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THE ROLE OF EPIGENOME-WIDE ASSOCIATION STUDIES (EWAS) IN IDENTIFYING EPIGENETIC BIOMARKERS FOR BREAST CANCER: A NARRATIVE REVIEW

Karunia[1], Rafika I. Paramita[2,3], Sonar S. Panigoro[4]

AFFILIATIONS

1. Master's Programme in Biomedical Science, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
2. Department of Medical Chemistry, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
3. Bioinformatics Core Facilities – IMERI, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
4. Division of Surgical Oncology, Department of Surgery, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

ABSTRACT

Breast cancer remains the most common malignancy among women worldwide and a leading cause of cancer-related mortality. Advances in epigenetics, particularly through the Epigenome-Wide Association Study (EWAS) approach, have provided new opportunities to identify epigenetic biomarkers for early detection, risk assessment, and therapeutic monitoring. This narrative review aims to describe the role of EWAS in identifying DNA methylation biomarkers relevant to breast cancer and to highlight current methodological challenges. Literature was retrieved from PubMed, ScienceDirect, and Google Scholar using the keywords "EWAS," "breast cancer," "DNA methylation," and "epigenetic biomarker," focusing on studies published between 2015 and 2025. Findings indicate that EWAS can reveal methylation patterns associated with cancer risk, prognosis, and potential for noninvasive detection, with alterations detectable even before clinical onset. Several candidate biomarkers identified include methylation changes in genes such as BRCA1, RASSF1A, CDH1, and APC, as well as specific CpG sites associated with hormonal exposure and lifestyle-related risk factors. Despite technological advances in microarray platforms and bioinformatics, many studies still face issues such as cross-sectional design, cellular heterogeneity, and limited replication. These challenges highlight the need for standardized analytical pipelines, larger longitudinal cohorts, and multi-omics integration to improve the reliability and clinical applicability of EWAS findings. Nevertheless, with growing standardization and integration of multi-omics data, EWAS holds significant promise for advancing precision medicine toward more predictive and preventive breast cancer care.

KEYWORDS:

EWAS, DNA Methylation, Epigenetic, Biomarker, Breast Cancer

CORRESPONDING AUTHOR:
Karunia
karunia31@ui.ac.id

INTRODUCTION

Breast cancer, also known as carcinoma mammae, is currently the most common type of cancer among women and the second leading cause of cancer-related death worldwide.[1] It is a malignant tumor that develops in breast tissue, including the mammary glands, fatty tissue, and connective tissue. Although it predominantly affects women, men can also develop this disease. Currently, breast cancer remains one of the most feared illnesses, particularly among women, due to its aggressive nature and potentially fatal outcome.[2]

According to the Global Cancer Observatory (GLOBOCAN), the incidence of breast cancer continues to rise across various countries. In 2020, there were 2,261,419 new cases worldwide (accounting for 11.7% of all cancer cases) with an incidence rate of approximately 44 cases per 100,000 population, making it the most common cancer globally. In Indonesia, breast cancer also ranks first among all cancer types, with 65,858 new

cases (16.6%) out of 396,914 total new cancer cases, and remains the leading cause of cancer-related mortality, with a death rate of 15.3 per 100,000 population. It is estimated that one in every eight diagnosed cancers worldwide is breast cancer, and projections suggest that by 2040, the number of new cases could reach three million annually, with deaths approaching one million per year.[3]

In line with this increasing trend, early detection and accurate diagnosis are essential to reduce breast cancer mortality. One promising approach is the utilization of biomarkers, which enable more precise diagnosis and therapy based on the molecular characteristics of each patient. Currently, several biomarkers such as estrogen receptor (ER), progesterone receptor (PR), and HER2 are widely used in clinical practice. However, new biomarkers are still needed to better capture the biological heterogeneity of breast cancer.[4]

The Epigenome-Wide Association Study (EWAS) plays an important role in analyzing the impact of genetic and environmental factors on epigenetic regulation that contributes to breast cancer risk. Epigenetics itself is a branch of genetics that studies changes in gene expression without altering the DNA sequence, such as DNA methylation and histone modification. In recent years, epigenomic variation has emerged as a new research direction, with DNA methylation at CpG sites recognized as the most common epigenetic mark. The EWAS approach distinguishes cancer samples from normal tissues by analyzing alterations in DNA methylation patterns that affect cellular phenotypes. Since its introduction about a decade ago, the number of EWAS studies on various common diseases has grown significantly. Similar to Genome-Wide Association Studies (GWAS), EWAS has been widely used to identify biomarkers in large populations and to elucidate the molecular mechanisms underlying disease development.[4]

However, studies specifically investigating EWAS-based epigenetic biomarkers for breast cancer in the Indonesian population remain limited, and research in this area is still in its early stages. This highlights the need for further investigation to understand population-specific epigenetic patterns and to support the development of precision medicine strategies in Indonesia.

