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DNA Methylation in the Regulation and Clinical Application of HER2-Positive Breast Cancer

Jiaying Feng

WUT-AMU Institute, Wuhan University of Technology, Wuhan, Hubei, 430070, China

ABSTRACT
HER2-positive breast cancer represents an aggressive form of the disease driven by excessive HER2 protein expression or genetic amplification, leading to accelerated tumor growth, heightened metastatic potential, and unfavorable clinical outcomes. Epigenetic dysregulation plays a pivotal role in the pathogenesis of this malignancy, with aberrant DNA methylation patterns serving as one of the key molecular hallmark. These methylation-based biomarkers hold promise for improving diagnostic accuracy, predicting disease outcomes, and assessing treatment efficacy in HER2-positive tumors. Consequently, investigating the relationship between methylation anomalies and disease progression in this subtype is critical for advancing translational research. This review comprehensively examines the molecular signatures and mechanistic underpinnings of DNA methylation dysregulation in HER2-positive breast cancer, its correlation with HER2 overexpression, and its clinical utility. By synthesizing current evidence and prospecting future research directions, this discussion aims to guide future studies and facilitate the development of epigenetically targeted interventions.

KEYWORDS
HER2-positivie breast cancer; DNA methylation; HER2; Epigenetics

1 Introduction

Breast cancer ranks among the most prevalent malignancies affecting women worldwide and represents a leading contributor to female cancer mortality. A distinct subset, comprising roughly 15% to 20% of cases, exhibits elevated expression levels of human epidermal growth factor receptor 2 (HER2) or genomic amplification of the HER2 locus. These tumors are clinically classified as HER2-positive due to their unique molecular profile. As a key molecule regulating signal transduction in cell growth and differentiation, the overexpression of HER2 can induce dysregulation of cell proliferation and drive tumorigenesis. Thus, HER2 overexpression is clinically recognized as a marker of aggressive tumor behavior and unfavorable clinical outcomes. As the first targeted driver molecule in breast cancer, the therapeutic value of HER2 was initially focused on breast cancer and has gradually expanded to other solid tumors such as gastric cancer, becoming an important target for cross-cancer research. Despite the clinical success of HER2-directed biological agents such as trastuzumab in transforming treatment outcomes, therapeutic challenges persist. A subset of patients demonstrates either intrinsic non-responsiveness or acquires resistance during the course of treatment, leading to suboptimal clinical responses. Furthermore, their malignant characteristics such as high metastatic potential remain problematic in terms of clinical management. Therefore, exploring the regulatory mechanisms underlying HER2 overexpression is of great significance for the development of new therapeutic strategies [1].

As a fundamental epigenetic modification, DNA methylation plays a pivotal role in gene expression control. Disruptions in this regulatory mechanism have been strongly implicated in both carcinogenesis and malignant transformation, constituting a hallmark of neoplastic epigenetic dysregulation. In malignancies, these alterations typically manifest as genome-wide hypomethylation and site-specific hypermethylation, with such abnormalities particularly impacting CpG-rich regions located in critical gene regulatory sequences [2]. In breast cancer with HER2 positivity, abnormal DNA methylation not only participates in tumor initiation and progression, but also may influence the maintenance of HER2 overexpression and therapeutic sensitivity by regulating HER2 pathway-related genes or upstream and downstream signaling molecules [1]. Therefore, deciphering the characteristics and functions of abnormal DNA methylation in such tumors constitutes a critical entry point for revealing their malignant phenotypes and drug resistance mechanisms.

Current investigations have revealed numerous genes exhibiting methylation alterations specifically in HER2-overexpressing breast tumors. These epigenetically modified genes participate in crucial cellular signaling pathways, including but not limited to the PI3K/AKT axis and Wnt/β-catenin pathways. However, questions remain to be elucidated, including how abnormal methylation directly regulates HER2 overexpression, and the obstacles in the clinical translation of such methylation abnormalities as markers for early diagnosis or drug resistance prediction. This article aims to systematically review the characteristics and regulatory mechanisms of abnormal DNA methylation in HER2-positive breast cancer, as well as its associations with HER2 overexpression and clinical applications. It summarizes the progress of existing research, prospects future research directions, and provides a reference for mechanism exploration and clinical

application in this field.

