Review Article

Epigenetic Modulation in Breast Cancer: From Mechanisms to Therapeutic Interventions

Abstract
Breast cancer, a complex and heterogeneous disease, remains a leading cause of global cancer-related morbidity and mortality. According to the World Health Organization 2020 report, breast cancer is responsible for over 685,000 deaths. This comprehensive review explores epigenetic modifications contributing to breast cancer initiation, progression, and therapeutic interventions. Dynamic modifications in DNA methylation, histone modifications, chromatin remodelling, and non-coding RNA expression patterns characterise the epigenetic landscape of breast cancer. Aberrant DNA methylation patterns, particularly in the promoter regions of tumour suppressor genes, lead to their silencing, providing a selective advantage for breast tumour cells. Histone modifications, mediated by enzymes such as histone acetyltransferases (HATs) and histone deacetylases (HDACs), influence chromatin structure, impacting gene accessibility and transcriptional activity. Dysregulation of chromatin remodelling complexes further disrupts gene expression patterns, contributing to tumour progression. Non-coding RNAs that play crucial roles in regulatory processes, such as microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), are essential in breast cancer biology. The therapeutic potential of targeting epigenetic modifications in breast cancer has gathered significant interest. DNA methylation inhibitors, histone deacetylase inhibitors, and agents targeting chromatin remodelling complexes have shown promise in preclinical and clinical settings. Additionally, identifying specific epigenetic biomarkers holds the potential for personalised therapeutic approaches. This article thoroughly analyses the relationship between epigenetics and breast cancer. Understanding the epigenetic alterations driving breast cancer offers promising avenues for developing targeted therapies, ultimately improving patient outcomes.

Keywords: Epigenetics, breast cancer, DNA methylation, histone modifications, medicine

INTRODUCTION

Embryologist Conrad Waddington first introduced epigenetics principles in 1942, focusing on changes in genetic phenotype without gene alterations. Through these principles, challenges bothering genetic malignancies and disorders can be resolved. This concept has evolved significantly based on the 17th-century idea of 'epigenesis'. The statements made by Conrad Waddington about epigenetics inspired further studies; however, its flaws resulted from a limited understanding and applications [1].

According to a current definition, epigenetics is the study of persistent, transferable, and heritable changes in gene expression brought on by chromosomal modifications as opposed to changes in DNA sequence [2]. In epigenetic mechanisms, the DNA sequence remains unchanged, but the activities of DNA are influenced by modifications in chemical compounds that affect gene expression. These modifications also impact other components of the chromosome other than the DNA. Notable modifications are biochemical changes in histone proteins. Histone octamers contain two copies of the histone proteins H2A, H2B, H3, and H4. The histone octamer plays a significant role in epigenetics because each histone has an N-terminal and C-terminal histone fold, a significant site for acetylation, methylation and other chemical interactions [3].

Epigenetics involves histone modification, ncRNA-associated gene silencing, and DNA methylation, controlling gene expression and silencing within cells, influencing cell types and functions [4]. During the early stages of development, specific patterns are created by DNA and histone modifications that remain consistent throughout multiple cell divisions. Disruption of these established epigenetic patterns in cancer may render tumour suppressor genes such as CDKN2A inactive while activating anti-apoptotic and pro-proliferative genes [5]. Epigenetics offers new insights into breast cancer development and progression, the second most common cause of cancer death among women worldwide, with over one in ten new cases diagnosed annually [6]. Routine screenings typically

detect breast cancer, which often develops without noticeable symptoms. Inherited DNA defects or pro-cancerous genes like BRCA1 and BRCA2, as well as exposure to oestrogen, can cause breast cancer. Breast cancer is, therefore, more likely to develop in families with ovarian or breast cancer history.

According to the World Health Organization (WHO), in 2020, there were an estimated 2.3 million new breast cancer cases globally, accounting for about 11.7% of all new cancer cases. Breast cancer is most common in developed countries but rapidly rises in developing countries [7]. The age distribution of cases and deaths varies globally, with Middle Africa having 43% of cases and 49% of deaths at postmenopausal ages, while Northern America and Western Europe have over 80% of cases and 90% of deaths [8]. Breast cancer rates vary due to genetic manipulations, lifestyle factors, and early detection and treatment access, making understanding risk factors crucial for early detection and treatment.

