

Investigation of DNMT-mediated DNA methylation and its role in adipogenesis and breast cancer

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Abstract

DNA methylation, which is mediated by DNMTs, plays crucial roles in regulating gene expression and cell differentiation. In this study, we identified adipogenesis-related genes and analyzed their coexpression with DNMT isoforms in breast cancer samples from the TCGA dataset. Our findings revealed that 114 genes were coexpressed with DNMTs, among which six genes, GATA3, IRS1, LPIN1, ME3, SREBF1, and STAT1, were significantly negatively correlated with methylation and expression levels, as determined using Spearman correlation with false discovery rate correction to account for multiple testing. The differential expression patterns of these genes across breast cancer subtypes and their associations with survival outcomes were examined. Specifically, ME3 and STAT1 showed distinct associations with survival outcomes, where high ME3 expression correlated with significantly better survival rates, whereas low STAT1 expression was associated with improved prognosis. ME3 expression was significantly elevated in tumors with high adipocyte enrichment, particularly in the luminal B subtype, suggesting a subtype-specific relationship between adipogenesis and tumor behavior. Conversely, STAT1 exhibited lower expression in samples with high adipocyte counts, reinforcing its role in the tumor microenvironment. These results underscore the importance of DNMT-mediated DNA methylation in adipogenesis and breast cancer.

Keywords: DNA methylation; DNA methyltransferase; adipogenesis; obesity-induced breast cancer; epigenetics; ME3; tumor microenvironment; STAT1; CpG islands; molecular subtypes.

1. Introduction

Adipogenesis is an intricately orchestrated process that plays a pivotal role in transforming mesenchymal stem cells (MSCs) into mature, lipid-laden adipocytes. Studies have demonstrated the substantial influence of epigenetic mechanisms on gene expression during adipogenesis [1]. Epigenetic modifications are genome-related modifications that do not alter the fundamental DNA sequence and are therefore useful biomarkers that can capture the outcomes of genetic and environmental effects in detail [2]. Among these mechanisms, DNA methylation is well characterized and is functionally relevant in adipogenic regulation. It involves the transfer of methyl groups to cytosine substrates in CpG from the S-adenosyl methionine cofactor, which is catalyzed by DNA methyltransferase (DNMT) [3]. The DNMT family includes DNMT1, which primarily maintains methylation

patterns during DNA replication, and DNMT3A and DNMT3B, which are responsible for establishing de novo methylation during development and differentiation [4,5]. Abnormal DNMT activity has been implicated in tumorigenesis by silencing tumor suppressor genes or altering metabolic pathways [6,7].

Interestingly, the same DNA methylation patterns observed in adipogenesis, including genome-wide hypomethylation and site-specific hypermethylation, are observed in breast cancer [8–10]. Several DNA methylation sites associated with body mass index (BMI) have been detected in breast tissue, suggesting a potential role for BMI in tumorigenesis through alterations in DNA methylation [11]. Studies have shown that DNA methylation regulates key adipogenic transcription factors, such as PPAR γ and C/EBP α , which also influence tumor metabolism. In breast cancer, obesity-driven inflammation alters the methylation patterns of genes such as LEP, ADIPOQ, and FABP4, impacting both adipocyte function and tumor progression. Additionally, methylation changes in DNMT3A, IRS1, and SREBF1 have been associated with

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disrupted lipid metabolism in breast tumors, supporting the emerging role of epigenetic regulation at the intersection of adipogenesis and breast cancer [12,13]. Obesity is correlated with a twofold increase in breast cancer risk in postmenopausal women, whereas it is associated with a decreased incidence in premenopausal women [14]. The prevalence of breast cancer and dietary fat consumption are closely associated [15]. Adipocytes act as dynamic partners to breast cancer cells, enhancing their invasiveness and metastatic potential through the secretion of various factors and modification of the tumor microenvironment [16]. Obesity-associated inflammation and adipocyte lipolysis release fatty acids that drive cancer cell proliferation and migration, highlighting the metabolic link between obesity and breast cancer [17]. Cancer-associated adipocytes (CAAs) lose lipid content and secrete inflammatory cytokines, promoting cancer invasion, metastasis, and metabolic reprogramming [16,18,19]. They alter cancer cell metabolism, enhancing fatty acid uptake and tumor growth [20,21]. In obese individuals, altered adipokines such as leptin and adiponectin affect signaling pathways, promoting breast cancer progression [22].