2 Abnormal DNA methylation leading to HER2 overexpression

HER2 amplification triggers the formation of both homodimers and heterodimeric complexes with related epidermal growth factor receptor proteins. This molecular interaction represents a critical step in the activation of downstream signaling pathways, which promote uncontrolled cellular growth, enhance transition through cell cycle checkpoints, and suppresses programmed cell death. These molecular alterations collectively contribute to the aggressive phenotype and unfavorable clinical outcomes observed in HER2-driven breast malignancies [3-4].

A significant body of evidence highlights the close relationship between recurrent DNA methylation events and HER2 gene amplification in mammary carcinomas. Specifically, studies have confirmed positive correlations between HER2 expression and the methylation levels of CDH13, PGR, HSD17B4, and MYOD1 genes [5-6].

An inverse relationship is observed between HER2 protein levels and DNA methylation patterns in the HER2 gene enhancer (HGE) domain in breast tumors exhibiting HER2 amplification. The presence of trimethylated histone H3 lysine 4 (H3K4me3) and acetylated histone H3 lysine 9 (H3K9ac) marks correlates with transcriptional activation. Reduced methylation in the HGE region enables enhanced binding of transcriptional regulators (including TFAP2C and ERRα), subsequently promoting the accumulation of activating histone marks like H3K4me1 and H3K27ac. This epigenetic reprogramming cascade culminates in elevated HER2 expression levels [7].

3 Other abnormal DNA methylation in HER2-positive breast cancer

The initiation and progression of HER2-positive breast cancer are closely associated with extensive epigenetic abnormalities, among which the disruption of gene methylation patterns constitutes one of the core features. These epigenetic alterations function cooperatively rather than independently, synergistically molding the aggressive characteristics of HER2-driven breast malignancies through coordinated regulation of critical genetic elements, signal transduction routes, and essential cellular mechanisms.

A comprehensive analysis of the DNA methylome in HER2-driven breast malignancies reveals a dual pattern of epigenetic dysregulation, characterized by focal promoter-associated CpG island hypermethylation occurring alongside pervasive genomic hypomethylation affecting specific gene subsets and intergenic chromosomal segments. Hypermethylation of promoter CpG islands leads to transcriptional silencing, serving as a mechanism for inactivating tumor suppressor genes. In contrast, global hypomethylation often leads to genomic instability, thereby promoting tumor progression. For instance, hypomethylation-induced expression of TSTD1 directly reduces patient survival rates and treatment efficacy [8].

The expression of homeobox genes is often subject to epigenetic regulation, and methylation of CpG islands in their promoters leads to silencing of these genes. In HER2-positive breast cancer, HOXA1, HOXA7, HOXA9, TLX3, and PAX3 exhibit hypermethylation alongside high expression, among which the high expression of HOXA1 is considered to exert oncogenic effects. In contrast, the CpG island in the promoter region of HOXC8 shows low methylation, with its expression being upregulated; this contributes to the proliferation, clonogenesis, and metastasis of HER2-positive breast cancer cells, reduces the apoptosis rate, and enhances resistance to trastuzumab [9].