PATHOLOGY OF BREAST CANCER

Breast cancer progression is influenced by genetic and environmental factors, including DNA damage, genetic mutations, epigenetic modifications, and exposure to certain substances [9]. Aside from these factors, the evasion of growth suppressors, which form a part of cancer hallmark, plays an important role. For many cancers, the ability to hinder the normal functions of genes that prevent carcinogenesis is vital for proliferation and metastasis. Tumour genes in breast cancer, such as the BRCA1 and BRCA2, are mutated, leading to defective or pro-cancerous genes that can be inherited; individuals with a family history of breast cancer may be at greater risk of developing the disease. Typically, the immune system attacks cells with abnormal DNA or abnormal growth. However, this process fails in individuals with breast cancer, leading to tumour growth and spread [9].

RISK FACTORS IN BREAST CANCER

As female populations age, breast cancer incidence increases, putting many women at risk. Gender is often overlooked, but most breast cancers occur in women, making it a risk factor. Abnormalities during breast biopsy, such as lobular carcinoma in situ (LCIS) and proliferative alterations with atypia, increase the risk. First-degree relatives are two to three times more likely to develop the disease [10]. Genetic influences are responsible for 5% to 10% of breast cancer cases, but this number increases to 25% in women under 30. The BRCA1 and BRCA2 genes are linked to increased breast cancer susceptibility, with oestrogen exposure increasing risk at reproductive milestones. Oestrogen receptors (ER) increase susceptibility as oestrogen affects cancerous cell proliferation, DNA damage, tumour growth, and angiogenesis [11]. Women who are exposed to disproportionate amounts of chemicals and radiation from the environment or work settings are also at risk of breast cancer [12].

EPIGENETICS MODIFICATIONS IN BREAST CANCER

Epigenetic factors, including alterations in tumour suppressor genes and DNA repair pathways, contribute to 90% of breast cancer cases, prompting researchers to explore new therapeutic strategies [13]. As previously stated, the common epigenetic modulations are DNA methylation, histone modifications, non-coding RNAs and chromatin remodelling.

DNA Methylation

DNA methylation is a common epigenetic modification frequently involved in breast cancer development. DNA methylation, a process where a methyl group is added to DNA, can modify gene expression without altering DNA sequences. The process of methylation can lead to the progression of breast cancer, particularly in younger women without inherited mutations in BRCA1 and BRCA2 genes. The methylation of other tumour suppressor genes, such as RASSF1A and CDKN2A, has also been linked to the development of breast cancer. DNA demethylating agents could be a promising therapeutic approach [14].

Breast cancer is a complex disease with varying gene methylation based on subtype and genetic makeup. Genes such as TP53, GSTP1, and CDH1 have been studied for their involvement in breast cancer because of their ability to undergo methylation. The TP53 gene helps in apoptosis by producing a protein, p53, and prevents altered DNA sequences from being passed to daughter cells [15]. The CDH1 gene, responsible for producing E-cadherin, functions as a tumour suppressor protein and regulatory molecule [16]. Methylation can turn it into an oncogene, promoting tumour cell growth, similar to GSTP1 and RASSFIA genes.

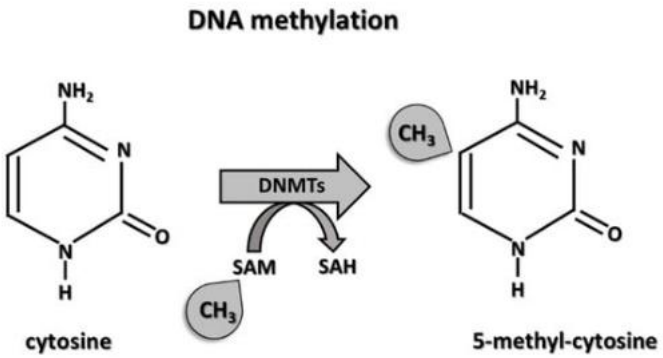

Figure 1: DNA methylation's mode of action. DNA methylation is carried out by DNA methyltransferases (DNMTs). DNMTs transfer methyl groups from SAM (S-adenosylmethionine) to SAH (S-adenosylhomocysteine) at the 5′-position of cytosine residues in CpG dinucleotide, resulting in the formation of 5-methylcytosine [17].

