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Review

Beyond genetics: Exploring the role of epigenetic alterations in breast cancer

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ABSTRACT

Breast cancer remains a major global health challenge. Its rising incidence is attributed to factors such as delayed diagnosis, the complexity of its subtypes, and increasing drug resistance, all contributing to less-than-ideal patient outcomes. Central to the progression of breast cancer are epigenetic aberrations, which significantly contribute to drug resistance and the emergence of cancer stem cell traits. These include alterations in DNA methylation, histone modifications, and the expression of non-coding RNAs. Understanding these epigenetic changes is crucial for developing advanced breast cancer management strategies despite their complexity. Investigating these epigenetic modifications offers the potential for novel diagnostic markers, more accurate prognostic indicators, and the identification of reliable predictors of treatment response. This could lead to the development of new targeted therapies. However, this requires sustained, focused research efforts to navigate the challenges of understanding breast cancer carcinogenesis and its epigenetic underpinnings. A deeper understanding of epigenetic mechanisms in breast cancer can revolutionize personalized medicine. This could lead to significant improvements in patient care, including early detection, precise disease stratification, and more effective treatment options.

1. Introduction

Breast cancer, the most diagnosed cancer globally, accounted for one in eight diagnoses in 2020, with a staggering 2.3 million new cases. This pervasive disease claimed roughly 685,000 lives that year, emphasizing the critical need for continuous research and improved treatment strategies [1]. The disease is characterized by a broad spectrum of complex and heterogeneous subtypes, each harboring a unique set of molecular characteristics and clinical trajectories. The increase in incidence, predicted to be over 3 million new cases and 1 million deaths every year by 2040, has significantly amplified the urgency for innovative and effective detection, treatment, and prognosis strategies [1].

Breast cancer, a highly heterogeneous disease, is classified into four primary molecular subtypes: Luminal A, Luminal B, HER2-positive, and Triple-Negative Breast Cancer (TNBC). This classification is crucial as it significantly influences the prognosis and treatment approach for patients. Among these, TNBCs are particularly notable, constituting 10–20% of breast tumors. Originating from basal cell lineage, TNBCs are characterized by the absence of estrogen receptor (ER), progesterone receptor (PR), and HER2 expression [2,3]. This subtype is notorious for its aggressive nature and heterogeneous presentation, posing substantial challenges in clinical management and leading to generally poorer prognoses for patients.

The expression levels of estrogen, progesterone, and HER2 are pivotal in stratifying breast tumors into these molecular subtypes. However, it's important to note that genetic variations play a significant role in the onset, response to treatment, and progression of the disease [4–7]. These genetic factors are critical in understanding patient-specific cancer characteristics and developing targeted therapies.

In addition to genomic abnormalities, which are undeniably central to clinical therapy in breast cancer, the epigenetic landscape offers another dimension of understanding. Epigenetic changes, which are

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dynamic and influential, can disrupt normal cellular processes in breast cancer and other malignancies. The interaction between tumor genomics and epigenomics is particularly noteworthy. Tumor genomics can influence epigenomic control by regulating the activity of chromatin-modifying enzymes [5,8]. These post-translational epigenetic alterations, affecting histones and DNA, play a critical role in modifying chromatin accessibility. They do so by altering histone–DNA interactions, facilitating the recruitment of transcriptional machinery to DNA, or interacting with transcription factors [9]. This complex interplay underscores the importance of both genomic and epigenomic factors in the pathogenesis and treatment of breast cancer.

Epigenetic pathways regulate multiple facets of cancer biology, encompassing the facilitation of primary tumor development and invasion, as well as the modulation of the immune response within the tumor microenvironment [10–12]. In contrast to genetic defects, which are challenging to rectify, dysregulated epigenetic pathways can be efficiently targeted with small molecule drugs. Furthermore, the alteration of the epigenome in various forms of solid tumors makes cancer cells susceptible to attacks from the immune system, hence increasing their ability to respond to immunotherapy. The advantages of these strategies have generated growing enthusiasm in the last ten years for the advancement of epigenetic methods to combat cancer [13,14]. The field of epigenetics presents itself as a beacon of hope in this relentless quest. Epigenetics, the study of heritable and reversible changes in gene expression that occur independently of alterations to the underlying DNA sequence, crucially influences a myriad of biological processes and underpins a multitude of diseases, notably cancer [15–17]. The potential of this biological domain to sculpt a diverse array of phenotypes from a single genetic blueprint is central to the tapestry of life.

This comprehensive review delves into the intricate realm of epigenetic modifications in cancer, exploring their interplay with genetic alterations and the consequential impact on tumor heterogeneity and therapeutic resistance, areas still shrouded in significant ambiguity [18,19]. Our primary focus is to elucidate the current understanding of epigenetic changes as drivers of cancer diversity and their role in fostering resistance to conventional treatments [20].

In this review, we dissect the multifaceted nature of epigenetic alterations in cancer. We scrutinize how these modifications influence the fundamental biology of cancer cells, potentially leading to varied responses to treatment. A significant portion of our discussion is dedicated to the emerging potential of epigenetic therapies. These novel approaches offer a promising avenue to counteract drug resistance, a pervasive challenge in cancer treatment. Moreover, we examine the complexities and challenges faced in translating these epigenetic discoveries from the laboratory to clinical practice, a critical step in realizing their therapeutic potential [21,22].

