

Expression and potential prognostic value of histone family gene signature in breast cancer

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Received December 28, 2018; Accepted October 11, 2019

DOI: 10.3892/etm.2019.8131

Abstract. Breast cancer (BC) is the most common type of malignancy among females worldwide. Histone modifications, which are the major post-translational modifications, have a significant role in cancer development and prognosis. However, whether histone family genes may serve as potential prognostic biomarkers for BC patients has remained elusive. In the present study, RNA-sequencing data were obtained from The Cancer Genome Atlas (TCGA). Differentially expressed genes were identified and Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway functional enrichment analysis was performed. As histone family genes have been reported to be associated with cervical cancer, the present study hypothesized that histone family genes are associated with gynecological tumors. Histone family genes, including histone cluster 1 H1A family member B (*HIST1H1B*), *HIST1H2AJ*, *HIST1H2AM*, *HIST1H2BI*, *HIST1H2BO*, *HIST1H3B*, *HIST1H3F*, *HIST1H3H*, *HIST1H4C* and *HIST1H4D*, were upregulated and identified as hub genes in the protein-protein interaction network. In addition, Oncomine and the Human Protein Atlas were used to further verify the expression levels of histone gene sets. The PrognScan database was then used to investigate the association between expression and prognostic value regarding cancer patient survival. The present results indicated that higher expression of histone gene sets was associated with poor overall survival, relapse-free

survival and distant metastasis-free survival of BC patients. The differential expression of histone family genes between BC and normal samples was validated by reverse transcription-quantitative PCR. Finally, to determine the clinical role of histone family genes in BC, the correlations between histone family genes expression and clinical characteristics were investigated through data collected from TCGA. Therefore, the present study indicates that histone gene sets may be used as prognostic factors for survival prediction for BC patients.

Introduction

Breast cancer (BC) is the most common type of malignancy among females and represents a serious public health issue. It is a heterogeneous disease that is the leading cause of cancer-associated death among females. For 2018, ~2.1 million newly diagnosed cases have been estimated (1). According to cancer statistics, the incidence of BC has increased from 2005 to 2014, including yearly increases by 0.3-0.4% per year among Hispanic and black females and 1.7% per year among Asians/Pacific islanders (2). Treatments include surgery, radiation and drug therapy. However, the treatment of patients with metastatic BC is challenging (3,4). Numerous biomarkers have been determined for BC but their application has rarely been implemented in clinical practice (5). Therefore, exploration of novel biomarkers for BC detection, screening, diagnosis, prognostication and treatment monitoring, is urgently required.

Epigenetic modifications are reversible and heritable processes, which are involved in mechanisms associated with the occurrence of cancer without causing any changes in the DNA sequence (6). Furthermore, epigenetic alterations may serve as biomarkers for the detection, prognosis and treatment of cancer (7). Histone modifications are the major type of epigenetic modifications (8). Normally, histone proteins with abundant lysine and arginine residues bind to negatively charged linear DNA to form nucleosomes. The histone family includes histones H1, H2A, H2B, H3 and H4. The four core histone proteins, H2A, H2B, H3 and H4, form an octamer. These histones may be modified by a large number of enzymes and are associated with multiple cancers. Histone variant H2A.Z.1 has been reported to have an oncogenic role in hepatocellular

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Key words: bioinformatics, histone family gene, breast cancer, the Cancer Genome Atlas

carcinoma via accelerating the cell cycle transition and epithelial to mesenchymal transition (9). Another previous study suggested that histone variant H2A.Z may be a novel target for BC therapy (10). The transforming growth factor- β /protein arginine methyltransferase 5/methylome protein 50 axis was indicated to regulate transcriptional activation and repression of cancer cell invasion pathways through histone H3 and H4 arginine methylation (11). Furthermore, loss of histone H4K20 trimethylation is associated with cell invasion *in vitro* and may be used as an independent marker to predict poor prognosis in BC patients (12). Although previous large-scale studies suggest that histone genes are involved in numerous types of cancer, a systematic, comprehensive analysis of histone family genes as prognostic markers in BC has not been previously performed.

In the present study, mRNA expression data of breast tumor and normal tissues were downloaded from The Cancer Genome Atlas (TCGA) database and differences in gene expression were assessed. The edgeR package of R software was used to determine significantly differentially expressed genes (DEGs). The molecular functional and pathway enrichment of these DEGs was assessed using the Database for Annotation, Visualization and Integrated Discovery (DAVID). Next, a closely connected cluster was constructed using the Molecular Complex Detection (MCODE) plug-in of Cytoscape. A previous study reported that histone family genes may serve as prognostic factors for cervical cancer and it can be hypothesized that they are associated with gynecological tumors (13). Thus, they were determined as hub genes in BC with the criterion of degrees ≥ 10 . To further validate the present results, the Oncomine online platform was used to assess the expression levels of histone family genes. In addition, the association between the expression levels and the prognostic value of histone genes in BC patients was analyzed. Finally, the differential expression of histone family genes between BC and normal samples was validated by reverse transcription-quantitative (RT-q)PCR.

Materials and methods

RNA expression data mining. The RNA sequencing data of 1,208 samples associated with breast carcinoma were obtained from TCGA (<https://cancergenome.nih.gov/>; accession date, September 14, 2018), and were retrieved using all of the following key words simultaneously: Primary site, breast; program name, TCGA; project ID, TCGA-BRCA; gender, female; workflow type, HTseq-counts; data category, transcriptome profiling; data type, gene expression quantification (14). The mRNA expression data were grouped into 1,096 BC samples and 112 normal breast tissues. These data are publicly accessible and there was no further ethical approval from the Ethics Committee.