Histone Modifications

Histone, as a nuclear material, plays a vital role in defining the integrity of the DNA and plays essential functions in epigenetic processes [18]. The modification of histones refers to the chemical alterations that occur to histone proteins, including acetylation, methylation, phosphorylation, and ubiquitination. Histone modifications can affect how DNA is transcribed, thus regulating gene expression and influencing cancer progression in some cases.

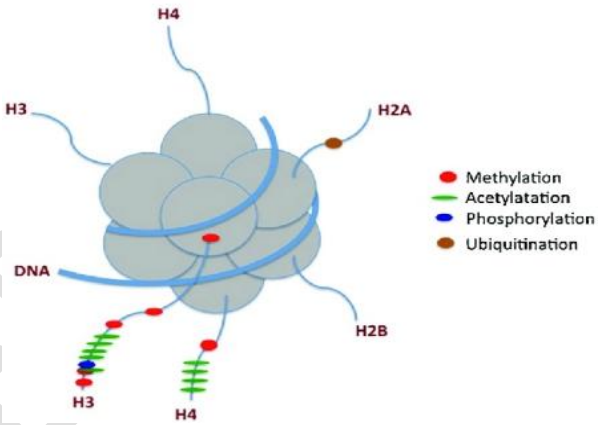

Figure 2:Schematic representation of histone modifications. Highlight the methylation sites in red at H3K4, H3K9, H3K27, H3K36, H3K79, and H4K20. The acetylation sites are green at amino acids H3K9, H3K14, H3K18, and H3K23 and H4K5, H4K8, H4K12, and H4K16. 73 The phosphorylation site is indicated in blue colour at H3S10.82. Aubiquitination site is randomly designated in brown colour or H2A [19].

The various histone modifications can result in gene activation or gene silencing. For example, acetylation of histones is associated with gene activation, while methylation can lead to gene silencing. Histone methyltransferase (HMT) catalyses methylation, affecting chromatin remodelling, chromosomal dysfunction, and disease progression, particularly in cancers, by transferring methyl groups onto histone lysine residues [20]. Epigenetics studies reveal that histone modification can disrupt DNA repair mechanisms, leading to genomic instability and the accumulation of genetic mutation, a common feature of most cancers – breast cancer [21]. Histone modifications promote

epithelial-to-mesenchymal transition (EMT), a critical process in cancer metastasis and breast cancer, transforming epithelial cells for migration and invasion [22].

Chromatin Remodelling

The expression and silencing of specific genes are fundamental in breast cancer progression. The chromatin is composed of DNA, RNA and nuclear proteins. The alteration of chromatin structure leads to cells controlling gene expression by either allowing or restricting access to specific regions of DNA. Researchers have associated the development of complex diseases and cancers with a malfunction in chromatin remodelling [23].
Breast cancer progression has been associated with specific chromatin proteins because they can encourage the invasion and spread of cancer cells. These chromatin proteins are SWI/SNF Complex (Switch/Sucrose Non-Fermentable), ATRX (Alpha Thalassemia/Mental Retardation Syndrome X-Linked) and INI1/SMARCB1 – (IntegraseInteractor 1/SWI/SNF-Related, Matrix-Associated, Actin-Dependent Regulator of Chromatin, Subfamily B, and Member 1) [24]. For example, mutations in genes encoding SWI/SNF subunits, such as ARID1A, ARID1B, and SMARCA4, have been identified in various cancer types, contributing to cancer development and progression that can be seen in breast cancer [25].

