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Review of DNA Methylation in Early Detection of Breast Cancer

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Abstract Breast cancer remains one of the leading causes of death for women worldwide, underscoring the importance of early detection. As one of the key epigenetic mechanisms, DNA methylation provides a promising marker for early breast cancer diagnosis. Various techniques for DNA methylation, including disulfiram sequencing, methylation-specific PCR, pyrosequencing, and microarrays, provide insights into the epigenetic changes that drive tumorigenesis. This study synthesizes the current knowledge on the clinical application of DNA methylation markers in non-invasive early detection, discusses challenges including variability in methylation patterns and technical limitations, and the clinical and ethical considerations that affect the implementation of these technologies. This study aims to uncover specific methylation patterns and their epigenetic changes in breast carcinogenesis, thereby exploring and validating new biomarkers. Improve the early detection rate of breast cancer and the quality of life of patients.

Keywords DNA methylation; Breast cancer; Early detection; Epigenetic biomarkers; Non-Invasive diagnosis

1 Introduction

Breast cancer remains one of the most prevalent and deadly cancers among women worldwide, with early detection being crucial for improving patient outcomes and survival rates. Traditional screening methods, such as mammography, have significantly reduced breast cancer mortality, particularly in women over the age of 50. However, these methods have limitations, including reduced sensitivity in certain populations and high false-positive rates, which can lead to unnecessary biopsies and anxiety (Brooks et al., 2009; Shan et al., 2016; Zhang et al., 2023). Therefore, there is a pressing need for more accurate, minimally invasive, and cost-effective screening techniques that can be used alongside existing methods to enhance early detection.

DNA methylation, an epigenetic modification involving the addition of a methyl group to the DNA molecule, plays a critical role in gene regulation and has been implicated in the early stages of carcinogenesis. Aberrant DNA methylation patterns, particularly the hypermethylation of tumor suppressor gene promoters, are common in various cancers, including breast cancer. These methylation changes can be detected in circulating cell-free DNA (cfDNA) in the blood, making them promising biomarkers for non-invasive cancer detection (Nunes et al., 2018; Constâncio et al., 2020; Liu et al., 2020; Liu et al.,2021). Recent advances in high-throughput sequencing and methylation-specific PCR techniques have enabled the development of sensitive and specific assays to detect these epigenetic alterations, offering a potential complementary tool to traditional imaging methods (Li et al., 2020; Roy et al., 2020).

This study evaluates the current status of DNA methylation as a biomarker for early detection of breast cancer. We provide a comprehensive overview of the methods used, the sensitivity and specificity of the various methylation markers, and the potential clinical applications of these findings. By synthesizing the available evidence, this study highlight the promise of DNA methylation-based assays in improving early breast cancer detection and identify areas where further research is needed to facilitate their integration into clinical practice.

2 Technologies for Detecting DNA Methylation

2.1 Bisulfite sequencing

Bisulfite sequencing is a widely used method for detecting DNA methylation. This technique involves treating DNA with sodium bisulfite, which converts unmethylated cytosines to uracil while leaving methylated cytosines

unchanged. The treated DNA is then sequenced to determine the methylation status of cytosines. Bisulfite sequencing is considered the "gold standard" for DNA methylation analysis due to its high sensitivity and specificity (Reed et al., 2010). It has been effectively used in various studies, including the detection of methylation patterns in circulating cell-free DNA (cfDNA) for early cancer detection (Li et al., 2016; Liu et al., 2020). Whole-genome bisulfite sequencing (WGBS) has also been employed to study methylation heterogeneity in metastatic breast cancer, providing insights into intra-tumor heterogeneity (Luo et al., 2023).

2.2 Methylation-Specific PCR (MSP)

Methylation-Specific PCR (MSP) is a PCR-based technique that allows for the rapid assessment of the methylation status of specific CpG sites. This method involves the initial modification of DNA by sodium bisulfite, followed by PCR amplification using primers specific for either methylated or unmethylated DNA. MSP is highly sensitive, capable of detecting as low as 0.1% methylated alleles, and can be performed on small quantities of DNA, including those extracted from paraffin-embedded samples. Variants of MSP, such as SMART-MSP, incorporate high-resolution melting (HRM) analysis to provide quantitative methylation detection and to distinguish between homogeneous and heterogeneous methylation (Kristensen et al., 2007).

