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Review Article

A Review on the Impact of Aberrant Methylation in Breast Cancer: Diagnostic, Prognostic, and Therapeutic Approaches

Majid Hosseini[1], Saba Feghhi-Najafabadi[2], Mehdi Azad[3*]

[1]Department of Medical Biotechnology, Biotechnology Research Center, Pasteur institute of Iran, Tehran, Iran.
[2]Department of Immunology, Pasteur Institute of Iran, Tehran, Iran
[3]Department of Medical Laboratory Sciences, Faculty of Allied Medicine, Qazvin University of Medical Sciences, Qazvin, Iran.

[*]*Corresponding author*: Mehdi Azad, Department of Medical Laboratory Sciences, Faculty of Allied Medicine, Qazvin University of Medical Sciences, Qazvin, Iran. Tel: +98-2833359501 Fax: +98-2833338034, E-mail: M.azad@qums.ac.ir

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Abstract: Breast cancer (BC) is still a major global health concern, and a key factor in its pathophysiology is epigenetic abnormalities, specifically DNA methylation and histone modifications. This review offers a thorough examination of current research on the effects of these epigenetic changes in BC, emphasizing significant discoveries in the fields of prognosis, diagnostics, and treatment strategies. In particular, the advancement of breast cancer and patient survival have been connected to promoter methylation of genes including *BRCA1*, *DAPK1*, and *RASSF1A*. Furthermore, there is a correlation between tumor size and grade and the methylation state of *APAF1*, *GSTP1*, and *ER*. Histone modifications, such as acetylation and methylation, are essential for controlling gene expression in breast cancer. Changes in these modifications are associated with the advancement of tumors and resistance to therapy. The analysis highlights the potential of methylation-targeting medicines to improve the effectiveness of traditional chemotherapy and reveals particular methylation indicators that differentiate malignant tissues from normal ones . Further clinical validation is necessary to confirm the efficacy of DNMT and HMT inhibitors in mitigating hormone resistance and epigenetic modifications in BC, despite encouraging outcomes. Large-scale trials are necessary to validate these results, and investigating combination therapy, including those targeting histone modifications, to enhance patient outcomes is one of the main recommendations..

Keywords: Biomarker, Breast cancer, DNA Methylation, Treatment

1. Background

Cancer is one of the most common diseases globally, which has a massive burden on society and causes many deaths worldwide (1, 2). In 2021, there were 2.3 million women diagnosed with breast cancer (BC) and 685,000 deaths globally. As of 2020, there were 7.8 million women alive who were diagnosed with BC in the past five years, making it the world's most prevalent cancer (3). Even with improvements in early diagnosis and treatment, BC continues to pose a major clinical challenge. The majority of the existing diagnostic techniques, which rely on imaging and histological analysis, can be intrusive and can fail to distinguish between benign and malignant tumors or identify cancers in their early stages. Furthermore, gene expression patterns play a major role in the molecular classification of BC, which separates it into subgroups like Luminal A (ER+/PR+/HER2-), Luminal B (ER+/

PR+/HER2+), HER2-positive (ER-/PR-/HER2+), Triple-negative (ER-/PR-/HER2-), and indeterminate BC, which is essentially Luminal A. According to these subtypes, invasive BC cases account for 50%, 20%, 15%, and 15% of cases, respectively (4-6). The obstacles of treatment are similarly difficult. Surgical procedures, chemotherapy, radiation therapy, and hormonal therapy are examples of conventional treatments that frequently encounter problems such medication resistance, unfavorable side effects, and patient variability in efficacy. Although tailored therapy regimens based on molecular profiles are beginning to take shape, finding and validating trustworthy biomarkers continues to be a major challenge (7, 8).

Cancer is known worldwide as a disease caused by progressive genetic abnormalities, including mutations in oncogenes, tumor suppressor genes, and chromosomal abnormalities. More importantly, epigenetic abnormalities are more common in most human cancers (9-11). BC is also caused by a combination of genetic and epigenetic aberrations. Numerous mutations are identified in early BC (12, 13). Inherited changes in gene expression due to epigenetic regulation can lead to the suppression or activation of specific genes without direct impairment in DNA sequence (14). In other words, most epigenetic changes are post-transcriptional and reversible events that do not target gene sequences (12, 15, 16).

The epigenetic profile is changed at different stages of tumor growth and development. As one of the main mechanisms of epigenetic regulation, DNA methylation can control gene expression and play an essential role in the pathogenesis of various cancers (17-19). Most DNA methylation occurs on cytosine that precedes guanine nucleotides or CpG sites (20-22).