Although this review focuses on studies published between 2015 and 2025 to reflect recent advances, earlier foundational research on DNA methylation and epigenetic regulation has established the biological relevance of epigenetic alterations in carcinogenesis.

Therefore, this review aims to discuss the role of the Epigenome-Wide Association Study (EWAS) in identifying epigenetic biomarkers of breast cancer and to explore the potential of this approach

in advancing more precise diagnosis and therapy in the future.

METHODS

This study employed a narrative review approach to examine the role of the Epigenome-Wide Association Study (EWAS) in identifying epigenetic biomarkers for breast cancer. Relevant scientific literature was collected through searches on PubMed, ScienceDirect, and Google Scholar databases.

The review included original research articles and systematic reviews published between 2015 and 2025, focusing on studies that investigated DNA methylation patterns, CpG site analyses, and candidate genes associated with breast cancer.

The keywords used in the search were "Epigenome-Wide Association Study (EWAS)", "breast cancer", "DNA methylation", and "epigenetic biomarker". Articles were selected based on their relevance to the topic, alignment with the study objectives, and the availability of complete data. Additional selection criteria included relevance to EWAS-based DNA methylation in breast cancer, full-text availability, and methodological clarity. Publications not directly related to breast cancer or not employing an EWAS approach were excluded from the review. Given the relatively limited number of EWAS studies specifically focusing on breast cancer, relevant studies were included to provide a comprehensive overview of the available evidence.

All selected articles were analyzed descriptively to identify general research trends, differences in findings among studies, and the potential development of specific epigenetic biomarkers for breast cancer.

Review

Epigenetic Biomarkers and the EWAS Approach

In cancer research, a biomarker is defined as a measurable biological molecule (found in blood, serum, or tissue) that reflects the presence of a pathological process, such as the transformation of normal cells into malignant ones. Unlike clinical screening methods such as mammography, MRI, PET, and biopsy, which are effective only in detecting masses or morphological changes, biomarkers can identify molecular alterations that occur at the earliest stages of breast cancer. This has driven researchers to search for biological markers capable of distinguishing normal from cancerous tissues, revealing tumor activity even before a detectable mass forms, and potentially serving as tools for early diagnosis, prognosis, and therapeutic monitoring.[5]

Biomarkers are generally classified into three major categories: protein biomarkers, gene-based biomarkers, and metabolite or small-molecule biomarkers.[5] With the advancement of research,

the focus of biomarker studies has expanded beyond conventional protein and gene molecules to include the epigenetic level, which reflects changes in gene regulation without alterations in the DNA sequence. Epigenetic modifications, such as DNA methylation in the promoter regions of tumor suppressor genes, have been shown to represent one of the earliest mechanisms in the process of carcinogenesis, making them ideal candidates for early cancer detection.[6]