Identification of DEGs. The DEGs between normal samples and BC were selected using the edgeR package in R (v3.5.1). EdgeR is a Bioconductor software package for selecting differences in replicated count data (15). Fold-change (FC) analysis was based on the two groups (tumor tissue and normal tissue). The DEGs were then obtained using an unpaired t-test. $P < 0.0001$ and $|\log FC| \geq 4$ were set as cut-off values based on

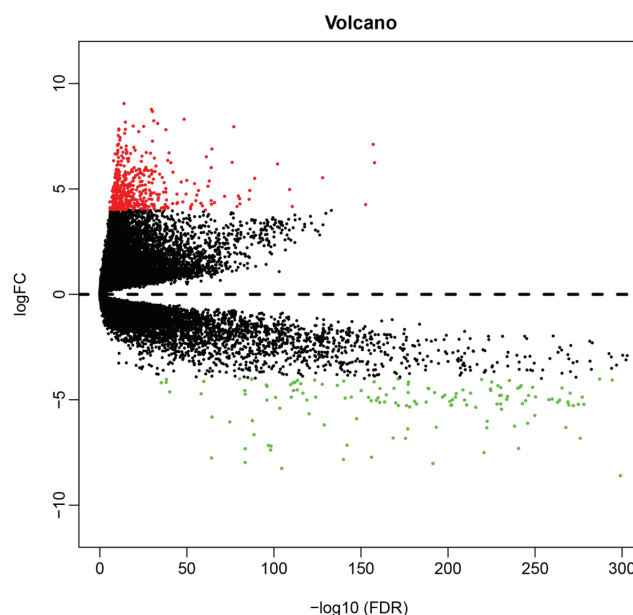


Figure 1. Volcano plots of DEGs in breast carcinoma and normal breast samples. Log FC is displayed on the y-axis and the $-\log_{10}(\text{FDR})$ on the x-axis. FDR refers to the calibrated P-value. Red and green dots represent up-regulated and down-regulated genes, respectively. $P < 0.0001$ and $|\log FC| \geq 4$ were used as cut-off criteria. DEGs, differentially expressed genes; FDR, false discovery rate; FC, fold-change.

the Benjamini-Hochberg method. A volcano plot was drawn to represent the DEGs.

Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) enrichment analyses of DEGs. GO functional enrichment analysis of DEGs and KEGG signaling pathway analysis was performed using DAVID (<https://david.ncifcrf.gov/>; version 6.8). DAVID is an online bioinformatics enrichment tool for comprehensive analysis of the functions of genes (16,17). GO enrichment analysis is an important bioinformatics tool to annotate genes accumulated in the categories 'biological process', 'molecular function' and 'cellular component' (18,19). KEGG is an encyclopedia of genes and genomes, which may be used for pathways enrichment analysis of lists of genes (20). $P < 0.05$ was set as the cut-off criterion.

Protein-protein interaction (PPI) network construction and analysis. The Search Tool for the Retrieval of Interacting Genes (STRING; <https://string-db.org/>; version 10.5) was applied to construct the PPI network (21). Furthermore, acknowledgment of interactions between proteins may provide further understanding of the complex mechanisms of tumor development. In the present study, the PPI network was built using STRING. A combined score of > 0.4 was considered to indicate statistical significance. Cytoscape (version 3.6.1), which is a software platform for bioinformatics analysis (22), was used for visualizing PPI.

Hub gene selection and analysis. In the present study, a degree of ≥ 10 was set as the criterion for selection of hub genes. MCODE (version 1.5.1) is a plugin of Cytoscape which can identify densely connected regions of a given network based on topology. The networks from STRING were visualized

Table I. GO and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis of differentially expressed genes in breast carcinoma samples.

Term	Description	Gene count	P-value
Upregulated			
GO:0032200	Telomere organization	9	6.97x10 ⁻¹⁰
GO:0044267	Cellular protein metabolic process	14	1.73x10 ⁻⁹
GO:0006335	DNA replication dependent nucleosome assembly	9	3.13x10 ⁻⁹
GO:0005576	Extracellular region	60	4.86x10 ⁻¹⁵
GO:0000786	Nucleosome	16	4.47x10 ⁻¹³
GO:0001533	Cornified envelope	10	3.60x10 ⁻⁹
GO:0046982	Protein heterodimerization activity	20	3.58x10 ⁻⁶
GO:0005198	Structural molecule activity	14	9.47x10 ⁻⁶
GO:0004890	GABA-A receptor activity	5	6.83x10 ⁻⁵
hsa05322	Systemic lupus erythematosus	16	7.53x10 ⁻¹¹
hsa05034	Alcoholism	17	4.24x10 ⁻¹⁰
hsa04080	Neuroactive ligand-receptor interaction	14	3.72x10 ⁻⁵
Downregulated			
GO:0008307	Structural constituent of muscle	13	1.43x10 ⁻¹⁶
GO:0003779	Actin binding	20	2.00x10 ⁻¹³
GO:0051373	FATZ binding	6	3.92x10 ⁻⁶
GO:0030018	Z disc	18	2.27x10 ⁻¹⁷
GO:0030017	Sarcomere	11	2.35x10 ⁻¹³
GO:0031674	I band	8	2.71x10 ⁻¹⁰
GO:0030049	Muscle filament sliding	18	1.64x10 ⁻²⁶
GO:0006936	Muscle contraction	20	9.30x10 ⁻²¹
GO:0060048	Cardiac muscle contraction	12	4.09x10 ⁻¹⁴
hsa04260	Cardiac muscle contraction	8	3.65x10 ⁻⁶
hsa04152	AMPK signaling pathway	9	1.01x10 ⁻⁵
hsa03320	PPAR signaling pathway	7	2.42x10 ⁻⁵

GO, Gene Ontology; GABA-A, γ -aminobutyric acid-a; PPAR, peroxisome proliferator-activated receptor; AMPK, AMP-activated protein kinase.

using Cytoscape and the subnetworks were drawn by MCODE. The selection criteria were set as follows: MCODE scores, >5; degree cut-off, 2; node score cut-off, 0.2; Max depth, 100; and k-score, 2.

Expression data analysis. The expression data of histone family genes in BC vs. normal tissue were obtained via the Oncomine online database (23). The parameters were set as follows: P-value < 10⁻⁴; FC, >2; and gene ranking, top 10%. The immunohistochemistry results on the expression of the histone family proteins in BC were retrieved from the Human Protein Atlas (HPA) database (24).

