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# BRCA1 IN CANCER, CELL CYCLE AND GENOMIC STABILITY

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## 1. ABSTRACT

The BRCA1 gene was isolated in 1994; germline mutations of this gene are known to confer susceptibility to breast and ovarian cancer in high-risk families. Since its discovery, several mutations have been identified in this gene; these are scattered throughout the gene, and include insertion and deletion frameshifts, base substitutions, and inferred regulatory mutations. It role in the pathogenesis of breast cancer, which accounts for almost 95%, although unproven to date, cannot be ruled out. The functional inactivation of both copies of this gene in sporadic tumor cells does not follow the traditional mode: the loss of function in BRCA1 is not accompanied by underlying mutation of the gene in tumor cells with loss of heterozygosity for the BRCA1 gene. Several studies now suggest that an alternate mechanism of inactivation, involving promoter hypermethylation that results in reduced expression of the gene, may be common to a significant proportion of sporadic breast and ovarian cancers. BRCA1 as a tumor suppressor plays an important role in maintaining genomic stability. BRCA1 has the ability to interact with numerous proteins and to form complexes that are involved in recognizing and subsequently repairing DNA. BRCA1 contains several functional domains that directly or indirectly interact with a variety of proteins via protein-protein interaction; these include tumor suppressors (BRCA2, p53, Rb and ATM), oncogenes (c-Myc, casein kinase II and E2F), DNA damage repair proteins (RAD50 and RAD51), cell cycle regulators (cyclins and cyclin dependent kinases), transcriptional activators and repressors (RNA polymerase II, RHA, histone deacetylase complex and CtIP), DNA damage-sensing complex and mismatch repair proteins (BRCA1- Associated Surveillance Complex; BASC) and signal transducer and activator of transcription (STAT) among others Formation of foci containing BRCA1 by inherited mutations, or epigenetic mechanisms (promoter methylation) in sporadic cancers leads to a loss of DNA repair ability, disrupts the potential to form complexes with other proteins that are crucial for DNA repair pathways. Thus, BRCA1 plays a significant role in maintaining genomic stability and serves as a tumor suppressor in breast cancer tumorigenesis.

## 2. INTRODUCTION

### 2.1. Mutation spectrum of BRCA1-associated tumors

The American Cancer Society estimated that a total of 203,500 new cases of breast cancer (out of 647,400 estimated cancers at all sites) would occur among US women in the year 2002 and assesses the probability that one in eight American women would develop breast cancer during their lifespan (American Cancer Society Facts and Figures, 2002). Taken together, cancers of the breast and ovaries constitute almost one fourth of all cancer-related mortality in this country. BRCA-1 and BRCA-2 genes (1,2,3) are known to be associated with early onset familial breast and ovarian cancer. Patients with a strong hereditary component account for only 5% of all breast cancers occurring in the Unites States (4,5); nevertheless, identification of genes responsible for hereditary cancers is important, as such genes have been shown to play a critical role in the much more common form of "sporadic" tumors (6) in a variety of cancers. The role of the BRCA1 gene in sporadic breast cancer, however is not well defined, as mutations of these two genes in tumors with loss of heterozygosity (LOH) for BRCA1 and 2 are very rare (6). As reviewed by Szabo and King (1995) (4), BRCA1 and BRCA2 combined contribute to only 6-10% of breast and ovarian cancer regardless of the family history. In addition, approximately 30% of high-risk families do not exhibit mutations in either BRCA1 and BRCA2 genes. Such observations are consistent with the fact that there may be other genes that may predispose individuals to breast cancer. A limited number of recurring mutations (BRCA1 185delAG, 5382insC; BRCA2 617delT) in the BRCA 1 and 2 genes account for a substantial fraction of the breast cancer burden in the Jewish population (4,7).

Molecular studies on hereditary cancer syndromes, such as retinoblastoma, adenomatous polyposis

coli, etc., have demonstrated that at least two genetic events are necessary for the development of tumors. One of these events must be mutation in the tissue-specific cancer-predisposing gene, whereas second event results in inactivation of the corresponding normal allele, which is normally accomplished by LOH.

A wealth of information is now available regarding the extent and type of germline mutations detected in various populations in the world. These studies clearly demonstrated that mutation in the BRCA1 gene are not localized to a specific exon.

The first breast cancer susceptibility gene discovered BRCA1, spans approximately 100 kb on the long arm of chromosome 17 (17q21.3) from which a 7.8-kb mRNA is transcribed that encodes a protein of 1863 amino acids (8), and consists of 24 exons, including a very large exon 11(8). The tumor suppressor gene BRCA1 was cloned through its linkage to inherited breast cancer (8).