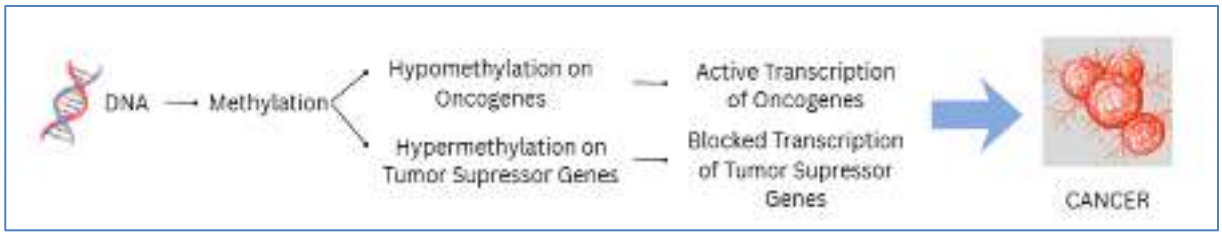

Figure 1. Effects of DNA Methylation on Gene Expression in Cancer

Several genes, such as GSTP1 and MGMT, have been clinically tested as promising epigenetic biomarkers in various cancers. Hypermethylation of GSTP1 serves as a strong diagnostic marker for prostate cancer, whereas MGMT methylation is used to predict therapeutic response to alkylating agents in glioblastoma.[7] Meanwhile, several genes such as BRCA1, RASSF1A, CDH1, and APC have been reported to undergo hypermethylation in the early stages of breast cancer and are considered potential candidates for specific diagnostic markers.[8] Besides acting as markers in tumor initiation, DNA methylation patterns have also been shown to reflect tumor progression and treatment response in breast cancer. For example, hypermethylation of the TGFBI gene is associated with resistance to trastuzumab therapy in the HER2-positive subtype, while methylation of the ESR1 gene in patient serum shows both diagnostic and prognostic potential in distinguishing different breast cancer subtypes.[9]

In addition to tissue-based approaches, epigenetic biomarkers can also be detected non-invasively through blood or other body fluids. This approach, known as liquid biopsy, enables the detection of cell-free DNA (cfDNA) or circulating RNA that carries specific epigenetic signatures of tumors. Non-invasive biomarkers such as methylated cfDNA, microRNAs, and plasma proteins are now recognized as potential tools to replace or complement conventional radiological screening, as they are fast, safe, and capable of identifying molecular changes long before clinical symptoms appear.[6]

Along with these developments, various DNA methylation analysis methods such as MethyLight qPCR, MS-HRM, and bisulfite

pyrosequencing have been widely used to identify epigenetic alterations in specific genes. However, advances in microarray and next-generation sequencing (NGS) technologies now make it possible to analyze DNA methylation comprehensively across the entire genome. Since the introduction of the EPIC BeadChip platform and genome-scale bisulfite sequencing, the Epigenome-Wide Association Study (EWAS) approach has become a popular method for discovering epigenetic biomarkers associated with both diseases and environmental exposures.[7,10]

A comprehensive review by Wei et al., titled "Ten Years of EWAS," explains that the Epigenome-Wide Association Study (EWAS) approach has undergone rapid development over the past decade and is now recognized as a key method for identifying epigenetic biomarkers.[4] The study by Campagna et al. (Clinical Epigenetics, 2021) also emphasized that advancements in Illumina microarray technologies (450K and EPIC) and bioinformatics pipelines such as Minfi and ChAMP have established EWAS as a standard approach for efficient, standardized, and reproducible genome-wide DNA methylation analysis across cohorts.[11]

Similarly, Sahoo and Sundararajan (2024) stated that microarray-based EWAS is an efficient, cost-effective, and wide-ranging approach for detecting CpG sites that serve as diagnostic and prognostic markers.[12] With its ability to map DNA methylation variations comprehensively and link them to disease phenotypes, EWAS has become one of the key drivers in the paradigm shift from genetic research toward epigenetic approaches in the discovery of clinical biomarkers.[4]

Specifically, the Epigenome-Wide Association Study (EWAS) is a genome-scale research approach used to identify the relationship between epigenetic variations, especially DNA methylation, and specific phenotypes or diseases. EWAS was developed because genetic and environmental factors alone cannot fully explain the variation in disease risk, making the epigenome a crucial biological link between the two. This approach works by comparing DNA methylation levels across the genome between two or more groups (for example, patients and controls) to identify CpG sites that exhibit differential methylation levels.[4,11]

The process involves bisulfite conversion to distinguish methylated from unmethylated cytosines, followed by measurement of

methylation signal intensity using microarray platforms such as Illumina 450K or EPIC. The resulting data are then analyzed to quantify the methylation level at each CpG site, expressed as a beta (β) value, which represents the ratio of methylated signal intensity to total signal intensity. A β value ≤ 0.25 indicates an unmethylated site, values between 0.25–0.75 are considered hemimethylated, and β ≥ 0.75 indicates a fully methylated site. These data are further analyzed to identify CpG positions or regions showing significant differences, which are then biologically interpreted through gene function or molecular pathway analysis. Today, EWAS is widely applied to identify diagnostic and prognostic biomarkers and to monitor therapeutic responses based on epigenetic changes.[4,11]