2.3 Pyrosequencing

Pyrosequencing is another method used to analyze DNA methylation. It involves sequencing by synthesis, where the incorporation of nucleotides is detected in real-time by the release of pyrophosphate. This method is advantageous for its ability to provide quantitative data on methylation levels at specific CpG sites. Pyrosequencing has been compared with bisulfite sequencing PCR (BSP) and found to be equally effective in detecting hypomethylation and mixed methylation, although BSP may be more sensitive for detecting strong hypermethylation (Kristensen and Hansen, 2019). Massively parallel bisulfite pyrosequencing has been used to reveal the molecular complexity of breast cancer-associated cytosine-methylation patterns in both tissue and serum DNA (Korshunova et al., 2007).

2.4 Microarrays

Microarrays are used for high-throughput analysis of DNA methylation across the genome. This technology involves hybridizing bisulfite-treated DNA to probes on a microarray chip, allowing for the detection of methylation at thousands of CpG sites simultaneously. Microarrays have been employed to evaluate the methylation levels of candidate genes in plasma cfDNA for breast cancer early detection, demonstrating the feasibility of using this method for non-invasive cancer diagnostics (Li et al., 2016).

2.5 Comparative analysis with methods

Each of these technologies has its own strengths and limitations. Bisulfite sequencing is highly sensitive and specific but can be labor-intensive and costly, especially for whole-genome applications (Reed et al., 2010; Luo et al., 20023). MSP is rapid and highly sensitive but is limited to the analysis of specific CpG sites and may produce false positives if not carefully controlled (Giridhar et al., 2023). Pyrosequencing provides quantitative data and is less labor-intensive than bisulfite sequencing but may be less sensitive for detecting strong hypermethylation. Microarrays offer high-throughput capabilities but may lack the single-base resolution provided by sequencing methods.

3 Advances in Understanding DNA Methylation in Breast Cancer

3.1 Methylation patterns in breast cancer

Recent studies have significantly advanced our understanding of DNA methylation patterns in breast cancer. Massively parallel bisulphite pyrosequencing has revealed the molecular complexity of cytosine-methylation patterns in both tissue and serum DNA from breast cancer patients. This comprehensive analysis demonstrated that tumor samples exhibit more variation in methylation levels compared to normal samples, highlighting the potential of these patterns as biomarkers for early detection (Korshunova et al., 2007). Additionally, genome-wide DNA methylation profiling has identified numerous hypermethylated loci/genes in breast tumors, which are associated with clinical features such as estrogen receptor and progesterone receptor status, tumor relapse, and lymph node metastasis (Hill et al., 2011). High-throughput MALDI-TOF mass array analysis has further identified

specific hypermethylated genes that distinguish between cancerous and normal tissues, suggesting their utility as biomarkers for clinical diagnosis and targeted treatments (Radpour et ala., 2019).

3.2 Epigenetic changes and tumorigenesis

Epigenetic changes, particularly DNA methylation, play a crucial role in the onset and progression of breast cancer. Abnormal DNA methylation is implicated in tumorigenesis by regulating key processes such as cell proliferation, apoptosis, differentiation, and cell cycle control (Pan et al., 2018). Studies have shown that global DNA hypomethylation and higher epigenetic age are associated with an increased risk of breast cancer, indicating that these epigenetic markers could serve as short-term predictors of breast cancer risk (Ennour-Idrissi et al., 2020). Furthermore, the identification of differentially methylated genes across various cancers has provided insights into cancer-specific methylation patterns, which could be used to develop individualized treatment strategies (Zhang et al., 2015).