Our aspiration with this review is to ignite further research in this rapidly evolving field. By shedding light on the nuanced interplay between epigenetic and genetic changes in cancer, we aim to contribute to the advancement of cancer management strategies. Ultimately, our goal is to positively impact the lives of cancer patients worldwide by paving the way for more effective and personalized treatment approaches.

2. Epigenetic mechanisms

The intricate nature and occurrence rate of the epigenetic alterations observed in cancer cells depends on several factors stemming from numerous genetic abnormalities and environmental influences, which disrupt the diverse mechanisms of epigenetic regulation [16,23] (Fig. 1). Epigenetic modifications such as DNA methylation, histone modifications, and non-coding RNAs are pivotal in breast cancer development and progression [24–26]. These modifications can potentially alter critical genes involved in cell growth, proliferation, and DNA repair. In creating unique patterns, they may serve as potential diagnostic and prognostic biomarkers. The inherent stability, high frequency, reversibility, and accessibility of body fluids offer an exciting opportunity to develop diagnostic assays and personalized medicine [27].

3. DNA methylations

DNA methylation is the process that adds a methyl group to cytosine within CpG dinucleotides, resulting in 5-methyl-cytosine (5mC). Donor S-adenosyl-L-methionine can transfer its methyl group to recipient DNA with the help of DNA methyltransferases (DNMTs) DNMT1, DNMT3A,

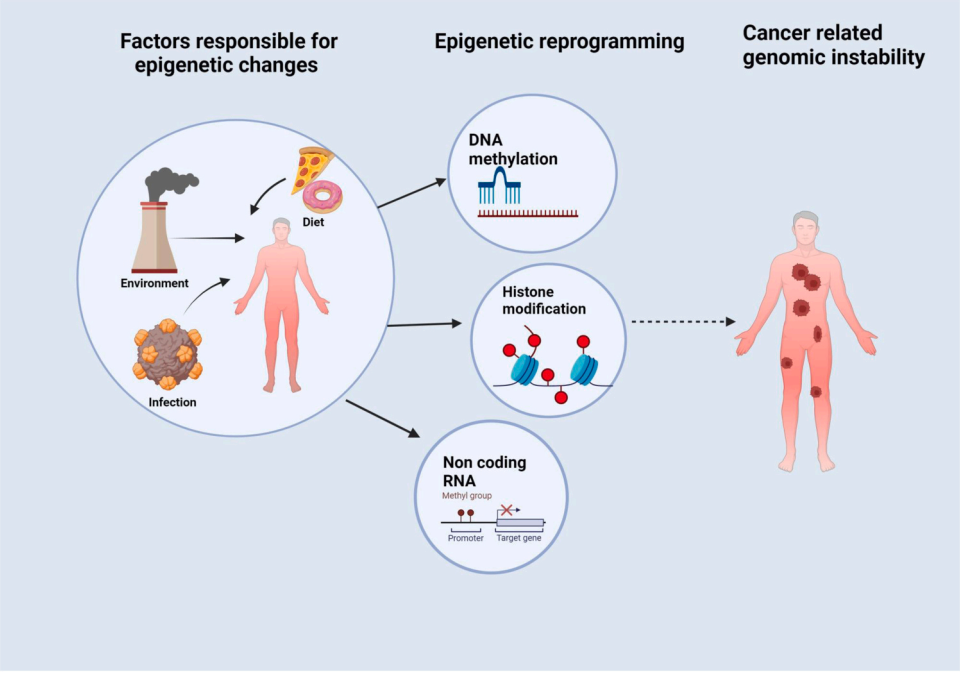

Fig. 1. Summary of Epigenetic Mechanisms Regulating Gene Expression. Various environmental factors that might cause abnormal alterations to DNA methylation, histone modifications, and non-coding RNAs (ncRNAs) causes the oncogene activation and/or silencing of tumor suppressor gene repression, which in turn affects the signaling pathways critical for cellular homeostasis, repair, and homeostasis.

and DNMT3B [28]. During DNA replication, DNMT1 preserves the existing methylation patterns, while DNMT3A and DNMT3B generate novel patterns. To reduce gene expression, methylation of DNA in promoter regions can either prevent transcriptional machinery from binding to promoter recognition sequences or enhance the binding of proteins with methyl-binding domains (MBDs) to promoters. Through their interactions with histone deacetylase complexes and chromatin remodeling factors, MBD proteins mediate the connection between DNA methylation and histone changes. The interaction leads to the compacting of chromatin, which subsequently hinders transcription [29]. DNA methylation regulates important biological processes such as chromatin remodeling, genomic imprinting, X-chromosome inactivation, post-translation, transcription, and post-transcription [30].

Cancer is characterized by abnormal DNA methylation [31]. Hyper-methylation, the process of adding methyl groups to promoter CpGs that are usually unmethylated, can lead to the inactivation of tumor suppressor genes. This procedure has the capacity to function as a biomarker for the prompt identification and prediction of cancer. Furthermore, it has been shown that many cancers display a reduction in DNA methylation throughout the entire genome. This reduction can result in instability in the genetic material, damage to DNA, and the reactivation of retroviruses and transposons [29,32].

Aberrant DNA methylation is a distinct and reversible epigenetic process linked to the initiation of breast cancer as a result of abnormal gene expression. These genes are crucial in determining the development of clinicopathological characteristics, such as the stage of the tumor, the grade of the tissue, and the TP53 status [29,32]. However, the discovery of ten-eleven translocation (TET) proteins changed this viewpoint. The TET family, consisting of TET1, TET2, and TET3 proteins, catalyzes the oxidation of 5mC to 5-hydroxymethylcytosine, 5-formylcytosine, and 5-carboxylcytosine, thereby promoting the process of active DNA demethylation [29,33].