Survival analysis of hub genes. For survival analysis for hub genes, PrognosScan (<http://www.prognoscan.org/>) was employed, which is a useful tool for researching the biological association between gene expression and clinical prognosis based on public cancer microarray datasets (dataset numbers provided in Table SV) (25). A Cox proportional hazards model P < 0.05 was considered to indicate statistical significance and associated data were displayed in the Kaplan Meier plot.

Ethics statement and clinical specimens. The acquisition of tissue specimens for the present study was approved by the Ethical Committee of Shanghai Tenth People's Hospital (approval no. 107 SHSY-IEC-4.0/19-24/01). Each patient provided written informed consent prior to participating in the study. Fresh BC samples and para-carcinoma tissues were collected from patients who had undergone surgical resection between April and May 2019 in Shanghai Tenth People's Hospital. The authors collected samples from a total of seven patients. There were seven cancer tissues and ten normal tissues, among which three normal tissues were the repetitive tissues belonging to the seven patients (Table SI). A total of seven BC primary tumor tissues and 10 adjacent non-tumor tissues were collected.

RNA isolation and RT-qPCR. According to the manufacturer's protocols, total RNA was isolated from 10 normal breast tissues and seven BC samples using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.). A NanoDrop ONE spectrophotometer (Thermo Fisher Scientific, Inc.) was used to measure the total RNA concentration. RNA was used

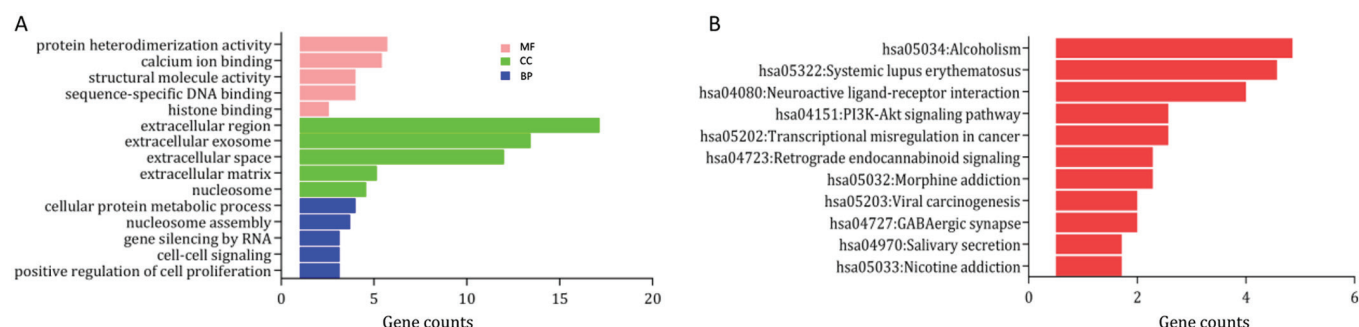


Figure 2. GO and KEGG analysis of upregulated DEGs. (A) GO analysis of upregulated DEGs. Gene counts are displayed on the x-axis and GO function enrichment on the y-axis. (B) KEGG analysis of upregulated DEGs. Gene counts are displayed on the x-axis and KEGG pathway analysis on the y-axis. KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology; DEG, differentially expressed gene; MF, molecular function; BP, biological process; CC, cellular component; Akt, protein kinase B; PI3K, phosphatidylinositol 3 kinase; GABA, γ -aminobutyric acid-a.

Table II. Subnetwork module analysis by Molecular Complex Detection plug-in.

Score	Nodes	Edges	Node IDs
17.167	37	309	MYBPC2, ACTA1, ATP2A1, HIST1H3B, TCAP, HIST1H2AI, TTN, NEB, ACTL8, HIST1H4C, HIST1H3J, HIST1H3F, HIST1H3H, HIST1H4D, MYL3, TNNC2, HIST1H4B, HIST1H4L, HIST1H2BI, TNNC1, HIST1H2AM, HIST1H1B, MYLPF, HIST1H2BO, MYL2, HIST1H3I, MYH7, TNNI1, MYL1, HIST1H2AJ, HIST1H2BM, DES, MYH1, TNNI2, TNNT3, MYH2, ACTN2
9.882	18	84	KISS1R, LIPE, FABP4, APOB, GNGT1, INS, CCKBR, TRH, OXTR, GHSR, LEP, GCG, LPL, ADRA1A, CD36, SLC2A4, ADIPOQ, GNG13
7	7	21	ASB10, ASB15, ASB11, ASB5, FBXO40, UBE2C, KBTBD10
5	5	10	GABRA5, GABRQ, GABRA3, GABRA1, GABRG2
4.5	5	9	CST4, HTN1, CST2, CST5, CST1
4	5	8	FBP2, PYGM, GYS2, PCK1, ENO3
4	4	6	LCE1F, LCE2C, LOR, LCE1A
3.333	4	5	NEUROD1, NKX2-2, PDX1, IAPP
3.333	4	5	ADH1A, ADH1B, HSD17B13, DHRS7C
3	3	3	LDB3, MYPN, MYOZ1

Node Score Cut-off: 0.2; Haircut: true; Fluff: false; K-Core: 2; Max. Depth from Seed: 100.

for first-strand cDNA synthesis in a reaction (final volume, 10 μ l) comprising 1 μ l RNA, according to the protocol of PrimeScript™ RT reagent kit (Takara Bio Inc.). The RT conditions were as follows: reverse transcription at 37°C for 5 min; inactivation of reverse transcriptase at 85°C for 5 sec; 4°C hold. qPCR was performed using the C1000 Thermal Cycler (Bio-Rad Laboratories, Inc.) in a reaction (final volume, 25 μ l) comprising 2 μ l cDNA with the following conditions: Initial denaturation for 1 cycle at 95°C for 30 sec; 40 cycles of 95°C for 5 sec and 60°C for 30 sec; PCR primer sequences are listed in Table SII. GAPDH was used as the endogenous control and the $2^{-\Delta\Delta C_q}$ method was used to analyze the relative expression levels (26).

