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Original Article

Exploring the critical role of SDHA in breast cancer proliferation: implications for novel therapeutic strategies

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Abstract: Objectives: This study aims to evaluate Succinate Dehydrogenase Complex Flavoprotein Subunit A (SDHA) expression across various breast cancer subtypes, its prognostic significance, and the impact of SDHA knockdown on breast cancer cell functions. Methods: To assess SDHA expression in breast cancer, we utilized multiple publicly available databases. Prognostic significance was also evaluated using relevant databases. Methylation status, and enrichment analysis were performed using the GSCA database. The mutational status of SDHA was examined using cBioPortal, and its relationship with immune infiltration and drug sensitivity was assessed. Functional assays, including cell proliferation, colony formation, wound healing, and SDHA knockdown, were performed using MCF-7 and SKBR3 breast cancer cell lines. Results: Our results showed that SDHA was significantly overexpressed in breast cancer tissues compared to normal tissues. High SDHA expression was correlated with worse survival in breast cancer patients. Pathological stage analysis revealed that SDHA expression increased as the disease progressed, with lower methylation levels in tumor tissues suggesting epigenetic regulation of its expression. Functionally, SDHA knockdown in MCF-7 and SKBR3 cells led to significant reductions in cell proliferation, colony formation, and migration, highlighting its role in supporting breast cancer cell growth and metastasis. Conclusion: SDHA was upregulated in breast cancer and associated with poor prognosis. Our findings also suggest that SDHA plays a crucial role in promoting breast cancer cell growth and migration, indicating its therapeutic potential. Targeting SDHA could provide a novel strategy for breast cancer treatment, particularly in overcoming chemoresistance and inhibiting tumor progression.

Keywords: Breast cancer, SDHA, prognosis, therapeutic target, proliferation

Introduction

Breast cancer is one of the most common and deadly cancers globally, representing a significant health burden, particularly for women [1, 2]. It is estimated that 1 in 8 women will be diagnosed with breast cancer during their lifetime, and the disease accounts for approximately 25% of all cancer cases and 15% of cancer-related deaths worldwide [3, 4]. Although the survival rates for early-stage breast cancer have improved significantly due to advances in screening, early detection, and personalized treatments, metastatic breast cancer remains a leading cause of cancer-related mortality [5]. The molecular landscape of breast cancer is highly complex and heterogeneous, encompassing multiple subtypes with distinct genetic,

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molecular, and clinical characteristics [6, 7]. These subtypes, such as luminal A, luminal B, HER2-positive, and triple-negative breast cancer (TNBC), differ in their prognosis and response to treatment [8], emphasizing the need for more precise diagnostic and therapeutic strategies tailored to the unique features of each subtype.

The pathogenesis of breast cancer involves a combination of genetic mutations, epigenetic changes, and environmental factors [9, 10]. Genetic predispositions, such as mutations in the BRCA1 and BRCA2 genes, increase the risk of developing breast cancer [11]. Hormonal factors, including prolonged exposure to estrogen, and environmental influences such as lifestyle choices (diet, physical activity, alcohol use) also contribute to disease development [12]. Despite extensive research into these factors, there is still much to be learned about the molecular mechanisms driving tumorigenesis, particularly those related to the cellular metabolism and energy regulation that are now recognized as key components in cancer progression [13, 14].

One such molecular pathway gaining attention is the role of Succinate Dehydrogenase Complex Flavoprotein Subunit A (SDHA), a critical enzyme in the mitochondrial respiratory chain and the citric acid cycle [15-17]. SDHA plays a pivotal role in cellular energy production, linking the tricarboxylic acid cycle to the electron transport chain, and its function is essential for mitochondrial respiration and cellular homeostasis [18]. In normal cells, SDHA ensures efficient energy production, but in cancer cells, metabolic reprogramming often leads to altered SDHA activity, contributing to tumor growth and metastasis [19]. While mutations and dysregulations in SDHA have been extensively studied in other cancers such as pheochromocytomas, paragangliomas, and gastrointestinal stromal tumors [20], the role of SDHA in breast cancer has not been thoroughly explored.

Recent research indicates that cellular metabolism is intricately linked to tumorigenesis [21, 22], with metabolic shifts facilitating the growth and survival of cancer cells in hostile environments [23, 24]. SDHA, as a central player in oxidative phosphorylation and cellular metabolism, may influence how breast cancer cells adapt to hypoxic and nutrient-deprived conditions, common features of solid tumors [25]. Furthermore, studies have suggested that mutations or alterations in SDHA expression could affect cancer cell behavior, including migration, invasion, and resistance to therapy [26]. However, its specific impact on breast cancer progression, prognosis, and treatment response remains largely unknown.

Given these gaps in knowledge, our study aims to explore the diagnostic, prognostic, and therapeutic significance of SDHA in breast cancer. Through bioinformatics analysis and experimental validation [27], we aim to identify the expression patterns and genetic alterations of SDHA in breast cancer tissues compared to normal breast tissues. We will investigate how these alterations correlate with key clinical parameters such as disease stage, metastasis, and overall survival. Additionally, the study will assess the potential of SDHA as a prognostic biomarker and therapeutic target, exploring how modulating SDHA expression or activity might affect the growth and treatment response of breast cancer cells. This study addresses a critical gap in breast cancer research by linking SDHA dysregulation to cancer progression and providing evidence for its potential as a novel therapeutic target. By elucidating the role of SDHA in breast cancer biology, we hope to uncover new insights into its metabolic contributions to tumorigenesis and propose strategies to enhance breast cancer treatment through targeted modulation of SDHA activity. Ultimately, our research aims to improve diagnostic precision, prognostic accuracy, and therapeutic outcomes for breast cancer patients, particularly those with more aggressive or metastatic forms of the disease.

Methodology

Expression analysis of SDHA in breast cancer

To assess the expression levels of SDHA across breast cancer and other cancer types, we utilized publicly available databases, including TIMER2, HPA, and TISIDB. TIMER2 (https://cistrome.shinyapps.io/timer/) is a comprehensive web tool that provides immune cell infiltration data across various tumor types, including breast cancer, based on gene expression profiles from multiple sources [28]. TIMER2 allows for pan-cancer analysis and facilitates the exploration of tumor-associated immune landscapes. The RNA sequencing data used in

TIMER2 were processed and normalized to ensure comparability across different datasets. Specifically, raw read counts were converted into fragments per kilobase per million (FPKM), which were then transformed into transcripts per million (TPM) for gene expression quantification. Following this, a log2 transformation was applied to ensure a more normalized distribution and to reduce the skewness in the data. For immunohistochemical analysis, the HPA database (https://www.proteinatlas.org/) was utilized, offering tissue-specific protein expression data from a wide variety of human tissues and cancer types. This resource provides information about the expression of proteins in normal versus tumor tissues, enabling us to investigate SDHA expression in both normal and breast cancer tissues [29]. To explore the expression of SDHA across immune and molecular subtypes of breast cancer, we used the TISIDB (http://cis.hku.hk/TISIDB/), which integrates information on immune cell infiltration and molecular subtypes across different cancers. TISIDB provides a comprehensive platform for the exploration of gene expression data, particularly in relation to tumor immunity and subtype-specific molecular characteristics [30]. The violin plots from TISIDB allowed us to compare SDHA expression across breast cancer subtypes, including Basal, Her2, LumA, and LumB, to understand its role in various molecular contexts.

Prognostic significance of SDHA

To evaluate the prognostic significance of SDHA expression in breast cancer, we performed survival analysis using publicly available data from the Kaplan-Meier (KM) plotter and GENT2 databases. The KM plotter tool (http://kmplot.com/analysis/) is an online resource that provides Kaplan-Meier survival curves for gene expression data in multiple cancers, including breast cancer [31]. This tool allows for the analysis of overall survival (OS) and disease-free survival (DFS) by stratifying patients based on their gene expression levels. We used this tool to generate Kaplan-Meier curves for high and low SDHA expression in breast cancer, with statistical significance determined using the log-rank test.

For further validation, we used the GENT2 database (https://gent2.applab.gist.ac.kr/) to perform a meta-analysis of SDHA expression across multiple independent breast cancer datasets. The GENT2 database provides access to gene expression data and survival outcomes from a large cohort of breast cancer patients, enabling robust survival analysis [32]. Using the GENT2 database, we generated forest plots to assess the pooled hazard ratio (HR) and confidence interval (CI) for OS and DFS across different studies, further supporting the findings obtained from KM plotter analysis.

Pathological stage-specific expression, methylation, and enrichment analysis of SDHA

To explore the expression of SDHA across different pathological stages, its methylation status, and the associated Gene Set Enrichment Analysis (GSEA) of SDHA in breast cancer, we utilized the GSCA database (http://bioinfo.life.hust.edu.cn/GSCA/). The GSCA database provides comprehensive genomic, transcriptomic, and methylation data across various cancer types, including breast cancer [33]. The database allows for the examination of gene expression and its relationship with different clinical and molecular characteristics, providing valuable insights into the role of SDHA in cancer progression.

Genetic alteration analysis of SDHA

To investigate the mutational and copy number variation (CNV) status of SDHA in breast cancer, we utilized the cBioPortal database (https://www.cbioportal.org/), which provides comprehensive genomic data from various cancer studies, including breast cancer. The cBioPortal database integrates multiple types of genomic data, such as single nucleotide variants (SNVs), copy number alterations (CNAs), and mutation frequency, allowing for detailed analysis of gene mutations and their potential impact on cancer progression [34].

