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High SET Domain Bifurcated 1 (SETDB1) Expression Predicts Poor Prognosis in Breast Carcinoma

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Background: SETDB1, an H3K9-specific histone methyltransferase, plays important roles in the progression of various human cancers. However, the expression patterns and its clinical roles of SETDB1 remain elusive in breast cancer (BC).





Material/Methods: The transcriptional level of SETDB1 and survival data in BC were analyzed through UALCAN, ONCOMINE, and Pan Cancer Prognostics Database. SETDB1 protein expression was assessed by immunohistochemistry (IHC) in 159 BC tissue samples. The associations between SETDB1 expression and clinical pathological characteristics of patients were analyzed. The GEO dataset GSE108656 was downloaded and analyzed to identify the differentially expressed genes (DEGs) between control and BC cells targeting interference with SETDB. The DEGs were further integrated by bioinformatics analysis to decipher the key signaling pathways and hub genes that are regulated by SETDB.

Results: The public databases showed the level of SETDB1 mRNA was significantly upregulated in BC. Our IHC results demonstrated the level of SETDB1 protein was associated with tumor size ($P=0.028$), histopathological grading ($P=0.012$), lymph node metastasis ($P<0.001$), and TNM stage ($P<0.001$). High expression of SETDB1 indicated worse overall survival ($P=0.015$) and shorter relapse-free survival ($P=0.027$). The bioinformatic analysis of GSE108656 suggested that the SETDB1-related DEGs was mainly enriched in antigen processing and presentation, as well as immune networks in BC. The cytoHubba analysis suggested the top 10 hub genes were IL6, BMP4, CD74, PECAM1, HLA-DPA1, HLA-DRA, LAMC1, CTSB, SERPINA1, and CTSD.

Conclusions: The results suggest that SETDB1 is an oncogene and can serve as a prognostic biomarker for BC. However, the mechanisms of SETDB1 in BC remain to be explored.

MeSH Keywords: **Immunohistochemistry • Prognosis • Triple Negative Breast Neoplasms**

Full-text PDF: <https://www.medscimonit.com/abstract/index/idArt/922982>

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Background

Breast cancer (BC), one of the most common malignancies in the world, is correlated with a high mortality rate [1]. In China, its incidence increased approximately 30%, and the related mortality has doubled over the past 30 years [2,3]. Although specific biomarkers are routinely used to predict response to therapy and prognoses, the underlying cause of development of this tumor is largely unknown, and clinically useful prognostic and predictive parameters are still insufficient. Thus, it is important to develop and broaden additional prognostic biomarkers.

SETDB1 (SET domain bifurcated 1), an H3K9-specific histone methyltransferase, locates on human chromosome 1q21.3 with a length of about 38.6 Kb. The SETDB1 protein belongs to the SET domain protein methyltransferase family and plays important roles in heterochromatin formation and gene expression [4]. As a H3K9 methyltransferase, the physiological function of SETDB1 has been associated with gene silencing in mammalian development [5], particularly involving heterochromatin formation [6], stem cell maintenance [7], and endogenous retrovirus genes repression [8]. In the field of oncology, SETDB1 has been observed to be deregulated in various human carcinogenesis, including colorectal cancer, lung cancer, melanoma, and BC [9–13]. Moreover, abnormal expression of SETDB1 has been found to be a diagnostic biomarker of patient survival in the 2 major forms of human non-small cell lung carcinoma (adenocarcinoma and squamous cell carcinoma) [11]. Thus, SETDB1 has been regarded as a key methyltransferase in the progression of human cancer.

Previous studies showed the level of SETDB1 was significantly elevated in BC, and SETDB1 was involved in BC tumorigenesis [13–15]. Most of these previous studies were performed on transcriptome or gene levels. A recent study performed immunohistochemical staining (IHC) of SETDB1 in 43 BC tissues and showed a significant increase in the SETDB1 level in BC samples [13]. The present study further investigated the expression of SETDB1 by IHC and its clinical performance in a cohort of 159 patients with BC. Our results demonstrated that the level of SETDB1 expression was associated with some pathological parameters in BC. Moreover, we deciphered the potential biological function of SETDB1 by bioinformatics analysis of GEO data, which was based on BC cells targeting interference with SETDB1. Therefore, it SETDB1 appears to play an important role in BC.

Material and Methods

Analysis of SETDB1 on the transcriptional level in public databases

The SETDB1 expression in BC was analyzed using public databases. UALCAN is a comprehensive and interactive web resource for analyzing cancer OMICS data, which includes the transcriptional and clinical data from TCGA and MET500 databases (<http://ualcan.path.uab.edu>) [16]. We used UALCAN to compare the transcriptional levels of SETDB1 among various cancer stages, and the SETDB1 expression across 20 cancer types was explored using the ONCOMINE database (www.oncomine.org) [17].