Statistical analysis. Values are expressed as the mean \pm standard deviation. Student's t-test was used to evaluate the differences between two groups. RNA expression profiling information was used to calculate the Median (M). The Mann-Whitney U test was used to evaluate the differences between two groups in SPSS Statistics version 20.0 software (IBM Corp.). $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Identification of DEGs in BC. The gene expression data of a total of 1,208 cases, including 1,096 BC samples and

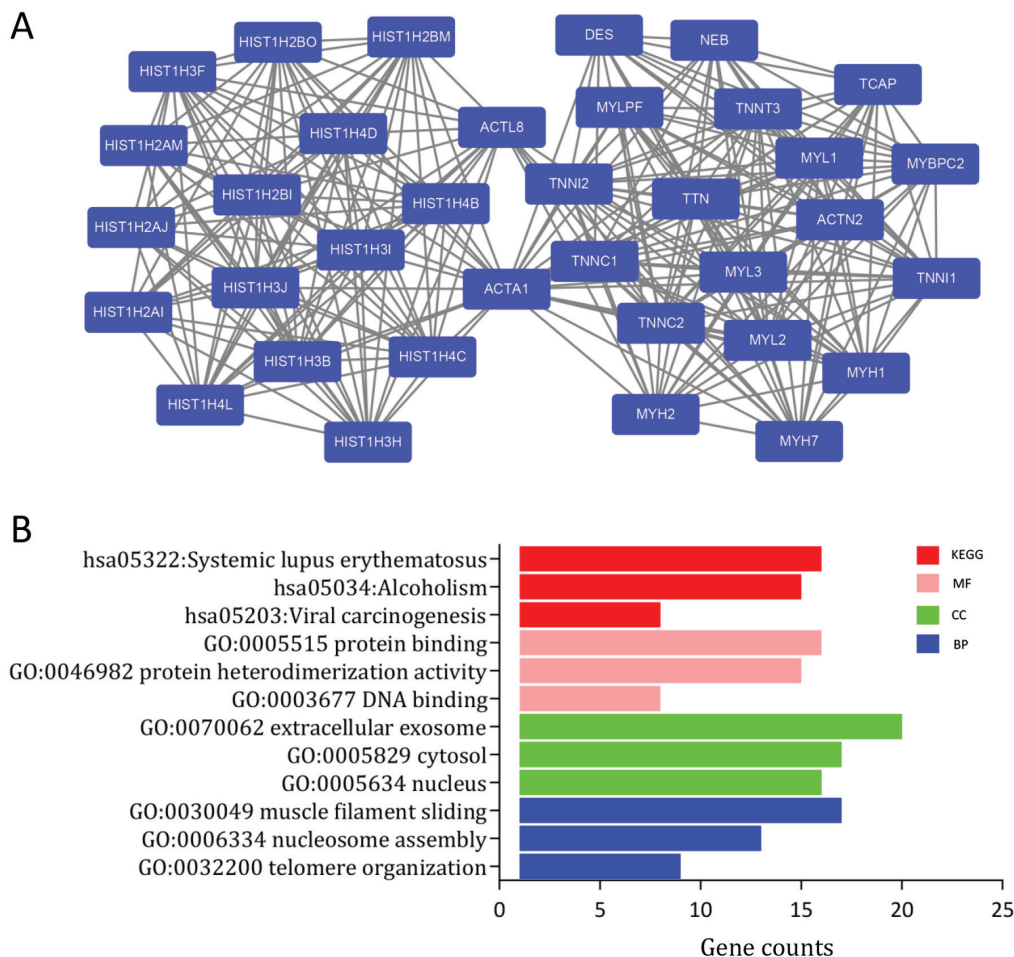


Figure 3. Sub-network construction and analysis. (A) The most significant cluster of DEGs determined using the Molecular Complex Detection plug-in. (B) GO and KEGG enrichment analysis of the most sub-cluster genes by the Database for Annotation, Visualization and Integrated Discovery online platform. KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology; DEG, differentially expressed gene; MF, molecular function; BP, biological process; CC, cellular component.

112 normal samples in multiple patients, were downloaded from TCGA. $P < 0.0001$ and $|\log FC| \geq 4$ were used as cut-off criteria. Through this analysis, a total of 525 DEGs were determined, of which 366 were upregulated and 155 were downregulated (Fig. 1).

GO and KEGG enrichment analysis of DEGs. DAVID was used to annotate the DEGs, including GO function and KEGG pathway enrichment. The results for the upregulated and downregulated genes are provided in Table I. In the category 'biological process' the upregulated DEGs were enriched in 'DNA replication-dependent nucleosome assembly', 'cellular protein metabolic process' and 'telomere organization' (Fig. 2A), and the downregulated DEGs were enriched in 'muscle filament sliding', 'muscle contraction' and 'cardiac muscle contraction' (Fig. S1A). In the category 'molecular function', the upregulated DEGs were significantly enriched in ' γ aminobutyric acid A receptor activity', 'structural molecule activity' and 'protein heterodimerization activity' (Fig. 2A), while the downregulated DEGs were enriched in 'actin binding', 'zinc ion binding', and 'structural constituent of muscle' (Fig. S1A). In addition, in the GO category 'cellular component', the upregulated DEGs were mainly enriched in the terms 'cornified envelope', 'nucleosome'

and 'extracellular region' (Fig. 2A), while the downregulated DEGs were significantly enriched in 'I band', 'sarcomere' and 'Z disc' (Fig. S1A). KEGG pathway analysis suggested that the upregulated DEGs were mainly enriched in 'systemic lupus erythematosus (SLE)', 'alcoholism' and 'neuroactive ligand-receptor interaction' (Fig. 2B), while the downregulated DEGs were mainly enriched in the 'peroxisome proliferator activated receptor signaling pathway', 'protein kinase AMP-activated catalytic subunit $\alpha 1$ signaling pathway' and 'cardiac muscle contraction' (Fig. S1B).