Immune infiltration and drug sensitivity analysis of SDHA

To investigate the relationship between SDHA expression and immune cell infiltration as well as drug sensitivity in breast cancer, we utilized the GSCA database. GSCA integrates comprehensive genomic data, including gene expression, immune infiltration scores, and drug sensitivity data across various cancer types [33]. This database allows for an in-depth exploration of gene expression patterns and their

impact on tumor immunology and therapeutic responses.

Protein-protein interaction (PPI) network construction and gene enrichment analysis of SDHA-associated partners

The GeneMANIA database (http://genemania.org/) was used to construct the PPI network of SDHA. GeneMANIA generates PPI networks based on a variety of data sources, including co-expression, protein interaction, and functional association data, allowing us to explore the key binding partners of SDHA [35]. For gene enrichment analysis, we utilized the DAVID tool (https://david.ncifcrf.gov/) to perform a detailed functional enrichment analysis of SDHA and its associated proteins. DAVID provides functional annotation of genes based on gene ontology (GO) terms, biological pathways, and cellular components [36].

Cell lines and SDHA knockdown

MCF-7 and SKBR3 breast cancer cell lines were obtained from the American Type Culture Collection (ATCC). These cell lines are commonly used in breast cancer research due to their well-documented characteristics and relevance to human breast cancer biology. The MCF-7 cell line, derived from a human breast adenocarcinoma, is estrogen receptor-positive (ER+) and has been widely used to study hormone-responsive breast cancer. The SKBR3 cell line, derived from a human breast carcinoma overexpressing the HER2 receptor, is an important model for studying HER2-positive breast cancer. Both cell lines were authenticated by ATCC through short tandem repeat (STR) profiling, ensuring their identity and purity. Both cell lines were maintained at low passage numbers (below passage 20) to avoid phenotypic drift, which could affect experimental reproducibility. MCF-7 and SKBR3 cells were cultured in RPMI-1640 medium (Gibco, Thermo Fisher Scientific) supplemented with 10% fetal bovine serum (FBS) (Gibco, Thermo Fisher Scientific), 1% penicillin-streptomycin (Gibco, Thermo Fisher Scientific), and 2 mM L-glutamine (Gibco, Thermo Fisher Scientific) under standard conditions of 37°C and 5% CO_2. Medium was changed every 2-3 days, and cells were subcultured at approximately 80-90% confluence using 0.25% trypsin-EDTA (Gibco, Thermo Fisher Scientific).

SDHA siRNA (catalog number: AM16708, Thermo Fisher Scientific) was used for gene silencing. Transfection of siRNA was performed using Lipofectamine RNAiMAX (Thermo Fisher Scientific), following the manufacturer's instructions, to ensure efficient delivery of siRNA into cells.

Confirmation of SDHA knockdown

To confirm the successful knockdown of SDHA expression, RNA extraction was performed using TRIzol reagent (Thermo Fisher Scientific), following the manufacturer's instructions. The quality and quantity of the RNA were determined using a NanoDrop spectrophotometer (Thermo Fisher Scientific), and cDNA synthesis was carried out using the SuperScript IV First-Strand Synthesis System (Thermo Fisher Scientific). For reverse transcription quantitative PCR (RT-qPCR), SYBR Green Master Mix (Thermo Fisher Scientific) was used to detect SDHA mRNA levels on Bio-Rad CFX96 Real-Time System (Bio-Rad). For the normalization of SDHA expression, GAPDH was used as an internal. Data were collected and analyzed using the ΔΔCt method. Experiments were performed in triplicates. The following primers were used for the amplification of SDHA and GAPDH: SDHA forward primer: 5'-GAGATGTGGTGTCTCGGTCCAT-3'; SDHA reverse primer: 5'-GCTGTCTCTGAAATGCCAGGCA-3'; GAPDH forward primer: 5'-GTCTCCTCTGACTTCAACAGCG-3'; GAPDH reverse primer: 5'-ACCACCCTGTTGCTGTAGCCAA-3'.

Additionally, Western blotting was performed to assess SDHA protein levels. Protein was extracted from cells using RIPA buffer (Thermo Fisher Scientific) containing protease inhibitors. The protein concentration was determined using the BCA Protein Assay Kit (Thermo Fisher Scientific 23225). SDS-PAGE was performed using 4-12% Bis-Tris gels (Thermo Fisher Scientific), and the proteins were transferred to PVDF membranes (Thermo Fisher Scientific). The membranes were blocked with 5% non-fat milk in PBS-T (0.1% Tween 20) for 1 hour at room temperature. The membranes were incubated with primary antibodies against SDHA (Thermo Fisher Scientific, dilution 1:1000) and GAPDH (Cell Signaling Technology, dilution 1:5000) overnight at 4°C. After washing, the membranes were incubated with the appropriate HRP-conjugated secondary antibodies

(Thermo Fisher Scientific, dilution 1:2000). Chemiluminescence was detected using the Clarity Western ECL Substrate (Bio-Rad), and images were captured using the Gel Documentation System (Bio-Rad Chemidoc XRS+).

Proliferation assay

To evaluate the effect of SDHA knockdown on cell proliferation, a cell viability assay was performed using the CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega). Briefly, 5×10^3 cells per well were seeded in 96-well plates and incubated for 24, 48, and 72 hours post-transfection. Absorbance at 490 nm was measured using a microplate reader to quantify cell proliferation. The results were analyzed to determine the effect of SDHA knockdown on cell growth. Experiments were performed in triplicates.

Colony formation assay

To assess the impact of SDHA knockdown on colony formation, 500 cells were seeded in 6-well plates and allowed to grow for 10-14 days. Colonies were fixed with methanol and stained with crystal violet (Sigma-Aldrich). Colonies were counted, and the results were presented as the average number of colonies per well. The colony formation ability of si-SDHA-treated cells was compared to control siRNA-treated cells. Experiments were performed in triplicates.

Wound healing assay

To evaluate the effect of SDHA knockdown on cell migration, we performed a wound healing assay. MCF-7 and SKBR3 cells were seeded in 6-well plates and grown to confluence. A wound was introduced using a p200 pipette tip, and the cells were then treated with either si-SDHA or control siRNA. The cells were cultured in serum-free medium (Thermo Fisher Scientific) to promote migration. Images were taken at 0 hour and 24 hours post-wounding using a Zeiss microscope, and the wound closure rate was calculated by measuring the gap size at each time point. Experiments were performed in triplicates.

Statistics

Statistical analyses were performed using Student's t-test for comparisons between two groups. For experiments involving more than two groups, one-way ANOVA followed by Tukey's post-hoc test was used to determine statistical significance. Kaplan-Meier survival curves were analyzed using the log-rank test to assess differences in survival between high and low SDHA expression groups. Hazard ratios (HR) and 95% confidence intervals (CI) were calculated for survival analysis. All experiments were conducted in triplicates, and statistical analyses were performed using GraphPad Prism 8 (GraphPad Software, Inc., San Diego, CA). $P^* < 0.05$, $P^{**} < 0.01$, and $P^{***} < 0.001$ were considered statistically significant.

Results

Expression analysis of SDHA in breast cancer across tumor, immune, and molecular subtypes

Firstly, we evaluated the expression levels of SDHA across a range of cancers, with a particular focus on breast cancer. **Figure 1A** presents a pan-cancer expression analysis of SDHA across various tumor types using the TIMER2 database. The boxplot indicates that SDHA was significantly overexpressed in breast cancer tissues compared to normal breast tissues, with a notable statistical significance ($P < 0.001$) (**Figure 1A**). **Figure 1B** shows the immunohistochemical staining of SDHA using the HPA database, with images from normal and breast cancer tissues. The immunohistochemical staining results clearly reveal a higher expression of SDHA in breast cancer samples compared to normal breast samples, with varying intensities (medium in normal breast tissue, high in breast cancer tissues) (**Figure 1B**). **Figure 1C** explores the expression of SDHA across immune subtypes of breast cancer using the TISIDB database. The violin plot illustrates distinct expression patterns of SDHA in different immune subtypes of breast cancer. Specifically, SDHA expression is shown to vary across immune subtypes, with Basal subtype exhibiting the highest expression levels, followed by Her2 and LumA subtypes (**Figure 1C**). Furthermore, **Figure 1D** investigates SDHA expression across different molecular subtypes of breast cancer, such as Basal, Her2, LumA, LumB, and Normal using the TISIDB database. The plot demonstrates that SDHA expression was notably higher in Basal and Her2 subtypes, with significant differences ($P < 0.001$) com-