Therapeutic strategies may involve disrupting the crosstalk between adipocytes and breast cancer cells to inhibit these protumorigenic effects. For example, targeting specific adipokines, such as leptin and resistin, which are involved in signaling pathways that support tumor growth and metastasis, could provide new avenues for treatment [23,24]. Additionally, interventions that modulate adipocyte metabolism, such as the use of PPAR γ agonists, may alter the tumor microenvironment to inhibit cancer cell proliferation and invasion [25,26]. Furthermore, leveraging the unique properties of adipose-derived stem cells to deliver therapeutic agents directly to the tumor site represents a promising approach for enhancing the efficacy of breast cancer treatments [27].

On the basis of this evidence, DNA methylation is known to regulate the expression of adipogenic genes in breast tumors, and specific adipogenic genes involved in this regulatory process can be identified by analyzing the coexpression of DNMT isoforms and the presence of CpG islands in their promoter regions. Our study investigated the regulatory effect of DNA methylation on the expression of adipogenic genes in breast tumors. By analyzing DNMT isoform coexpression, we identified adipogenic genes with CpG islands in their promoters to study the regulatory effects of methylation on these genes in breast tumors.

2. Materials and Methods

2.1. Selection of genes involved in adipogenesis

Adipogenesis-related genes were identified from four key data sources: the Molecular Signature Database for Gene Set Enrichment Analysis (GSEA) [28], the Kyoto Encyclopedia of Genes and Genomes (KEGG) PATHWAY database [29], the Human Protein Atlas [30], and WikiPathway [31]. The specific pathways and datasets utilized included HALLMARK-Adipogenesis from the GSEA database, the PPAR signaling pathway from the KEGG database, Adipogenesis, Transcription factor regulation in adipogenesis, and Transcriptional cascade regulating adipogenesis from

WikiPathway, and Adipogenesis from the Human Protein Atlas. All the genes obtained from these pathways were merged, and a unique subset of genes was further considered for analysis. The detailed pathways selected and the corresponding numbers of genes are outlined in Supplementary file 1. To ensure comprehensive annotation, all selected genes were mapped to the CpG island database downloaded using the Table Browser functionality of the UCSC Genome Browser [32]. The downloaded CpG island coordinates were intersected with RefSeq gene coordinates using Bedtools to obtain genes with overlapping CpG islands [33]. The complete methodology followed for the analysis is depicted in Fig. 1.

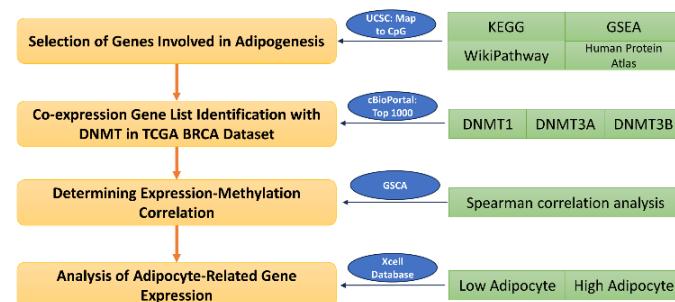


Fig. 1. Workflow for selecting and analyzing genes involved in adipogenesis and their coexpression with DNMT isoforms in breast cancer

2.2. Identification of genes coexpressed with DNMTs in The Cancer Genome Atlas (TCGA) BRCA dataset

To identify genes coexpressed with DNMT isoforms in the context of adipogenesis, we utilized cBioPortal to gather data [34]. We compiled a comprehensive list of 20,000 genes associated with DNMT isoforms (DNMT1, DNMT3A, and DNMT3B), and the top 1,000 genes with positive and negative coexpression of each DNMT isoform were selected for further analysis. Emphasis was placed on genes negatively correlated with methylation on the well-established principle that promoter hypermethylation is often associated with transcriptional repression. This strategy enabled the identification of epigenetically silenced genes potentially involved in adipogenic regulation and breast cancer progression, thereby narrowing our focus to the most biologically relevant candidates for downstream analysis.

2.2 Determining expression-methylation correlation

The Gene Set Cancer Analysis (GSCA) module was used to assess the associations between gene expression and methylation levels [35]. Specifically, RNA-Seq by expectation-maximization (RSEM)-normalized mRNA expression data and Illumina Methylation 450k level 3 data from the TCGA database were utilized. Spearman correlation analysis was conducted on mRNA expression and methylation data merged with TCGA barcodes to examine the relationships. Our primary objective was to identify the genes that presented the most significant negative correlation between gene expression and methylation. To ensure robust statistical analysis, p values were adjusted using the false discovery rate (FDR) method to account for multiple testing.