Non-Coding RNAs

Non-coding RNAs are RNA nucleotides of short base pairs that do not play any role in protein synthesis; however, they play a specific function in epigenetic processes by regulating gene expression. This regulation occurs at the transcriptional and translational levels, making non-coding RNAs important players in fine-tuning the function of protein-coding genes. They can be divided into housekeeping and regulatory non-coding RNAs [26]. These non-coding RNAs are transcribed from a portion of the human DNA sequence, often called RNA genes. As a result of their roles in regulating gene expression, they play roles in breast cancer progression.

Non-coding RNA, including transfer RNA, ribosomal RNA, microRNA, siRNA, piRNA, snoRNA, snRNA, exRNA, scaRNA, and long ncRNAs, are abundant and essential in the human genome [27]. Research by Fanfan Li in 2019 found a positive correlation between the expression levels of miR-124 and miR-126 in breast cancer tissues, indicating that these miRNAs promote cancer development [28]. Also, miRNAs expressed in cancers have the potential to serve as diagnostic or therapeutic targets. Circulated RNAs, including circCDYL variants, have been identified as tumour suppressor genes, down-regulating oncogenes in bladder, colon, and triple-negative breast cancer, and positively correlated with patient survival [29].

Long non-coding RNAs (lncRNAs) and piwiRNAs influence breast cancer progression, playing crucial roles in gene expression regulation. Dysfunction in regulatory processes can lead to the formation of oncogenes [29]. However, lncRNAs can also serve as tumour suppressor genes to develop breast cancer. In addition, several miRNAs, such as miR-1275, are known to be highly upregulated in breast cancer. This includes miR-7, miR-30c, miR-135b and miR-16 [30].

Taurine Unregulated Gene 1 (TUG1) and piwi RNAs, small non-coding RNAs in the piRNA pathway [31], have been linked to transposon silencing and germline development. Recent research suggests they may play a role in breast cancer through deregulation and altered tissue expression.

EPIGENETICS BIOMARKERS IN BREAST CANCER

Research on epigenetic processes in tumorigenesis, cancer progression, invasion, and diagnosis uses biomarkers as epigenetic regulators, enabling personalised patient management and treatment outcomes [31].In order to address the challenges surrounding breast cancer, the most common cause of cancer-related death among women worldwide, several interventions using epigenetic strategies have to be developed. Molecular cancer biomarkers measure cancer risk, occurrence, and patient outcomes. Modern tools and technologies enable gene characterisation to measure other biomarkers besides traditional plasma proteins [32]. Measured epigenetic biomarkers may include transcriptional alterations, proteomic signatures, genetic variations, and epigenetic signatures [33]. These biomolecules are in tissue samples, blood, saliva, buccal swabs, stool, or urine [33]. Epigenetic biomarkers, including DNA methylation-based, histone modification-based, and non-coding RNA-based biomarkers, are crucial for the early detection of breast cancer, enabling quicker and more efficient therapeutic interventions.

DNA Methylation as a Biomarker in Breast Cancer

The use of DNA methylation as a biomarker for breast cancer diagnosis has shown to be relatively stable compared to other biomarkers, and it can also be easily gotten from routinely collected formalin-fixed paraffin-embedded (FFPE) clinical materials, as stated by Martens et al. [32]. The main emphasis of prognostic and diagnostic methods for breast cancer has been genetic alterations in gene sequences. On the other hand, DNA methylation silencing causes hypermethylation of tumour suppressor genes, such as BRCA 1 and 2, to be valuable biomarkers in clinical settings. Quantitative PCR-based technology and methylation-specific polymerase chain reaction (MSP) are diagnostic approaches that can aid identification [34].

As previously hinted, several DNA sources exist for methylation biomarker detection, as stated by Martens and colleagues [32]. Detecting DNA methylation using exfoliated tumour cells, cellular DNA, and plasma, as well as urinary sediments, saliva, and breast lavage, can increase the sensitivity of methylation assays by identifying specific methylated DNA, such as cell-free plasma DNA and cDNA [35]. According to Martens et al. [32], DNA methylation is present in the blood of BRCA1 mutant carriers who have developed breast cancer; this can be used for screening purposes in those mutation carriers who have not yet developed the disease. Since BRCA1 gene hypermethylation is not seen in healthy breast tissue, it appears to be tumour-specific and is reliable in identifying sporadic breast and ovarian cancer cases. Aside from its reliability as a tool in medical diagnosis, it can also be applied in prognosis and monitoring therapy. Among the often utilised DNA methylation genes as biomarkers to detect breast cancer are RASSF1A, BRCA1, GSTP1, TWIST1, and CDH1 genes.

Histone Modification-Based Biomarkers

Histone modifications are significant in gene transcriptional processes, DNA repair, and DNA replication [36]. Specific histone-modified biomarkers such as H3K4me3, H3K27me3, and H3K9me3 are potential tools and markers for detecting breast cancer and monitoring treatment. Understanding histone modifications can help understand the aggression of breast cancer and aid with its prognosis. Acetylation and methylation are the significant reactions between functional chemical groups and histone, generating different histone isoforms. Meng et al. [37] investigated the relationship between H3K9me3 and estrogen receptor status in women; they found a significant level of interaction among H3K9me3 and ER-positive tumours, particularly patients who expressed high ER-positive tumours. Histone 3 Lysine 9 Trimethylation (H3K9me3) and H3K27ac, two processes involved in gene transcription, are linked to breast cancer development and spread, with altered levels potentially affecting prognosis [38].

Non-Coding RNA-Based Biomarkers

Breast cancer is a multiplex disease with different clinical outcomes and treatment responses. Non-coding RNAs, particularly microRNAs and lncRNAs, are being explored as potential biomarkers due to their regulatory role in gene expression. Common ncRNAs studied as useful epigenetic biomarkers for breast cancer include miR21, miR155, MALAT1, exosomalmiRNA and miR210. The miR21 is the most studied microRNA in breast cancer and has been identified as an oncogenic miRNAupregulated in various types of cancers, including breast cancer. It also has implications in carcinogenic processes; therefore, its inhibition confers protection against breast cancer and different types of cancer [39].Using lncRNA HOTAIR (HOX transcript antisense RNA) has proven to be a well-established biomarker for several cancers, including breast cancer [40]. Different research studies on lncRNA HOTAIR show that it was upregulated in cancers and associated with metastasis, poor prognosis and reduced survival rates [41].

Another lncRNA that serves as a biomarker is the MALAT1 (Metastasis Associated Lung Adenocarcinoma Transcript 1), which is one of the most studied lncRNA that is expressed in cells and tissues and shown to play significant roles in regulating genes at both transcriptional and post-transcriptional stages [42]. As a result of the involvement of this lncRNA in breast cancer, their detection serves as promising biomarkers in the prognosis and diagnosis of breast cancer. The presence of circulating miRNA, like miR-210, has shown promise in breast cancer diagnosis due to its predictive value and association with chemotherapy resistance in breast cancer patients [43]; hence, its importance as a biomarker.

EPIGENETIC THERAPIES FOR BREAST CANCER

Epigenetic techniques often employ inhibitors as therapeutic approaches for breast cancer, as they help prevent abnormal gene transcription or chromatin processes [44]. The use of inhibitors has paved the way for developing drugs that prevent DNA methylation, histone deacetylation, chromatin remodelling and non-coding RNA activities.

Understanding the molecular mechanisms behind initiating and maintaining epigenetic changes in cancer has excellent potential for clinical use [45]. Epigenetics-based diagnostic and prognostic methods significantly impact

precision oncology [46]. Epigenetic treatment for oncogenesis focuses on molecular changes affecting cancer cell gene activity, restoring regular expression of tumour suppressor genes to prevent cancer cell growth. Epigenetic therapy, including HDACi, DNMTi, and RNA inhibitors, is the most commonly used treatment for breast cancer, alongside other forms of treatment [47][48].

DNA Methylation Inhibitors (DMNTi)

DNA methylation, a reversible process in human cells, is a critical epigenetic change in breast cancer; the use of DNA methylation inhibitors has shown promise in reactivating silenced genes involved in breast cancer; this has demonstrated potential therapeutic benefits, especially in triple-negative breast cancer - the absence of oestrogen, progesterone and HER2 receptors [49].