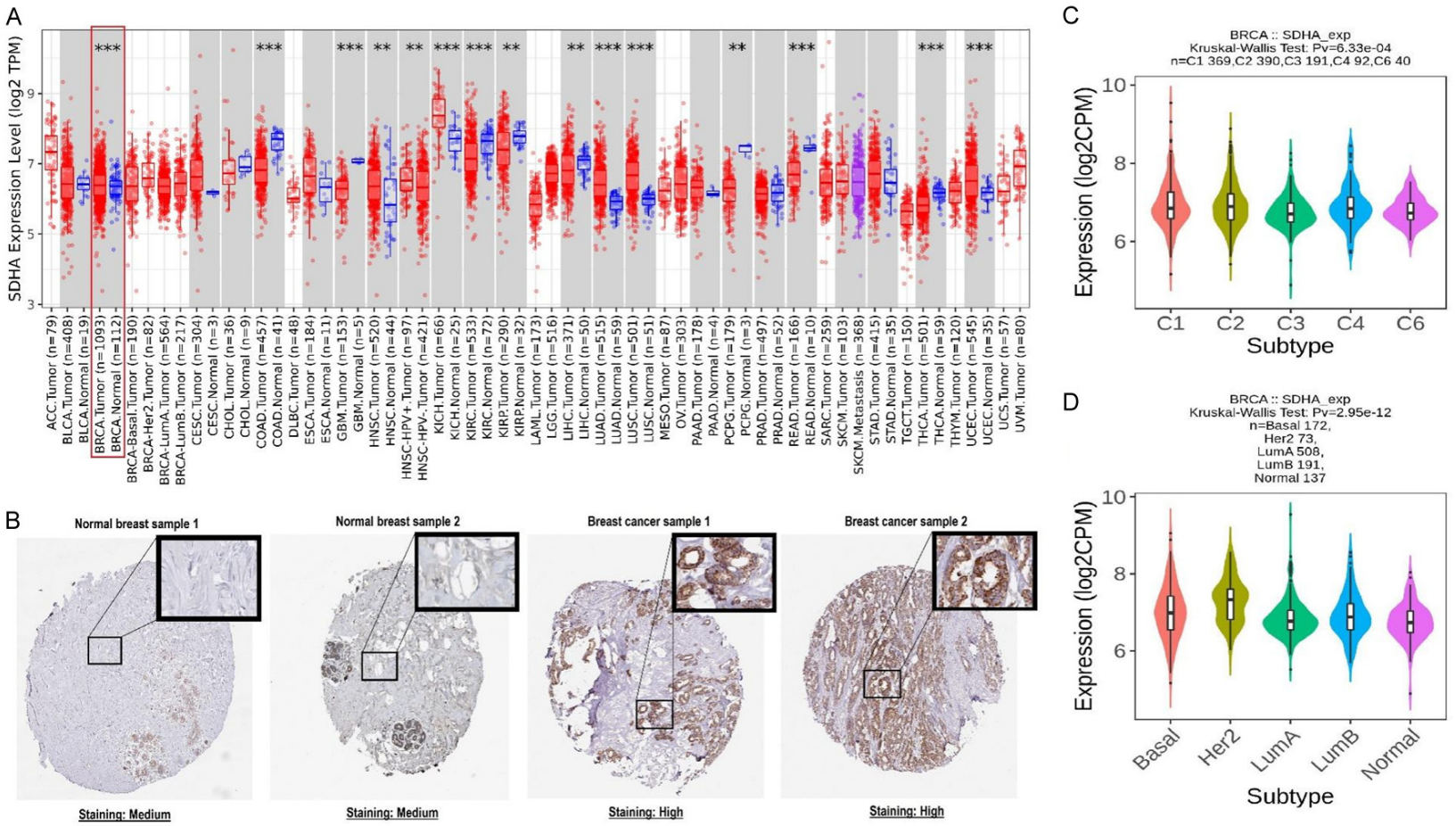


Figure 1. Expression analysis of Succinate Dehydrogenase Complex Flavoprotein Subunit A (SDHA) across tumor, immune, and molecular subtypes of breast cancer. A. Pan-cancer expression analysis of SDHA across various tumor types using the TIMER2 database. B. Immunohistochemical staining of SDHA using the HPA database. C. Expression of SDHA across immune subtypes of breast cancer using the TISIDB database. D. SDHA expression across different molecular subtypes of breast cancer. P** < 0.01 and P*** < 0.001.

pared to the Normal and LumA/LumB subtypes (**Figure 1D**).

Prognostic significance of SDHA expression in breast cancer

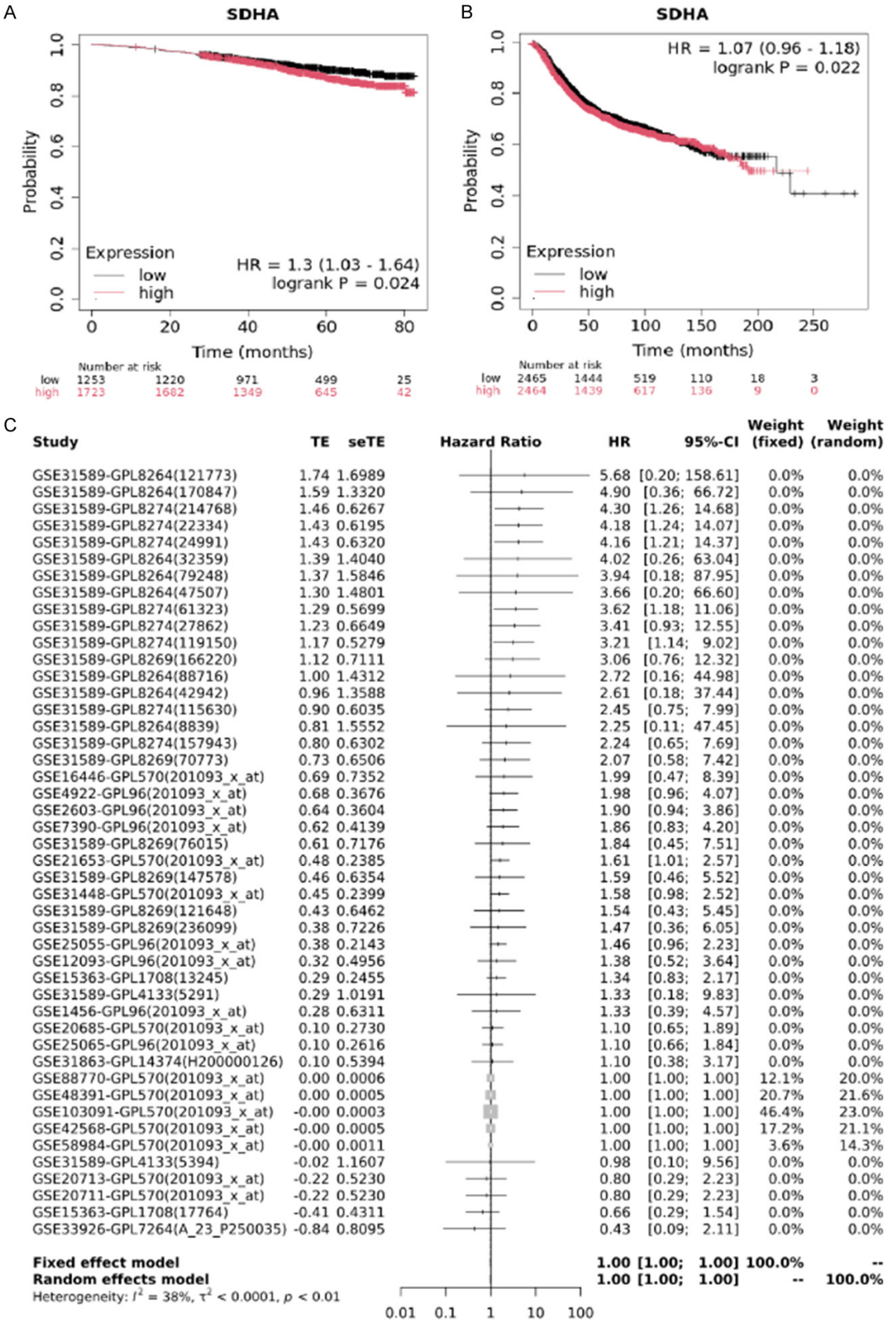
In this section, we presented the survival analysis results of SDHA expression in breast cancer, focusing on both OS and DFS. **Figure 2A** illustrated the OS analysis of SDHA using the KM plotter tool, where Kaplan-Meier survival curves for high and low SDHA expression were shown. The results indicated that high SDHA expression was associated with significantly worse OS outcomes (HR = 1.3, 95% CI = 1.03-1.64, log-rank P = 0.024) (**Figure 2A**). **Figure 2B** presented the DFS analysis of SDHA, using the KM plotter tool. Here, we observed that high SDHA expression was also associated with a worse DFS (HR = 1.07, 95% CI = 0.96-1.18, log-rank P = 0.022) (**Figure 2B**). **Figure 2C** provided a forest plot showing the OS results from the GENT2 database, presenting multiple studies with their corresponding hazard ratios (HR), confidence intervals (95% CI), and weights. Most of the studies reviewed indicated a positive association between SDHA expression and worse OS, with the majority of the studies showing a HR > 1, signifying that higher SDHA expression correlated with poorer survival outcomes. The combined hazard ratio from the fixed effect model was 1.75 (95% CI: 1.18-2.59), further confirming SDHA as a strong predictor of worse overall survival in breast cancer (**Figure 2C**). **Figure 2D** presented the DFS results from the GENT2 database, which again showed consistent findings. The hazard ratios across different studies ranged from 1.22 to 1.91, indicating that higher SDHA expression correlated with a higher risk of disease recurrence. The combined hazard ratio for DFS was 1.75 (95% CI: 1.18-2.59), and the overall weight distribution of studies further supported the significance of SDHA as a predictor of DFS (**Figure 2D**).

Pathological stage-specific expression, methylation, and enrichment analysis of SDHA in breast cancer

Next, we provide an in-depth analysis of the gene expression across pathological stages, methylation status, and enrichment of SDHA in breast cancer tissues compared to normal tissues using GSCA database. **Figure 3A**, **3B** presents SDHA mRNA expression across various pathological stages of breast cancer, showing that SDHA expression increases significantly as the disease progresses from Stage I to Stage IV (*p*-values indicated) (**Figure 3A**, **3B**). **Figure 3C** demonstrates SDHA methylation levels across normal and breast cancer tissues. The plot reveals that SDHA methylation was significantly lower in breast cancer tissues compared to normal tissues (P = 4.9e-07), indicating that hypomethylation of SDHA may contribute to its overexpression in cancer (**Figure 3C**). **Figure 3D** shows a negative correlation between SDHA methylation and SDHA expression in breast cancer (correlation coefficient = -0.1), suggesting that decreased methylation leads to increased expression of SDHA. This relationship is statistically significant (FDR = 9.8e-03) (**Figure 3D**), supporting the idea that epigenetic regulation plays a key role in modulating SDHA levels. **Figure 3E** illustrates the results from GSEA analysis, showing that SDHA-related pathways are significantly enriched in breast cancer development (**Figure 3E**).