With the increasing number of EWAS-based studies, a centralized data resource known as The EWAS Catalog has now been established to integrate and compare findings across studies. This database compiles thousands of associations between DNA methylation and various phenotypes or diseases from diverse populations and is publicly accessible through a web portal and the R package ewascatalog. The catalog serves as a primary repository for researchers to review, analyze, and validate EWAS results in a more comprehensive and standardized manner.[13]

Application of EWAS in Breast Cancer Biomarker Discovery

With the rapid advancement of EWAS technologies and the increasing availability of public databases, this approach has been widely applied to identify DNA methylation patterns associated with breast cancer. Numerous studies have demonstrated how this comprehensive epigenomic analysis contributes to a deeper understanding of the molecular mechanisms of cancer, ranging from early risk assessment to prognosis and non-invasive detection

Table 1. Summary of EWAS studies investigating epigenetic biomarkers in breast cancer

No	Author(s) (Year)	Study Title	Key Findings
1.	van Veldhoven K et al. (2015)	Epigenome-Wide Association Study reveals decreased average DNA methylation levels years before breast cancer diagnosis	Global hypomethylation found in gene body and 3'UTR regions; higher methylation associated with reduced breast cancer risk, suggesting potential as an early blood-based biomarker.
2.	Johansson A & Flanagan JM (2017)	Epigenome-wide association studies for breast cancer risk and risk factors	EWAS revealed global hypomethylation and lifestyle-associated CpGs—AHRR, F2RL3 (smoking), HIF3A, PHGDH (BMI), ER-α, E-cadherin (alcohol)—with accelerated epigenetic aging increasing postmenopausal breast cancer risk.
3.	Johansson A et al. (2019)	Epigenome-Wide Association Study for lifetime estrogen exposure identifies an epigenetic signature associated with breast cancer risk	EWAS identified 694 CpGs associated with ELEE; a 31-CpG Methylation Index predicted breast cancer risk, with higher scores increasing risk (top quartile OR = 1.45). Key CpGs included CTNNA2, GRB10, RPH3AL, and TINCR.
4.	Ennour-Idrissi K et al. (2020)	DNA Methylation and Breast Cancer Risk: An Epigenome-Wide Study of Normal Breast Tissue and Blood	7,315 CpGs identified in normal breast tissue (52 Bonferroni-adjusted); key genes LHX2, TFAP2B, JAKMIP1, SEPT9, POM121L2, KCNQ1, CLEC4C. Pathways involved fatty acid metabolism and Wnt/β-catenin signaling; blood replication supported potential pre-diagnostic biomarkers.
5.	Wei S, Tao J, Xu J, Chen X, Wang Z, Zhang N, dkk. (2021)	Ten Years of EWAS	EWAS revealed CpGs associated with age, BMI, and hormone therapy use, including >800 age-related, 694 estrogen-exposure, and 527 hormone therapy–linked sites (ARHGEF4). Hypermethylation at cg46801642 and hypomethylation at cg27091787 (HYAL2) were both associated with elevated breast cancer risk.
6.	Massi MC et al. (2022)	A Deep Survival EWAS approach estimating risk profile based on pre-diagnostic DNA methylation: An application to breast cancer time to diagnosis	Deep Survival EWAS identified the F120 CpG island cluster (20 sites) linked to time-to-diagnosis; higher methylation correlated with earlier onset. GSEA showed enrichment in cancer-related, PI3K/Akt/mTOR, and calcium signaling pathways. AI-based model outperformed conventional EWAS in detecting biologically relevant pathways.
7.	Kim et al. (2023)	DNA methylation patterns associated with breast cancer prognosis that are specific to tumor subtype and menopausal status	Fifteen significant CpGs were linked to patient survival, differing by molecular subtype and menopausal status. Key CpGs (DVL1, SH3PXD2A, ESYT2) were enriched in Wnt and insulin signaling pathways.
8.	Corsaro L et al. (2023)	Notch, SUMOylation, and ESR-Mediated Signalling Are the Main Molecular Pathways Showing Significantly Different Epimutation Scores between Expressing or Not Oestrogen Receptor Breast Cancer in Three Public EWAS Datasets	Significant subtype differences in promoter epimutation scores were observed. ER-positive tumors exhibited hypomethylation in estrogen signaling pathways, whereas ER-negative tumors showed hypermethylation in Notch and SUMOylation pathways, reflecting distinct epigenetic regulation involving estrogen signaling and EMT.
9.	Hillary RF et al. (2023)	Blood-based epigenome-wide analyses of 19 common disease states: A longitudinal, population-based linked cohort study of 18,413 Scottish individuals	100 CpGs identified across common diseases, including breast cancer. Hypomethylation at cg06072257 (UBIAD1) and cg06123699 (TPRG1) associated with breast cancer risk; pathways involved vitamin K metabolism and estrogen regulation. Blood methylation showed potential as a non-invasive biomarker.
10.	Herzog CMS et al. (2025)	Systems epigenetic approach towards non-invasive breast cancer detection	Cervical and buccal samples showed 21,614 and 585 significant CpGs (FDR < 0.05), while blood showed none. Methylation-based classifiers (WID-buccal, WID-cervical, WID-blood) achieved AUCs of 0.75, 0.66, and 0.51, respectively, with buccal methylation most closely reflecting breast tissue (AUC > 0.88), highlighting its potential for non-invasive detection.

Based on the findings summarized in Table 1, it can be observed that the Epigenome-Wide Association Study (EWAS) approach has made a substantial contribution to understanding the dynamics of DNA methylation associated with the risk, progression, and potential early detection of breast cancer. In general, these studies reveal that alterations in DNA methylation patterns do not occur solely at advanced stages of cancer but can also be detected long before the onset of clinical manifestations, even among individuals who appear to be healthy.

Furthermore, the studies included in Table 1 vary considerably in their objectives, sample sources, and analytical approaches. While some investigations focus on identifying risk-associated methylation signatures detectable in peripheral blood prior to diagnosis, others emphasize tumor-specific alterations related to prognosis, molecular subtypes, or treatment response. This diversity reflects the multifaceted role of EWAS in breast cancer research but also complicates efforts to establish universally applicable biomarkers.

One of the pioneering studies conducted by van Veldhoven et al. in 2015 represents an important milestone in breast cancer epigenetic research, as it demonstrated that the average level of DNA methylation in peripheral blood was lower in women who subsequently developed breast cancer compared to the control group. The observed global hypomethylation of approximately 0.2%, predominantly occurring within gene bodies and 3'UTRs, indicates a broad epigenetic deregulation that may reflect genomic instability during the early stages of carcinogenesis. Interestingly, higher levels of methylation were associated with a reduced risk of cancer (OR = 0.61; p = 0.0004), suggesting that this hypomethylation pattern has potential as an early risk biomarker. This finding marked a shift in research focus from single-gene analysis toward comprehensive epigenome-wide investigations.[14]

These findings were subsequently expanded by Johansson and Flanagan in 2017, who confirmed that global hypomethylation patterns were consistently observed across six large prospective studies. They highlighted that these epigenetic alterations are strongly influenced by lifestyle and environmental factors such as smoking, obesity, and alcohol consumption, with specific methylation markers including AHRR (cg05575921), F2RL3, HIF3A, and PHGDH. In addition, they introduced the concept of epigenetic age acceleration, demonstrating that an accelerated "epigenetic aging" process is associated with an increased risk of cancer, particularly among postmenopausal women. This study reinforced the notion that DNA methylation profiles also serve as cumulative reflections of environmental exposures and an individual's biological status.[15]

Following this line of research, Johansson et al. (2019) explored the relationship between lifetime estrogen exposure (ELEE) and breast cancer risk through a multi-cohort EWAS approach. By analyzing more than 694 significant CpG sites, they developed a Methylation Index (MI) based on 31 CpGs that was able to predict breast cancer risk with a 1.4–1.5-fold increase. Key contributing genes included CTNNA2, GRB10, RPH3AL, and TINCR, which are known to be involved in the regulation of cell growth and differentiation. These findings further support the concept that blood DNA methylation profiles can serve as an "epigenetic record" of lifelong hormonal exposure, rather than merely representing a consequence of disease.[16]

A study focusing on normal breast tissue was conducted by Ennour-Idrissi et al. (2020), aiming to identify pre-neoplastic methylation changes. They reported 7,315 significant CpG sites, of which 52 remained significant after Bonferroni correction. Genes such as LHX2, TFAP2B, JAKMIP1, SEPT9, and KCNQ1 were implicated in fatty acid metabolism and Wnt/β-catenin signaling pathways, which represent two key mechanisms involved in cellular proliferation and differentiation. These results strengthen the hypothesis that epigenetic alterations may precede neoplastic transformation, suggesting that even histologically normal tissue may harbor "early epigenetic marks" reflecting a predisposition to breast cancer and offering potential utility as early screening biomarkers.[17]

