part of transcriptional complex. Mutations at 3' end of BRCA1, which are found in patients, reduce the ability of BRCA1 to form a complex with RNA helicase A. The C-terminus of BRCA1 (amino acids 1528-1863) when fuses in-frame with DNA binding domain of GAL4 protein induces transcription. Clinically, relevant mutations that truncate BRCA1 C-terminus are inactive in such transcription assays. Fan et al. have shown that BRCA1 protein suppresses estrogen dependent mammary epithelial proliferation by inhibiting ER-alpha mediated transcriptional pathways related to cell proliferation. ER-alpha is one of the two ligand-activated nuclear receptor type of transcription factors through which estrogen elicits its response. This study probably throws a light on functions of BRCA1 in mammry epithelium, an estrogen responsive tissue. BRCA1 has also been shown to be associated with the histone deacetylase complex. Histone deacetylases are a group of proteins that deacetylate histones thereby modulating their structure and hence the expression of the genes that they bind. BRCA1 might repress transcription by recruiting histone deacetylase complex to specific promoters, where the complexes deacetylating certain core histones, modulating local structure of chromatin and making DNA less accessible to transcription enzymes.

The question that arises that BRCA1 is a caretaker or a gatekeeper. Cell proliferation is usually regulated by transcription factors for which these are classified as gatekeepers. Similarly, since BRCA1 controls cell proliferation, it may be classified as gatekeeper. There are direct evidences to support the proliferation hypothesis, such as neutralization of BRCA1 gene transcript by antisense mRNA increases the proliferation rate of benign and malignant cells in culture. Moreover, overexpression of BRCA1 in ovarian cancer cells and MCF-7 cells inhibits their growth. However, the
repair function of BRCA1 indicates that it can be a caretaker as it maintains the integrity of the genome. Analyzing the sequence of events which occurs as a result of mutation or down regulation will throw some light about its function as a gatekeeper and/or caretaker.

As roles for BRCA1 and BRCA2 in DNA repair and cell cycle check points have been revealed, one paradox of their biology might be closer to resolution, why BRCA1 and BRCA2 null mice die in early embryonic life, whereas, BRCA1 and BRCA2 null breast and ovarian cancer cells develop tumours. Evidences from conditional knock-out mice suggest that loss of BRCA1 in mammary cells leads to incomplete proliferation, apoptosis and tumor at low frequencies. In these mice the additional heterozygous mutations of \(^{p3}\) leads to many more mammary tumors, most of which loose the remaining \(^{p3}\) alleles. Genetic instability caused by the loss of BRCA1 or BRCA2 could trigger mutation including mutations in checkpoint genes such as \(^{p53}\). Mutation in \(^{p53}\) would overcome incomplete proliferation to uncontrolled proliferation and basic growth. Rapid proliferation of breast epithelium during puberty and pregnancy would result in a loss of remaining allele in women inheriting a BRCA1 gene mutation. This may later lead to a loss of a gatekeeper like \(^{p3}\) leading to tumor formation. Thus, BRCA1 mutation is an earlier event in the development of the disease.

In conclusion, it appears that BRCA1 gene participates in growth, development and differentiation of mammary gland abnormality manifestation as mutation seen mostly in familial breast and breast/ovarian cancer and lower expression that would lead to development and progression of the disease even in sporadic cancer. Since several reports indicate that patients carrying BRCA1 germline mutation show increased incidence of \(^{p3}\) mutation in their tumors, it appears that probably both genes play in concert for oncogenesis. However, germline mutation of \(^{p3}\) is rare in breast or breast ovarian cancer families indicating that \(^{p3}\) mutation is a later somatic event occurring in the breast tissue and probably BRCA1 germ line mutation might render the cells more susceptible for \(^{p3}\) mutation by causing a genome wide instability. Since both caretaker and gatekeeper genes are affected, tumors of BRCA1 mutant carriers are more aggressive. However, since BRCA1 carrier tumors are more radiosensitive and chemosensitive, a timely treatment with these therapies would improve the life of the patient. Again since \(^{p3}\) mutation which is known to be a later event in the breast tissue of BRCA1 mutant carrier patient, it would be interesting to see whether \(^{p3}\) mutations can be studied from breast fluid cells presymptomatically in carrier individuals. Such findings would lead to an early diagnosis and treatment of the disease.

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