Cancer stemness features are also significantly impacted by aberrant DNA methylation. A comprehensive analysis of DNA methylation throughout the whole genome was carried out in recent research. In breast cancer, the results showed that circulating tumor cells (CTCs) clustering significantly promotes metastasis and tumor progression. This occurs due to uncontrolled methylation binding sites for transcription factors that are associated with proliferation and stemness. This provides further evidence that cluster targeting may be an effective method of preventing cancer from spreading to other organs, a process known as metastasis [34]. Furthermore, research has shown that the alteration of DNA methylation is a significant factor in the process of mesenchymal stem cells transforming into tumor-forming cells during the development of breast cancer [35].

4. Histone modifications

Histones, positively charged proteins, have a pivotal role in DNA packaging and regulation. They form the structural core of nucleosomes, the fundamental units of chromatin, where approximately 146 base pairs of DNA are wound around an octamer of core histones. This octamer consists of pairs of H3, H4, H2A, and H2B histones, compacting the DNA into a dense protein-DNA complex [36]. Beyond their structural role, histones undergo extensive covalent post-translational modifications on their side chains or tails, which critically influence chromatin dynamics and gene expression.

These histone modifications include the addition or removal of various functional groups. Such alterations can significantly impact the charge density between histones and DNA, thereby modulating the chromatin architecture and, consequently, the transcription process [37]. There are multiple types of histone modifications, but four have been most extensively studied for their profound effects on gene expression: acetylation, methylation, phosphorylation, and alterations affecting DNA coiling. Crucially, specific patterns of these histone post-translational modifications have been strongly associated with the occurrence of cancer [21,38]. Dysregulation of these modifications can lead to aberrant gene expression patterns, contributing to cancer initiation and progression. For instance, alterations in histone acetylation and methylation patterns are frequently observed in various cancer types, leading to the misregulation of genes critical for cell cycle control, apoptosis, and DNA repair.

Histone modifications have been found to impact essential physiological activities, including gene transcription, DNA replication, DNA recombination, and DNA repair [39]. Furthermore, there is a recognized association between histone alterations and the onset and advancement of cancer. Multiple histone changes are anticipated to be present, acting as diagnostic indicators in different forms of cancer [40]. Notable variations in the histone modification profile of frozen tissue compared to primary cell lines were observed. Breast cancer cell lines showed the presence of K36me1 or K9me3/K14Ac deposition, whereas primary cells were generally lacking the K14Ac signature [41,42]. Recently, several studies have been conducted to attribute histone alteration to the development of tumors, the advancement of cancer, and the spread of breast cancer [43–46]. Based on the acquired results, some 650 genes were identified as responsible for driving normal cells towards neoplastic transformation. The neoplastic transformation in breast cancer patients is influenced by the posttranslational alteration of H3K9. This effect is observed in a transformation model that expresses the Large T antigen, TERT, and RAS (V12). Moreover, the process of tumorigenic transformation might be characterized by a notable decrease in the levels of H3K9me2/me3, accompanied by an accumulation of H3K9ac [41]. Increased expression of LSD1, a histone methylation eraser targeting H3K4 and H3K9, has been linked to early breast cancer progression [47]. In the intricate landscape of cancer biology, mutations in histone acetyltransferases (HATs) emerge as a recurring theme across various cancer types [48]. These HATs play a pivotal role in modulating gene expression by adding acetyl groups to histone proteins, thereby influencing chromatin structure and accessibility. The mutation of these enzymes can disrupt normal acetylation patterns, potentially leading to oncogenesis.

Conversely, histone deacetylases (HDACs) remove acetyl groups from lysine residues on histones, a counterbalancing act that is also implicated in cancer development. When the function of HDACs is altered, either through mutations or dysregulation, it can lead to aberrant deacetylation, a phenomenon observed in various cancer types [49]. This imbalance between acetylation and deacetylation is a key factor in the epigenetic regulation of gene expression and can contribute to tumorigenesis.

Beyond acetylation, methylation of histone tails, particularly on lysine and arginine residues, represents a more complex layer of chromatin modification with profound implications in cancer. Histone methylation, unlike acetylation, does not simply switch gene expression on or off but rather contributes to a more nuanced regulation of chromatin dynamics [38]. The patterns of histone methylation, whether low or high, have been linked to cancer development. This correlation suggests that both hypo- and hypermethylation of histones can play distinct roles in the genesis and progression of cancer [50].

This complex interplay of histone modifications—acetylation, deacetylation, and methylation—highlights the nuanced and multifaceted nature of epigenetic regulation in cancer. Understanding these mechanisms is crucial for developing targeted therapies that can modify these epigenetic alterations and potentially halt or reverse cancer progression.

5. Non-coding RNAs

Non-coding RNAs (ncRNAs) are a diverse group of RNA molecules produced by RNA polymerase that do not encode proteins but are crucial in various biological functions. ncRNAs can be considered as epigenetic aberrations, particularly in the context of cancer. This is based on their significant role in dysregulating the epigenetic landscape of cancer cells.

ncRNAs, including microRNAs (miRNAs), are known to recruit and direct chromatin-modifying protein complexes to specific genomic loci. This action results in notable changes in DNA methylation, histone modifications, and overall chromatin structure, as evidenced in several studies [51,52]. These alterations directly impact gene expression by modulating the accessibility of DNA to transcription factors, either activating or repressing the transcription of target genes. Moreover, the role of ncRNAs extends to a complex network of regulation through their interactions with other ncRNAs, adding further depth to their epigenetic influence [51].