IHC of SETDB1 expression in BC tissues

All of the specimens, fixed with 10% formalin and paraffin-embedded in wax blocks, were retrospectively collected from patients who had undergone BC resection in Huashan Hospital of Fundan University between January 2000 and December 2010. These tissues were obtained from 159 patients with an average age of 55.23 years (range 34.4–85.5). All the patients met the following criteria: i) patients were diagnosed as primary mammary carcinoma; and ii) patients received chemotherapy or radiation therapy before surgery [18]. All the patients were diagnosed according to the AJCC/UICC TNM Classification and Stage grouping system [19]. Prior to the study, all patients provided informed consent approved by the local ethics committee. The expression of SETDB1IHC was determined by IHC. Antigen retrieval was performed with 0.01 M citrate buffer at 95°C for 30 min, followed by washing with PBS buffer. Then, slides were incubated with diluted anti-human primary antibodies against SETDB1 (1: 80; Proteintech, IL), anti-ER (1: 100, Sigma-Aldrich, MO), anti-PR (1: 100, Sigma-Aldrich, MO), and anti-HER-2 (1: 100 Newcastle upon Tyne, UK) at 4°C overnight, followed by incubations with HRP-conjugated secondary antibody and diaminobenzidine as chromogen. Slides were again counterstained with hematoxylin. The negative controls were incubated with PBS in place of primary antibody in the slides. Each slide was assessed by 2 independent pathologists to obtain average percentage and the intensity of immunostained cells. The average percentage was scored as follows: 0 (0%); 1 (1–25%); 2 (26–50%); 3 (51–75%); and 4 (76–100%). The intensity of stained cells was scored according to the following criteria: 0 (negative); 1 (weak); 2 (moderate), and 3 (strong). The final IHC scores were calculated by multiplying the staining intensity (0 to 3) by percentage of tumor cells [20].

Bioinformatics analysis of GSE108656

To explore the potential mechanism, the GEO database was searched to study the roles of SETDB1 in BC. The search details

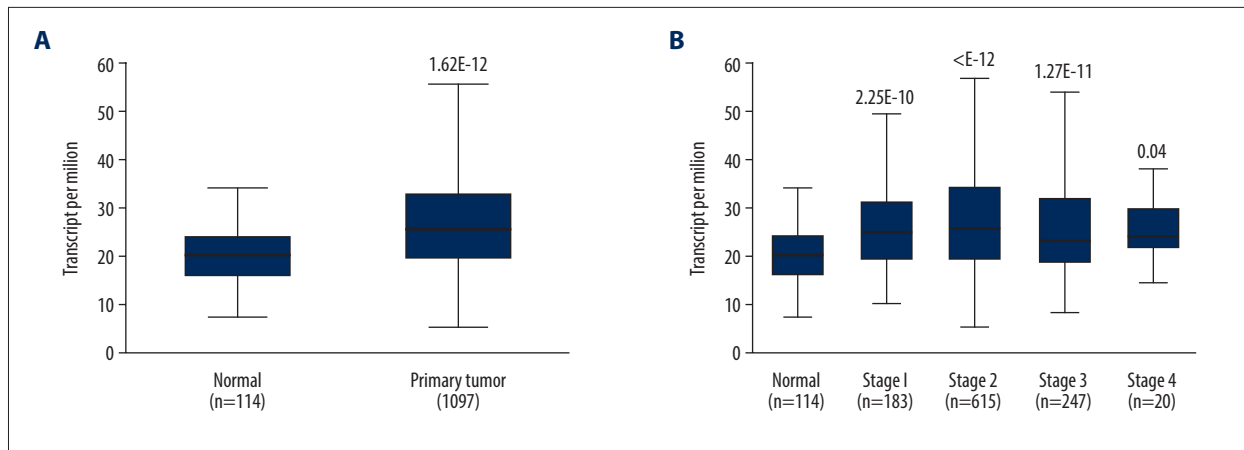


Figure 1. Expression levels of SETDB1 gene are upregulated in breast cancer. (A) Box plot showed the relative expression of SETDB1 in tumor tissues compared with non-tumor tissues from the UALCAN database. (B) Box plot shows relative expression of SETDB1 in normal individuals or in BC patients with different clinical stages. * $P < 0.01$.

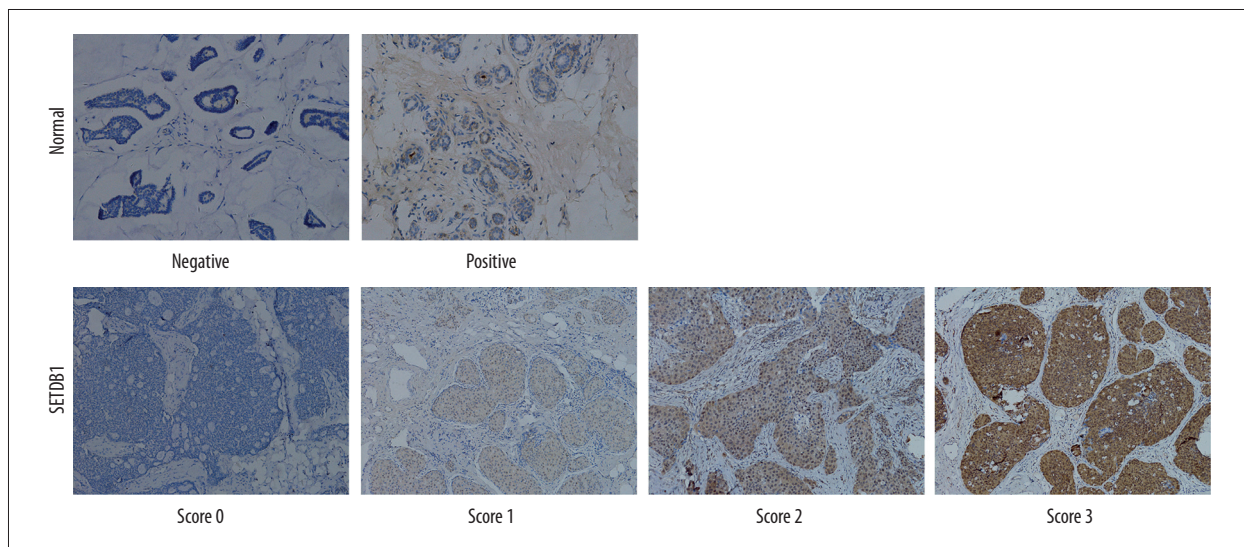


Figure 2. Representative immunostaining of SETDB1 in breast cancer. Representative immunostaining of SETDB1 scaled 0, 1, 2, 3, along with positive and negative controls. Sections of human normal breast tissues were used as control. Negative controls were incubated with immunoglobulin fraction in place of polyclonal primary antibody. All representative images were taken at power of $\times 100$.