In line with these findings, Wei et al. (2021), through their systematic review "Ten Years of EWAS," highlighted more than 800 age-associated CpG sites and 694 CpG sites influenced by estrogen exposure, including ARHGEF4 (cg01382688) and HYAL2 (cg27091787). This cross-study analysis emphasized that aging and hormonal exposure are two major biological factors that leave measurable epigenetic imprints, thereby establishing epigenetic biomarkers as one of the most promising approaches for early detection and risk prediction of breast cancer.[4]

A more advanced computational approach was implemented by Massi et al. (2022), who introduced the Deep Survival EWAS model to predict time-to-diagnosis based on pre-diagnostic blood DNA methylation profiles. By applying survival neural networks and SHAP analysis, they identified a CpG island cluster, F120, characterized by high methylation levels that correlated with earlier diagnosis and were functionally linked to the PI3K/Akt/mTOR and calcium signaling pathways. This study opened a new direction in the application of artificial intelligence and machine learning for the interpretation of complex methylation data, while also demonstrating the predictive potential of epigenetic biomarkers for disease onset timing.[18]

From a prognostic perspective, Kim et al. (2023) identified 15 significant CpG sites in tumor tissues that were associated with patient survival, particularly among Luminal A subtypes and postmenopausal women. Most of these CpG sites, such as those located in DVL1, ESYT2, and SH3PXD2A, showed that higher methylation levels were correlated with lower risks of recurrence and mortality, indicating a more favorable prognosis. Conversely, CpG sites within MZF1 and ELAC1 exhibited the opposite trend (HR > 1), where hypermethylation was associated with an increased risk of disease progression, reflecting a poorer prognosis. These findings highlight that DNA methylation patterns can serve as prognostic biomarkers, as methylation levels at specific sites have the potential to predict clinical outcomes and therapeutic responses in breast cancer patients.[19]

Meanwhile, a comparative analysis was conducted by Corsaro et al. (2023) to distinguish the epigenetic profiles between breast cancer subtypes, namely ER-positive and ER-negative. The ER-positive subtype exhibited hypomethylation in ESR-mediated signaling pathways, whereas the ER-negative subtype displayed hypermethylation in Notch and SUMOylation pathways, which are associated with cellular differentiation and drug resistance. These findings reinforce the notion that the molecular heterogeneity of breast cancer is largely reflected in its DNA methylation patterns, suggesting that the EWAS approach can facilitate more precise subtype characterization.[20]

In a large population-based study, Hillary et al. (2023) analyzed blood methylation profiles from more than 18,000 individuals and identified two novel sites, cg06072257 (UBIAD1) and cg06123699 (TPRG1), which were associated with a history of breast cancer. The UBIAD1 gene is involved in vitamin K metabolism, whereas TPRG1 is regulated by estrogen signaling; both play roles in cellular proliferation and homeostasis. These findings suggest that blood DNA methylation alterations may reflect breast cancer risk at a systemic level rather than being limited to tumor tissue, thereby offering potential as non-invasive risk biomarkers for early detection. [21]

Furthermore, the study conducted by Herzog et al. (2025) represents the most recent advancement in this field by evaluating the potential of non-invasive detection through EWAS using cervical, buccal, and blood samples. Thousands of significant CpG sites were identified, with the highest classification performance observed in the buccal WID-index (AUC 0.75–0.88). These findings confirm that peripheral epithelial tissues, such as buccal mucosa, can reflect systemic epigenetic alterations, thereby opening new opportunities for the development of non-invasive screening tests for the early detection of breast cancer.[22]

Overall, these studies illustrate the evolution of EWAS applications from population-based risk analyses toward predictive modeling and clinical implementation. EWAS not only enables the identification of specific methylation signatures associated with genetic, hormonal, and environmental factors but also facilitates the development of multidimensional biomarkers encompassing diagnosis, prognosis, and non-invasive detection. With the expanding integration of multi-omics data and advances in analytical technologies, the EWAS approach is increasingly recognized as a key pillar in the pursuit of precise epigenetic biomarkers for breast cancer, offering great potential for the development of non-invasive screening tests for early detection.

