Review Article

BRCA1 promoter methylation in peripheral blood cells and predisposition to breast cancer

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Abstract

Early onset breast cancer is a common malignancy and cause of death among young women in KSA. In addition, the data from women have demonstrated that most patients present late with an advanced stage. The early detection of this disease would not only save patients’ lives but would also have the potential to reduce the budget and the time required for treating and nursing advanced breast cancer patients. This review highlights the risk of developing breast cancer in women with the methylated BRCA1 promoter in their white blood cells and proposes the potential use of this epigenetic modification as a powerful molecular marker for the early detection of breast cancer.

Keywords: BRCA1; Breast cancer; Epigenetic; Epigenetic modification; Methylation

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Introduction

Breast cancer among Arab women, as elsewhere in the world, is a common malignancy and cause of death, and its incidence is increasing. In KSA, 26.4% of all female breast cancers develop before the age of 40 compared to 6.5% in the USA. The breast cancer susceptibility gene, BRCA1, was discovered in 1994 as the first major gene associated with breast cancer.1 The hereditary type of breast cancer has been found to be attributed to germline mutations in BRCA1.2,3 Furthermore, DNA methylation is the mechanism by which BRCA1 is inactivated during sporadic carcinogenesis.4 Both types of tumours occur at an early age and exhibit poor histological differentiation, Oestrogen and Progesterone receptor negativity and similar global gene expression profiles.5

The detection of the methylated BRCA1 promoter in DNA from peripheral blood and tumour tissues in breast cancer patients6 has suggested the involvement of this epigenetic modification, which occurs in normal non-epithelial tissue, in the development of breast cancer with...
*BRCA1*-like characteristics. However, it is still undetermined whether women carrying the methylated *BRCA1* promoter in their WBC are at a high risk of breast cancer predisposition.

In this review, we explore the possible implication of *BRCA1* promoter methylation in the development of breast cancer and propose the potential use of this aberrant methylation as a powerful non-invasive molecular marker for detecting predisposed individuals at an early age.

**Breast cancer susceptibility gene: *BRCA1***

The human *BRCA1* gene is a tumour suppressor gene that is located on the long (q) arm of chromosome 17. *BRCA1* is expressed in cells in the breast and other tissues. *BRCA1* plays a crucial role in the process of DNA repair, the control of cell cycle checkpoints and transcription. The loss of *BRCA1* activity leads to tumour formation in specific target tissues. As *BRCA1* is involved in the potentially error-free pathway of homologous recombination, which repairs double-strand breaks, cells that lack the *BRCA1* protein tend to repair DNA damage by alternative error-prone mechanisms. This results in the generation of mutations and gross chromosomal rearrangements that can lead to carcinogenesis. Hence, females carrying germline *BRCA1* mutations are at an increased risk of developing aggressive breast and ovarian tumours characterized by poor histologic differentiation, high grade, aneuploidy, and hormone receptor negativity at an early age (<50%).

**DNA methylation is an alternative mechanism for *BRCA1* inactivation**

Both *BRCA1* mRNA and protein levels were found to be under-expressed in a subset of sporadic human breast cancers. These sporadic early onset breast cancers have aggressive pathologic features that are similar to those observed with mutated *BRCA1*. This finding suggested that, in the nonhereditary forms of breast cancer, alterations in *BRCA1* or *BRCA1*-related pathway(s) might also play a role in the aggressiveness and pathogenesis of sporadic breast cancer. As no somatic mutations in *BRCA1* were detected in the sporadic form of breast cancer, it was suggested that an epigenetic mechanism might be an alternative means by which *BRCA1* is inactivated during this form of breast carcinogenesis. Indeed, the results from several studies revealed that 9–44% of sporadic breast cancer samples harboured methylated *BRCA1* promoter.

**Structure of the 5' regulatory region of *BRCA1***

The 5' regulatory promoter region of *BRCA1* has been shown to contain 30 CpG sites overlying the area from −567 to +44 relative to the exon 1A transcription start site (Figure 1). A bi-directional core promoter (−218 to +1), which is located within this region, has been found to regulate the transcription of both the *BRCA1* and the *NBR2* genes. This 218 bp region is a CpG-rich area containing 11 CpG sites with a strong promoter activity that has been shown to be aberrantly hyper-methylated in human breast cancer cells and tissues but not in normal human mammary epithelial cells.

**Methylated *BRCA1* promoter in peripheral blood DNA from breast cancer female patients**

In 2008, Snell et al. have demonstrated the presence of the methylated *BRCA1* promoter in normal non-epithelial tissues in patients from breast-ovarian cancer families. This finding suggested that the methylated *BRCA1* promoter occurring in this tissue of the body is linked with *BRCA1*-like breast cancer development. This led the author to hypothesize that the deactivation of *BRCA1* by promoter hyper-methylation might occur as a germline or an early somatic event, leading to breast cancer predisposition with a phenotype that is similar to that linked with *BRCA1* germ-line mutations. Subsequent to Snell’s study, several investigators have reported the detection of methylated *BRCA1* in very young breast cancer patients suggesting the potential use of methylated *BRCA1* as a predictor of cancer risk.

In 2011, we have reported that 27.6% of primary sporadic breast carcinomas in Arab women comprise the hyper-methylated *BRCA1* promoter. This occurrence is in the higher end of previously reported incidences of 7–44%. Notably, the methylation of the *BRCA1* promoter was found to be strongly associated with an early age onset of ≤40 years and is more common in high-grade tumours.