3.3 Biomarker discovery and validation

The discovery and validation of DNA methylation biomarkers for breast cancer have been a focal point of recent research. Whole-blood DNA methylation markers have been suggested as potential biomarkers for early detection, although their diagnostic value remains modest, with only a few markers showing significant sensitivity and specificity (Guan et al., 2018). A systematic review and meta-analysis have identified common DNA methylation signatures across different breast cancer subtypes, reflecting deregulation of the immune system and alterations to the cell cycle, which could support the identification of novel biomarkers and therapeutic targets (Trasierras-Fresco et al., 2023). Additionally, the automatic detection of circulating cell-free methylated DNA patterns, such as those of CCDC181, GCM2, and ITPRIPL1, has shown promise in improving the accuracy of early breast cancer detection and monitoring surgical treatment responses (Wang et al., 2021).

4 Clinical Applications of DNA Methylation in Early Detection

4.1 Biomarkers for early detection

DNA methylation-based biomarkers have shown significant promise in the early detection of breast cancer. Aberrant DNA methylation is an early event in cancer development and can be detected in circulating cell-free DNA (cfDNA), making it a valuable biomarker for cancer detection and prognosis (Cheuk et al., 2017; Constâncio et al., 2020). Studies have identified specific methylation markers, such as RASSF1A and HOXA10, which show significant differences in methylation between breast cancer patients and healthy controls, enhancing the positive predictive value for breast cancer detection. Additionally, the use of multi-gene panels rather than single-gene methylation status has been suggested to increase the sensitivity and specificity of breast cancer screening.

4.2 Non-Invasive detection methods

Non-invasive methods for detecting DNA methylation involve analyzing cfDNA from blood samples, which offers a less invasive alternative to traditional biopsy methods. Liquid biopsies, particularly the analysis of cfDNA, have emerged as a promising approach for the non-invasive diagnosis of early-stage cancers (Figure 1) (Luo et al., 2021). Whole-genome bisulfite sequencing and methylation-specific PCR are among the techniques used to detect methylation markers in cfDNA, providing a stable and quantifiable means of early cancer detection (Constâncio et al., 2020; Liu et al., 2021). The combination of liquid biopsy with traditional diagnostic imaging has been shown to improve diagnostic accuracy and reduce false-positive rates, thereby avoiding unnecessary biopsies (Roy and Tiirikainen, 2020; Zhang et al., 2023).

The CCGA study demonstrated the clinical potential of DNA methylation analysis by using cfDNA methylation analysis for early multi-cancer detection. By examining fragment-level methylation patterns, the test is able to distinguish cancer from non-cancer based on methylation signatures unique to cancer cells. For non-cancer participants, cfDNA was derived from cells throughout the body, including leukocytes, and their methylation markers reflected the characteristics of blast cells. As shown in the example of chromosome 10 region in the figure, most of the cfDNA fragments appear turquoise, indicating that they are predominantly unmethylated. In lung cancer patients, plasma contains a mixture of methylated (burgundy) and unmethylated (turquoise) cfDNA

fragments, reflecting that circulating cfDNA is a mixture of tumor cfDNA and cfDNA from other cells in the body. Sequencing of tissue samples confirmed that this region was almost completely methylated. This approach utilizes an extensive database to identify genomic regions that reliably indicate the presence of cancer, aiding in early diagnosis and potentially improving patient outcomes. The use of cfDNA methylation not only provides a non-invasive diagnostic tool, but also enhances our understanding of cancer biology at the molecular level.