DNA methylation inhibitors like 5-azacytidine and decitabine (5-aza-2'-deoxycytidine; Dacogen; SuperGen) [50] function by incorporating themselves into DNA during replication, leading to the depletion of DNA methyltransferases (DNMTs). This results in DNA demethylation and reactivation of silenced tumour suppressor genes. Recently, nucleoside analogues have been produced with DNA methylation inhibitors to function as anticancer treatments. Nevertheless, studies have shown that nucleoside analogues can have serious side effects, albeit comprehension of their mechanism can lead to better results. Low dosages have been created to improve patients' DNA methylation inhibition [51]. Zebularine is another potent and chemically stable demethylating agent belonging to the family of nucleoside analogues; its prospects are promising for future clinical trials [52].

DNMTi therapy can overall improve hormone therapy efficacy, inhibit cancer growth, and prevent metastasis spread; however, clinical trials are ongoing, and more studies are needed to establish its effectiveness in treating breast cancer. [53]. Other drugs, such as guadecitabine (SGI-110), a prodrug whose active metabolite is decitabine, also have promising prospects owing to its ability to resist degradation by cytidinedeaminase and thereby prolong in vivo exposure, increasing its efficacy [54].

Histone Deacetylase Inhibitors

According to some studies by Guo et al. [55], histone acetylation modification is one of the most understandable epigenetic changes. It has an essential role in the emergence of breast cancer. Histone deacetylases (HDACs) are a family of specialised enzymes that remove acetyl groups from the amino group of proteinogenic histone lysine residues, compressing the chromatin structure and suppressing the transcription of genes [56][57]. HDAC is a potential and effective target for anticancer drugs due to its overexpression and abnormal activity in several cancer subtypes. For the treatment of haematological malignancies, the FDA has currently approved various HDAC inhibitors like Vorinostat (SAHA), Belinostat (PXD-101), Romidepsin (FK-228), and Panobinostat (LBH589) are some examples [58 – 60].

HDAC inhibitors have been researched for treating solid tumours, including triple-negative breast cancer (TNBC). Published research has shown that HDAC inhibitors can repress breast cancer cell proliferation [61] and angiogenesis [62], regulate anti-tumour immunity [63][64], invasion and metastasis [65], reduce DNA repair, impact mitosis, and prevent the growth of breast cancer cells in preclinical models [66 –68]; however, the efficacy of HDAC inhibitors as monotherapy in breast cancer remain disappointing [69]. Researchers are conducting several clinical trials to assess how effective HDAC inhibitors are in treating breast cancer.

Chromatin Remodeling Inhibitors

Chromatin remodelers, crucial for DNA damage repair, recombination, replication, and transcriptional control, are frequently mutated genes in human cancers [70]. Through the interaction between subunits, chromatin remodelling complexes change chromatin structure, and this determines gene expression levels via the regulation of the interaction between proteins with double-stranded DNA [71]. Chromatin remodelling complexes, such as SWItch/Sucrose Non-Fermentable, contain subunits like ARID1A and BRG1, which are linked to breast cancer. Mutations in this complex occur in 20-25% of human cancers [72]. A high mutation rate in cancer cells allows them to adapt to environmental changes. SWI/SNF complexes remodelling chromatin structure, allowing transcription factor binding and serving as a therapeutic strategy in breast cancer [73]. ER-positive breast cancer frequently has mutations in ARID1A, a SWI/SNF complex component that increases resistance to endocrine treatment. The

response to endocrine therapy for breast cancer treatment, intrinsic proliferative capacity, and HDAC1/BRD4 activity are all impacted by ARID1A [74]. BRG1, a subset of human SWI/SNF chromatin remodelling enzymes, is crucial for breast cancer cell proliferation, with most tumours overexpressing BRG1 and BRM [75][76]. Studies by Wu et al. [77] show that BRG1 knockdown in triple-negative breast cancer cells generated a delayed proliferation phenotype by inhibiting de novo lipid synthesis in breast cancer cells [77].