BRCA1 is well conserved among species. Approximately 50% of inherited breast cancer cases are the result of germ-line mutation in the BRCA1 gene, and virtually all families have a history of both ovarian and breast cancer carry mutation in the gene (6,9)

Hereditary breast cancers account for 10-15% of all breast cancer cases whereby ~50% of these are associated with the susceptibility genes BRCA1 and BRCA2. Mutations in the BRCA1 gene are rare in sporadic breast cancer. LOH and mutation in BRCA1 has been observed in ovarian cancers (10). Loss of the wild-type allele is often seen in the tumor of a carrier with germline BRCA1 mutation, qualifying BRCA1 as a tumor suppressor gene. Inheritance of a mutated copy of the BRCA1/2 genes increases the lifetime risk of breast cancer 5-to 8-fold and that of ovarian cancer 20-to 40- fold. Genetically-predisposed individuals typically present with cancer at an earlier age, i.e., >50% of BRCA1 mutant carriers develop cancer by age 50. Members of breast cancer-prone families often seek genetic counseling to assess their relative risk for cancer development. High-risk patients are then evaluated by identifying germline mutations in the BRCA1 (and/or the BRCA2) gene. Most of these individuals carry a nucleotide sequence alteration in the BRCA1 gene (~80%) that results in a frameshift or missense mutation, whereas a subset of patients (~10%) possess chromosomal rearrangements affecting the gene. A third subset, ~10% of high-risk patients, lack discernable mutations in either BRCA1 or BRCA2, despite a calculated high probability for mutation on the basis of family history. Recent studies show that methylation-dependent epigenetic silencing of BRCA1 can contribute to the development of breast cancer that is indistinguishable from that of patients with BRCA1 mutation.

### 2.2. Mutation spectrum and phenotypes of BRCA1-associated tumors

BRCA1-associated tumors are largely ductal type, histological grade III, and show significant lymphocytic infiltration (11). BRCA1-associated tumors display distinct histological and biological features indicating that these tumors are under distinct genetic control. A strong correlation is seen between the position of a BRCA1 mutation and the ratio of breast to ovarian cancer incidence within the families (12). In particular, mutations located upstream of exon 13 are more likely to give a high proportion of ovarian and breast cancer while mutations at the 3' end of this exon give an opposite phenotype. Furthermore, BRCA1 mutations at the 5' or 3'-ends of the coding region correlate with highly proliferative tumors (13).This may suggest that inactivation of a functional domain of the BRCA1 protein, such as the RING finger or BRCT domain, results in a more severe phenotype than mutation occurring somewhere else. Since the discovery of the BRCA1 gene in 1994 {8), about 113,705 cases have been reported in OMIM (Online Mendelian Inheritence in Man). Unique coding region mutations have been identified as listed in the Human Gene Mutation data base (www.nhgri.nih.gov/intramural.research). Almost all kinds of mutations seen in the BRCA1 gene, include inversion, deletion, insertion, missense/nonsense and aberrant splicing. Most of these caused frameshift of the coding region leading to a truncated BRCA1 protein. The relatively low number of point mutations found in BRCA1 suggest that a significant portion of the encoded protein has to be inactivated to confer susceptibility to breast cancer. Most of the tumor-associated point mutations are found in conserved domains such as the RING finger and the BRCT domain. No obvious signs of clustering or mutational hotspots have been seen in BRCA1, but certain sequences and nucleotides of BRCA1 may be more susceptible to mutations than others (14). Certain polymorphisms and unclassified variants also have been reported. Most mutations generate a premature stop codon, which results in the production of a truncated protein. Many BRCA1 mutations occur in repeated motifs, including strings of homonucleotides, short direct repeats, and inverted repeats. A good example is the mutation 185delAG, prevalent among Ashkenazi Jews, which precedes a second AG pair (TCTTAGAGTGTC) (15). The allelic frequency of this mutation (185delAG) in the Ashkenazi Jew population in USA is about 1% (16). However, among Ashkenazi Jews in Australia the prevalence of this breast carcinoma-predisposing allele is even greater (17). This mutation as well as BRCA1 5382incC are considered to be founder mutations, associated with the migration of carriers with this mutation. The length of the Ashkenazi Jewish founder mutation, 185delAG, has been estimated to be 760 years old (18). Similarly, among African-Americans, three recurrent BRCA1 mutations, namely 943ins10, 1832del5, and 5296del4, have been described and it has been considered that these are likely to arise from a common ancestory (reviewed by Olopade *et al.*, 2003) (19). Similarly, many recurrent mutations have been described in many populations. These mutations may occur due to slippage and misalignment in DNA replication. The physiological and biological alterations caused by this mutation can lead to breast cancer. The individual heterozygote for germ-line mutation in BRCA15382incC developed breast cancer at a relatively early age. A breast tumor cell line (HCC1937; ATCC) that is homozygous for BRCA1 5382insC mutation (20), has been used to

decipher the physiological role of BRCA1 in DNA repair pathways. These aspects are discussed later in this chapter. There have been cases of breast cancer-prone families where rearrangements involving 3.4 and 11.5 Kb of the BRCA1 gene resulted in loss of amino acid in the C-terminus of the gene, which is involved in DNA repair pathways (21;22). Since then, many more novel ethnic mutations have been described.

Recent studies have suggested that inherited BRCA1 mutations are able to promote an oncogenic event, perhaps by masking the functions of the remaining wild-type BRCA1 allele (23).Mutations in BRCA1 gene sporadic cases are absent or rare. Nonetheless, BRCA1 has been shown to play a role in the development of breast cancer in sporadic breast cancers. In case of early onset of breast cancer without a family history, germline mutation of the BRCA1 gene has also been reported (6). Sporadic breast cancers account for about 90% of all cases, with hereditary breast cancer accounting for the balance. A great many studies have examined the molecular pathogenesis of sporadic and hereditary breast cancer, but very few have examined the epigenetic contributions to this process. The possible contribution of methylation-dependent epigenetic regulation of BRCA1 in sporadic and hereditary breast cancer remains to be determined**.** BRCA1-associated tumors are generally estrogen Receptor (ER-) negative (24,25). Some studies have shown that loss of ER positivity in tumors is due to methylation of ER, specifically of ER-alpha. ER-alpha-negative cancers arising in BRCA1 mutation carriers were more extensively methylated than ER-alpha negative cancers from women without a BRCA1 mutation (26).

### 2.3. Methylation in the promoter region of BRCA1

In many sporadic breast cancers methylation of the BRCA1 promoter has been reported. In non-hereditary breast cancers, which account for 90% of cases, the involvement of BRCA1 has not been so clearly elucidated. A great variety of genetic mutations was found in BRCA1-linked families. Inactivation of a tumor suppressor gene by loss of one allele, linked to mutation of the remaining allele, is a common mechanism. BRCA1, in some ways, puts itself on the brink of ignoring the two hit model proposed by Knudson that is generally followed by other tumor suppressor genes; because no sporadic mutations was seen in BRCA1, an alternative mechanism to genetic mutations, namely hypermethylation of the BRCA1 promoter resulting in a loss of expression, has been shown. Methylation of DNA represents a significant epigenetic alteration in humans. It occurs most frequently on the 5'-methylcytosine residue of 5'-CpG-3' dinucleotides. De novo enzymatic methylation of 5'-CpG islands can lead to the inactivation of the gene. CpG sites are generally clustered into islands, called "CpG islands", usually cover 0.2 to sevaral KB and are found in the promoter region of the genes (27). Enzymatic methylation of 5'-CpG islands can lead to the inactivation of the contiguous gene. DNA methylation inhibits transcription by interfering with transcription initiation. This repression can arise by several means.

In human cancer, this epigenetic (non-genetic) event has been shown to be a powerful mechanism by which tumor suppressor gene activity is inhibited. Local promoter hypermethylation in human cancer is often part of global genomic hypermethylation. DNA methylation inhibits transcription by interfering with transcription initiation. Thus the potential mechanism is a reduction of binding affinity of sequence-specific transcription factors. The BRCA1 gene is regulated by two promoters. Two distinct transcripts differing by the alternative use of the first exon have been described (28). BRCA1 promoter lacks TATA , but has several CAAT boxes, GC boxes and PEA3 binding sites, and a CREB binding site (29,30). Sequence homology searches reveal that a classic estrogen response element (ERE) sequence was not present in either BRCA1-α or BRCA1-β promoter regions (31). An alternative ERE was observed in BRCA1 β promoter (30) which is believed to be responsive to estrogen stimulation via the classical ER pathway to stimulate transcription. Estrogen may regulate BRCA1 α promoter via some complex or indirect mechanism(s) since BRCA1-α lacks a conventional ERE. A putative AP1 site is present in the BRCA1 promoter (28,32). Several CpG sites exist in BRCA1 promoter; the 5' CpG island of BRCA1 encompasses about 2 Kb. The region of the BRCA1 promoter shares the first exon of another gene, NBR2, and its bi-directional promoter with exon 1a and 1b of BRCA1 (28,33). Hypermethylation of BRCA1 promoter has been detected in about 11-31% of breast cancer cases and about 5-15% of ovarian cancer cancer. Several methods have been used to identify methylation, namely Southern analysis with methylation-specific PCR (MSP) and sodium bisulfite, followed by PCR. Normal tissues or cell lines did not show BRCA1 methylation; it has been observed exclusively in malignant breast and ovarian tissues. BRCA1 hypermethylation varies according to histological subtypes and is common in mucinous and medullary subtypes (34).. Interestingly, these histological sub-types are also highly represented in inherited BRCA1 mutant (35). These cancers display a distinct phenotype, such as loss of ER and PR positivity, and often have mutated p53 (25). Hypermethylation was more frequent in high-grade breast cancer (24,36). There is a strong correlation between promoter hypermethylation and decrease in gene expression and protein expression (37). On the other hand, in one study, 37 tumors that showed a reduction in BRCA1 expression gave no evidence of hypermethylation (38). This indicates, that a mechanism other than methylation is also responsible for suppression of BRCA1 expression. Methylation specifically inhibited the binding of the CREB protein to the CRE site within the 5' regulatory region of the BRCA1 promoter (39). The CRE site appears to play a constitutive role in BRCA1 expression. One possible mechanism by which methylation could abrogate gene expression is by impairing the interaction of transcription factors with DNA binding sites. In this regard the putative CREB binding motif present in α promoter of BRCA1 has been shown to be sensitive to methylation (37). The 5' SmaI site was found to be in close proximity to a Sp1 binding motif (28) and it has been suggested that the Sp1 element plays a role in protecting CpG islands from de novo methylation (40). A strong correlation has been observed between loss of ER positivity and BRCA1 methylation (41,42). In a study of 96 sporadic breast cancers, 10 out of 11 BRCA1 methylated cases were ER and PR negative (ER- and PR-). On the