PPI network construction and hub gene screening. Next, the PPI network of the DEGs was constructed using STRING with visualization by Cytoscape (Fig. S2). The MCODE plug-in, which is based on topology, was used to identify close clusters in order to locate highly connected regions. The score of cluster modules are presented in Table II. The score of the most significant cluster was 17.167 and it included 37 nodes and 309 edges (Fig. 3A). Furthermore, genes involved in this module were analyzed using the DAVID online platform for GO and KEGG analysis. The results indicated that genes in this module were significantly enriched in 'SLE', 'muscle filament sliding' and 'nucleosome' (Fig. 3B, Table I).

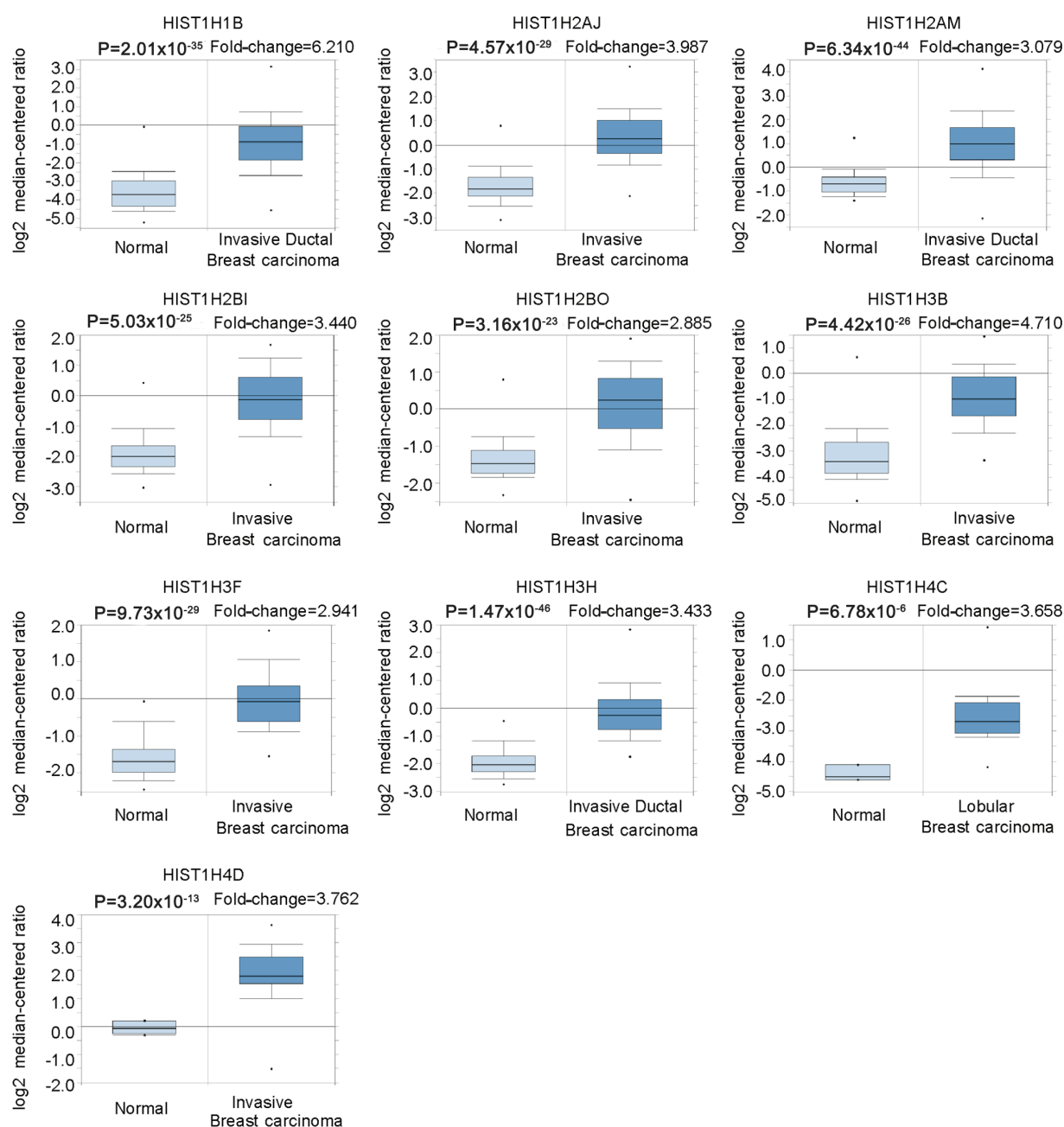


Figure 4. Expression levels of hub genes compared between different types of breast cancer and normal tissues from the Oncomine platform. Fold-changes and P-values of each hub gene are displayed in the plot.

From the MCODE plug-in, a total of 10 genes were selected as hub genes with degrees ≥ 10 . The further analysis focused on histone family genes, which were all upregulated in BC in the present results (Table SIII). The names of the hub genes were as follows: Histone cluster 1 H1 family member B (*HIST1H1B*), *HIST1H2AJ*, *HIST1H2AM*, *HIST1H2BI*, *HIST1H2BO*, *HIST1H3B*, *HIST1H3F*, *HIST1H3H*, *HIST1H4C* and *HIST1H4D*.

Hub gene analysis. Oncomine was used to further verify the expression levels of 10 hub genes in BC vs. normal breast tissues. The results indicated that the histone family genes selected were significantly upregulated in invasive breast carcinoma, invasive ductal breast carcinoma and lobular breast carcinoma, with $P < 0.05$ considered to indicate statistical

significance (Fig. 4; Table SIV). Analysis using the HPA database indicated that the 10 hub genes were slightly elevated in BC tissues (Fig. 5; Table III).