Non-Coding RNA-Targeted Therapies

Non-coding RNAs (ncRNAs) are functional RNA molecules that control the expression of genes at the transcriptional or post-transcriptional levels rather than being translated into polypeptides or protein sequences. Only 1% of the whole genome's RNA comprises non-coding molecules [78]. MicroRNAs (miRNAs), long non-coding RNAs (lncRNAs), circular RNAs (circRNAs), small nucleolar RNAs (snoRNAs), and PIWI-interacting RNAs (piRNAs) are some of the ncRNAs that fall under this category [79]. There are now three groups of "classic ncRNAs" that have been studied the most: microRNAs, long-non-coding RNAs (lncRNAs), and circular RNAs (circRNAs) [80]. The role of ncRNAs as potential therapeutic targets in cancer is highlighted by mounting evidence that these molecules are dysregulated and connected to several cancer-related processes, including cancer stem cell (CSC) initiation, metastasis, and drug resistance [81 – 83]. Numerous ncRNAs are linked to various biological processes in breast cancer, including cell proliferation, apoptosis, migration, invasion, angiogenesis, and drug resistance, with potential diagnostic, prognostic, and therapeutic applications [84][79][85].

MiRNAs were the first ncRNAs to be examined concerning cancer, particularly breast cancer [86]. MicroRNAs play a critical role in investigating non-coding RNAs (ncRNAs) as biomarkers and therapeutic targets for breast cancer. Dysregulation of ncRNA expression in cancer tissues and ncRNA expression targeting to modify cancer phenotype are necessary for efficient usage [87]. MiRNAs are crucial in cancer treatment, serving as both therapeutic prospects and targets. Two main approaches are miRNA inhibition using anti-miRNAs and miRNA replacement treatment using miRNA mimics [88][89]. Furthermore, miRNA replacement treatment, or miRNA mimicry, restores tumour-suppressive miRNAs using synthetic oligonucleotides. MiRNA mimics are chemically modified and delivered via exosomes, liposomes, polymeric NPs, and expression vectors [90].

EPIGENETIC RESEARCH CHALLENGES IN BREAST CANCER AND FUTURE DIRECTIONS

Researchers encounter difficulties such as tumour heterogeneity, limited technologies, research costs, absence of standardised assaying methods, and interpretation of epigenetic data in the complexity of breast cancer. Epigenetic tools offer therapeutic approaches for combating breast cancer and promoting resistance [91]. Breast cancers exhibit intra-tumoral heterogeneity, causing different epigenetic profiles in different areas; this presents challenges for researchers, as different subtypes have distinct epigenetics. As a result of this challenge, a nuanced approach to epigenetic therapies is necessitated, leading to low treatment response rates [92]. Epigenetic profiling technologies are crucial in studying breast cancer, but their resolution and sensitivity are essential for accurately identifying epigenetic modifications. Advanced sequencing technologies are needed to accurately map these modifications, which contribute to the pathophysiology, diagnosis, and treatment of breast cancer [93]. However, these technologies can be expensive and resource-intensive, making them less accessible to all research groups, potentially limiting adequate research. However, Companies like Illumina offer cost-effective, next-generation sequencing and array-based epigenetic analysis tools to address these limitations [94].

The need for standardised assays and protocols in epigenetic research is a significant challenge. While resources like 'Epigenomics: Methods and Protocols provide a comprehensive collection of protocols, harmonising them across different labs remains challenging due to variations in techniques, sample types, and the rapidly evolving nature of epigenetic research. Establishing a standardized epigenome reference, particularly for breast cancer, could significantly aid comparative studies [95]. Epigenetic data interpretation is complex, requiring a thorough understanding of functional consequences and integrating it with genomic data [96]. Correctly understanding epigenetic data will require collaboration across disciplines, technological advancements, and standardised protocols. Overcoming these obstacles will improve breast cancer diagnosis, prognosis, and treatment strategies.