Mutational and CNV analysis of SDHA in breast cancer

In this section, we present the mutational and CNV analysis results of SDHA in breast cancer using the cBioPortal database. **Figure 4A** shows the SNV percentage heatmap of SDHA in breast cancer. The heatmap indicates that SDHA has a relatively low mutation frequency of 4% in breast cancer (**Figure 4A**). **Figure 4B** provides a detailed breakdown of the mutational types and variant classifications for SDHA. The data reveals that most mutations are missense mutations (green), with a few frame shift deletions (blue) (**Figure 4B**). These results suggest that missense mutations are the most common form of SDHA mutation in breast cancer, and these mutations could contribute to changes in SDHA function that affect cancer progression. **Figure 4B**, **4C** illustrates the specific mutation types in SDHA, showing the prevalence of C>T transitions, which are typically linked to genetic instability or environmental factors such as exposure to carcinogens (**Figure 4B**, **4C**). There are no mutations in other classes (e.g., T>G, T>A, T>C), indicating that SDHA mutations in breast cancer are mostly confined to this specific transition type (**Figure 4B**, **4C**).


A
SDHA
Probability
Expression
low
high
HR = 1.3 (1.03 - 1.64)
logrank P = 0.024
Time (months)
Number at risk
low 1253 1220 971 499 25
high 1723 1682 1349 645 42
B
SDHA
HR = 1.07 (0.96 - 1.18)
logrank P = 0.022
Probability
Expression
low
high
Time (months)
Number at risk
low 2465 1444 519 110 18 3
high 2464 1439 617 136 9 0
C
Study TE seTE Hazard Ratio HR 95%-CI Weight (fixed) Weight (random)
GSE31589-GPL8264(121773) 1.74 1.6989 5.68 [0.20; 158.61] 0.0% 0.0%
GSE31589-GPL8264(170847) 1.59 1.3320 4.90 [0.36; 66.72] 0.0% 0.0%
GSE31589-GPL8274(214768) 1.46 0.6267 4.30 [1.26; 14.68] 0.0% 0.0%
GSE31589-GPL8274(22334) 1.43 0.6195 4.18 [1.24; 14.07] 0.0% 0.0%
GSE31589-GPL8274(24991) 1.43 0.6320 4.16 [1.21; 14.37] 0.0% 0.0%
GSE31589-GPL8264(32359) 1.39 1.4040 4.02 [0.26; 63.04] 0.0% 0.0%
GSE31589-GPL8264(79248) 1.37 1.5846 3.94 [0.18; 87.95] 0.0% 0.0%
GSE31589-GPL8264(47507) 1.30 1.4801 3.66 [0.20; 66.60] 0.0% 0.0%
GSE31589-GPL8274(61323) 1.29 0.5699 3.62 [1.18; 11.06] 0.0% 0.0%
GSE31589-GPL8274(27862) 1.23 0.6649 3.41 [0.93; 12.55] 0.0% 0.0%
GSE31589-GPL8274(119150) 1.17 0.5279 3.21 [1.14; 9.02] 0.0% 0.0%
GSE31589-GPL8269(166220) 1.12 0.7111 3.06 [0.76; 12.32] 0.0% 0.0%
GSE31589-GPL8264(88716) 1.00 1.4312 2.72 [0.16; 44.98] 0.0% 0.0%
GSE31589-GPL8264(42942) 0.96 1.3588 2.61 [0.18; 37.44] 0.0% 0.0%
GSE31589-GPL8274(115630) 0.90 0.6035 2.45 [0.75; 7.99] 0.0% 0.0%
GSE31589-GPL8264(8839) 0.81 1.5552 2.25 [0.11; 47.45] 0.0% 0.0%
GSE31589-GPL8274(157943) 0.80 0.6302 2.24 [0.65; 7.69] 0.0% 0.0%
GSE31589-GPL8269(70773) 0.73 0.6506 2.07 [0.58; 7.42] 0.0% 0.0%
GSE16446-GPL570(201093_x_at) 0.69 0.7352 1.99 [0.47; 8.39] 0.0% 0.0%
GSE4922-GPL96(201093_x_at) 0.68 0.3676 1.98 [0.96; 4.07] 0.0% 0.0%
GSE2603-GPL96(201093_x_at) 0.64 0.3604 1.90 [0.94; 3.86] 0.0% 0.0%
GSE7390-GPL96(201093_x_at) 0.62 0.4139 1.86 [0.83; 4.20] 0.0% 0.0%
GSE31589-GPL8269(76015) 0.61 0.7176 1.84 [0.45; 7.51] 0.0% 0.0%
GSE21653-GPL570(201093_x_at) 0.48 0.2385 1.61 [1.01; 2.57] 0.0% 0.0%
GSE31589-GPL8269(147578) 0.46 0.6354 1.59 [0.46; 5.52] 0.0% 0.0%
GSE31448-GPL570(201093_x_at) 0.45 0.2399 1.58 [0.98; 2.52] 0.0% 0.0%
GSE31589-GPL8269(121648) 0.43 0.6462 1.54 [0.43; 5.45] 0.0% 0.0%
GSE31589-GPL8269(236099) 0.38 0.7226 1.47 [0.36; 6.05] 0.0% 0.0%
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GSE15363-GPL1708(13245) 0.29 0.2455 1.34 [0.83; 2.17] 0.0% 0.0%
GSE31589-GPL4133(5291) 0.29 1.0191 1.33 [0.18; 9.83] 0.0% 0.0%
GSE1456-GPL96(201093_x_at) 0.28 0.6311 1.33 [0.39; 4.57] 0.0% 0.0%
GSE20685-GPL570(201093_x_at) 0.10 0.2730 1.10 [0.65; 1.89] 0.0% 0.0%
GSE25065-GPL96(201093_x_at) 0.10 0.2616 1.10 [0.66; 1.84] 0.0% 0.0%
GSE31863-GPL14374(H200000126) 0.10 0.5394 1.10 [0.38; 3.17] 0.0% 0.0%
GSE88770-GPL570(201093_x_at) 0.00 0.0006 1.00 [1.00; 1.00] 12.1% 20.0%
GSE48391-GPL570(201093_x_at) 0.00 0.0005 1.00 [1.00; 1.00] 20.7% 21.6%
GSE103091-GPL570(201093_x_at) -0.00 0.0003 1.00 [1.00; 1.00] 46.4% 23.0%
GSE42568-GPL570(201093_x_at) -0.00 0.0005 1.00 [1.00; 1.00] 17.2% 21.1%
GSE58984-GPL570(201093_x_at) -0.00 0.0011 1.00 [1.00; 1.00] 3.6% 14.3%
GSE31589-GPL4133(5394) -0.02 1.1607 0.98 [0.10; 9.56] 0.0% 0.0%
GSE20713-GPL570(201093_x_at) -0.22 0.5230 0.80 [0.29; 2.23] 0.0% 0.0%
GSE20711-GPL570(201093_x_at) -0.22 0.5230 0.80 [0.29; 2.23] 0.0% 0.0%
GSE15363-GPL1708(17764) -0.41 0.4311 0.66 [0.29; 1.54] 0.0% 0.0%
GSE33926-GPL7264(A_23_P250035) -0.84 0.8095 0.43 [0.09; 2.11] 0.0% 0.0%
Fixed effect model 1.00 [1.00; 1.00] 100.0% --
Random effects model 1.00 [1.00; 1.00] -- 100.0%
Heterogeneity: I^2 = 38%, τ^2 < 0.0001, p < 0.01
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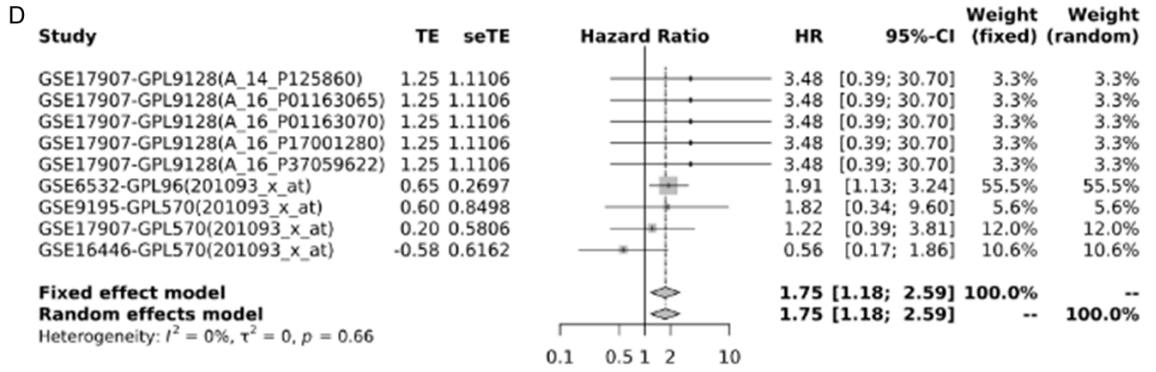

Figure 2. Prognostic significance of Succinate Dehydrogenase Complex Flavoprotein Subunit A (SDHA) expression in breast cancer. A. Kaplan-Meier overall survival (OS) analysis of SDHA expression in breast cancer. B. Kaplan-Meier disease-free survival (DFS) analysis of SDHA expression in breast cancer. C. Forest plot from the GENT2 database showing the OS results of SDHA in breast cancer. D. Forest plot from the GENT2 database showing DFS results of SDHA in breast cancer. $P < 0.05$.