These anticipated clinical implications are consistent with the perspective presented by Skinner (2024), who emphasized that EWAS findings hold great potential to transform medical practice from a reactive approach toward preventive precision medicine. DNA methylation profiles identified through EWAS can serve as risk-indicating biomarkers, enabling early screening and personalized intervention before the onset of clinical symptoms. In the context of breast cancer, this opens opportunities for utilizing specific methylation signatures to identify high-risk groups and to establish more targeted strategies for prevention and disease control.[23]

This perspective is further supported by Sahoo and Sundararajan (2022), who highlighted that EWAS has now evolved into an essential tool for diagnosis, prognosis, and therapeutic monitoring. Methylation profiles of genes such as BRCA1 and SEPT9 demonstrate clinical potential for non-invasive detection and monitoring of treatment response. Furthermore, they emphasized the importance of standardizing analytical workflows, integrating multi-omics data, and applying machine learning approaches to enhance the accuracy and reproducibility of EWAS findings.[12]

Overall, despite the growing number of EWAS studies investigating breast cancer, several gaps remain in the current body of evidence. Findings across studies are often heterogeneous due to differences in study populations, sample sources (blood versus tissue), analytical platforms, and statistical approaches. While some studies report global hypomethylation associated with cancer risk, others identify site-specific hypermethylation patterns linked to prognosis or molecular subtypes. Moreover, many studies rely on cross-sectional designs and relatively small sample sizes, limiting causal inference and generalizability. These inconsistencies highlight the need for large-scale longitudinal studies, standardized analytical pipelines, and cross-population validation to establish robust and clinically applicable epigenetic biomarkers.

Challenges and Limitations of EWAS Research

Although EWAS has opened vast opportunities for the discovery of epigenetic biomarkers, several challenges still limit its broad application. Most current EWAS studies employ cross-sectional designs, making it difficult to distinguish whether DNA methylation changes are a cause or a consequence of disease (reverse causation). Moreover, cellular heterogeneity within tissues such as blood often results in methylation signals that reflect changes in cell composition rather than true epigenetic alterations. In addition, the effect sizes of methylation changes are generally small and highly influenced by genetic, environmental, and technical batch effects; therefore, EWAS findings should be interpreted carefully.[24]

From a methodological perspective, it is also necessary to standardize analytical pipelines and increase sample sizes to improve the accuracy and reliability of EWAS results. Tissue heterogeneity, batch effects, and the lack of cross-population replication remain major challenges. Furthermore, integration of multi-omics data and longitudinal analyses is needed to better understand the temporal dynamics of DNA methylation while minimizing bias arising from environmental factors.[4]

From a technical standpoint, EWAS based on cell-free DNA still faces several challenges due to the limited quantity of DNA, high fragmentation, and contamination that reduce detection sensitivity. In addition, the lack of pre-analytical standardization, degradation of genetic material, and insufficient inter-laboratory clinical validation remain major barriers to the successful translation of EWAS findings into medical practice.[25,26]

Overall, the future success of EWAS will depend on the integration of multi-omics data, cross-population clinical validation, and the harmonization of international protocols to ensure that the resulting epigenetic biomarkers can be accurately implemented and feasibly applied in routine clinical practice.

CONCLUSION

Advances in microarray technology and the increasing standardization of bioinformatics algorithms have established EWAS as a primary method for exploring epigenetic biomarkers. This approach deepens the understanding of interactions among genetic, hormonal, and environmental factors, and also opens opportunities for the implementation of predictive and preventive precision medicine.

The Epigenome-Wide Association Study (EWAS) approach has made a significant contribution to understanding the epigenetic basis of breast cancer. Through genome-scale DNA methylation analysis, EWAS has been able to identify epigenetic patterns associated with risk, prognosis, and the potential for non-invasive detection of the disease. Findings from various studies discussed also

indicate that DNA methylation changes do not occur solely at advanced stages of cancer but may emerge years before clinical diagnosis, thereby providing strong predictive value.

With the expanding integration of multi-dimensional data and the support of modern computational analyses, EWAS findings are expected to be translated into clinical practice in the form of early screening, individualized risk assessment, and more personalized and accurate therapeutic monitoring.

This review has several limitations. The literature specifically addressing the application of EWAS for the discovery of breast cancer biomarkers remains limited, resulting in a predominantly descriptive analysis. In addition, differences in terminology and methodology across studies make it challenging to present the findings in a uniform manner. Nevertheless, this review provides a comprehensive overview of the direction and potential development of EWAS in the field of early breast cancer detection.

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