Histone modifications, including those mediated by HMTs, can influence DNA methylation patterns by recruiting DNA methyltransferases to specific genomic regions, thus contributing to aberrant DNA methylation of cancer-associated genes. Some Histone Methyl Transferases (HMTs), such as EZH2 and MLL, act in large complexes with cofactors that may also be mutated in BC (23, 24). EZH2 has a significant role in cancer growth and metastasis development (24, 25). High expression of EZH2 has been discovered in many solid tumors, including BC (26, 27). The EZH2 level shows a negative relationship with the patients' outcomes. In addition, EZH2 can promote breast tumor-initiating cell proliferation (28, 29). Dysregulation of methylation-associated enzymes, including both HMTs and DNMTs, affects the methylome of breast cancer, leading to irregular expression of genes and cancer progression through direct aberrant DNA methylation of cancer-associated genes.

2. DNA Methylation as A Biomarker for the Diagnosis and Prognosis of BC

Aberrant DNA methylation plays a vital role in cell cycle regulation, apoptosis, tissue invasion and metastasis, angiogenesis, and hormone signaling involved in BC (30). Many genes have been hypermethylated in breast tumors or BC cell lines (31). Diagnosis and prognosis of BC are associated with aberrant methylome, especially hypermethylation. Most sites were hypermethylated and located upstream of transcriptional regulatory regions, including promoters. The studies emphasized the importance of the role of methylation profiles in the diagnosis and prognosis of genes and identified specific methylation markers to distinguish tissues from normal tissues. Promoter methylation of *BRCA1, DAPK1,* and *RASSF1A* genes may be associated with BC progression and survival. The *BRCA1* gene, which is widely recognized as a tumor suppressor gene, plays a critical role in repairing DNA through a process called homologous recombination. The process of adding a methyl group to the promoter region of a gene causes the gene to become inactive, which hinders the ability of DNA to be repaired. This in turn leads to an increase in genetic instability and the advancement of cancer (26). The methylation of *DAPK1*, a gene that induces programmed cell death, leads to a decrease in apoptosis and an increase in cell survival, hence facilitating the formation of tumors (27). *RASSF1A* functions as a tumor suppressor that plays a role in regulating the cell cycle and inducing apoptosis. The loss of function caused by promoter methylation leads to uncontrolled cell proliferation and the development of tumors. The methylation of *GSTP1* and *ER* genes shows a favorable correlation with tumor grade and size. The methylation of GSTP1, a gene involved in detoxification, results in the buildup of harmful substances and oxidative stress, which in turn promotes the development of cancer. When the ER gene undergoes methylation, a process important for hormone communication, it enables cancer cells to proliferate independently of hormones, hence

facilitating tumor growth. Malignant breast tumors exhibit a substantial proportion of hypermethylated genes, such as *RARβ2*, *APC*, and *Cyclin-D2*. The process of methylation affects the RARβ2 protein, which plays a role in retinoic acid signaling. This interference hinders the normal processes of apoptosis and cellular differentiation (32). Methylation-induced silencing of APC, a key element of the Wnt signaling pathway, leads to unregulated cellular proliferation and growth. When methylated, cyclin-D2, an essential factor for cell cycle progression, leads to uncontrolled cell proliferation (33).

Therefore, these genes are important biomarkers in the initial diagnosis and management of BC. Jeronimo et al. reported that the promoter of *14-3-3 σ* is methylated in BC, benign lesions, and standard tissue samples, with no significant association (34). Jing et al. (35) and Meng et al. (36) reported that *14-3-3 σ* promoter methylation was significantly higher in BC than in benign lesions and normal tissues. There is no significant correlation between *14-3-3 σ* promoter methylation status and clinical-pathological features, including age, tumor grade, clinical stage, lymph node status, histological subtype, and ER/PR/HER2 status. Several studies have evaluated the potential clinical implications of E-cad inactivation in BC (37, 38). The aberrant DNA methylations of tumor suppressors and growth-regulating genes in BC are shown in **Table 1**.