Figure 4D presents the CNV analysis of SDHA in breast cancer. The pie chart shows that SDHA has a significant copy number alteration, with a notable proportion of heterozygous amplification (Hete. Amp.) (red) observed in breast cancer cases. This amplification could lead to increased SDHA expression, contributing to its overactivation in tumor cells (**Figure 4D**).

Immune infiltration and drug sensitivity analysis of SDHA in breast cancer

This study performed immune infiltration and drug sensitivity analysis of SDHA using GSCA database. In **Figure 5A**, the plot reveals that SDHA expression was negatively correlated with several immune cell types, including CD4+ T cells, CD8+ T cells, Th17, and Th2 cells. The negative correlation (blue) observed in these immune cells suggests that higher SDHA expression may be associated with lower infiltration of certain immune cells in the breast cancer microenvironment (**Figure 5A**). **Figure 5B** presents the correlation between SDHA expression and the sensitivity to therapeutic drugs using the GDSC database via the GSCA. The plot indicates that SDHA expression was positively correlated with resistance to various drugs (red color represents resistance). Notably, drugs such as AP-24534, BHG712, and Dabrafenib show a strong positive correlation with SDHA, suggesting that higher SDHA expression is associated with reduced drug sensitivity (**Figure 5B**). This highlights that SDHA overexpression may contribute to chemoresistance, making breast cancer cells less responsive to these specific therapeutic agents.

PPI network construction and gene enrichment analysis of SDHA-associated partners

To better understand the biological roles and clinical relevance of SDHA in breast cancer, we constructed PPI network of SDHA via GeneMANIA database and performed gene enrichment analysis of SDHA and its other associated partners using DAVID tool. **Figure 6A** presents the PPI network of SDHA, which highlights its interactions with key mitochondrial proteins, including SDHB, SDHC, SDHD, and FH. **Figure 6B** provides evidence that SDHA and its associated partners were involved in cellular respiration and mitochondrial activity related cellular component terms. **Figure 6C** shows the gene molecular function enrichment analysis of SDHA and its binding partners. This analysis identifies mitochondrial respiratory complexes as significantly enriched, particularly the Succinate-CoA ligase complex and succinate dehydrogenase complex (**Figure 6C**). **Figure 6D** further explores the biological processes associated with SDHA, including cellular respiration and energy derivation by oxidation of organic compounds. Additionally, there is enrichment in purine metabolism, which is vital for nucleotide synthesis and cell proliferation (**Figure 6D**). **Figure 6E** presents disease-associated pathways enriched in SDHA and its binding partners. The analysis reveals significant involvement in metabolic disorders, such

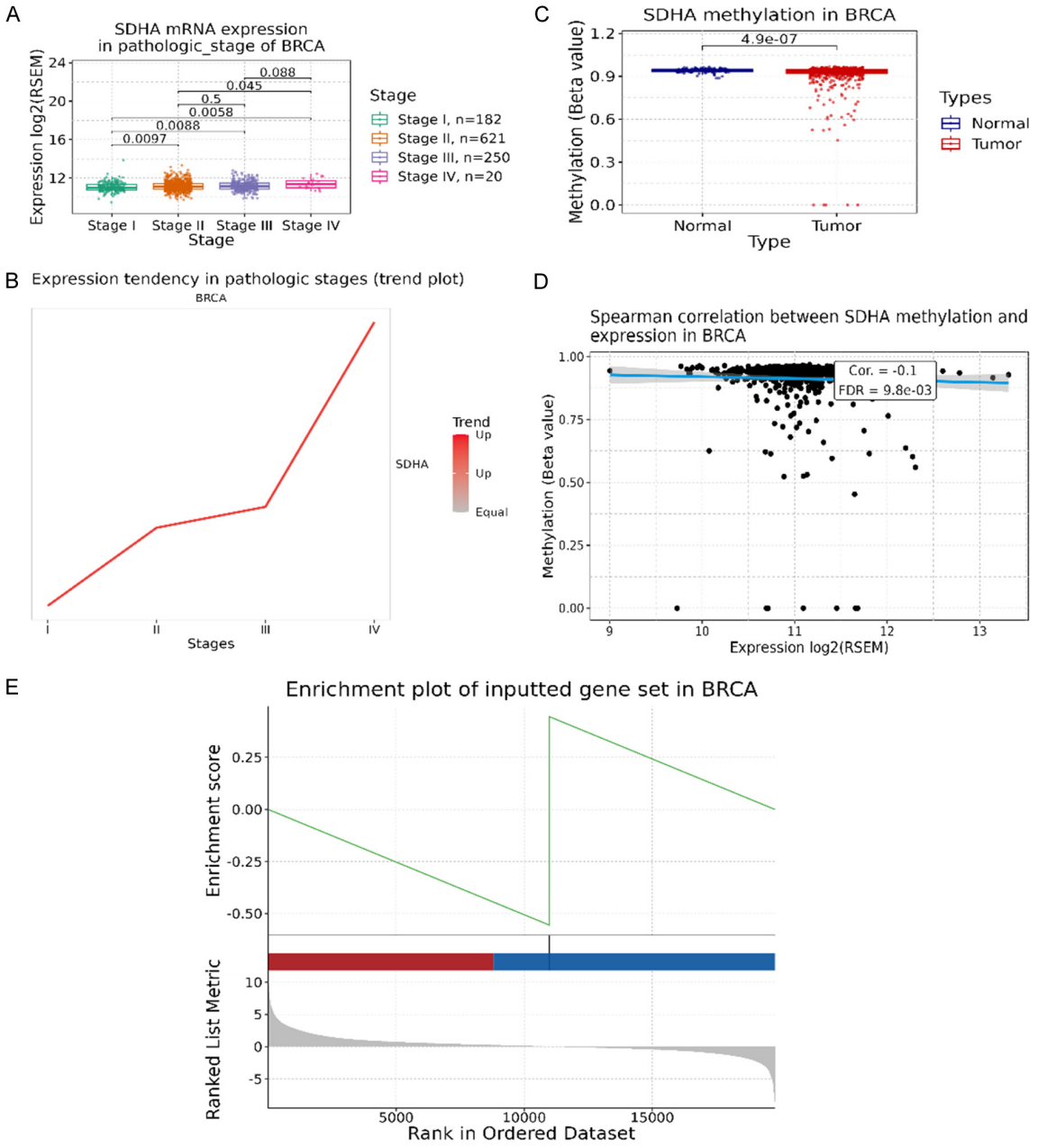

Figure 3. Pathological stage-specific expression, methylation, and enrichment analysis of Succinate Dehydrogenase Complex Flavoprotein Subunit A (SDHA) in breast cancer. A, B. SDHA mRNA expression across various pathological stages of breast cancer. C. SDHA methylation levels in normal and breast cancer tissues. D. Negative correlation between SDHA methylation and SDHA expression in breast cancer. E. Gene Set Enrichment Analysis (GSEA) showing significant enrichment of SDHA in breast cancer development. $P < 0.05$.

as non-alcoholic fatty liver disease, Parkinson's disease, and Alzheimer's disease, highlighting the broad relevance of SDHA in conditions related to mitochondrial dysfunction (**Figure 6E**). These findings indicate that SDHA's role extends beyond cancer metabolism and may influence the pathophysiology of neurodegenerative diseases.

Functional implications of SDHA knockdown in MCF-7 and SKBR3 breast cancer cell lines

To explore the functional implications of SDHA in MCF-7 and SKBR3 breast cancer cell lines, we performed a series of functional assays following SDHA knockdown using siRNA. The goal was to assess how SDHA depletion affects key