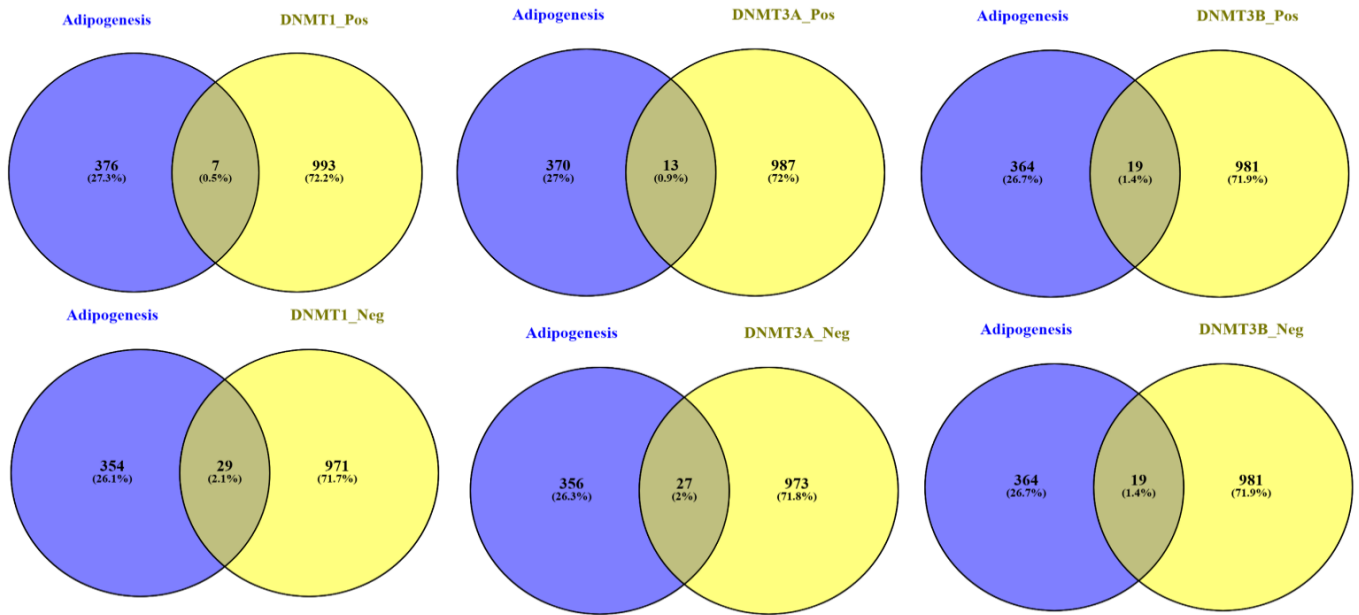


Fig. 2. Venn diagrams illustrating the overlap between adipogenesis-related genes and genes coexpressed with DNMT isoforms (DNMT1, DNMT3A, and DNMT3B) in breast cancer samples

2.3 Analysis of adipocyte-related gene expression

Precalculated adipocyte enrichment scores for each TCGA BRCA sample were obtained from the Xcell database [36]. These scores were used to categorize the samples into high- and

low-adipocyte groups. Specifically, samples with adipocyte scores below the 25th percentile were classified into the low-adipocyte group, whereas those with scores above the 75th percentile were classified into the high-adipocyte group. Additionally, expression, subtype, and survival data for these