Prognostic value of histone family genes in BC. PrognScan was used to further investigate the survival of hub genes in BC patients. The present results demonstrated that a higher expression of *HIST1H2AJ* (Cox $P=0.002962$), *HIST1H2AM* (Cox $P=0.019590$), *HIST1H2BI* (Cox $P=0.019661$), *HIST1H2BO* (Cox $P=0.016204$), *HIST1H3H* (Cox $P=0.049099$) and *HIST1H4C* (Cox $P=0.012216$) were associated with poorer overall survival for BC patients. Higher expression of *HIST1H2BI* (Cox $P=0.043480$), *HIST1H2BO* (Cox $P=0.048887$), *HIST1H3H* (Cox $P=0.026703$), *HIST1H3F* (Cox $P=0.024383$) and

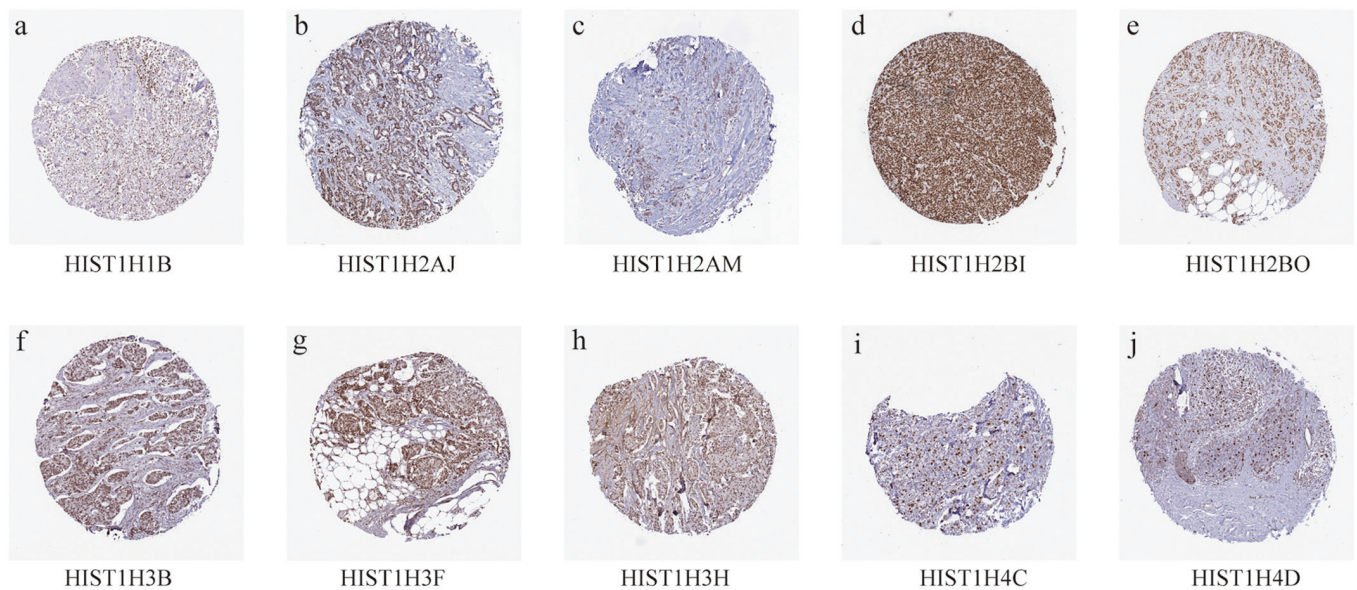


Figure 5. Immunohistochemical analysis of the histone family gene in breast cancer tissues from the Human Protein Atlas. (a) The protein expression of HIST1H1B. (b) The protein expression of HIST1H2AJ. (c) The protein expression of HIST1H2AM. (d) The protein expression of HIST1H2BI. (e) The protein expression of HIST1H2BO. (f) The protein expression of HIST1H3B. (g) The protein expression of HIST1H3F. (h) The protein expression of HIST1H3H. (i) The protein expression of HIST1H4C. (j) The protein expression of HIST1H4D. HIST1, histone cluster 1.

Table III. Immunohistochemistry analysis of histone family gene.

Gene	Patient ID	Age	Sex	Cancer type	Intensity	Quantity
HIST1H1B	1910	61	Female	Breast Duct carcinoma	Moderate	>75%
HIST1H2AJ	1939	87	Female	Breast Duct carcinoma	Strong	>75%
HIST1H2AM	2091	40	Female	Breast Duct carcinoma	Moderate	>75%
HIST1H2BI	1775	55	Female	Breast Duct carcinoma	Strong	>75%
HIST1H2BO	2115	73	Female	Breast Duct carcinoma	Moderate	>75%
HIST1H3B	2160	83	Female	Breast Duct carcinoma	Moderate	>75%
HIST1H3F	2428	75	Female	Breast Duct carcinoma	Strong	>75%
HIST1H3H	1874	80	Female	Breast Duct carcinoma	Strong	>75%
HIST1H4C	2805	59	Female	Breast Lobular carcinoma	Strong	75-25%
HIST1H4D	3546	58	Female	Breast Lobular carcinoma	Strong	<25%

HIST1, histone cluster 1.

HIST1H4D (Cox $P=0.031189$) was associated with poorer relapse-free survival. Higher expression of *HIST1H1B* (Cox $P=0.021894$), *HIST1H3H* (Cox $P=0.02670$), *HIST1H3F* (Cox $P=0.000753$), *HIST1H3B* (Cox $P=0.000020$), *HIST1H4C* (Cox $P=0.000046$), *HIST1H4D* (Cox $P=0.031189$) was associated with poorer distant metastasis-free survival (Fig. 6). Cox P-values and hazard ratios with 95% confidence intervals are displayed in Table SV.

Expression of hub genes in BC. Next, seven of the 10 hub genes were selected to analyze the expression levels in seven BC samples and 10 para-carcinoma tissues by qPCR. When compared with that in normal breast tissues, the levels of *HIST1H1B*, *HIST1H2BI*, *HIST1H2BO* and *HIST1H3F* were significantly increased in BC samples compared to paracarcinoma samples ($P=0.0016$, $P=0.0220$, $P=0.0323$ and $P=0.0184$,

respectively; Fig. 7). However, the expression levels of *HIST1H3B*, *HIST1H4C* and *HIST1H4D* in these samples were not significantly different from those in the adjacent tissues ($P>0.05$; Fig. S3).