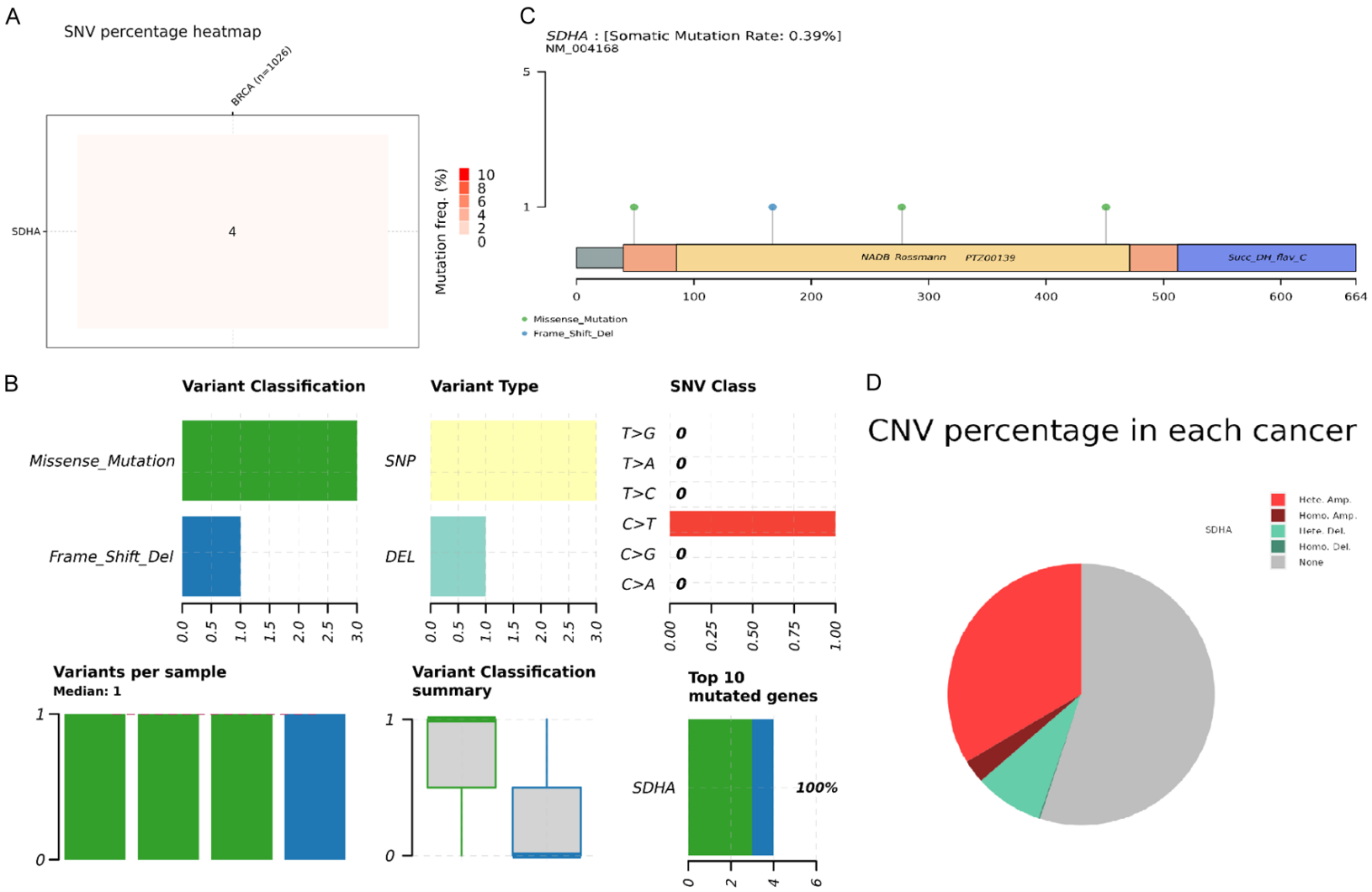

Figure 4. Mutational and copy number variation (CNV) analysis of Succinate Dehydrogenase Complex Flavoprotein Subunit A (SDHA) in breast cancer. A. SNV percentage heatmap of SDHA in breast cancer. B. Breakdown of SDHA mutation types and variant classifications. C. Specific mutation types in SDHA, showing the prevalence of C>T transitions. D. CNV) analysis of SDHA in breast cancer, showing significant heterozygous amplification (Hete. Amp.) in a notable proportion of breast cancer cases. $P < 0.05$.

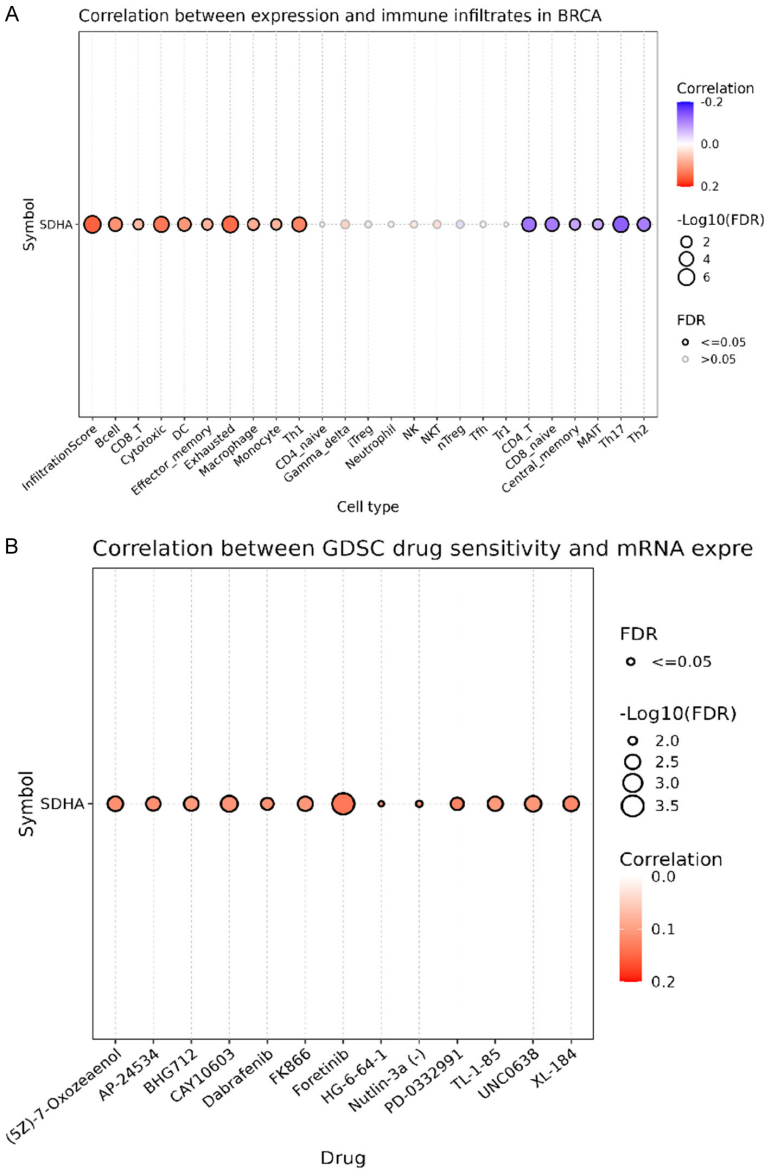

Figure 5. Immune infiltration and drug sensitivity analysis of Succinate Dehydrogenase Complex Flavoprotein Subunit A (SDHA) in breast cancer. A. Correlation between SDHA expression and immune cell infiltration in breast cancer. B. Correlation between SDHA expression and drug sensitivity. $P < 0.05$.

cellular processes involved in proliferation, colony formation, and wound healing, which are crucial for tumor growth and metastasis. In both MCF-7 and SKBR3 cell lines, successful SDHA knockdown was confirmed at both the mRNA and protein levels, leading to the significant ($P < 0.01$) depletion in its expression (**Figures 7A**, **7B**, **8A**, **8B** and Supplementary Figure 1). Proliferation and colony formation assays (**Figures 7C**, **7D**, **8C**, **8D**) demonstrated a profound impact on cell growth following SDHA depletion. In both MCF-7 and SKBR3 cells, SDHA knockdown resulted in a significant reduction in cell proliferation ($P < 0.01$) and colony formation ($P < 0.01$), indicating that SDHA is vital for sustaining cell growth and survival (**Figures 7C-E**, **8C-E**). Wound healing assay results (**Figures 7F-H**, **8F-H**) showed a significant decrease in cell migration after SDHA knockdown. Wound closure was significantly impaired in the si-SDHA-treated cells in both MCF-7 and SKBR3 cell lines ($P < 0.01$).

Discussion

Breast cancer remains one of the most common and aggressive malignancies worldwide, with high incidence rates and significant mortality, particularly due to metastasis and relapse in advanced stages [5, 37, 38]. According to the American Cancer Society, breast cancer is the most diagnosed cancer in women, accounting for approximately 30% of all cancers in women globally [39]. Despite considerable advancements in early detection, chemotherapy, and targeted therapies, the prognosis for patients with advanced breast cancer, especially those with metastatic or recurrent disease, remains poor [40, 41]. The high variability in treatment response and the lack of targeted therapies for certain subtypes underscore the need for deeper molecular insights into the mechanisms driving breast cancer progression, metastasis, and therapeutic resistance [42, 43].

SDHA is a crucial component of the mitochondrial succinate dehydrogenase complex, which links the TCA cycle to the electron transport chain in cellular respiration [15]. SDHA plays a pivotal role in cellular energy production and is integral to maintaining metabolic homeostasis