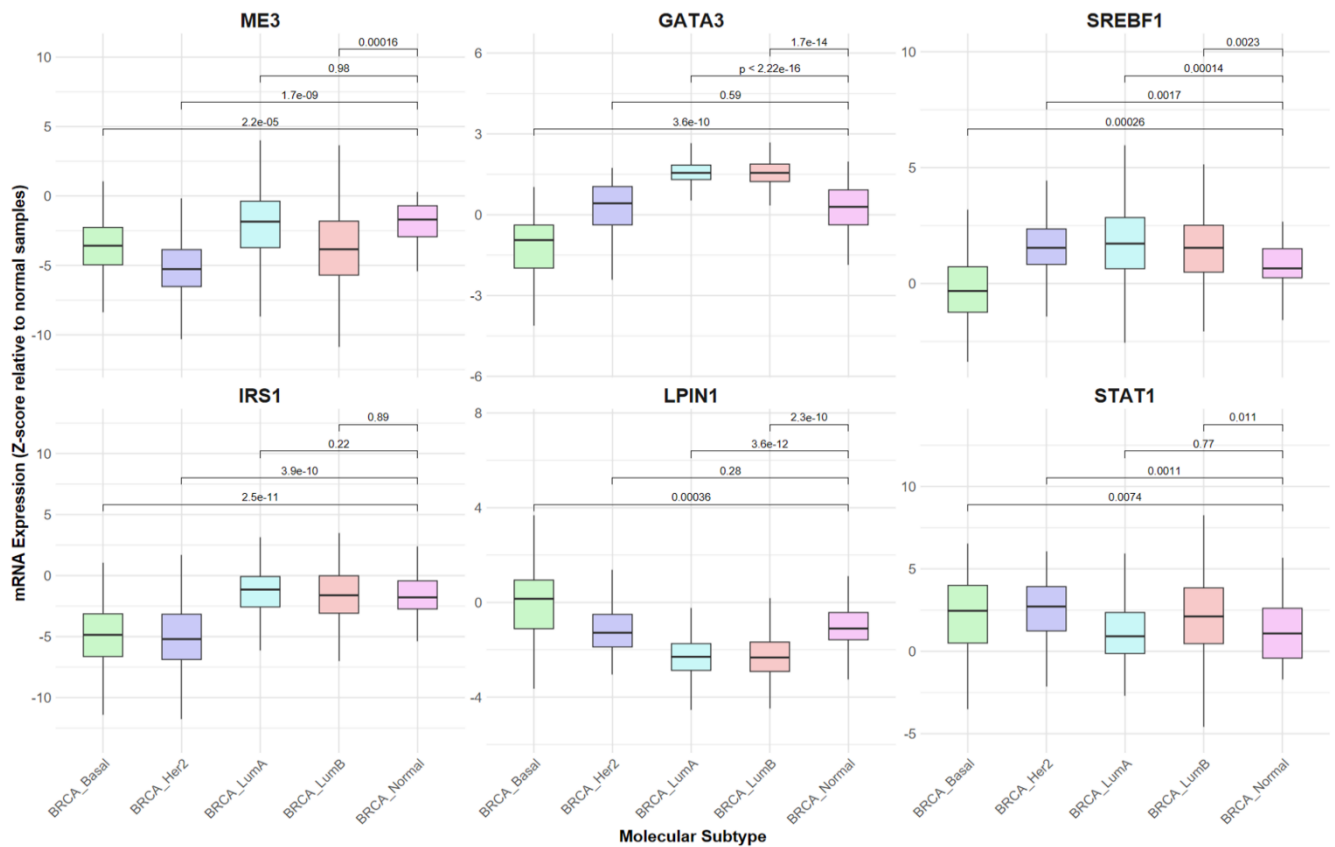


Fig. 3. Differential expression of six genes (ME3, GATA3, SREBF1, IRS1, LPIN1, and STAT1) across breast cancer molecular subtypes. Box plots representing mRNA expression Z scores relative to normal samples for each gene across five breast cancer subtypes

Abbreviations: LumA – Luminal A; LumB – Luminal B; HER2 – HER2-enriched; Basal – Basal-like. The expression is expressed as TPM (transcripts per million). Significance was tested using Wilcoxon rank-sum test

samples were downloaded from cBioPortal for comprehensive analysis. This stratification allowed us to investigate the associations between adipocyte content and gene expression, subtype distribution, and survival outcomes in patients with breast cancer.

3. Results and Discussion

3.1 Coexpression analysis

A total of 386 genes were identified or hypothesized to play a role in the regulation or implementation of adipogenesis, with 384 of these genes harboring CpG islands. A comparative study was conducted between the list of coexpressed genes and adipogenesis-related genes to determine the common gene pool (Fig. 2). A total of 114 genes were found to be correlated with DNMT isoforms. Specifically, 7, 13, and 19 genes were positively correlated, and 29, 27, and 19 genes were negatively correlated with DNMT1, DNMT3A, and DNMT3B, respectively.

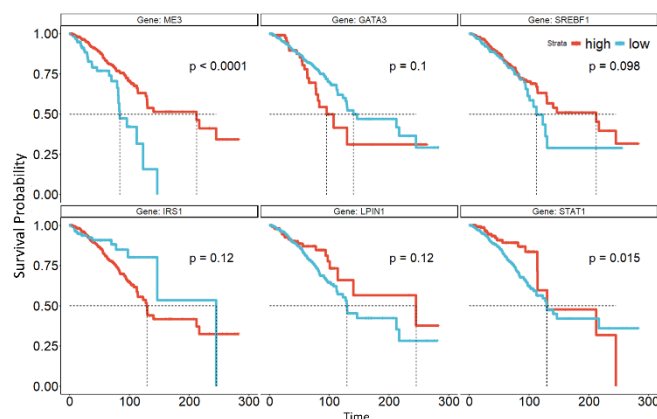


Fig. 4. Kaplan–Meier survival curves illustrating the survival probabilities of breast cancer patients with high (red) versus low (blue) expression levels of six genes (ME3, GATA3, SREBF1, IRS1, LPIN1, and STAT1), revealing significant associations between high expression of ME3 ($p < 0.0001$) and STAT1 ($p = 0.015$) and better survival outcomes

3.2 Expression methylation correlation analysis

Using the GSCA module, we analyzed the correlation between methylation levels and mRNA expression for our genes of interest. Spearman correlation analysis revealed varying degrees of negative correlations with high confidence ($FDR \leq 0.05$) between methylation at specific sites and gene expression levels for the following six genes: GATA3, IRS1, LPIN1, ME3, SREBF1, and STAT1. For the ME3 gene, the methylation site with the most significant negative correlation had a Spearman correlation value of -0.79 . This strong negative correlation indicates that higher methylation levels at this site are associated with lower mRNA expression of ME3.

3.3 Molecular subtype analysis

Analysis of the mRNA expression levels of the six identified genes across different breast cancer molecular subtypes, namely, the basal, Her2, luminal A (LumA), luminal B (LumB), and normal subtypes, revealed significant differential expression patterns, as shown in Fig. 3. Compared with those in the other groups, the basal and Her2 subtypes in ME3 presented the greatest differences. There are extremely

significant differences in the expression of GATA3, particularly between LumA and other subtypes. There were also significant differences in SREBF1 expression, especially between basal and other subtypes. For IRS1, very significant differences were observed, notably between the basal and Her2 subtypes and the other subtypes. LPIN1 was significantly different, particularly between LumB and the other subtypes. Finally, there were significant differences in the expression of STAT1, especially basal STAT1, and Her2 compared with that of other subtypes. These findings indicate that the expression levels of these genes vary significantly across different breast cancer subtypes, highlighting their potential roles in the molecular characterization and treatment of breast cancer.

As shown in Fig. 4, the survival analysis further supported these findings, demonstrating that patients with high expression levels of ME3 had significantly better survival outcomes ($p < 0.0001$). Similarly, for STAT1, low gene expression was associated with better survival outcomes ($p = 0.015$). In contrast, SREBF1 and LPIN1 did not show significant correlations in survival analyses or exhibited weaker correlations with expression levels and methylation status.

3.4 Relationship of adipocyte counts in TCGA BRCA samples

Two genes, ME3 and STAT1, that were significantly associated with survival outcomes were further analyzed for their expression levels in relation to the adipocyte count in TCGA BRCA samples, aiming to clarify their potential roles in breast cancer prognosis. Fig. 5(a) shows that the samples with a high adipocyte count had significantly greater median mRNA expression of ME3 than those with a low adipocyte count did, suggesting a strong association between the adipocyte count and ME3 expression in breast cancer samples. The distribution of ME3 expression in the high-adipocyte count group was tighter with less variability, indicating more consistent expression levels across these samples. In contrast, STAT1 expression was significantly lower in high-adipocyte-count samples than in low-adipocyte samples (Fig. 5(d)), implying a potential inverse relationship between adipocyte content and STAT1 expression.

To determine whether these adipocyte-associated expression differences were specific to particular breast cancer molecular subtypes, we analyzed ME3 and STAT1 expression stratified by subtype (basal-like, HER2-enriched, luminal A, luminal B, and normal-like). ME3 expression was significantly elevated in high-adipocyte-count samples in the luminal B (LumB) and normal-like subtypes but not in the basal-like, HER2-enriched, or luminal A subtypes (Fig. 5(b)). For STAT1, a significant reduction in expression in high-adipocyte samples was observed only within the luminal B subtype (Fig. 5(e)), whereas other subtypes did not significantly differ. These findings suggest that the link between adipocyte content and gene expression may be subtype specific, with the luminal B subtype showing the strongest associations with both ME3 and STAT1. Furthermore, Kaplan–Meier survival analysis revealed that patients with high ME3 expression had significantly better survival outcomes than those with low ME3 expression did (Fig. 5(c)), whereas low STAT1 expression was associated with improved prognosis (Fig. 5(f)), reinforcing the potential prognostic roles of these genes in the context of adipocyte

content and breast cancer subtype.

3.5 Discussions

DNA methyltransferases (DNMTs) play critical roles in breast cancer pathophysiology through multiple tumor-specific mechanisms. DNMT1 has been shown to promote breast cancer progression and metastasis by activating breast stromal fibroblasts via AUF1 upregulation, enhancing cancer stemness through FOXO3a/FOXN1/SOX2 signaling, and contributing to tumor initiation, particularly in triple-negative breast cancer (TNBC) [37–40]. Elevated DNMT1 expression has been correlated with poor patient survival. Similarly, DNMT3B overexpression leads to a hypermethylator phenotype, silences tumor suppressor genes such as BRCA1, and drives breast cancer progression [41,42]. These findings highlight the significant role of DNMT1 and DNMT3B in breast cancer pathophysiology through their influence on fibroblast activation, cancer progression, metastasis, and gene expression through hypermethylation mechanisms.

Our study elucidated the role of DNA methylation mediated by DNMT isoforms in adipogenesis and its implications in

breast cancer. DNMTs have both inhibitory and promoting effects on adipogenesis by modulating gene expression through epigenetic processes. Reduced DNMT1 levels can lead to disorganized adipogenesis, affecting the proper differentiation and function of adipocytes. This is evident in increased de novo methylation, which disrupts adipocyte aging [43]. DNMT1 is crucial for maintaining methylation patterns during adipocyte differentiation, and its knockout results in profound changes in adipocyte metabolism and differentiation, underscoring its role in metabolic fitness [44,45]. Additionally, DNMT3A expression in adipose tissue is associated with obesity and insulin resistance because it affects the methylation of genes involved in metabolic processes [46]. The dynamic methylation landscape during adipogenesis involves DNMTs regulating gene expression, which is essential for this process [47].

By integrating data from multiple genomic and epigenomic sources, we identified key genes and their associations with adipogenesis and breast cancer subtypes. These findings provide valuable insights into the complex regulatory mechanisms underlying these processes. Among the six key genes identified, STAT1 and ME3 were significantly negatively correlated, indicating that increased methylation is