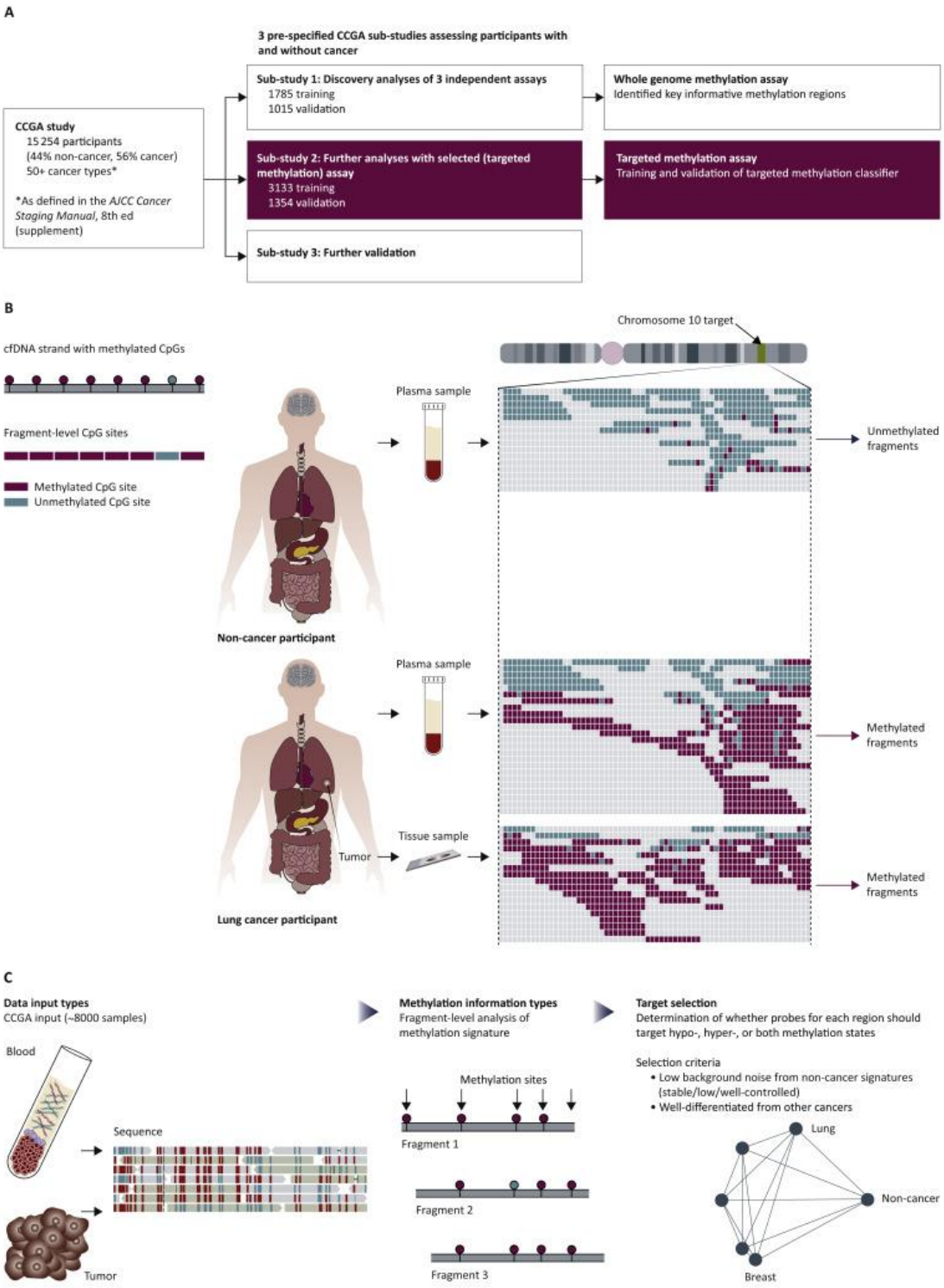

Figure 1 The CCGA study for development and validation of a cfDNA-based assay for multi-cancer detection (Adopted from Liu et al., 2020)

Image caption: (A) CCGA study design. (B) Methylation biology discriminates cancer from non-cancer. (C) Target selection (Adopted from Liu et al., 2020)

4.3 Comparative effectiveness with traditional methods

Comparative studies have demonstrated that DNA methylation-based detection methods can be more effective than traditional imaging techniques alone. For instance, the integration of methylation markers with breast ultrasound has been shown to significantly improve the accuracy of early breast cancer diagnosis, particularly in patients with indeterminate breast nodules categorized under BI-RADS. This combined approach has been found to enhance diagnostic accuracy and specificity, reducing the rate of unnecessary biopsies and surgeries (Guan et al., 2018). Furthermore, computational models using DNA methylation data have been developed to predict breast cancer invasiveness (Figure 2), offering a potential tool for guiding clinical decision-making and improving patient outcomes (Wang et al., 2020).