Relationship between genes and clinical pathological parameters. A total of 1,096 BC samples from TCGA were investigated to explore the relationship between gene expression and clinical pathological characteristics. As can be seen in Table SVI, a significant difference in *HIST1H1B* was due to age ($P<0.001$), estrogen responsive (ER) growth status ($P<0.001$), progesterone responsive growth (PR) status ($P<0.001$), human epidermal growth factor 2 (HER2) status ($P=0.003$) and primary tumors (T) ($P=0.032$). BC patients in the group aged <60 (median=5.85) had an increased expression of *HIST1H1B* compared with those aged ≥ 60

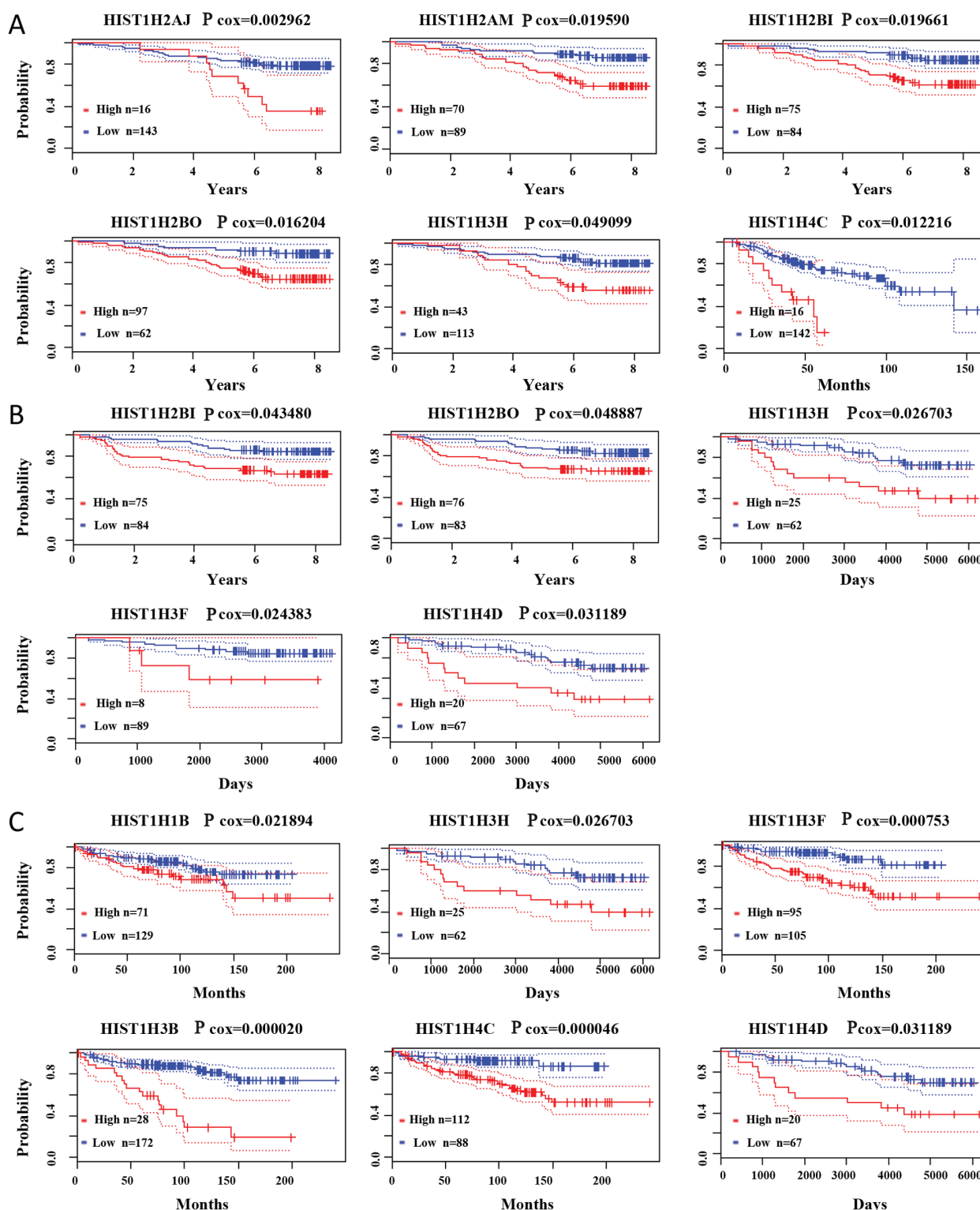


Figure 6. Association between histone family gene set expression and prognosis in breast cancer patients from the PrognScan database. Kaplan-Meier curve analysis for (A) overall survival, (B) relapse-free survival and (C) distant metastasis-free survival. Cox $P < 0.05$. HIST1, histone cluster 1.

(median=3.83). The expression of HIST1H1B in ER negative BC patients (median=7.99) was more significantly increased than that in ER positive patients (median=4.07). The expression of HIST1H1B in PR negative BC patients (median=7.32) was increased when compared with expression in PR positive patients (median=4.02). Patients at an early stage (T1-T2; median=5.15) had increased expression compared with those

at an advanced stage (T3-T4; median=4.17). As can be seen in Table SVII, a significant difference in HIST1H2BI expression was due to ER Status ($P=0.047$). ER positive BC patients (median=0.95) had increased expression of HIST1H2BI compared with the ER negative group (median=0.82). Furthermore, as is shown in Table SVIII, a significant difference in HIST1H2BO was found to be related to age

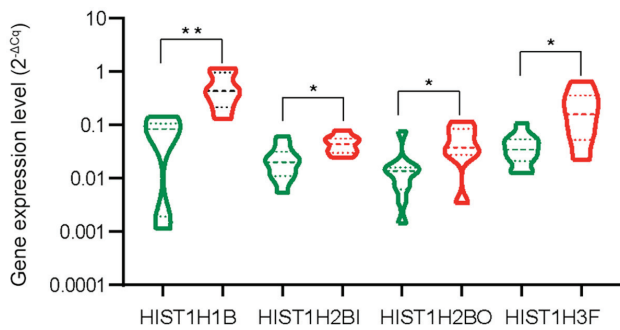


Figure 7. Violin plot of hub gene expression levels in breast cancer and normal tissues. The x-axis represents hub genes and the y-axis represents gene expression. * $P<0.05$ and ** $P<0.01$. HIST1, histone cluster 1.