other hand, another 31 of 96 cases , that were ER- and PR- failed to show methylation of BRCA1. In our study, we found all ER- and PR- cases to be BRCA1 methylated. It is interesting that the ER CpG island has been found to be methylated in 25% of ER- breast cancers (43), and the same percentage of ER- cases was found to be hypermethylated in the BRCA1 promoter region (41). An abnormal methylation was detected in approximately ten percent of sporadic breast tumors, it indicated that this mechanism alone cannot account for the reduction in BRCA1 mRNA levels observed in the majority of sporadic invasive breast cancer cases (44).

It would be intriguing to see whether a strong corrletaion exists between loss of ER positivity and BRCA1 hypermethylation. Reduced BRCA1 expression levels in sporadic cancer are strongly correlated with negative ER status (45). Furthermore, an increased DNA methyltransferase activity in ER- cell lines compared to ER+ cell lines, suggests that loss of ER positivity is due to increased methylation. This may mean that methylation of the BRCA1 promoter in ER- tumours may also be secondary to increased DNA methyltransferase activity. BRCA1 expression was low or completely lost in invasive carcinoma, however an opposite, namely higher expression was seen in non-invasive breast cancers (45). The highest levels were observed in samples from non-comedo ductal carcinoma *in situ*, a premalignant breast lesion with a finite, but relatively low rate of progression to invasion. Determination of hypermethylation of BRCA1 may play an important role in early detection of tumors. It has been observed recently that one case of sporadic breast cancer, misclassified for a BRCA1 mutation, turned out to be hypermethylated BRCA1 (46). These investigators used microarray analysis to identify genes associated with BRCA1 hereditary tumors that could contribute to BRCA1 positivity of breast tumors. Direct interaction of BRCA1 with known DNA methyltransferase, or demethylase complex has not been demonstrated. However, BRCA1 has been shown to be physically associated with a component of the histone deacetylase complex (47). It is possible that histone acetylation and CpG methylation may be inter related epigenetic processes. It is also feasible that methylation reflects gene activation rather than being the cause of it. Thus, restoring gene expression by treating cells with the demethylating agent 5' aza-deoxycytosine could restore BRCA1 expression. Such studies remain to be explored.

### 2.4. Physiological role of BRCA1

It is a phospho-nuclear protein, generally residing in the nucleus; however, BRCA1 may "shuttle" between nucleus and cytoplasm (48) . BRCA1 consists of the N-terminal Really Interesting New Gene (RING) finger domain and a C-terminal acidic domain termed BRCA1 C terminus (BRCT) (8;49). BRCA1 has both nuclear localization signals (NLSs), nuclear export signals (NES), and 2 C-terminal BRCT domains of about 100 residues (50). BRCT domains are a common protein-protein interaction motif involved in DNA damage response and repair. BRCA1 may participate in mammalian heat shock response pathways (51). BRCA1 has been shown to localize in the mitotic centrosome, where it interacts with γ-tubulin (Liu *et al.*, 2002), suggesting its role in mitosis. BRCA1 is shown to be associated with a hyperphosphorylated form of retinoblastoma protein (pRb), an interaction that is crucial for BRCA1-induced growth arrest in the G1/S phase of the cell cycle (53). pRB interacts with E2F to inhibit cell proliferation and it is possible that BRCA1 keeps RB in the hyperphosphorylated state to achieve growth arrest. Furthermore, the BRCA1-RB complex interacts with a histone-deacetylase complex (47) to suppress the transcription of E2F-responsive genes, perhaps to inhibit cell growth.