were (SETDB1[Description] AND breast cancer[Description]) AND “Homo sapiens”[porgn: __txid9606]) AND “Homo sapiens”[porgn: __txid9606]. The GSE108656 dataset was included in our analysis. GSE108656 is an expression profile of the human breast cancer cell line MDA-MB-231 upon silencing of SETDB1. The dataset was based on the GPL10558 Illumina Human HT-12V4.0 expression bead chip platform. The raw data were analyzed using the GEO2R tool. To screen differentially expressed genes (DEGs) between SETDB1-silenced cells and control, the adjusted p -value < 0.05 and $|\log_2FC| > 2.0$ were used as cutoff criteria [21]. A volcano plot was applied to show the DEGs in GSE108656.

For the function annotation of SETDB1, the DEGs were analyzed for Gene Ontology (GO) terms (biological process) using the GO database, an online database that provides a comprehensive interpretation of summed genes. Kyoto encyclopedia of genes and genomes (KEGG) pathway was enriched by using the DAVID database (Version 6.7). $P < 0.05$ was set as a cutoff for significance.

STRING database was used to obtain the networks of DEGs. To evaluate the interactive associations among SETDB1-related DEGs, the obtained DEGs were analyzed by STRING to get the PPI network, which was visualized by Cytoscape software (version 3.4.0). Moreover, the hub genes were screened by using

Table 1. Correlation of SETDB1 with clinicopathological factors (n=159).

Variable	Total	SETDB1 expression		r	p-Value
		Low	High		
All cases	159	78	81		
Age (year)					
<50	58	25	33	-0.09	0.258
≥50	101	53	48		
Tumor size(cm)					
<2.5	90	51	39	0.174	0.028*
≥2.5	69	27	42		
Lymph-node metastasis					
Negative	97	64	33	0.423	<0.001*
Positive	62	14	48		
TNM classification					
I	59	41	18	0.409	<0.001*
II	66	33	33		
III	32	4	28		
IV	2	0	2		
Histological grade					
I	19	15	4	0.198	0.012*
II	114	53	61		
III	26	10	16		
ER status					
Negative	65	27	38	-0.125	0.116
Positive	94	51	43		
PR status					
Negative	86	43	43	0.020	0.798
Positive	73	35	38		
HER-2 status					
Negative	61	29	32	-0.024	0.765
Positive	98	49	49		
Subtype					
Luminal A	37	20	17	0.134	0.093
Luminal B	63	35	28		
HER-2overexpression	35	14	21		
TNBC	24	9	15		

the Cytoscape plugin cytoHubba. The set parameters in cytoHubba were as follows: hubba nodes: top 10 nodes ranked by degree, display options: check the first-stage nodes, display the shortest path, and display the expanded subnetwork.

Statistical analyses

Statistical analyses were performed using SPSS 22.0 software (IBM SPSS Statistics, Armonk, NY, USA). Using web-based bioinformatics tools, statistical analysis was automatically obtained. To assess the relationship between SETDB1 expression and clinical parameters, categorical variables were analyzed using

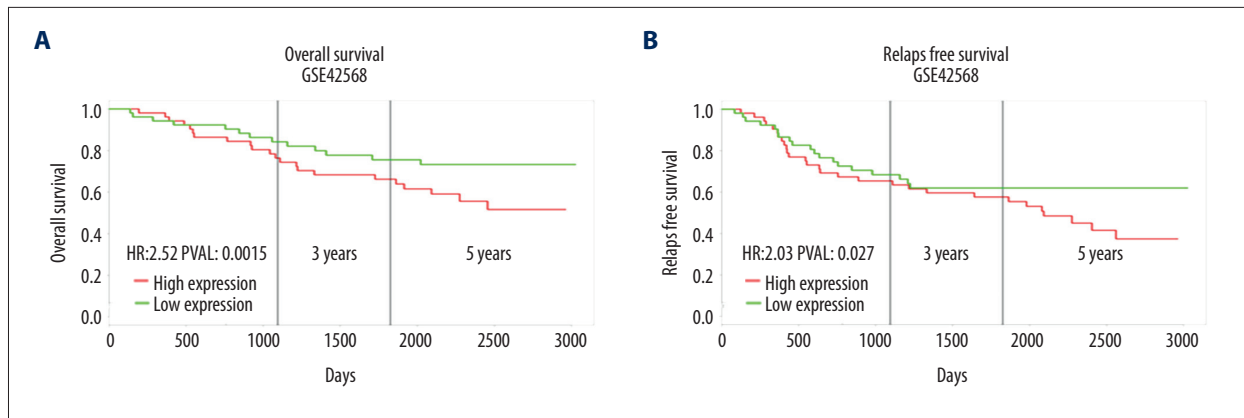


Figure 3. The prognostic value of SETDB1 in breast cancer. Kaplan-Meier curves showed high mRNA expression levels of SETDB1 was associated with unfavorable overall survival (A) and relapse-free survival (B) in patients with breast cancer.

the chi-squared tests or Fisher's exact test [22]. $P < 0.05$ was considered a statistically significant difference.

Results

SETDB1 overexpression in BC within the public databases

The transcriptional level of SETDB1 in BC was analyzed within the UALCAN database. The results showed the levels of SETDB1 were relatively upregulated in BC (Figure 1A). In terms of tumor stages, the levels of SETDB1 were significantly elevated in all stages compared with normal counterparts (Figure 1B). Intriguingly, SETDB1 was not only upregulated in BC, but was also induced in other types of cancers, including cervical cancer, liver cancer, lymphoma, and sarcoma according to the ONCOMINE database (Supplementary Figure 1).

Correlation of SETDB1 expression with patient survival in BC

To verify the transcriptional levels of SETDB1 in BC, 159 BC tissues were subjected to IHC assay. Expectedly, SETDB1 was upregulated in BC tissues relative to the normal controls. Representative immunostaining of SETDB1 with different staining levels (score: 0~3) along with negative and positive controls are shown in Figure 2. Next, we determined the relationship between the protein levels of SETDB1 and the clinical parameters of patients with BC. Our results demonstrated that high level of SETDB1 expression was correlated with tumor size ($P=0.028$), lymph node metastasis ($P < 0.001$), clinical stage ($P < 0.001$), and histological grading ($P=0.012$). No significant correlation was observed between SETDB1 expression levels and age and receptor status ($P > 0.05$, Table 1).

To explore the prognostic value of SETDB1 in BC, the relationship between the level of SETDB1 and survival time was assessed

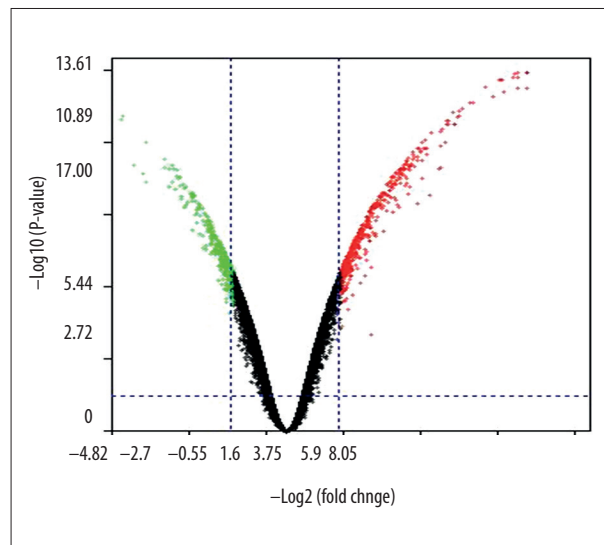


Figure 4. Identification of the DEGs between SETDB1-silenced BC cells and control. The volcano plot shows the DEG distributions in both up- and downregulated genes. The green color shows the downregulated genes, while the red indicates the upregulated genes.

by using an online database (<http://genomics.jefferson.edu>). SETDB1 overexpression was significantly correlated with poor overall survival ($P=0.015$; Figure 3A). Then, we analyzed the correlation of relapse-free survival with SETDB1 expression among patients with BC. These results also showed that patients with high SETDB1 expression experienced significantly shorter relapse-free survival than those with low SETDB1 expression ($P=0.027$; Figure 3B). Taken together, our results suggest that the expression of SETDB1 is a prognostic indicator for BC patients.

Identification of DEGs in GSE108656

To explore the potential mechanism underlying SETDB1-promoted BC, we searched the GEO database and obtained the

Table 2. Go analysis of differentially expressed genes in Biological processes.

Term	Count	P value	Genes
GO: 0009605~response to external stimulus	40	1.24E-08	KYNU, SHROOM3, F2RL1, ITGB2, GPR68, MDK, TIMP3, TYMP, SAA2, MAP1LC3A, CXCR4, BCHE, GSN, SAA1, SERPINA3, SERPINA1, CFD, SREBF1, TXNIP, BMP4, MUC1, IL6, CMKLR1, CFB, RXRA, SAA4, ITGA2, CHST4, NLRP3, COL5A1, AOX1, ECSCR, CTSD, PLLP, ID3, CTSB, ENG, CD14, IGFBP4, F2R
GO: 0009611~response to wounding	28	8.70E-08	F2RL1, ITGB2, GPR68, TIMP3, MDK, SAA2, CXCR4, SAA1, GSN, SERPINA3, SERPINA1, CFD, IL6, CFB, RXRA, SAA4, ITGA2, CHST4, NLRP3, COL5A1, AOX1, PLLP, ID3, CTSB, ENG, CD14, IGFBP4, F2R
GO: 0051223~regulation of protein transport	13	2.52E-07	BMP4, PRKCZ, IL6, SAA2, SAA1, ANG, PYCARD, BCL3, NLRP3, PKIA, NLRP2, LCP1, SRGN
GO: 0070201~regulation of establishment of protein localization	13	4.85E-07	BMP4, PRKCZ, IL6, SAA2, SAA1, ANG, PYCARD, BCL3, NLRP3, PKIA, NLRP2, LCP1, SRGN
GO: 0032880~regulation of protein localization	13	1.99E-06	BMP4, PRKCZ, IL6, SAA2, SAA1, ANG, PYCARD, BCL3, NLRP3, PKIA, NLRP2, LCP1, SRGN
GO: 0048513~organ development	53	3.68E-06	S100A4, GYPC, PGF, JAG1, HLA-DMA, SPRY1, ANG, CXCR4, GSN, AR, RXRA, PTPRU, TNS3, G6PD, TAGLN, CST6, LAMC2, LAMC1, SHROOM3, ALDOC, SOX4, EHF, MDK, TIMP3, TAGLN3, CD74, FOXQ1, TYMP, CRISPLD2, PPL, BCL3, AHNK2, ZC3H12A, LFNG, SRGN, DCLK1, COL18A1, BMP4, TXNIP, IL6, EPAS1, FOXA1, ITGA2, COL5A1, GJB2, WNT7B, ID1, LAMA5, ECSCR, PHGDH, ID3, ENG, F2R
GO: 0001525~angiogenesis	13	4.15E-06	COL18A1, BMP4, TYMP, EPAS1, CXCR4, ID1, ANG, LAMA5, PGF, ECSCR, ZC3H12A, JAG1, ENG
GO: 0051674~localization of cell	18	8.21E-06	IL6, S100P, ITGB2, DNAH2, COL5A1, TNS3, TNS1, SAA2, CXCR4, ID1, SAA1, LAMA5, ANG, PRSS3, LAMC1, ENG, DCLK1, LRP5
GO: 0048870~cell motility	18	8.21E-06	IL6, S100P, ITGB2, DNAH2, COL5A1, TNS3, TNS1, SAA2, CXCR4, ID1, SAA1, LAMA5, ANG, PRSS3, LAMC1, ENG, DCLK1, LRP5
GO: 0016477~cell migration	17	8.61E-06	IL6, S100P, ITGB2, COL5A1, TNS3, TNS1, SAA2, CXCR4, ID1, SAA1, LAMA5, ANG, PRSS3, LAMC1, ENG, DCLK1, LRP5

transcriptome of human BC cell lines by silencing of SETDB1 and GSE108656. We used the GEO2R tool to screen the DEGs between control and MDA-MB231 BC cells upon silencing of SETDB1. A total of 323 DEGs were identified from the dataset (available on request). As shown in Figure 4, there were 248 upregulated DEGs and 75 downregulated DEGs.

Functional annotation for DEGs

To identify the DEGs functions, all the DEGs were imported into DAVID. As shown in Table 2, the top 10 significant biological process GO terms were response to external stimulus (GO: 0009605), response to wounding (GO: 0009611), regulation of protein transport (GO: 0051223), regulation of establishment of protein localization (GO: 0070201), regulation of protein localization (GO: 0032880), organ development (GO: 0048513),

angiogenesis (GO: 0001525), localization of cell (GO: 0051674), cell motility (GO: 0048870), and cell migration (GO: 0016477).

For KEGG pathway enrichment analysis (Table 3), a total of 11 pathways were significantly enriched (P≤0.05). Among these pathways, the most significant term was antigen processing and presentation, containing 9 DEGs (KLRC2, KLRC3, HLA-DRB4, HLA-DPA1, CTSB, HLA-DMB, HLA-DMA, CD74, and HLA-DRA). Other enriched terms were: hsa04672: Intestinal immune network for IgA production, hsa05332: Graft-versus-host disease, hsa04940: Type I diabetes mellitus, hsa05310: Asthma, hsa05330: Allograft rejection, hsa05416: Viral myocarditis, hsa04514: Cell adhesion molecules (CAMs), hsa05320: Autoimmune thyroid disease, hsa04512: ECM-receptor interaction, and hsa05200: Pathways in cancer.

Table 3. KEGG pathway analysis of differentially expressed genes.

Term	Count	P value	Genes
hsa04612: Antigen processing and presentation	9	0.0002	KLRC2, KLRC3, HLA-DRB4, HLA-DPA1, CTSB, HLA-DMB, HLA-DMA, CD74, HLA-DRA
hsa04672: Intestinal immune network for IgA production	7	0.0003	IL6, CXCR4, HLA-DRB4, HLA-DPA1, HLA-DMB, HLA-DMA, HLA-DRA
hsa05332: Graft-versus-host disease	6	0.0008	IL6, HLA-DRB4, HLA-DPA1, HLA-DMB, HLA-DMA, HLA-DRA
hsa04940: Type I diabetes mellitus	6	0.0012	CPE, HLA-DRB4, HLA-DPA1, HLA-DMB, HLA-DMA, HLA-DRA
hsa05310: Asthma	5	0.0021	HLA-DRB4, HLA-DPA1, HLA-DMB, HLA-DMA, HLA-DRA
hsa05330: Allograft rejection	5	0.0048	HLA-DRB4, HLA-DPA1, HLA-DMB, HLA-DMA, HLA-DRA
hsa05416: Viral myocarditis	6	0.0115	HLA-DRB4, ITGB2, HLA-DPA1, HLA-DMB, HLA-DMA, HLA-DRA
hsa04514: Cell adhesion molecules (CAMs)	8	0.0132	PECAM1, HLA-DRB4, ITGB2, HLA-DPA1, CLDN11, HLA-DMB, HLA-DMA, HLA-DRA
hsa05320: Autoimmune thyroid disease	5	0.0162	HLA-DRB4, HLA-DPA1, HLA-DMB, HLA-DMA, HLA-DRA
hsa04512: ECM-receptor interaction	6	0.0224	LAMA5, ITGB4, ITGA2, LAMC2, LAMC1, COL5A1
hsa05200: Pathways in cancer	13	0.0228	JUP, BMP4, AR, IL6, WNT7B, EPAS1, LAMA5, PGF, RXRA, ITGA2, LAMC2, LAMC1, CRK

Analysis of protein-protein interactions (PPI) and hub genes

In addition to the DEGs, the PPI network and hub genes were further analyzed. The interactions among the 323 DEGs were analyzed by STRING with a combined score ≥ 0.4 and visualized by Cytoscape software. The PPI network of the DEGs consisted of 278 nodes and 451 edges (Figure 5). CytoHubba analysis showed 10 hub genes: IL6, BMP4, CD74, PECAM1, HLA-DPA1, HLA-DRA, LAMC1, CTSB, SERPINA1, and CTSD (Figure 6). Of the 10 hub genes, only SERPINA1 was downregulated, while the other 9 hub genes were upregulated. The MCODE result showed the most significant module contained 11 nodes with 8 hub genes (Figure 7).

Discussion

SETDB1 is involved in heterochromatin formation and gene expression through trimethylation of H3K9 (H3K9me3). Gene amplification of SETDB1 is observed in various cancer cell types, suggesting the role of SETDB1 in carcinogenesis. However, the clinical performance of SETDB1 expression is not fully characterized, and the potential mechanism(s) of SETDB1 contributing to breast tumorigenesis remain unexplored. Here, we initially explored SETDB1 expression in BC by integrating the public online databases UALCAN and ONCOMINE. Both databases showed that the levels of SETDB1 transcription in BC were significantly higher than in normal tissues. Consistent

with the public datasets, our IHC results showed the levels of SETDB1 protein were significantly elevated and high levels were strongly associated with poor clinical prognosis. To the best of our knowledge, our study is the first to investigate the clinical characteristics of SETDB1 protein by IHC in a relatively large cohort of BC patients. To this end, we conducted different bioinformatics using the GEO microarray dataset to compare the expression profiles between SETDB1-silenced BC cells and control. Our results showed SETDB1 mainly participates in regulating antigen processing and presentation, as well as the immune network in BC. Moreover, our results indicated the function of SETDB1 in BC could be associated with the hub genes IL-6, BMP4, CD74, PECAM1, HLA-DPA1, and HLA-DRA. Taken together, our results indicated that SETDB1 overexpression was correlated with poor prognosis in BC patients and SETDB1 might be involved in BC development by its regulation of immune response.

SETDB1 is an important histone methyltransferase (HMT) specific for lysine 9 position on histone H3 and is involved in transcription repression through H3K9me3 [4,23]. The SETDB1 gene was amplified in these cancers. The SETDB1 gene exhibited increased amplification frequency in basal-like BC conditions compared with HER2 and Luminal-A and -B cancer subtypes [15]. In agreement with the public transcriptome data, a recent study reported that SETDB1 protein was significantly increased in 43 BC tissues [14]. To extend these findings, we performed IHC assay in a cohort of 159 patients with BC and showed that SETDB1 expression was associated with tumor

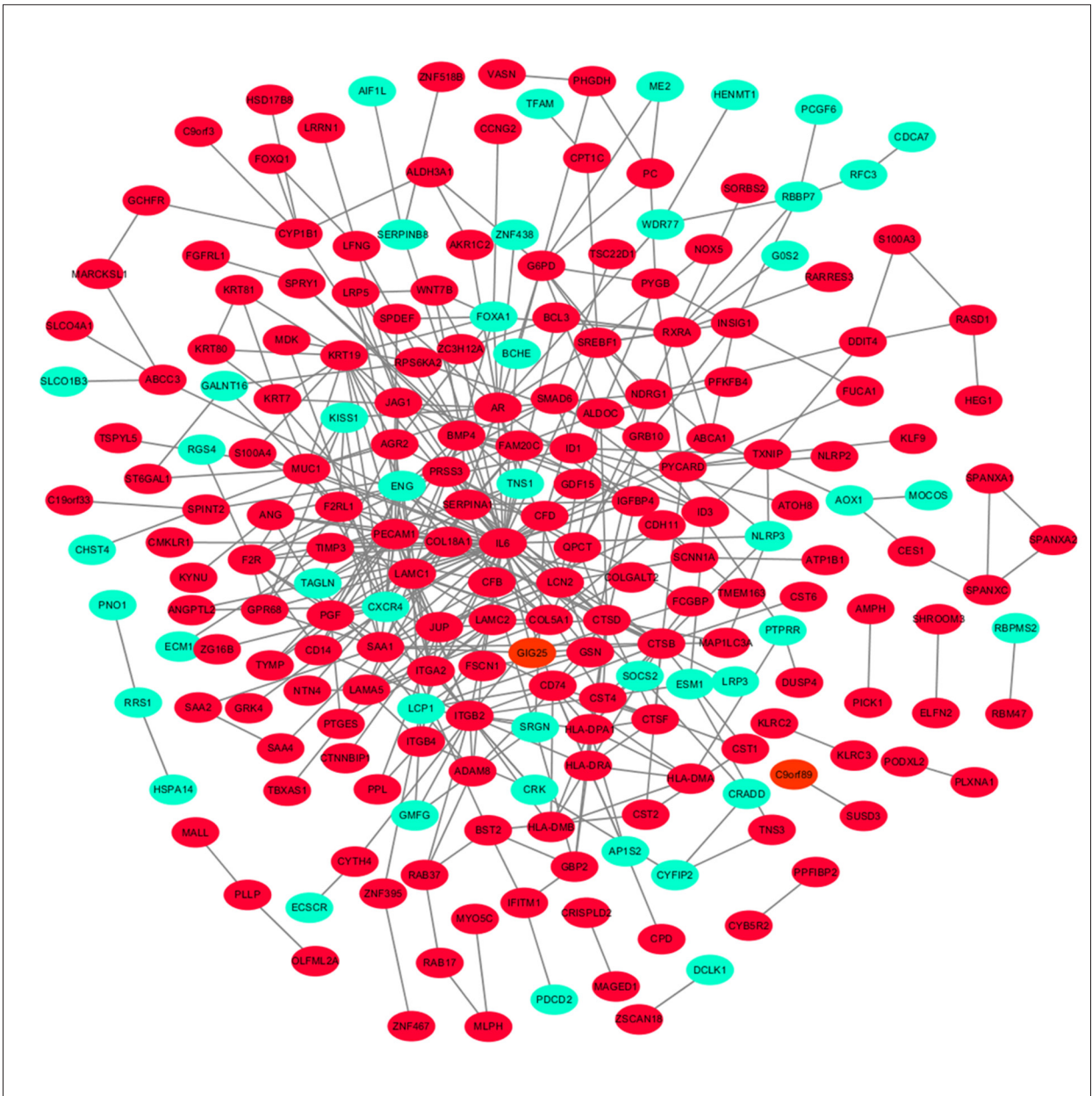


Figure 5. Protein-protein interaction network of DEGs. The green color shows the downregulated genes, while the red indicates the upregulated genes.

size ($P=0.028$), lymph node metastasis ($P<0.001$), histological grading ($P=0.012$), and clinical stage ($P<0.001$). Furthermore, SETDB1 was shown to be a prognostic biomarker for BC in subsequent survival analyses. Therefore, it appears that SETDB1 is involved in the BC progression, but its molecular mechanism needs further investigation.