($P<0.001$), ER Status ($P=0.001$), PR Status ($P=0.003$) and metastasis (M) ($P=0.001$). Patients aged <60 (median=15.95) exhibited increased expression of HIST1H2BO compared with patients aged ≥ 60 (median=11.02). The expression of HIST1H2BO in ER negative BC patients (median=17.52) was more significantly increased than that in ER positive patients (median=12.91). The expression of HIST1H2BO in PR positive patients (median=18.09) was more significantly increased than in PR negative patients (median=13.94). The expression of HIST1H2BO in patients without metastasis (median=14.31) was more significantly increased than in patients with metastasis (median=8.88). However as is revealed in Table SIX, there was no significant difference in HIST1H3F for clinicopathological parameters.

Discussion

Breast carcinoma is the most common type of malignant tumor in women worldwide. It has been classified into multiple subtypes according to the molecular status and its incidence has increased in recent years (2). Gene mutations, which may be inherited, are thought to be the most common etiological factor for BC (27). However, epigenetic reprogramming, which includes DNA methylation, histone modifications and RNA-mediated gene silencing, has gained vast interest from researchers investigating its role in BC development, drug resistance and clinical prognosis (28). Histone modifications occurring on lysine residues include acetylation, methylation, phosphorylation, sumoylation, biotinylation and ubiquitination (29).

In the present study, data were extracted from TCGA and 366 upregulated DEGs and 155 downregulated DEGs between BC and normal tissue samples were identified using bioinformatics. The PPI network of these DEGs was constructed and MCODE was used to construct clusters, which are closely and highly connected regions. The cluster with the highest score was selected and 37 genes were contained in this cluster. These genes were obviously enriched in SLE. Therefore, histone family genes were determined as hub genes in BC. Previous studies revealed that histone family genes are involved in multiple cancer types. Copy number variations of *HIST1H1B* were reported to be associated with cellular development and growth, and with proliferation in melanoma (30). *HIST1H3B*, as an amplification-dependent driver oncogene, was reported to be overexpressed in liver cancer (31). *HIST1H3F*, as a clas-

sifier gene, was indicated to be able to predict the prognosis of laryngeal cancer patients (32). Furthermore, the mutation of histone H3 variants may be a potential specific therapeutic target for diffuse intrinsic pontine glioma (33). Downregulation of histone H2A and H2B may be a possible means of reversing clinical anthracycline resistance in BC (34). In addition, histone modification profiling may provide valuable classification biomarkers and predict the risk of BC subtypes (35). Li *et al* (13) revealed that the histone family of genes may serve as prognostic factors for survival prediction in patients with cervical cancer. The authors of the present study searched PubMed and found that the use of TCGA data for histones gene family in BC has not been studied, which means data on histones has not been investigated before in flagship TCGA papers to the best of our knowledge. The present analysis indicated that histone family genes may also be used as prognostic factors for BC patients. It is suggested that the histone family of genes is closely associated with gynecological cancer types.

According to the KEGG functional pathway analysis, the set of upregulated histone variant genes were mainly enriched in the SLE pathway. Histone modification-mediated chromatin changes and gene expression have a vital role in the pathophysiology of SLE, which is a systemic autoimmune disease (36). Global histone H3 and H4 hypoacetylation were associated with active cluster of differentiation 4⁺ T cells in SLE (37). Deoxyribose-modified H2A histone bound by serum anti-DNA autoantibodies may trigger immune responses in SLE (38). Of note, an international multicenter cohort study suggested a small increased risk for cancer in general in SLE; however, a decreased risk was estimated for breast, endometrial and ovarian cancers (39). However, the specific molecular biological mechanisms of the roles of SLE pathways in BC require further study.

In the present study, 7 clinical BC and 10 adjacent non-cancerous tissues were used to examine the levels of histone members using qPCR. *HIST1H1B*, *HIST1H2BI*, *HIST1H2BO* and *HIST1H3F* expression in BC had a tendency to be upregulated, which was consistent with the results of the analysis of TCGA data. However, the small number of samples is a limitation. In further studies, larger cohorts of BC patients are required to demonstrate the prognostic value of the genes identified by analysis of in-house data.

To the best of our knowledge, the present study provides the first preliminary screening to indicate the predictive value of histone members regarding the prognosis of BC patients. Through retrieval and analysis of gene expression and survival data of multiple patients with BC, the present study enhances the understanding of histone members and their predictive value in BC prognosis. The present study provides evidence that the histone gene set may act as prognostic factors for survival in BC patients.

However, correlations between the clinical features and the histone gene set of BC have been seldom reported. The present study used a larger scale sample from TCGA breast cancer for a systematic investigation of the relationships. Therefore, based on the TCGA data, the *HIST1H1B*, *HIST1H2BI*, *HIST1H2BO* expression level in BC was related to age, ER status, PR status, HER2 status, stage, T and M.

In conclusion, the present study identified differentially expressed mRNAs in BC. Of note, histone family genes were

identified as the hub genes, which may have a significant impact on the survival and prognosis of BC patients. However, the biological function of histone family genes in BC requires further research.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Shanghai Talents Development Foundation (grant no. 2017103), the National Key R&D Program of China (grant no. 2016YFC0104303) and the National Natural Science Fund (grant no. 81771859).

Availability of data and materials

All data generated or analyzed during this study are included in this published article or are available from the stated public repository.

Authors' contributions

FY and CM designed the study. WX, JZ and PZ wrote the manuscript and analyzed the data. SQ, HZ, XF and YY performed the experiments. RL, HL, YH, YL, XY and ZL collected patient samples. All authors agree with the results and conclusions of this manuscript.

Ethics approval and consent to participate

The acquisition of tissue specimens for the present study was approved by the Ethical Committee of Shanghai Tenth People's Hospital (approval no. 107 SHSY-IEC-4.0/19-24/01). Each patient provided written informed consent prior to participating in the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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