The BRCT domain is found in various proteins, including 53BP1, RAD9, RAD4, crb2 and RAP1(54). The recently described MDC1 (mediator of DNA damage checkpoint protein 1) also possesses a BRCT domain (55). The NH2-terminal RING domain of BRCA1 mediates association with protein BARD1, which is similar in structure to BRCA1 (56) and BAP1that binds to the wild-type BRCA1-RING finger, but not to germline mutants of the BRCA1-RING finger found in breast cancer kindreds (57; 58). The BRCA1 and BARD1 complex was shown to exhibit ubiquitin ligase activity, which is lost by mutations in the BRCA1 gene in the RING finger region (59,60,61). In fact, recent studies have indicated that BARD1 plays a critical role in preventing nuclear export of BRCA1 by masking NES (48). BRCA1-BARD is the first example of a RING-dependent Ubiquitin Ligase that depends on the heterodimer to exhibit ubiquitin activity.

BRCA1 has a protein interaction domain for p53 (62), and BRCA1 expression is modulated by p53 (63). Levels of BRCA1 are down-regulated in response to p53 induction by DNA damage in cells that undergo either growth arrest or apoptosis (63). It has been suggested that, once phosphorylated, BRCA1 acts synergistically with p53 to cause cell cycle arrest after DNA damage; it then is degraded in a p53-dependent manner, when its is no longer required. BRCA1 initially participates in the accumulation of p53 protein but later p53 acts to reduce BRCA1 expression, perhaps via a feedback loop (64,65).

### 2.5. BRCA1 in Cell Cycle Control and DNA Repair

BRCA1, also called the caretaker of the genome (66), is involved in maintaining genome stability by virtue of its important role in cell cycle control, DNA double strand break repair, and transcription-coupled repair (reviewed by Deng and Brodie, 2000(50)). The BRCA1 RING domain has a direct link with Ub ligase, and mutations in these regions of BRCA1 have been shown to predispose to cancer perhaps by altering the Ub ligase activity (61,67). The BRCA1 protein and its interacting proteins are shown in Figure 1.

The BRCA1 protein shows no homology to any known protein and it is expressed widely (8). The functional motifs in the BRCA1 protein that have been described include a RING finger domain, a carboxy-terminal domain called BRCT, binding sites for tumor suppressor p53 and DNA repair protein RAD51, a human homolog of RecA. BRCA1 physically interacts with the proto-oncogene, c-Myc, and it may function as a tumor