![Figure 1: Schematic representation of the *BRCA1* promoter region.](image)
Subsequently, in 2014, we have reported that 14.2% of breast cancer patients harboured the methylated BRCA1 promoter in their WBC. This was also significantly associated with the early onset of the disease. A high proportion of those patients (66.7%) exhibited methylated BRCA1 in matching tumour DNA. This result suggests that the presence of BRCA1 promoter methylation in WBC may elicit the development of breast cancer. Certainly, it has been postulated that constitutional BRCA1 promoter methylation may represent the “first-hit” predisposing and initiating tumourigenesis with morphologic features similar to those associated with BRCA1 germline mutations.

**Methylated BRCA1 promoter in peripheral blood DNA from cancer-free women**

Snell et al. were the first to observe the presence of BRCA1 methylation in WBC DNA from a healthy female. This result led to the question of whether this female has a high risk of breast cancer predisposition in the future. Subsequently, several studies have reported the detection of methylated BRCA1 in WBC from normal healthy individuals. We also have shown the presence of the methylated BRCA1 promoter in WBC of 9.7% of healthy cancer-free women (carriers). The majority of those carriers are ≤40 years old, and 77% of them have cancer family histories, including breast and/or ovarian cancer.

**Detection of methylation-related mutations throughout the BRCA1 promoter CpG Island**

The use of high-resolution sodium bisulfite genomic sequencing of the BRCA1 promoter region has shown the presence of methylation-related mutations in WBC DNA from carriers and breast cancer patients. These types of mutations involve an association between cytosine methylation and T>C transitions, leading to the formation of novel CpG methylated sites. A number of these methylation-related mutations were found throughout the entire CpG Island, including the BRCA1 core promoter region (Figure 1). Although the functional significance of these mutations remains unknown, these mutations contribute to the overall methylation of the BRCA1 promoter region, suggesting their possible involvement in carcinogenesis. Indeed, several methylation-related mutations in the TP53 gene, which included those leading to the formation of new CpG sites, were found to predominate during lung carcinogenesis. Recently, the origin of T>C transition mutations in breast cancer has been revealed. It has been shown that these transition mutations are caused by DNA damage induced by Nitric Oxide, which is synthesized by the enzyme Nitric Oxide Synthase. This enzyme is enhanced in certain inflammatory environments and by oestrogen, and it is found to be over-expressed in the normal tissue adjacent to breast cancer. DNA damage caused by nitric oxide leads to the deamination of adenine to form hypoxanthine, which is then excised by the thymine DNA glycosylase base excision repair enzyme and repaired to C, resulting in the T>C transition. The majority of these mutations are observed in histologically normal tissues adjacent to breast cancer, and they occur most frequently in the 5'-ATG-3', 5'-CTG-3', and 5'-ATA-3' sites.

**Methylated BRCA1 promoter in peripheral blood DNA and the risk of breast cancer predisposition**

The following is an important question that still awaits a definite answer: Are carriers of the methylated BRCA1 promoter at a high risk of breast cancer predisposition? To answer this question, we hypothesized that if BRCA1 methylation in WBC presents a high risk of breast cancer predisposition, WBC from carriers should demonstrate molecular changes that are comparable, to some extent, to those identified in BRCA1-methylated WBC from breast cancer patients. Interestingly, we have demonstrated that cancer-free females harbouring the methylated BRCA1 promoter in their WBC have several breast cancer-related molecular changes that may provoke their potential predisposition for the development of breast cancer. We have reported that nine different breast cancer-related genes, in addition to BRCA1, were found to be epigenetically modified in WBC from both breast cancer patients and carriers. These genes are involved in various aspects of breast carcinogenesis, including tumour suppression (HIC1, CDH129, CDH130, CDKN2), DNA repair (MGMT, apoptosis (PYCARD, TNFRSF10C), and cell cycle regulation (CCNA1, CDH13). Furthermore, we have also reported that fifteen cancer-related genes in addition to BRCA1 were found to be differentially expressed in the WBC from breast cancer patients and carriers. Two of these genes, ATM and insulin-like growth factor receptor (IGFIR), were found to be highly expressed in the WBC from carriers compared to that from the breast cancer cases. An elevation in the expression of either of these genes has been reported to be associated with an increase in the risk of future breast cancer. We have also investigated the signature of plasma proteins in the carriers group and compared it with those in breast cancer patients and controls. In total, 35 proteins were found to be differentially expressed in the plasma from breast cancer patients, carriers, and controls. One of these proteins is Apolipoprotein CIII, which has been found to be down regulated in the plasma from pancreatic patients compared to that from controls. Hence, this protein was reported to be a potential marker for the early detection of pancreatic cancer. Intriguingly, we have reported the down regulation of Apolipoprotein CIII to be 3- and 1.5-fold in plasma from breast cancer patients and carriers compared to controls, respectively. Altogether, these findings suggest the existence of a robust correlation between the methylated BRCA1 promoter in WBC and breast cancer-related molecular changes. Accordingly, these findings may infer that women carrying the methylated BRCA1 promoter in their peripheral blood DNA are at a high risk of breast cancer predisposition.

**Conclusions**

BRCA1 promoter methylation occurring in WBC appears to be linked with a high risk of BRCA1-like breast cancer development. The high prevalence of this epigenetic modification in WBC DNA of cancer-free women may contribute to...
the high proportion of early onset breast cancer in women in KSA. Recently, a meta-analysis involving 40 studies, including our 2011 study, was performed to obtain a more precise estimate of the association between BRCA1 methylation and sporadic breast cancer. The study indicated that BRCA1 promoter methylation emerged as a useful predictive biomarker for breast cancer in clinical assessments. This strongly suggests the potential use of BRCA1 promoter methylation in WBC as a molecular biomarker for the early prediction of breast cancer predisposition.

**Author’s contribution**

NM is the sole author who conceived the idea of this review, revised the literature, wrote the initial draft, and edited the second draft. NM proofread the article and approved the final draft. NM is solely responsible for the content and the similarity index of this article.

**Conflict of interest**

The author has no conflict of interest to declare.

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