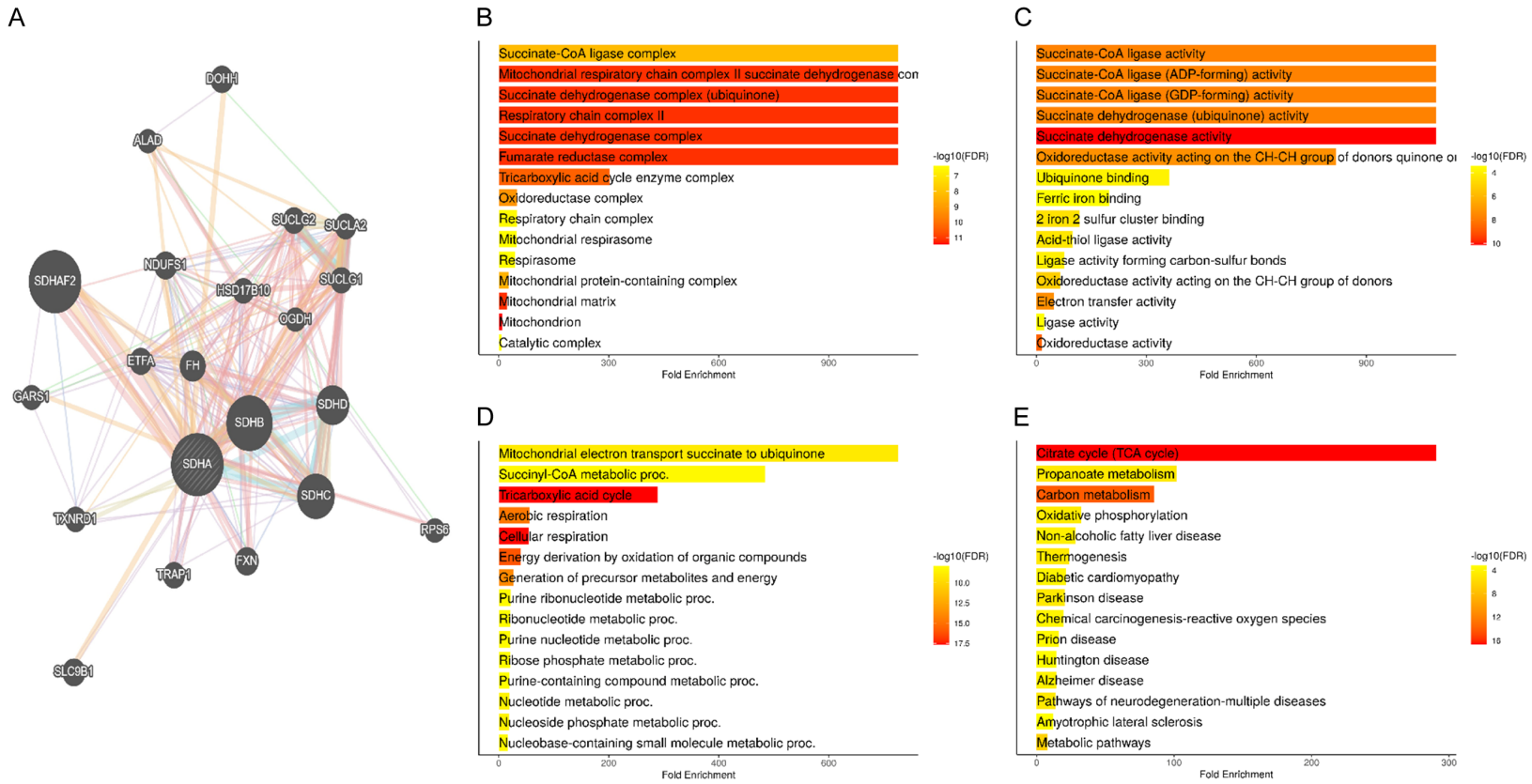

Figure 6. PPI network construction and gene enrichment analysis of Succinate Dehydrogenase Complex Flavoprotein Subunit A (SDHA)-associated partners. A. PPI network of SDHA constructed via GeneMANIA database. B. Cellular component terms. C. Molecular function terms. D. Biological processes terms. E. Disease-associated pathways enriched in SDHA and its binding partners. $P < 0.05$.

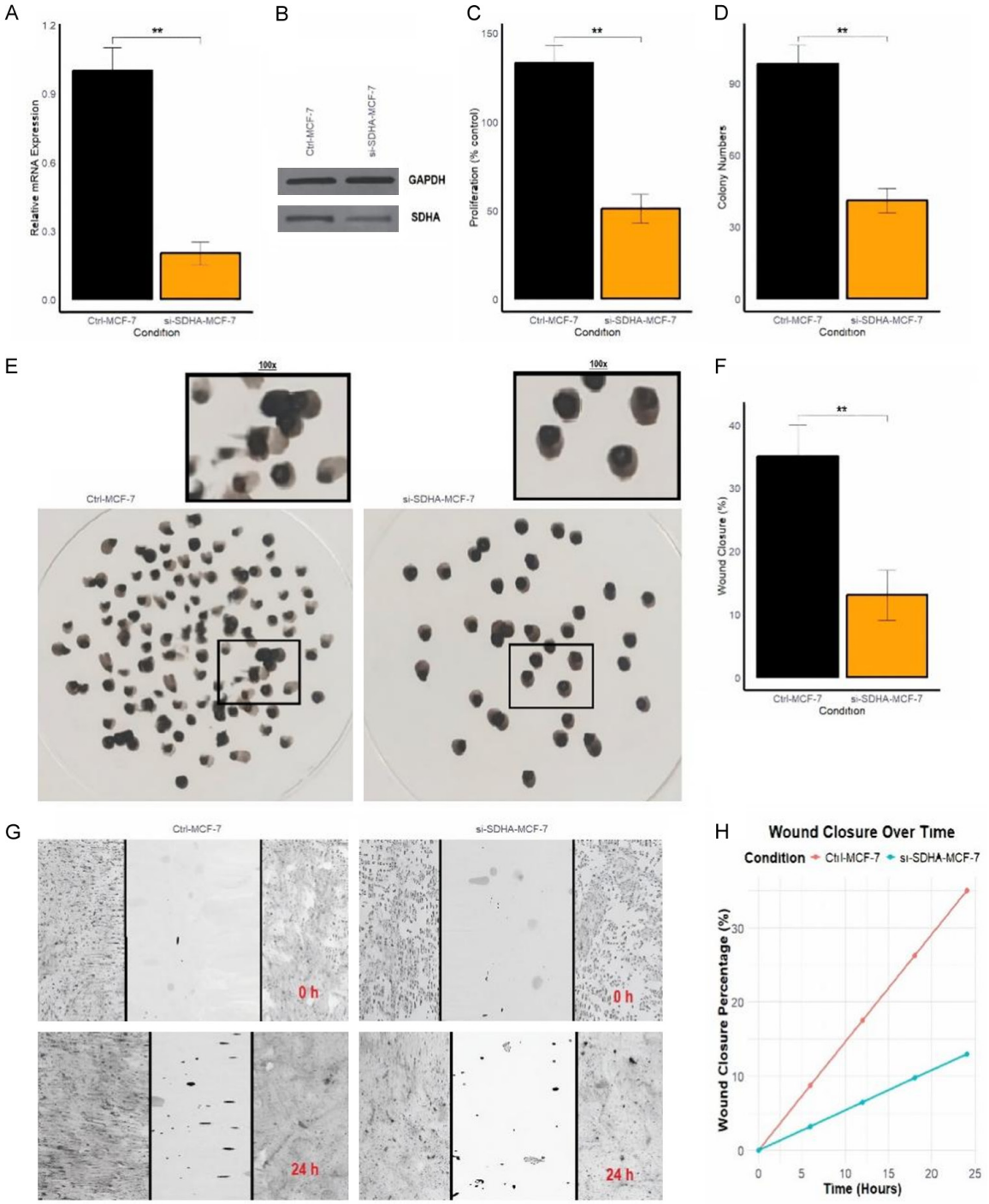

Figure 7. Functional implications of Succinate Dehydrogenase Complex Flavoprotein Subunit A (SDHA) knockdown in MCF-7 breast cancer cell line. A, B. Confirmation of SDHA knockdown at both mRNA and protein levels in MCF-7 cells. C-E. Proliferation and colony formation assays demonstrating that SDHA knockdown in MCF-7 cells leads to a significant reduction in cell proliferation and colony formation. F-H. Wound healing assay results showing significantly impaired wound closure in si-SDHA-treated MCF-7 cells. P** < 0.01.

[44]. In cancer cells, altered metabolism is a hallmark feature, known as the Warburg effect, where cells preferentially undergo aerobic glycolysis over oxidative phosphorylation, even in the presence of oxygen [45, 46]. This metabolic shift is often associated with the activation of specific enzymes like SDHA, which may enable tumor cells to adapt to the increased energy