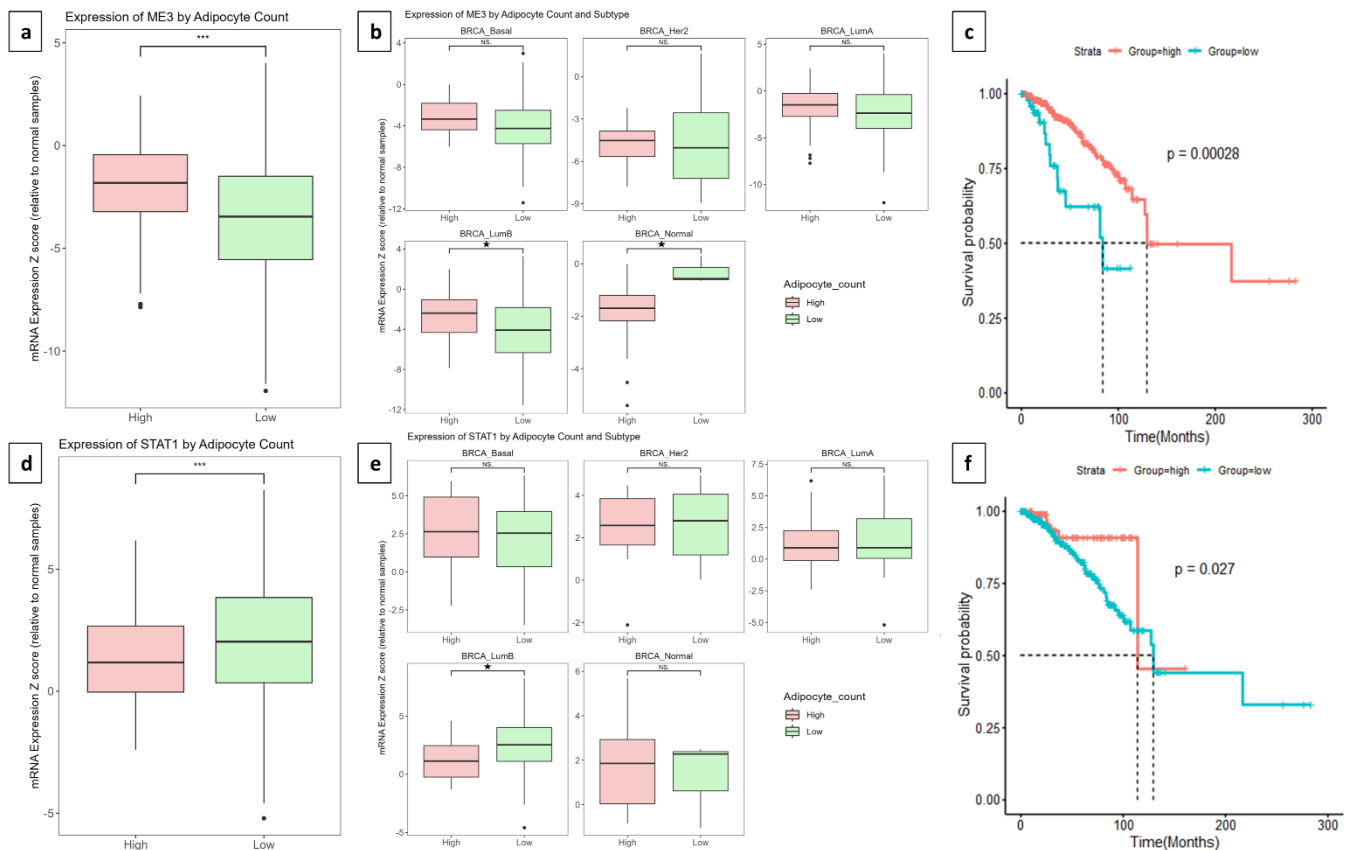


Fig. 5. Expression and survival analysis of ME3 and STAT1 in relation to adipocyte count in breast cancer samples from the TCGA BRCA dataset. (a) Box plot showing ME3 mRNA expression levels in samples with high versus low adipocyte counts. ME3 expression was significantly greater in samples with high adipocyte counts ($p < 0.0001$). (b) Box plots displaying ME3 mRNA expression levels across breast cancer subtypes with high versus low adipocyte counts. Significant differences in ME3 expression were observed between the LumB and normal subtypes. (c) Kaplan–Meier survival curve illustrating that the survival probabilities of patients with higher expression of ME3 are associated with better survival outcomes. (d) Box plot showing STAT1 mRNA expression levels in samples with high versus low adipocyte counts. STAT1 expression is significantly greater in samples with low adipocyte counts. (e) Box plots displaying STAT1 mRNA expression levels across breast cancer subtypes with high versus low adipocyte counts. Significant differences in STAT1 expression were observed among the basal subtypes. (f) Kaplan–Meier survival curve showing that lower STAT1 expression is associated with better survival outcomes

Abbreviations: BRCA: breast invasive carcinoma; LumA/LumB: luminal A/luminal B subtype; HER2: HER2-enriched subtype; NS: not significant; TPM: transcripts per million; $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

associated with decreased expression, which may have functional implications for breast cancer progression.

Interestingly, our survival analysis revealed that high ME3 expression and low STAT1 expression were both associated with improved prognosis. The survival associations observed for ME3 and STAT1 highlight the complex and context-specific roles these genes may play in breast cancer biology. High ME3 expression was linked to improved overall survival, which aligns with its known function in promoting mitochondrial redox balance and limiting oxidative stress. A recent study in gastric cancer similarly reported poorer survival outcomes in patients with low ME3 expression, supporting a tumor-suppressive role for ME3 through metabolic regulation [48]. Conversely, low STAT1 expression was associated with better prognosis in our study, which may initially seem contradictory, given the role of STAT1 as a tumor suppressor. In certain contexts, low STAT1 expression may also be associated with a more aggressive cancer phenotype. This apparent paradox highlights the dual nature of STAT1, which can function as an oncoprotein under specific conditions [49]. This may explain its negative prognostic impact in our adipocyte-stratified cohort.

ME3 is one of the three isoforms of malic enzymes and plays a role in cellular energy regulation, redox homeostasis, and biosynthetic processes. ME3 overexpression is linked to pancreatic tumor proliferation, invasion, and metastasis, suggesting that ME3 may play a similar role in breast cancer [50]. Analysis across different breast cancer subtypes revealed that ME3 was upregulated in the Her2 subtype and downregulated in the basal subtype, whereas GATA3 was upregulated in the LumA and LumB subtypes. These patterns align with the known roles of these genes in estrogen receptor-positive cancers and other subtypes, supporting their relevance in breast cancer classification. Increased adipocyte counts were associated with increased ME3 expression, particularly in the basal and LumB subtypes, suggesting a potential interaction between adipogenesis and the breast cancer microenvironment.

The STAT1 gene regulates various aspects of the tumor microenvironment and immune response in breast cancer. It downregulates NQO1, increasing oxidative stress and sensitivity to mitochondrial complex I inhibitors [51]. STAT1 acts as a tumor suppressor, and its deficiency leads to increased tumor growth and metastasis [52]. Our survival curve analysis also revealed that patients with low STAT1 expression had significantly better survival outcomes. Stromal STAT1 expression promotes tumor progression, indicating that it is a potential therapeutic target [53].

The results underscore the importance of DNMT-mediated DNA methylation in regulating genes involved in adipogenesis and breast cancer; however, this study is not without its limitations. First, it is based on publicly available datasets, which may introduce batch effects or biases not accounted for in our analysis. Second, while correlation-based methods can identify potential regulatory associations between methylation and gene expression, they do not establish causality. Third, clinical covariates such as treatment regimens, body mass index, and menopausal status, which can influence both methylation and adipogenesis, were not uniformly available in the TCGA dataset and therefore could not be included in our

analysis.

While accumulating studies have highlighted the critical role of tumor-associated adipocytes in breast cancer progression [54–56], the epigenetic regulation of these adipocytes remains largely unexplored. Our study revealed several genes, including ME3 and STAT1, that are correlated with DNA methyltransferases, suggesting a potential role for epigenetic regulation in modulating tumor-adipocyte interactions. Future studies are warranted to elucidate (a) the differential epigenetic regulation of these genes across breast cancer molecular subtypes and their role in tumor progression; (b) the contribution of adipocyte-specific gene regulation to cancer survival and metastasis; (c) therapeutic modulation of gene expression to inhibit tumor growth; and (d) novel strategies for targeting noncancerous stromal cells to reduce tumor burden. Moreover, investigating gene–environment interactions, such as dietary patterns, physical activity, and pharmacologic exposures, may further illuminate the mechanisms of adipocyte-driven tumor progression. Given the abundance of adipocytes in breast tissue, understanding these epigenetic mechanisms may also enable the design of innovative drug delivery strategies for targeted therapy.

The differential expression and methylation patterns of genes such as ME3 and STAT1 offer potential targets for therapeutic intervention. Drugs that target DNMT activity or specific methylation sites could be explored to modulate gene expression and improve patient outcomes. However, further mechanistic studies are needed to understand the precise roles of these genes in adipogenesis and breast cancer. Investigating the downstream effects of their methylation and expression changes could reveal new pathways and targets for intervention. These findings also have broader implications for understanding the role of epigenetics in other cancers and diseases involving adipogenesis. Comparative studies across different cancer types could reveal common regulatory mechanisms and potential cross-disease biomarkers.

4. Conclusion

This study presents a comprehensive analysis of the relationship between DNMT-mediated DNA methylation, adipogenesis, and breast cancer pathogenesis. ME3 and STAT1 have emerged as key candidate genes demonstrating subtype-specific expression and methylation patterns, suggesting their potential utility as biomarkers. ME3, which helps control redox balance and metabolism, may be a target for therapies that adjust these processes. STAT1, involved in both immune response and cancer growth could be used in immunomodulatory therapies. The findings also highlight the potential of epigenetic agents that modulate epigenetic regulators like DNMTs to selectively alter gene expression patterns and provide novel therapeutic approaches. Further research is needed to elucidate these molecular mechanisms and validate their clinical applicability. These findings underscore the complex interplay between genes involved in adipogenesis and breast cancer progression, highlighting new avenues for biomarker development, therapeutic intervention, and mechanistic studies.

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List of abbreviations

ADIPOQ	Adiponectin
AUF1	AU-Rich Element RNA-Binding Protein 1
BMI	Body Mass Index
BRCA1	Breast Cancer 1, Early Onset
CAA	Cancer-Associated Adipocyte
C/EBP α	CCAAT/Enhancer-Binding Protein Alpha
DNMT	DNA Methyltransferase
DNMT1	DNA Methyltransferase 1
DNMT3A	DNA Methyltransferase 3 Alpha
DNMT3B	DNA Methyltransferase 3 Beta
FABP4	Fatty Acid Binding Protein 4
FOXM1	Forkhead Box M1
FOXO3a	Forkhead Box O3
GATA3	GATA Binding Protein 3
GSCA	Gene Set Cancer Analysis
GSEA	Gene Set Enrichment Analysis
Her2	Human Epidermal Growth Factor Receptor 2
IRS1	Insulin Receptor Substrate 1
KEGG	Kyoto Encyclopedia of Genes and Genomes
LEP	Leptin
LPIN1	Lipin 1
LumA	Luminal A
LumB	Luminal B
ME3	Malic Enzyme 3
MSC	Mesenchymal Stem Cell
NQO1	NAD(P)H Quinone Dehydrogenase 1
PPAR γ	Peroxisome Proliferator-Activated Receptor Gamma
RSEM	RNA-Seq by Expectation-Maximization
SREBF1	Sterol Regulatory Element Binding Transcription Factor 1
SOX2	SRX-Box Transcription Factor 2
STAT1	Signal Transducer and Activator of Transcription 1
TCGA	The Cancer Genome Atlas
TNBC	Triple-Negative Breast Cancer

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