Precision medicine and epigenetic profiling are critical future directions in breast cancer research, offering personalised treatment, biomarkers for predictive medicine [97], improved therapeutic options, and whole-genome epigenetic profiling. Combination therapies targeting multiple epigenetic pathways are necessary due to the complexity of breast cancer epigenetics. Novel epigenetic therapies, such as small molecule inhibitors and RNA-

based methods, are being explored to target abnormal epigenetic alterations in breast cancer. However, these therapies are still in the experimental stages, and more research is needed to fully understand their potential benefits and risks.

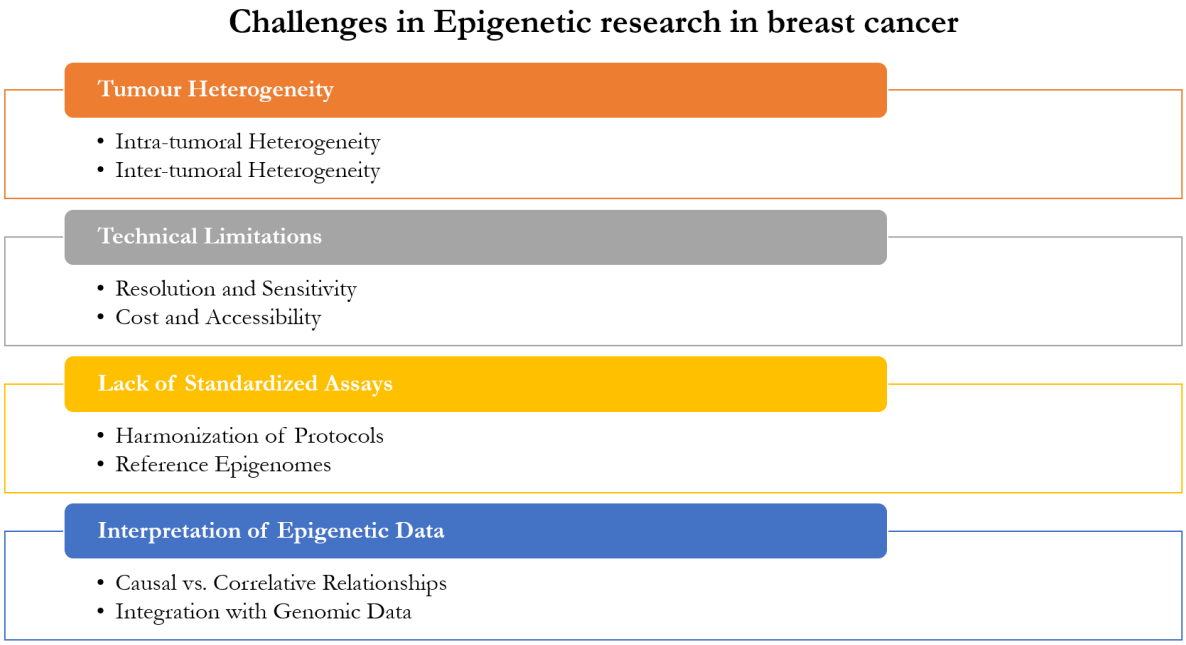

Figure 3: The challenges in epigenetic research lie in the diversity in cells that make the tumour, which can be intra-tumoral or intermoral heterogeneity. Also, other challenges exist in the form of technical limitations – resolution and sensitivity as well as cost and accessibility; lack of standardised assay and limitations in the interpretation of epigenetic data also make up part of the challenges in epigenetic research in breast cancer.

CONCLUSION

Breast cancer remains a prevalent disease globally, mainly due to factors like late diagnosis, limited treatments, and drug resistance. Future research in epigenetics could help develop diagnostic and prognostic biomarkers to predict risk, prognosis, and treatment response. Understanding how environmental factors influence epigenetic changes can help develop preventive measures. Epigenetics is crucial in breast cancer treatment and prevention, as it aids early detection, identifies at-risk individuals, determines successful therapies, and offers reversible therapies. Epigenetic drugs, like DNA methyltransferase and histone deacetylase inhibitors, have shown promise in clinical trials. Research could also reveal new therapeutic targets for breast cancer, potentially with fewer side effects than conventional chemotherapy. Integrating epigenetic information into clinical practice could improve outcomes and prevent high-risk individuals.

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