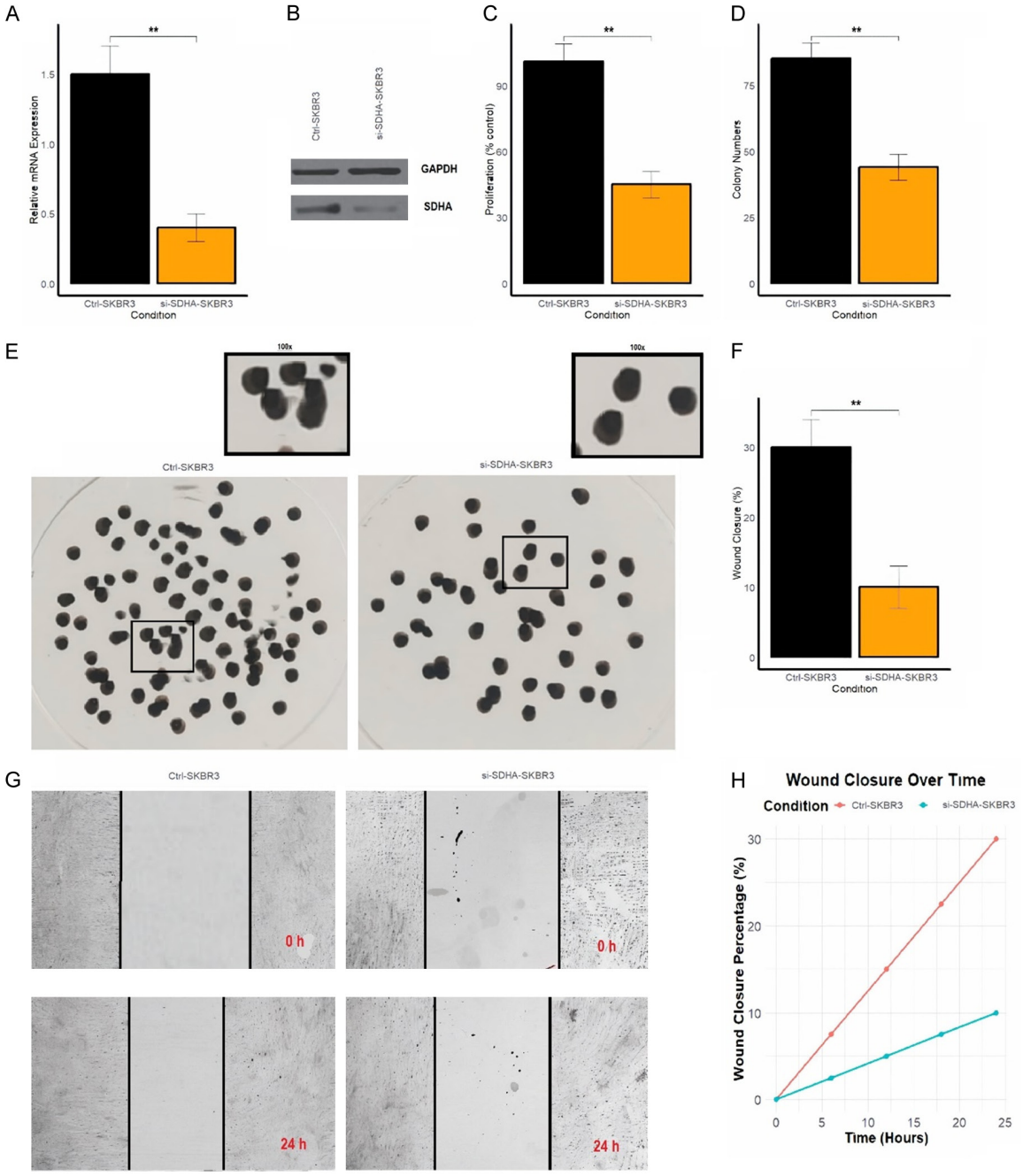

Figure 8. Functional implications of Succinate Dehydrogenase Complex Flavoprotein Subunit A (SDHA) knockdown in SKBR3 breast cancer cell line. A, B. Confirmation of SDHA knockdown at both mRNA and protein levels in SKBR3 cells. C-E. Proliferation and colony formation assays demonstrating that SDHA knockdown in SKBR3 cells leads to a significant reduction in cell proliferation and colony formation. F-H. Wound healing assay results showing significantly impaired wound closure in si-SDHA-treated SKBR3 cells. P** < 0.01.

demands and hypoxic conditions typical of growing tumors. Recent studies have highlighted the potential role of SDHA in various cancers, including gastrointestinal tumors, pheochromocytomas, and paragangliomas [47, 48]. These studies suggest that mutations and dysregulation of SDHA may lead to altered metabolic pathways, contributing to tumorigenesis and progression [49, 50]. Notably, SDHA mutations have been linked to familial paraganglioma syndrome, a condition that predisposes individuals to specific types of tumors [51].

However, the role of SDHA in breast cancer remains underexplored, and its exact contribution to the progression and prognosis of this disease was not fully understood prior to this study.

In this study, we focused on investigating the expression, functional significance, and therapeutic potential of SDHA in breast cancer. We performed a comprehensive analysis of SDHA expression across different breast cancer subtypes using databases like TIMER2, TISIDB, and GSCA, alongside survival analysis tools. Our results indicate that SDHA was significantly overexpressed in breast cancer tissues compared to normal breast tissues, with higher expression levels observed particularly in the Basal and Her2 molecular subtypes. These findings are consistent with previous studies showing elevated levels of SDHA in other cancers [47, 50, 52, 53], though our study extends this to breast cancer, particularly the more aggressive subtypes. Furthermore, our survival analysis indicated that high SDHA expression correlates with poor prognosis in breast cancer patients, both in terms of OS and DFS. In line with our results, several studies have demonstrated that high expression of SDHA is associated with poor prognosis and therapy resistance in various cancers, including gastrointestinal tumors and neuroendocrine cancers. For instance, SDHA overexpression has been linked to worse clinical outcomes in gastrointestinal cancers and renal cell carcinoma due to its role in enhancing cellular metabolism and survival under stressful conditions [54, 55]. However, our findings should be interpreted with caution, as Kaplan-Meier survival analysis, which we used to assess the relationship between SDHA expression and prognosis, can be influenced by several confounding factors, such as patient age, tumor stage, and treatment regimen. If these factors differ significantly between the high and low SDHA expression groups, the observed survival differences may not be entirely attributable to SDHA expression levels alone. While SDHA's role in cancer cell metabolism is well-established, its relationship with patient prognosis may vary across different cancer types. For example, in pancreatic cancer, SDHA expression was found to correlate with better prognosis, suggesting that the prognostic impact of SDHA may be context-dependent and influenced by the specific metabolic pathways active in different tumors [56]. The role of SDHA as a potential tumor suppressor or oncogene might also vary according to the cancer type and its underlying metabolic reprogramming. Therefore, further investigation is required to account for potential confounding variables, and future studies should consider multivariate survival models that adjust for patient demographics, tumor stage, and treatment history to more accurately assess the prognostic value of SDHA in breast cancer.

To explore the functional implications of SDHA in breast cancer, we utilized siRNA-mediated knockdown in MCF-7 and SKBR3 cell lines. Our functional assays revealed that SDHA depletion significantly impaired cell proliferation, colony formation, and cell migration, suggesting that SDHA is vital for the tumorigenic potential and metastatic capability of breast cancer cells. These findings align with previous studies in other cancer types, where SDHA inhibition resulted in reduced cell growth and motility [57, 58]. Notably, our study also demonstrated that SDHA knockdown in MCF-7 and SKBR3 cells led to reduced wound closure, indicating a potential role of SDHA in tumor migration and metastasis.

Additionally, SDHA methylation and CNVs were analyzed in breast cancer. Our results showed that SDHA exhibited significant hypomethylation in breast cancer tissues, which correlated with increased expression, supporting the notion that epigenetic regulation plays a critical role in SDHA dysregulation in breast cancer [59-62]. Furthermore, heterozygous amplification of SDHA was observed in a subset of breast cancer cases, contributing to its overexpression, which has been linked to chemoresistance in several cancers [55]. This aligns with our findings from the drug sensitivity analysis, where higher SDHA expression was associated with resistance to multiple therapeutic agents.

The findings of this study corroborate previous reports in other cancer types that highlight the importance of SDHA in cellular metabolism, especially in metabolic reprogramming during tumorigenesis. Studies in gastrointestinal tumors and neuroendocrine tumors have shown that SDHA mutations or dysregulation can lead to altered metabolism, promoting tumor growth and metastasis [19]. However, our study uni-

quely contributes to the existing body of research by showing that SDHA overexpression in breast cancer correlates with poor prognosis and is associated with reduced sensitivity to chemotherapy, indicating that SDHA may act as both a diagnostic and prognostic marker. Our analysis also extends the role of SDHA beyond its metabolic function, providing evidence for its involvement in immune evasion and chemoresistance, which are critical barriers in the treatment of breast cancer. The negative correlation between SDHA expression and immune cell infiltration suggests that high SDHA levels may suppress immune response in the tumor microenvironment, similar to other metabolic reprogramming strategies employed by cancer cells to evade immune surveillance.

The novelty of our study lies in the comprehensive multi-dimensional analysis of SDHA expression, molecular subtypes, pathological staging, and functional assays in breast cancer. By correlating SDHA expression with prognostic outcomes (OS and DFS), immune infiltration, and drug sensitivity, our study provides valuable insights into the broader role of SDHA in breast cancer metabolism and its potential as a target for therapeutic interventions. Furthermore, our findings regarding SDHA's involvement in chemoresistance and immune modulation represent an important new avenue for future research, suggesting that SDHA may not only be a key metabolic regulator but also an important player in cancer progression and therapy resistance.

While in vitro models like MCF-7 and SKBR3 breast cancer cell lines provide valuable insights, they have several limitations that may affect the generalization of the findings to in vivo conditions. These models lack the complexity of the tumor microenvironment (TME), including interactions with immune cells, vascularization, and extracellular matrix components, which are crucial for tumor progression and metastasis. Additionally, the lack of tumor heterogeneity in these cell lines and the inability to study organ-specific metastasis or long-term therapeutic responses limit the accuracy of the results. In vivo models, such as xenografts or patient-derived xenografts (PDX), are necessary to better replicate the immune interactions, drug resistance mechanisms, and metastatic processes, ensuring that the therapeutic implications of SDHA targeting are fully validated.

Conclusion

In summary, our study provides compelling evidence that SDHA overexpression plays a significant role in breast cancer progression and chemoresistance, particularly in aggressive subtypes like Basal and Her2. The data from our expression analysis, functional assays, and molecular studies suggest that SDHA is a promising prognostic biomarker and therapeutic target for improving the treatment of breast cancer. Future studies should focus on developing SDHA-targeted therapies to enhance treatment efficacy and overcome chemoresistance in breast cancer patients.

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Disclosure of conflict of interest

None.

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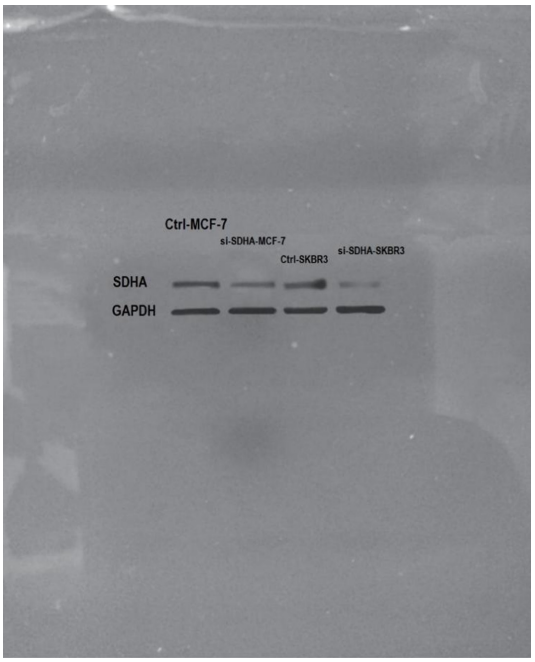

Supplementary Figure 1. Uncut Western blot bands of Succinate Dehydrogenase Complex Flavoprotein Subunit A (SDHA) and Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH).