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Current Advances in Breast Cancer: Implications for Developing New Treatment Strategies Through Epi-Drugs on the Road to Modifying the Epigenome

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ABSTRACT

Breast cancer is one of the most commonly diagnosed neoplasms affecting women worldwide, and it remains a leading cause of both mortality and morbidity. While genetic predisposition plays a critical role in the development of this neoplasm, significant epigenetic dysregulations accompany existing variants. The emergence of acquired drug resistance to current chemotherapeutics poses a significant challenge in managing therapy. However, progress has been made in developing novel agents that directly target epigenetic modifications. These agents, called "epi-drugs," can be used alone in the clinic or in combination with current treatment regimens, offering the potential to create diversified effects on the disease's predictive process. Within the scope of this review, general information about the major epigenetic dysregulations in breast cancer will be provided, and their effects on the molecular mechanisms in the carcinogenesis process will be discussed. Furthermore, current treatment approaches for breast cancer will be explored, classifying these epi-drugs, such as DNA methyltransferase inhibitors (DNMTIs), histone deacetylase inhibitors (HDACIs), histone acetyltransferases (HATIs), and others that have been developed to target these mechanisms. Predictions regarding the future prospects of these epi-drugs are highlighted, and their contributions to the field of personalized medicine are emphasized based on the results obtained from clinical studies.

Keywords: Breast cancer, DNA methyltransferase inhibitors, epi-drugs, epigenetics, histone deacetylase inhibitors.

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INTRODUCTION

Breast cancer (BC) ranks first among all neoplasms detected in women worldwide in terms of mortality and morbidity.[1] While the importance of genetic background in this type of cancer is evident from family history, recent studies have revealed the significant contributions of various epigenetic modifications to tumorigenesis.[2] Similar to the majority of cancers, alterations in epigenetic mechanisms in breast cancer, characterized by its complex and high intratumor heterogeneity, have emerged as key hallmarks of the disease. These alterations have also prompted the

exploration of new strategies to modulate the dysregulated epigenome and overcome acquired resistance to therapeutic agents.[3] In this context, the introduction of "epi-drugs", a new class of medical drugs that directly target the epigenome, has added a new dimension to treatment approaches.[4] Given that epigenetic modifications are reversible and primarily involve DNA methylation, histone modifications, and non-coding RNA (ncRNA), epi-drugs exert their mechanism of action through direct interference with these processes. While the clinical use of epi-drugs alone is still in its early stages, their combination with existing therapy regimens holds promise for significant improvements in preventing metastasis and enhancing disease response and prognosis. This review aims to discuss the classification criteria of epi-drugs and explore the main pathways and application areas primarily targeted by DNA methyltransferase inhibitors (DNMTIs) and histone deacetylase inhibitors (HDACIs) in breast cancer.

MAJOR EPIGENETIC DYSREGULATIONS PROMINENT IN BREAST CANCER

DNA Methylation

Genome-wide association studies (GWAS) have revealed that various variants contributing to the carcinogenesis process cause significant alterations in the metabolic activities of cancer cells, which are of high importance. However, besides these alterations, epigenetic events occurring independently of changes in the nucleotide sequence demonstrate the undeniable role of many genes affecting cell cycle control, apoptosis, and cell signaling in their gene expression levels.[1] Epigenetic modifications involving the activation of oncogenes and the inactivation of tumor suppressor genes, which play a crucial role throughout the carcinogenesis process, predominantly occur at the intersection of the genome and epigenome. Among these modifications, DNA methylation is the most extensively elucidated over the decades, mediated by DNA methyltransferases (DNMTs). This modification, which is part of the normal developmental process, predominantly occurs at the promoters of related genes rich in Cytosine-phosphate-Guanine (CpG) dinucleotides, leading to the conversion of cytosines to 5-methlylcytosines (5mC) through S-adenosyl methionine (SAM). Subsequently, it suppresses gene expression by inhibiting transcription factors.[5] DNA methylation, primarily carried out by DNMTs, plays a vital role in downregulating gene expression and also affects the chromatin complex's structure. This can be achieved by precisely inhibiting the binding of transcriptional factors to target DNA and by binding methyl-CpG-binding proteins (MBPs) that function in gene expression silencing. Ultimately, modifications in the chromatin structure involve the binding of MBPs and DNMTs to histone deacetylase (HDAC) and histone methyltransferase (HMT) complexes.[2]

The effects of DNA methylation in cancer cells were initially observed with the loss of methylation in repetitive regions of specific genes, including tumor antigens, oncogenes, and metastasis-associated genes, known as global genomic DNA hypomethylation. This phenomenon has been further associated with chromosomal instability in cancer.[3] It is noteworthy that the ratio of global DNA hypomethylation increases with age as genomic instability accumulates in cells throughout the life cycle.[6] In contrast to DNA hypomethylation, another phenomenon known as gene-locus hypermethylation impedes the functionality of tumor suppressor genes, such as proapoptotic genes, inhibitors of cell cycle genes, and genes involved in DNA repair.[1] Studies conducted in breast cancer have revealed significantly increased methylation in tumor suppressor genes, including Adenomatosis Polyposis Coli (*APC*), Cadherin 1 (*CDH1*), and Catenin Beta 1 (*CTNNB1*). Conversely, hypomethylation has been reported in the promoter regions of two most prominent genes, Alu and LINE-1, which have been linked to Human epidermal growth factor receptor-2 (HER2) breast cancer.[7,8] Additionally, some genes involved in cell differentiation, homeobox proteins, and transcription signaling appear to be actively subjected to DNA methylation.[9] Subsequently, it has been shown that these modifications cause significant changes in many genes related to cell adhesion, tissue invasion, and metastasis in breast cancer.[10]

Post-Translational Histone Modifications

Another epigenetic mechanism involved in the development and progression of cancer is post-translational histone modifications. Histone proteins, which act as DNA packaging proteins, play a crucial role in the formation and maintenance of chromatin structure. These histone proteins (H2A, H2B, H3, and H4), contribute to wrapping the 147 base pairs (bp) long DNA around octamers, forming nucleosomes.[3] Histone tails undergo various modifications, such as methylation, acetylation, ubiquitination, phosphorylation, sumoylation, and poly-ADP ribosylation, which neutralize their electrostatic charges and regulate the structure of euchromatin or heterochromatin.[5] Euchromatin, associated with gene activation, facilitates the binding of transcription factors to the relevant region, while heterochromatin, characterized as a closed form, restricts gene transcription.[3] While DNA methylation has been extensively studied in cancer, histone acetylation and methylation have consistently been at the forefront among histone modifications. Histone acetylation, carried out by histone acetyltransferases (HATs), is associated with euchromatin structure, while histone deacetylases (HDACs), which have the opposite effect, are associated with heterochromatin formation.[1] Regarding the implications of these modifications in breast cancer, it has been reported that DNMTs and HDACs are highly expressed in the promoter region of

the Estrogen receptor 1α (*ESR1*) gene, directly contributing to gene inactivation in Estrogen receptor-negative (ER-) breast cancer.[11] Additionally, additional studies have demonstrated the significant involvement of neoplastic epithelial cells in the Epithelial-Mesenchymal Transition (EMT) process, which is crucial for invading surrounding tissues and acquiring metastatic potential.[3,12]

Non-Coding RNAs

The alterations in the epigenome and their implications on cancer are not limited to DNA methylation and histone modifications. Another significant phenomenon is known as non-coding RNAs (ncRNAs), which constitute the majority of the genome, accounting for around 62–75%. Understanding their role in various diseases, ncRNAs have emerged as a substantial source of biomarkers and specific therapeutic targets.[13] A wide spectrum of ncRNAs has been identified, playing various regulatory functions, including microRNA (miRNA), small-interfering RNA (siRNA), piwi-interacting RNA (piRNA), short hairpin RNA (shRNA), small nucleolar RNA (snoRNA), circular RNA (circRNA), and long non-coding RNA (lncRNA). Among these ncRNAs, the dysregulation of miRNAs has gained visibility in the development of multifactorial diseases, including cancer. miRNAs are short endogenous single-stranded RNA molecules, approximately 18–22 nucleotides in length, and they inhibit translation by binding to the target mRNA.[3] With a precise and dynamic regulatory profile, miRNAs are key players in various biological processes, such as development, cell proliferation, apoptosis, fat metabolism, and brain morphogenesis.[14] The core mechanism of miRNAs is based on two principles. Firstly, the target mRNA is cleaved by several ribonucleases in the cytosol and, upon completion of the maturation process, incorporated into the RNA-induced silencing complex (RISC), leading to mRNA degradation. Alternatively, translation can be inhibited without mRNA degradation. The common feature in both conditions is the suppression of translation.[14] Many studies have associated the expression levels of miRNAs with distinct subtypes of breast cancer, and the number of such studies continues to grow. For instance, overexpression of miR-21 and miR-155 has been linked to metastatic breast cancer.[15,16] Similarly, miR-497 has been shown to play a crucial role in tumor cell division dynamics by targeting cyclin E1.[17]

THE EMERGENCE OF EPI-DRUGS AND THEIR UTILIZATION IN BREAST CANCER TREATMENT

In the 21st century, innovative strategies that focus on the effect of epigenetic regulators on shaping the prognosis of the disease and the tumor microenvironment have gained prominence in cancer treatment. These strategies have opened up possibilities for the utilization of various epi-drugs in the clinical settings. Epigenome intervention studies in cancer cells, using natural or synthetic compounds, have attracted attention for accelerating the development of epi-drugs and their application in cancer, given the reversibility of epigenetic alterations.[18] Epi-drugs are defined as drugs that can modify the epigenome and are developed to reactivate tumor suppressor genes or inactivate oncogenes by targeting enzymes involved in dysregulated epigenetic pathways of cancer.[19] While clinical-stage research supports the use of epi-drugs as single agents in optimal treatment regimens, numerous preclinical and clinical studies demonstrate that their combined use with existing treatment regimens enhances the synergistic efficacy of the drugs.[20] However, when utilizing these agents, factors such as the benefit-loss ratio for the patient and treatment sensitivity should be carefully evaluated and considered in the context of personalized medicine.

Likewise, in all types of cancer, including breast cancer, histopathological and molecular grades are used to categorize the disease, leading to distinct treatment regimens for each patient. For instance, hormone-receptor-positive patients may respond to estrogen receptor modulators such as tamoxifen, fulvestrant, or aromatase inhibitors like anastrozole and letrozole. However, patients with Triple Negative Breast Cancer (TNBC), where none of these receptors are expressed, do not benefit from these drugs or monoclonal antibodies.[18] Therefore, it is crucial to consider parameters such as tumor mutation burden and immune checkpoints, which play a significant role in immunotherapy, to enhance cytotoxic mechanisms and improve treatment sensitivity. Addressing these factors can help overcome potential obstacles and enhance the potential ameliorating implications of cancer treatment.[18] In the next section of this review, pioneer epi-drugs approved for use in breast cancer treatment will be classified based on the epigenetic mechanism they target and discussed in more detail.

The Classification of Epi-Drugs and Initial Implications for their Use as Antineoplastic Agents

Epi-drugs, which have become the focal point of both clinical and experimental studies, are primarily classified based on the epigenetic mechanism they target. Initially used for treating hematological malignancies, these agents have been categorized into three generations, aiming to increase stability and improve pharmacokinetic properties while minimizing off-target effects and cytotoxicity in each generation.[3] It can be explicitly seen that different agents, such as Histone methyltransferase inhibitors (HMTIs), Histone demethylase inhibitors (HDMIs), Bromodomain and extra-terminal domain inhibitors (BETIs), Histone acetyltransferase inhibitors (HATIs), and non-coding RNAs (ncRNAs), were

added to the third-generation of epi-drugs. The first two generations mainly consisted of DNMTi and HDACi drugs. Although the summary of all these epi-drug categories and their mechanisms of action is presented in Table 1, this article will mainly emphasize the therapeutic efficacy of agents that inhibit these enzymes, considering the overexpression of DNMTs and HDACs in carcinogenesis.

DNMTIs are broadly categorized as nucleoside analogs and non-nucleoside analogs. Among the nucleoside analogs, 5-azacitidine (AZA, Vidaza®) and 5-aza-2′-deoxycytidine (Decitabine, DAC, Dacogen®) were the first epi-drugs approved by the US Food and Drug Administration (FDA) for the treatment of Myelodysplastic syndrome (MDS) and Acute Myeloid Leukemia (AML) in 2004 and 2006, respectively.[33] Non-nucleoside analogs, which emerged in the second generation of DNMTIs, offer the advantage of using such epi-drugs in solid tumors as they do not require active DNA synthesis, unlike nucleoside analogs.[3] HDACIs, which inhibit histone deacetylation, include a wide range of drugs with antineoplastic properties. They are classified into four main classes: hydroxamic acid, cyclic peptides, benzamide derivatives, and small-chain fatty acids consisting of various types of natural or synthetic derivatives. Among these HDACIs, vorinostat (suberoylanilide hydroxamic acid, SAHA, Vorinostat, Zolinza®) and FK228 (romidepsin, Istodax®) are among the first drugs approved by the FDA.[34] However, it should also be noted that due to the implications of HDACs on T-cell homeostasis, the efficacy of HDACIs is being improved by supporting their combined use with immunotherapy.[12] Last but not least, third-generation epi-drugs are defined by their high degree of selectivity, allowing them to target specific cancer types at their specific biological signaling pathways.[3] Therefore, the importance and necessity of well-designed computational strategies to estimate the loci-specific sensitivities of the factors of interest for the agents collected in this group cannot be denied. Throughout the entire drug discovery process, third-generation epi-drugs, which are considered the third wave of epigenetic drug discovery, have opened a new window to transform dreams of new treatment strategies into phenotypic reality.[34] In this context, pinometostat, developed as an inhibitor of the Disruptor of Telomeric Silencing-1 (DOT1)-like Histone Lysine Methyltransferase gene, is the first Lysine N-Methyltransferase (KMT) inhibitor to enter phase I clinical trials and be tested against leukemia. Similarly, the potential use of agents such as Tazemetostat (Tazverik®), which inhibit other histone methyltransferases like Enhancer of Zeste 2 Polycomb Repressive Complex 2 Subunit (EZH2), highly expressed in breast cancer, becomes possible.[28,34,35] This clearly shows that current clinical studies are focused on these inhibitors, and third-generation drugs hold promise for personalized medicine.

NOVEL THERAPEUTIC STRATEGIES FOR THE COMBINATORIAL USE OF EPI-DRUGS IN THE TREATMENT OF BREAST CANCER

Following the classification of epi-drugs according to generations and the epigenetic mechanisms they target, it has been reported that nine epi-drugs have been approved by the FDA for use in the treatment of breast cancer.[12] The majority of these are inhibitors of DNMTs and HDACs, as well as inhibitors of HMTs such as EZH2, which cause significant changes throughout the epigenome of cancer cell. Although the therapeutic efficacy of these drugs has been tested in various clinical phase studies, current treatment approaches focus more on the rationale and combinatorial use of these epi-drugs to demonstrate their synergistic effects and reduce the side effects caused by current chemotherapeutic agents.[36] For instance, the suppression of EZH2, along with the antiproliferative effect of resveratrol, one of the polyphenols and an important component of the Mediterranean Diet, attracts attention in terms of providing this synergistic effect. Additionally, due to its natural compound properties, it holds the potential to eliminate potential side effects during treatment.[37]

In view of the fact that DNMTIs exert antineoplastic activity mainly by targeting Major Histocompatibility Complex (MHC) molecules or immune mechanisms such as tumor antigens,[12] DNMTIs can potentially reverse promoter methylation of the *MHC1* gene and modulate gene expression. The combined use of epi-drugs finds wide application with existing chemotherapeutics as well as other classes of epi-drugs. It has been observed that the efficiency of T cells improved positively with the combined use of DNMTIs such as azacitidine and decitabine.[38] After recognizing that immune mechanisms are targeted by various epigenetic agents, it should be emphasized that this effect is not only provided by DNMTIs but also by HDACIs. Considering the examples of combinatorial treatment strategies in estrogen receptor-positive (ER^+) breast cancer, an improvement in the clinical benefit rate was observed in phase II clinical trials (NCT00676663 and NCT00828854) with the co-administration of exemestane (an aromatase inhibitor) and Entinostat (an HDACi).[36] In addition, another ongoing clinical trial (NCT04315233) aims to examine the tolerance level of ribociclib, a Cyclin Dependent Kinase (CDK4/6) inhibitor, and Belinostat (another HDAC inhibitor) in inhibiting cell line growth in patients diagnosed with metastatic TNBC. Trichostatin A (TSA), which keeps the cell cycle in the G1 and G2 phases and has a strong effect on tumor growth inhibition, is considered among the leading epi-drugs categorized under hydroxamic acids in this regard.[38] Previous studies have revealed that the effect of TSA on breast cancer cell viability leads to a significant increase in reactive oxygen species production via disruption of mitochondrial membrane potential, leading to

Table 1. Classification of epi-drugs and their mechanism of action

Generation	Category	Sub-category	Epi-drug	Epigenetic/molecular mechanism	Ref.
First & Second	DNMTIs	Nucleoside analogs	5-Azacitidine (AZA, Vidaza®)	Inhibition of DNMTs in the promoter region and induction of MHC molecules and tumor antigens	19, 21, 22
			5-aza-2′ deoxycytidine (Decitabine, DAC, Dacogen®)	Increasing chemosensitivity by induction of apoptosis-related pathways	19, 22, 23
		Non-Nucleoside analogs	Hydralazine	Reactivation of methylated genes in tumor cells beyond its ability to induce caspase-dependent apoptosis	24
			Procainamide	Restoration of expression of TSGs silenced by DNA hypermethylation in tumor cells	25
	HDACIs	Bi-substrate analogs	Quinolone-Quinazoline conjugates	Downregulation of DNMT	26
		Hydroxamic acid	Suberoylanilide hydroxamic acid, (SAHA, Vorinostat, Zolinza®)	Alteration of acetylation levels in H3K27 and regulation of p21WAF1/CIP1 through alteration in H4	22
			Trichostatin A (TSA)	Induction of ERa expression in MCF-7 and MDA-MB-231 cell lines through the reduction of miR-204	19, 22
			Belinostat (Beleodaq®)	Inhibition of cell line growth and activation of the process of regulatory T cells	12
			Panobinostat (Farydak®)	Increasing the membrane expression of E-cadherin without significantly affecting ERα and ERα-related signaling in TNBC cells. Regulation of EMT by inhibiting cell migration and invasion.	19, 22
		Cyclic peptides	FK228 (romidepsin, Istodax®)	Promotion of cell death through activating caspase cascades and increasing NK cell activity	12
		Benzamide derivatives	Entinostat (MS-275)	Decreasing tumor-initiating cells such as CD44high/CD24low in TNBC cell lines	22
			Chidamide (CHI, Epidaza®)	Promotion of apoptosis via activating the caspase cascade	27
		Small-chain fatty acids	Valproic acid	Reducing cell viability and telomerase activity of MCF-7 cells. Induction of apoptotic pathways via increasing the Bax/Bcl-2 ratio.	19, 22
			Sodium butyrate	Inhibition of cell growth by arresting cell cycle in G2/M phase	22
Third	HMTIs	DOT1LIs	Pinometostat	Reducing effect on H3K79 methylation	28
		EZH2Is	Tazemetostat (Tazverik®)	Inhibition of EZH2 activity and increasing Myeloid-Derived Suppressor Cells (MDSCs) in the tumor microenvironment	12
	HDMIs	Jumonji C inhibitor	N-oxalylglycine (NOG)	Reactivation of histone lysine methlyation	29
		LSD1Is	Tranylcypromine	Reprogramming of tumor-associated macrophages (TAMs) into M1	12
	BETIs	–	JQ1	Declining NF-κB activity and cytokine generation	12
	HATIs	–	PF-9363 (CTx-648)	Anti-tumor activity through targeting KAT6A/KAT6B	30, 31
	ncRNAs	–	CALAA-01	Targeting RRM2 regulated in a cell cycle-dependent manner and influencing the down-regulation of cancer-related genes	32
			MesomiR-1	Demonstrating antineoplastic activity by targeting overexpressed miRNAs	3
			MRG-106	Demonstrating antineoplastic activity by targeting overexpressed miRNAs	3

DNMTIs: DNA methyltransferase inhibitors; HDACIs: Histone deacetylase inhibitors; HMTIs: Histone methyltransferase inhibitors; HDMIs: Histone demethylase inhibitors; BETIs: bromodomain and extra-terminal domain inhibitors; HATIs: Histone acetyltransferase inhibitors; ncRNAs: non-coding RNAs; DOT1Lis: DOT1-like histone lysine methyltransferase inhibitors; EZH2Is: Enhancer of Zeste Homolog 2 inhibitors; LSD1Is: lysine-specific histone demethylase 1A inhibitors.

the downregulation of Cyclin D1 by increasing transcription of BH3-containing Bcl-2 family genes, ultimately triggering the intrinsic apoptosis pathways.[39,40] In a study designed *in vivo* and *in vitro*, it has been reported that the combinatorial application of the HDAC inhibitor Chidamide (CHI, Epidaza®) with doxorubicin (DOX) induced apoptosis by activating the caspase cascade and ultimately suspended the cell cycle in the G0/G1 phase.[27]