In the realm of oncology, and particularly in breast cancer, ncRNAs are frequently found to be aberrantly expressed or mutated. Such aberrations disrupt normal epigenetic mechanisms, contributing to a cancer-specific epigenetic signature. For instance, various ncRNAs in breast cancer have been implicated in the modulation of key epigenetic factors like DNA methyltransferases (DNMTs), histone deacetylases (HDACs), and the polycomb repressive complex 2 (PRC2) [51]. These interactions significantly affect the methylation and acetylation status of DNA and histones, thereby influencing key cellular processes such as proliferation, differentiation, invasion, and metastasis.

Furthermore, the potential of ncRNAs as biomarkers for cancer diagnosis, prognosis, and therapy response – owing to their detectability in biological fluids like blood, urine, and saliva – underlines their critical role in the epigenetic landscape of cancer.

ncRNAs can be broadly categorized into two functional groups: regulatory ncRNAs and housekeeping ncRNAs. Regulatory ncRNAs include long non-coding RNAs (lncRNAs), small interfering RNAs (siRNAs), PIWI-interacting RNAs (piRNAs), and microRNAs (miRNAs). LncRNAs, characterized by their length of over 200 nucleotides, are involved in epigenetic control of gene expression, regulation of gene promoters, genomic imprinting, and maintaining nuclear architecture [21,53]. SiRNAs and miRNAs can bind to target messenger RNAs (mRNAs) and, in conjunction with other proteins, either degrade them or inhibit their translation, effectively silencing genes [52,54,55]. This process can also involve the recruitment of RNA-binding proteins that block transcription factors or promote histone deacetylation.

In the context of cancer, both small and long regulatory ncRNAs play significant roles. For example, miR-126 is highly expressed in colorectal and breast tumors and has been shown to downregulate p53 and related genes, contributing to cancer progression [56–58]. Similarly, miR-155, identified as an oncogene, is overexpressed in various cancers ranging from colon to liver and has been linked to increased proliferation in plexiform neurofibromas and reduced tumor levels when inhibited [21,53]. miR-215, another oncogene, is overexpressed under hypoxic conditions in glioblastoma cells.

On the other hand, housekeeping ncRNAs include transfer RNAs (tRNAs) and ribosomal RNAs (rRNAs). tRNAs are essential for translating mRNA into proteins, recognizing specific three-nucleotide sequences in mRNAs, and correctly assembling amino acids in the ribosome. rRNAs, which constitute the majority of RNA molecules in a cell, form the structural framework of ribosomes and are integral to protein translation [59]. Additionally, short nucleolar RNAs (snoRNAs) introduce chemical modifications in many housekeeping RNAs, further illustrating the complexity of RNA functions in the cell [60].

Alterations in miRNA expression, such as mutations, amplifications, or deletions on miRNA loci, have been found to be associated with human cancers [61,62], emphasizing the regulatory function of miRNAs in human diseases. MiR-21 is a microRNA that is significantly overexpressed and linked to a negative prognosis [63]. miR-21 has been found to target PTEN, resulting in breast cancer cell proliferation. miR-155 exerts a negative regulatory effect on SOCS-1 and FOXO3a, two transcription factors that have a role in modulating the development of breast cancer. MiR-10b forms interactions with oncogenic miRNAs HOXD10 and Krüppel-like factor 4 (KLF4), in metastatic breast cancer [64]. MiR-335, a group of miRNAs, is known for its ability to block metastasis by interacting with the transcription factor SOX4 and the extracellular matrix protein TNC. It has also been found to be suppressed in cases of breast cancer [65]. Similarly decrease in levels of miR34 and miR-205 have been known to play major role in pivotal roles in advancement of breast cancer [66,67].

Lethal-7 (let-7) is a kind of miRNA that acts as a tumor suppressor and is known to target various oncogenes including LIN28 [68]. Singh et al. [69] demonstrated that Mirlet7d a member of let-7 family plays a role in regulating the epigenome and organizing the genome by binding to non-coding RNAs (ncRNAs) in the nucleus. Furthermore, miR-200 is a group of miRNAs that act as tumor suppressors. When miR-200 is suppressed during the process of epithelial to mesenchymal transition (EMT), it has been observed to significantly increase the likelihood of developing breast cancer and invasiveness [69,70]. In summary, the diverse family of ncRNAs, both regulatory and housekeeping, plays crucial roles in cellular functions and disease pathology, particularly in the regulation of gene expression and the development and progression of various cancers.

6. Epigenetic drugs and breast cancer prevention

Epigenetic drugs, primarily DNA methyltransferase inhibitors (DNMTi) and histone deacetylase inhibitors (HDACi), represent a promising class of therapeutic agents in cancer treatment and prevention [71,72]. These drugs work by reversing aberrant epigenetic modifications, which are frequently observed in cancer cells, including breast cancer. DNMTi, such as azacitidine and decitabine, have shown promise in the treatment of hematologic malignancies and are being investigated for their preventive potential in breast cancer [73,74]. These inhibitors can demethylate hypermethylated tumor suppressor genes, potentially restoring their normal function. Early-phase clinical trials have been exploring the efficacy of these drugs in reducing breast cancer risk, particularly in patients with a high genetic predisposition.

HDACi, including trichostatin A, vorinostat, and romidepsin, function by inhibiting histone deacetylases, leading to increased acetylation of histones and consequently affecting gene expression [75,76]. In breast cancer, HDACi have been shown to inhibit tumor growth and induce apoptosis in cancer cells. Research is ongoing to determine their role in cancer prevention, with several clinical trials evaluating their efficacy in combination with other chemopreventive agents.

There are ongoing clinical trials investigating the preventive potential of epigenetic drugs in breast cancer. For instance, trials are exploring the use of DNMTi and HDACi in patients with BRCA mutations or those at high risk due to family history. These trials aim to assess not only the efficacy of these drugs in preventing breast cancer but also their safety and optimal dosing.

Despite the promise, several challenges impede the development of epigenetic drugs for breast cancer prevention. One major challenge is the specificity of these drugs. As epigenetic modifications are ubiquitous and vital for normal cellular functions, there is a risk of off-target effects leading to unintended consequences [77,78]. Another challenge is understanding the long-term effects of these drugs, given that cancer prevention requires prolonged use [79]. Additionally, identifying the patient population that would most benefit from these preventive strategies is crucial yet challenging.

The future of epigenetic drugs in breast cancer prevention lies in personalized medicine [80]. Ongoing research is focusing on identifying biomarkers that can predict response to these drugs. Furthermore, combining epigenetic drugs with other preventive strategies, such as lifestyle modifications and hormonal therapies, might enhance their efficacy [81]. There is also a growing interest in developing more targeted epigenetic therapies with fewer side effects.

The exploration of epigenetic drugs in the prevention of breast cancer holds significant promise. While current research and clinical trials are paving the way, a deeper understanding of epigenetic mechanisms in breast cancer and the development of more targeted and safer epigenetic therapies will be key to fully realizing this potential.

7. Chromatin remodeling and its role in gene expression and cancer

Chromatin, a complex of DNA and proteins, is fundamental to chromosome structure. In its condensed form, known as heterochromatin, chromatin fibers coil tightly, fitting compactly within the cell nucleus. This dense arrangement inhibits gene transcription, necessitating chromatin remodeling to transition into a more relaxed state, called euchromatin, for gene expression to occur [21].

Chromatin remodeling involves transforming this condensed chromatin into a transcriptionally accessible state, thus facilitating the binding of transcription factors [82]. This process can entail various changes in nucleosome positioning, including shifts in their relative locations or alteration in spacing across regions of the DNA. Crucially, chromatin remodeling also encompasses the eviction of histones or their replacement with variants associated with active transcription [83]. Such modifications are executed by nucleosome remodeling complexes like SWI/SNF, ISWI, Mi2/Chd, and INO80, which are characterized by their dependence on ATP [84].

Beyond facilitating normal cell function and transcriptional regulation, chromatin remodeling has significant implications in the pathogenesis of diseases, particularly cancer [21]. One notable aspect is the alteration in DNA methylation patterns observed in tumor cells compared to normal cells. Cancer cells often exhibit a dual mechanism of hypomethylation and hypermethylation. Hypomethylation, particularly at CpG sites, can lead to genomic instability and the activation of oncogenes, while hypermethylation often results in the silencing of tumor suppressor genes [85]. These aberrant methylation patterns contribute to uncontrolled cell growth and cancer progression, and they can influence the effectiveness of treatments targeting these pathways.

In summary, chromatin remodeling is a complex process integral to gene expression. Its dysregulation, particularly through altered methylation patterns and changes in nucleosome positioning and composition, plays a critical role in cancer development and progression. Understanding these mechanisms is key to developing targeted therapies that can modify these epigenetic alterations and potentially halt or reverse the progression of cancer.

8. Challenges in epigenetic research and translational strategies

In the rapidly evolving field of epigenetics, researchers continually uncover the profound impact of epigenetic mechanisms on human health and disease. While these discoveries hold immense promise, they also present unique challenges, particularly when translating epigenetic research into clinical applications. This section aims to delve into the multifaceted challenges inherent in epigenetic research, ranging from the complexity of epigenetic modifications and the identification of reliable biomarkers to the development of specific and safe epigenetic therapies. **1. Complexity of Epigenetic Mechanisms:** The inherent complexity of epigenetic mechanisms lies in their dynamic and context-dependent nature. Unlike genetic mutations, epigenetic alterations do not change the DNA sequence but rather affect how genes are expressed. This dynamic nature makes it challenging to pinpoint specific epigenetic changes as causal factors in disease development. For instance, the same epigenetic modification may play different roles in various types of cells or under different environmental conditions.

Addressing this complexity requires advanced methodologies that can capture the temporal and spatial dynamics of epigenetic changes. High-throughput technologies like chromatin immunoprecipitation sequencing (ChIP-seq) and whole-genome bisulfite sequencing provide comprehensive insights but also generate vast amounts of data that require sophisticated bioinformatics tools for analysis. Integrative approaches combining genomic, epigenomic, transcriptomic, and proteomic data are essential to understand the multifaceted nature of epigenetic regulation in disease. **2. Identification and Validation of Epigenetic Biomarkers:** The discovery of reliable epigenetic biomarkers is pivotal for early detection, prognosis, and personalized treatment of diseases. However, the variability in epigenetic modifications across different tissues and individuals poses a significant challenge. For example, DNA methylation patterns observed in cancerous tissues may not be the same in blood or other easily accessible tissues, complicating the development of non-invasive diagnostic tests.

To overcome these challenges, large-scale studies involving diverse populations are required to establish the specificity and sensitivity of proposed biomarkers. Multi-cohort studies, meta-analyses, and cross-validation across independent datasets are crucial for robust biomarker validation. Additionally, emerging technologies like liquid biopsies, which detect epigenetic changes in circulating tumor DNA, hold promise for non-invasive cancer diagnostics. **3. Drug Development and Specificity:** Developing epigenetic drugs that target specific epigenetic modifications without causing off-target effects is a major hurdle. Given the ubiquitous nature of epigenetic processes in normal cellular function, drugs targeting these mechanisms risk altering gene expression in unintended ways. For instance, inhibitors targeting DNA methyltransferases (DNMTs) or histone deacetylases (HDACs) can affect a wide array of genes, leading to potential side effects.

Efforts to develop more specific epigenetic modulators focus on understanding the structure and function of epigenetic enzymes and their interaction with the genome. Structure-based drug design and high-throughput screening are being used to identify compounds with higher specificity. Additionally, precision medicine approaches, where treatment is tailored based on an individual's epigenetic landscape, are emerging as a promising strategy to mitigate the risks of off-target effects. **4. Translating Epigenetic Research into Clinical Practice:** Bridging the gap between epigenetic research and clinical application involves several challenges. Translating laboratory findings into effective therapies requires not only a deep understanding of epigenetic mechanisms but also extensive clinical trials to assess efficacy and safety. For example, while epigenetic drugs may show promise in cell culture or animal models, their effectiveness and safety in humans can only be determined through rigorous clinical trials.

Furthermore, regulatory hurdles are significant when introducing novel epigenetic therapies. Regulatory agencies require comprehensive data on the long-term effects of these therapies, which can be particularly challenging given the reversible and dynamic nature of epigenetic modifications. Collaborative efforts between researchers, clinicians, and regulatory bodies are essential to facilitate the translation of epigenetic research into effective and safe clinical treatments. **5. Ethical, Legal, and Social Implications:** The rapid advancements in epigenetic research, especially in the context of epigenetic editing technologies like CRISPR/Cas9, raise several ethical, legal, and social questions. One of the primary concerns is the potential for germline modifications, which could have heritable effects. The prospect of 'designer babies' and the ethical implications of editing human embryos are subjects of intense debate.

To navigate these ethical challenges, robust frameworks and international guidelines are needed to govern research and clinical applications in this field. This includes clear policies on the permissible use of epigenetic editing, informed consent for patients, and regulations to prevent misuse. Public engagement and education are also crucial to address societal concerns and foster a well-informed dialogue on the ethical aspects of epigenetic research. **6. Strategies for Overcoming Challenges:** Encouraging public-private partnerships can provide the necessary funding and resources for large-scale clinical trials and the development of novel therapies. These collaborations can also help in navigating the regulatory landscape, ensuring that new treatments meet safety and efficacy standards. By addressing these challenges and implementing strategies to overcome them, the field of epigenetics can move forward, translating its findings from bench to bedside effectively and ethically.

9. Conclusion and future perspectives

The potential for early detection of breast cancer by identifying epigenetic markers represents a significant breakthrough. However, translating intriguing laboratory findings into practical clinical applications is challenging. The performance of these markers in terms of sensitivity, specificity, and predictive value must undergo rigorous validation through broad-based population studies. Moreover, the potential for false positives or negatives could result in overdiagnosis, overtreatment, or complacency [86,87].

Therapeutic applications of epigenetics present another captivating avenue [88]. The idea of using reversible epigenetic alterations to selectively target cancer cells is undoubtedly enticing. However, we must remember that these epigenetic drugs, while potentially life-saving, are no magic bullets [89]. They have a systemic nature, affecting not just cancer cells but also normal cells [90]. The dilemma of maintaining a precarious balance between therapeutic effectiveness and harmful side effects adds a complex layer to this pursuit. As Feinberg et al. [25] noted, this challenge necessitates a more detailed understanding of the nuances of epigenetic modifications in various cell types, their interplay with genetic alterations, and the role of the tumor microenvironment.

Moreover, the dynamic and context-dependent nature of epigenetic modifications presents another layer of complexity. Personalized epigenetic therapy, while appealing in concept, requires an intimate understanding of these complexities. Decoding the epigenomic landscape of breast cancer at different stages of the disease, understanding individual variability, and unraveling the intricacies of the interplay with the tumor microenvironment are prerequisites. Technologies like next-generation sequencing offer comprehensive insights into the epigenome, allowing for the identification of disease-specific epigenetic patterns. The development of these biomarkers can facilitate early detection, improve prognostic accuracy, and enable personalized treatment approaches.

The potential role of epigenetics in prognostication presents a ray of hope [91]. The identification of specific epigenetic signatures that could predict disease course or response to therapy could significantly enhance our ability to tailor treatment strategies [79]. However, as with diagnosis, the reliability and reproducibility of these markers need to be established in rigorous, well-controlled studies. The complexities and interindividual variability of epigenetic modifications add to this challenge, warranting cautious optimism.

The advent of "liquid biopsies" – analyzing circulating tumor cells or cell-free DNA in the blood – offers a dynamic, less invasive alternative for disease monitoring [92]. Detecting alterations in CTCs or cfDNA allows clinicians to gain valuable insights into the tumor's molecular landscape, therapeutic response, and progression dynamics. This innovative approach could significantly enhance the existing paradigm of breast cancer management (Fig. 2).