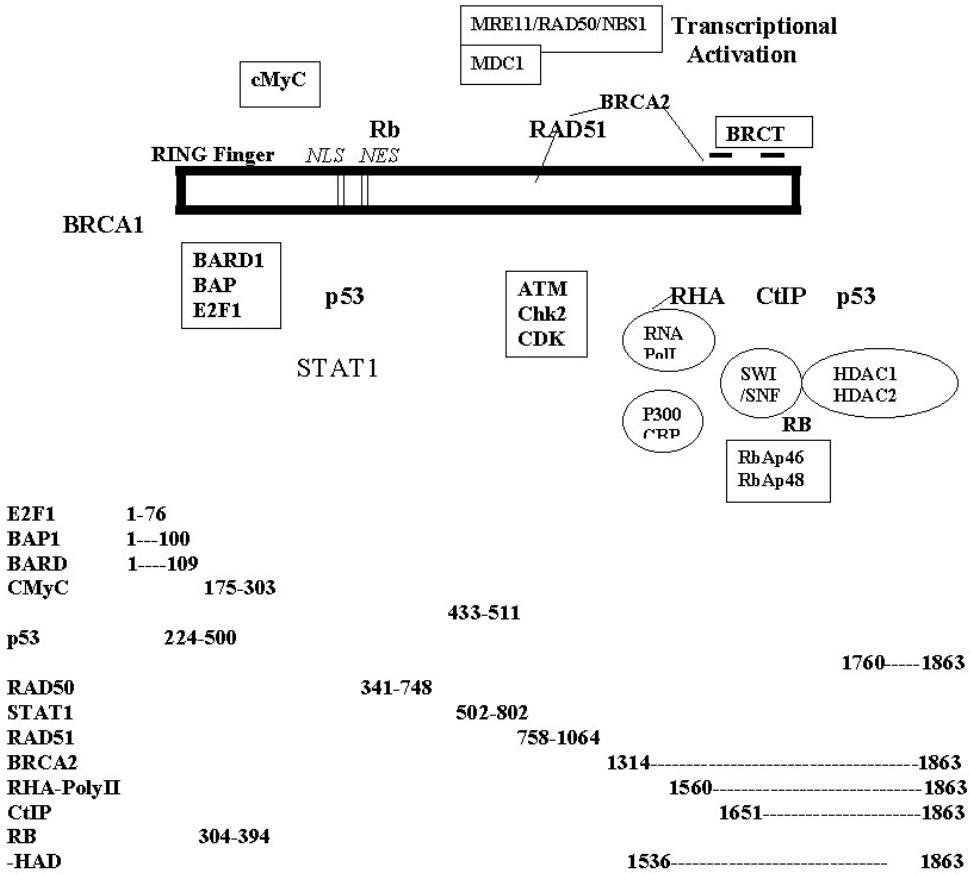


**Figure 1.** Diagrammatic representation of the BRCA1 protein. The interacting proteins are shown. (See text for detail also see Ref. 50, 77,78).

suppressor by regulating the activity of c-Myc (68). The region of BRCA1 between amino acids 502 and 802 interacts with the C-terminal transcriptional activation domain of the signal and activator of transcription 1(STAT1) and this interaction contributes to its IFN-γ activation (69). Each domain interacts with specific protein(s) pivotal for distinct functions in cellular processes. Thus, BRCA1 may directly control various assigned functions and also influence these functions by modulating their interaction with other proteins.

Evidence of a BRCA1/BARD1 interaction stems from immonofluorescence localization studies which show that both proteins co-localize to S-phase nuclear dots or foci (70). Neither BRCA1 nor BARD1form nuclear foci in G1; however, they come together just before the S phase. Unlike BARD1, which shows constant levels throughout the cell cycle, the expression of BRCA1 is generally absent or low during G1, but it peaks before the S-phase. BARD1 and RAD51 localize to PCNA nodules following treatment with hydroxyurea or UV (71,72).

Double strand breaks (DSB) are considered to be highly dangerous lesions in cells, such breaks can be generated by various genotoxic agents, from exogenous and endogenous sources. Exogenous sources include ionizing radiation (IR), radiomimetic agents, and chemotherapeutic agents. Endogenous agents are generated by mechanical stress and reactive oxygen species. In addition, endogenous topoisomerase, DNA cleavage, replication, meiosis, and fragile site formation can also generate DSB. Two distinct mechanisms have been established for the repair of DSB: I) Homologous recombination (HR) uses a sister chromosome as a template for repair; II**)** non-homologous end-joining (NHEJ), rejoins two broken ends of DNA directly.

One of the clues linking BRCA1 to DNA repair was its association with Rad51, the primary RecA homolog in eukaryotic cells (70,71,73,). RAD51 shares significant homology with bacterial RecA, which has been shown to mediate the pairing and ATP-dependent exchange of DNA strands in recombination (74). RAD51 interacts with the C-terminal region of the BRCA1 protein, between amino acid 758 and 1064. The BRCA1 protein co-localizes with Rad51 in nuclear dots during the S-phase and in response to DNA damage, suggesting that it may also be involved in homologous recombination and recombinational repair. BRCA1 null mice suffer embryonic lethality and are very similar in phenotype to mice lacking Rad51 or BRCA2

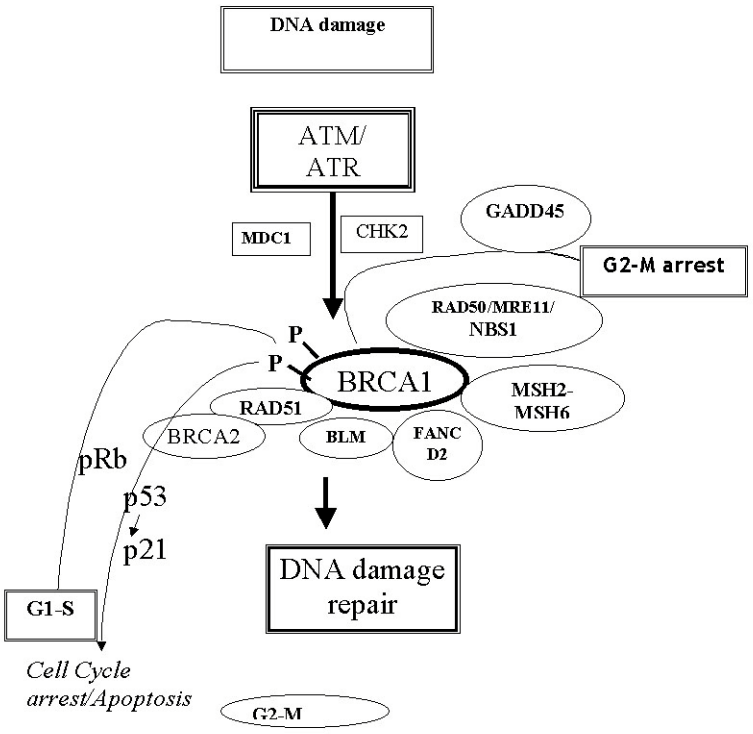


**Figure 2.** A model showing interaction of BRCA1 with other proteins in response to DNA damage. In this case, BRCA1 is phosphorylated by ATM/ATR. BRCA1forms complexes to repair Double Strand Breaks (DSB) by homologous recombination. BRCA1 also has a role in cell cycle arrest at G2-M checkpoint, possibly via upregulation of GADD45. The BRCA1-associated genome surveillance complex (BASC)-containing RAD50/MRE11/NBS1, DNA mismatch repair proteins, DNA helicase BLM (Bloom's syndrome protein) and others are in sensing and repair of abnormal DNA. BRCA1 may interact with p53 pathways to initiate cell cycle arreats/ apoptosis, if necessary, or interacts with Rb to control G1-S stage of cell cycle (Ref. 50, 77,79).

genes. These embryos display cellular proliferation defects, are sensitive to ionizing radiation, and exhibit high levels of chromosomal abnormalities; the latter can be partially rescued by p53 mutation.

BRCA1 is associated with Rad50 as a part of the Mre11/Rad50/Nbs1 (nibrin) complex (MRN) (75,76), which is involved in both nonhomologous end joining (NHEJ) and homologous recombination in yeast and vertebrate cells (74,77,78,79). The MRN complex localizes to the sites of DBS *in vivo* and plays a critical role in DNA metabolism, including DSB repair, meiotic recombination, and telomere maintenance. Cells deficient in Mre11 or Nbs1 continue DNA replication following X-ray damage, and thus display defective checkpoint signaling during the S-phase. In response to double strand breaks BRCA1 is phosphorylated by ATM, a kinase that phosphorylates the multiple protein complex (75,80,81,). ATM also phosphorylates Nbs1 in response to DNA damage (75,82,83). The BRCA1 foci that appear after ionizing radiation, are co-localized with the subset of foci formed by the MRN complex, re-stating a role for BRCA1 in the cellular response to DSB. In addition, BRCA1 is also involved in another kind of DNA repair, namely base excision repair (BER) (84). Embryonic stem cells of BRCA1-deficient mice exhibit defects in transcription-coupled repair, which are generally the result of accumulation of oxidized bases following insult by ionizing radiation. BRCA1 may manifest its role in BER through its association with mismatch repair enzyme (85) or by transcription via its interaction with RNA polymerase II holoenzyme. During the S-phase of the mitotic cell cycle, BRCA1 colocalizes with RAD51 in subnuclear structures, Scully *et al.* (1997)(70,71), known as "BRCA1 nuclear dots" that are succulent in nature. However, in response to DNA damage these dots appear to disperse (70,71). BRCA1 interacts with BRCA2, which directly binds with RAD51 (77,79). Recently it has been shown that BRCA1 co-purified with several proteins that are associated with the DNA damage machinery, including mismatch repair enzyme, MSH2, MSH6, MLH1, ATM, BLM, as well as MRN protein complex, in a single complex referred as the BRCA1-Associated Surveillance Complex (BASC) (75). BASC contains at least 15 subunits and is involved first in sensing damaged DNA and then in repairing it. All of these BRCA1-associated proteins may have BRCA1-dependent as well as independent functions and BRCA1 may regulate such proteins for a specialized repair. Figure 2 illustrates that BRCA1 forms various complex(es) with DNA-repair proteins to participate in repair of damaged

DNA or accelerate cell cycle arrest. Also, DNA replication factor C, itself a protein complex that recruits PCNA onto DNA polymerase δ, was found to be a component of BASC. Many proteins in BASC are tumor suppressors, indicating that loss of integrity of BASC may be a central mechanism in tumor development. The MSH mismatch proteins are involved in repair of mismatched DNA lesions is due to spontaneous errors during DNA replication or during repair of DSBs. It is possible that a MSH2-MSH6 complex may signal regulation of down-stream events, such as apoptosis or cell cycle arrest via BRCA1. In this manner, MSH2-MSH6 as a multi-protein complex interacts with the repair machinery and modulates cell cycle checkpoints and apoptosis decisions. The DNA helicase gene Bloom-syndrome (BLM) may be involved in sensing abnormal double strand DNA structures formed during replicatio). R/M/N protein complex is involved in DNA repair at stalled replication forks (87). Both ATR and ATM are components of BASC, which can phosphorylate numerous proteins of the BASC complex (89,90). BRCA1 can participate in DNA repair in many ways. One possibility is that BRCA1 acts as a scaffold protein. BRCA1 may also exert local activities at DSB sites via its interaction with enzymes that alter chromatin and DNA structures. BRCA1 interacts with SWI/SNF and other proteins that remodel chromatin with regulators of histone acetylation/deacetylation (reviewed by 77,78,79). BRCA1 also interacts with DNA helicases, including the RecQ homolog encoded by Bloom's syndrome gene, BLM and the helicase BACH1 (88). Perhaps these interactions are required for the accessibility of the repair machinery. One response of BRCA1 to DNA damage is to monoubiquitinate histones (H2A and H2B) in conjunction with BARD1; in this way chromatin remodeling takes place that could allow the DNA repair machinery to gain access to the damaged DNA.

The two kinases, CHK1 and CHK2 are responsible for the maintenance of the G2-M DNA damage checkpoint. CHK2 phosphorylates BRCA1 in response to DNA damage induced by IR. Chk1 and Chk2 are classic serine-threonine kinases that are required for cell cycle arrest in response to DNA damage. As downstream kinases, they are phosphorylated by an ATM/ATR-dependent process and then Chk2 phosphorylates BRCA1 (87,89). BRCA1 phosphorylates CHK1 to control G2-M transition. Reciprocal co-immunoprecipitation of BRCA1 and CHK1 has been shown in HeLa and MCF7 cells. In BRCA1 mutant HCC1937 cells, where BRCA1 is expressed, colocalization of BRCA1 and CHK1 is maintained even after gamma-radiation (90). It has been proposed that, in response to DNA damage, BRCA1 controls cell cycle progression to mitosis via CHK1, which regulates Cdc2 kinase, Cdc25C and WEE1 (91). Some studies suggest that BRCA1 forms a complex with the transcriptional co-repressor complex CtP and CtBP through its BRCT domain, and during the DNA damage response, this complex gets dissociated from BRCA1, which, in turn, activates GADD45 and p21.

Specific sites in BRCA1 are responsive to DNA damage repair stimuli. BRCA1 becomes phosphorylated in response to treatment of cells with a variety of DNA damaging agents, such as, UV, IR, adriamycine, hydroxyurea, mitomycin C, and hydrogen peroxide. Multiple phosphorylation sites at the serine (S) residue, including S1330, S1423, S1466, S1466, S1524 and S1542, have been detected by mass spectrometry analysis of recombinant BRCA1 peptides, phosphorylated *in vivo* in an ATM-dependent manner (89). These phosphorylations may lead to a change in BRCA1 subnuclear localization. Among specific BRCA1 phosphorylation sites that have been responsive to DNA damage. For example, in MCF-7 cells, IR-and UV-induced phosphorylation of BRCA1 at Ser-988/-1524 and Ser-988, respectively, was seen during the S-phase (92); however, in the G2/M phase, IR and UV treatment induced phosphorylation of Ser-988/ser-1423 and Ser-1423, respectively (92). In HCC1937 cells, with specific BRCA1 mutation where the functional C-terminal BRCT domain is lost, phosphorylation of Ser-1423 and –1524 was not induced. It is possible that allosteric change of the BRCA1 structure due to phosphorylation, may affect its interaction with other proteins involved in DNA damage repair pathways (93).

## 3. CONCLUSIONS

Thus, in summary, it is evident from the discussion presented above that BRCA1 serves as one of the important tumor suppressor genes in the etiology of the breast cancer, particularly in high risk families. The wide spectrum of mutations observed in the gene in various populations of the world, with a few exceptions, is not specific to any particular population and mutations are scattered throughout the coding region of the gene. While BRCA1 follows Knudson's "two hit" hypothesis, in familial early on set cancers, the mode of its inactivation, in the much more common in sporadic cancers, is poorly understood; the promoter hypermethylation as a mechanism of inactivation is prevalent only in less than half of the cases. A significant amount of work, therefore, is needed to elucidate the role of BRCA1 in sporadic cancers. Its physiological role in DNA damage sensing and repair sheds light on its function as a caretaker to maintain the genomic stability. Inactivation of BRCA1 confer on cells, new genetic abnormalities; this, in turn, leads to tumorigenesis. Future studies will focus on elucidating the mechanisms of BRCA1 in the multistep process of tumorigenesis relating to sporadic cancers, and eventually means to prevent cancers.

## 4. ACKNOWLEDGMENTS

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