3. Treatment of BC By Regulating Methylation

Building upon the prognostic significance of DNA methylation markers, their role in guiding therapeutic strategies cannot be overstated. The integration of these markers into treatment planning offers potential avenues for personalized medicine in BC. The main treatments for BC include surgery, radiotherapy, and medication therapy which can be used solo or in combination (58). The type or combination of treatments depends on how the cancer was diagnosed (58, 59). Mastectomy includes women with *BRCA1* or *BRCA2* gene mutations and women with cancer in both breasts (60, 61). Adjuvant radiation therapy may be given to women with BC with *BRCA1* or *BRCA2* mutations (62, 63). Medication therapy includes chemotherapy, hormone therapy, targeted therapy, and immunotherapy (59, 60). Therapies that aim for HER2 receptors may be given with chemotherapy for HER2-positive and Luminal B types of BC (64). Hormonal therapy (endocrine therapy) is an effective treatment for most tumors with ER-positive, PR-positive, or Luminal A (65). Immunotherapy uses Pembrolizumab and Dostarlimab in triple-negative to boost the body's natural defenses to fight against cancer cells (66-68). Hormone therapy generally would not apply for this type of cancer because these cancer cells are not responsive to estrogen, progesterone, or HER2. Different types of immunotherapy can cause several side effects containing skin rashes, flu-like symptoms, diarrhea, and weight changes (69, 70). Also, targeted therapy using monoclonal antibodies like Trastuzumab, Kadcyla, and Entinostat, which attach to the HER2 protein on cancer cells, can help stop the cells from growing (71, 72).

Epigenetic biomarkers can be helpful as predictor markers for predicting drug responses (73). Also, therapies that target aberrant methylation by inhibitors of methyltransferases are now available and make better therapeutic efficiencies possible (74). Many biomarker genes have been evaluated for BC detection; however, in the absence of a unique biomarker that has sufficient specificity and sensitivity, a panel of multiple genes must be used (75). Although epigenetic changes are inherited, they are potentially reversible (17). Therefore, the prospect of intervention to reverse these changes as a possible means of restoring the malignant phenotype is adsorbent. The availability of DNMT and HMT inhibitors makes this a testable strategy (17, 76, 77).

Lower DNA methyltransferase activity inhibits tumor growth by increasing the expression of silenced genes such as tumor suppressor genes, alpha estrogen receptors, E-cadherin, and SFRPs (78). Cytidine analogs such as decitabine (5-aza-2'-deoxycytidine) and 5-azacytidine act as DNMT inhibitors and reactivate critical gene expression through DNMT depletion (74, 79, 80).

These drugs have severe limitations for the treatment because of their poor durability, lack of specificity for cancer cells, and rapid inactivation by cytidine deaminase (74, 81). These undesirable properties led to new DNMT and HMT inhibitors (81, 82).

3.`1. Dnmt Inhibitors

DNMT inhibitors act directly on activated endothelial cells and inhibit angiogenesis in *in-vitro* and *in-vivo* conditions (83). Decitabine and its analog, zebularine, show significant angiostatic activity. It is associated with a substantial effect on the expression level of angiogenesis inhibitory genes (84, 85).

Table 1: Frequency of methylation of some tumor suppressor and growth regulator genes in BC.

Function	Gene	Incidence (%)	Result	Ref
DNA damage Repair	*BRCA1*	The methylated status was detected in 44.4% of the malignant group. The methylated status frequency was 9.7% in the benign group	Findings suggest that promoter hypermethylation of the BRCA-1 gene may have prognostic value in patients with BC. Hypermethylation in the BRCA-1 promoter was significantly associated with poor overall survival in patients with BC.	(39)
		The methylated status was observed in 51.66% of DNA extracted from mononuclear cells (MNCs) in newly diagnosed peripheral blood samples of 60 histopathological patients.	No significant association was found between *BRCA-1* methylation status and age, menopausal status, histological grading, lymph node status, chemotherapy, ER/PR/HER2 status, and distant metastasis. Results indicate that promoter methylation of *BRCA-1* genes in DNA may be associated with BC progression and poor overall survival	(40)
	DAPK1	The methylated status was observed in 55% of DNA extracted from MNCs in peripheral blood samples of 60 histopathologically confirmed.	No significant association was found between *DAPK1* methylation status and age, menopausal status, histological grading, lymph node status, chemotherapy, ER/PR/HER2 status, and distant metastasis. Results indicate that promoter methylation of *DAPK1* genes in DNA may be associated with BC progression and poor overall survival.	(40)
		The methylated status of *DAPK1* was seen in BC tissue (36%) of 28 patients with BC.	They detected a significant correlation between the changed methylation status of the DAPK1 gene and mRNA levels. The role of hypermethylation in the inactivation of this gene in BC is in line with its onco-suppressor and proapoptotic gene functions.	(41)
	RASSF1A	The methylated status was observed in 46.6% of DNA extracted from MNCs in peripheral blood samples of 60 histopathologically confirmed	No significant association was found between the RASSF1A gene and age, menopausal status, histological grading, lymph node status, chemotherapy, ER/PR/HER2 status, or distant metastasis. Results indicate that promoter methylation of RASSF1A genes in DNA may be associated with BC progression and poor overall survival.	(40)
		In 134 of 149 (89.9%) primary breast carcinomas, the RASSF1A promoter was methylated.	A significant association between the methylation statuses of RASSF1A and known clinic pathological characteristics of BC patients was seen. DNA methylation features have a possible association with BC subtypes and may improve the management of BC patients.	(42)
	APAF1	The methylated status in BC tissue (32%) of 28 patients with BC was seen. The methylated status of this gene was not found in any of the histologically intact tissue specimens.	They detected a significant correlation between the changed methylation status of the APAF1 gene and mRNA expression levels. The role of hypermethylation in the inactivation of this gene in BC is in line with its onco-suppressor and proapoptotic gene functions.	(41)
		The methylated status of APAF1 was seen at 57% in tumor tissues and 21% in normal tissue.	There was no association between *APAF1* promoter methylation and the patients' age, menopausal age, and tumor stage. However, the methylation status was correlated with *APAF-1* expression level and tumor grade.	(43)
Steroid receptor	*ER*	The methylated status of *ER3*, *ER4*, and *ER5* occurred in 65%, 26.7%, and 61.7% of malignant BC tissues, respectively, and 57.5%, 21.7%, and 55.8% of serum DNA samples of BC cases, respectively.	This study demonstrated the potential utility of serum DNA methylation of the *ERα* gene promoter as a noninvasive diagnostic and/or prognostic marker in patients with BC.	(44)
		The methylated status of *ER3* and *ER5* occurred in 71% and 56% of BC tumor tissues, respectively. Overall, *ERα*-methylated status was detected in 88% of BC cases. The methylated status in the *ER3* was 1.3 times higher than in the *ER5*.	The higher prevalence of *ER* methylation in Iranian patients may be due to environmental exposures or carcinogenic lifestyles. A strong correlation was found between *ERα* methylation and ER-negativity in tumors. Also, *ERα* methylation has been associated with PR-negativity and ER/PR-negative status in breast tumors.	(45)
	RARβ2	Among 137 BC malignant tissues, the methylated status was detected in 36.5% of cases.	It was observed that *RARbeta2* hypermethylation was inversely correlated with age at diagnosis.	(46)
		The methylated status in 95.9% of malignant BC cases and 14.5% of the benign group was detected. The *RARβ2* methylated pattern was significantly reported in all triple-negative BC patients.	*RARβ2* promoter hypermethylation is a potential serum-based biomarker for the early detection of BC patients.	(32)

regulate the cell cycle	*P16INK4a*	The methylated status in the promoter of 33% of MNCs in patients with BC was detected.	Promoter hypermethylation of P16 may be a possible mechanism accounting for sporadic breast carcinoma. Lymph node involvement, cancer grade, and histopathological findings did not show any difference with the methylation status of the P16 promoter.	(47)
		The methylated status in 37.5% of benign samples and in 48% of malignant patients' MNCs was detected.	with high hypermethylation rate in *P16INK4a* gene of malignant BC cases suggests the silencing in the repair pathways, senescence, and cell cycle control in the impalpable breast lesions establishment.	(48)
Tumor Suppressor	*APC*	*APC* promoter methylated status was detected in 30.67% of BC tissues.	Methylation of *APC* was associated with low histological grade. The methylation status of *APC* can be a predictive marker for early detection and better management of BC patients.	(49)
		The methylated status of the *APC* promoter was detected in 93.4% and 7.8% of the malignant and benign cases, respectively, while it was not detected in normal individuals. *APC* methylated pattern was reported in 59% of TNBC patients	No significant difference was detected among clinic pathological factors apart from triple-negative BC. The methylated *APC* gene might be a valuable serum-based molecular marker for the early detection of BC.	(32)
	HIN-1	In general, the methylated status of *HIN-1* was detected in 53% of BC tumor samples	The methylation status of the *HIN-1* promoter was correlated with the age of the patients. *HIN-1* promoter methylation can be considered the best suitable biomarker for detecting field cancerization.	(33)
		The methylated status of *HIN-1* was seen in 13.6% of early-stage BC tumors.	Methylation of *HIN-1* was associated with clinical characteristics. Hypermethylated *HIN-1* was not associated with overall survival and time to the first recurrence.	(50)
	Cyclin D2	The methylated status of *HIN-1* was seen in 62.1% of malignant BC tumors.	DNA hypermethylation of *Cyclin-D2* increased the possibility of malignant transformation. *Cyclin-D2* hypermethylation could be the potential biomarker for prognosis and early diagnosis of BC patients.	(51)
		The frequency of methylated status of *Cyclin-D2* was 19.6% in women with primary invasive BC.	No significant association was found between methylation frequencies and clinic pathological parameters of BC patients.	(52)
Carcinogen detoxification	*GSTP1*	Promoter-methylated status was detected in 34.4% of BC cases. 37.9% of methylated cases belonged to the early stage of the BC group, while 62.1% were from the advanced disease group.	*GSTP1* polymorphism was not associated with increased promoter hypermethylation. The results suggest that *GSTP1* methylation is a significant event in breast carcinogenesis and may be a tumor-specific biomarker.	(53)
		The methylated status frequency of *GSTP1* was 59.49% in tumor tissues. The methylated status frequency of *GSTP1* was 35.44%.	No significant association between the methylation frequency of *GSTP1* and clinic pathological parameters of BC patients were shown. The methylation frequency of *GSTP1* can be a potential biomarker for diagnosing and classifying BC.	(54)
		The methylated status frequency of *GSTP1* was 37.2% in tumor tissues. The methylated status frequency of *GSTP1* in standard adjacent tissue samples was 12.79%.	This study demonstrated a significantly elevated methylation frequency of the *GSTP1* gene in BC tissues, which was positively correlated with tumor grade and size, and negatively correlated with ER/PR expression.	(55)
Transcription factor	*TWIST*	The methylated status frequency of the *TWIST* gene was 25.0% in primary BC tumor tissue samples.	Tumor grade and age were meaningless with the *TWIST* gene's hypermethylation but found to be significant with lymph node positivity, ER positivity, PR negativity, and HER2/NEU negativity.	(56)
		The methylated status frequency of the *TWIST1* promoter was 34% in invasive BC cases.	*TWIST1* promoter methylation was not associated with the mitotic index, tumor size, grade, lymph node, or *TWIST1* protein or RNA expression.	(57)

3.2.HMT Inhibitors

HMT inhibitors are emerging as a promising epi-genetic therapeutic approach in clinical oncology (86). In recent years, HMTs have been targeted with a wide range of small molecule inhibitors. A list of potential investigational and approved DNMT and HMT inhibitor drugs for BC therapy is listed in **Table 2** (77, 84, 87-93). The potential epi-drug molecules listed in **Table 2** have shown promising anticancer effects against BC. Several clinical trials were performed using an epigenetic modifier and showed promising anticancer effects against BC.

Table 2. List of potential investigational or approved methylome-regulating drugs for BC therapy

Drug Category	Drug Name	BC Subtype	Approval	Current Indication	Research result	Ref
DNMT inhibitors	Azacytidine	TNBC	FDA approved 2020	Myelodysplastic Syndrome	Safe drugs such as low-dose 6-mercaptopurine singly or combined with 5-azacitidine, which are suitable for use before disease relapse, can inhibit highly resistant triple-negative BC cells.	(94)
					Epi-drugs such as Azacytidine possess the demethylation effect and changes in the expression of the innate immunity component involved in inflammation, metastasis, and tumor cell proliferation. This results in increased sensitivity of previously resistant cells to treatment with anticancer drugs.	(95)
	Zebularine	TNBC	Under trials	Colon Cancer	Zebularine treatment inhibits cell growth in a dose- and time-dependent manner. The combination of zebularine with decitabine or vorinostat significantly inhibits cell proliferation and colony formation in MDA-MB-231 cells compared with either drug alone.	(96)
					Scriptaid and Zebularine are potential anticancer drugs, either single or in combination, for the therapy of BC. After the treatments with zebularine, the variety of Scriptaid and zebularine exhibited a significant reduction in cell migration of BC cells (MDA-MB-231).	(97)
	Decitabine (5-aza-deoxycytidine)	TNBC	FDA approved 2006	Myelodysplastic Syndrome	5-aza-deoxycytidine increases the chemical sensitivity of anticancer drugs in BC cells and may be a promising approach to increasing the chemotherapeutic potential of these anticancer agents for more effective management of BC.	(98)
					Taxol-resistant BC cells expressing high levels of the multidrug resistance transporter remain sensitive to decitabine, suggesting that this drug could be used as a second-line treatment for chemo-resistant patients.	(99)
	5-Fluoro-2-deoxycytidine (FdCyd)	TNBC	Phase II clinical trials	Solid tumors	Increased FdCyd exposure allows it to be taken up intracellularly and converted to its triphosphate, which is incorporated into deoxyribonucleic acid (DNA) and inhibits the action of the DNMT. Inhibition of DNMT and DNA methylation can result in the re-expression of tumor suppressor genes.	(100)
	Hydralazine	ER+ (luminal and normal)	FDA approved 1997. For BC, it is in Phase II clinical trials	Hypertension	Hydralazine treatment significantly reduces tumor growth and volume in the mouse model. These findings suggest that the drug hydralazine has a slight anti-tumor effect on BC growth and progression in cell and mouse models.	(101)
					Hydralazine produces a decrease in DNA methylation in clinical cancer specimens. The effect of this demethylation on the expression of biologically important genes such as ER and p16 must be evaluated and presented. The biological effect of such an alteration in genomic DNA methylation would require subsequent investigation to prove its therapeutic impact.	(102)
	Guadecitabine	ER+/HER2+ (Luminal B) TNBC	Phase III clinical trials	Myelodysplastic Syndrome	Guadicitabine depletes and alters Myeloid-derived suppressor cells, inhibits the growth of two different murine mammary carcinomas in-vivo, and augments immunotherapeutic efficacy. The immunomodulatory effects of Guadicitabine can help rescue anti-tumor immune responses and contribute to the overall effectiveness of current cancer immune therapies. The immunosuppression relief provided by Guadecitabine may also enhance responsiveness to other cancer treatments such as checkpoint inhibitors.	(103)
	Epigallocatechin-3-gallate (EGCG)	Newly diagnosed BC TNBC	Phase I clinical trials	colon, kidney, breast, and brain cancers	EGCG can promote apoptosis in various malignant tumor cells. The apoptotic rate in MCF-7 cells treated with EGCG was significantly higher than the control group. EGCG can provide a theoretical basis for the new target drug development for BC.	(104)
					EGCG suppresses human BC cells' growth, migration, and invasion by inhibiting vascular endothelial growth factor expression. A better understanding of this mechanism may lead to an improved strategy for tumor therapy based on the inhibition of angiogenesis.	(105)
	Liraglutide	TNBC cell-like	FDA approved 2014	chronic weight management	Liraglutide and the combined treatment of Liraglutide, paclitaxel, or methotrexate effectively reduced tumor growth. The modulation of Cdh1 and Adam33 mouse gene expression by DNA demethylation suggests a role for Liraglutide in DNMT activity. In vivo results indicate that Liraglutide may be further analyzed as a new adjuvant treatment for BC.	(106)
					Liraglutide may function as a miR-27a inhibitor, potentially providing a novel method for the clinical prevention and treatment of BC.	(107)

HMT inhibitors	EPZ004777	TNBC	Pre-clinical studies	Mixed lineage leukemia	EPZ004777 shows remarkable selectivity against other HMTs, using SAM as a cofactor. EPZ004777 was able to selectively kill MLL-rearranged leukemia cells in culture while having little effect on non-MLL translocated cells and prolonging survival in a mouse model of MLL-rearranged leukemia.	(108)
	UNC0638	TNBC	Pre-clinical studies	TNBC & Lung Cancer	UNC0638 suppresses the migration and invasion of TNBC cells (MDA-MB-231 and BT-549 cells), inhibits cancer stem-like cell properties, and reduces the size and number of the tumor shape.	(92)
	Tazemetostat	HER2+ Luminal B	FDA approved 2020	Epithelioid sarcoma	Tazemetostat may be a novel treatment to improve the sensitivity of tumors to 5-FU in therapy. In conclusion, the combination of 5-FU and Tazemetostat shows high therapeutic possibility with reduced unfavorable effects.	(109)
					The histone-lysine N-methyltransferase enzyme (EZH2) Tazemetostat reasonably affected syngeneic xenograft tumor growth, increased the taxane-chemotherapy efficacy, significantly decreased overall tumor bulk, and increased intra-tumor T cells.	(110)

TNBC: Triple-negative breast cancer

They also reported positive results favoring epigenetic combination drugs with or without anticancer therapy compared to single-agent therapy. Various drugs have been approved or tested for the treatment of BC through epigenetic changes, especially methylation. These include Hydralazine, Tazemetostat, Pertuzumab, Trastuzumab, and Tamoxifen, which are effective against luminal subtype BC. Most DNMT inhibitors such as Azacitidine, Decitabine, FdCyd, Guadecitabine, and Liraglutide and some HMT inhibitors (EPZ004777 and UNC0638) can be used to treat triple-negative BCs through epigenetic changes. To treat HER2-enriched BC, histone methyltransferase inhibitors such as Tazemetostat and monoclonal antibodies such as Pertuzumab, Trastuzumab, Tamoxifen, and chemotherapy drugs (Kadcyla) are used for epigenetic changes. (**Fig. 1**)

Evidence suggests that epigenetic therapies can work synergistically to increase therapeutic effects when combined with conventional chemotherapy. DNMT and/or HMT in the treatment of BC must be tested in various trials to evaluate its effectiveness in overcoming epigenetic changes and hormonal resistance.

Most combined DNMT inhibitor therapies evaluated have included a combination of decitabine and 5-azacytidine with other drugs. Vijayaraghavalu *et al*. noted a significant increase in doxorubicin treatment efficiency in MCF-7 and MDA-MB-231 cells when combined with decitabine (111). This treatment stopped the cell cycle phase for more than 90% of the cells and restored doxorubicin sensitivity by highly regulating p21 proto-oncogene expression to overcome the drug resistance of these BC cells. A recent study reported that combination therapy with methyltransferases (5-azacytidine) inhibitors and suppression of *MBD2* expression using RNA interference technology resulted in activation of apoptosis and reduced cell growth and inactivation of invasive and metastatic processes in BC cells (112).

4. Clinical Challenges

The heterogeneity of breast cancer is a significant difficulty in the application of DNA methylation for diagnostic, prognostic, and therapeutic purposes. There can be substantial variation in the epigenetic landscape among patients and even between various regions of the same tumor. The presence of diversity poses challenges in the development of universally applicable standardized diagnostic and prognostic markers. Another obstacle pertains to the stability and identification of methylation marks. Although tissue biopsies offer direct access to tumor DNA, they can be invasive and may not always be possible. Liquid biopsies, which examine the presence of circulating tumor DNA (ctDNA) in blood samples, provide a less intrusive option but may exhibit reduced sensitivity and specificity (30). Moreover, the expense and intricacy of epigenetic profiling can hinder the general adoption of this technique in clinical settings. Precise identification and measurement of DNA methylation alterations necessitate the use of sophisticated sequencing methods and bioinformatics tools, which can incur significant costs and demand substantial

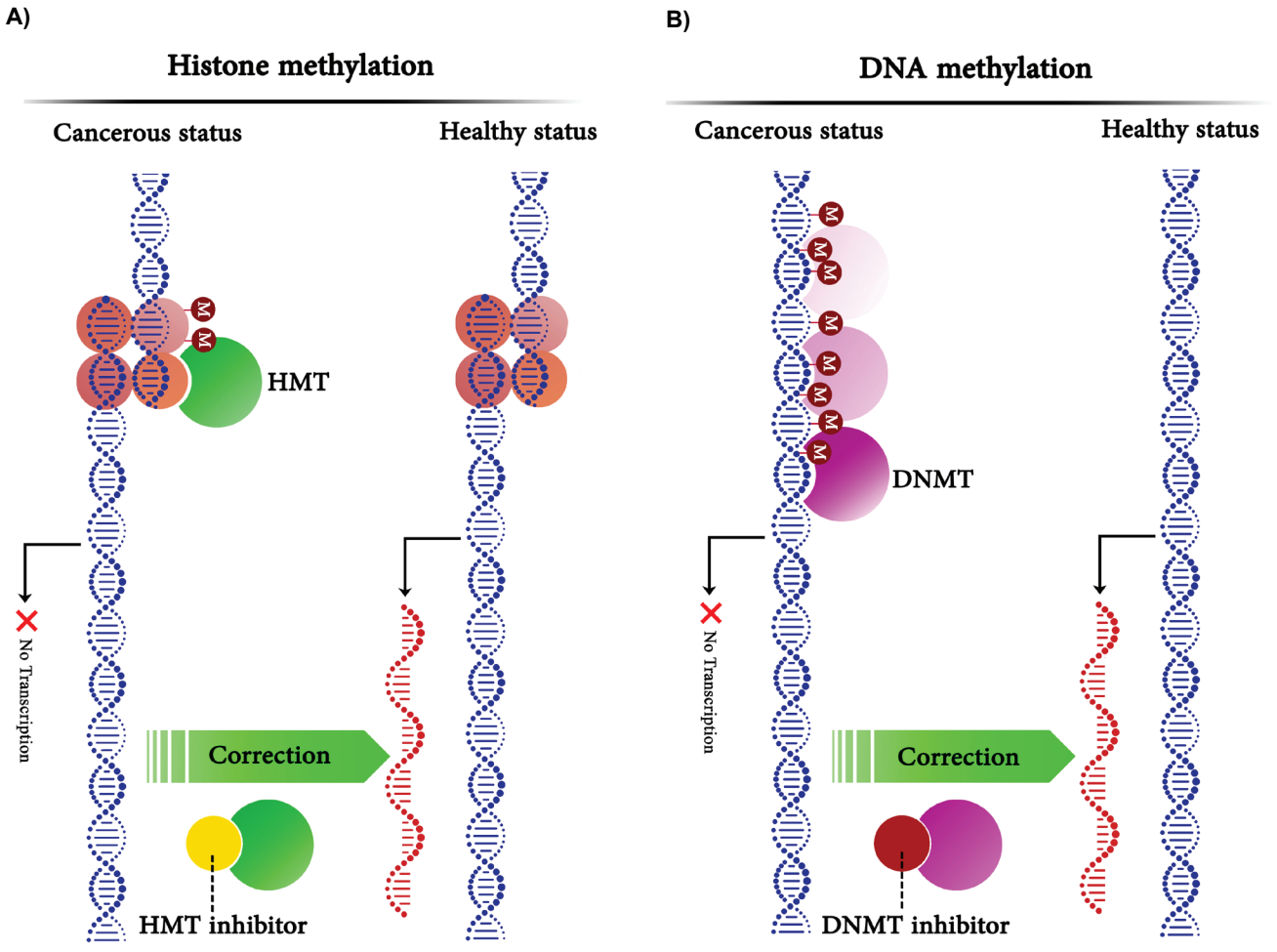

Figure 1. Aberrant methylations in breast cancer and relevant therapeutic approaches. A) The aberrant histone methylation leads to down-regulation of essential genes associated to healthy status of cells. HMT inhibitors correct the methylation status of histones throught inhibiting HMT. **B)** The abberant DNA methylation leads to down-regulation of essential genes associated to healthy status of cells. DNMT inhibitors correct the methylation status of DNA (specially hypermethylated promoters) throught inhibiting DNMT.

resources. Furthermore, it is imperative to conduct rigorous clinical trials in order to authenticate the clinical usefulness of these indicators. Although initial investigations have displayed encouraging outcomes, it is necessary to conduct bigger, multi-center trials to validate their efficacy and dependability in various patient populations (94). Although there are obstacles, the potential benefits of using DNA methylation indicators in the therapy of breast cancer are significant. They provide the opportunity for early identification, customized treatment strategies, and enhanced prognostic precision. To fully harness the promise of these promising indicators, we need to tackle the challenges that prevent their practical use in clinical settings.

5. Conclusion and Future Insights

In conclusion, most hypermethylated genes with biomarking potency were located in transcriptional regulatory regions, including the promoter. In the early management and diagnosis of malignant BC, detecting hypermethylated genes is very important for the most effective treatment, which can be investigated by methylation of *RARβ2*, *APC*, and

Cyclin-D2 genes. Methylation of several biomarkers was associated with BC survival, including *BRCA1*, *DAPK1*, and *RASSF1A*. In addition, the correlation between methylation of different genes with clinical parameters can be used to ensure the effectiveness of treatment; for example, methylation of *APAF1*, *GSTP1*, and *ER* genes positively correlate with tumor grade and size. The examination of potential methylation-modulating drugs researched or approved for the treatment of BC concludes that most approved DNMT/ HMT inhibitors, i.e., Azacitidine, Decitabine, FdCyd, and Liraglutide or in-trial drugs, i.e., EGCG, Guadecitabine, EPZ004777, and UNC0638, can treat triple-negative BC through methylome correction. There are still many challenges in using DNMT inhibitors such as 5-azaC and 5-azadC, as monotherapy for solid tumors. Several factors, i.e., toxicity, lack of specificity, low stability, and the simultaneous activation of proto-oncogenes, promote the necessity of developing new inhibitors. Second-generation DNMT inhibitors such as Guadicitabine (SGI-110) may provide a superior response generation DNMT inhibitors. With recent advances in omics, future identification of effective biomarkers will increase our ability to use these agents most appropriate for each patient. Epigenetics combined with more extensive access to noninvasive biological material will be crucial over the increased understanding of the biology of BC and the development of personalized therapy to provide a better outlook for patients with BC.

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Conflict of interests

There is no conflict of interest in this manuscript.

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