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Epigenetic changes in BRCA1-mutated familial breast cancer

Bradley Downs and San Ming Wang*

Department of Genetics, Cell Biology and Anatomy, College of Medicine, University of Nebraska Medical Center, Omaha, NE 68198

Abstract

Familial breast cancer occurs in about 10% of breast cancer cases. Germline mutation in *BRCA1* is the most penetrant predisposition for the disease. The mutated *BRCA1* leads to the disease by causing genome instability via multiple mechanisms including epigenetic changes. This review summarizes recent progress in studying the correlation between *BRCA1* predisposition and epigenetic alterations in *BRCA1*-type familial breast cancer.

Breast cancer is the most frequent cancer in women (1). In the United States, the chance of a woman being diagnosed with breast cancer is 1 in 8 in her lifetime (2). Of the breast cancer cases, about 10% or so are familial breast cancer, in which multiple members across generations in the family are affected in an autosomal dominant manner. Germline predisposition has been determined as the major factor for familial breast cancer. The best-known predisposition is the germline mutation in *BRCA1* (*BRCA1+*) (3, 4, 5). A woman who has inherited a pathogenic mutation in *BRCA1* (*BRCA1+*) has up to 80% probability of developing breast cancer by the age of 70 (6).

BRCA1 is a tumor suppressor gene that plays essential roles in maintaining genomic stability through multiple mechanisms including homologous double strand DNA break repair, transcriptional regulation, chromatin remodeling, ubiquitin ligation, cell cycle and apoptosis regulation (7). Germline mutations in *BRCA1* cause premature truncation, translational frame-shift, altered splice sites, and nonsense-mediated RNA decay. These changes damage the normal function of *BRCA1*, cause genome instability, and lead to the development of breast and ovarian cancer (8, 9). It was previously shown that the *BRCA1* gene can be regulated by promoter DNA methylation (10). Additionally, more recent studies show that *BRCA1* also plays important roles in multiple types of epigenetic modifications and mutated *BRCA1* can cause epigenetic abnormalities, which contribute to the development of breast cancer.

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*Corresponding author: San Ming Wang, MD, Department of Genetics, Cell Biology and Anatomy, College of Medicine, University of Nebraska Medical Center, 986805 Nebraska Medical Center, Omaha, NE 68198, sanming.wang@unmc.edu.

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The role of BRCA1 in DNA methylation

Of the four types of DNA methyltransferases, DNMT1, DNMT3a DNMT3b and DNMT3L, DNMT1 is essential in methylation of the nascent replicated DNA strand to maintain the original DNA methylation pattern of DNA (11). In contrast, DNMT3a and DNMT3b act as *de novo* methyltransferases to methylate previously unmethylated DNA (12, 13). DNMT3L is involved in maternal genomic imprints (14 13). Approximately 60% of protein-coding genes harbor CpG islands in their promoters (15). In general, hypomethylated promoters are transcriptionally more active than hypermethylated promoters, which are often silenced (16). Many of those genes silenced by DNA methylation are involved in important functions including cell cycle control, steroid receptors, metastasis, oncogene and tumor suppressor. The relationship between BRCA1 and DNA methylation has been extensively studied in familial breast cancer (17–18).

Followings are examples showing the relationship between BRCA1 and methylation:

- BRCA1 and hypermethylation

Abnormal expression of estrogen receptor plays important role in breast cancer development, and the majority of BRCA1+ familial breast cancer is estrogen receptor negative. The promoter of estrogen receptor alpha (ER α) in *BRCA1*+ familial breast cancer is highly methylated (19), explaining why ER α is low in *BRCA1*+ familial breast cancer. Certain tumors exhibit aberrant concurrent hypermethylation of specific genes, a phenomenon known as CpG island methylator phenotype (CIMP) (20). Hypermethylation across the genome of blood cells in *BRCA1*+ carriers had significant correlation with breast cancer development and can be used accurately for disease prediction (21).

- BRCA1 and hypomethylation

In BRCA1+ familial breast cancer, DNA methylation is less abundant compared to sporadic breast cancers with normal BRCA1 (22). Hypomethylation leads these genes, such as the oncogene Rad9, to have increased expression, and thus promotes breast cancer proliferation and invasion (23). Hypomethylation can also cause unbalanced chromosomal translocation with breakpoints in pericentromeric DNA of specific chromosomes (24). Hypomethylation is also common in noncoding Alu and LINE-1 repetitive sequences in breast cancer (25). Studies in a *Brca1*-deficient mouse model showed that Brca1 can bind directly to the OCT1 site of *DNMT1* promoter to activate *DNMT1* expression at transcriptional level to maintain genomic methylation. Mutated BRCA1 lost this function, led to global DNA hypomethylation, loss of genomic imprinting, and over-expression of multiple oncogenes including *c-Fos*, *Ha-Ras*, and *c-Myc* (26).

BRCA1 can regulate gene expression through both DNA hypomethylation and hypermethylation. In general, BRCA1-regulated hypermethylation or hypomethylation is not a uniformed changes for all genes but gene type-dependent. For example, hypermethylation can suppress the expression of tumor suppressor genes and

hypomethylation can promote the expression of oncogenes (26). It remains unclear for how BRCA1 plays such a selective role.