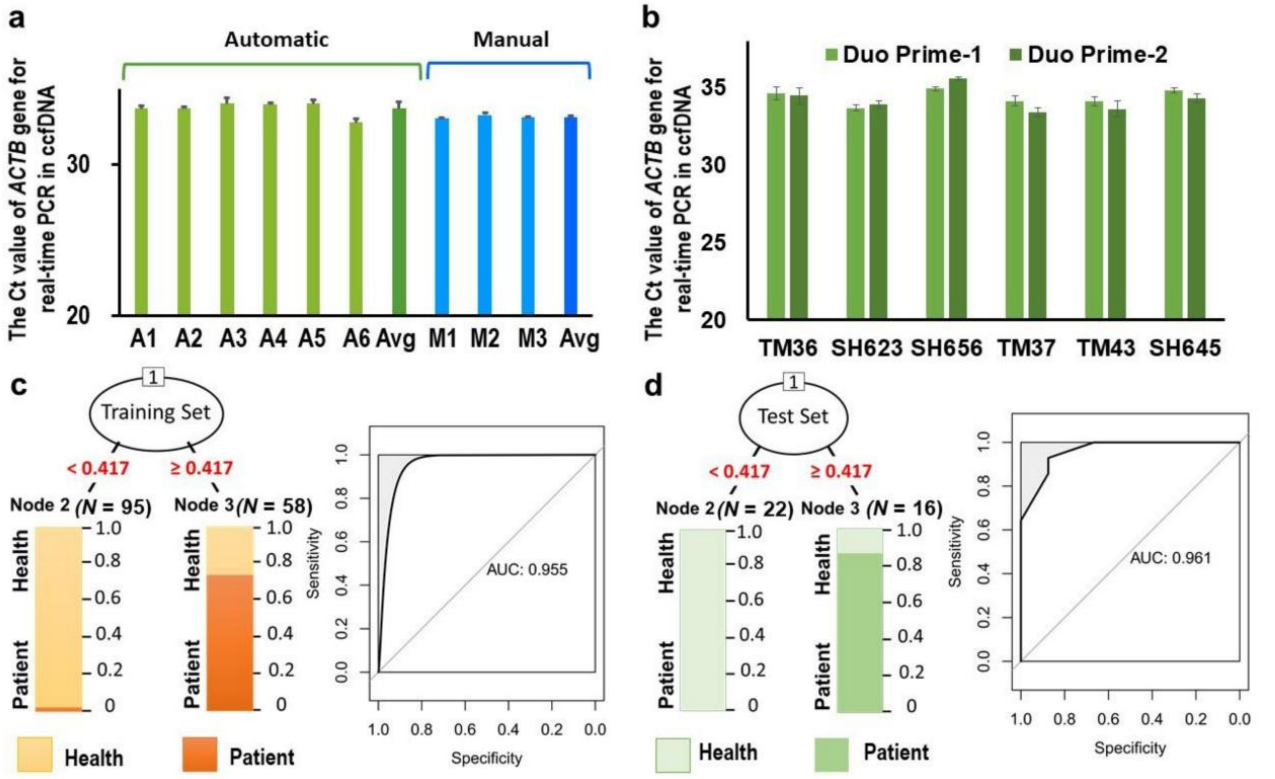

Figure 2 Stability and clinical validation of the automatic detection process for circulating methylated ccfDNA (Adopted from Wang et al., 2021)

Image caption: (a) PCR Ct values of ACTB gene relative to ccfDNA methylation levels were used to compare the repeatability of automatic and manual processes. (b) PCR Ct values using the relative ccfDNA methylation levels of the ACTB gene. (c) Decision tree model and ROC curve for breast cancer prediction in recursive partitioning and regression tree training sets. (d) Decision tree model and ROC curve for predicting breast cancer in the recursive partitioning and regression tree test set (Adopted from Wang et al., 2021)

The study by Wang et al. (2021) demonstrated the clinical application of DNA methylation in early detection through automated ccfDNA testing. The graph shows a comparison of Ct values between automated and manual processes (a) and between machines (b), showing the consistency and accuracy of the replication, indicating a high degree of reliability. The decision tree model and ROC curves (c and d) demonstrated high sensitivity and specificity in breast cancer prediction, highlighting the potential of DNA methylation in early cancer detection, providing a pathway for non-invasive and efficient diagnosis.

DNA methylation biomarkers hold great potential for the early detection of breast cancer. Non-invasive detection methods, such as liquid biopsies, provide a promising alternative to traditional diagnostic techniques, with the added benefit of reducing unnecessary invasive procedures. Comparative studies indicate that integrating DNA methylation analysis with traditional methods can enhance diagnostic accuracy and improve clinical outcomes for breast cancer patients.

5 Current Challenges and Limitations

5.1 Variability in methylation patterns

One of the primary challenges in utilizing DNA methylation for early detection of breast cancer is the significant variability in methylation patterns across different studies and populations. For instance, while some studies have identified specific methylation markers with potential diagnostic value, the reproducibility of these markers remains inconsistent. In a systematic review, it was noted that although 276 CpG sites were identified in two prospective studies, there was no overlap between the CpGs reported, highlighting the variability in findings (Widschwendter et al., 2017). Additionally, the complexity of methylation patterns in both tissue-derived and circulating DNA further complicates the development of reliable biomarkers, as demonstrated by the extensive variation observed in methylation levels across different samples (Ennour-Idrissi et al., 2020).

5.2 Technical and methodological limitations

The detection and analysis of DNA methylation patterns are fraught with technical and methodological challenges. High-throughput methods such as bisulfite sequencing and methylation arrays, while powerful, require rigorous standardization and validation to ensure accuracy and reproducibility. For example, the use of massively parallel bisulphite pyrosequencing revealed a high degree of molecular complexity in methylation patterns, which poses a significant hurdle for the development of clinical tests (Liu et al., 2020). Moreover, the sensitivity and specificity of methylation-based assays can vary widely. A study on a six-gene methylation panel achieved sensitivities of 79.6% and 82.4% with specificities of 72.4% and 78.1%, respectively, indicating room for improvement in assay performance (Shan et al., 2016). Furthermore, the lack of large-scale validation studies limits the clinical applicability of these biomarkers (Constâncio et al., 2020).

5.3 Clinical and ethical considerations

The clinical implementation of DNA methylation markers for early breast cancer detection also raises several ethical and practical concerns. Overdiagnosis is a significant issue, particularly in breast cancer screening, where the detection of indolent tumors that may not progress to clinical significance can lead to unnecessary treatments and patient anxiety (Wittenberger et al., 2014). Additionally, the integration of methylation-based tests into existing screening programs requires careful consideration of cost-effectiveness and accessibility, especially in resource-limited settings where traditional screening methods like mammography may already be challenging to implement (Stastny et al., 2020). Ethical considerations also extend to the management of incidental findings and the potential psychological impact on patients who test positive for methylation markers but do not have detectable tumors on imaging (Wang et al., 2021).

In conclusion, while DNA methylation holds promise for the early detection of breast cancer, significant challenges related to variability in methylation patterns, technical limitations, and clinical and ethical considerations must be addressed to realize its full potential. Future research should focus on standardizing methodologies, validating biomarkers in large, diverse cohorts, and carefully evaluating the clinical and ethical implications of implementing these tests in practice.

6 Future Directions

6.1 Advances in detection technologies

The future of breast cancer detection lies in the continuous advancement of detection technologies, particularly those focusing on DNA methylation. Recent studies have highlighted the potential of methylated circulating cell-free DNA (cfDNA) as a non-invasive biomarker for early breast cancer detection. Techniques such as whole-genome bisulfite sequencing and methylation-specific PCR have shown promise in improving the sensitivity and specificity of breast cancer screening (Cheuk et al., 2017; Constâncio et al., 2020; Liu et al., 2021). Additionally, the development of high-throughput methods for methylation quantification and the integration of advanced computational frameworks to identify optimal methylation markers are paving the way for more accurate and early diagnosis (Wang et al., 2021). Future research should focus on refining these technologies and validating them through large-scale, prospective clinical trials to ensure their efficacy and reliability in clinical settings (Tzanikou and Lianidou, 2020).

6.2 Personalized medicine and tailored screening

Personalized medicine is becoming increasingly important in the management of breast cancer. The heterogeneity of breast cancer necessitates tailored screening and treatment approaches. DNA methylation markers offer a promising avenue for personalized screening strategies. By identifying specific methylation patterns associated with different subtypes of breast cancer, it is possible to develop individualized screening protocols that can detect cancer at its earliest stages (Wittenberger et al., 2014). Moreover, the integration of methylation markers with genetic and environmental risk factors can enhance risk stratification and enable more precise monitoring of disease progression and treatment response (Hoque et al., 2006; Guan et al., 2018). Future research should aim to identify and validate additional methylation markers that can be used in combination with existing screening methods to improve the accuracy and personalization of breast cancer detection (Suijkerbuijk et al., 2021).

6.3 Integration with other biomarkers and imaging techniques

The integration of DNA methylation markers with other biomarkers and imaging techniques holds significant potential for improving breast cancer detection. Combining methylation analysis with traditional imaging methods, such as mammography and ultrasound, can enhance the accuracy of early-stage breast cancer diagnosis and reduce false-positive rates (Liu et al., 2021). Additionally, the use of multi-gene methylation panels can increase the sensitivity and specificity of screening, providing a more comprehensive assessment of cancer risk. Future studies should explore the synergistic effects of combining methylation markers with other molecular and imaging biomarkers to develop robust, multi-modal screening approaches that can be implemented in clinical practice (Roy and Tiirikainen, 2020). This integrated approach has the potential to revolutionize breast cancer screening, leading to earlier detection, better prognosis, and improved patient outcomes.

7 Concluding Remarks

The systematic review of DNA methylation in early detection of breast cancer has highlighted several key findings. Whole-blood DNA methylation markers have shown potential as biomarkers, but their diagnostic value remains modest, with only a few markers like HYAL2 and S100P being independently validated. Combining breast ultrasound with methylation markers in circulating tumor DNA has improved diagnostic accuracy, particularly in younger women and those with indeterminate nodules. Studies have also demonstrated that panels of methylated genes in plasma can detect early-stage breast cancer with varying degrees of sensitivity and specificity. Epigenome-wide association studies have identified numerous CpG sites related to breast cancer risk, although there is little overlap between studies. Additionally, cell-free DNA methylation assays have shown promise in distinguishing between benign and malignant breast lesions, potentially reducing unnecessary biopsies.

The findings from this study suggest that DNA methylation markers could significantly enhance early detection of breast cancer, leading to better patient outcomes. The integration of methylation markers with traditional imaging techniques like ultrasound can improve diagnostic accuracy and reduce false positives, thereby minimizing unnecessary invasive procedures. Early detection through non-invasive methods such as cell-free DNA methylation assays could lead to earlier interventions, potentially improving survival rates and reducing the burden of advanced disease. Moreover, the ability to predict metastatic potential through methylation markers could aid in patient surveillance and personalized treatment planning.

Future research should focus on the validation of promising methylation markers and the development of robust, multi-marker panels that can be reliably used in clinical settings. High-throughput methods and large-scale prospective studies are needed to identify and validate new markers with higher sensitivity and specificity. Additionally, integrating methylation data with other omics data and clinical parameters could enhance the predictive power of these biomarkers. Clinical practice could benefit from the adoption of methylation-based assays as complementary tools to existing screening methods, potentially leading to more accurate and less invasive breast cancer detection strategies. Further exploration into the role of DNA methylation in different subtypes of breast cancer and its relationship with other genetic and environmental factors will also be crucial for advancing personalized medicine in oncology.

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Conflict of Interest Disclosure

The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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