According to functional enrichment analysis, SETDB1 was mainly involved in the response to external stimulus, and its closely related pathway was associated with antigen processing and presentation as well as the immune network. Consistent with

these results, Adoue et al. demonstrated that SETDB1 resulted in H3K9me3 deposition at a cell-type-specific set and ensured Th cell lineage integrity by inhibiting a repertoire of endogenous retroviruses [24]. Moreover, a recent observation showed that SETDB1 significantly prevented the expression of the proinflammatory cytokine (IL-6) through its methyltransferase activity in macrophages, and macrophage-specific Setdb1-knockout mice had higher serum interleukin-6 concentrations [25]. Additionally, the knockdown of SETDB1 interfered with the interaction between PIWIL4 and HP1 proteins, indicating SETDB1 is important for the differentiation

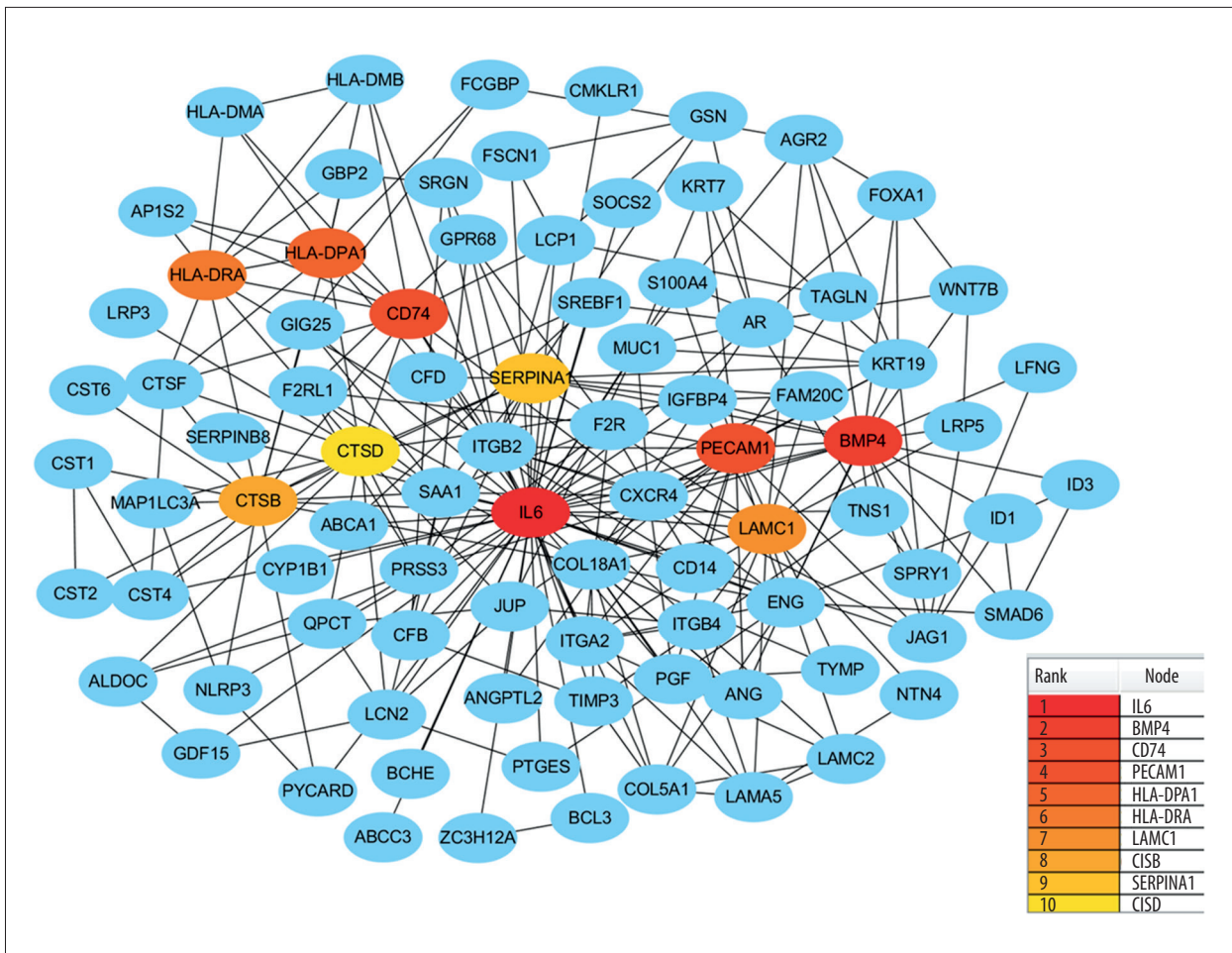


Figure 6. Identification of hub DEGs by cytoHubba plugin. The rank of connection degree is represented by different degrees of color (from red to yellow).

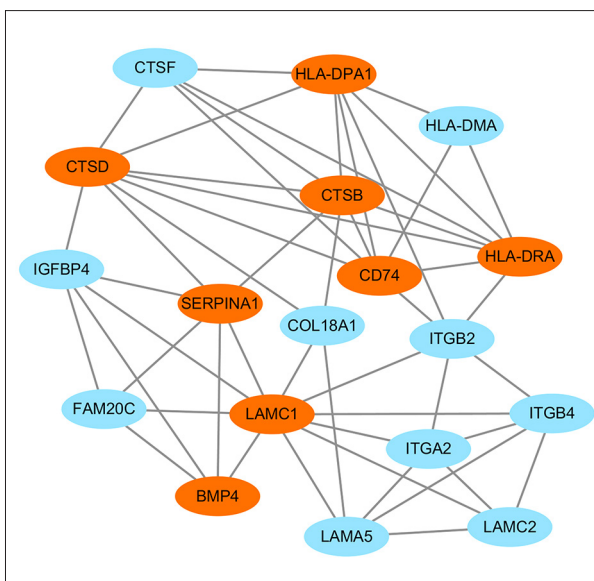


Figure 7. The significant module network analyzed by MCODE. The orange color represents hub genes.

of monocytes [26]. Thus, our results, together with the above-mentioned observations, indicate SETDB1 regulates the innate immune cells during environmental and intrinsic stress.

In addition to function enrichment, our bioinformatics analysis also showed SETDB1 was significantly associated with 10 hub genes: IL-6, BMP4, CD74, PECAM1, HLA-DPA1, HLA-DRA, LAMC1, CTSB, SERPINA1, and CTSD. Among the hub genes, IL-6, BMP4, CD74, PECAM1, HLA-DPA1, and HLA-DRA were shown to play a role in the immune response. IL-6 plays an important role in the development and progression of inflammatory responses. Our results suggest that specific knockdown of SETDB1 induced the expression of IL-6. In line with the above phenomena, Hachiva et al. reported Setdb1 induced TLR4-mediated IL-6 expression in macrophages and macrophage-specific knockout mice [25], suggesting SETDB1 is involved in IL-6 transcription and the inflammatory response in BC. Except for IL-6, the other 9 hub genes have not been shown to be linked to SETDB1. For example, CD74 is widely expressed in human immune cells, including B cells, activated T cell subsets,

monocytes, macrophages, and dendritic cells [27]. Although CD74 was originally identified as an MHC class II chaperone, accumulating evidence shows that CD74 has diverse roles in immune responses [28]. Given CD74 as a hub gene in our study, we hypothesized that the interplay between SETDB1 and CD74 has a crucial role in immune responses and thus regulates the development of BC.

Our study has certain limitations. First, we only detected the expression of SETDB1 in BC by IHC. Further validation using PCR and Western blotting should be performed in the future. Second, the biological functions of SETDB1 in BC should be investigated. Based on the biological mechanism of SETDB1 in immune response, we will focus on the SETDB1-mediated IL-6 expression in BC, which needs to be confirmed.

Conclusions

In summary, our study showed that the elevated SETDB1 expression was strongly correlated with poor prognosis in BC, and SETDB1 expression could be a promising biomarker for the prognosis of BC patients. Our future works will concentrate on identification of the hub gene that is regulated by SETDB1. By exploring the functional aspects of SETDB1 and its hub genes, we will aim to evaluate its eligibility as a potential target in BC therapy.

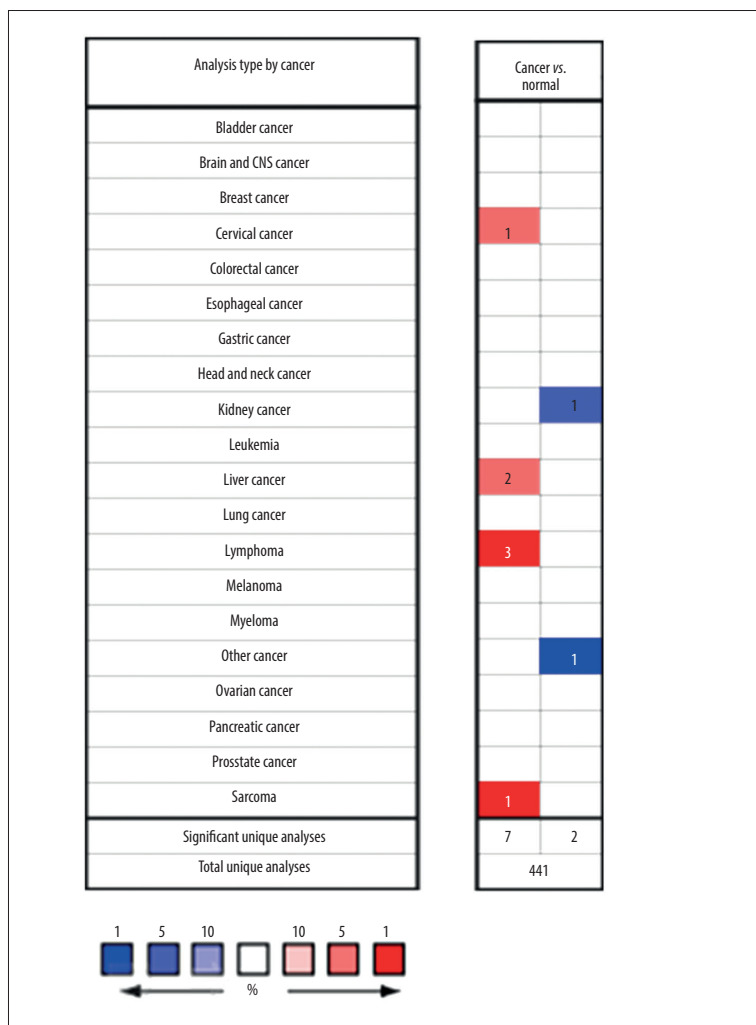
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Conflicts of interests

None.

Supplementary Data



Supplementary Figure 1. ONCOMINE database showed SETDB1 expression in various types of tumors.

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