The systemic nature of epigenetic drugs, impacting not just cancer cells but also normal cells, presents a unique challenge. The development of drugs targeting epigenetic regulators, such as DNA methyltransferases (DNMTs) and histone deacetylases (HDACs), aims to reverse aberrant epigenetic changes. Currently, the effectiveness of using inhibitors for histone deacetylases (HDACs) or DNA methyltransferases (DNMTs) in the treatment of breast cancer has been evaluated in multiple clinical trials (Table 1). The purpose of these trials is to determine the efficiency of these medications in addressing epigenetic changes. These potential therapies can reactivate tumor suppressor genes and block oncogenes, thus offering a revolutionary approach to breast cancer treatment [93]. Furthermore, by modulating the epigenetic landscape, these drugs could sensitize tumor cells to traditional chemotherapy or targeted therapies, enhancing overall treatment efficacy [16].

Interestingly, it has been demonstrated that estrogen receptor (ER) or progesterone receptor (PR) can be suppressed by epigenetic mechanisms involving DNMT and HDAC in breast cancer [94]. Recently, a comprehensive study on assessing the effects of decitabine in breast cancer revealed a range of responses. Many alterations in gene expression caused by decitabine are either an indirect consequence of its demethylation or the consequences of induced cell death, DNA damage, and immunological responses. The anticancer effects of hypomethylating medicines include cytotoxicity, apoptosis, growth inhibition, differentiation, and angiogenesis inhibition [95]. Hence, the effectiveness of using epi-drugs as a sole treatment is unsatisfactory, and it is more advantageous to combine epigenetic medications with other treatments as immunotherapy or chemotherapy for solid tumors [96]. Combining epigenetic drugs with other treatments, such as chemotherapy, targeted therapy, or immunotherapy, can enhance therapeutic efficacy. For instance, combining DNMTis with traditional chemotherapeutic agents has shown increased effectiveness in some cancers by sensitizing tumor cells to chemotherapy. Personalized medicine approaches, where treatments are tailored based on an individual's genetic and epigenetic

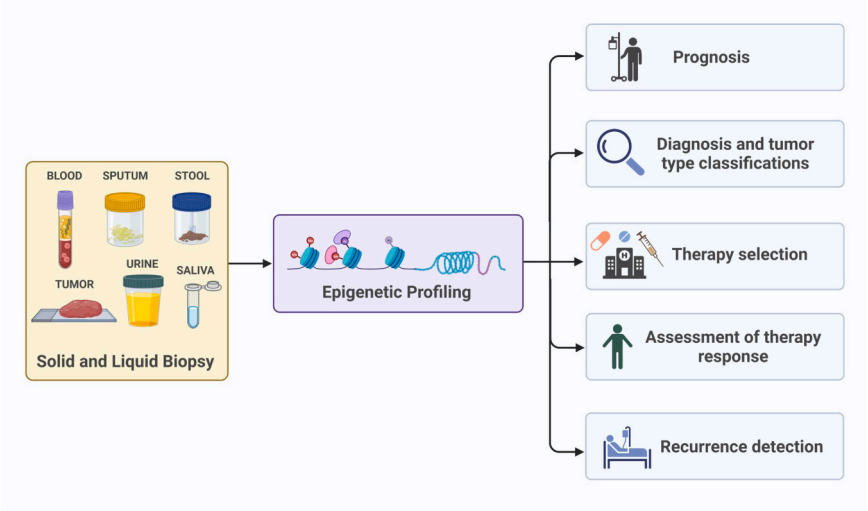

Fig. 2. Utilization of Epigenetic Biomarkers in Clinical Care: Epigenetic profiles, distinguished by their unique characteristics in both solid tumor tissues and various body fluids (including blood, urine, stool, and sputum), serve as valuable biomarkers. These markers contribute significantly to the processes of disease identification, ongoing monitoring, and the selection of treatment strategies. The enduring stability of DNA methylation within circulating tumor DNA has encouraged the creation of DNA methylation-based tests, positioning them as an optimal, non-invasive method for consistent patient observation.

Table 1
Impact of various class of agents undergoing clinical trial for treatment of breast cancer, data obtained from ClinicalTrials.gov (retrieved on 21st December 2023).