Both the PI3K/AKT and Wnt signaling pathways can independently regulate the glycolytic pathway, and they exhibit synergistic effects to collectively modulate cellular glucose metabolism. In HER2-positive breast cancer, HER2 is overexpressed and mediates downstream signaling through the PI3K/AKT pathway. However, to date, there have been no reports on the methylation status of HER2 or AKT. Hypomethylation has been detected in HK1 and PFKP, two crucial checkpoints in the glycolytic pathway. Enhanced glycolysis under aerobic conditions is a characteristic of cancer, and increased hexokinase expression facilitates this phenomenon [10]. Additionally, PRKACA, a gene involved in the PI3K pathway, is differentially expressed in trastuzumab-resistant breast cancer tissues, leading to PI3K dysfunction, which studies have shown to be associated with drug resistance in patients [11]. Hypermethylation of the promoter of the tumor suppressor gene PTEN results in reduced expression, impairing its ability to effectively regulate HER2 and the downstream PI3K/AKT pathway. Sustained activation of the PI3K/AKT pathway subsequently induces resistance to trastuzumab in tumor cells. The Wnt signaling cascade, a highly conserved pathway governing cellular proliferation and stem cell maintenance, plays a pivotal role in regulating stemness properties. Within HER2-amplified breast malignancies, epigenetic perturbations—specifically hypomethylation of the Wnt coactivator Bcl9 coupled with promoter hypermethylation of negative regulators including SOX17, APC, SFRP2, SFRP5, and DKK3—collectively drive aberrant pathway activation. Notably, reduced DKK3 expression exhibits a strong correlation with advanced disease stage and unfavorable clinical outcomes [12]. Such cross-regulation between pathways extends the impact of abnormal methylation from individual genes to core cellular processes such as metabolism and proliferation.

Additionally, in HER2-positive breast cancer, several other genes exhibit abnormal methylation patterns. The Ras-association domain family (RASSF) members RASSF1 and RASSF10 are hypermethylated: RASSF1 is silenced due to

promoter hypermethylation, losing its ability to regulate the MAPK pathway as well as cell proliferation, apoptosis, and cycle; epigenetic dysregulation of RASSF10 is also observed in other cancers [1]. The CCDN1 gene frequently exhibits promoter hypomethylation, and its subsequent amplification or elevated expression correlates with advancing disease severity. Concurrently, IGFBP7—a modulator of p53-mediated signaling—is commonly silenced through promoter hypermethylation. This epigenetic repression results in diminished IGFBP7 transcription, compromising its capacity to constrain proliferation and trigger programmed cell death. These findings indicate that dysregulated methylation of IGFBP7 may represent a contributory mechanism in oncogenesis [13]. FOXF2 is often silenced by promoter hypermethylation or histone deacetylation, and its low expression correlates with poor prognosis; while it exerts tumor-suppressive effects by inhibiting the CDK2-RB-E2F pathway, its regulation of DNA replication and cell cycle is weakened or lost due to p53 dysfunction [14].

HSD17B4 displays highly specific methylation, which can silence the gene and cause its specific downregulation, potentially affecting trastuzumab sensitivity through sex hormone metabolism. In HER2-positive BT-474 cells with HSD17B4 knockout, unsaturated fatty acids are reduced, the PI3K/Akt pathway is activated, and the cells show enhanced glucose dependence and sensitivity to lapatinib. This mechanism may be applicable to similar cells, and inhibiting PI3K/Akt may be an effective strategy. However, the study only used one cell line, which has limitations [15].

SLC25A43 can undergo heterozygous deletion, and its knockdown impairs cell renewal and metabolism; with no mutations detected in breast cancer, CpG island methylation may serve as an alternative silencing mechanism in the absence of deletion. Associations between specific site methylation and clinicopathological parameters highlight the significance of SLC25A43 epigenetic changes in carcinogenesis [16].

4 Clinical application value of methylation markers

Aberrant methylation patterns hold multidimensional value, offering critical implications for determining prognosis and optimizing treatment strategies in HER2-enriched breast malignancies.

In the context of predicting treatment response, HSD17B4 methylation can serve as a key biomarker to determine the sensitivity of HER2-positive tumor to trastuzumab combined with chemotherapy. Patients exhibiting hypermethylation of this gene show a significantly increased propensity for achieving pathological complete response (pCR) when treated with combination therapy [17-18]. Meanwhile, regarding the clinically intractable issue of trastuzumab resistance, hypermethylation of transforming growth factor β1 (TGFβ1), B-cell lymphoma 6 (BCL6), p53-regulated DNA replication inhibitor (KILLIN), and cathepsin Z (CTSZ) provides important clues: these genes are downregulated in drug-resistant cells due to hypermethylation, and demethylation treatment can restore their expression, suggesting that they can serve as potential markers for drug resistance prediction and provide a basis for timely adjustment of treatment strategies [19].

The clinical utility of DNA methylation markers extends to prognostic assessment and monitoring of disease status. In HER2-positive breast cancer, promoter hypermethylation of PTPRO—a frequent epigenetic alteration in primary tumors—demonstrates dual applicability: it is readily detectable in circulating blood and correlates strongly with reduced overall survival. These characteristics establish PTPRO methylation as a robust prognostic biomarker capable of supporting clinical decision-making by stratifying progression risk [20]. Mechanisms such as inactivation of tumor suppressor genes (e.g., RASSF1A) caused by promoter hypermethylation and promotion of genomic instability by global hypomethylation jointly participate in the progression of HER2-related tumors, providing a new reference dimension for the early identification and typing of HER2-positive breast cancer [1,21]. Moreover, silencing of FOXF2 and epigenetic inactivation of SLC25A43, among others, are closely linked to poor patient prognosis by impairing cell cycle regulation and disrupting metabolic balance [14,16]. In addition, the frequency of methylation of specific genes is significantly positively correlated with the degree of HER2 amplification; for example, the methylation levels of genes such as CDH13 and PGR are positively correlated with HER2 expression, so they can serve as epigenetic markers for HER2 status.

Therapeutic strategies based on abnormal methylation are also under continuous exploration. The reversibility of epigenetic changes not only provides possibilities for gene therapy but also makes the development of drugs targeting epigenetic alterations an important direction in cancer treatment [22]. Among these, DNA methyltransferases (DNMTs) are core targets. Given that excessive DNMT activity induces abnormal DNA hypermethylation, and such hypermethylation is associated with HER2 amplification, interventions targeting DNMTs have thus become an important research direction. For example, siDNMT targeting DNMT1 and DNMT3b can be specifically delivered to HER2-positive breast cancer cells; by silencing DNMTs, they promote demethylation of the tumor suppressor gene RASSF1A promoter, thereby restoring its expression to inhibit tumor cell proliferation. In addition, DNMT inhibitors are also used to target tumor cells. When combined with traditional chemotherapeutic drugs, DNMT inhibitors such as azacitidine and decitabine (DAC) can produce a highly synergistic effect. Decitabine is the most effective specific inhibitor of DNA methylation, which can inhibit DNMTs to reduce DNA methylation, hinder tumor cell proliferation, and prevent the development of drug resistance [23]. The small-molecule inhibitor DI-1 exhibits effective inhibitory activity against DNMT1 and can produce a synergistic effect with trastuzumab, enhancing the anti-tumor activity of HER2 antagonists [24].

5 Discussion

Overall, methylation markers not only act as effective predictive indicators for assessing therapeutic response, drug resistance, and prognosis in HER2-positive breast cancer, but the epigenetic regulatory mechanisms they involve also provide potential targets for the development of novel therapeutic strategies, thereby laying a theoretical and experimental foundation for precision diagnosis and treatment. Despite the current limited range of relevant therapeutic approaches and the significant challenges in translational application. Given that methylation interventions or demethylation operations may extensively affect the methylation profiles of multiple genes in the genome, resulting in a critical bottleneck of insufficient specificity in their translational application. How to achieve selective regulation of the methylation status of a single gene without interfering with the epigenetic modifications of other genes remains a core problem to be urgently addressed. Nevertheless, it cannot be denied that the complex network of abnormal methylation in HER2-positive breast cancer still holds tremendous research and development potential and ample exploration space in the future.

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