DRUG DELIVERY-FOCUSED STRATEGIES IN THE COMBINATORIAL USE OF EPI-DRUGS IN BREAST CANCER TREATMENT

Despite the ongoing clinical studies on the toxicity of first and second-generation epi-drugs in cancer cells, researchers have recently explored the antineoplastic activities of third-generation epi-drugs. In this regard, suggesting different nano-carrier formulations to enhance the solubility and specificity of these agents has emerged as a potential approach to improve the success of combinatorial treatments.[3] For example, the combination of phosphatidylinositol 3-kinase (PI3K) inhibitors and endocrine therapy, along with chromatin regulators such as Lysine Methyltransferase 2D (KMT2D) and Lysine Acetyltransferase 6A/6B (KAT6A/KAT6B), has offered a new perspective in drug discovery, particularly for ER^+ breast cancer. As a matter of fact, considering the KAT6A amplification observed in 10–15% of breast cancers, it has been reported that new molecules such as PF-9363 (CTx-648) have been developed as HATIs against KAT6A/KAT6B. These molecules have shown potent anti-tumor activity as a result of analyses conducted in both cell lines and xenografts.[30,31]

Beyond the therapeutic applications targeting primary epigenetic mechanisms such as DNA methylation and histone modifications, it is worth mentioning the utilization of ncRNAs. Different ncRNAs, including miRNAs and lncRNAs, participate in coordinated networks related to cell motility, proliferative signaling pathways of cancer cells, and the process of EMT, which is an important criterion for invasion and metastasis. Although this article does not extensively cover the application areas of current ncRNAs in breast cancer, it aims to draw attention to the existence of only a few phase clinical trials with these agents. Recent clinical trials in mesothelioma and lymphoma have begun exploring miRNA-based drug applications, such as MesomiR-1 and MRG-106.[18] However, the delivery of these RNA molecules to the cytoplasm of the target cell without nuclease degradation remains a significant obstacle in this treatment approach. As a result, their utilization is generally limited to hematological malignancies, and further studies are ongoing to address the treatment of solid tumors, including breast cancer.[18] In a phase I/II clinical trial conducted in solid tumors, CALAA-01, an siRNA targeting Ribonucleotide Reductase Regulatory Subunit M2 (RRM2) in a cell cycle-dependent manner, demonstrated the ability to influence the down-regulation of cancer-related genes.[32] This research has undoubtedly shed light on investigating the synergistic effects of ncRNAs and the combinatorial use of current chemotherapy and immunotherapy strategies.

CONCLUDING REMARKS AND PERSPECTIVES

At this stage of oncological treatment development over the past two decades, the use of epi-drugs, either alone or in combination with current therapeutics, has brought new hope for the treatment of various malignancies, including breast cancer. However, the low efficacy levels and limited tolerance of many epi-drugs developed so far continue to be major obstacles in clinical studies. It is therefore crucial to integrate data from genome-wide association studies (GWAS) with epigenetic modifications of tumor-related genes, as the current clinical approach moves away from a "one size fits all" approach and requires more accurate and consistent data for personalized medicine.

Throughout this review, we aimed to summarize the current approaches and implications of epi-drugs in breast cancer, focusing on DNMTIs and HDACIs and their mechanisms of action. Synthetic compounds targeting various epigenetic modifications alone or in combination with each other or with other chemotherapeutic agents have demonstrated antineoplastic activity in numerous pre-clinical studies. In addition to synthetic compounds, natural-based molecules derived from botanical sources, such as polyphenols and omega fatty acids abundant in the Mediterranean Diet, have shown profound effects on the regulation of epigenetic mechanisms like chromatin remodeling and DNA methylation.

Despite the significant clinical implications of epi-drug utilization in recent decades, notable limitations remain. The impact of these drugs on the immune system and the associated high costs present significant challenges. Furthermore, the dynamic nature of epigenetic reprogramming during disease progression, combined with the inherent genetic variability among individual cancer patients, adds complexity to disease management. To overcome these challenges, further *in vitro* and *in vivo* studies are needed. Additionally, the implementation of consortium studies that capture and record comprehensive clinical parameters in a centralized database is expected to yield faster and more conclusive results. By leveraging robust bioinformatics tools, this approach would enable a more nuanced evaluation of the risk-benefit ratio, ultimately leading to a more informed and judicious treatment selection process.

In conclusion, considering the dynamics of cell division and the interaction of cell viability with the carcinogenesis process, it is possible to develop nature-based epi-drugs with high efficiency and minimal toxicity in the clinic, in addition to fur-

ther studies with existing chemotherapeutics. Future research in the field of epi-drugs for breast cancer treatment should explore novel therapeutic targets, such as third-generation epi-drugs, and investigate their potential synergistic effects when combined with existing treatment modalities. Furthermore, unraveling the complex interplay between epigenetic alterations and the tumor microenvironment could reveal new therapeutic strategies and personalized approaches for patients with breast cancer.

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