Class	Drugs	Status	Phase	Clinical trial number	Study population (Patient enrollment)
Hypomethylation agents	Decitabine	Active, not recruiting	2	NCT02957968	HER2 negative with either hormone receptor-negative or positive
Methyltransferase inhibitors	Azacitidine	Active, not recruiting	2	NCT01349959	advanced stage
Methyltransferase inhibitors	Azacitidine	Completed	2	NCT02811497	ER positive and HER2 negative
Methyltransferase inhibitors	Azacitidine	Completed	1,2	NCT00748553	Advanced or metastatic stage with HER2-negative
Histone deacetylase inhibitors	Valproic acid	Active, not recruiting	1	NCT01552434	Advanced malignancy
Histone deacetylase inhibitors	Entinostat	Completed	1	NCT03473639	Advanced malignancy with either positive or negative ER/PR
Histone deacetylase inhibitors	Entinostat	Completed	2	NCT00676663	Female patients who have reached menopause, have ER positive, recurrence or progression of the disease after previous treatment with an aromatase inhibitor.
Histone deacetylase inhibitors	Entinostat	Active, not recruiting	3	NCT02115282	Adenocarcinoma of the breast
Histone deacetylase inhibitors	Entinostat	Completed	2	NCT03291886	Patients with progression or relapse after treatment with non-steroidal aromatase inhibitors
Histone deacetylase inhibitors	Entinostat	Completed	1	NCT01434303	Metastatic cancer, HER2 positive.
Histone deacetylase inhibitors	Entinostat	Active, not recruiting	1	NCT02453620	Invasive adenocarcinoma, HER-2-negative
Histone deacetylase inhibitors	Entinostat	Active, not recruiting	1,2	NCT03280563	locally advanced or metastatic stage, hormone receptor-positive, HER2-negative and have had disease progression while receiving or after treatment with a cyclin-dependent kinase (CDK) 4/6 inhibitor.
Histone deacetylase inhibitors	Romidepsin	Active, not recruiting	1,2	NCT02393794	BRCA1 or BRCA2 mutation
Histone deacetylase inhibitors	Romidepsin	Completed	2	NCT00098397	Patients who have received anthracycline and/or taxane as adjuvant therapy or for metastatic disease
Histone deacetylase inhibitors	Vorinostat	Recruiting	1,2	NCT03742245	Breast cancer with the HER2 positive.
Histone deacetylase inhibitors	Vorinostat	Completed	1,2	NCT00574587	Patients with adenocarcinoma of the breast, HER2/neu positive and with no prior treatment or surgery
Histone deacetylase inhibitors	Vorinostat	Completed	NA	NCT01720602	Histologically or cytologically proven diagnosis of breast cancer.
Histone deacetylase inhibitors	Vorinostat	Completed	1, 2	NCT00258349	Patients diagnosed with Metastatic or Locally Recurrent Breast Cancer
Histone deacetylase inhibitors	Vorinostat	Completed	NA	NCT01153672	Histologically or cytologically proven diagnosis of breast cancer.
Histone deacetylase inhibitors	Vorinostat	Completed	1	NCT00719875,	Advanced stage breast cancer
Histone deacetylase inhibitors	Vorinostat	Completed	2	NCT00262834	Newly diagnosed cases who have undergone surgery
Histone deacetylase inhibitors	Vorinostat	Completed	1	NCT00788112	Womens diagnosed with Ductal Carcinoma in Situ of the Breast
Histone deacetylase inhibitors	Vorinostat	Completed	2	NCT00365599	Metastatic breast cancer and either ER or PR positive
Histone deacetylase inhibitors	Vorinostat	Active, not recruiting	2	NCT00616967	Infiltrating ductal breast cancer, HER2-negative
Histone deacetylase inhibitors	Vorinostat	Completed	1	NCT01084057	Patients with stage IV adenocarcinoma of the breast
Non-selective histone deacetylase inhibitor	Panobinostat`	Completed	2	NCT00777049	HER2 positive, metastatic breast cancer
Non-selective histone deacetylase inhibitor	Panobinostat`	Completed	1, 2	NCT01105312	ER, PR, or HER2 level
Non-selective histone deacetylase inhibitor	Panobinostat`	Completed	1	NCT00788931	HER2 positive
Non-selective histone deacetylase inhibitor	Panobinostat`	Completed	1	NCT00632489	Metastatic stage
Histone deacetylase inhibitors	Belinostat	Recruiting	1	NCT04315233	In Patients TNBC& Recurrent Ovarian Cancer

HER2- human epidermal growth factor receptor 2; TNBC-triple negative breast cancer; ER-Estrogen receptor; PR-Progesterone receptor; BRAC1- Breast Cancer gene 1; BRAC2- Breast Cancer gene 2

profile, are becoming increasingly feasible with advancements in genomics and bioinformatics. This approach promises more effective and less toxic therapeutic strategies. The impact of diet and lifestyle on epigenetic mechanisms is another growing area of interest. Nutrients like folate, vitamin B12, and polyphenols can influence DNA methylation. For example, folate is essential for the synthesis of S-adenosylmethionine, a key methyl donor for DNA methylation. Lifestyle factors such as physical activity and stress management have been shown to impact histone modifications and miRNA expression. Integrating dietary and lifestyle changes into therapeutic strategies could offer a non-invasive approach to modulate epigenetic alterations in disease. Emerging technologies like CRISPR/dCas9-based epigenome editing offer the potential to directly modify epigenetic marks. This technology can be used to reactivate silenced genes or silence overactive ones, providing a targeted approach to treat diseases with known epigenetic alterations. Artificial intelligence and machine learning are

playing an increasing role in interpreting complex epigenomic data, predicting disease risk, and identifying potential therapeutic targets. These technologies are at the forefront of personalized medicine and hold great promise for the future management of epigenetic diseases.

Despite these advancements, there are significant challenges in applying epigenetic therapies. One major concern is the specificity and long-term effects of epigenetic modifications. Since epigenetic changes are reversible and dynamic, understanding the timing and dosage of interventions is crucial. There are also ethical considerations, especially in the context of epigenome editing, which could potentially lead to heritable changes. Navigating these challenges requires a careful and considered approach, with ongoing research and ethical oversight [97].

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Ethical Statement

Not Applicable.

CRediT authorship contribution statement

Prabhu Kirti S: Writing – review & editing, Writing – original draft, Resources, Formal analysis, Data curation. **Sadida Hana Q:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Kuttikrishnan Shilpa:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Junejo Kulsoom:** Writing – review & editing, Resources, Investigation, Data curation. **Bhat Ajaz A:** Writing – review & editing, Writing – original draft, Supervision, Resources, Investigation, Data curation, Conceptualization. **Uddin Shahab:** Writing – review & editing, Writing – original draft, Supervision, Resources, Investigation, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author Contributions

HQS and AAB performed a bibliographical search. AAB and SU designed the manuscript content. AAB, KSP, HQS, SK and SU wrote the manuscript. KJ edited and reviewed the content. All authors read and approved the final manuscript before submission.

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