Regulation of *BRCA1* expression by promoter DNA methylation

In certain familial breast cancer, *BRCA1* gene coding structure remains intact but the CpG islands in *BRCA1* promoter can be highly hypermethylated (27). Methylation in multiple CpG islands of the *BRCA1* promoter was observed in nearly a third of breast cancer cases. The event was more frequent in breast cancer in younger women, with high-grade pathology, and triple-negative breast cancer (estrogen receptor, progesterone receptor, Her2/Neu) (28). Compared with the *BRCA1* promoter without methylation, hypermethylation led to decreased or even completely abrogated expression of *BRCA1*. This event correlated with poor survival, and is present in both *BRCA1*+ familial breast cancer and sporadic breast cancer (29, 30). The significance of *BRCA1* methylation is that *BRCA1* promoter methylation in sporadic breast cancer can lead to functional consequence of loss of *BRCA1* function similar to *BRCA1* mutation in hereditary breast cancer (31, 32).

Relationship between *BRCA1* and histone modification

Histone proteins constitute the nucleosome core around which DNA is tightly packaged. Amino-terminal tails which protrude from the histone core harbor numerous sites for protein post-translational modification, such as acetylation, methylation, phosphorylation, sumoylation, ubiquitylation, or ADP-ribosylation (33). The type of modification and the affected amino acid residue determine the DNA-histone or other protein-histone interactions, leading to either an open chromatin state (active transcription) or compact chromatin (transcriptional repression). *BRCA1* ubiquitylates histone H2A in both major and minor satellite regions and the RING finger domain in the N-terminal of *BRCA1* is determined to be essential for this function (34). Mutation in the RING finger domain of *BRCA1* results in the loss of histone H2A ubiquitylation, leading to increased expression of satellite DNA, deficient homologous recombination and genome instability. *BRCA1* also deacetylates H2A and H3 through interaction between its BRCT domain and histone deacetylases HDAC1 and HDAC2 (35). Without *BRCA1*, HDAC2 cannot properly deacetylate H2A and H3, leaving histones in their active form. *BRCA1* also interacts with HDAC1 to deacetylate the genes involved in Ku70-involved non-homologous recombination pathway for DNA damaging repair (36). Lysine-specific histone demethylase 5B (KDM5B) is involved in DNA homologous damage repair through recruiting *BRCA1* to the damaged sites (37). *BRCA1*+ abolishes this interaction. Without functional *BRCA1*, It is likely that KDM5B will not be able to facilitate repair the damaged DNA.

BRCA1 and microRNAs

Certain miRNAs have been implicated in *BRCA1*-related regulation of histone modification. Studies show in breast cancer, at least 16 miRNAs were downregulated (miR-9-1, miR-29a-c, miR-148, let-7, miR-15/16, miR-17-5p, miR-27b, miR-125a/b, miR-126, miR-130a, miR-143, miR-145, miR-155, miR-200c, miR-205, miR-335) and 5 were up regulated (miR-10b, miR-18a, miR-21, miR-27a, miR-206)(38). However, this pattern may not be conclusive. Taking miR-155 as a typical example: miR-155 regulates many genes involved

in apoptosis, differentiation, angiogenesis, proliferation, and epithelial-mesenchymal transition (39–40), such as *SOCS1*, a member of the STAT-induced STAT inhibitor in negative regulators of cytokine signaling (41). BRCA1 represses miR-155 expression by deacetylating histone H2A and H3 on the miR-155 promoter via HDAC2. The mutated BRCA1 leads to acetylation of miR-155 promoter, overexpression of miR-155, dysregulation of cytokine signaling pathways, and promotes cellular transformation (38–41, Figure 2).

In conclusion, epigenetic abnormality in *BRCA1*+ breast cancer provides new avenues to understand the mechanism of *BRCA1*+ caused familial breast cancer, and could provide potentially new targets, for example, targeting the overexpressed miRNAs and DNA methyltransferases, for treatment of familial breast cancer.

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Figure 1.

BRCA1 and heterochromatin methylation. BRCA1 silences heterochromatin transcription by methylating heterochromatin through interaction with Wip1 phosphatase, HP1, and DNA methyltransferase. BRCA1+ loses heterochromatin demethylation, releases the transcriptional silencing, leads overexpression of heterochromatin transcripts, and causes genome instability. CH3: methyl group.

Figure 2.

BRCA1 regulates miR-155 expression. A. BRCA1 maintains intact structure of promoter of miR-155 by suppressing HDAC2 activity, block transcriptional factors binding to promoter elements, resulting in decreased miR-155 transcription; B. Mutated BRCA1 loses its suppression on HDAC2 activity, leading to increased acetylation of miR-155 promoter, increased transcriptional factors binding to promoter elements, and increased miR-155

transcription. The overexpressed miR-155 causes dis-regulation of cytokine signaling pathways, and promotes cellular transformation.

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